



Does radiation interception or radiation use efficiency limit the growth of wheat inoculated with tan spot or leaf rust?



Matías Schierenbeck^{a,b,*}, María Constanza Fleitas^{a,b}, Daniel Julio Miralles^{c,d},
María Rosa Simón^a

^a Cerealicultura, Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, Av. 60 y 119, La Plata, Argentina

^b CONICET CCT La Plata, Calle 8 N° 1467, La Plata, Buenos Aires, Argentina

^c Departamento de Producción Vegetal, Cátedra de Cerealicultura, Universidad de Buenos Aires, Av. San Martín 4453, C1417DSE Buenos Aires, Argentina

^d IFEVA-CONICET, Universidad de Buenos Aires, Av. San Martín 4453, C1417DSE Buenos Aires, Argentina

ARTICLE INFO

Article history:

Received 30 January 2016

Received in revised form

12 September 2016

Accepted 19 September 2016

Available online 25 September 2016

Keywords:

Wheat

Radiation use efficiency

Radiation interception

Biomass

Pyrenophora tritici-repentis

Puccinia triticina

ABSTRACT

Tan spot [*Pyrenophora tritici-repentis* (Died.) Drechs., anamorph *Drechslera tritici-repentis* (Died.) Shoem.] and leaf rust (*Puccinia triticina* Eriks) are major diseases worldwide and some of the main biotic causes of yield and quality reduction in wheat (*Triticum aestivum* L.). Although wheat crop losses due to foliar diseases have already been studied based on an ecophysiological approach, none of these studies analyzed the independent effects of foliar pathogens with different nutritional habits. The aim of the present study was to determine the independent effects of (i) *Py. tritici-repentis* (necrotroph) and (ii) *Puccinia triticina* (biotroph) on the physiological components of biomass production: accumulated intercepted by green tissue photosynthetic active radiation (IGPAR), radiation use efficiency calculated by intercepted radiation (RUE_{int}) as well as by intercepted by green tissue (RUE_{gt}) in a wide range of Argentine commercial bread wheat cultivars growing in two field environments. Field experiments were carried out during two consecutive years combining a large range of wheat bread commercial cultivars and two levels of inoculation to promote infection of Tan spot and Leaf rust diseases, including a control without inoculation. Treatments were arranged in an experimental split-split plot design with three replications, where the main plots were both diseases, subplots corresponded to inoculation treatments 1- without inoculation (WI), 2- low concentration of inoculum of each disease (LC), 3- high concentration of inoculum of each disease (HC) and ten Argentine bread wheat commercial cultivars were the sub-subplots. Area under disease progress (AUDPC), area under percentage of non-green leaf area (AU %NGLA), crop growth rate (CGR) and healthy area duration (HAD) were calculated. Green leaf area index (GLAI), above-ground biomass (AGB), IGPAR and RUE were measured at three different crop stages (GS39, GS61 and GS82). Increases of inoculum concentration decreased AGB between 8 and 20% mainly explained by reductions in HAD and decreases on IGPAR by 14–18% with higher reductions when the crop was inoculated with *Py. tritici-repentis* than with *P. triticina*. Although both diseases reduced the physiological components of accumulated biomass related to radiation interception (IGPAR), RUE_{int} (–23%), RUE_{gt} (–11%) and CGR (–29%) were more reduced, respect to WI, when plants were inoculated with *P. triticina* compared to *Pyrenophora tritici-repentis*. The differential responses in RUE could be associated with the nutritional habit of *P. triticina* that reduces leaf nitrogen concentration, enhance assimilates consume by leaf respiration, reducing radiation use efficiency.

© 2016 Elsevier B.V. All rights reserved.

Abbreviations: AGB, aboveground biomass; IGPAR, accumulated intercepted by green tissue photosynthetic active radiation; AUDPC, area under disease progress; CGR, crop growth rate; FL, flag leaf layer; GLAI, green leaf area index; HAD, healthy area duration; HC, high concentration of inoculum; leg, interception efficiency by green tissue; IPAR, intercepted photosynthetic active radiation; LC, low concentration of inoculum; %NGLA, proportion of non-green leaf area; RUE_{int}, intercepted radiation use efficiency; RUE_{gt}, intercepted by green tissue radiation use efficiency; WI, control without inoculation.

* Corresponding author at: Cerealicultura, Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, Av. 60 y 119, La Plata, Argentina.

E-mail addresses: m.schierenbeck@hotmail.com, matt_svp2@hotmail.com (M. Schierenbeck), constanzafleitas@gmail.com (M.C. Fleitas), miralles@agro.uba.ar (D.J. Miralles), mrsimon@agro.unlp.edu.ar (M.R. Simón).

<http://dx.doi.org/10.1016/j.fcr.2016.09.017>

0378-4290/© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Wheat (*Triticum aestivum* L.) is grown in most regions of the globe due to its importance as a food source, and its enormous genetic phenological plasticity (Slafer and Rawson, 1994). Airborne diseases, and particularly leaf rust (*Puccinia triticina* Eriks) and tan spot [*Pyrenophora tritici-repentis* (Died.) Drechs., anamorph *Drechslera tritici-repentis* (Died.) Shoem.] are the main foliar diseases in Argentina and in many wheat production areas in the world, causing important yield and quality reduction in this crop. Leaf rust, is the most common rust disease of wheat (Bolton et al., 2008; Huerta-Espino et al., 2011). The fungus is an obligate parasite that cannot carry over from one season to the next on seed, stubble or soil. The most important sources of inoculum are susceptible volunteer wheat plants growing during the summer/autumn known as the 'green bridge'. The urediniospores produced by *P. triticina* can be wind-disseminated and infect host plants hundreds of kilometers from their source plant, which can result in wheat leaf rust epidemics on a continental scale (Bolton et al., 2008). Leaf rust requires moisture (rain or heavy dew) or high humidity for spores to germinate and infect leaves (Beard et al., 2015).

Unlike *P. triticina*, the principal sources of inoculum of tan spot are the seeds, the straw and collateral hosts (Rees and Platz, 1980; Wright and Sutton, 1990). For inoculum dispersal and infection development, temperature among 10–30 °C and moisture among 6–48 h are needed (Sah, 1994). The increasing inoculum of this pathogen has been attributed to the use of conservation tillage systems, shorter crop rotations, continuous wheat cultivation and use of susceptible cultivars (Moreno et al., 2012). Disease severity and time of infection depends on the presence of inoculum, carried over from last season (leaf rust) and/or present in the stubble (tan spot); favorability of seasonal conditions; pathotype virulence and varietal susceptibility (Cotuna et al., 2015). For example, under reduced tillage, where infested residues with *P. tritici-repentis* remains, the onset of tan spot epidemic occurs earlier than under conventional tillage (Mehta and Gaudencio, 1991). Both diseases alter the formation of different yield components, depending on the crop developmental stage at which infection occurs (Madden and Nutter, 1995) as well as the duration and severity of the epidemic (Robert et al., 2004; Serrago et al., 2009). Under severe infections of *P. triticina* yield losses can be up to 50% (Huerta-Espino et al., 2011), whereas under *Py. tritici-repentis* infections losses can range between 3 and 53% (Rees and Platz, 1983), 17–27% (Simón et al., 2011) and 27–42% (Wegulo et al., 2012).

Yield can be described by the cumulated biomass throughout the growing period and the proportion of biomass that is partitioned to the grain (Harvest index). Biomass production depends on the ability of the canopy to (i) intercept the incident radiation, which is a function of leaf area index (LAI) and canopy architecture (extinction coefficient $-k-$) and (ii) the efficiency at which the solar energy is converted into biomass (radiation use efficiency-RUE-) (Miralles and Slafer, 1997; Reynolds et al., 2005). Damage to leaf tissue through infection by foliar pathogens modifies plant's carbon metabolism due to reductions in the capacity of the crop to intercept and absorb the photosynthetic active radiation (IPAR-IGPAR) and/or as a consequence of reductions in RUE, response that is associated with the nutritional habit of the pathogen (Boote et al., 1983; Johnson, 1987; Bingham et al., 2009). According to their nutritional relationship with the host, fungal pathogens can be classified as necrotrophs, biotrophs or hemi-biotrophs (Oliver and Ipcho, 2004). Necrotrophs (like *Py. tritici-repentis*) destroy tissue during the colonisation by fungal hyphae through the secretion of toxins and cell wall degrading enzymes (PtrToxA, PtrToxB and PtrToxC), leading to a loss of green area and shrinkage of the leaf surface (Gooding et al., 2000; Wegulo, 2011). When necrotic regions are retained on leaves, they continue to intercept some light but

without contributing to photosynthesis determining reductions in IGPAR (Dimmock and Gooding, 2002). On the other hand, biotrophs (like *P. triticina*) derive their resources for growth and sporulation from living host cells (Voegelé and Mendgen, 2011), altering source-sink relations within the leaf and directing host nutrients to the fungal mycelium (Scholes and Rolfe 2009; Bancal et al., 2012; Ney et al., 2013). The host response to biotrophs tends to be more complex than that of necrotrophs. Infected leaves by biotrophs generally determine increases in respiration rate, reductions in the rate of photosynthesis and losses of chlorophyll (McNew, 1960; Scholes and Rolfe, 1995; Robert et al., 2005; Carretero et al., 2011) that could be associated with decreases on RUE. Most of the studies conducted to analyze the effect of foliar diseases in wheat have documented detrimental effects on IPAR/IGPAR but not in RUE, however, these studies have been focused on the effect of a complex of diseases (Serrago et al., 2009; Carretero et al., 2010) or natural infections (Bryson et al., 1997; Bancal et al., 2007), so it is difficult to ascertain the individual effects of a necrotroph pathogen (*Py. tritici-repentis*) or a biotroph pathogen (*P. triticina*) on the IGPAR and RUE. In the present study, both pathogens were inoculated independently to assess the individual effects of each disease on the physiological attributes related to biomass accumulation in wheat.

Traditionally, diseases management decisions on wheat are based on the use of thresholds constructed from empirical relationships relating the percentage of reduction of yield and the level of disease in a given time, often calculated based on incidence, severity and AUDPC parameters (Gaunt, 1995; McRoberts et al., 2003; Serrago et al., 2009). The problem that arises from this view is that they do not consider ecophysiological aspects related to biomass and yield formation (LAI, GLAI, % non green leaf area -%NGLA-), limiting the extrapolation to a wide range of genotypes, environments, years and locations (Gaunt, 1995; Bryson et al., 1997; Paveley et al., 1997; Savary et al., 2006). An eco-physiological approach focused on the impact of pathogens with different nutritional habits (biotrophs-necrotrophs) on attributes associated with the biomass production could be useful to improve the quantification and modelling of crop losses and thus contribute to the management of these diseases. Several authors reported that the use of this approach to predict the diseases effects is more accurate and robust than those models that only consider a phytopathological perspective (Waggoner and Berger, 1987; Serrago et al., 2009; Carretero et al., 2010). However, well-known models as WHEAT-PEST (Willcoquet et al., 2008) does not include the effects of tan spot and consider only partially the effects of leaf rust. We hypothesize that *Pyrenophora tritici-repentis* is mostly a light stealer reducing radiation interception while *Puccinia triticina* is a light stealer and also removes soluble assimilates from host, therefore it reduces the green LAI (lesion area + virtual lesion area) and outflows assimilates from the pool of assimilates, processes that could be associated not only with reductions in intercepted radiation but also in RUE.

The present study was designed to determine the effects of independent artificial inoculations of foliar diseases with different nutritional habit 1) *Pyrenophora tritici-repentis* (necrotroph) and 2) *Puccinia triticina* (biotroph) on the physiological components of biomass production: Accumulated intercepted by green tissue photosynthetic active radiation (IGPAR) and radiation use efficiency (RUE) in bread wheat crops.

2. Materials and methods

2.1. General conditions

Field experiments were carried out in 2012 and 2013 at the Experimental Station J. Hirschhorn, Faculty of Agriculture and Forestry Sciences, National University of La Plata, Province of

Table 1
Environmental conditions during the wheat growing seasons of 2012 and 2013 at Los Hornos, La Plata, Argentina.

	Temperature (°C)			Humidity (%)		Radiation (Watt/m ²)		Precipitation (mm)		
	2012	2013	1969–2009	2012	2013	2012	2013	2012	2013	1969–2009
June	10.0	10.0	10.7	77	84	1759	1937	22.2	10.4	54.3
July	7.1	9.8	10.3	71	87	2210	1860	13.4	46.0	62.1
August	12.3	10.0	10.7	89	73	1803	2990	222.8	7.2	59.2
September	13.6	11.9	13.5	88	84	3334	2967	52.4	150.8	67.9
October	16.6	16.1	16.5	90	83	3594	4263	192.0	28.8	96.1
November	20.1	18.9	19.3	82	83	5191	4775	41.2	140.6	98.6
December	22.6	24.2	22.3	84	71	5641	6052	143.6	30.3	80.6

Buenos Aires, Argentina (34° 52' LS; 57° 58' LO). The soil was a Typic Argiudoll according to the USDA taxonomy (Soil Survey Staff, 1999). Analysis of the soil samples (top-0.20 m) indicated the following values by weight: organic matter 3.3%; N 0.167%; P 16.1 mg kg⁻¹, and pH 6.15. Incident global radiation, mean daily temperature, relative humidity and total monthly precipitation during the experimental periods were recorded in a standard meteorological station located ca. 200 m away from the experimental site (Table 1).

2.2. Treatments and experimental design

In each year, treatments consisted of a combination of three levels of infection (including a control without inoculation) with tan spot (*Py. tritici-repentis*) and leaf rust (*P. triticina*) diseases inoculated separately in 10 bread wheat commercial varieties. The experimental design was a split-split-plot with three replications. Main plots were the diseases: 1- tan spot and 2- leaf rust. Inoculation treatments were considered as subplots: 1- without inoculation, protected treatment (WI), 2- unprotected treatment infected with a low concentration of inoculum (LC), 3- unprotected treatment infected with a high concentration of inoculum of each causal pathogen (HC). The sub-sub-plots corresponded to ten Argentine wheat cultivars with similar heading date (late heading), widely used in the Argentine wheat belt including varieties from moderately resistant (MR) to moderately susceptible (MS) to both diseases. The varieties were: Klein Yará (MR to leaf rust and tan spot), Klein Guerrero (MR to leaf rust and tan spot), Baguette 11 (MS to leaf rust and tan spot), Baguette 17 (MS to leaf rust, MS-MR to tan spot), Baguette 18 (MS to leaf rust, MR to tan spot), ACA 303 (MR to leaf rust, MS to tan spot), ACA 315 (MR to leaf rust, MS to tan spot), BioINTA 3004 (MR to leaf rust, MS to tan spot), SY100 (MS to leaf rust, MR to tan spot) and Sursem LE2330 (MR to leaf rust and tan spot). Sowing was carried out in mid-June with a seeding rate of 250 seeds m⁻² using conventional tillage. Plots were 7.7 m² (5.50-m long by 1.4-m wide), containing 7 rows (0.2 m between rows). The entire experiment was fertilized with 50 kg P ha⁻¹ as calcium triple superphosphate and 50 kg N ha⁻¹ as urea at the time of sowing plus 50 kg N ha⁻¹ as urea at Growth Stage (GS) 30 (Zadoks et al., 1974). Weed control was made by Glyphosate (2 L ha⁻¹) 15 d before sowing. In addition, Misisil® herbicide [(metsulfuron metil, dry flowable 60% + Dicamba (Dimethylamine 2-Metoxi-3,6 diclorobenzoic acid) soluble liquid 57.1%, Dupont, Rosario, Argentina)] at 100 cm³ Dicamba + 6.7 g metsulfurón metil in 120 L water ha⁻¹ was applied at the three-leaf stage (GS13). The application of insecticides was not required for these trials as the incidence of insects was not important throughout the crop cycle. Fertilization with K and micronutrients is not an usual practice in our wheat region as deficiencies are not common.

2.3. Inoculation treatments

A mixture of virulent isolates of *Drechslera tritici-repentis* provided by the Laboratory of Cereals, Faculty of Agriculture and

Forestry Sciences, National University of La Plata was used to prepare the inoculum. The isolates were grown on V8 media at 23 °C +/- 2 °C with 12 h alternating light and dark cycles (Raymond and Bockus, 1982). The inoculum was prepared by aseptically scraping sporulating colonies with a scalpel and suspending conidia in deionized water. The conidial suspension was adjusted to 3 × 10² spores mL⁻¹ (LC) and 3 × 10³ spores mL⁻¹ (HC) using a Neubauer hemacytometer. The highest concentration is usually recommended by several authors (Jordahl and Francl, 1992; Ali and Francl, 2003), thus, the lowest concentration was selected as ten times less than the highest to obtain a big difference between both concentrations. One milliliter of Tween 20 per liter was added as a surfactant. Plants were sprayed with the inoculum suspension until runoff (800 mL per plot). Leaf rust was artificially promoted using a mixture of virulent races (provided by Pablo Campos, INTA Bordenave-Argentina) using talc as a vehicle. Border rows of susceptible cultivars (Baguette 10 and Baguette 21) were dusted with 0.5 mg spores per plant as HC (Shtaya, 2015) and 0.2 mg spores per plant on LC, in order to generate a gradient of disease between inoculation treatments. Oat plots (*Avena sativa* L.) were sown between subplots to avoid the spread of inoculum between inoculated and non-inoculated treatments. The main plots were spaced at a distance of at least 200 m to minimize the transfer of inoculum. For both pathogens, inoculations were done at the beginning of tillering (GS 21) and at the beginning of shoot development (GS 31). After inoculations, plants were kept moist by spraying with water several times a day with sprinklers during a period of two days. In the protected sub-plots (WI), fungicide Orquesta Ultra™ (fluxapyroxad 50 g/L, epoxiconazole 50 g/L and pyraclostrobin 81 g/L) was applied at tillering (GS 23) and at flag leaf (GS39) using an application rate of 140 L ha⁻¹ with a dose of 1.2 L ha⁻¹ product.

2.4. Measurements and calculations

To estimate the effects of foliar diseases on biomass production, samples of aboveground biomass (AGB) along 1.5 m were taken from central rows of the plots at particular developmental stages: flag leaf (GS39); anthesis (GS61) and early dough stage (GS82) and weighted. An approximate 100 g sub-sample was oven-dried at 60 °C for 72 h and dry weights determined to relate with the total weight sample. The severity (necrotic and chlorotic area of wheat leaves) of the two main diseases, leaf rust or tan spot, in each plot was estimated visually on seven randomized selected plants per plot on the upper six leaves at GS 39, on the upper four leaves at GS61 and on the upper leaf at GS 82. The area under disease progress curve (AUDPC) which is estimated by the formula

$$AUDPC = \sum_{i=1}^{n-1} \left(\frac{X_i + X_{i+1}}{2} \right) (t_{i+1} - t_i) \text{ where } X_i = X(t_i), n \text{ is the number}$$

of assessments, X is the disease severity (%) and (t_{i+1} - t_i) is the interval between two consecutive assessments was calculated to summarize the progress of the disease in the three evaluations (Shaner and Finney, 1977).

The total leaf area index (TLAI) was estimated by counting the main stems and tillers in two linear meters from each plot and measuring the length and wide of all leaves with at least a portion of green tissue of a total of seven tillers. The individual leaf area was calculated multiplying length and wide affecting by a correction factor of 0.835 (Miralles and Slafer, 1990). Through estimates of severity, green leaf area index (GLAI) and the proportion of non-green leaf area index (%NGLA) were calculated at GS39, GS61 and GS82. GLAI and %NGLA were used to estimate the healthy area duration (HAD) and area under proportion of non-green leaf area index [AU (%NGLA)], estimated in the same way as AUDPC but using %NGLA instead of the disease severity (Waggoner and Berger, 1987).

The proportion of incident PAR intercepted was measured at GS39, GS61 and GS82. For this purpose, a lineal ceptometer (1 m) was located 20 cm above the top of the canopy (I_o) and at ground level (I_t) at five positions parallel to the rows in order to minimize canopy disturbance (Gardner and Auma, 1989; Slafer et al., 1990; Miralles and Slafer, 1997). The intercepted photosynthetic active radiation (PRI) was calculated as the ratio of the difference between incident radiation (I_o) and (I_t) to (I_o). The amount of radiation intercepted by the crops each day was calculated as the product of PRI and incident global radiation. Considering severity determinations, interception efficiency by green tissue (I_{eg}) was calculated as the percentage of radiation intercepted by functional tissue (GLAI) of total incident radiation assuming an equal loss of green area from each layer, being the formula for a given time ($I_{eg} = PRI * [1 - \% \text{Severity}/100]$) as it was described by Bergamin Filho et al. (1997). By adding the daily data, accumulated intercepted PAR (IPAR) and accumulated intercepted PAR by green tissue (IGPAR) for the whole crop cycle (GS1–GS82), for the flag leaf-anthesis period (GS39–GS61) and for the grain filling period (GS61–GS82) were calculated, assuming a linear interpolation between evaluations.

RUE (gMJ^{-1}) was calculated as the slope of the regression between accumulated biomass at every developmental stage evaluated (GS39, GS61 and GS82) and IPAR (RUEint) or IGPAR (RUEgt). Crop growth rate at GS39–GS82 (CGR) was calculated as the slope of the regression between the accumulated biomass at GS39, GS61 and GS82 and the days from emergence to the date of these evaluations and expressed as g day^{-1} .

2.5. Statistical analysis

Data were analyzed by ANOVA for a split-split plot design using GenStat 12th Edition software. Means were compared using the LSD test ($P=0.05$). Data fitted normal distribution, for that reason they were not transformed. In spite of the risk of false positives, for some variables LSD test ($P<0.1$) was considered as significant, due to visible important agronomic differences between traits.

3. Results

3.1. Area under disease progress (AUDPC), area under percentage of non-green leaf area (AU %NGLA) and healthy area duration (HAD)

AUDPC was significantly influenced by the Years, Pathogens, Inoculation treatments, Cultivars and Years \times Pathogens, Years \times Cultivars, Inoculation \times Cultivars, Years \times Pathogens \times Cultivars and Years \times Pathogens \times Inoculation interactions (Table 2).

AUDPC showed higher levels in 2012 than in 2013. In both years, the WI treatment had lower values of AUDPC, differing significantly from LC and HC, however, WI also showed diseases infection by tan spot and leaf rust probably due to the high level of induced inoculum in the plots as a consequence of artificial inoculations. In 2012, AUDPC for *Py. tritici-repentis* was 40% higher in HC than

in WI, while the AUDPC for *P. triticina* was 25% higher in the HC respect to the WI treatment. In 2013, the plots inoculated with *Py. tritici-repentis* presented 81% (HC) more infection than WI, while the HC treatments inoculated with *P. triticina* showed an increase of 64% when compared to WI. In both years, the AUDPC values for leaf rust were higher than for tan spot in the WI treatment (Fig. 1).

AU (%NGLA) was influenced by the Years, Inoculation treatments, Pathogens, Cultivars and Years \times Inoculation treatments, Years \times Cultivars, Inoculation \times Cultivar and Pathogens \times Cultivars interactions (Table 2). *Py. tritici-repentis* increased the AU (%NGLA) between 31% (LC) and 42% (HC), respect to the WI treatment (1299), while *P. triticina* increased AU (%NGLA) up to 27% (HC) compared to WI (1623).

The interaction Pathogen \times Inoculation \times Cultivar was not significant, despite the higher increases in the AU (%NGLA) with the inoculum application in some cultivars as Baguette 17 and Baguette 18 with both pathogens (Fig. 2a and b)

HAD was influenced by Inoculation treatments, Cultivars and Years \times Pathogens, Pathogens \times Inoculation, Years \times Cultivars, Pathogens \times Cultivars, Inoculation \times Cultivars, Year \times Pathogens \times Inoculation and Year \times Pathogens \times Cultivars interactions (Table 2)

In both years, increases in the inoculum concentration decreased HAD. HC inoculation with *Py. tritici-repentis* reduced HAD by 36% (2012) and 41% (2013) respect to the control (WI). When the same treatment, i.e. HC, was compared using *P. triticina* inoculum, HAD decreased 32% (2012) and 32% (2013) (Fig. 3).

The Years \times Pathogens \times Cultivar interaction for HAD showed differences between *P. triticina* and *Py. tritici-repentis* in five genotypes. ACA 303, Baguette 11 and BioINTA 3004 showed lower HAD when inoculated with *Py. tritici-repentis* in 2012, while ACA 303, ACA 315 and SY 100 showed lower HAD under *P. triticina* inoculations in 2013 (data not shown).

Taking into account an average over years and cultivars, increases in the inoculum concentration of both pathogens decreased GLAI at all the leaf layers evaluated during the crop cycle, with more significant reductions when the crop was inoculated with *Py. tritici-repentis*, mainly on the lower leaf layers. At GS39, GLAI was reduced by 26% (FL); 26% (FL-1); 30% (FL-2); 39% (FL-3) and 70% (FL-4) in HC respect to the WI treatment when inoculated with *Py. tritici-repentis*. At the same growth stage, GLAI decreased 22% (FL); 21% (FL-1); 21% (FL-2); 29% (FL-3) and 48% (FL-4) in the HC treatment compared to WI when plants were inoculated with *P. triticina*.

A similar trend was detected on GS61. Under *Py. tritici-repentis* inoculations, GLAI of each leaf layer decreased by 33% (FL), 38% (FL-1), 49% (FL-2) and 75% (FL-3) on the HC when compared to the WI treatment. On the other hand GLAI was reduced by 32% (FL), 33% (FL-1), 44% (FL-2), and 26% (FL-3) due inoculations with HC of *P. triticina* respect to the WI treatment. Decreases on GLAI on FL layer were also detected in GS82. Taking into account an average over years, GLAI reduction on FL at GS82, was similar in both diseases ca. 70% when HC was compared to the WI treatment. Results showed slight differences between pathogens at all growth stages on GLAI reductions at FL, however those differences were higher for *Py. tritici-repentis* in the lower leaves when HC was contrasted to WI.

3.2. Crop growth rate (CGR), accumulated intercepted PAR (IPAR), accumulated intercepted by green tissue PAR (IGPAR) and radiation use efficiency

The CGR for GS39–GS82 period was significantly influenced by the Pathogens, Inoculation treatments, Cultivars and Years \times Pathogens interaction (Table 3).

Increments on inoculum concentration of both pathogens reduced crop biomass at all growth stages evaluated ($P<0.001$).

Table 2

Means square (values $\times 10^3$) and p-value (ANOVA) of the area under disease progress (AUDPC), area under percentage of non-green leaf area (AU %NGLA) and healthy area duration (HAD) for the three inoculations treatments of *Py. tritici-repentis* and *P. triticina* on ten Argentine wheat cultivars during two years.

Source of variation	D.F	AUDPC ($\times 10^3$)	AU %NGLA ($\times 10^3$)	HAD ($\times 10^3$)
Years (Y)	1	42443.98 (0.008)	86114.48 (0.004)	20.85 (0.358)
Error A	2	348.62	310.09	14.87
Pathogens (Pa)	1	3484.14 (0.023)	7178.75 (0.011)	0.01 (0.965)
Y \times Pa	1	182.04 (0.023)	377.37 (0.358)	44.51 (0.054)
Error B	4	270.11	350.29	6.08
Inoculation (In)	2	794.33 (<0.001)	8163.25 (<0.001)	148.91 (<0.001)
Y \times In	2	113.65 (0.59)	415.05 (0.004)	0.01 (0.967)
Pa \times In	2	41.54 (0.315)	132.01 (0.113)	1.84 (0.060)
Y \times Pa \times In	2	106.81 (0.068)	74.13 (0.273)	1.78 (0.064)
Error C	16	33.41	52.61	0.54
Cultivars (Cu)	9	946.29 (<0.001)	1068.84 (<0.001)	6.78 (<0.001)
Y \times Cu	9	587.71 (<0.001)	930.08 (<0.001)	3.92 (<0.001)
Pa \times Cu	9	58.24 (0.180)	135.54 (0.003)	0.72 (0.071)
In \times Cu	18	124.49 (<0.001)	104.57 (0.004)	0.60 (0.089)
Y \times Pa \times Cu	9	92.23 (0.019)	77.65 (0.104)	1.43 (<0.001)
Y \times In \times Cu	18	114.86 (<0.001)	103.75 (0.004)	0.50 (0.223)
Pa \times In \times Cu	18	32.84 (0.697)	47.13 (0.463)	0.26 (0.858)
Y \times Pa \times In \times Cu	18	21.93 (0.939)	49.97 (0.397)	0.39 (0.497)
Error D	216	40.96	47.21	0.40
Total	359			

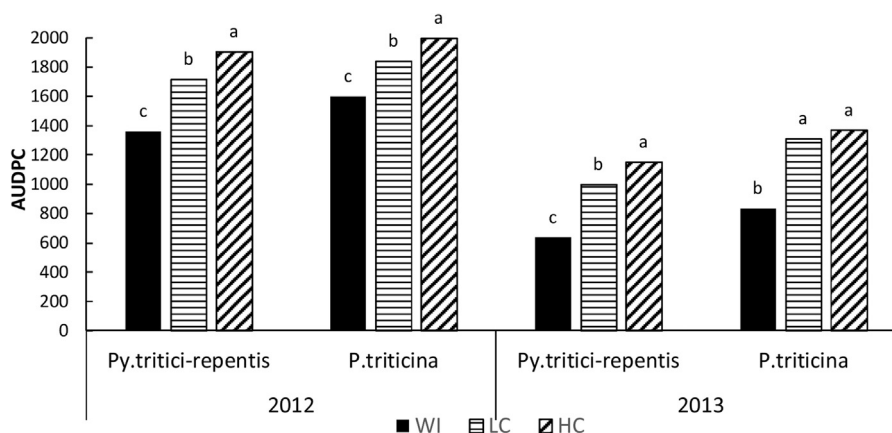


Fig. 1. Area under disease progress for Years \times Pathogens \times Inoculation treatment interaction. WI: Protected treatment, without inoculation; LC: Unprotected treatment with low concentration of inoculum and HC: Unprotected treatment with high concentration of inoculum of each pathogen. Means followed by the same letter within the same year and pathogen are not statistically different (LSD, $P < 0.10$).

Table 3

Means square and p-value (ANOVA) of intercepted photosynthetic active radiation (IPAR), intercepted photosynthetic active radiation by green tissues (IGPAR), intercepted radiation use efficiency (RUEint), intercepted radiation use efficiency by green tissues (RUEgt) and Crop growth rate (CGR) between GS39 and GS82 in an experiment with three inoculations treatments of *Py. tritici-repentis* and *P. triticina* on ten Argentine wheat cultivars during two years.

Source of variation	D.F	IPAR GS1–GS82 ($\times 10^3$)	IGPAR GS1–GS82 ($\times 10^3$)	RUEint	RUEgt	CGR GS39–GS82
Years (Y)	1	3426.49 (0.046)	1845.88 (0.051)	0.00 (0.995)	3.69 (0.367)	3.82 (0.753)
Error A	2	169.53	102.49	0.64	2.77	29.41
Pathogens (Pa)	1	93.10 (0.394)	212.71 (0.21)	24.19 (0.002)	33.48 (0.017)	4517.15 (<0.001)
Y \times Pa	1	2.69 (0.879)	23.82 (0.644)	2.22 (0.089)	4.53 (0.223)	331.23 (0.019)
Error B	4	102.24	95.82	0.44	2.18	23.15
Inoculation (In)	2	219.02 (<0.001)	436.54 (<0.001)	3.07 (0.018)	2.53 (0.286)	876.22 (0.001)
Y \times In	2	52.23 (0.022)	44.43 (0.001)	0.17 (0.751)	2.33 (0.314)	36.40 (0.655)
Pa \times In	2	34.33 (0.067)	38.45 (0.002)	0.35 (0.556)	2.55 (0.283)	27.46 (0.725)
Y \times Pa \times In	2	9.27 (0.439)	0.43 (0.902)	0.02 (0.962)	0.20 (0.896)	18.36 (0.806)
Error C	16	10.69	4.16	0.58	1.87	83.81
Cultivars (Cu)	9	73.12 (<0.001)	84.85 (<0.001)	0.43 (0.086)	3.25 (<0.001)	109.85 (0.001)
Y \times Cu	9	24.32 (<0.001)	30.54 (<0.001)	0.35 (0.185)	2.84 (<0.001)	61.75 (0.065)
Pa \times Cu	9	11.67 (<0.001)	8.11 (0.005)	0.40 (0.124)	1.35 (0.099)	50.28 (0.154)
In \times Cu	18	2.23 (0.862)	6.56 (0.005)	0.06 (0.99)	0.54 (0.845)	12.07 (0.993)
Y \times Pa \times Cu	9	6.37 (0.063)	4.89 (0.107)	0.24 (0.466)	0.63 (0.639)	23.53 (0.713)
Y \times In \times Cu	18	2.04 (0.905)	4.42 (0.1)	0.13 (0.93)	0.60 (0.761)	23.24 (0.822)
Pa \times In \times Cu	18	1.84 (0.941)	1.26 (0.982)	0.13 (0.944)	0.63 (0.726)	20.74 (0.888)
Y \times Pa \times In \times Cu	18	2.03 (0.907)	2.51 (0.654)	0.09 (0.993)	0.33 (0.984)	16.03 (0.967)
Error D	216	3.47	2.99	0.25	0.81	33.84
Total	359					

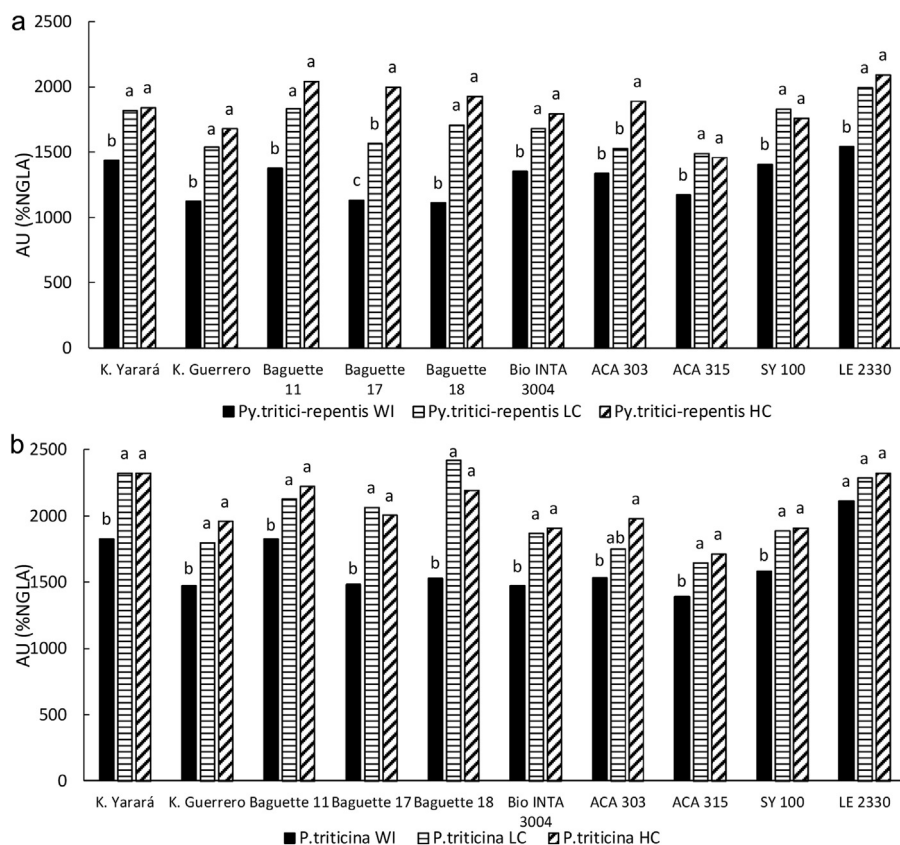


Fig. 2. (a and b) Means of area under proportion of non-green leaf area index [AU (%NGLA)] for Pathogen \times Cultivars \times Inoculation treatments interaction for a) *Py. tritici-repentis* and b) *P. tritici*. WI: Protected treatment, without inoculation; LC: Unprotected treatment with low concentration of inoculum of each pathogen; HC: Unprotected treatment with high concentration of inoculum of each pathogen. Means followed by the same letter within the same cultivar and pathogen indicate not statistically differences between inoculation treatments (LSD, $P < 0.05$).

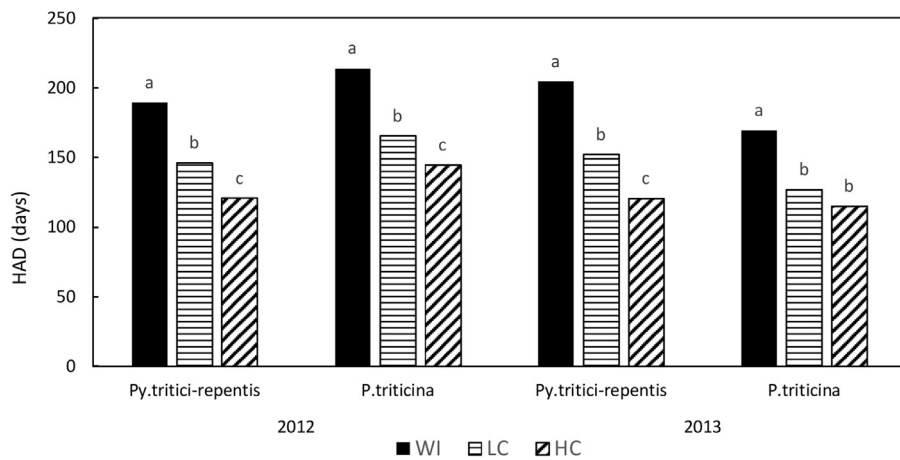


Fig. 3. Healthy area duration (HAD) for Years \times Pathogens \times Inoculation treatment interaction. WI: Protected treatment, without inoculation; LC: Unprotected treatment with low concentration of inoculum of each pathogen; HC: Unprotected treatment with high concentration of inoculum of each pathogen. Means followed by the same letter within the same year and pathogen are not statistically different (LSD, $P < 0.05$).

In relative terms, AGB at GS39 was reduced 11.5% in HC in relation to the WI treatment. At GS61 and GS82 diseases reduced AGB ca. 8 and 20% in LC and HC, respectively, when compared to WI (Fig. 4).

CGR was significantly reduced by *P. tritici* compared to *Pyrenophora tritici-repentis* in both years and taking into account an average over inoculations treatments and cultivars. During 2012, *P. tritici* decreased CGR 39% (22.8 g day^{-1} vs. 13.8 g day^{-1}), while reductions of 25% (20.7 g day^{-1} vs. 15.5 g day^{-1}) were detected on 2013, when compared to *Pyrenophora tritici-repentis* inocula-

tions. High concentration of inoculum reduced CGR on the period GS39–GS82 by ca. 29% and 24% respect to the WI treatment, when the crop was inoculated with *P. tritici* and *Py. tritici-repentis*, respectively (Fig. 5).

IGPAR and IPAR were significantly influenced by main effects and Years \times Inoculation treatments, Pathogens \times Inoculation treatments, Years \times Cultivars and Pathogens \times Cultivars interactions were detected (Table 3). In addition, significant differences were detected in Inoculation treatments \times Cultivars interaction for

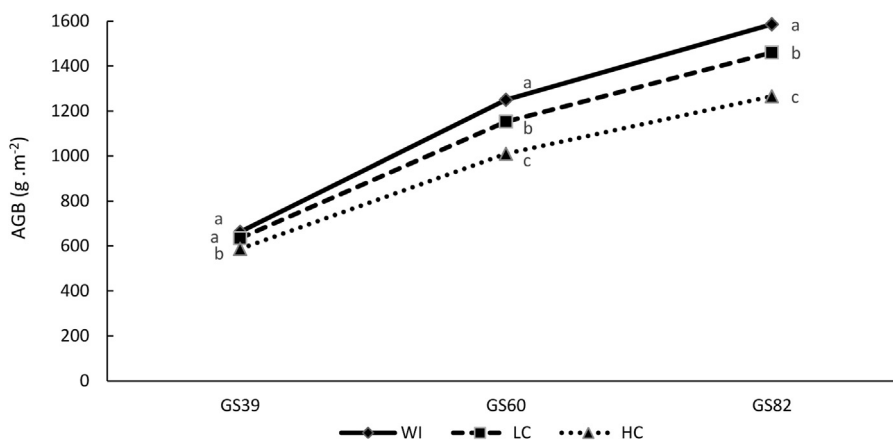


Fig. 4. Aboveground biomass (AGB) at three growth stages under three inoculations treatments (g m^{-2}) (means of plots inoculated with *P. triticina* and *Py. tritici-repentis*). WI: Protected treatment, without inoculation; LC: Unprotected treatment with low concentration of inoculum of each pathogen; HC: Unprotected treatment with high concentration of inoculum of each pathogen. Means followed by the same letter within each growth stage are not statistically different (LSD, $P < 0.001$).

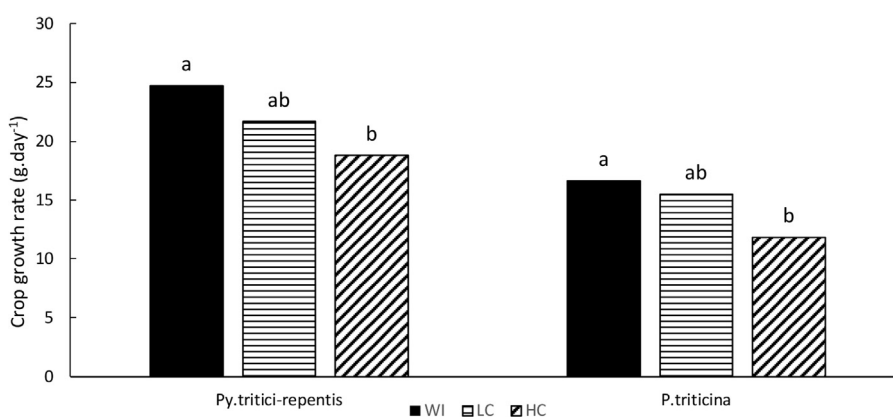


Fig. 5. Means of Crop growth rate (CGR) during GS39–GS82 (g day^{-1}) for Pathogens \times Inoculation treatments interaction. WI: Protected treatment, without inoculation; LC: Unprotected treatment with low concentration of inoculum of each pathogen; HC: Unprotected treatment with high concentration of inoculum of each pathogen. Means followed by the same letter within each pathogen indicate not significant differences between inoculum concentration treatments (LSD, $P < 0.05$).

IGPAR, while the triple interaction Years \times Pathogens \times Cultivars significantly influenced the IPAR (Table 3). RUEint was significantly modified by pathogens, inoculum and, in less degree, by cultivars while RUEgt was mostly affected by pathogens and cultivars (Table 3).

IPAR and IGPAR during GS1–GS82 period showed a decline with increases in the inoculum concentration of the pathogens in both years. In 2012, the IPAR showed a decrease of 5% in HC, compared to WI (905.8 MJ m^{-2}), while IGPAR was reduced 6% (LC) and 14% (HC) with respect to the WI (624.2 MJ m^{-2}). In 2013, IPAR was reduced 5% (LC) and 11% (HC), when compared to WI treatment (1146.2 MJ m^{-2}), while IGPAR showed decreases of 14% (LC) to 18% (HC) respect to WI (811.1 MJ m^{-2}) (data not shown).

Pathogens \times Inoculation interaction showed reductions on IPAR when HC was compared to WI treatment for the two tested pathogens. *Py. tritici-repentis* reduced up to 10% IPAR (i.e. 1046 MJ m^{-2} on WI vs. 940 MJ m^{-2} on HC), while reductions by *P. triticina* were ca. 6% (i.e. 1006 MJ m^{-2} on WI vs. 945 MJ m^{-2} on HC) (Table 3). IGPAR was reduced between 11% (LC) and 20% (HC), respect to the WI treatment under *Py. tritici-repentis* inoculations, while non-differences on IGPAR were detected under *P. triticina* inoculations (Table 3, Fig. 6).

Under *Py. tritici-repentis* inoculations IPAR (MJ m^{-2}) and AGB (g m^{-2}) for the GS1–GS82 period in the cultivars considered in the present study, ranged from $1031 \text{ MJ m}^{-2}/1550 \text{ g m}^{-2}$ (INTA 3004) to $1102 \text{ MJ m}^{-2}/1806 \text{ g m}^{-2}$ (Klein Yará) on WI treat-

ment. For the LC treatment both IPAR and AGB ranged from $993 \text{ MJ m}^{-2}/1260 \text{ g m}^{-2}$ (INTA3004) to $1075 \text{ MJ m}^{-2}/1682 \text{ g m}^{-2}$ (K. Yará) and from $920 \text{ MJ m}^{-2}/1123 \text{ g m}^{-2}$ (INTA3004) to $969 \text{ MJ m}^{-2}/1445 \text{ g m}^{-2}$ (Baguette 18) on HC treatment. When *P. triticina* was the primary pathogen IPAR and AGB ranged from $1036 \text{ MJ m}^{-2}/1357 \text{ g m}^{-2}$ (SY100) to $1055 \text{ MJ m}^{-2}/1761 \text{ g m}^{-2}$ (K. Yará) on WI treatment; while when diseases appeared the ranges for IPAR and AGB were from $1000 \text{ MJ m}^{-2}/1277 \text{ g m}^{-2}$ (SY100) to $1026 \text{ MJ m}^{-2}/1597 \text{ g m}^{-2}$ (K. Yará) on LC treatment and between $800 \text{ MJ m}^{-2}/1104 \text{ g m}^{-2}$ (LE2330) and $1013 \text{ MJ m}^{-2}/1378 \text{ g m}^{-2}$ (Klein Yará) on HC treatment (Fig. 7). Fig. 7 shows, considering all cultivars, that for both diseases the higher the inoculum concentration the lower the IPAR and AGB as a general trend, however, when both diseases are compared for the same range of values of IPAR the AGB observed for *P. triticina* was lower than that observed for *Pyrenophora tritici-repentis*. Thus, averaging across the genotypes in both years, the RUEint values were 1.38 and 1.09 g MJ^{-1} for LC and HC, respectively when crops were infected by *P. triticina*, and 1.77 and 1.68 g MJ^{-1} for LC and HC respectively when infected by *Pyrenophora tritici-repentis*.

In line to that described above, RUEint and RUEgt were significantly reduced by *P. triticina* in both years. *P. triticina* decreased RUEint between 36% (2012) and 21% (2013), while RUEgt was reduced 25% (2012) and 13% (2013) respect to *Pyrenophora tritici-repentis* inoculations (Fig. 8). For RUEgt, there was a significant

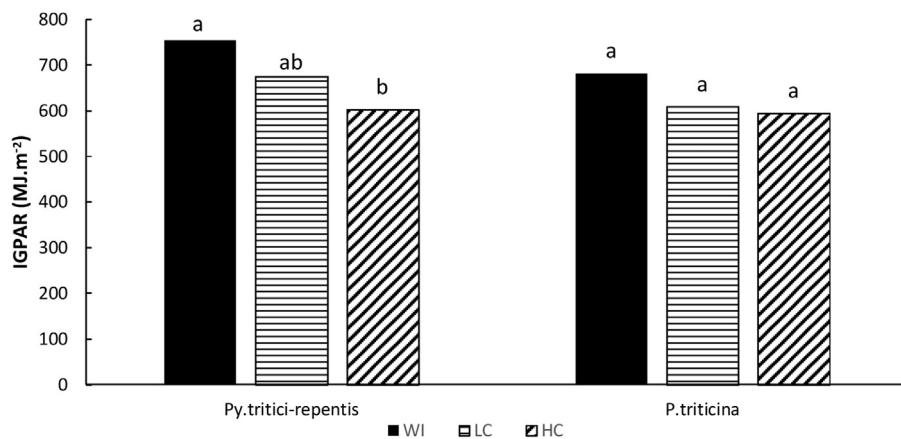


Fig. 6. Means of accumulated intercepted by green tissue photosynthetic active radiation (IGPAR) during GS1-GS82 (MJ m^{-2}) for Pathogens \times Inoculation treatments interaction. WI: Protected treatment, without inoculation; LC: Unprotected treatment with low concentration of inoculum of each pathogen; HC: Unprotected treatment with high concentration of inoculum of each pathogen. Means followed by the same letter within each pathogen are not statistically different (LSD, $P < 0.05$).

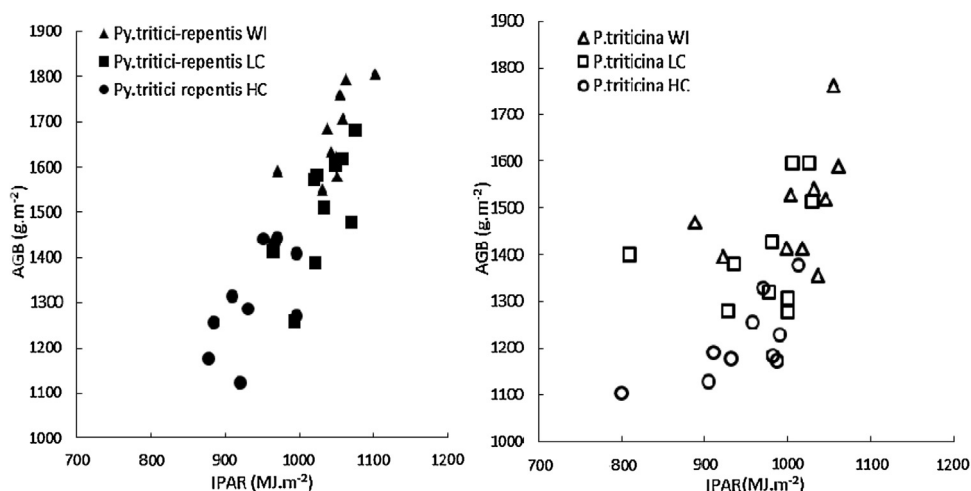


Fig. 7. Aboveground biomass (g m^{-2}) at GS82 vs. IPAR (MJ m^{-2}) for the GS1–GS82 period for *Py. tritici-repentis* (left) and *P. triticina* (right). WI: Protected treatment, without inoculation (triangles); LC: Unprotected treatment with low concentration of inoculum of each pathogen (squares); HC: Unprotected treatment with high concentration of inoculum of each pathogen (circles).

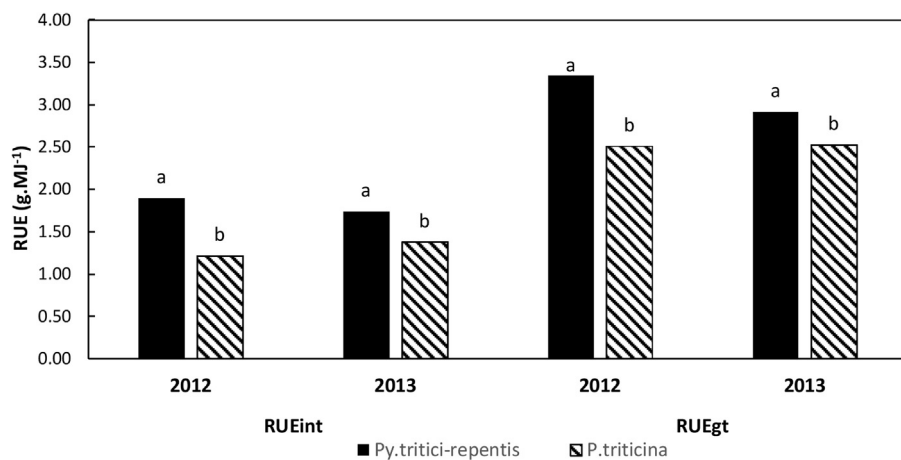


Fig. 8. Intercepted radiation use efficiency (RUEint) and intercepted radiation use efficiency by green tissue (RUEgt) for Years \times Pathogens interaction (g MJ^{-1}). Means followed by the same letter within each year and variable are not statistically different (LSD $P < 0.10$).

Pathogen \times Cultivar interaction because there was a tendency to a higher reduction with *P. triticina* in some cultivars (data not shown).

Although Pathogen \times Inoculation interaction was not significant, both RUEint and RUEgt showed a tendency to higher reductions under *P. triticina* inoculations. When *P. triticina* was

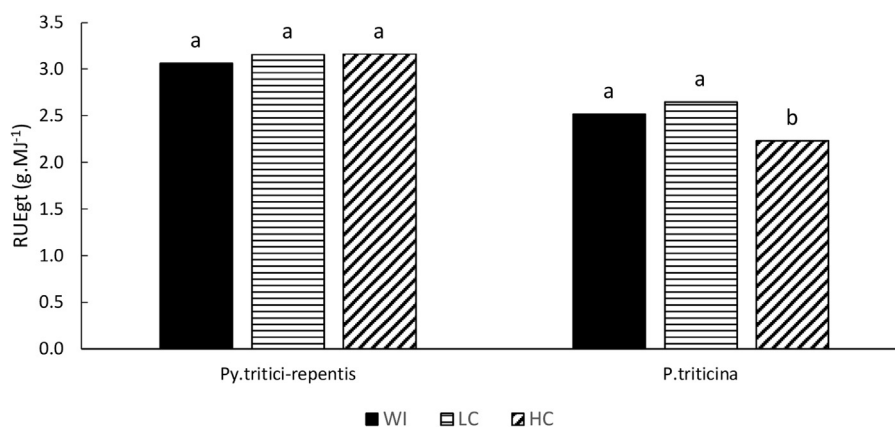


Fig. 9. Means of intercepted by green tissue radiation use efficiency (g MJ^{-1}) for Pathogens \times Inoculation treatments interaction. WI: Protected treatment, without inoculation; LC: Unprotected treatment with low concentration of inoculum of each pathogen; HC: Unprotected treatment with high concentration of inoculum of each pathogen. Means followed by the same letter within each pathogen are not statistically different (LSD, $P < 0.05$).

the primary pathogen, the HC treatment decreased 11% and 23% the values of RUEgt and RUEint, respectively. However, *Py. tritici-repentis* inoculation did not produce reductions on the RUEgt (Fig. 9) and decreased 16% RUEint when HC was compared to WI (data not shown).

As was described in Fig. 7 the genotypes followed a similar trend when AGB was plotted against IPAR, however, genotypic variation were detected on RUEint and RUEgt, showing higher reductions when plots were inoculated with *P. triticultura* than with *Py. tritici-repentis* for both inoculum levels. Genotypic variations in RUEint (g MJ^{-1} intercepted) for WI ranged from 1.76 (ACA303) to 2.12 (Baguette 17 and SY100). When plots were inoculated with LC, RUEint ranged from 1.34 (INTA3004) to 2.01 (Klein Yará) while under HC the range of RUEint was between 1.46 (INTA3004) and 1.93 (Baguette 18) when *Py. tritici-repentis* was evaluated. Under *P. triticultura* inoculations, RUEint fluctuated between 1.04 (SY100) to 1.81 (Klein Yará) on WI treatment; from 0.92 (SY100) to 1.64 (Baguette 18 and LE2330) on LC and from 0.86 (SY100) to 1.24 (ACA315) when HC treatment was considered. Similar trend was evident for RUEgt (g MJ^{-1} intercepted by green tissue) where values under *Py. tritici-repentis* oscillated between 2.79 (INTA3004) and 3.59 (LE2300) for WI, while for the LC treatment the values ranged from 2.39 (INTA3004) to 3.68 (Klein Yará) and from 2.51 (ACA315) to 3.82 (Baguette 17) on the HC treatment. When plants were inoculated with *P. triticultura*, RUEgt values ranged for WI between 1.84 (SY100) and 3.24 (Klein Yará); from 1.88 (SY100) to 3.79 (LE2330) for LC treatment and between 1.73 (SY100) and 2.74 (Klein Yará) for HC treatments.

4. Discussion

In this study independent artificial inoculations with *Py. tritici-repentis* and *P. triticultura* were done to ensure a better separation, than under natural infection, of their effects on the physiological components of biomass (IPAR, IGP, RUE). The profile of the infection under this type of artificial inoculations (especially in necrotrophs) may vary respect to natural conditions, where the infection starts in the infected stubble (Rees and Platz, 1980; Wright and Sutton, 1990) and splashed from lower to upper leaves during the crop cycle (Duveiller et al., 2005). For that reason, early inoculations during the crop cycle (GS21 and GS31) were done to simulate the natural conditions of infections. For rusts, where the primary inoculum comes from the dispersion of the spores originated in weeds or other crops, the profile of natural and artificial inoculations are similar (Eversmeyer and Kramer, 1992).

In line to that reported by Campbell and Madden (1990) and Wegulo et al. (2012), climatic variations between years played a major role in the epidemiological development of both diseases evaluated. In this sense, in 2012 climatic conditions were more favorable for disease development during post-anthesis than in 2013, due to increases in rainfall, relative humidity and higher average temperatures. The weather conditions promoted higher levels of AU (%NGLA) and AUDPC by increases in the rate of leaves senescence, determining lower values of IPAR and IGP on GS1–GS2 in 2012 than in 2013. The area under proportion of non-green leaf area index was similar in all cultivars for *Py. tritici-repentis*. For *P. triticultura*, Baguette 11, Baguette 17 and Baguette 18, which were reported as the most susceptible, showed higher AU (%NGLA), although some others cultivars reported as moderately resistant, as Klein Yará and LE 2330, also showed high values AU (%NGLA). This could be caused by the fact that the inoculum was prepared with a mix of races, chosen on the basis of their virulence to some cultivars. In this work, in both levels of inoculation, *Py. tritici-repentis* and *P. triticultura* reduced IGP by affecting LAI, GLAI and HAD compared to the protected treatment, although the HC level of inoculation produces more damage in those traits than LC. In both levels of inoculum concentration diseases produced early leaf senescence in main stems as well as in tillers (Lim and Gaunt, 1986; Waggoner and Berger, 1987; Whelan et al., 1997) although the highest reductions in the number of tillers was found in the HC (–12.5%) compared to WI treatment (data not shown). Those findings are in line with Bancal et al. (2007) who studied natural infections of *P. triticultura* and *S. tritici* and with evidence reported by Serrago et al. (2009) who evaluated a complex of diseases composed by *P. triticultura*, *Py. tritici-repentis* and *S. tritici*. Consequently, it is expected that independently of the differences in genotypic resistance, foliar diseases cause losses in efficiency of interception during the crop cycle. Moreover, in the diseased genotypes, part of the radiation intercepted by the crop is captured by diseased or senescent leaf area (%NGLA) without photosynthetic activity decreasing HAD. Sharma et al. (2004) and Rosyara et al. (2006) showed reductions in HAD under natural infections of *Bipolaris sorokiniana* and *Py. tritici-repentis*. Thus, the reduction in IGP, due to the loss of radiation interception by green and functional leaf area, reduce the crop growth rate and thereby the dry matter accumulation (Carretero et al., 2010). The results of the present study demonstrated that both pathogens, and especially *Py. tritici-repentis*, reduced the physiological components of radiation interception (IPAR and IGP) associated with losses of green area (GLAI and HAD), the shrinkage of the leaf surface and an acceleration of leaf senescence due to destruction of the tissues during the colonization by fungal hyphae through the secretion of toxins and

cell wall degrading enzymes (Gooding et al., 2000; Wegulo, 2011). However, when RUE was considered, *P. triticina* showed higher reductions than *Py. tritici-repentis*. The effect of foliar diseases on RUE presents contrasting results in the literature. As it was shown for rust in this work, in some pathosystems photosynthetic activity of the remained healthy tissues can be reduced affecting negatively RUE (Rabbinge et al., 1985; Bastiaans, 1991) even with greater effects than that observed in IPAR (Madeira et al., 1994; Béasse et al., 2000; Shah et al., 2004). Although some evidences (Johnson 1987; Olesen et al., 2003; Robert et al., 2005; Bingham et al., 2009) showed differential and negative effects of the diseases on RUE, others reports did not show effects on RUE under natural infections or a complex of foliar pathogens in wheat (Bryson et al., 1997; Bancal et al., 2007; Serrago et al., 2009; Carretero et al., 2010). Part of the explanation for the controversial results in the literature respect of the differential effects of the diseases on RUE could be associated with the type of pathogen that determines the infection. In our work, and especially under HC treatment, *P. triticina* reduced RUE in a higher magnitude than *Py. tritici-repentis* supporting that the differential effects on the physiological attributes that determine biomass accumulation throughout the crop cycle are dependent on the type of pathogen that produces the infection. Thus, despite that *Py. tritici-repentis* produced higher negative effects on HAD, IPAR and IGPARG than *P. triticina*, the latter reduced more the RUE, suggesting that *P. triticina* affects more negatively the photosynthetic activity of the green sectors of healthy tissues in damaged leaves than *Py. tritici-repentis*. This is in line to that reported in various pathosystems by Rabbinge et al. (1985) (*Erysiphe graminis*-wheat) and Bastiaans (1991) who studied the effect of *Pyricularia oryzae* C. in rice (*Oryza Sativa* L.) and Rossing et al. (1992) who reported detrimental effects on photosynthetic activity under leaf rust and powdery mildew on cereals. The decreases on RUEint and RUEgt under *P. triticina* inoculations could be due to the particular interaction that biotrophic pathogens established with the host cells (Voegelé and Mendgen, 2011). This interaction produces important changes in the physiology of the host, infecting living cells and causing reductions in the photosynthetic capacity of leaves, increasing respiration rate and decreasing the rate of assimilates translocation of the affected organs (McNew, 1960; Boote et al., 1983; Zuckerman et al., 1997; Robert et al., 2004). According to Dimmock and Gooding (2002), the lack of effects of necrotrophic pathogens on RUE could be explained by their limited action on photosynthesis, since *P. tritici-repentis*, differently that occurs with *P. triticina*, do not interact with living cells. Thus, biotrophic pathogens as *P. triticina* are characterized not only by a reduction in green leaf area accelerating leaf senescence but also by a significant reduction in the photosynthesis apparatus decreasing assimilates production that can be used for the synthesis of dry matter (Gooding et al., 2000; Carretero et al., 2011).

In coincidence with Robert et al. (2004), Bancal et al. (2007) and Carretero et al. (2010) increases in the inoculum concentration decreased GLAI at all the leaf layers evaluated during the crop cycle, with more significant reductions on the lower leaf layers (FL-4, FL-3 and FL-2) under *Py. tritici-repentis* inoculations, while *P. triticina* infection pattern tended to be more homogeneous and presented smaller reductions than *Py. tritici-repentis*. Apart from the early artificial inoculum, natural inoculum was also present which may have caused an increase in the severity of *Py. tritici-repentis* in the lower leaves, originated in the stubble. In addition *P. triticina* infections started late in the crop cycle due to the higher temperature requirements of the pathogen in relation to *Py. tritici-repentis*, thus affecting upper leaves. Findings of this work about the vertical distribution of both diseases can be used in simulation models to differential quantification of both diseases in the different leaf layers and its influence in yield. Carretero et al. (2010) showed that assuming a uniform distribution of the diseases lead to underestimations of

accumulated absorbed radiation up to 21%, and as a consequence to an overestimation of radiation use efficiency (RUE) up to 29% when diseases were concentrated in the lower leaf layers into the canopy. In the present study, regression between RUE and percentages of GLAI, as independent variable in each leaf layer for each cultivar, showed that RUE, as dependent variable, was not related to the distribution of GLAI (data not shown). In that sense, those genotypes that presented the lowest percentage of leaf area of FL and FL-1 did not show the lowest RUE values, suggesting that reductions in radiation interception due to diseases infection appear to be more important that reductions in RUE, probably due to photosynthetic compensation in other tissues and/or retranslocation of assimilates from reserve organs to maintain the biomass production (Carretero et al., 2011). According to the relation between IPAR/IGPAR and AGB, genotypic differences were detected for RUE among wheat genotypes. In addition, RUE was more variable among genotypes and environments when the crop was affected by both diseases. This would indicate a differential effect of the pathogens on the photosynthesis of the remaining green tissues of each cultivar, which can be considered a tolerance mechanism to the diseases appearance (Bingham et al., 2009; Ney et al., 2013)

The physiological negative effects of foliar diseases on IGPARG and RUE can be incorporated into models modifying the components of crop growth, e.g., canopy photosynthesis (Bastiaans and Kropff, 1993), and assimilate partitioning (Bancal et al., 2012) to simulate their effect on yield. Because of Beer's Law relationship between LAI and RI, pathogens that affect LAI as *Py. tritici-repentis* will have a small effect on yield reduction at low disease intensity. On the other hand, pathogens that affects negatively not only LAI, also RUE (like *P. triticina*) will have a large effect even at low disease intensity, and this effect will decrease relatively as disease intensity and injury increases, response that could be associated to the effect that the pathogen produces on the non-green leaf area (Johnson, 1987; Bastiaans, 1991). For some diseases as leaf rust, the variability found in the estimations of β (MacGrath and Pennypacker, 1990; Spitters et al., 1990; Bastiaans, 1991; Shtienberg, 1992) suggest that the relationship between leaf photosynthesis and the severity of the disease needs clarification.

5. Conclusions

This study showed that foliar diseases caused by *Py. tritici-repentis* and *P. triticina* decreased the crop growth rate mainly by reductions in HAD (due to foliar necrosis or accelerated death of tillers), decreasing the capacity of the crop to intercept and accumulate PAR, and as a consequence, reducing the cumulated biomass. Artificial inoculations proved to be useful to imitate the profile of infection of *Py. tritici-repentis* and *P. triticina* and allowed the separation of their effects on the physiological components of biomass. Inoculations of *Py. tritici-repentis* produced greater reductions in IPARG and IGPARG compared to *P. triticina*. Conversely, *P. triticina* affected more RUEint, RUEgt and CGR than *Py. tritici-repentis* suggesting that the photosynthetic system of the remaining healthy tissues infected by *P. triticina* is more negatively affected than under *Py. tritici-repentis* infections. The negative effect of leaf rust on RUE and CGR could be associated with the nutritional habit of the pathogen that produces important changes in the physiology of the host, causing reductions in the photosynthetic capacity, increasing respiration rate and decreasing the rate of assimilates translocation of affected organs that confirmed the hypothesis of this study.

Results of this work about how pathogens as *Pyrenophora tritici-repentis* and *Puccinia triticina* affect attributes associated with the biomass production could be useful to improve the quantification and modelling of crop losses and predict the diseases effects more

accurately and robustly than those models that only consider a phytopathological perspective.

Acknowledgements

This study was funded by projects from ANPCYT, PICT 2181/2010 and UNLP 227. We wish to thank the staff from the J. Hirschhorn Experimental Station, Faculty of Agricultural and Forestry Sciences, National University of La Plata, Argentina for technical assistance during the field experiments. The authors gratefully acknowledge Dr Richards for his valuable assistance for improving the manuscript.

References

- Ali, S., Francl, L.J., 2003. Population race structure of *Pyrenophora tritici-repentis* prevalent on wheat and noncereal grasses in the Great Plains. *Plant Dis.* 87, 418–422.
- Béasse, C., Ney, B., Tivoli, B., 2000. A simple model of pea (*Pisum sativum*) growth affected by *Mycosphaerella pinodes*. *Plant Pathol.* 49, 187–200.
- 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.
- Bancal, M.O., Hansart, A., Sache, I., Bancal, P., 2012. Modelling fungal sink competitiveness with grains for assimilates in wheat infected by a biotrophic pathogen. *Ann. Bot.* 110, 113–123.
- Bastiaans, L., Kropff, M.J., 1993. Effects of leaf blast on photosynthesis of rice. 2. Canopy photosynthesis. *Neth. J. Plant Pathol.* 99, 205–217.
- Bastiaans, L., 1991. The 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.
- Beard, C., Thomas, G.J., Jayasena, K., 2015. Managing Stripe Rust and Leaf Rust in Wheat in Western Australia. Department of Agriculture and Food, Government of Western Australia, Available from: <https://www.agric.wa.gov.au/grains-research-development/managing-stripe-rust-and-leaf-rust-wheat-western-australia?page=0%2C1> (last accessed: April, 2016).
- Bergamin Filho, A., Carneiro, S.M.T.P.G., Godoy, C.V., Amorim, L., Berger, R.D., Hau, B., 1997. Angular leaf spot of Phaseolus beans: relationships between disease, healthy leaf area, and yield. *Phytopathology* 87, 506–515.
- Bingham, I.J., Walters, D.R., Foulkes, M.J., Paveley, N.D., 2009. Crop traits and the tolerance of wheat and barley to foliar disease. *Ann. Appl. Biol.* 154, 159–173.
- Bolton, M.D., Kolmer, J.A., Garvin, D.F., 2008. Wheat leaf rust caused by *Puccinia triticina*. *Mol. Plant Pathol.* 9, 563–575.
- Boote, K.J., Jones, J.W., Mishoe, J.W., Berger, R.D., 1983. Coupling pests to crop growth simulators to predict yield reductions. *Phytopathology* 73, 1581–1587.
- Bryson, R.J., Paveley, N.D., Clark, W.S., Sylvester-Bradley, R., Scott, R.K., 1997. Use of in-field measurements of green leaf area and incident radiation to estimate the effects of yellow rust epidemics on the yield of winter wheat. *Eur. J. Agron.* 7, 53–62.
- Campbell, C.L., Madden, L.V., 1990. Introduction to Plant Disease Epidemiology. John Wiley & Sons, New York, NY, USA.
- 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. <http://dx.doi.org/10.1016/j.fcr.2009.12.009>.
- Carretero, R., Bancal, M.O., Miralles, D.J., 2011. 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. *Eur. J. Agron.* 35, 237–246.
- Cotuna, O., Paraschiv, M., Paraschiv, A.M., Sărățeanu, V., 2015. The influence of tillage, crop rotation and residue management on tan spot (*Drechslera tritici-repentis*. Died. Shoemaker) in winter wheat. *Res. J. Agric. Sci.* 47, 13–21.
- Dimmock, J.P.R.E., Gooding, M.J., 2002. The effects of fungicides on rate and duration of grain filling in winter wheat in relation to maintenance of flag leaf area. *J. Agr. Sci. Camb.* 138, 1–16.
- Duveiller, E., Kandel, Y.R., Sharma, R.C., Shrestha, S.M., 2005. Epidemiology of foliar blights (Spot blotch and Tan spot) of wheat in the plains bordering the Himalayas. *Phytopathology* 95, 248–256.
- Eversmeyer, M.G., Kramer, C.L., 1992. Local dispersal and deposition of fungal spores from a wheat canopy. *Grana* 31, 53–59.
- Gardner, F.P., Auma, E.O., 1989. Canopy structure, light interception, and yield and market quality of peanut genotypes as influenced by planting pattern and planting date. *Field Crops Res.* 20, 13–29.
- Gaunt, R.E., 1995. The relationship between plant disease severity and yield. *Annu. Rev. Phytopathol.* 33, 119–144.
- Gooding, M.J., Dimmock, J.P., France, R.E., Jones, J., 2000. Green leaf area decline of wheat flag leaves: the influence of fungicides and relationships with mean grain weight and grain yield. *Ann. Appl. Biol.* 136, 77–84.
- Huerta-Espino, J., Singh, R.P., German, S., McCallum, B.D., Park, R.F., Chen, W.Q., Bhardwaj, S.C., Goyeau, H., 2011. Global status of wheat leaf rust caused by *Puccinia triticina*. *Euphytica* 179, 143–160.
- Johnson, K.B., 1987. Defoliation, disease and growth: a reply. *Phytopathology* 77, 1495–1497.
- Jordahl, J.G., Francl, L.J., 1992. Increase and storage of cultures of *Pyrenophora tritici-repentis*. In: Francl, L.J., Krupinsky, J.M., McMullen, M.P. (Eds.), *Advances in Tan Spot Research*. Agric. Exp. Stn., Fargo, North Dakota, p. 109.
- Lim, L.G., Gaunt, R.E., 1986. The effect of powdery mildew (*Erysiphe graminis* f. sp. *hordei*) and leaf rust (*Puccinia hordei*) on spring barley in New Zealand. I. Epidemic development, green leaf area and yield. *Plant Pathol.* 35, 44–53.
- MacGrath, M.T., Pennypacker, S.P., 1990. Alteration of physiological processes in wheat flag leaves caused by stem rust and leaf rust. *Phytopathology* 80, 677–685.
- Madden, L.V., Nutter, F.W.J., 1995. Modeling crop losses at field scale. *Can. J. Plant Pathol.* 17, 124–137.
- Madeira, A.C., Clark, J.A., Rossall, S., 1994. Growth and light interception in field bean (*Vicia faba*) infected by *Ascochyta fabae*. *J. Agric. Sci.* 123, 225–232.
- McNew, G., 1960. The nature, origin and evolution of parasitism. In: Horsfall, J.G., Dimond, A.E. (Eds.), *Plant Pathology: An Advanced Treatise*, vol. 2. Univ. Wisconsin press, Madison, WI, pp. 19–69.
- McRoberts, N., Hughes, G., Madden, L.V., 2003. The theoretical basis and practical application of relationships between different disease intensity measurements in plants. *Ann. Appl. Biol.* 142, 191–211.
- Mehta, Y.R., Gaudencio, C.A., 1991. The effects of tillage practices and crop rotation on the epidemiology of some major wheat diseases. In: Saunders, D.F. (Ed.), *Wheat for the Nontraditional Warm Areas*. CIMMYT, Mexico, D.F, pp. 266–283.
- Miralles, D.J., Slafer, G.A., 1990. Estimación del área foliar en trigo: Generación y validación de un modelo. 11vo Congreso Nacional de Trigo. Pergamino. Cap.1, 76–85.
- Miralles, D.J., Slafer, G.A., 1997. Radiation interception and radiation use efficiency of near-isogenic wheat lines with different height. *Euphytica* 97, 201–208.
- Moreno, M.V., Stenglein, S.A., Perelló, A.E., 2012. In: Caliskan, Mahmut (Ed.), *Pyrenophora tritici-repentis*, Causal Agent of Tan Spot: A Review of Intraspecific Genetic Diversity, The Molecular Basis of Plant Genetic Diversity. InTech, 10.5772/33516 (last accessed: April, 2016).
- Ney, B., Bancal, M.O., Bancal, P., Bingham, I.J., Foulkes, J., Gouache, D., Paveley, N., Smith, J., 2013. Crop architecture and crop tolerance to fungal diseases and insect herbivory: mechanisms to limit crop losses. *Eur. J. Plant Pathol.* 135, 561–580. <http://dx.doi.org/10.1007/s10658-012-0125-z>.
- Olesen, J.E., Jørgensen, L.N., Petersen, J., Mortensen, J.V., 2003. Effects of rates and timing of nitrogen fertilizer on disease control by fungicides in winter wheat: 2. Crop growth and disease development. *J. Agric. Sci.* 140, 15–29.
- Oliver, R.P., Ipcho, S.V.S., 2004. Arabidopsis pathology breathes new life into the necrotrophs-vs.-biotrophs classification of fungal pathogens. *Mol. Plant Pathol.* 5, 347–352.
- Paveley, N.D., Lockley, K.D., Sylvester-Bradley, R., Thomas, J., 1997. Determinants of fungicide spray decisions for wheat. *Pestic. Sci.* 49, 379–388.
- Rabbinge, R., Jorritsma, I.T.M., Schans, J., 1985. Damage components of powdery mildew in winter wheat. *Neth. J. Plant Pathol.* 91, 235–247.
- Raymond, P.J., Bockus, W.W., 1982. An in vitro technique for profuse sporulation of *Drechslera tritici-repentis*. *Phytopathology* 72, 934 (Abstr.).
- Rees, R.G., Platz, G.J., 1980. The epidemiology of yellow leaf spot of wheat in Southern Queensland. *Aust. J. Agric. Res.* 31, 259–267.
- Rees, I.G., Platz, G.J., 1983. Effects of yellow spot of wheat in Southern Queensland. *Aust. J. Agric. Res.* 34, 39–46.
- Reynolds, M.P., Pellegrineschi, A., Skovmand, B., 2005. Sink-limitation to yield and biomass: a summary of some investigations in spring wheat. *Ann. Appl. Biol.* 146, 39–49.
- Robert, C., Bancal, M.O., Lannou, C., 2004. Wheat leaf rust ureidospore production on adult plants: influence of leaf nitrogen content and *Septoria tritici* blotch. *J. Phytopathol.* 94, 712–721.
- 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.
- Rossing, W.A.H., van Oijen, M., van der Werf, W., Bastiaans, L., Rabbinge, R., 1992. Modelling the effects of foliar pests and pathogens on light interception, photosynthesis, growth rate and yield of field crops. In: Ayres, P.G. (Ed.), *Pests and Pathogens. Plant Responses to Foliar Attack*. Bios Scientific Publishers, Oxford, UK, pp. 161–180.
- Rosyara, U.R., Sharma, R.C., Duveiller, E., 2006. Variation of canopy temperature depression and chlorophyll content in spring wheat genotypes and association with foliar blight resistance. *J. Plant Breed. Group* 1, 45–52.
- Sah, D.N., 1994. Effects of leaf wetness duration and inoculum level on resistance of wheat genotypes to *Pyrenophora tritici-repentis*. *J. Phytopathol.* 142, 324–330.
- Savary, S., Teng, P.S., Willcoquet, L., Nutter Jr., F.W., 2006. Quantification and modeling of crop losses: a review of purposes. *Annu. Rev. Phytopathol.* 44, 89–112.
- Scholes, J.D., Rolfe, S.A., 1995. How do biotrophic pathogens affect the photosynthetic metabolism of their host? *Aspects Appl. Biol.* 42, 91–99.
- Scholes, J.D., Rolfe, S.A., 2009. Chlorophyll fluorescence imaging as tool for understanding the impact of fungal diseases on plant performance; a phenomics perspective. *Funct. Plant Biol.* 36 (11), 880–892.
- Serrago, R.A., Carretero, R., Bancal, M.O., Miralles, D.J., 2009. Foliar diseases affect the ecophysiological attributes linked with yield and biomass in wheat (*Triticum aestivum* L.). *Eur. J. Agron.* 31, 195–203.
- Shah, S.F.A., McKenzie, B.A., Gaunt, R.E., Marshall, J.W., Frampton, C.M., 2004. Effect of production environments on radiation interception and radiation use

- efficiency of potato (*Solanum tuberosum*) grown in Canterbury, New Zealand. *N. Z. J. Crop Hortic.* 32, 113–119.
- Shaner, G., Finney, R.E., 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. *Phytopathology* 67, 1051–1056, <http://dx.doi.org/10.1094/Phyto-67-1051>.
- Sharma, R.C., Duveiller, E., Gyawali, S., Shrestha, S.M., Chaudhary, N.K., Bhatta, M.R., 2004. Resistance to *Helminthosporium* leaf blight and agronomic performance of spring wheat genotypes of diverse origins. *Euphytica* 139, 33–44.
- Shtaya, M.J.Y., 2015. Sources of partial resistance to leaf rust in hard wheat landraces cultivated in Palestine. *Walailak J. Sci. Technol.* 12 (3), 245–250.
- Shtienberg, D., 1992. Effects of foliar diseases on gas exchange processes: a comparative study. *Phytopathology* 82, 760–765.
- Simón, M.R., Ayala, F.M., Golik, S.I., Terrile, I., Cordo, C.A., Perelló, A.E., Moreno, V., Chidichimo, H.O., 2011. Integrated foliar disease management to prevent yield loss in argentinian wheat production. *Agron. J.* 103, 1441–1451, <http://dx.doi.org/10.2134/agronj2010.0513>.
- Slafer, G.A., Rawson, H.M., 1994. Sensivity of wheat phasic development to major environmental factors: a re-examination of some assumptions made by physiologists and modellers. *Aust. J. Plant Physiol.* 21, 393–426.
- Slafer, G.A., Andrade, F.H., Satorre, E.H., 1990. Genetic improvement effects on pre-anthesis physiological attributes related to wheat grain-yield. *Field Crops Res.* 23, 255–263.
- Soil Survey Staff, 1999. *Soil taxonomy: a basic system of soil classification for making and interpreting soil surveys*. In: Natural Resources Conservation Service, 2nd edition. U.S. Department of Agriculture Handbook, pp. 436.
- Spitters, C.J.T., Roermund, H.J.W.V., Nassau, H.G.M.G.V., Scheppers, J., Mesdag, J., 1990. Genetic variation in partial resistance to leaf rust in winter wheat: disease progress, foliage senescence and yield reduction. *Neth. J. Plant Pathol.* 96, 3–15.
- Voegelé, R.T., Mendgen, K.W., 2011. Nutrient uptake in rust fungi: how sweet is parasitic life? *Euphytica* 179, 41–55.
- Waggoner, P.E., Berger, R., 1987. Defoliation, disease and growth. *Phytopathology* 77, 393–398.
- Wegulo, S., Stevens, J., Zwingman, M., Baenziger, P.S., 2012. In: Dhanasekaran, Dr. Dharumadurai (Ed.), *Yield Response to Foliar Fungicide Application in Winter Wheat, Fungicides for Plant and Animal Diseases*. InTech, <http://dx.doi.org/10.5772/25716>, ISBN: 978-953-307-804-5.
- Wegulo, S.N., 2011. Tan spot of cereals. *Plant Health Instr.*, <http://dx.doi.org/10.1094/PHI-I-2011-0426-01>.
- Whelan, H.G., Gaunt, R.E., Scott, W.R., 1997. The effect of leaf (*Puccinia hordei*) on yield response in barley (*Hordeum vulgare* L.) crops with different yield potentials. *Plant Pathol.* 46, 397–406.
- Willoquet, L., Aubertot, J.N., Lebard, S., Robert, C., Lannou, C., Savary, S., 2008. Simulating multiple pest damage in varying winter wheat production situations. *Field Crops Res.* 107, 12–28.
- Wright, K.H., Sutton, J.C., 1990. Inoculation of *Pyrenophora tritici-repentis* in relation to epidemics of tan spot of winter wheat in Ontario. *Can. J. Plant Pathol.* 12, 149–157.
- Zadoks, J.C., Chang, T.T., Konzak, C.F., 1974. A decimal code for the growth stages of cereals. *Weed Res.* 14, 415–421.
- Zuckerman, E., Eshel, A., Eyal, Z., 1997. Physiological aspects related to tolerance of spring wheat cultivars to *Septoria tritici* blotch. *Phytopathology* 87, 60–65.