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Breadmaking quality and yield response to the green leaf area duration caused by fluxapyroxad under three nitrogen rates in wheat affected with tan spot

María Constanza Fleitas[a,b,*], Matías Schierenbeck[a,b], Guillermo Sebastián Gerard[a,b], Juan Ignacio Dietz[a,b], Silvina Inés Golik[a], María Rosa Simón[a,c]

[a] Cerealicultura, Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, La Plata (1900), Buenos Aires, Argentina
[b] CONICET CCT La Plata, La Plata (1900), Buenos Aires, Argentina
[c] CIC, La Plata (1900), Buenos Aires, Argentina

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ABSTRACT

Tan spot caused by the necrotroph pathogen *Pyrenophora tritici-repentis* (Died.) Drechs. [anamorph *Drechslera tritici-repentis*) (Died.) Shoem] causes reductions in yield and grain quality of wheat (*Triticum aestivum* L.) by affecting the photosynthetically active area of the crop which could affect grain protein content (GPC) and breadmaking parameters. Nitrogen (N) fertilization is required for achieving high yields and quality in wheat but can exert a profound effect on disease development and fungicide efficacy. The active ingredient fluxapyroxad, belonging to the carboxamides chemical group, not only control fungal pathogens and reduce disease progression but also, might increase green leaf area duration of the crop. We evaluated the effect of fungicide applications containing a carboxamide, in a mixture with triazole and strobilurin (TSC), above a double-mixture (triazole and strobilurin) (TS) under three N rates, on tan spot severity, healthy area duration (HAD), flag leaf healthy area duration (FLHAD) and grain yield. We also assessed its impact on GPC, wet gluten content, loaf volume and dough rheological parameters in wheat. Two field experiments were conducted during 2014 and 2015 in a split-split plot design with three fungicide treatments as main plots and three N fertilization rates as sub-plots using a susceptible cultivar (Baguette 11, Nidera). Treatment TSC significantly reduced the area under disease progress curve (AUDPC) and this was associated with increased HAD and FLHAD resulting in higher yields when compared to the TS treatment and the untreated control. The AUDPC values were lower with higher N rates in the untreated plots. The GPC and wet gluten content increased in untreated plots under 0 kg N ha^{-1} and 70 kg N ha^{-1} rate and was reduced following fungicide applications, however, this was reverted with the maximum N rate (140 kg N ha^{-1}). Increases in GPC and wet gluten content in the untreated plots did not improve loaf volume and breadmaking parameters of wheat which only enhanced following fungicide application and N fertilization.

1. Introduction

Tan spot caused by the necrotroph pathogen *Pyrenophora tritici-repentis* (Died.) Drechs. [anamorph *Drechslera tritici-repentis*) (Died.) Shoem] is a key foliar disease of wheat (*Triticum aestivum* L.) in Argentina and in many wheat production areas in the world, causing yield and quality reductions in this crop (Schierenbeck et al., 2016). The increasing importance of the disease has been attributed to the use of susceptible cultivars under conservation tillage systems, shorter crop rotations and continuous wheat cultivation that leads to inoculum increments (Moreno et al., 2012). Symptoms include well-defined necrotic blotching, often surrounded by yellow halos (Lamari and Bernier, 1991), through the secretion of toxins and cell wall degrading enzymes (Ney et al., 2013). According to Boote et al. (1983), *Py. tritici-repentis* is classified as "light stealer" because the pathogen not only stops carbon uptake in the affected areas, but also, interfere with photosynthesis in other leaves by intercepting light before it reaches those leaves. Thus, tan spot may cause grain shrivelling which are undesirable because they are associated with low flour extraction rates in milling (Rose et al., 2001). Some studies have hypothesized that other impacts on wheat quality might be increases in grain protein content (GPC) given a much larger effect of the pathogen on carbon

* Corresponding author. Cerealicultura, Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, La Plata (1900), Buenos Aires, Argentina.
E-mail address: constanzafleitas@gmail.com (M.C. Fleitas).

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accumulation than on nitrogen (N) accumulation (Dimmock and Gooding, 2002). This same response has been confirmed in several studies focused on Septoria leaf blotch caused by the hemibiotrophic fungus *Zymoseptoria tritici* (formerly *Mycosphaerella graminicola*) (Mc Kendry et al., 1995; Rodrigo et al., 2015; Castro and Simón, 2016), but there is very little information considering the necrotic pathogen *Py. tritici-repentis*. In fact, in an important review (Dimmock and Gooding, 2002) regarding the influence of foliar diseases and their control by fungicides on GPC in wheat, from the thirty-six reports from North America, Australia, Africa and Europe, only one (Rees et al., 1982) corresponded to tan spot, which tended to decrease GPC under fungicide applications. Contrary, in the same review, the control of Septoria leaf blotch by fungicides caused increases, decreases or had no effects on GPC, probably due to the hemibiotrophic conditions of the pathogen. Therefore, it would be expected that the effect of tan spot on GPC be more consistent, given that Septoria leaf blotch is considered a hemibiotrophic, whilst tan spot is a pure necrotroph.

On the other hand, N fertilization is required for achieving high yields but is particularly important in wheat as the content and composition of the grain proteins determine the suitability and quality for producing bread and other food products (Godfrey et al., 2010). However, N fertilization may also influence the development of foliar diseases such as tan spot and the effectiveness of fungicide applications. Few studies have been carried out so far to investigate the effect of N on the severity of the disease. Increased N fertilization has been reported to decrease tan spot severity (Krupinsky et al., 2007; Carignano et al., 2008; Simón et al., 2011; Gerard et al., 2015).

In Argentina, the most common fungicides used to control foliar fungal diseases of wheat are triazoles [a Quinone Outside Inhibitor (QoI)], strobilurins [a Demethylation Inhibitor (DMI)] and more recently, carboxamides [a Succinate Dehydrogenase Inhibitor (SDHI)]. Yield increases attributed to the application of certain fungicides such as strobilurins have been observed in wheat and have been reported to be greater than those arising purely from fungicidal properties of the product (Koehle et al., 2002). Gerhard (2001) reported that the application of strobilurins not only prevented the fungal disease but also, had a direct effect on plant physiology inducing an increase in assimilation intensity, optimize transpiration and improve water use efficiency as well, compared with other fungicides. Recent studies have shown that similar to strobilurins, the yield increase in a crop treated with a carboxamide is often greater than the explained simply through good disease control. Evidence collected by Smith et al. (2013) from across multiple trials suggested that, in addition to controlling visible disease symptoms, the SDHI fluxapyroxad could exert positive physiological effects on the plant including increased leaf greening, delayed senescence, reduced cell damage, reduced stomatal conductance, improved photosynthetic rate and increased water use efficiency. Similarly, Berdugo et al. (2012) verified that another SDHI (bixafen), delayed senescence of spikes and leaves in spring wheat during grain filling by enhancing physiological activities leading to increased grain yield. However, this evidence has been obtained under greenhouse conditions which differ from field environments since plants do not grow as communities under fluctuating environmental conditions. Extending canopy life would be of great importance to moderns high-yielding cultivars that have become terminally source-limited and this aspect could be improved with fungicide use (Pepler et al., 2005).

Despite the positive influence of fungicides on grain yield and milling quality, there are concerns that substantial yield gains may compromise GPC, with the suggestion that protein levels might be diluted by additional carbohydrate production which in turn, could affect breadmaking quality. Concerns are heightened by the recent introduction and widespread use of carboxamides which offers unprecedented levels of disease control, delays in senescence and yield responses. Grain yield has often been found to be negatively associated with GPC, but this relation is not true for a single genotype because N supply and irrigation strongly influence the relation between grain yield and GPC (Fischer et al., 1993). In this sense, some authors have suggested that when *Z. tritici* is the dominant pathogen, the use of fungicides can reduce GPC, however, such losses can be diminished or eliminated through application of foliar urea during grain filling (Dimmock and Gooding, 2002). Nevertheless, no information is available regarding this situation when *Py. tritici-repentis* is the main pathogen controlled.

There are many articles covering the effect of foliar diseases on breadmaking parameters of wheat such as Septoria leaf blotch or leaf rust caused by the biotroph *Puccinia triticina* Eriks. However, most of these studies have been carried out under natural infections where the effect of the pathogen type cannot be distinguished individually. In addition, the majority of them, have assessed the disease through the area under disease progress curve (AUDPC) which does not provide aspects related to crop canopy size, limiting the extrapolation to a wide range of genotypes, environments, years and locations. Moreover, the effect of fungicides containing triazoles and their combination with strobilurins on GPC and breadmaking quality parameters have been extensively studied (Ruske et al., 2003; Wang et al., 2004; Castro, 2016). However, there is no evidence of the effect of a fungicide treatment containing the SDHI fluxapyroxad.

From the above said, the aim of this study was to evaluate the influence of adding the SDHI fluxapyroxad (carboxamide) to a triazole-strobilurin combination under three N fertilization rates on the progress of tan spot, healthy area duration, flag leaf healthy area duration and its effect on grain yield, GPC and breadmaking parameters of wheat.

2. Materials and methods

2.1. Field trials and experimental design

Two field experiments were conducted under artificial inoculations at the J. Hirschhorn Experimental Station, Faculty of Agricultural and Forestry Sciences, National University of La Plata, Argentina; during 2014 and 2015. The trials were sown on July 28 and June 16 respectively under conventional tillage. The soil was a typic Argiudoll, analysis of the soil samples indicated the following mean values by weight in each year: (i) top-0.20 m, organic matter: 3.59%; N: 0.20%; N-NO_3: 10,4 ppm; P: 28.3 ppm and pH: 5.8; (ii) 0.20–0.40 m, N-NO_3: 5.1 ppm and pH: 6.0. Weather data (monthly precipitation; relative humidity and minimum, maximum and mean temperatures) were recorded at a Davis Meteorological Station located 100 m from the experiments.

The experimental design was a split-split plot with three replications. Within every year, main plots were the fungicide treatments and sub-plots were the N rates applied as granulated urea (Table 1). The genotype used in the experiments was Baguette Premium 11 (Nidera), moderately susceptible to tan spot (according to the information provided by the breeder) and classified in the Argentinean breadmaking quality grade as quality group 2 which corresponds to traditional breadmaking cultivars suitable for major long fermentations (higher than eight hours) (Steffolani et al., 2007). Each sub-subplot was 7.7 m^2 (5.5 m long × 1.4 m wide). The experiments were fertilized with 50 kg P_2O_5 ha^{-1} as calcium triple superphosphate at sowing. Glyphosate (2 L ha^{-1}) was used for weed control 15 days before sowing. In addition, Misil® herbicide [(metsulfuron methyl, dry flowable 60% + Dicamba (Dimethylamine 2-Metoxi-3,6 diclorobenzoic acid) soluble liquid 57.1%, Dupont, Rosario, Argentina)] at 100 cm^3 Dicamba +6.7 g metsulfuron methyl in 120 L water ha^{-1} was applied at the three-leaf stage (GS 13).

2.2. Fungicide applications and inoculation

Fungicides were applied at tillering (GS 23) and at flag leaf (GS 39) using an application rate of 140 L ha^{-1}. The experiment was inoculated at the beginning of tillering (GS 21) and at the beginning of shoot development (GS 31) with a mixture of virulent isolates of *Py. tritici-repentis* grown on V8® media at 23 °C ± 2 °C with 12 h alternating light

Table 1
Treatment list (TS: triazole + strobilurin; TSC: triazole + strobilurin + carboxamide).

Treatments	Active ingredient (g a.i. L^{-1})	Fungicide timing[a] (L ha^{-1})		Nitrogen timing (kg N ha^{-1})		
		GS 23	GS 39	Sowing	GS 33	Total
Untreated control	–	–	–	–	–	0
Untreated control	–	–	–	35	35	70
Untreated control	–	–	–	70	70	140
Opera® (TS)	epoxiconazole (50) + pyraclostrobin (133)	1	1	–	–	0
Opera® (TS)	epoxiconazole (50) + pyraclostrobin (133)	1	1	35	35	70
Opera® (TS)	epoxiconazole (50) + pyraclostrobin (133)	1	1	70	70	140
Orquesta Ultra® (TSC)	epoxiconazole (50) + pyraclostrobin (81) + fluxapyroxad (50)	1.2	1.2	–	–	0
Orquesta Ultra® (TSC)	epoxiconazole (50) + pyraclostrobin (81) + fluxapyroxad (50)	1.2	1.2	35	35	70
Orquesta Ultra® (TSC)	epoxiconazole (50) + pyraclostrobin (81) + fluxapyroxad (50)	1.2	1.2	70	70	140

[a] Zadoks et al. (1974).

and dark cycles (Raymond and Bockus, 1982). The inoculum was prepared by aseptically scraping sporulating colonies with a scalpel and suspending conidia in deionized water. The conidial suspension was adjusted to 3×10^3 spores mL^{-1} (Jordahl and Francl, 1992; Ali and Francl, 2003) using a Neubauer hemocytometer. 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) during three days.

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

Disease severity was assessed by visual estimation of the percentage of leaf area affected by *Py. tritici-repentis* on seven to ten plants in each plot on the upper four leaves at GS 39 and at anthesis (GS 60); and on 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 disease progression through three evaluations, according to the formula provided by Shaner and Finney (1977):

$$AUDPC = \sum_{i=1}^{n-1} \left(\frac{X_i + X_{i+1}}{2} \right) (t_{i+1} - t_i)$$

where $X_i = X(t_i)$, n is the number of assessments, X is the disease severity (%) and $(t_{i+1} - t_i)$ is the interval between two consecutive assessments. The total leaf area index was estimated by counting the main stems and tillers in two linear meters from each plot and measuring the length and wide of all leaves layers with at least 10% or more of green tissue of seven tillers. The individual leaf area was calculated multiplying length and wide and corrected with a correction factor of 0.835 (Miralles and Slafer, 1990). Through estimates of severity, green leaf area index (GLAI) was calculated at GS 39, GS 60 and GS 82 and the healthy area duration (HAD) was estimated in the same way as AUDPC but using GLAI instead of disease severity (Waggoner and Berger, 1987). Flag leaf healthy area duration (FLHAD) was calculated with the same method as HAD but using flag leaf GLAI.

2.4. Yield and breadmaking parameters determinations

Four meters of the three central rows in each plot were harvested and threshed (2.4 m^2) and the grain yield (kg ha^{-1}) was calculated. Grain samples from each sub-plot were conditioned to 15.5% moisture (IRAM 15854–1:1982) and milled using a laboratory mill (Bühler MLU-202), extracting flour at a rate of about 70% (IRAM 15854–2:1984). Nitrogen concentration was determined by Micro-Kjeldahl method and GPC was expressed as crude protein multiplying the value of N obtained by 5.7 (AOAC, 1970).

Wet gluten content was determined in accordance with the IRAM standard method 15864:2007 by washing a piece of dough made using 10-g of flour and 4.8 mL of salt solution with a Glutomatic®. Bread loaf volume was determined from mini loaves obtained from 100-g of flour sample baked in 9.5 × 5.5 × 5.5 cm tins following the IRAM 15858–1:1996 procedure.

Dough rheological properties were evaluated using a Chopin® Alveograph and a Brabender® Farinograph in accordance with the IRAM standard method 15857:1995 and 15855:2000, respectively. The Chopin's alveogram parameters were measured with a 250-g sample of flour. Dough tenacity (P) measured in mm, is the maximum height along the y-axis and estimates the dough aptitude to resist deformation. Its length along the x-axis, which is the maximum volume of air that the bubble is able to contain, is referred as extensibility (L) measured in mm; and the area under the curve is proportional to the energy required to cause the dough bubble to break named gluten strength (W) expressed as $J \times 10^{-4}$.

Brabender's farinogram parameters were measured with a 50-g sample of flour. Flour water absorption (A) measured in mL is the necessary water quantity to produce dough with a peak development of 500 Brabender units (BU). Dough development time (B) measured in min, is the time from the beginning of mixing until the highest point on the curve and indicates when the dough has reached its maximum viscosity before gluten strands begin to break down. Dough stability (D), measured in min as well, is the time between the top of the resistance curve meeting the maximum consistency measurement (500 BU) and the point at which it drops below this measurement during dough softening. Dough softening degree (E) is the difference between the resistance of the dough at its peak (500 BU) and 12 min later measured in BU.

2.5. Statistical analysis

Data were analyzed by a combined analysis of variance (ANOVA) for both years with a split-split plot design with GenStat 12 Ed. program (2009). Mean values were compared with LSD test.

3. Results

3.1. Weather conditions and area under disease progress curve

Data from environmental conditions over the two years are presented in Table 2, including the historical climatic values (period 1969–2009) for monthly rainfall and temperature. Precipitation varied greatly between both years under study. In 2014, the sum of rainfall during crop cycle was 755 mm (+45.5% higher than historical values), while in 2015 was 543 mm. Differences of temperature and relative moisture values over the two growing seasons were relatively modest compared to the historic values (Table 2).

Analysis of variance and averages values of AUDPC are presented in Tables 3 and 4, respectively. There were significant differences for *Year*, *Fungicide*, *N* and the *Fungicide* × *N* interaction (Table 3). The AUDPC

Table 2
Environmental conditions during the wheat growing seasons of 2014 and 2015 and historical values (1969–2009) at Los Hornos, La Plata, Argentina.

	Temperature (°C)			Humidity (%)			Precipitation (mm)		
	2014	2015	1964–2009	2014	2015		2014	2015	1969–2009
June	10.2	11.3	10.6	82	69		59	41	54.3
July	10.5	10.3	10.2	78	77		134	61	62.1
August	12.7	13.0	11.5	68	81		62	157	59.2
September	14.1	12.0	13.5	74	66		94	48	67.9
October	18.1	13.7	16.5	71	72		162	68	96.1
November	18.9	18.0	19.2	65	52		193	130	98.6
December	21.5	22.1	22.3	64	69		41	39	80.6
Mean	**15.1**	**14.3**	**14.8**	**71.7**	**69.4**	**Total**	**755**	**543**	**519**

Table 3
Mean squares of the area under disease progress curve (AUDPC), healthy area duration (HAD), flag leaf healthy area duration (FLHAD), grain yield (GY) and grain protein content (GPC) of wheat under three fungicide treatments and three nitrogen rates in two years.

Source of variation	Df	AUDPC	HAD	FLHAD	GY	GPC
Year (Y)	1	523174*	261*	69.6*	114030	0.095
Error a	2	125	1.0	0.037	3600132	0.286
Fungicide (Fu)	2	690724***	12455***	970***	29941821***	6.1***
Y × Fu	2	24390	1.4	0.701	380523	0.332
Error b	8	17609	118	21.5	1262760	0.176
Nitrogen (N)	2	83200***	14330***	1632***	9987390***	16.6***
Y × N	2	2971	3.2	1.1	854157	0.598
Fu × N	4	15460**	445***	37.9***	1998794**	3.1*
Y × Fu × N	4	554	0.200	0.043	171130	0.692
Error c	24	2607	51.9	4.0	340526	0.644
Total	53					

Significant at: *$p = .05$, **$p = .01$ and ***$p = .001$.

Table 4
Mean values of the area under disease progress curve (AUDPC), healthy area duration (HAD), flag leaf healthy area duration (FLHAD), grain yield (GY) and grain protein content (GPC) of wheat under three fungicide treatments and three nitrogen rates in two years (TS: triazole + strobilurin; TSC: triazole + strobilurin + carboxamide).

Main factors	AUDPC	HAD (days)	FLHAD (days)	GY (kg ha^{-1})	GPC (%)
Year					
2014	629 a	110 a	36.6 a	6732 a	10.8 a
2015	432 b	106 b	34.4 b	6641 a	10.9 a
LSD	13.1	1.7	0.225	2222	0.627
Fungicide					
Untreated	754 a	78.8 c	27.6 c	5317 c	11.5 a
TS	450 b	114 b	36.8 b	6863 b	10.8 b
TSC	389 b	130 a	42.1 a	7879 a	10.3 c
LSD	102	8.4	3.6	864	0.323
Nitrogen					
0 kg N ha^{-1}	591 a	78.9 c	25.4 c	5895 c	10.1 b
70 kg N ha^{-1}	545 b	109 b	36.7 b	6784 b	10.5 b
140 kg N ha^{-1}	457 c	135 a	44.4 a	7378 a	11.9 a
LSD	35.1	4.9	1.4	401	0.552

value was significantly higher in 2014 (+45.6%) in relation to 2015 (Table 4). Increments in N rates significantly reduced AUDPC values in the untreated plots, while in the TSC treatment, no differences were detected (Fig. 1a). The higher the N rate, the lowest the AUDPC values. Thus, total AUDPC averaged 591 at low N rate (0N), 545 at 70N and 457 at high N rate (140N) (Table 4). Averaged over all treatments, AUDPC was significantly reduced with fungicide applications which averaged 389, 450 and 754 in the TSC, TS and control treatment respectively (Table 4).

3.2. Healthy area duration and flag leaf healthy area duration

The HAD and FLHAD were significantly influenced by the main factors *Year*, *Fungicide, N* and the *Fungicide* × *N* interaction (Table 3). In the wettest year (2014), HAD was significantly higher (+4.8%) compared to 2015 (mean 106 days) (Table 4). Similarly, FLHAD was more extended in 2014 (+6.5%) than in 2015 (mean 34.4 days) (Table 4). Greater HAD responses to N fertilization occurred in the untreated control when compared to the fungicide protected treatments (Fig. 1b). Thus, HAD was +90.1% higher in untreated plots when 0N (56.8 days) and 140N (108 days) were compared, a greater response in relation to TS (+61.4%) and TSC (+69.6%) treatments (Fig. 1b). Averaged over all treatments, HAD was significantly more extended with the TSC treatment (mean 130 days), followed by the TS mixture (mean 114 days) and the untreated control (mean 78.8 days) (Table 4). Regarding FLHAD, the *Fungicide* × *N* interaction arose because the FLHAD increased from 0N to 140N, to a much greater extent with the TSC treatment (+78.8%) compared to the TS treatment (+67.7%) and the untreated plots (+77.4%) (Fig. 1c). Averaged overall treatments, mean values of FLHAD were significantly more prolonged under TSC treatment (42.1 days), followed by TS (36.8 days) and the untreated control (27.6 days) (Table 4).

3.3. Grain yield and grain protein content

Analysis of variance and averages values of grain yield and GPC are presented in Tables 3 and 4, respectively. Grain yield significantly varied with *Fungicide* and *N* treatments (Table 3). Moreover, a significant *Fungicide* × *N* interaction occurred (Table 3). There was a higher response to N fertilization when TSC was applied, reaching 7879 kg ha^{-1} compared to TS which averaged 6863 kg ha^{-1} (Fig. 1d). In contrast, no differences in grain yield were detected among N rates in the untreated plots (average 5317 kg ha^{-1}). The rate of grain yield increase per day of extra leaf life under fungicide treatment containing the SDHI fluxapyroxad was 34.2 kg ha^{-1} day^{-1} ($R^2 = 0.627$***), followed by the TS treatment with 18.1 kg ha^{-1} day^{-1} ($R^2 = 0.514$**) and the untreated control with 10.1 kg ha^{-1} day^{-1} ($R^2 = 0.447$*) (Fig. 1f).

There were significant differences in GPC for *Fungicide*, *N* and the *Fungicide* × *N* interaction (Table 3). Although fungicide treatments reduced GPC at 0N and 70N, this effect was reverted at 140N, where all treatments showed similar GPC values (Fig. 1e). Following TSC treatments, GPC ranged from 9.0% (0N) to 12.2% (140N), a relative higher increment than the TS treatment, where values fluctuated from 10.0 (0N) to 11.8% (140N), and the untreated control (11.3–11.8% for 0N and 140N respectively).

3.4. Wet gluten content, breadmaking parameters and loaf volume

Analysis of variance and averages values of wet gluten content, breadmaking parameters and loaf volume are given in Tables 5 and 6,

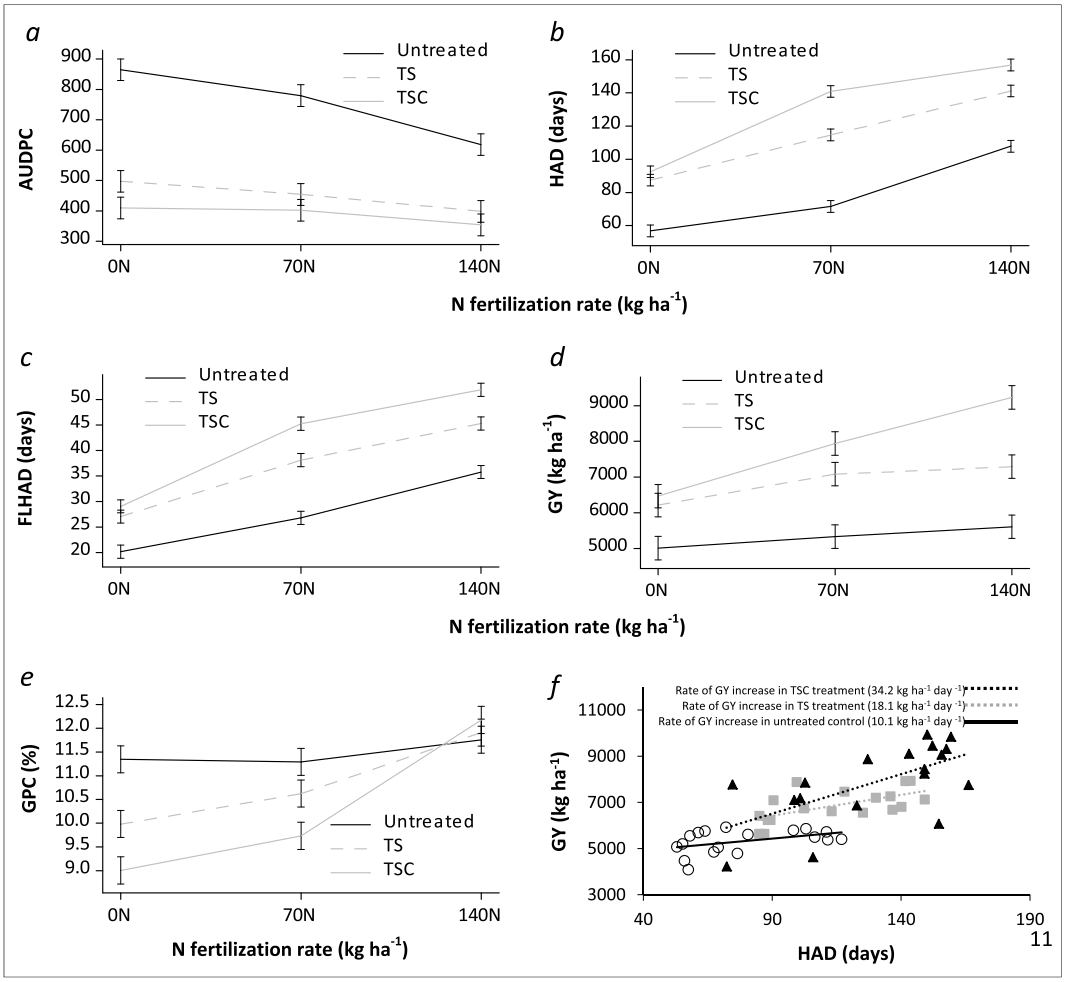

Fig. 1. Mean values of (a) area under disease progress curve (AUDPC), (b) healthy area duration (HAD), (c) flag leaf healthy area duration (FLHAD), (d) grain yield (GY) and (e) grain protein content (GPC) in the *Fungicide* × *N* interaction; (f) Regression between HAD and GY for every fungicide treatment. TS: triazole + strobilurin fungicide, TSC: triazole + strobilurin + carboxamide fungicide). Error bars indicate standard errors ($p = .05$).

respectively. Wet gluten content was significantly influenced by the fungicide applications and the N rate, as well as by the *Fungicide* × *N* interaction (Table 5). Similar to GPC, fungicide treatments reduced wet gluten content at 0N and 70N, this effect was also reverted at 140N, where the TS treatment and the untreated control showed similar values, contrary to the TSC fungicide, which evidenced significant higher values (Fig. 2a). Following TSC treatments, wet gluten content fluctuated from 21.3% (0N) to 27.8% (140N), a relative higher increment when compared to the TS treatment, where values ranged from 22.6% (0N) to 26.4% (140N), and the untreated control (24.2%–26.5% for 0N and 140N respectively) (Fig. 2a).

Alveogram parameters *P*, *L*, and *W* were influenced by *Fungicide*, *N*

Table 5
Mean squares of wet gluten content, tenacity (*P*), extensibility (*L*), *P/L* ratio, dough strength (*W*), water absorption (*A*), dough development time (*B*), stability (*D*), softening degree (*E*) and bread loaf volume of wheat under three fungicide treatments and three nitrogen rates in two years.

Source of variation	Df	Wet gluten content	Alveogram parameters				Farinogram parameters				Loaf volume
			P	L	P/L ratio	W	A	B	D	E	
Year (Y)	1	5.5	64.4	51.4	8.0E-05	13050	26.0	0.822	0.987	3270	11787
Error a	2	1.8	155	1.0	2.3E-02	201	34.9	0.500	0.082	1051	378
Fungicide (Fu)	2	5.9*	8282**	3455**	5.3E-02**	22622**	64.9**	2.4**	13.8**	5581	26592**
Y × Fu	2	0.196	17.9	6.8	3.2E-03	546	1.2	0.326	0.005	390	244
Error b	8	1.0	35.7	27.4	2.1E-03	1616	8.5	0.055	0.923	1363	1772
Nitrogen (N)	2	84.8***	11177***	1577***	3.6E-01***	38249***	570***	1.4**	18.6***	2507**	165133***
Y × N	2	1.2	121	3.3	9.3E-03	135	0.866	0.065	0.011	23	292
Fu × N	4	6.7**	244*	275*	4.8E-02	3380*	11.5	0.297	4.0*	194	8029**
Y × Fu × N	4	1.4	8.1	6.0	1.6E-03	100	1.1	0.027	0.073	465	825
Error c	24	1.5	44.0	40.4	1.1E-02	595	7.6	0.164	0.976	285	1403
Total	53										

Significant at: *$p = .05$, **$p = .01$ and ***$p = .001$.

Table 6
Mean values of wet gluten content, tenacity (*P*), extensibility (*L*), *P/L* ratio, dough strength (*W*), water absorption (*A*), dough development time (*B*), stability (*D*), softening degree (*E*) and bread loaf volume of wheat under three fungicide treatments and three nitrogen rates in two years (TS: triazole + strobilurin; TSC: triazole + strobilurin + carboxamide).

Main factors	Wet gluten content (%)	Alveogram parameters				Farinogram parameters				Loaf volume (cm^3)
		P (mm)	L (mm)	P/L ratio	W (J × 10^{-4})	A (mL)	B (min)	D (min)	E (BU)	
Year										
2014	24.1 a	118 a	97.3 a	1.21 a	356 a	58.3 a	2.5 a	5.4 a	64.6 a	479 a
2015	24.8 a	120 a	99.3 a	1.20 a	387 a	59.7 a	2.8 a	5.6 a	49.1 a	509 a
LSD	1.5	14.6	2.2	0.159	33.6	6.9	0.626	0.335	38.0	32.8
Fungicide										
Untreated	25.1 a	87.8 c	82.3. a	1.07 c	326 c	57.2 b	2.3 b	4.5 b	75.6 a	450 b
TS	24.0 b	128 b	105 b	1.22 b	377 b	58.8 b	2.7 b	5.9 a	54.4 a	510 a
TSC	24.2 b	141 a	108 b	1.31 a	412 a	61.0 a	3.0 a	6.1 a	40.6 a	522 a
LSD	0.769	4.6	4.0	0.020	30.9	2.2	0.421	0.738	28.3	32.3
Nitrogen										
0 kg N ha^{-1}	22.7 c	98.4 c	88.1 c	1.12 c	328 c	52.7 c	2.4 b	4.4 c	68.3 a	392 c
70 kg N ha^{-1}	23.8 b	117 b	100 b	1.17 b	366 b	60.7 b	2.7 ab	5.7 b	57.6 ab	510 b
140 kg N ha^{-1}	26.9 a	141 a	106 a	1.33 a	420 a	63.6 a	2.9 a	6.4 a	44.7 b	581 a
LSD	0.844	4.6	4.4	0.070	16.8	1.9	0.432	0.680	11.6	25.8

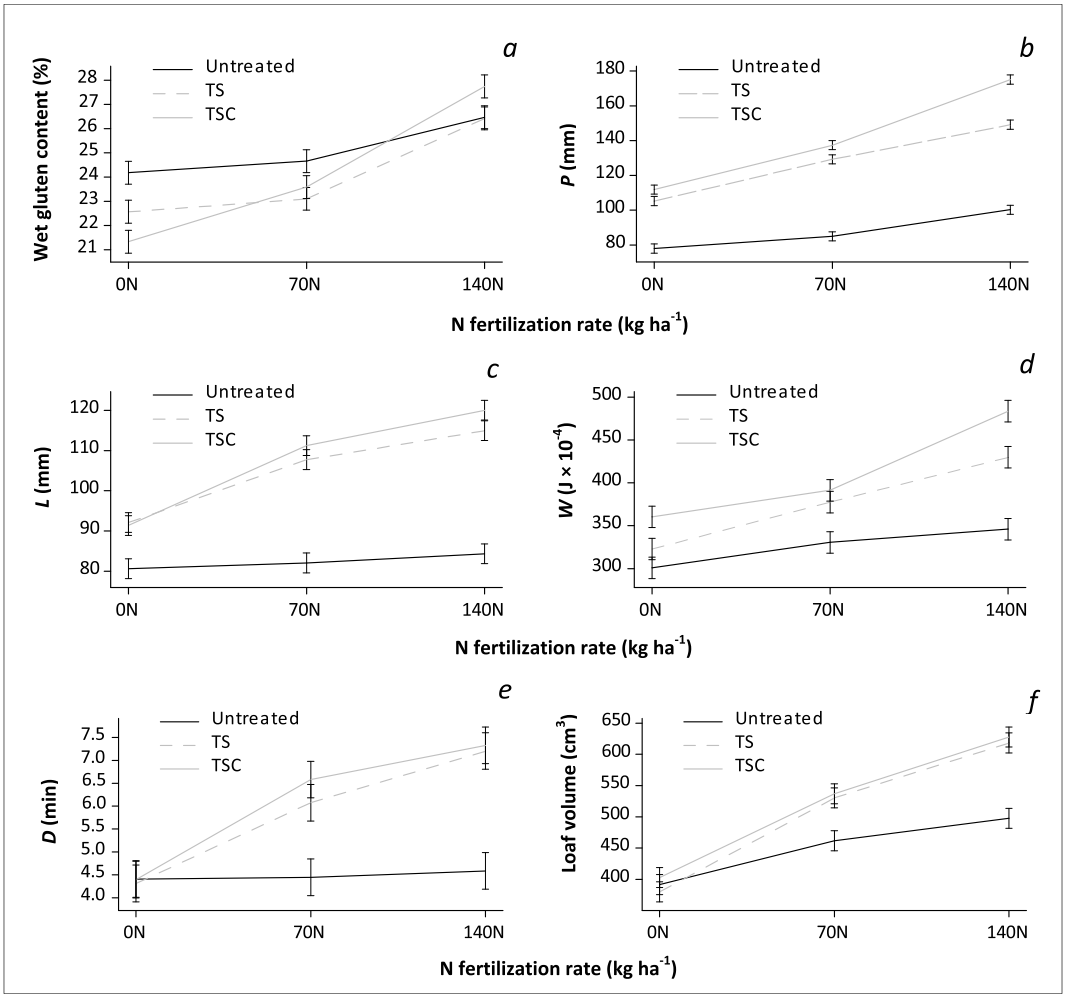

Fig. 2. Mean values of (a) wet gluten content, (b) tenacity (*P*), (c) extensibility (*L*), (d) gluten strength (*W*), (e) stability (D) and (f) loaf volume in the *Fungicide* × *N* interaction. TS: triazole + strobilurin fungicide, TSC: triazole + strobilurin + carboxamide fungicide). Error bars indicate standard errors ($p = .05$).

and the *Fungicide* × *N* interaction (Table 5), mainly explained by different responses across the three N fertilization rates under fungicide treatments. Dough rheological parameters *P* and *W* significantly increased with N fertilization in a much higher magnitude when sprayed with TSC than with TS fungicide compared to the untreated plots (Fig. 2b and d). The same behaviour was observed for *L* values, which significantly augmented following fungicide treatments independently of the fungicide type (Fig. 2c). Contrary, no differences were observed in the untreated plots across N fertilization rates. The *P/L ratio* significantly improved under fungicide applications (Table 6) compared to

the untreated plots (1.07), which reached the highest values with TSC treatment (1.31), followed by TS (1.22) (Table 6). Moreover, *P/L* ratio increased with rising N rates that rose from 1.12 (0N) to 1.33 (140N) (Table 6).

Farinogram parameters *A* and *B* were only influenced by the main factors *Fungicide* and *N* (Table 5). The application of TSC resulted in a further significant increase of *A* and *B* values over the TS treatment and the untreated control which did not differ between them (Table 6). In addition, these parameters evidence a significant increment with rising N rates. Thus, *A* values rose a +20.6% when comparing the 0N rate with the maximum rate (140N), while *B* values rose a +22.9% (Table 6). Parameter *D* was significantly influenced by main factors *Fungicide, N* and the *Fungicide* × *N* interaction (Table 5). No differences of *D* were detected among the N rates in the untreated plots (average 4.5 min). In contrast, under fungicide treatments, values increased with rising N rates independently of fungicide type. However, no differences between 70N and 140N were observed (Fig. 2e). Parameter *E* only varied across N fertilization rates (Table 5), which tended to fall from 68.3 BU to 44.7 BU as the N fertilization rate increased representing a decrease of −34.5% (Table 6).

Finally, the ANOVA showed a significant effect of fungicide treatments and N fertilization rate on loaf volume, as well as for its interaction *Fungicide* × *N* (Table 5). Fungicide applications brought about significantly larger increments of loaf volume with rising N rates (regardless fungicide type), compared with the untreated plots (Fig. 2f). Under TSC treatments, loaf volume fluctuated from 403 cm^3 (0N) to 628 cm^3 (140N), a relative higher increment when compared to the TS treatment, where values ranged from 380 cm^3 (0N) to 618 cm^3 (140N), and the untreated control (392 cm^3–498 cm^3 for 0N and 140N respectively).

4. Discussion

The AUDPC caused by tan spot was significantly lower in 2015 than in the previous year, probably explained by the scarce precipitation and lower mean temperatures after inoculations done at GS 21 (September) and GS 31 (October) (Table 2). By contrast, tan spot was initiated earlier in 2014 due to the more conducive conditions for disease development after inoculations.

Disease severity was significantly higher in the untreated plots than in the sprayed ones, showing the fungicide treatment containing the SDHI fluxapyroxad (in a mixture with epoxiconazole and pyraclostrobin) the highest levels of control, probably associated with a reduced possibility of reinfection given a more durable residual efficacy of the triple-mixture compared to the double-one. Some evidences in the literature suggest that fluxapyroxad might also reduce phylloplane fungi, which could in turn, diminish the level of cell damage from infection attempts by these non-target pathogens (Smith et al., 2013). Recent studies have shown that fluxapyroxad gives an excellent control of visible disease symptoms and is considered superior to many current fungicide products (Waterhouse and Semar, 2012). This was recently supported by neighboring experiments where tan spot and leaf rust severity was significantly lower with TSC (0.3%) compared to TS (7.7%) regarding the untreated control (24%), even after 36 days of spraying at GS 41 (flag leaf sheath extending) (Maddaloni, 2016). In addition, Smith et al. (2013), using the winter wheat-stripe rust (*Puccinia striiformis* f. sp. *tritici*) pathosystem, verified that disease severity was significantly reduced when fluxapyroxad was applied in relation to other active ingredients.

Under fungicide applications, AUDPC tended to decrease, but did not differ across rising N rates, whilst in untreated plots, values decreased in a much greater extent when N was applied. Mineral nutrition has long been recognized as an important component of disease control practices (Huber and Jones, 2013). However, levels of disease under N fertilization were still higher than those observed in the plots sprayed with fungicides, probably because cultivar Baguette 11 is a susceptible genotype and high levels of disease can be expected even under N fertilization. Therefore, in a highly susceptible cultivar, disease management by modifying fertilizer practice, might not be enough to limit tan spot development and fungicide applications would be needed. Since the trend in farming today is to reduce production costs using conservation tillage, in order to lower the risk of tan spot and the use of fungicides, it is important to use resistant cultivars and include crop rotations, all of which have a major impact on the risk of developing this disease.

Some studies have suggested that the morphological response of a fungal pathogen would depend on nutrient levels of the plant tissue (Walters and Bingham, 2007). In the present study, under artificial inoculations of *Py. tritici-repentis*, N fertilizer significantly reduced tan spot severity. Our findings are in line with those reported by Huber et al. (1987), Krupinsky et al. (2007), Carignano et al. (2008) Simón et al. (2011) and Gerard et al. (2015) but in conflict with results observed by Bockus and Davis (1993). Some authors hypothesized that in the case of saprophytic fungi, the fungus adopt an exploration strategy under low nutrient levels, where fungal resources are allocated to radial growth in search of nutrients, whilst under higher nutrient levels, colony structure results in a denser and more branched mycelium enabling the fungus to exploit the substrate (Dowson et al., 1989). This might explain why some authors (Snoeijers et al., 2000) pointed out that tan spot colonizes weak and senescent tissues. Furthermore, Bockus and Davis (1993) found that N fertilization did not directly reduce tan spot severity, but rather due to an apparent benefit by reducing leaf senescence. Another possible explanation is that under N fertilized practices, a dense canopy might limit the *Py. tritici-repentis*, inoculum movement rather than a sparse one as suggested by Lovell et al. (1997) for *Z. tritici*.

Significant differences in HAD and FLHAD were noted between years, less extended values were obtained in 2015, due to lower rainfall and poor distribution over the growing season (Table 2). As expected, HAD and FLHAD tended to increase under fungicide applications but the main finding of this study is that fungicide treatment containing the SDHI fluxapyroxad consistently increased these physiological parameters respect to the TS mixture. The extension of FLHAD per day of extra leaf life was about 9 days under TS spraying, whilst under TSC applications resulted in more than 14 days. Evidence collected from multiple trials suggests that, in addition to controlling visible disease symptoms, carboxamides are able to exert positive physiological effects on the host. Such effects include increased leaf greening, delayed senescence, reduced cell damage, reduced stomatal conductance, improved photosynthetic rate and increased water use efficiency (Berdugo et al., 2012; Smith et al., 2013; Ajigboye et al., 2014). Under the combined effect of fungicide applications and N fertilization, HAD and FLHAD were more extended than in the untreated plots. This response could be explained because, in the fungicide sprayed plot under N applications, values not only increased due to the fertilizer effect but also, because of the lower severity of tan spot. Thus, the combination of N fertilization and fungicide applications simultaneously may result in a higher level of physiological response in the plant than those caused by each individual factor.

Differences in HAD and FLHAD reflected different fungicide effects on yield with TS improving about 1500 kg ha^{-1}, whilst TSC gained nearly 2500 kg ha^{-1} ascribed to the extra leaf life (rate of grain yield increase, Fig. 1f) and the lower levels of disease. Furthermore, fungicide applications brought about significantly larger increments in GPC with rising N rates, compared with the untreated plots probably due to both, increased N uptake into the above ground crop, and more efficient remobilization of N from leaf laminas. Under the minimum N rate (0N), GPC was higher in the untreated plots (attributed to the endosperm weight reduction due to the higher levels of disease inducing shrivelled grains) compared to the sprayed ones, where substantial yield gains compromised GPC that was diluted by additional carbohydrate production. Our findings are in line with those reported by Rees et al.

(1982) who observed grain N concentrations augments from wheat plants severely affected by tan spot. Data reported by Dimmock and Gooding (2002) and Castro (2016) showed that small reductions in GPC following fungicide-use are common when *Z. tritici* is the main pathogen controlled. Conversely, Blandino and Reyneri (2009) did not find any effects of fungicide sprays on GPC. However, the disease pressure was generally low as the experiment was carried out under natural infections.

There have been some suggestions that N and fungicide applications interact with respect to protein concentrations. Penny et al. (1978) found positive interactions between fungicide and liquid N (solution of ammonium nitrate and urea) applications at ear emergence on GPC in conflict with Kelley (1993), who verified that applying additional topdress N significantly increased grain protein, but foliar fungicide had no effect. In the present study, under the maximum N rate (140N), no differences in GPC were observed among the fungicide treatments, because in untreated plots, the diseased was limited. However, in the protected ones, substantial yield gains did not dilute GPC due to greater N accumulation in the grain. Castro (2016) also found a tendency, although non-significant, to decrease GPC following fungicide applications at low N rates (0 kg N ha^{-1}) yet have the opposite effect at high N application rates (140 kg N ha^{-1}) under artificial inoculations of *Z. tritici*.

In this experiment, although GPC increased under the effect of tan spot, due to limitations in carbohydrate supply, and this resulted in higher levels of wet gluten content, loaf volume and dough rheology properties did not necessary improve, most likely due to the weak correlation of GPC and loaf volume ($r = 0.545$***) and breadmaking quality parameters *P* ($r = 0.230$**), *L* ($r = 0.220$), *W* ($r = 0.343$*), *P/L* ratio ($r = 0.217$), *A* ($r = 0.327$***), *B* ($r = 0.357$**), *D* ($r = 0.436$***) and *E* ($r = 0.106$*) in the cultivar Baguette 11. Previous studies (Pechanek et al., 1997), showed that the effect of grain N on protein composition was not consistent but varied between cultivars under study, in conflict with others (Kindred et al., 2008) where increases in GPC were associated with higher *L* values. On the other hand, the application of fungicides increased loaf volume, alveogram parameters of *P*, *L*, *W*, *P/L* ratio, and the farinogram parameters *A*, *B* and *D*, showing the fungicide treatment containing the SDHI fluxapyroxad consistently higher values. Previous studies (García et al., 2005) observed a deleterious effect of fungicide spray (triazole-only) on dough rheology, specifically *P*, in several cultivars differing in crop cycle. Blandino and Reyneri (2009) found consistent benefits in *W* following fungicide applications (triazole + strobilurin) with no significant differences in *P/L* values. Other studies performed in Argentina (Fleitas, 2011; Castro, 2016), verified that *W* and *P* parameters decreased following fungicide applications to control *Z. tritici* but only in the wettest year, likely ascribed to GPC reductions derived from higher yields, indicating that both, *W* and *P* were more sensitive to the fungicide applications than other parameters of Chopin's alveogram.

Data of this research confirm that GPC increases in plants affected by tan spot resulting in higher wet gluten content, but this was not associated with improvements in other breadmaking parameters of wheat. In addition, higher grain yield augments caused by adding fluxapyroxad came about largely through increasing the healthy area duration of the canopy. Moreover, delaying leaf senescence caused by fungicide applications, reduced the decline in physiological activity assuring higher grain yield and flour quality. The prolongation of the green period of the flag leaf by SDHI fungicides could collaborate in the stability of the yield particularly in modern cultivars whose higher yield potential has been mainly explained by increasing grains per spike (sink), and where the amount of photosynthetic source is not well balanced (sink/source relation).

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