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Evaluation of different fungicides and nitrogen rates on grain yield and bread-making quality in wheat affected by Septoria tritici blotch and yellow spot

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ABSTRACT

This study aims to evaluate the influence of different fungicide mixtures under three nitrogen (N) fertilization rates on the area under disease progress curve (AUDPC) of STB (Septoria tritici blotch, *Zymoseptoria tritici*) (hemibiotrophic) and YS (yellow spot, *Pyrenophora tritici-repentis* (Died) Drechs (anamorph *Drechslera tritici-repentis* (Died) Shoem) (necrotrophic) separately inoculated, green leaf area duration, and its effect on grain yield, grain protein concentration (GPC) and bread-making traits. Fungicide applications decreased AUDPC of both diseases compared to the control treatment, without significant differences between fungicide types. Fungicides increased green leaf area duration and grain yield compared to the control treatment, although no differences were found between fungicide types when STB was the prevalent disease. The GPC was increased by STB and YS, whereas fungicides decreased it. Those reductions with triazole-strobilurin tended to be lower as N increased for STB and were significantly lower for YS. Conversely, under 0 to 70 kg N/ha, GPC decreased when fungicides were used, particularly under the triazole-strobilurin-carboxamide treatment. Cultivars differed with respect to bread-making quality traits. In spite of the increase in GPC with both pathogens, gluten content and bread volume increased with STB but decreased with YS, indicating that the nutritional habit of both pathogens may have a different impact on protein composition.

1. Introduction

Bread wheat (*Triticum aestivum* L.) is a staple food because is the most versatile grain among cereals for the preparation of diverse foods (Peña-Bautista et al., 2007) determined by the quantity and quality of grain protein concentration (GPC). The amount of grain protein is usually an indicator of the gluten in flour providing an index of flour strength. Gluten proteins, consisting of gliadins and glutenins, play an important role in the bread-making quality of wheat flour as gliadins mainly contribute to dough viscosity and extensibility, while glutenins to dough strength and elasticity (Wieser, 2007).

Most of the studies concerning wheat quality under abiotic stresses have focused on heat stress, water stress or nutrient deficiency. Conversely, studies investigating the effect of biotic stresses such as foliar diseases on bread-making quality are scarce or focused on milling quality because they are associated with the occurrence of grain shriveling (Gooding and Davies, 1997). Among wheat foliar diseases, Septoria tritici blotch (STB) caused by the fungus *Zymoseptoria tritici* and yellow spot (YS) caused by *Pyrenophora tritici-repentis* (Died) Drechs anamorph *Drechslera tritici-repentis* (Died) Shoem are the most devastating since both reduce grain yield and affect quality worldwide. The lifestyle of *Z. tritici* is hemibiotrophic, which means it is biotrophic early in the infection process, deriving its nutrition from the apoplast around living cells, and then kills the surrounding host cells becoming necrotrophic (utilizing dead tissue) during the later stages of infection (Ponomarenko et al., 2011). On the other hand, *P. tritici-repentis* is a

Abbreviations: STB, Septoria tritici blotch; YS, yellow spot; N, nitrogen; AUDPC, area under disease progress curve; GS, growth stage; TLAI, total leaf area index; GLAI, green leaf area index; GLAD, green leaf area duration; GPC, grain protein concentration; BV, bread volume; WGC, wet gluten content; T, triazole; TS, triazole-strobilurin; TSC, triazole-strobilurin-carboxamide; SDHI, succinate dehydrogenase inhibitor

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Table 1
Main characteristics of cultivars used in the Septoria tritici blotch (STB) and the yellow spot (YS) experiments (MS: moderately susceptible; MR: moderately resistant).

Cultivars	Reaction to STB	Quality group[a]	Description
Buck Guapo (BGuapo)	MS	1	Corrector cultivars for industrial bread-making
Klein Escorpión (KEscorp)	MR to MS	2	Traditional bread-making cultivars suitable for major long fermentations (> 8 h)
Nidera Baguette 10 (Bag 10)	MS	3	Cultivars for direct bread-making methods (< 8 h)
Cultivars	**Reaction to YS**	**Quality group[a]**	**Description**
ACA 315 (A315)	MS	1	Corrector cultivars for industrial bread-making
Nidera Baguette 11 (Bag 11)	MS to MR	2	Traditional bread-making cultivars suitable for major long fermentations (> 8 h)
ACA 303 (A303)	MS	3	Cultivars for direct bread-making methods (< 8 h)

[a] Argentinian bread -making quality grade (Argentine Winter Cereals Committee of the National Seeds Institute) (INASE).

pure necrotroph, killing the cell host by the production of host-selective toxins (Manning and Ciuffetti, 2015). A foliar disease as STB have been reported to influence GPC and other bread-making characteristics with contradictory results (Arabi et al., 2007; Castro and Simón, 2016), while YS was reported to cause increases (Rees et al., 1982). In particular, literature concerning the effect of YS on GPC is scarce compared to STB (See Dimmock and Gooding, 2002), especially with reference to other bread-making traits as gluten content and bread volume.

Nitrogen (N) nutrition is widely considered as one of the main factor affecting GPC as well as the technological quality of the grain. However, some studies have reported that N fertilization may also affect the expression of foliar diseases. This literature is characterized by many inconsistencies; for instance, both increases and decreases of STB and YS severity of plants were reported following N applications (Jones et al., 1990; Simón et al., 2003; Krupinski et al., 2007; Carretero et al., 2010).

Photosynthesis in wheat during grain filling period plays a key role in dry matter accumulation in the grain, including N. Strategies to protect leaves, particularly the flag leaf, and delay the senescing process are therefore important to assure higher yield and quality (Blandino and Reyneri, 2009). Fungicide application is a common disease management practice in the Argentinean wheat region, particularly in environments of high yield potential. A few years ago, strobilurins and triazoles and their mixtures were the most wide-spread active ingredients applied. However, during the last few years, new fungicide groups which control pathogens by succinate dehydrogenase inhibitor (SDHI) such as carboxamides have been incorporated. Prior studies found that increased grain yield by SDHI was associated with extended green leaf area duration (GLAD) due to positive effects in plant physiology (Berdugo et al., 2012; Fleitas et al., 2018); nonetheless, more studies are needed. Furthermore, fungicide applications have been reported to influence GPC and other bread-making characteristics with contradictory results (Cuniberti et al., 2004; Blandino and Reyneri, 2009; Rodrigo et al., 2015). In this sense, there are concerns that under low to medium N-soil conditions, significant yield gains may decrease GPC mainly caused by the dilution of protein by more carbohydrates and thus, compromise bread-making characteristics. This also can interact with the genotype since varietal differences in the N-use efficiency have been reported (Puppala et al., 1998). Therefore, more studies investigating the influence of fungicide applications and N fertilization on GPC and bread-making quality combined are needed. In addition, most of the studies concerning the effects of foliar diseases on grain yield and quality have been carried out under natural infections or where the disease severity has been assessed only throughout the area under disease progress curve (AUDPC) which does not consider green canopy size that can vary among genotypes, seasons, and environments. Given the difference between *Z. tritici* and *P. tritici-repentis* regarding nutrition habit (hemibiotrophic or necrotrophic), we would predict that GPC increases caused by YS, might be more consistent than STB. Therefore, this study sought to evaluate the influence of different fungicide mixtures under three N fertilization rates on the progress of STB and YS (separately inoculated), GLAD, and to find out its effect on grain yield, GPC, and other bread-making traits.

2. Materials and methods

2.1. Septoria tritici blotch experiments

Two field experiments were conducted under artificial inoculations at the Estación Experimental J. Hirschhorn (EEJH), Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, Argentina; during 2009 and 2010. Weather data (monthly precipitation; relative humidity and minimum, maximum and mean temperatures) were recorded at a Davis® Meteorological Station situated 100 m from the experiments. Trials were sown in adjacent fields in July under conventional tillage for each year. The EEJH has a silt loam soil, classified as Typic Argiudoll, analysis of the soil samples indicated the following values by weight in each year: (i) top-0.20 m, organic matter: 3.65 and 3.49%; Nt: 0.18 and 0.10%; $N\text{-}NO_3^-$: 43 and 29 ppm; P: 19 ppm and 15 ppm and pH: 5.80 and 6.00; (ii) 0.20–0.40 m, $N\text{-}N\text{-}NO_3^-$: 30 ppm and 28 ppm and pH: 5.70 and 6.3 for 2009 and 2010, respectively.

The experimental design was a split-split plot with three replications. Within every year, the main plots were the fungicide treatments [1-with fungicide Nativo® which is a combination of a triazole (tebuconazole, 480 cm^3/ha) and a strobilurin (trifloxystrobin, 120 cm^3/ha in a total dose of 600 cm^3/ha) (TS), 2- with fungicide Folicur® (triazole-only: tebuconazole, 750 cm^3/ha) (T) and 3- Untreated (control treatment)]. Sub-plots were the N treatments [1-N_0 (0 kg N/ha), N_{70} (70 kg N/ha) and N_{140} (140 kg N/ha), applied as granulated urea one-half at sowing and the other half at growth stage (GS) 33 (Zadoks et al., 1974)]. Sub-sub plots were three registered cultivars with similar heading time and varying from moderate resistance to moderate susceptibility to STB according to the information provided by their respective breeders (Table 1). Each sub-sub plot was 7.7 m^2 (5.5 m long by 1.4 m wide). The entire experiment was fertilized with 50 kg P_2O_5/ha as calcium triple superphosphate at sowing.

2.2. Yellow spot experiments

Two field experiments were conducted under artificial inoculations at the EEJH; during 2014 and 2015. Weather data were also recorded. Trials were sown in July and June respectively under conventional tillage. The type of soil was the same described for the STB experiments, values of soil samples were similar for both years, on average: (i) top-0.20 m, organic matter: 3.59%; Nt: 0.20%; $N\text{-}NO_3^-$: 10.4 ppm; P: 28 ppm and pH: 5.80; (ii) 0.20–0.40 m, $N\text{-}NO_3$: 5.1 ppm and pH: 6.0.

The experimental design was a split-split plot with three replications, as the same described for the STB. Within every year, the main plots were the fungicide treatments [1-with fungicide Orquesta Ultra® which is a combination of a triazole (epoxiconazole, 60 g/ha) and a

strobilurin (pyraclostrobin, 97.2 g/ha) and a carboxamide (fluxapyroxad 60 g/ha) (TSC), 2- with fungicide Opera® which is a combination of a triazole (epoxiconazole, 50 g/ha) and a strobilurin (pyraclostrobin, 133 g/ha) (TS) and 3- Untreated (control treatment)]. The sub-plots were the N treatments as described for the STB experiments (N_0, N_{70} and N_{140}). Sub-sub plots were three registered cultivars with similar heading time and varying from moderate resistance to moderate susceptibility (Table 1). The size of sub-sub plots and the phosphorus fertilization was the same as described for the STB experiments.

2.3. Inoculations and fungicide applications

The experiments of STB were inoculated with a mixture of four virulent isolates of *Z. tritici* grown on malt extract agar. The inoculum was prepared by aseptically scraping sporulating colonies with a scalpel and suspending conidia in deionized water. Experiments of YS were inoculated with a mixture of *P. tritici-repentis* grown on V8® media at 23 °C ± with 12 h alternating light and dark cycles. The spore concentration was measured with a Neubauer hemocytometer. The conidial suspension of *Z. tritici* was adjusted to 5×10^6 spores/ml, while for *P. tritici-repentis* was adjusted to 3×10^3 spores/ml. Tween 20 (0.5 ml per liter) was added as a surfactant. Plants were sprayed with the inoculum suspension until runoff. After inoculations, plants were kept moist by spraying water several times a day (for 15 min every 2 h) for three days. In the STB experiments , inoculations and fungicide were applied three times at the beginning of tillering (GS 22), three nodes stage (GS 33) and at the beginning of flowering (GS 61) in both years. In the YS experiments, inoculations were performed at GS 22 and beginning of shoot development (GS 31), while fungicides were applied at tillering (GS 23) and flag leaf (GS 39).

2.4. Evaluations of disease severity, area under disease progress curve and dynamics of green leaf area index

In both experiments, disease severity was assessed by visual estimation of the percentage of leaf area affected by STB and YS on seven to ten plants in each plot at the following growth stages: on the upper four leaves at GS 39 and at GS 60; and in the upper two leaves (flag leaf and the leaf below flag leaf) at early dough stage (GS 82). The AUDPC for each treatment was calculated to summarize the progress of the disease, according to Shaner and Finney (1977). In the STB experiment, the green leaf area index (GLAI, green leaf m^2/soil m^2) was determined at every growth stage by counting the numbers of tillers in two linear meters from each plot and measuring the individual green leaf area by separating and pasting leaves from seven tillers with at least 10% green on paper sheets, which were scanned and measured by the image J program. In the YS experiments, GLAI was estimated at the three growth stages by discounting the disease severity to the total leaf area index (TLAI, total leaf m^2/soil m^2). TLAI was measured by counting the main stems and tillers in two linear meters from each plot and measuring the individual leaf area (length × wide) affected by a correction factor of 0.835 (Miralles and Slafer, 1991) of seven tillers. Finally, GLAD (days) was calculated by the trapezoidal method in both experiments according to Waggoner and Berger (1987).

2.5. Grain yield and quality assessments

Four meters of the three central rows in each plot were harvested and threshed (2.4 m^2) and the grain yield (kg/ha) was calculated. For both experiments, grain samples from each sub-sub-plot were conditioned to 15.5% moisture and milled using a laboratory mill (Bühler® MLU-202), extracting the flour at a rate of about 70%. Nitrogen concentration was determined by the Micro-Kjeldahl method and GPC was expressed as crude protein (N × 5.7) (IRAM 15852-1, 2002). The wet gluten content (WGC, %) was determined with 10 g of flour by Glutomatic 2200® (IRAM 15864-1:2002). The bread-making process was carried out using the direct dough method with 100 g of flour (IRAM 15858–1:1996), and then bread loaf volume (BV, cm^3) was determined by rapeseed displacement using a volume-meter.

2.6. Statistical analysis

For both experiments, data were analyzed using a combined analysis of variance (ANOVA) for both years with a split-split plot design with GenStat 12 Ed. program. In the STB experiments, WGC (log-transformed) and BV (exponential-transformed) were converted using functions that best-fitted in order to meet the assumptions of the ANOVA. In the same way, for the YS experiments, transformations were performed for AUDPC, WGC (log-transformed), GLAD (square root-transformed), and BV (exponential-transformed). Mean values were compared with LSD test ($p < 0.05$).

3. Results

3.1. Meteorological conditions

In the STB experiments, rainfall varied greatly between years. The sum of rainfall during the crop cycle was 631 mm and 344 mm for 2009 and 2010 respectively (data not shown). Mean temperature and mean relative moisture were similar for both years, which averaged 14.5 °C and 72.0% respectively. In the YS experiments, the sum of rainfall during the crop cycle in 2014 was 755 mm and in 2015 was 543 mm. Differences in temperature and relative moisture values over the two growing seasons were relatively modest for both years averaging 14.7 °C and 70.5%, respectively (data not shown).

3.2. Area under disease progress curve and green leaf area duration

Analysis of variance and mean values of AUDPC and GLAD for both diseases are presented in Tables 2 and 3 respectively. For both experiments, AUDPC of STB and YS was significantly higher in the wettest year (2009 and 2014, respectively). Fungicide applications decreased AUDPC of both diseases compared to the control treatment, without significant differences between fungicide types (Table 3). In the STB experiment, there were differences of AUDPC among wheat cultivars; KEscorp was the most affected cultivar while Bag 10 was the least affected (Table 3). In this experiment, the significant *Year* × *N* interaction was mainly ascribed to a higher decrease of AUDPC across the N rate in the wettest year (2009) compared to the driest (2010). In 2009, values fluctuated from 2683 (N_{140}) to 3789 (N_0), while in 2010 values ranged from 1696 (N_{140}) to 1726 (N_0). In the YS experiments, the *Fungicide* × *Cultivar* arose given the differences in the magnitude of the effect of the fungicide treatments across cultivars. Thus, AUDPC of Bag 11 decreased a −48.5% when comparing the TSC treatment with the untreated control, while in A315 and A303, the magnitude of the decrease was −39.4% and −37.3% respectively.

The GLAD was significantly influenced by the main factors and some interactions (Table 2). In the STB experiments, GLAD means were significantly higher (+51.7%) in the wettest year. Fungicide treatments increased GLAD (+16.0%) compared to the control treatment, although no differences were found between fungicide types (Table 3). Interaction *Year* × *Cultivar* was mainly explained by the higher values of GLAD in KEscorp followed by BGuapo and Bag 10 in the driest year, while in 2009 no differences were detected. The *N* × *Cultivar* and *Fungicide* × *N* × *Cultivar* interactions were mainly due to differences in the response magnitude of the three cultivars among fungicide treatments with rising N rates. Averaged over all cultivars, KEscorp had the highest values of GLAD (115 days), followed by BGuapo (108 days) and Bag 10 (101 days) (Table 3).

In the YS experiments, GLAD values were statistically higher in 2014 (+3.9%) in relation to the driest year (2015) (Table 3). The *Fungicide* × *N* × *Cultivar* interaction was significant (Table 2) because

Table 2
Mean squares for the combined ANOVA for the area under disease progress curve (AUDPC), green leaf area duration (GLAD), grain yield (GY), grain protein concentration (GPC), wet gluten content (WGC) and bread volume (BV) for three wheat cultivars under three fungicide treatments and three N rates inoculated with *Zymoseptoria tritici* and *Pyrenophora tritici-repentis* in two years.

Source of variation	df	Septoria tritici Blotch						Yellow spot					
		AUDPC	GLAD	GY	GPC	WGC	BV	AUDPC	GLAD	GY	GPC	WGC	BV
Year (Y)	1	95082551***	79313**	91858260	4.22	3.35E-2**	1.60E+8	1.06E+00***	1.61E+0**	139244	0.11	7.69E-3	3.01E-4
Error a	2	115780	1017	14378967	0.53	9.95E-4	1.35E+10	1.40E-05	4.20E-3	6633324	0.02	3.24E-3	5.54E-4
Fungicide (Fu)	2	14739275***	4725***	46836660***	5.44 ***	4.76E-3*	5.09E+9*	8.55E-01***	4.58E+1***	54309404***	9.68***	2.82E-2***	4.34E-3***
Y × Fu	2	1460295	16.3	5854610	0.47	1.05E-3	8.67E+8	1.21E-04	5.00E-4	94299	0.10	2.47E-3**	8.77E-5
Error b	8	1109458	317	4075683	0.29	1.23E-3	1.01E+9	2.47E-02	2.73E-1	455639	0.35	1.54E-4	2.61E-4
Nitrogen (N)	2	4766972***	56083***	68611219***	45.0***	7.78E-2***	3.94E+10***	1.26E-01***	1.29E+2***	31862644***	26.2***	9.01E-2***	6.83E-2***
Y × N	2	3554934***	242	32103403***	1.16	3.71E-4	1.83E+9	1.52E-04	2.30E-3	2725061	0.13	2.19E-4	3.43E-5
Fu × N	4	864997	161	1285039	1.38	3.20E-3	1.07E+9	6.36E-04	8.21E-1**	1889698**	3.16***	2.94E-3***	5.01E-4**
Y × Fu × N	4	227989	218	955170	0.18	1.08E-3	1.66E+9	1.56E-04	5.00E-4	161790	0.18	8.50E-5	6.03E-5
Error c	24	368526	149	1269429	0.69	2.36E-3	1.10E+9	6.39E-03	2.43E-1	440232	0.22	1.56E-4	1.58E-4
Cultivar (Cv)	2	16145104***	2459***	15081439***	13.7***	5.66E-2***	9.24E+9**	1.77E-01**	6.04E+0***	6970161***	44.7***	4.01E-2***	5.30E-3***
Y × Cv	2	1269472	1140***	1528465	0.48	2.35E-2***	2.19E+8	1.10E-05	3.40E-3	14445	1.86	7.69E-3***	1.45E-4
Fu × Cv	4	516842	162	2150108	1.18	4.91E-3*	8.37E+8	1.13E-02**	2.08E+0***	2373336***	1.31*	4.67E-3***	4.27E-4
N × Cv	4	866273	2109***	811058	0.76	7.00E-5	2.96E+9*	3.85E-03	9.72E-1**	148681	1.16	6.51E-4	9.19E-4**
Y × Fu × Cv	4	162672	129	2666773	0.48	8.24E-4	2.84E+9	4.00E-06	2.00E-4	192298	0.41	2.47E-3**	1.63E-4
Y × N × Cv	4	252894	282	4373020	0.47	1.09E-3	2.24E+9	5.00E-06	1.00E-4	12730	0.26	2.19E-4	1.05E-5
Fu × N × Cv	8	227641	506***	1734232	0.78	2.25E-3	1.16E+9	3.00E-03	7.20E-1**	585500	0.44	4.80E-4	2.68E-4
Y × Fu × N × Cv	8	136998	58.3	1100039	0.31	3.82E-3	1.43E+9	2.00E-06	3.00E-4	50129	0.30	8.50E-5	4.33E-5
Error d	72	459641	153	2473828	1.40	2.05E-3	1.33E+9	2.50E-03	2.20E-1	392903	0.51	4.89E-4	3.41E-4

*p < 0.10, **p < 0.05, ***p < 0.001.

Table 3
Area under disease progress curve (AUDPC), green leaf area duration (GLAD), grain yield (GY), grain protein concentration (GPC), wet gluten content (WGC) and bread volume (BV) for three wheat cultivars under three fungicide treatments and three N rates inoculated with *Zymoseptoria tritici* and *Pyrenophora tritici-repentis* in two years. Different letters in the same column indicate significant differences according to LSD test ($p < 0.05$).

Septoria tritici blotch							Yellow spot						
Main factors	AUDPC	GLAD	GY	GPC	WGC	BV	Main factors	AUDPC	GLAD	GY	GPC	WGC	BV
		(days)	(kg/ha)	(%)	(%)	(cm^3)			(days)	(kg/ha)	(%)	(%)	(cm^3)
Year													
2009	3231 b	130 b	7098 a	11.9 a	23.5 b	375 a	2014	537 b	109 b	6658 a	11.2 a	27.5 a	501 a
2010	1699 a	85.7 a	5592 a	12.2 a	21.9 a	373 a	2015	370 a	105 a	6599 a	11.2 a	26.6 a	492 a
Fungicide													
Untreated	3053 b	97.4 a	5573 a	12.3 b	23.0 b	389 b	Untreated	607 b	86.2 a	5494 a	11.7 b	25.4 a	470 a
T	2290 a	115 b	6957 b	12.2 b	22.9 b	364 a	TS	404 a	112 b	6994 b	11.0 a	27.5 b	495 ab
TS	2053 a	111 b	6805 b	11.7 a	22.2 a	369 a	TSC	349 a	122 c	7397 c	10.9 a	28.2 c	519 b
Nitrogen rate													
N_0	2758 c	75.1 a	5066 a	11.3 a	21.3 a	335 a	N_0	498 b	75.2 a	5829 a	10.6a	24.6 a	398 a
N_{70}	2474 b	109 b	6777 b	11.8 b	21.8 ab	380 b	N_{70}	461 ab	107 b	6695 b	11.0 b	26.8 b	503 b
N_{140}	2164 a	140 c	7193 c	13.1 c	24.9 b	407 c	N_{140}	401 a	138 c	7361 c	12.0 c	29.8 c	582 c
Cultivar													
BGuapo (QG1)	2575 b	108 a	6193 c	12.4 b	21.7 a	383 b	A315 (QG1)	425 b	99.5 a	6227 a	12.2 c	29.0 b	520 b
KEscorp (QG2)	2949 c	115 b	5909 a	12.3 b	24.7 b	385 b	Bag 11 (QG2)	531 c	108 b	6920 b	10.9 b	26.0 a	500 ab
Bag 10 (QG3)	1872 a	101 a	6933 b	11.5 a	21.8 ab	354 a	A303 (QG3)	405 a	113 c	6738 b	10.5 a	26.2 a	463 a

UT: untreated control, T: triazole, TS: triazole + strobilurin, TSC: triazole + strobilurin + carboxamide, QG: quality group.

in the untreated control, the increment of GLAD values was significantly higher when comparing the N_{140} with the N_0 rate, in relation to the TS and the TSC treatments where the response tended to be lower. Although the same tendency was observed in the three cultivars, A315 evidenced the highest increment in the untreated control followed by Bag 11 and A303 (data not shown).

3.3. Grain yield and grain protein concentration

Analysis of variance and averages values of grain yield of both experiments are presented in Tables 2 and 3 respectively. In the STB experiments, grain yield significantly increased (+30.5%) following fungicide applications compared to the control treatment, although, no differences were found between fungicide types (Table 3). Cultivar Bag 10 evidenced the highest grain yield value, followed by BGuapo and KEscorp which did not statistically differ (Table 3). The significant interaction *Year* × *N* arose given differences in the grain yield increase through rising N rates between years. In 2010, values ranged from 3682 kg/ha (N_0) to 7299 kg/ha (N_{140}), a higher increment (+98.2%) than in 2009, where values fluctuated from 6449 kg/ha (N_0) to 7086 kg/ha (N_{140}), showing a lower increment of +9.90% (Fig. 1a). In the YS experiments, the *Fungicide* × *N* interaction arose because in the TSC treatment, grain yield increased +34.3% when comparing the N_0 with the N_{140} rate, a greater response than when comparing the TS (+19.4%) and the untreated control (+24.7%) (Fig. 1b). Regarding the *Fungicide* × *Cultivar* interaction (Table 2), grain yield of Bag 11 significantly increased (+48.2%) comparing the untreated plots with the TSC treatment, in a much greater extent than for A315 (+29.3%) and A303 (+27.2%) (Fig. 1c).

Grain protein concentration was increased by STB and YS, whereas fungicide applications caused significantly decreases (Table 3). In addition, GPC tended to increase greatly across the rising N rates (Table 3). In the STB experiments, despite the *Fungicide* × *N* interaction was not significant, GPC reductions with the TS treatment tended to be lower as the dose of N increased (Fig. 2a). In the YS experiment, the *Fungicide* × *N* interaction occurred because under the maximum N status (N_{140}), no differences of GPC were detected among the three fungicide treatments (Fig. 2b). Conversely, under N_0 and N_{70}, following fungicide applications, mean values of GPC significantly decreased, particularly under the TSC treatment (Fig. 2b). Regarding the *Fungicide* × *Cultivar* interaction, fungicide applications statistically decreased GPC among cultivars. Mean values were significantly lower in the TSC treatment compared to the TS fungicide in ACA 315 and Bag 11, however, in ACA 303, GPC tended to be lower following the TS treatment (Fig. 2d).

In the STB experiments, there were differences among cultivars. Thus, GPC was significantly lower in Bag 10 (mean 11.5%) compared to BGuapo and KEscorp which did not differ statistically between them

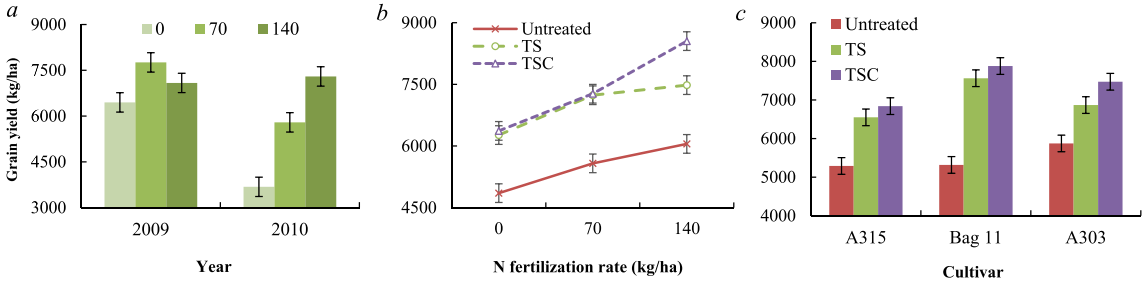

Fig. 1. Mean values of grain yield in (a) the *Year* × *N* interaction for the Septoria tritici blotch experiment, (b) the *Fungicide* × *N* and (c) the *Fungicide* × *Cultivar* interaction for the yellow spot experiment. 0, 70 or 140 is the N fertilization rate (hg/ha), T: triazole, TS: triazole + strobilurin fungicide, TSC: triazole + strobilurin + carboxamide fungicide. Vertical bars indicate LSD ($p = 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

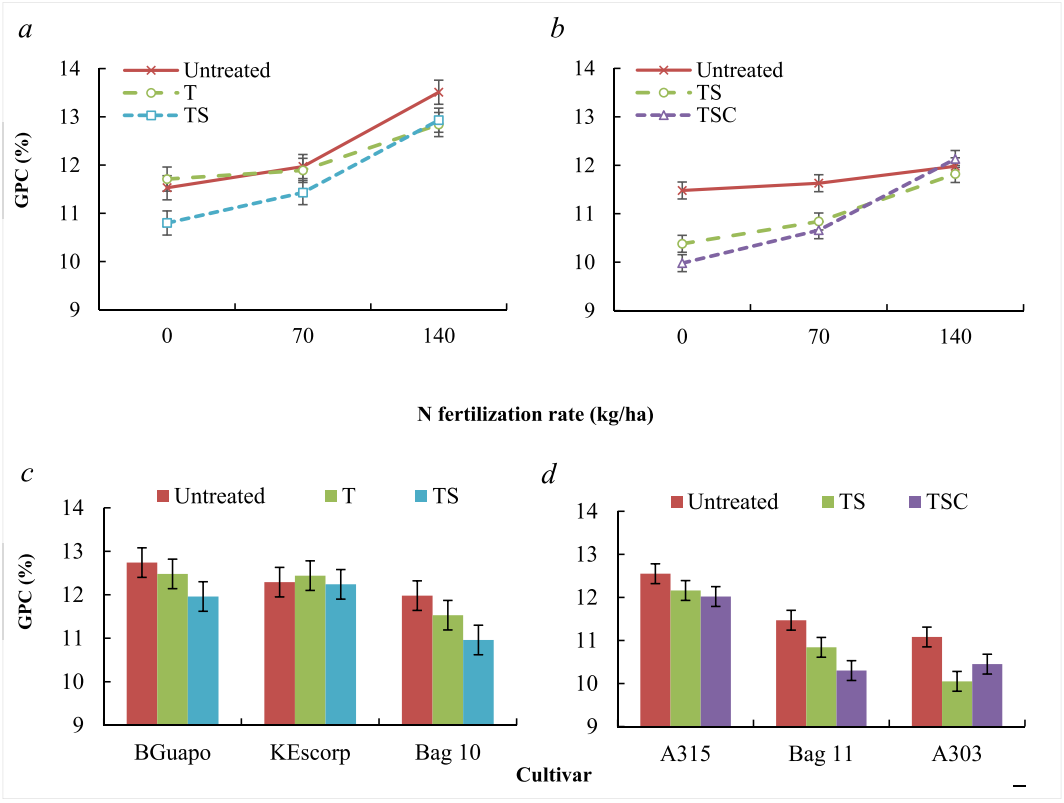

Fig. 2. Mean values of grain protein concentration (GPC) in (a) the *Fungicide* × *N* interaction for the Septoria tritici blotch experiment, (b) the same interaction for the yellow spot experiment, (c) the *Fungicide* × *Cultivar* interaction for the Septoria tritici blotch experiment and (d) the same interaction for the yellow spot experiment. T: triazole, TS: triazole + strobilurin fungicide, TSC: triazole + strobilurin + carboxamide fungicide. Vertical bars indicate LSD ($p = 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

(means 12.4% and 12.3% respectively) (Table 3). Even though the *Fungicide* × *Cultivar* interaction was not significant (Table 2), cultivar Bag 10 tended to have the highest reduction of GPC with both fungicides (−8.5% with TS and −3.8% with T treatment) followed by BGuapo (−6.1% with TS and −2.0% with T treatment) and KEscorp (−0.41% with TS and −1.2% with T treatment) (Fig. 2c).

3.4. Wet gluten content and bread volume

In the STB experiments, the wet gluten content was affected by *Year*, *Fungicides*, *N*, *Cultivar*, *Year* × *Cultivar* and *Fungicide* × *Cultivar* interaction (Table 2). Mean values were statistically higher in 2009 (+7.3%) compared to 2010 (Table 3). Wet gluten content tended to decrease following fungicide applications in relation to control treatment although only TS showed significant differences. The higher the N rate, the higher the WGC (Table 3). The *Year* × *Cultivar* interaction arose given that Bag. 10 had the lowest means in the wettest year (2009) whereas in the driest (2010), values were significantly higher (Fig. 3a). The *Fungicide* × *Cultivar* interaction arose because the TS fungicide significantly decreased WGC in cultivar Bag 10 (−7.62%) compared to the untreated control, while cultivars BGuapo and KEscorp showed lower decreases following fungicide applications (−1.35% and −2.03% respectively).

Fungicide × *N* interaction for WGC for the YS experiments is shown in Fig. 3b. Under the maximum N rate (N_{140}), WGC was significantly higher with the TSC treatment, followed by the TS fungicide and the control. Conversely, under N_0 and N_{70}, means did not statistically differ between fungicide types, although both were significantly higher than the untreated control (Fig. 3b).

The *Year* × *Fungicide* × *Cultivar* interaction arose (Table 2) because values of gluten content did not statistically differ in cv. Bag 11 across the fungicide treatments in 2015 (average 24.7%), whereas in the previous year (2014), values significantly increased from 25.1% to 30% when comparing the untreated control with the TSC treatment (data not

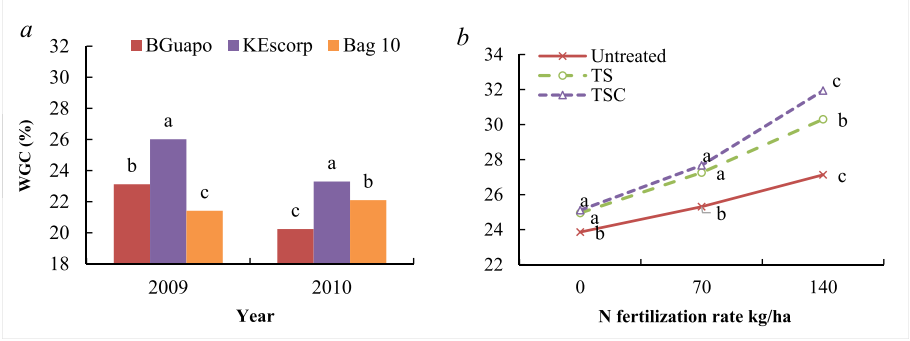

Fig. 3. Mean values of wet gluten content (WGC) in (a) the *Year* × *Cultivar* interaction for the Septoria tritici blotch experiment and (b) the *Fungicide* × *N* interaction for the yellow spot experiment. T: triazole, TS: triazole + strobilurin fungicide, TSC: triazole + strobilurin + carboxamide fungicide. Different letters within the same year or N rate, do not statistically differ (LSD $p = 0.05$). Letters were used instead of bars to indicate LSD differences, as mean values were compared using the transformed means. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

shown).

Finally, BV was modified by *Fungicide*, *N* and *Cultivar* factors in both, STB and YS experiments (Table 2). In the STB experiments, the application of fungicide significantly decreased BV, although there were no differences between fungicide types (Table 3). Conversely, in the YS experiments, *Fungicide* × *N* interaction showed that the TSC application with higher rates of N significantly increased BV (+54.4%) compared to TS (+46.8%) and the untreated control (+37.0%). Regarding N × *Cultivar* interaction, in both diseases the higher the N rate, the higher the BV means in all cultivars. Cultivars BGuapo and KEscorp (quality group 1 and 2 respectively) had the highest increases (+29.3% and +15.8% respectively) followed by Bag 10 (+20.3%) (quality group 3) in the STB experiments (Table 3). The same pattern was observed in the YS experiments, where means were significantly higher with higher doses of N in the highest quality group cultivar (A315) (+51.7%) followed by Bag 11 (+51.0%) (quality group 2) and finally, A303 (+35.2%) (quality group 3) (Table 3).

4. Discussion

4.1. Area under disease progress curve, green leaf area duration and grain yield

There was a high number of necrotic blotches caused by *Z. tritici* and *P. tritici-repentis*, particularly in the wettest years (2009 and 2014 respectively) where diseases were noted in high intensity explained by the higher precipitations occurred during those years. Rodrigo et al. (2015), Castro and Simón (2016) and Fleitas et al. (2018) also reported that the weather played a key role on how intense the symptoms of the pathogens were. Weather conditions also explained the differences in the effect of N fertilization on the AUDPC found in the STB experiments between years, because in the wettest year (2009) with more disease pressure, N fertilization significantly decreased disease severity. Conversely, in the driest year (2010) no differences were detected probably because *Z. tritici* requires a moist leaf surface for successful infection and is spread throughout the crop canopy *via* rain splash originating than the effect caused by the N fertilization is diminished.

In the present study, STB and YS severities significantly decreased as the N rate increased. Coincidently, Jones et al. (1990) observed that a susceptible cultivar to YS decreased AUDPC with rising N rates and Krupinsky et al. (2007) concluded that higher N fertilization levels apparently had a positive effect in reducing disease severities under environmental conditions of Great Plains Region in North Dakota of the United States. Their results highlighted evident differences among N treatments in the winter wheat and spring wheat in annual cropping system evaluations, higher levels of disease severity of the causal agent of YS, STB and other necrotrophic pathogens were usually associated with the low N treatment. In contrast, Simón et al. (2003) found that under N fertilization, AUDPC of STB was higher. In this sense, Carretero et al. (2010) registered that the composition of the diseases complex was different depending on the N treatment. Thus, under low N availability, necrotrophic pathogens (mainly YS) predominated in the diseases complex reaching values up to 9% while leaf rust (*Puccinia triticina* Eriks.) did not exceed 3%. Therefore, the disease severity curve following fertilization is complex and more studies are needed to confirm the trends above mentioned.

The amount of green-healthy tissue is an important parameter of crop growth models (Bancal et al., 2007). In the present study, lower AUDPC extended the green surface of the upper leaves delaying GLAD. Foliar pathogens shorten GLAD by reducing green leaf area because of the lesions themselves and by stimulating senescence in infected leaves. An adequate application of fungicides makes the duration of the green leaf area last longer, and as a consequence, higher yields can be obtained. Recent neighboring experiments have demonstrated efficacy differences between fungicides containing TSC or TS active ingredients on disease control and the GLAD. For instance, Maddaloni (2016) reported better levels of YS and leaf rust control using a TSC treatment compared to a fungicide containing TS active ingredients. Furthermore, Fleitas et al. (2018) reported lower AUDPC and longer GLAD values following TSC applications compared to a TS treatment in the wheat-leaf rust pathosystem, obtaining higher grain yields with the triple mixture regarding the double one. In addition, Berdugo et al. (2012) showed that after the application of preparations representing the carboxamide group (bixafen), the aging process of the green organs of wheat is prolonged and as a result, a higher yield is produced. Moreover, it is important to mention that fungicide mixtures with different modes of action applied at the recommended dose and at the right moment, are often most effective and reduce the likelihood that fungicide resistance will develop in target pathogens.

Several factors influenced the severity of the main foliar diseases of wheat, among them the resistance of the cultivars, tillage systems, N fertilization and fungicide applications (Krupinsky et al., 2007; Simón et al., 2011). In wheat regions of high yield potential, N fertilization is inevitable in order to obtain better grain yields and quality. As fungicides, N application also increases both, green leaf area index and GLAD, therefore is not unreasonable to suppose that fungicide and N combined may have a synergistic effect on canopy size and duration and in turn, on grain yield. The *Fungicide* × *N* interaction for grain yield was not found in the experiments diseased with STB. In addition, the *Year* × *N* interaction in the STB experiment was probably ascribed to higher precipitations in 2009 (631 mm compared to 344 mm in 2010) with higher N-soil availability as nitrates.

4.2. Grain protein concentration, wet gluten content and bread volume

The effect of STB and YS augmented GPC but the application of fungicide decreased it, although no differences were found among fungicide types. This result is in line with Dimmock and Gooding (2002) who reported that small reductions in GPC following fungicide-use are common when *Z. tritici* is the principal disease controlled. Also, Ruske et al. (2004) showed that disease control decreases GPC and BV. On the other hand, Blandino and Reyneri (2009) found that the fungicide-only application (triazole and a mixture of triazole-strobilurin) had no significant effect on the GPC compared to the untreated control. Rodrigo et al. (2015) did not find changes in GPC following fungicide use. In contrast, Cuniberti et al. (2004) observed an increment in GPC, gluten content but a decrease in BV following fungicide application in most cultivars affected by natural infections of leaf rust and YS, although the authors do not specify accurately disease levels. One possible explanation is that the increase in grain yield due to the fungicide application leads to a decrease in the protein content causing a dilution effect (Rodrigo et al., 2015) and that the magnitude of that increase could be conditioning the discrepancies between those findings. Likely, the severity of the disease and how it affects the GLAD, which is also conditioned by genotype characteristics, could have been influencing these results.

The application of fungicides in the STB experiments caused decreases in WGC and BV but the opposite occurred in YS experiments, where an increment of those quality parameters was evidenced. In the first case, the lower protein obtained in the fungicide treatments could have had a negative impact on quality parameters. These results are in accordance with Ruske et al. (2004) as was mentioned above. Nevertheless, when reductions in protein did occur, they could be compensated by applying extra late-season N as foliar urea at anthesis or ammonium nitrate at flag leaf emergence. In the second case (YS), an increment of quality parameters despite the lower GPC obtained was verified. It is probably that despite the lower grain protein obtained in fungicides treatments, the protein did not suffer changes in their composition. In this sense, Arabi et al. (2007) found that despite STB caused protein reductions, storage proteins that play an important role in bread-making quality, were not affected by the disease. This result reflected that the end product may not be negatively affected, though

lower in protein content. Also, Gooding et al. (1994) found that, despite GPC reductions due to STB control, the overall quality of the bread improved with the application of fungicides. Similarly, although speculative, the increased in GPC due to YS, may not have produced changes in their protein composition. Information evaluating how diseases affect protein composition is scarce and more studies are needed.

It is well known that an increase in the N fertilization rate has a favorable effect on grain quality by producing an increase in protein concentration (Gooding and Davies, 1997). In our study, the GPC, WGC and BV significantly increased through N rates in both experiments. The interaction *Fungicide* × *N* was not significant in STB experiments, as it was reported by Ruske et al. (2004) although the higher the N, the lower the reductions in GPC caused by fungicide applications. In contrast, in YS experiments *Fungicide* × *N* was significant, the application of N combined with TSC caused much smaller reductions in GPC respect to the untreated control than when no N fertilization was applied. Moreover, the higher N rate (140 N) did not show differences among fungicides types.

The interaction *N* × *Cultivar* showed in both diseases that cultivars belonging to quality group 1 improved BV better than cultivars of quality group 3 with rising N rates. The cultivars responded differentially with the application of fungicides, cultivars belonging to quality group 1 improved BV better than cultivars of quality group 3. These results coincide with Puppala et al. (1998) who reported larger increases in protein concentration following fungicide use on a cultivar specifically bred for high protein concentration and Clark (1993) who mentioned that protein concentration reductions following fungicide use were less in bread-making cultivars.

5. Conclusions

Fungicides increased green leaf area duration and grain yield compared to the control treatment. The results obtained in this study allow to conclude that necrotrophic and hemibiotrophic pathogens cause reductions in yield and GLAD and tended to increase GPC, whereas N fertilization diminishes the reductions caused by the fungicide applications. Although YS increased a little more GPC than STB as we predicted, those differences were very small indicating the preponderance of the necrotrophic phase of STB on its effect on GPC, as biotrophic pathogens tend to decrease GPC. However, the effect of STB and YS on WGC and BV may depend on some other factors as the differential impact of both pathogens in the protein composition, as gluten and BV increased with STB but decreased with YS, in spite of the increase in GPC caused by both diseases. This indicates that it is necessary to deepen the investigations on this subject to better understand the effects of these foliar diseases in bread-making quality. Furthermore, the reductions in GPC caused by the fungicides under both diseases effect were diminished when the higher dose of N was applied.

Once again, the importance of the classification of varieties in quality groups was observed, showing clear differences among groups, reinforcing the aim to classify Argentine wheat production based on these characteristics.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jcs.2018.07.014.

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