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Effect of tolerance to Septoria tritici blotch on grain yield, yield components and grain quality in Argentinean wheat cultivars

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ABSTRACT

Septoria tritici blotch (STB) caused by *Zymoseptoria tritici* (*Mycosphaerella graminicola*) is a major disease of wheat worldwide due to significant losses in grain yield and quality. Disease tolerance is the ability to maintain yield performance in the presence of disease symptoms. Therefore, it could be a useful tool in the management of the disease. Although it is known, that there is disease tolerance to STB in some wheat cultivars, this aspect has not been studied among Argentinean cultivars. The aims of this study were to evaluate genotypic differences in tolerance to STB among Argentinean cultivars, considering the relationship between the area under disease progress curve or the green leaf area or the non-green leaf area duration with the grain yield. In addition the effect of the disease on yield, yield components, test weight, grain protein concentration, wet and dry gluten concentration and the influence of tolerance on these traits was investigated. Field experiments were carried out with ten cultivars in a split-split-plot design during 2010 and 2011. Inoculation treatments were the main plots and cultivars, the subplots. STB significantly reduced grain yield, their components, test weight and increase grain protein and gluten concentration. Cultivar Baguette 10 showed major tolerance to STB, indicated by a consistent low regression slope between the green area duration and yield, while Klein Chaja was non-tolerant due to a high regression slope. However, many cultivars such as Buck Brasil, Buck 75 Aniversario, Klein Escorpion and Klein Flecha had considerably similar regression slopes to Baguette 10, provided good levels of tolerance. Other cultivars presented no significant differences. The correlation coefficient between tolerance and grain yield potential was not significant, suggesting that tolerant high-yielding cultivars can be obtained. No relationship was found between quality group or tolerance with the increase in protein and gluten concentration due to STB either.

1. Introduction

Bread wheat (*Triticum aestivum* L.) is a major cereal crop grown in most regions of the globe due to its importance as a food source, and its enormous genetic variability in phenological response to photoperiod and temperature, including vernalization (Slafer and Rawson, 1994). Foliar diseases are among factors that reduce yield and quality in wheat crops in the Argentinean Pampas (Annone et al., 2001) and in many other regions around the world characterized by mild climate and high rainfall conditions during the growing season. Septoria tritici blotch (STB) caused by *Zymoseptoria tritici* (Desm.) (teleomorph: *Mycosphaerella graminicola* (Fuckel) J. Schröt. in Cohn) is one of the most important diseases worldwide because of yield reduction and quality loss (Gilbert and Tekauz, 1990, 1992; Bailey et al., 1993; Rodrigo et al., 2015). Severe infections can cause losses of up to 60% of total yield (Arraiano et al., 2001). In Argentina, Annone et al. (1991) and Simón et al. (2002) reported yield losses from 20 to 50%, and Simón et al. (1996, 2002) found reductions in thousand kernel weights (TKW) up to 22%.

Breeding for resistance to STB is complicated by variability of the pathogen, partly caused by the presence of both asexual and sexual reproduction (Simón et al., 2012), and because of a large effective population size and substantial gene flow (Zhan and McDonald, 2004). These traits enable an adaptation to the host resistance (Mundt et al., 1999; Mundt, 2002) and fungicides (Torriani et al., 2009; Cools and Fraaije, 2013). A continuous increase in azole resistance has been reported in European populations of *Zymoseptoria tritici* during the last 10–15 years, as it has also been observed to quinone-outside inhibitors (strobilurins) (Estep et al.,

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2015). Consequently, STB tolerance could be a useful tool in the management of the disease. Tolerance is a quantitative trait, and its expression depends on both the genotype and the environment (Parker et al., 2004). It has been demonstrated that there is disease tolerance in some wheat cultivars (Ziv and Eyal, 1978), although progress towards understanding and exploiting the mechanisms that confer tolerance has been slow (Parker et al., 2004) due to the wide and inconsistent use of the term tolerance and the practical difficulties in quantifying it. Many authors have defined tolerance maintaining multiple interpretations (Schafer, 1971) (Clarke, 1984; Parker et al., 2004; Foulkes et al., 2006). Tolerance to STB has not been studied in Argentinean wheat cultivars until now, which in this study is considered, as the ability to maintain yield performance in the presence of disease symptoms (Foulkes et al., 2006).

In spite of the fact that tolerance has been quantified from the slope of the relationship between area under disease progress curve (AUDPC) and grain yield (GY) (Kramer et al., 1980; Inglese and Paul, 2006), this approach provides no information on the absolute size of the canopy, which is likely to differ between sites and seasons and hence affects the remaining area of functional healthy tissue.

Damage functions, which quantify the relationship between injuries and yield loss (Zadoks, 1985), can be determined experimentally. Statistical models of disease-induced yield loss based on absolute measurements of green leaf area duration (GLAD) or light interception have shown to be more robust across sites and seasons than those based on percentage AUDPC or non-green leaf area index (NGLAI) scores (Johnson, 1987; Waggoner and Berger, 1987; Madden and Nutter, 1995; Bryson et al., 1997). Tolerance of several wheat varieties to STB has been quantified as the slope of the relationship between GLAD and GY across treatments of contrasting disease pressure within each cultivar (Parker et al., 2004; Paveley et al., 2005; Foulkes et al., 2006).

Statistical models provide information about the relationship between AUDPC or GLAD with GY under specific conditions. However, simulation models can obtain information about the effect of wheat diseases on mechanisms of biomass generation and its influence on GY, even though they need validation. Damage function can be determined from crop loss simulation models, because they represent processes that are underpinned by subprocesses: damage mechanisms. Different mechanisms can usually be described (Rabbinge and Rijsdijk, 1981; Boote et al., 1983; Rabbinge et al., 1989), in relation to the nutritional habit of the pathogens. WHEATPEST is a simulation model developed in order to simulate yield losses caused by pests (diseases, insects, weeds), individually or in combination, under a range of production situations (Willocquet et al., 2008; Savary and Willocquet, 2014). The WHEATPEST simulation model has incorporated damage functions to simulate the effects caused by *Zymoseptoria tritici* on yield.

Furthermore, tolerance against STB in wheat could have an impact on other variables such as the parameters of wheat quality due to a lower reduction in yield. The STB influence on grain proteins has received little attention despite the fact that these proteins are important in determining the quality and end use of the grain (Shewry and Halford, 2002). Nutritional strategies of pathogens produce different effects on the physiology of crops and thus influence grain protein concentration (GPC) and both wet (WGC) and dry gluten concentration (DGC).

Dimmock and Gooding (2002) observed that when classic biotrophs are controlled, the concentration of grain protein often increases. Therefore, the pathogen has a more damaging effect on the accumulation and partitioning of nitrogen to the grain than it does on the accumulation and partitioning of the dry matter. GPC is often reduced with infection by rusts and, therefore, increased by methods adopted to control rusts (Phipps, 1938; Keed and White, 1970; Clare et al., 1990). On the other hand, Myram and Kelly (1981), Penny et al. (1983) found that the use of fungicide reduces GPC, indicating that the pathogen increases it.

Conversely, most reports of the effect of controlling necrotrophic pathogens as *Drechslera tritici repentis* or hemibiotrophic pathogens (becomes necrotrophic after an initial biotrophic phase) such as *Zymoseptoria tritici* found that fungicide use is associated with a reduction in protein concentration, thus the pathogen increases it (Rees et al., 1982; Ishikawa et al., 2001; Ruske et al., 2001). However, Puppala et al. (1998) reported large increases in protein concentration following fungicide use on a cultivar specifically bred for high protein concentration. Thus, it is reasonable to suppose that cultivars specially bred for bread making may be able to maintain grain nitrogen accumulation more effectively as senescence is delayed and yield increases compared with cultivars for biscuits where protein concentration is much less important (Dimmock and Gooding, 2002). This indicates that Argentinean wheat cultivars belonging to a high quality group could have less reduction in quality variables when affected by STB. The hypothesis of this work is that there is tolerance to STB among wheat cultivars cropped in Argentina and that the STB infection leads to losses in GY and increased protein and gluten concentration.

The aims of the present study are: 1-to evaluate genotypic differences in tolerance to STB tested taking into account the relationship between the AUDPC, GLAD and NGLAD with the GY; 2-to test the effect of the disease on GY, yield components, TW (test weight), GPC, WGC and DWC; and 3-to investigate if tolerance and the quality group of the wheat cultivars can influence these traits.

2. Materials and methods

2.1. Field trials and experimental design

Two experiments were conducted at the Experimental Station Julio Hirschhorn in La Plata, Faculty of Agricultural and Forestry Sciences, National University of La Plata during 2010 and 2011. The trials were sown on 15 July and 16 June respectively under conventional tillage. The soil was a Typic Argiudoll. Analysis of the soil samples (top-0.20 m) indicated the following values by weight: organic matter: 3.55%, N: 0.139%, P: 15 ppm, pH: 5.75. Weather data were recorded at a meteorological station situated 100 m from the experiments.

The experimental design was a split-split-plot design with three replications. Main plots were the inoculum concentrations: 1-Non-inoculated treatment (NI), 2-Low concentration (LC) (5×10^5 spores ml^{-1} suspension) and 3-High concentration (HC) (5×10^6 spores ml^{-1}suspension). Sub-plots were the cultivars: Klein Zorro (K. Zorro), Buck 75 Aniversario (B.75 Aniversario), Buck Brasil (B. Brasil), Buck Guapo (B. Guapo) (all of them belonging to quality group 1, G1), Klein Escorpion (K. Escorpion), Klein Flecha (K. Flecha), ACA 801 and Relmo Centinela (R. Centinela) (G2), Nidera Baguette 10 (Bag 10) and Klein Chaja (K. Chaja) (G3). In Argentina, the Committee of Winter Grain classifies wheat cultivars into three groups, GC 1 corresponds to the highest quality cultivars, suitable for industrial bread making, GC 2 includes traditional bread making cultivars suitable for major long fermentations higher than eight hours, while GC 3 includes cultivars with the lowest quality with short fermentation times up to eight hours (PRONACATRI, 2006). Between the main plots, plots of oats were sown to diminish interplot interferences (James et al., 1973). The entire experiment was fertilized with 50 kg P_2O_5 ha^{-1} as calcium triple superphosphate plus 100 kg ha^{-1} N as urea at the time of sowing and 80 kg ha^{-1} at the end of tillering.

2.2. Inoculum preparation

A mix of virulent isolates (FALP14707, FALP20107-FALP20207, and FALP20507-) of *Zymoseptoria tritici* was used to prepare the inoculum. The isolates were grown on malt extract agar at 19 °C with 12 h alternating light and dark cycles. The inoculum was prepared by aseptically scraping sporulating colonies with a scalpel and suspending conidia in deionized water. The spore concentration was measured with a Neubauer hemacytometer. Conidial suspensions were adjusted to the required concentrations. One milliliter of Tween 20 per liter was added as a surfactant.

Two inoculations were performed: at the beginning of tillering, GS (growth stage) 21-GS22, and at flag leaf emergence, GS 39 (Zadoks et al., 1974). Plants were sprayed with the inoculum suspension until runoff, using a backpack sprayer for manual application in evening hours. After inoculations, plants were kept moist by spraying with water several times a day with sprinklers during a period of three days.

2.3. Evaluations of disease severity, area under disease progress curve and dynamics of leaf area

Disease severity evaluations were done by visual estimation of the symptoms as a percentage of the two to four uppermost leaves of seven to ten plants, depending on the growth stage of each plot at three growth stages (GS 39, 60, 82). The AUDPC values of STB measured in the two upper leaves were calculated according to the formula of Shaner and Finney (1977). GLAI, NGLAI, GLAD and NGLAD were determined by the following procedure. The leaves with at least 10% green of tillers of every plot were separated and pasted in sheets of paper. Then, leaves were scanned and their area was measured by the software image J (Rasband, 2014). Both, GLAD and NGLAD values were calculated according to the formula developed by Waggoner and Berger (1987) $LAD = \sum[(LAI_i + LAI_{i+1}/2)] \times (t_{i+1} - t_i)]$ where LAD (it is GLAI plus NGLAI) and $(t_{i+1} - t_i)$ is the interval between two consecutive assessments.

2.4. Determinations of grain yield and their components

Yield-components, spikes per square meter (SPM^2), kernels per spike (KPS), TKW and TW were evaluated in each plot. Three 1 m long sections in each plot were harvested at random and the numbers of spikes counted to determine SPM^2. From that sample, KPS were determined on 20 spikes, threshed, and the grains counted by means of a mechanical counter. The grains counted in the 20 spikes were weighed to determine TKW (g). TW was determined with a Schopper scale, which weighs a volume of 250 cm^3 and converts it to 1 hl by means of a table. The GY (kg ha^{-1}) was estimated by harvesting the plots.

2.5. Milling of the samples and determination of quality variables

The samples of grain from each subplot were cleaned, conditioned to 15.5% moisture and milled using a Buhler laboratory mill (MLU 202), extracting flour at a rate of about 70%. The percentage of N on the grains was determined by Microkjeldahl method (A.O.A.C 11 Ed, 1970), and GPC was estimated by multiplying Kjeldahl N by a factor of 5.7. WGC and DWC were determined by Glutomatic 2000 (IRAM 15864, 2007).

2.6. Statistical analysis

Severity, AUDPC, GLAI, NGLAI, GLAD, NGLAD, GY, yield components, and quality data were analyzed by ANOVA for a split-split-plot design in a combined analysis for both years with Genstat 12 Ed (VSN, 2011). Inoculation treatments and cultivars were considered as fixed effects whereas replications were considered as random effects. Mean values were compared with Fisher's Protected LSD test ($P < 0.05$). Regression analysis was used to detect differences in tolerance among varieties using AUDPC-GY, GLAD-GY and NGLAD-GY according to Foulkes et al., 2006. The significance of the slopes of the regression equation was compared by ANOVA. Correlation coefficients were determined between tolerance and yield potential or variations in GPC and among cultivars quality groups and quality data.

3. Results

3.1. Meteorological conditions during crop cycle

Precipitation varied greatly between both years. In 2010, the sum of rainfall during crop cycle was 344 mm and in 2011 was 481.3 mm. Although mean temperatures were similar for both years, in 2011, the amplitude was lower than in 2010 as minimum temperature was higher and maximum temperature was lower. Mean humidity was similar for both years (71 and 72% for 2010 and 2011 respectively).

3.2. Disease severity and area under disease progress curve

Analysis of variance of severity caused by STB at GS 39 and GS 60 (four leaves), GS 82 (two upper leaves), and AUDPC, for each cultivar and inoculation treatment for both years, is shown in Table 1. There was a tendency to a higher severity in 2011 compared to 2010 at all GS, but it was only significant at GS 39, GS 60 and for the AUDPC. In addition, inoculation treatments and cultivars also affected the severity at all GS.

Disease severity increased with increases in inoculum concentration for all cultivars in both years of study (Fig. 1, Annex 1). In 2011, AUDPC was higher than in 2010 for all cultivars and the increase of inoculum concentration caused a marked elevation of AUDPC in both years (Annex 1). ACA 801, R. Centinela and B. Brasil were the most susceptible cultivars while Bag. 10 was the most resistant for both growing seasons.

3.3. Dynamics of reen leaf area index, non-green leaf area index, reen leaf area duration and non-green leaf area duration

GLAI was influenced by years and the inoculum concentration at all GS (Table 2). In addition, cultivars significantly affected GLAI at GS 39 and GS60. GLAI values were lower in 2010 than in 2011 and decreased with increasing inoculum concentration in all cultivars for both years (Annex 2). Interactions year × inoculum concentration and inoculum concentration × cultivar influenced GLAI at GS 39. GLAD was modified by all main effects. Lower GLAD values were observed in 2010 compared to 2011 and GLAD decreased with the increase in the inoculum concentration (Fig. 2). The highest reduction in GLAD was observed in K. Escorpion (65% and 36% for 2010 and 2011 respectively) and ACA 801 (53% and 36% for each year, respectively). The lowest reductions in GLAD were shown by B. 75 Aniversario (21%), R. Centinela (27%) and Bag. 10 (29%) in 2010 while B. Brasil, R. Centinela and Bag. 10 (19–22%); K. Flecha and B. 75 Aniversario (24%) in 2011. NGLAI was significantly affected by inoculum concentration at all GS ($P \leq 0.10$) and by years except at GS82 (Table 2). Furthermore, the interaction year × cultivar, inoculum concentration × cultivar and year × inoculum concentration × cultivar influenced NGLAI. NGLAI increased with a raise in inoculum concentration. In 2010, most of the cultivars greatly increased NGLAD with increasing in inoculum concentration, except for K. Flecha, B. 75 Aniversario and K. Escorpion which showed a slight increase in NGLAD. In 2011 R. Centinela, K. Escorpion and K. Flecha did not modify NGLAD with the increase in inoculum concentration, while ACA 801 and K. Chaja highly

Table 1
Mean squares (MS) from the combined analysis of variance for disease severity at GS 39, 60, 82 and area under disease progress curve (AUDPC) for ten wheat cultivars under three inoculation treatments with *Zymoseptoria tritici* in two years.

Source of variation	df	Disease severity						AUDPC	
		GS 39		GS 60		GS82			
		MS	P > F	MS	P > F	MS	P > F	MS	P > F
Year (Y)	1	0.747	0.014	0.214	0.034	5.080	0.129	31,788,758	0.022
Error a	2	0.002		0.017		0.160		121,246	
Inoculum (I)	2	0.206	<0.001	0.624	<0.001	1.460	<0.001	10,291,204	<0.001
Y × I	2	0.005	0.479	0.001	0.895	0.130	0.127	422,342	0.043
Error b	8	0.003		0.001		0.040		70,855	
Cultivar (C)	9	0.079	<0.001	0.048	<0.001	0.400	<0.001	507,382	<0.001
Y × C	9	0.017	0.001	0.007	0.778	0.070	0.092	162,162	0.157
I × C	18	0.001	0.999	0.005	0.982	0.030	0.654	71,474	0.842
Y × I × C	18	0.003	0.941	0.008	0.808	0.010	0.979	48,357	0.973
Error c	108	0.005		0.012		0.050		120,760	
Total	179								

increased NGLAD. The rest of cultivars augmented with little variations (Annex 3).

3.4. Yield and yield components

Yield and SPM^2 were affected by year, inoculum concentration and cultivar effects (Table 3). Inoculation treatment was significant for all yield components. There were significant differences for year × inoculum concentration and year × cultivar interactions for SPM^2. The inoculation with *Zymoseptoria tritici* reduced significantly GY (Fig. 3) and its components, SPM^2 (data not shown), KPS and TKW (Fig. 4) in both years. Yield reduction fluctuated between 18% and 49.6% when the maximum inoculum concentration was applied, depending on the cultivar. SPM^2 were more reduced by inoculation treatments in 2010 (21.5%) compared to 2011 (6.9%). Moreover, in 2011 all cultivars had higher SPM^2. Decreases in KPS were variable between cultivars and lower in 2010 (7.2%) than in 2011 (12.9%). TKW was more reduced in 2011 (13.3%) than in 2010 (7.3%) (Annex 4).

TW was significantly affected by year, cultivar and the interaction year × cultivar. In 2011 TW was lower in all cultivars with respect to 2010, except for B. Guapo, Bag. 10, and K. Escorpion, which showed similar values in both years (Annex 5).

3.5. Tolerance among wheat cultivars

Slopes of the linear regression between AUDPC or NGLAD as independent variables and GY as dependent variables did not show significant differences among cultivars. Nevertheless, a tendency to differences in tolerance to *Zymoseptoria tritici* among cultivars could be observed (Table 4). In contrast, the slopes of the regression lines between GLAD and GY values experienced significant differences among cultivars in both years ($P = 0.014$). Bag. 10 was the cultivar which showed major tolerance to STB, indicated by its consistent low regression slope, while K. Chaja, in turn, was intolerant due to its considerably higher regression slope (Table 4). However, many cultivars had considerably similar slopes to Bag.10, such as B. Brasil, B. 75 Aniversario, K. Escorpion and K. Flecha, evidencing high levels of tolerance. Moreover, R. Centinela showed better tolerance in 2011 than 2010 and other cultivars presented no significant differences from both groups. The correlation coefficient between tolerance and GY potential were not significant: $r = 0.38$ in 2010 ($P = 0.28$) and $r = 0.58$ in 2011 ($P = 0.08$). This indicates a tendency (not significant) to a relationship between intolerance (higher slopes) with high yields. However, Bag. 10 and K. Escorpion were tolerant and high yielding cultivars in both years, while B. 75 Aniversario, K. Flecha and B. Brasil (tolerant) and K. Chaja (non-tolerant) had variable yields between years.

3.6. Quality parameters: grain protein concentration and gluten concentration

As a consequence of lower GY, GPC, WGC and DWC significantly increased under STB infection (Table 5 and Fig. 5). In 2011 averages of GPC were lower than in 2010 whereas cultivars were significantly different. Bag. 10 had the lowest GPC, followed by B. Guapo, K. Zorro, B. 75 Aniversario, K. Flecha, whereas K. Chaja, R. Centinela, K. Escorpion, B. Brasil and ACA 801 were the cultivars with the highest GPC (Annex 5). The interaction year × cultivar was significant because five cultivars decreased GPC in the second year (ACA 801, K. Flecha, B. Brasil, B. 75 Aniversario, R. Centinela) while the rest were not significantly different. The WGC was significantly higher in 2010 (21.8%) than in 2011 (21.6%) Year × cultivar interaction influenced WGC and DWC as some cultivars had higher values of WGC in 2010 (Annex 5).

The correlation coefficient between the quality group of each cultivar and the variations in GPC and gluten concentration due to

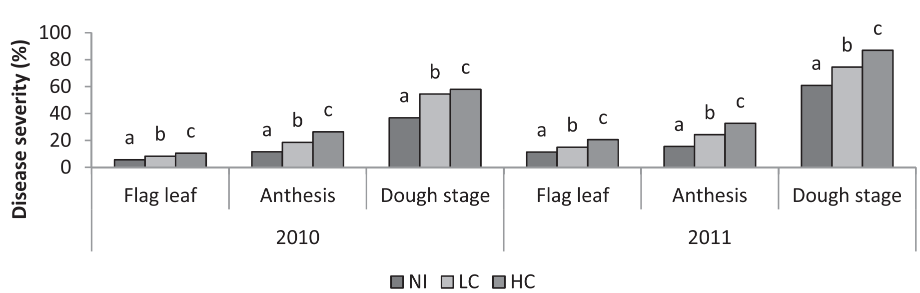

Fig. 1. Disease severity on ten wheat cultivars under three inoculation treatments: NI (non-inoculated), LC (low concentration) and HC (high concentration) with *Zymoseptoria tritici* in two years through crop cycle: Flag leaf (GS 39), Anthesis (GS 60) and Early dough stage (GS 82). Different letters among inoculation treatments indicate significant differences within GS 39: LSD = 1.3; GS 60 LSD = 3.0 and GS 82 LSD = 7.5, $P < 0.05$.

Table 2
Mean squares (MS) from the combined analysis of variance for green leaf area index (GLAI), non-green leaf area index (NGLAI) at GS 39, 60, 82, green leaf area duration (GLAD) and non green leaf area duration (NGLAD) on ten wheat cultivars under three inoculation treatments with *Zymoseptoria tritici* in two years.

Source of variation	df	GLAI						NGLAI						GLAD		NGLAD	
		GS 39		GS 60		GS 82		GS 39		GS 60		GS 82					
		MS	P > F	MS	P > F	MS	P > F	MS	P > F	MS	P > F	MS	P > F	MS	P > F	MS	P > F
Year (Y)	1	366	<0.001	124	0.020	5.53	0.044	4.69	0.006	7.01	0.066	0.47	0.146	308,483	0.001	8767	0.055
Error a	2	0.31		0.25		0.26		0.03		0.52		0.09		446		591	
Inoculum (I)	2	42.9	<0.001	11.76	<0.001	2.55	<0.001	1.24	0.006	0.60	0.093	1.78	<0.001	40,172	<0.001	2248	0.009
Y × I	2	1.89	0.024	0.05	0.850	0.00	0.999	0.28	0.155	0.09	0.620	0.01	0.699	542	0.302	231	0.430
Error b	8	0.31		0.28		0.08		0.12		0.18		0.03		388		246	
Cultivar (C)	9	3.53	<0.001	1.34	0.006	0.08	0.570	0.38	<0.001	0.23	0.006	0.19	<0.001	2234	<0.001	367	<0.001
Y × C	9	1.45	0.061	0.54	0.362	0.09	0.502	0.37	<0.001	0.64	<0.001	0.10	0.001	614	0.208	832	<0.001
I × C	18	1.69	0.007	0.26	0.932	0.09	0.477	0.08	0.015	0.19	0.006	0.09	<0.001	599	0.176	176	0.029
Y × I × C	18	0.67	0.617	0.23	0.968	0.05	0.924	0.12	<0.001	0.17	0.014	0.08	0.001	232	0.943	225	0.004
Error c	108	0.77		0.49		0.09		0.04		0.08		0.03		446		95.6	

Zymoseptoria tritici was not significant for both years. The relationship among tolerance and variations in GPC due to *Zymoseptoria tritici* was not significant either. However, in 2010, cultivars belonging to G1 and G2 quality highly increased or maintained GPC with inoculum concentration, while G3 cultivars tended to decrease GPC. In 2011, G1 cultivars decreased GPC with inoculum concentration, while B. Guapo and B. 75 Aniversario (GC1) increased GPC.

4. Discussion

This study aimed to address the relationship between the effect of the AUDPC caused by *Zymoseptoria tritici* or NGLAD or GLAD on GY in wheat through statistical models in ten wheat cultivars. Meteorological conditions (higher temperature, precipitation and relative moisture) observed during post-inoculation with *Zymoseptoria tritici* done during 2011 were more conducive for the development of the disease than in 2010, causing thus an increase in the disease severity. Hess and Shaner (1987), Simón et al., 2003 found that disease severity increased as post-infection temperature or as post-inoculation moist periods were higher, and the greatest severities resulted from combinations of long moist periods (96 h) and high temperatures (20–25 °C). Predisposing weather conditions in 2011 produced a higher disease severity and AUDPC. But these conducive weather conditions influenced the dynamics of GLAI, and GLAD, which, therefore, produced higher values of GLAI in 2011 with respect to 2010.

In our work, yield and the yield components were reduced by inoculation with *Zymoseptoria tritici*. Other researchers, Leitch and Jenkins (1995); Simón et al. (1996, 2002); Leyva-Mir et al. (2006); Rodrigo et al. (2015) also found, significant reductions in yield, SPM^2, TKW and TW due to *Zymoseptoria tritici*. STB is a light stealer and assimilates sapper, which reduces the intercepted radiation and removes soluble assimilates from the host. It reduces the GLAI and outflows assimilate from the pool of assimilates (Robert et al., 2006). Indeed, yield is reduced predominantly through effects on the duration of green area and restrictions on the number of grains per spike and the average grain weight (Cornish et al., 1990; Parker et al., 2004; Robert et al., 2004; Blandino et al., 2009; Serrago et al., 2011). Additional works about quantification of damage mechanisms will allow a better understanding of the underlying mechanisms of the effects of pests on crop growth. Simulation models as WHEATPEST also estimate yield losses caused by diseases, incorporating damages mechanisms produced by *Zymoseptoria tritici* individually or in combination with other diseases, under a range of production situations. These models need appropriated trials to validate the results, though.

The loss of yield components varied between growing seasons: in 2010 there was a higher loss in SPM^2, which may be due to a higher water stress impacting in GLAI compared to 2011. In 2011 KPS and TKW were more affected, due to greater precipitations which caused higher disease severity.

The presence of tolerance to *Zymoseptoria tritici* could be only established among our cultivars when the linear regression between GLAD - GY was analyzed. These findings are supported by several studies which have confirmed that measurements of canopy size, and in particular, the effect of disease on GLAI, correlate more closely to yield loss than estimates of the percentage of disease severity alone (Lim and Gaunt, 1981; Waggoner and Berger, 1987; Whelan and Gaunt, 1990; Bryson et al., 1995; Parker et al., 2004; Foulkes et al., 2006). In our study the model including

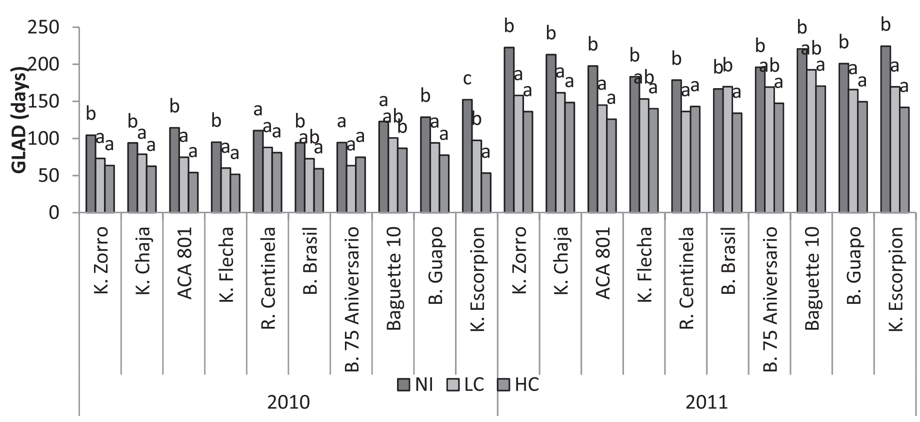

Fig. 2. Green leaf area duration (GLAD) on ten wheat cultivars under three inoculation treatments with *Zymoseptoria tritici*: NI (non-inoculated), LC (low concentration) and HC (high concentration) in two years. Different letters between inoculation treatments within each cultivar indicate significant differences (LSD = 8.3 P < 0.05).

Table 3
Mean squares (MS) from the combined analysis of variance for grain yield (GY), spikes per square meter (SPM^2), kernel per spike (KPS), thousand kernel weight (TKW) and test weight (TW) for ten wheat cultivars under three inoculation treatments with *Zymoseptoria tritici* in two years.

Source of variation	df	GY		SPM^2		KPS		TKW		TW	
		MS	P > F	MS	P > F	MS	P > F	MS	P > F	MS	P > F
Year (Y)	1	96,222,632	0.004	909,142	0.010	283	0.183	850	0.101	566	0.020
Error a	2	341,376		8882		70.5		100		11.7	
Inoculation (I)	2	52,464,302	<0.001	62,785	<0.001	235	<0.001	261	<0.001	21.1	0.154
Y × I	2	1,893,810	0.103	11,142	0.047	23.5	0.181	17.9	0.095	7.22	0.475
Error b	8	618,229		2418		11.1		5.58		8.84	
Cultivar (C)	9	4,608,409	<0.001	28,690	<0.001	44.9	0.112	21.86	0.076	65.6	<0.001
Y × C	9	1,547,207	0.078	23 249	0.002	33.6	0.286	18.60	0.145	22.9	<0.001
I × C	18	605,998	0.802	1831	0.999	4.00	1.000	10.98	0.573	2.91	0.969
Y × I × C	18	281,878	0.996	1933	0.999	4.22	1.000	1.68	1.000	3.05	0.961
Error c	108	863,086		7247		27.4		12.12		6.34	

GLAD has achieved better results than the models using AUDPC or NGLAD to detect the presence of tolerance to STB. Coincidently, Bryson et al. (1997) did not find differences in tolerance using measurements of AUDPC and Parker et al. (2004) found that the relationship between NGLAD and GY fitted poorly.

Some other authors have also identified the presence of tolerance to STB in different germplasms. Zuckerman et al. (1997) indicated that photosynthesis in the remaining green tissues of the tolerant cultivars was higher than in non-infected cultivars, while Simón et al. (2002) found that some cultivars (K. Centauro and K. Dragon) had increased AUDPC values in 1997, despite the fact that yield reductions recorded in 1996 were similar. In our research, TKW in tolerant cultivars was also less affected by STB than in intolerant cultivars. Zilberstein et al. (1985) demonstrated that the tolerant bread wheat cultivar Miriam maintained kernel weight under severe epidemics of STB and suggested that a possible mechanism responsible for grain filling in tolerant cultivars under STB epidemic is the compensation by carbohydrate supply from unaffected tissues. There was evidence for tolerance being associated with lower yield potential (Parker et al., 2004; Paveley et al., 2005). However, in this work correlations between both variables were not found, indicating that it is possible to breed cultivars with high tolerance and high yield potential.

The lower GPC observed in 2011 could be explained by the

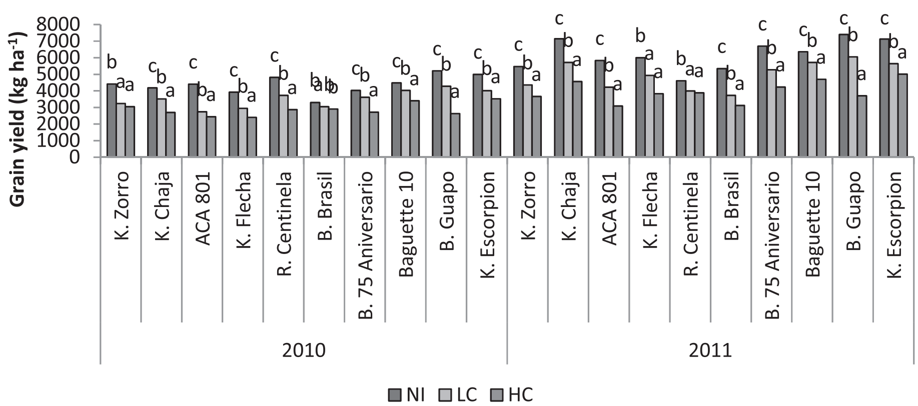

Fig. 3. Grain yield (GY) (kg ha^{-1}) for ten wheat cultivars under three inoculation treatments with *Zymoseptoria tritici*: NI (non-inoculated), LC (Low concentration), HC (High concentration) in two years. Different letters between inoculation treatments within each cultivar indicate significant differences (LSD = 331; P < 0.05).

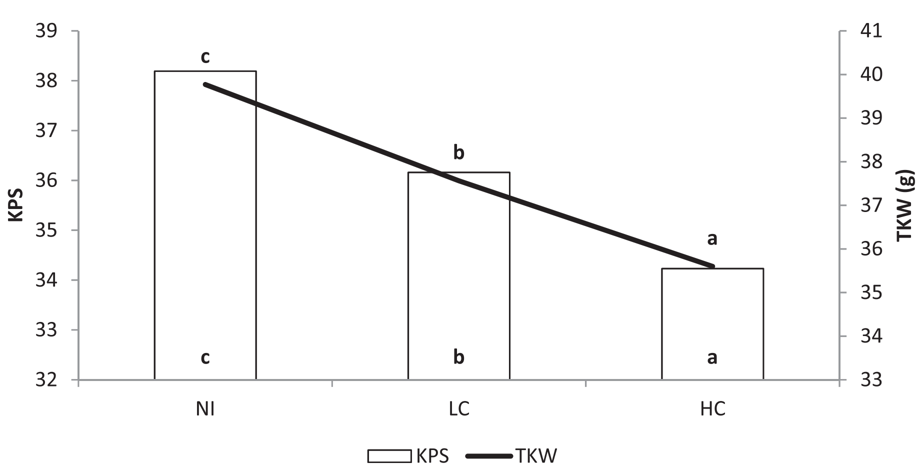

Fig. 4. Kernels per spike (KPS) and thousand kernel weight (TKW) for ten wheat cultivars under three inoculation treatments with *Zymoseptoria tritici* NI (Non-inoculated), LC (Low concentration), HC (High concentration) in two years. Different letters between inoculation treatments indicate significant differences (LSD for KPS = 1.40, TKW = 0.99, P < 0.05).

Table 4
Slopes of the regression between the area under disease progress curve (AUDPC) and grain yield (GY) (left) and between green leaf area duration (GLAD) and grain yield (GY) (right) for ten wheat cultivars under three inoculation treatments with *Zymoseptoria tritici* in 2010 and 2011.

	AUDPC-GY		Mean	GLAD-GY		Mean
	2010	2011		2010	2011	
K. Zorro	−1.96	−1.30	−1,63 a	34.3	33.8	30.1 abc
K. Chaja	−2.56	−2.38	−2,47 a	47.1	47.3	43.8 c
ACA 801	−2.26	−1.86	−2,06 a	33.8	36.6	30.3 abc
K. Flecha	−0.76	−2.83	−1,79 a	25.8	25.9	25.4 ab
R. Centinela	−2.51	−0.92	−1,71 a	51.7	16.5	35.5 bc
B. Brasil	−1.20	−1.60	−1,40 a	11.3	14.3	18.7 ab
B. 75 Aniversario	−0.52	−2.93	−1,72 a	20.0	21.8	18.6 ab
Baguette 10	−2.79	−3.96	−3,37 a	17.6	15.5	16.1 a
B. Guapo	−3.22	−4.25	−3,74 a	46.5	49.6	43.5 bc
K. Escorpion	−1.80	−2.18	−1,99 a	15.0	25.7	20.7 ab

Different letters in the same column indicate significant differences (LSD = 17.5, $P < 0.05$).

Table 5
Mean squares (MS) from the combined analysis for grain protein concentration (GPC), wet gluten concentration (WGC), dry gluten concentration (DGC) on ten wheat cultivars under three inoculation treatments with *Zymoseptoria tritici* in two years.

Source of variation	df	GPC		WGC		DGC	
		MS	P > F	MS	P > F	MS	P > F
Year (Y)	1	37.8	0.068	2.44	0.084	0.67	0.050
Error a	2	2.88		0.23		0.04	
Inoculation (I)	2	22.8	0.002	38.8	0.002	35.5	0.001
Y x I	2	6.47	0.063	0.33	0.886	0.07	0.723
Error b	8	1.62		2.73		0.19	
Cultivar (C)	9	11.0	<0.001	60.3	<0.001	84.7	<0.001
Y × C	9	2.90	0.039	9.68	<0.001	0.74	0.009
I × C	18	1.63	0.312	2.40	0.222	0.32	0.341
Y × I × C	18	1.13	0.691	1.37	0.779	0.29	0.422
Error c	108	1.41		1.89		0.28	

respect to *Zymoseptoria tritici*, Arabi et al. (2007) found a protein reduction due to STB depending on the susceptibility of the cultivar used. Puppala et al. (1998) report large increases in GPC following fungicides use on a cultivar, specifically bred for high protein concentration. However, other investigations found increases of protein concentration when wheat cultivars were affected by STB. In that way, Watson et al., 2010 found an increase in GPC of 0.004% for every 1% increase in STB severity, whereas Clark (1993) mentions that protein concentration reductions following fungicide use were less in bread making cultivars, but this interaction also reflected varietal differences in disease susceptibility and yield responses.

The correlation coefficient between the quality group of the wheat cultivar and the variations in GPC and gluten concentration due to *Zymoseptoria tritici* was not significant for both years. However, cultivars belonging to G1 and G2 quality highly increased or maintained GPC with inoculum concentration, while G3 cultivars tended to decrease in both years. This tendency coincides with Dimmock and Gooding (2002) who suggested that cultivars specifically bred for bread-making, where high protein concentration is a selection criterion together with high grain yield, may be able to maintain grain nitrogen accumulation more effectively as senescence is delayed and yields increase, compared with cultivars suited to biscuit and livestock feed markets, where protein concentration is much less important. Also, the relationship among tolerance and variations in GPC due to *Zymoseptoria tritici* was not significant.

Our results indicate that yield can be improved using tolerant cultivars when wheat is affected by STB. Tolerance mechanism is a potentially durable form of defense, placing little or no selection pressure on pathogen populations, because they do not interfere with pathogen multiplication (Walters et al., 2012) and farmers are guaranteed stable yields, despite severe epidemics of STB. Minimizing the extent of yield loss per unit of disease expression or visible leaf damage could help to reduce the need for fungicides by increasing the thresholds for an application or reducing the dose

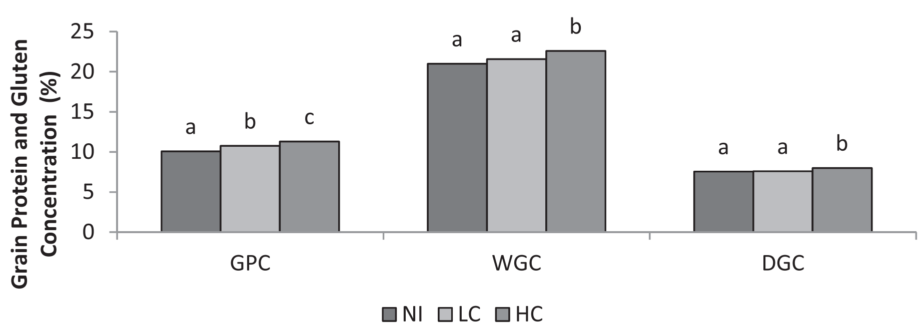

Fig. 5. Mean values of grain protein concentration (GPC), wet gluten concentration (WGC) and dry gluten concentration (DGC) on ten wheat cultivars under three inoculation treatments with *Zymoseptoria tritici*: NI (non-inoculated), LC (low concentration) and HC (high concentration) in two years. Different letters between inoculation treatments indicate significant differences (LSD for GPC = 0.54, WGC = 0.70 and DGC = 0.19, $P < 0.05$).

higher GY which may have caused a dilution effect. Regarding the effect of STB in GPC, WGC and DWC, the results of this work evidenced an increment of these variables with the inoculum concentration. In this aspect, there are contrasting results. *Zymoseptoria tritici* has been observed to exhibit some characteristics of biotrophy in the early stages of infection before a necrotrophic phase (Royle et al., 1995; Kema and van Silfhout, 1996) reason why it is considered hemibiotrophic. When biotrophs are controlled, GPC often increases, indicating that the pathogen reduces it. In contrast, the effect of controlling necrotrophic pathogens is associated with a reduction in protein concentration because pathogens increase it (Dimmock and Gooding, 2002). With required. Furthermore, the effect of STB in increasing the GPC was demonstrated in these cultivars, although no effect of tolerance was seen in this increase. No association was observed between tolerance and grain yield either, indicating that tolerant high yielding cultivars can be obtained.

Acknowledgments

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Annex

Annex 1
Mean values for disease severity and area under disease progress curve (AUDPC) for ten wheat cultivars under three inoculation treatments: NI (non-inoculated), LC (low concentration) and HC (high concentration) with *Z. tritici* in two years through crop cycle: Flag leaf (GS 39), Anthesis (GS 60) and Early dough stage (GS 82).

	Disease severity																		AUDPC					
	GS 39						GS 60						GS 82											
	2010			2011			2010			2011			2010			2011			2010			2011		
	NI	LC	HC	NI	LC	HC	NI	LC	HC	NI	LC	HC	NI	LC	HC	NI	LC	HC	NI	LC	HC	NI	LC	HC
K. Zorro	2.6	5.6	7.4	11.5	14.9	22.8	12.5	19.8	26.0	12.1	19.9	32.1	42.2	60.2	74.5	53.2	68.7	92.1	792	1207	1538	1256	1743	2535
K. Chaja	1.8	4.4	4.6	8.6	13.5	15.0	10.2	12.5	27.2	7.3	20.2	31.6	49.0	61.1	65.8	70.1	82.1	100.0	795	1017	1437	1315	1920	2519
ACA 801	7.4	7.2	7.8	7.8	14.0	20.1	14.8	22.6	29.3	19.5	44.5	47.1	37.9	66.4	64.3	74.3	100.0	100.0	877	1367	1520	1709	2871	3035
K. Flecha	5.8	7.6	6.9	10.0	13.3	18.9	4.5	17.8	23.6	16.8	19.6	19.4	38.3	54.7	48.3	61.1	78.7	97.6	598	1130	1196	1480	1852	2193
R. Centinela	2.0	3.9	9.1	13.3	18.3	23.9	9.7	24.0	27.1	20.9	27.4	31.8	45.6	67.9	60.6	73.5	80.0	99.1	751	1368	1445	1816	2165	2641
B. Brasil	2.9	5.9	5.8	4.5	5.2	12.5	16.3	21.0	26.5	16.3	29.8	40.4	49.7	67.4	64.1	57.8	72.8	84.5	971	1317	1420	1339	1942	2515
B. 75 Aniversario	3.0	6.9	7.1	9.5	12.5	19.7	13.1	18.8	24.0	13.3	17.8	30.3	39.0	68.3	69.9	64.1	74.1	82.8	779	1287	1436	1413	1725	2309
Bag. 10	7.7	12.6	17.5	13.3	18.0	23.0	9.3	12.7	21.4	14.0	17.3	26.0	28.6	31.7	39.4	58.0	61.3	69.0	642	833	1203	1404	1614	2041
B. Guapo	12.0	13.3	18.5	17.7	19.0	24.3	14.0	19.7	33.8	18.3	24.0	37.7	19.4	22.1	30.3	48.7	52.0	60.0	729	918	1431	1460	1688	2266
K. Escorpion	11.5	15.5	20.9	17.3	21.3	26.0	11.6	17.1	25.7	16.7	22.3	31.0	18.4	44.7	62.9	48.0	74.7	84.3	651	1121	1606	1398	1991	2441
LSD Y	1.6						5.9						2.0						550.2					
LSD I	1.3						3.0						7.5						125.2					
LSD Y × I	1.7						4.8						15.2						450.4					
LSD C	2.8						5.5						12.2						217.2					
LSD Y × C	3.8						7.9						19.2						431.7					
LSD I × C	4.7						9.4						21.0						372.5					
LSD Y × I × C	6.7						13.4						30.7						589.0					

Annex 2
Mean values for green leaf area index (GLAI) and green leaf area duration (GLAD) for ten wheat cultivars under three inoculation treatments: NI (non-inoculated), LC (low concentration) and HC (high concentration) with *Z. tritici* in two years through crop cycle: Flag leaf (GS 39), Anthesis (GS 60) and Early dough stage (GS 82).

	GLAI																		GLAD					
	GS 39						GS 60						GS 82											
	2010			2011			2010			2011			2010			2011			2010			2011		
	NI	LC	HC	NI	LC	HC	NI	LC	HC	NI	LC	HC	NI	LC	HC	NI	LC	HC	NI	LC	HC	NI	LC	HC
K. Zorro	2.2	2.0	1.8	6.0	5.3	4.1	2.7	1.4	1.4	5.1	3.1	2.9	1.3	0,8	0,3	0,3	0,1	0,1	104.5	73.2	63.6	222.7	158.0	136.3
K. Chaja	2.1	1.9	1.8	6.3	4.7	4.6	2.3	1.8	1.3	4.3	3.5	3.1	0.6	0,6	0,5	0,8	0,2	0,1	94.1	78.9	62.6	213.0	161.7	148.6
ACA 801	3.1	1.5	1.1	6.7	4.3	3.8	2.4	1.8	1.3	3.6	2.7	2.7	0.8	0,6	0,6	0,4	0,1	0	114.5	74.8	54.2	197.8	145.1	126.1
K. Flecha	2.2	1.2	1.3	6.0	4.8	3.4	2.2	1.5	1.2	3.5	3.1	3.5	0.7	0,5	0,3	0,3	0,2	0	95.2	60.2	51.8	183.2	153.4	140.3
R. Centinela	2.9	2.5	2.5	6.3	4.3	5.1	2.4	1.8	1.5	3.1	2.7	2.6	0.8	0,6	0,7	0,4	0,2	0	110.6	88.0	81.0	178.7	136.4	143.2
B. Brasil	3.1	2.1	1.4	4.1	5.7	3.7	1.7	1.4	1.3	3.9	3.2	3.0	0.7	0,6	0,5	0,5	0,3	0,2	94.2	72.8	59.4	166.9	169.8	134.2
B. 75 Aniversario	2.4	1.8	1.6	6.6	4.9	4.6	2.1	1.3	1.8	3.7	3.7	3.0	0.8	0,4	0,5	0,3	0,2	0,1	94.6	63.4	74.7	196.0	169.2	147.5
Baguette 10	2.7	2.4	2.1	6.5	5.7	4.9	2.9	2.2	2.1	4.2	4.0	3.8	0.8	0,7	0,2	0,6	0,5	0	123.0	100.9	86.9	220.8	192.6	170.7
B. Guapo	4.8	3.4	2.8	6.9	5.4	4.8	1.8	1.4	1.3	3.6	3.1	3.1	0.9	0,7	0,2	0,7	0,5	0	128.8	94.0	77.6	200.9	166.0	149.6
K. Escorpion	5.2	3.0	1.4	7.2	5.0	3.4	2.5	1.9	1.0	4.2	3.6	3.4	1.0	0,4	0,6	0,8	0,2	0,4	152.4	97.6	53.4	224.5	169.7	142.0
LSD Y	0.4						0.3						0.3						13.6					
LSD I	0.2						0.2						0.1						8.3					
LSD Y × I	0.3						0.3						0.3						11.9					
LSD C	0.6						0.5						0.2						14.0					
LSD Y × C	0.8						0.6						0.3						19.8					
LSD I × C	1.0						0.8						0.4						24.0					
LSD Y × I × C	1.4						1.1						0.5						34.0					

Annex 3

Mean values for non green leaf area index (NGLAI) and non green leaf area duration (NGLAD) for ten wheat cultivars under three inoculation treatments: NI (non-inoculated), LC (low concentration) and HC (high concentration) with *Z. tritici* in two years through crop cycle: Flag leaf (GS 39), Anthesis (GS 60) and Early dough stage (GS 82).

	NGLAI																		NGLAD					
	GS 39						GS 60						GS 82											
	2010			2011			2010			2011			2010			2011			2010			2011		
	NI	LC	HC	NI	LC	HC	NI	LC	HC	NI	LC	HC	NI	LC	HC	NI	LC	HC	NI	LC	HC	NI	LC	HC
K. Zorro	0.0	0.1	0.1	0.2	0.8	0.3	0.2	0.2	0.3	0.5	1.0	1.1	0.3	0.6	0.6	0.4	0.5	0.6	9.0	11.1	15.8	18.7	40.9	37.6
K. Chaja	0.0	0.1	0.1	0.2	0.7	0.9	0.2	0.1	0.4	0.2	1.0	1.0	0.3	0.3	0.5	0.1	0.3	0.7	7.9	8.2	16.2	9.1	40.5	45.0
ACA 801	0.3	0.2	0.2	0.6	1.5	1.5	0.3	0.4	0.2	0.6	1.5	1.5	0.5	0.8	0.4	0.5	0.4	0.7	17.5	20.8	10.8	28.2	64.2	67.6
K. Flecha	0.1	0.1	0.1	0.5	0.7	0.9	0.1	0.2	0.1	0.6	0.7	0.5	0.4	0.7	0.4	0.5	0.6	0.7	7.3	13.8	9.3	26.7	33.4	33.3
R. Centinela	0.0	0.1	0.3	0.1	0.7	0.4	0.1	0.1	0.5	1.3	0.7	0.8	0.4	0.4	1.3	0.2	0.3	0.5	6.3	8.3	29.9	37.7	32.7	29.8
B. Brasil	0.0	0.1	0.2	0.3	0.5	0.2	0.1	0.2	0.2	1.0	1.2	0.5	0.3	0.5	0.7	0.2	0.3	0.8	6.4	11.3	14.7	32.7	42.7	25.4
B. 75 Aniversario	0.1	0.3	0.2	0.2	0.3	0.7	0.2	0.4	0.3	0.5	0.4	0.8	0.6	0.4	0.8	0.1	0.3	0.4	13.0	18.7	18.3	15.2	17.7	35.8
Baguette 10	0.1	0.2	0.5	0.1	0.2	0.7	0.4	0.6	0.7	0.3	0.5	0.6	0.2	0.3	0.6	0.1	0.2	0.5	12.8	21.3	31.8	10.3	18.4	31.0
B. Guapo	0.3	0.7	0.4	0.1	0.6	0.5	0.4	1.1	0.7	0.3	1.0	0.6	0.2	0.1	0.7	0.1	0.0	0.6	16.8	40.7	30.4	11.5	35.4	29.8
K. Escorpion	0.2	0.4	0.6	0.6	0.4	0.5	0.7	0.5	0.5	0.6	0.4	0.5	0.1	0.4	0.6	0.0	0.3	0.4	22.2	23.3	29.4	25.0	21.0	22.4
LSD Y	0.1						0.5						0.2						14.7					
LSD I	0.1						0.2						0.1						6.6					
LSD Y × I	0.2						0.4						0.1						11.5					
LSD C	0.1						0.2						0.1						6.5					
LSD Y × C	0.2						0.4						0.2						11.9					
LSD I × C	0.3						0.4						0.2						12.1					
LSD Y × I × C	0.3						0.5						0.3						17.9					

Annex 4

Mean values for grain yield (GY), spikes per square meter (SPM^2), kernels per spike (KPS) and thousand kernel weight (TKW) for ten wheat cultivars under three inoculation treatments: NI (non-inoculated), LC (low concentration) and HC (high concentration) with *Z. tritici* in two years.

	GY (kg ha^{-1})						SPM^{-2}						KPS						TKW (g)					
	2010			2011			2010			2011			2010			2011			2010			2011		
	NI	LC	HC	NI	LC	HC	NI	LC	HC	NI	LC	HC	NI	LC	HC	NI	LC	HC	NI	LC	HC	NI	LC	HC
K. Zorro	4409	3238	3049	5473	4358	3661	442.1	310.9	323.3	518.8	531.3	485.7	36.2	36.7	33.8	41.4	39.6	38.1	41.3	41.1	40.5	40.3	36.8	36.2
K. Chaja	4183	3512	2701	7143	5711	4561	462.2	370.0	307.8	472.1	472.3	463.7	34.8	34.6	33.7	44.0	40.1	36.6	39.9	39.3	37.8	39.6	35.9	32.9
ACA 801	4404	2745	2440	5834	4220	3088	449.8	309.9	305.3	600.7	567.7	555.4	34.6	33.9	31.4	37.9	33.3	31.9	40.0	37.6	36.5	34.7	33.0	28.2
K. Flecha	3924	2946	2402	5999	4934	3831	362.2	263.3	227.8	464.4	422.2	412.7	37.1	39.1	36.2	42.7	40.1	36.7	41.7	41.0	41.3	36.2	34.4	32.5
R. Centinela	4817	3723	2864	4611	3993	3886	425.3	364.4	317.6	482.2	423.5	442.7	40.8	37.5	33.7	36.8	34.2	30.9	39.9	38.7	39.0	39.8	37.4	34.7
B. Brasil	3297	3047	2906	5347	3730	3116	371.1	378.7	313.2	480.3	493.3	450.6	30.8	30.3	32.9	39.3	35.7	33.5	41.7	40.5	40.3	36.2	34.6	34.4
B. 75 Aniversario	4029	3616	2721	6701	5272	4235	373.3	355.6	300.0	691.0	626.3	639.7	35.9	34.5	33.5	40.9	40.0	38.7	43.8	42.2	39.6	36.6	34.6	31.7
Baguette 10	4480	4027	3402	6364	5712	4693	436.7	408.7	401.7	537.2	502.4	505.0	37.8	35.4	34.0	39.8	35.7	33.7	40.4	40.4	37.1	37.2	37.5	33.4
B. Guapo	5206	4277	2634	7398	6051	3703	420.7	429.3	396.7	521.0	531.6	500.0	37.3	34.3	32.5	39.3	36.3	34.5	44.0	37.9	34.7	42.0	34.3	30.9
K. Escorpion	4997	4013	3523	7122	5642	5014	447.7	404.7	393.9	547.7	504.8	491.1	36.9	34.9	33.0	39.2	36.9	35.4	41.5	38.2	36.5	38.6	35.8	33.7
LSD Y	375						60.5						5.4						6.4					
LSD I	331						20.7						1.4						1.0					
LSD Y × I	468						46.6						4.3						2.3					
LSD C	614						56.3						3.5						5.7					
LSD Y × C	868						80.9						5.4						4.9					
LSD I × C	1063						94.1						5.8						3.9					
LSD Y × I × C	1604						135.2						8.5						6.4					

Annex 5

Mean values for grain protein concentration (GPC), wet gluten and dry gluten concentration (WGC-DGC) and test weight (TW) for ten wheat cultivars under three inoculation treatments: NI (non-inoculated), LC (low concentration) and HC (high concentration) with *Z. tritici* in two years.

	GPC (%)						WGC (%)						DGC (%)						TW (Kg Hl^{-1})					
	2010			2011			2010			2011			2010			2011			2010			2011		
	NI	LC	HC	NI	LC	HC	NI	LC	HC	NI	LC	HC	NI	LC	HC	NI	LC	HC	NI	LC	HC	NI	LC	HC
K. Zorro	10.0	11.1	11.3	10.8	10.1	10.1	22.5	24.0	24.3	23.5	23.4	23.1	7.9	8.2	8.5	8.3	8.2	8.1	82.4	81.5	81.9	76.6	79.0	78.8
K. Chaja	10.9	11.0	12.3	11.1	10.6	11.0	20.3	20.7	24.2	18.4	21.4	22.0	7.4	7.3	8.2	6.7	7.4	7.3	81.6	82.4	83.7	72.5	77.5	76.6
ACA 801	10.1	13.0	13.4	9.3	11.1	11.4	21.5	22.3	22.4	23.5	24.3	24.6	7.8	8.0	7.8	8.3	8.3	8.4	82.5	82.8	84.1	77.4	78.8	77.2
K. Flecha	9.3	12.5	12.8	10.0	9.6	11.4	21.7	23.0	23.5	21.3	22.4	25.1	7.7	8.1	8.3	7.4	7.7	8.7	82.6	84.2	83.4	77.9	79.2	76.8
R. Centinela	11.5	13.0	11.8	9.7	9.7	11.9	21.5	21.5	21.1	21.2	22.7	23.5	7.4	7.3	7.5	7.4	7.1	8.5	81.6	82.2	81.9	78.4	78.9	78.2
B. Brasil	11.1	12.5	12.5	10.6	10.5	10.6	22.3	22.8	23.1	21.9	21.0	22.6	7.8	8.0	8.0	7.4	7.1	7.9	83.4	82.8	84.4	79.8	80.1	77.7
B. 75 Aniversario	10.6	11.3	12.9	9.3	9.6	9.8	22.3	22.4	23.5	18.2	18.9	20.6	7.7	7.7	8.2	6.8	6.6	7.4	82.7	83.4	81.5	76.9	78.9	78.7
Baguette 10	9.0	9.6	10.0	9.3	8.3	9.1	21.9	22.0	23.5	22.1	21.7	23.4	8.5	8.5	8.9	9.2	8.7	8.2	76.2	77.8	77.7	75.1	77.7	77.5
B. Guapo	8.6	9.2	10.2	8.8	9.0	10.8	16.5	16.1	18.2	16.8	15.9	18.6	5.8	5.8	6.8	5.9	5.8	6.6	75.1	76.5	76.4	73.7	75.8	76.3
K. Escorpion	10.6	11.7	11.1	10.9	11.7	11.7	21.3	22.3	22.4	21.5	22.7	22.2	7.7	8.0	8.5	7.8	8.3	8.1	77.0	77.9	77.9	78.1	77.9	74.9
LSD Y	1.09						0.31						0.12						2.2					
LSD I	0.54						0.70						0.19						1.3					
LSD C	0.78						0.91						0.35						1.9					
LSD Y × I	0.76						0.98						0.26						1.7					
LSD Y × C	1.11						1.28						0.50						2.5					
LSD I × C	1.36						1.57						0.61						3.0					
LSD Y × I × C	1.92						2.22						0.86						4.2					

References

Annone, J.G., Calzolari, A., Polidoro, O., Conta, H., 1991. Efecto de la mancha de la hoja causada por Septoria tritici sobre el rendimiento. Informe 122. INTA EEA Pergamino, Pergamino, Argentina, p. 4. Informe N° 122.

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. Agropecu. VI, 21–23.

Arabi, M.I., Jawar, M., Mir Ali, N., 2007. The effect of *Mycosphaerella graminicola* infection on wheat protein content and quality. Cereal. Res. Commun. 35, 81–88.

Arraiano, L.S., Brading, P.A., Brown, J.K.M., 2001. A detached seedling leaf technique to study resistance to *Mycosphaerella graminicola* (anamorph *Septoria tritici*) in wheat. Plant. Pathol. 50, 339–346.

Association of official Analytical Chemists (A.O.A.C), 1970. Micro Kjeldahl Technique, 11.

Bailey, K.L., Duczek, L.J., Jones-Flory, L., Kutcher, R., Fernandez, M.R., Hughes, G.R., Kirkham, C., Mortensen, K., Boyetchko, S., Burnett, P., Orr, D., 1993. Saskatchewan/central alberta wheat diseases survey 1992. Can. Plant Dis. Surv. 73, 75–78.

Blandino, M., Pilati, A., Reyneri, A., 2009. Effect of foliar treatments to durum wheat on flag leaf senescence, grain yield, quality and deoxynivalenol contamination in North Italy. Field Crop. Res. 14, 214–222.

Boote, K.J., Jones, J.W., Mishoe, J.W., Berger, R.D., 1983. Coupling pests to crop simulators to predict yield reductions. Phytopathology 73, 1581–1587.

Bryson, R.J., Sylvester-Bradley, R., Scott, R.K., Paveley, N.D., 1995. Reconciling the effects of yellow rust on yield of winter wheat through measurements of green leaf area and radiation interception. Asp. Appl. Biol. 42, 9–18.

Bryson, R.J., Paveley, N.D., Clark, N.D., 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.

Clare, R.W., Jordan, V.W.L., Smith, S.P., Davies, W.P., Bulman, C., Evans, C.J., Stevens, D.B., Nuttal, M., Naylor, R.E.L., 1990. The effects of nitrogen and fungicide treatment on the yield and grain quality of Avalon winter wheat. Asp. Appl. Biol. 25, 375–386. Cereal Quality II.

Clark, W.S., 1993. Interaction of winter wheat varieties with fungicide programmes and effects on grain quality. Asp. Appl. Biol. 36, 397–406. Cereal Quality III.

Clarke, D.D., 1984. Tolerance of parasitic infection in plants. In: Wood, R.K.S., Jellis, G.J. (Eds.), Plant Diseases: Infection Damage and Loss. Blackwell Scientific Publications, Oxford, UK, pp. 119–127.

Cools, H.J., Fraaije, B.A., 2013. Update on mechanisms of azole resistance in *Mycosphaerella graminicola* and implications for future control. Pest. Manag. Sci. 69, 150–155.

Cornish, P., Baker, G., Murray, G., 1990. Physiological responses of wheat (*Triticum aestivum*) to infection with *Mycosphaerella graminicola* causing *Septoria tritici* blotch. Aust. J. Agric. Res. 41, 317–327.

Dimmock, J.P.R.E., Gooding, M.J., 2002. The influence of foliar diseases, and their control by fungicides, on the protein concentration in wheat grain: a review. J. Agric. Sci. 138, 349–366.

Estep, L.K., Torriani, S.F.F., Zala, M., Anderson, N.P., Flowers, M.D., McDonald, B.A., Mundt, C.C., Brunner, P.C., 2015. Emergence and early evolution of fungicide resistance in North American populations of *Zymoseptoria tritici*. Plant Pathol. 64, 961–971.

Foulkes, M.J., Paveley, N.D., Worland, A., Welham, S.J., Thomas, J., Snape, J.W., 2006. Major genetic changes in wheat with potential to affect disease tolerance. Phytopathology 96, 680–688.

Gilbert, J., Tekauz, A., 1990. Foliar pathogens of spring wheat in Manitoba in 1989. Can. Plan. Dis. Surv. 70, 46.

Gilbert, J., Tekauz, A., 1992. Foliar pathogens of spring wheat in Manitoba in 1991. Can. Plant. Dis. Surv. 72, 60–61.

Hess, D.E., Shaner, G., 1987. Effect of moisture and temperature on development of *Septoria tritici* Blotch in wheat. Am. Phytopathol. Soc. Phytophatol 77, 215–219.

Inglese, S.J., Paul, N.D., 2006. Tolerance of *Senecio vulgaris* to infection and disease caused by native and alien rust fungi. Phytopathology 96, 718–726.

IRAM 15864, 2007. Determination of Wet Gluten, Dry Gluten and Gluten Index.

Ishikawa, S., Hare, M.C., Kettlewell, P.S., 2001. Nitrogen accumulation in grains of wheat in response to strobilurins fungicides. Asp. Appl. Biol. 64, 235–236. Wheat Quality.

James, W.C., Shih, C.S., Callbeck, L.C., Hodgson, W.A., 1973. Interplot interference in field experiments with late blight of potato (*Phytophtora infestans*). Phytopathology 63, 1269–1275.

Johnson, K.B., 1987. Defoliation, disease and growth: a reply. Phytopathology 77, 1495–1497.

Keed, B.R., White, N.H., 1970. Quantitative effects of leaf and stem rusts on yield and quality of wheat. Aust. .J Exp. Agric. Animal Husb. 11, 550–555.

Kema, G.H.J., van Silfhout, C.H., 1996. Histology of the pathogenesis of *Mycosphaerella graminicola* in wheat. Phytopathology 86, 777–786.

Kramer, T., Gildemacher, B.H., van der Ster, M., Parlevliet, J.E., 1980. Tolerance of spring barley cultivars to leaf rust, *Puccinia hordei*. Euphytica 29, 209–216.

Leitch, M.H., Jenkins, P.D., 1995. Influence of nitrogen on the development of *Septoria* epidemics in winter wheat. J. Agric. Sci. 124, 361–368.

Leyva-Mir, S.G., Gilchrist-Saavedra, L., Zavaleta-Mejía, E., Khairallah, M., 2006. Yield losses in wheat (T*riticum aestivum L.*) genotype inoculated with single and mixed isolates of *Septoria tritici* rob ex. Desm. Agrocienc. 40, 315–323.

Lim, L.G., Gaunt, R.E., 1981. Leaf area as a factor in disease assessment. J. Agric. Sci. 97, 481–483.

Madden, L.V., Nutter, F.W., 1995. Modelling crop losses at the field scale. Can. J. Plant Pathol. 17, 124–137.

Mundt, C.C., Hoffer, M.E., Ahmed, H.U., Coakley, S.M., DiLeone, J.A., Cowger, C., 1999. Population genetics and host resistance. In: Lucas, J.A., Bowyer, P., Anderson, H.M. (Eds.), Septoria on Cereals: a Study of Pathosystems. CAB International, Wallingford, UK, pp. 115–130.

Mundt, C.C., 2002. Use of multiline cultivars and cultivar mixtures for disease management. Annu. Rev. Phytopathol. 40, 381–410.

Myram, C., Kelly, J.R., 1981. Prediction of the economic need for a fungicide programme on winter wheat and the effects of this programme on some parameters of grain quality. In: Proceedings of the British Crop Protection Conference – Pests and Diseases, pp. 241–248.

Parker, S.R., Welham, S., Paveley, N.D., Foulkes, J., Scott, R.K., 2004. Tolerance of Septoria leaf blotch in winter wheat. Plant Pathol. 53, 1–10.

HCGA Paveley, N.D., Foulkes, J., Sylvester-Bradley, R., Parker, S., Lovell, D., Snape, J., Farrar, J., Neumann, S., Nason, J., Ellerbrook, C., 2005. Maximising Disease Escape, Resistance and Tolerance in Wheat through Genetic Analysis and

Agronomy. Project report N° 358.
Penny, A., Widdowson, F.V., Jenkyn, J.F., 1983. Experiments with solid and liquid N-fertilizers and fungicides on winter wheat at Saxmundham, Suffolk 1976–9. J. Agric. Sci. 100, 163–173. Cambridge.
Phipps, I.F., 1938. The effect of leaf-rust on yield and baking quality of wheat. J. Aust. Inst. Agric. Sci. 4, 148–151.
PRONACATRI, 2006. Calidad industrial del trigo argentino: análisis de la Red de Ensayos Comparativos de Trigo (RET), comparación de subregiones y variedades comerciales en cultivo. In: Programa Nacional de Calidad de Trigo, SAGPyA, Bs. As, p. 34.
Puppala, V., Herrman, T.J., Bockus, W.W., Loughin, T.M., 1998. Quality responses of twelve hard red winter wheat cultivars to foliar disease across four locations in central Kansas. Cereal Chem. 75, 148–151.
Rabbinge, R., Rijsdijk, P.H., 1981. Disease and crop physiology: a modeler's point of view. In: Ayres, P.G. (Ed.), Effects of Disease on the Physiology of the Growing Plants. Cambridge Univ. Press, Cambridge, UK, pp. 201–220.
Rabbinge, R., Ward, S.A., Van Laar, H.H. (Eds.), 1989. Simulation and Systems Management in Crop Protection. Pudoc, Wageningen, p. 420.
Rasband, W.S., 2014. ImageJ. U. S. National Institutes of Health, Bethesda, Maryland, USA, p. 1997. http://imagej.nih.gov/ij.
Rees, R.G., Platz, G.J., Mayer, R.J., 1982. Yield losses in wheat from yellow spot: comparison of estimates derived from single tillers and plots. Aust. J. Agric. Res. 33, 899–908.
Robert, C., Bancal, M.O., Nicolas, P., Lannou, C., Ney, B., 2004. Analysis and modelling effects of leaf rust and *Septoria tritici* blotch on wheat growth. J. Exp. Bot. 55, 1079–1094.
Robert, C., Bancal, M.O., Lannou, C., Ney, B., 2006. Quantification of the effects of *Septoria tritici* blotch on wheat leaf gas exchange with respect to lesion age, leaf number, and leaf nitrogen status. J. Exp. Bot. 57, 225–234.
Rodrigo, S., Cuello Hormigo, B., Gomes, C., Santamaría, O., Costa, R., Poblaciones, M.J., 2015. Influence of fungicide treatments on disease severity caused by *Zymoseptoria tritici*, and on grain yield and quality parameters of bread- making wheat under Mediterranean conditions. Eur. J. Plant Pathol. 141, 99–109.
Royle, D.J., Parker, S.R., Lovell, D.J., Hunter, T., 1995. Interpreting trends and risks for better control of Septoria in winter wheat. In: Hewitt, H.G., Tyson, D., Hollomon, D.W., Smith, J.M., Davies, W.P., Dixon, K.R. (Eds.), A Vital Role for Fungicides in Cereal Production. Bios, Oxford, pp. 105–115.
Ruske, R.E., Gooding, M.J., Pepler, S., Froggatt, P., 2001. Nitrogen accumulation in grains of winter wheat in response to strobilurin fungicides. Asp. Appl. Biol. 64, 227–234. Wheat Quality.
Savary, S., Willocquet, L., 2014. Simulation modeling in botanical epidemiology and crop loss analysis. APSnet Plant Health Instr. http://dx.doi.org/10.1094/PHI-A-2014-0314-01. http://www.apsnet.org/edcenter/advanced/topics/BotanicalEpidemiology/Pages/default.aspx.
Schafer, J., 1971. Tolerance to plant disease. Annu. Rev. Phytopathol 9, 235–252.
Serrago, R.A., Carretero, R., Bancal, M.O., Miralles, D.J., 2011. Grain weight response to foliar diseases control in wheat (*Triticum aestivum* L.). Field Crop. Res. 120, 352–359.
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.
Shewry, P.R., Halford, N.G., 2002. Cereal seed storage proteins: structures, properties and role in grain utilization. J. Exp. Bot. 53, 947–958.
Simón, M.R., Perelló, A.E., Cordo, C.A., Arriaga, H.O., 1996. Influencia de la infección tardía de Septoria tritici Rob Ex Desm sobre el peso de mil granos y algunos parámetros de calidad en *Triticum aestivum* L. Invest. Agrar. Prod. Prot. Veg. 11, 161–171.
Simón, M.R., Perelló, A.E., Cordo, C.A., Struik, P.C., 2002. Influence of *Septoria tritici* on yield, yield components, and test weight of wheat under two nitrogen fertilization conditions. Crop Sci. 42, 1974–1981.
Simón, M.R., Cordo, C.A., Perelló, A.E., Struik, P.C., 2003. Influence of nitrogen supply on the susceptibility of wheat to *Septoria tritici*. Phytopathology 151, 283–289.
Simón, M.R., Cordo, C.A., Castillo, N.S., Struik, P.C., Borner, A., Simón, M.R., Castillo, N., Struik, P.C., Borner, A., 2012. Recent advances on the population structure of *Mycosphaerella graminicola* in Argentina and location of genes for resistance in several populations. Int. J. Agron. Special Issue on Integrated. Wheat Management. 2012, 7. Article ID 680275. www.hindawi.com.
Slafer, G.A., Rawson, H.M., 1994. Sensitivity 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.
Torriani, S.F.F., Brunner, P.C., McDonald, B.A., Sierotzki, H., 2009. QoI resistance emerged independently at least 4 times in European populations of *Mycosphaerella graminicola*. Pest Manag. Sci. 65, 155–162.
VSN International, 2011. GenStat for Windows, fourteenth ed. VSN International, Hemel Hempstead, UK. Web page. GenStat.co.uk.
Waggoner, P.E., Berger, R.D., 1987. Defoliation, disease and growth. Phitopathology 77, 393–398.
Walters, D.R., Avrova, A., Bingham, I.J., Burnett, F.J., Fountaine, J., Havis, N.D., Hoad, S.P., Hughes, G., Looseley, M., Oxley, S.J.P., Renwick, A., Topp, C.F.E., Newton, A.C., 2012. Eur. J. Plant Pathol. 133, 33–73.
Watson, A.M., Hare, M.C., Kettlewell, P.S., Brosnan, J.M., Agu, R.C., 2010. Relationships between disease control, green leaf duration, grain quality and the production of alcohol from winter wheat. J. Sci. Food Agric. 90, 2602–2607.
Whelan, H.G., Gaunt, R.E., 1990. Yield loss: disease relationship in barley crops with different yield potentials. In: Proceeding of the 43rd NZ Weed and Pest Control Conference 1990, pp. 159–162.
Willocquet, L., Aubertot, J.N., Lebard, S., Robert, C., Lannou, C., Savary, S., 2008. Simulating multiple pest damage in varying winter wheat production situations. Field Crop. Res. 107 (1), 12–28.
Zadoks, J.C., Chang, T.T., Konzak, C.F., 1974. A decimal code for the growth stages of cereals. Weed Res. 14, 415–421.
Zadoks, J.C., 1985. On the conceptual basis of crop loss assessment: the threshold theory. Annu. Rev. Phytopathol. 23, 455–473.
Zhan, J., McDonald, B.A., 2004. The interaction among evolutionary forces in the pathogenic fungus *Mycosphaerella graminicola*. Fungal Genet. Biol. 41, 590–599.
Zilberstein, M., Blum, A., Eyal, Z., 1985. Chemical desiccation of wheat plants as a simulator of post anthesis speckled leaf blotch stress. Phytopathology 75, 226–230.
Ziv, O., Eyal, Z., 1978. Assesment of yield component losses caused in plants of spring wheat cultivars by selected isolates of *Septoria tritici*. Phytopathology 68, 791–796.
Zuckerman, E., Eshel, A., Eyal, Z., 1997. Physiological aspects related to tolerance of spring wheat cultivars to *Septoria tritici* blotch. Phytopathology 87, 60–65.