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Quality and yield response to the control of *Mycosphaerella graminicola* in wheat as affected by nitrogen rate and cultivar bread-making characteristics

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Abstract. Septoria leaf blotch (SLB), caused by *Mycosphaerella graminicola*, reduces yield and grain quality of wheat (*Triticum aestivum* L.) by affecting the photosynthetically active area of the crop. This might influence grain protein concentration (GPC) and affect bread-making parameters. Nitrogen (N) fertilisation is required to achieve high yields in wheat; however, it may enhance the development of foliar diseases such as SLB. The aim of this study was to evaluate the effect of fungicide and N rate on SLB severity, green-leaf-area duration, grain yield and bread-making parameters in three wheat cultivars differing in bread-making characteristics. Two field experiments were conducted during 2009 and 2010 in a split-split-plot design with three fungicide treatments (triazole, triazole–strobilurin, nil) as main plots, three N fertiliser rates as subplots and three cultivars as sub-subplots. Fungicides significantly reduced the area under disease-progress curve (AUDPC) and this was associated with increased yield, which varied among cultivars. The AUDPC was lower in the higher N-rate treatments. Fungicide applications and increasing N rates extended green-leaf-area duration. GPC increased in untreated plots and it was reduced with applications of triazole–strobilurin fungicide. GPC reduction caused by this type of fungicide tended to be lower when the rate of N increased. The two cultivars with low bread-making characteristics showed a tendency to greater reductions in GPC with both fungicide types. Regarding quality variables, only tenacity and dough strength were reduced by the triazole-strobilurin fungicide. On average, for all treatments, tenacity, water absorption and dough development time were higher in the best quality group cultivars.

Additional keywords: alveogram, dough rheology, farinogram, foliar diseases, wheat quality, *Zymoseptoria tritici*.

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Introduction

Septoria leaf blotch (SLB), caused by the hemibiotrophic fungus *Zymoseptoria tritici* (Desm.) Quaedvl. and Crous (teleomorph *Mycosphaerella graminicola* (Fuckel) J. Schröt, in Cohn), is an important foliar disease that may cause significant yield losses in many wheat-producing areas of the world (Eyal *et al*. 1985; Jørgensen *et al*. 2014; Rodrigo *et al*. 2015). The pathogen infects mainly leaves, but also stems, leaf sheaths and internodes. Symptoms include necrotic blotching, hastened senescence of leaves, delayed leaf expansion during spring, and reduction of leaf size in some later formed leaves (Rodrigo *et al*. 2015). The influence of the disease on yield may depend on the intensity of the disease, tillage system, environmental conditions and growth stage at the time of infection. Early infections can reduce the number of grains per spike, whereas late infections can reduce the 1000-kernel weight (Adolf *et al*. 1993; Milus 1994; Simón *et al*. 2002). Yield losses of 20–50% in high-yielding cultivars have been reported in Argentina by Annone *et al*. (1991) and Annone (2001). Castro and Simón (2016) verified reductions of 18–49.6% depending on the cultivar under high inoculum pressure.

Nitrogen (N) fertilisation is necessary to achieve yield potential and grain quality in wheat. Even in conditions of high soil fertility, N uptake is important because it is positively correlated with grain protein concentration (GPC) (Stone and Savin 1999). Despite this positive influence on wheat quality, N availability may also affect the development of some foliar diseases as SLB, but the magnitude and direction of these effects are inconsistent. Increased N fertility has been reported to increase the severity of SLB (Gheorghies 1974; Prew *et al*. 1983; Howard *et al*. 1994; Leitch and Jenkins 1995; Simón *et al*. 2002, 2003). By contrast, Johnston *et al*. (1979) reported a decrease in the severity of the disease with increased N in one year of their experiments.

Fungicides are particularly important in the Argentinian wheat-growing area for the control of SLB, because cultivars with high yield potential but a lack of adequate genetic resistance are used in the region. However, the effectiveness of the fungicides may interact with N fertilisation and cultivar. Triazoles, which are characterised by active inhibition of ergosterol, are one of the most common groups of fungicides used to control foliar diseases such as SLB. Triazoles are usually used in mixtures with strobilurins, which are synthetic derivatives of natural compounds produced by the fungus *Strobilurus tenacellus* (Pers.), with a broad-spectrum antifungal activity as an inhibitor of mitochondrial respiration. Bayles (1999) stated that strobilurins could lead to substantial yield increases, ~10–20% above those produced by conventional fungicides. These increases would be associated with the maintenance of canopy photosynthesis during the grain-filling period, but also with prolonged duration of green flag leaf area (Dimmock and Gooding 2002*a*). Gerhard (2001) suggested that the application of strobilurins not only prevents the side effect of fungal disease, it also induces an increase in assimilation intensity, optimal transpiration and improved water use efficiency compared with other fungicides.

Dimmock and Gooding (2002*b*) indicated that when classic biotrophs are controlled by fungicides, the GPC often increases. This is because the pathogen has a more damaging effect on the accumulation and partitioning of N to the grain than it does on the accumulation and partitioning of the dry matter. Conversely, most reports of the effect of controlling necrotrophic fungi found that fungicide use is associated with a reduction in protein concentration. In this case, the pathogen interrupts the supply of assimilates by reducing the photosynthetic capacity of the plant via destruction of leaf tissue, therefore having a much larger effect on carbon accumulation than on N (Dimmock and Gooding 2002*b*).

Septoria leaf blotch, which is caused by a hemibiotroph, exhibits some characteristics of biotrophy in the early stages of infection (Royle *et al*. 1995). Thus, *M. graminicola* can either increase GPC or have no effect (Clare *et al*. 1993; Gooding *et al*. 1994; Ishikawa *et al*. 2001; Ruske *et al*. 2001; Blandino and Reyneri 2009). By contrast, Morris *et al*. (1989), Hedke and Verret (1999) and Arabi *et al*. (2007) found that SLB caused reductions in GPC depending on cultivar susceptibility. Besides its behaviour as a hemibiotrophic fungus, these inconsistences might be partly due to the fact that some cultivars, specifically bred for bread-making, exhibit high GPC together with high grain yield (GY) and would maintain high protein concentration more easily than other cultivars where the protein is less important (Dimmock and Gooding 2002*b*). GPC is the factor that more widely influences the quality of the flour derived from that grain (Fuertes-Mendizábal *et al*. 2010) and is affected by the genotype and the environment (Garrido-Lestache *et al*. 2004).

Wheat quality is generally assessed on the basis of its suitability for a particular end-use. Numerous wheat cultivars exist that differ not only in terms of yield and tolerance or resistance to diseases, but also in terms of grain quality suitable for processing, such as milling and baking. In Argentina, the Winter Cereals Committee of the National Seeds Institute classifies wheat cultivars according to their industrial end-use properties into three quality groups. Quality group 1 corresponds to the highest quality cultivars, suitable for industrial bread making; quality group 2 corresponds to traditional bread-making cultivars suitable for major, long fermentations (>8 hours); and quality group 3 includes cultivars with the lowest quality and short fermentation times (<8 hours) (Cuniberti 2005).

Studies on yield and quality parameters as influenced by the interactions of fungicide, N availability and cultivar bread-making characteristics when affected by SLB are scarce. Some studies take into account only some of these factors (Cox *et al*. 1989; McKendry *et al*. 1995; Herrman *et al*. 1996; Simón *et al*. 2002; Ruske *et al*. 2003, 2004; Garrido-Lestache *et al*. 2004; Arabi *et al*. 2007; Blandino and Reyneri 2009; Godfrey *et al*. 2010; Rodrigo *et al*. 2015). Furthermore, these studies often quantify disease severity on a proportional scale (i.e. as percentage severity), which gives no information about crop-canopy area (Parker *et al*. 2004). Therefore, green-leaf-area duration (GLAD) is a better measure of disease pressure than the area under disease-progress curve (AUDPC), because it quantifies the spatial and temporal effects of foliar diseases taking into account factors affecting disease development such as crop-canopy size and genotype.

We hypothesised that SLB severity increases GPC depending on the quality group of the cultivars, and consequently some bread-making parameters are affected. Therefore, the control of SLB with fungicide applications decreases GPC, and such decreases are higher with fungicides containing triazole and strobilurins, which would cause greater increases in GLAD and/or yield than triazole-only fungicides. We further hypothesised that N availability increases disease severity but also might prevent the reduction in GPC caused by fungicide applications.

Therefore, the aim of this work was to investigate the influence of two fungicide products (containing triazole or triazole–strobilurin combination), three N fertiliser rates and three cultivars of contrasting bread-making characteristics on the progress of *M. graminicola*, GLAD, and its effect on GY, GPC and bread-making parameters of wheat.

Materials and methods

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 2009 and 2010. Weather data (monthly precipitation, relative humidity, and minimum, maximum and mean temperatures) were recorded at the Davis Meteorological Station situated 100 m from the experiments. The trials were sown in adjacent fields in July under conventional tillage each year. The soil was a Typic Argiudoll (Soil Survey Staff 1999). Analysis of soil samples indicated the following values by weight in 2009 and 2010, respectively. In the top 0.20 m, organic matter was 3.65% and 3.49%, total N 0.18% and 0.10%, NO_3-N 43 and 29 µg/g, P 19 and 15 µg/g, and pH 5.8 and 6.0. At 0.20–0.40 m depth, NO_3-N was 30 and 28 µg/g, and pH 5.7 and 6.3.

The experimental design was a split-split-plot with three replications. Within every year, the main plots were the fungicide treatments: (*i*) Nativo, a product with a combination of a triazole (tebuconazole, 480 cm^3/ha) and a strobilurin (trifloxystrobin, 120 cm^3/ha) with a total dose of 600 cm^3/ha; (*ii*) Folicur, a triazole-only product (tebuconazole, 750 cm^3/ha);

(*iii*) nil fungicide (control treatment). The subplots were the N treatments: nil N (0N); and 70 and 140 kg N/ha (70N, 140N) applied as granulated urea, half at sowing and the other half at Zadoks growth stage Z33 (Zadoks *et al*. 1974). The sub-subplots were three registered cultivars with similar heading time and varying from moderate resistance to moderate susceptibility (information provided by their respective breeders): Buck Guapo (quality group 1, Guapo), moderately susceptible to SLB; Klein Escorpión (quality group 2, Escorpion), moderately resistant to moderately susceptible to SLB; Nidera Baguette 10 (quality group 3, Baguette 10), moderately susceptible. Each sub-subplot was 7.7 m^2 (5.5 m long by 1.4 m wide). The entire experiment was fertilised with 50 kg P_2O_5/ha as calcium triple superphosphate at sowing.

Inoculations and fungicide treatments were applied three times between the end of August and the beginning of November, at the beginning of tillering (Z22), three-node stage (Z33) and the beginning of flowering (Z61) in both years. The whole experiment was sprayed with a mixture of four virulent isolates of *M. graminicola* (FALP14707, FALP20107, FALP20207 and FALP20507) grown on malt extract agar. The inoculum was prepared by aseptically scraping sporulating colonies with a scalpel and suspending conidia in deionised water. The spore concentration was measured with a Neubauer hemocytometer. The conidial suspension was adjusted to 5×10^6 spores/mL. Tween 20 (0.5 mL/L) was added as a surfactant. Plants were sprayed with the inoculum suspension until runoff. After inoculations, plants were kept moist by spraying with water several times a day (15 min every 2 h) for 3 days.

In both years, disease severity was assessed by visual estimation of the percentage of leaf area affected by *M. graminicola* on 7–10 plants in each plot at the following growth stages: on the upper four leaves at the flag leaf stage (Z39) and at flowering (Z60); and on the upper two leaves (flag leaf and the leaf below flag leaf) at early dough stage (Z82). The AUDPC for each treatment was calculated to summarise the progress of the disease, according to Shaner and Finney (1977). The green-leaf-area index was determined at every growth stage by separating and pasting leaves from seven tillers with at least 10% green on paper sheets. These sheets were scanned and the leaf area was measured by the image J program (Schneider *et al*. 2012). Finally, GLAD was calculated by the trapezoidal method according to Waggoner and Berger (1987).

Plants along 4 m of the three central rows in each plot were harvested and threshed (2.4 m^2) and the GY (kg/ha) was calculated. Grain samples from each subplot were conditioned to 15.5% moisture and milled by using a laboratory mill (Bühler MLU-202; Bühler Holding, Uzwil, Switzerland), extracting the flour at a rate of ~70%. 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).

Dough rheological properties were evaluated by using a Chopin Alveograph (Chopin Technologies, Villeneuve-la-Garenne, France) and a Brabender Farinograph (Brabender, Duisburg, Germany) in accordance with the IRAM (*Instituto Argentino de Normalización*) standard methods 15855 and 15857, respectively. The alveograph parameters were measured with a 250-g sample of flour. Tenacity (*P*, mm) is the maximum height along the *y*-axis and estimates the ability of the dough to resist deformation. The length along the *x*-axis, which is the maximum volume of air that the bubble is able to contain, is referred as extensibility (*L*, mm). The area under the curve is proportional to the energy required to cause the dough bubble to break, named dough strength (*W*, as $J \times 10^{-4}$).

Farinograph parameters were measured with a 50-g sample of flour. Water absorption (*A*, mL) is the quantity of water necessary to produce dough with a peak development of 500 Brabender units (BU). Dough development time (*B*, 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. Stability (*D*, min) 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. The degree of softening (*E*) is the difference between the resistance of the dough at its peak (500 BU) and 12 min later, measured in BU.

Data were analysed by a combined analysis of variance (ANOVA) for both years with a split-split-plot design with GENSTAT 12 Edn (GENSTAT 2009), considering *Year*, *Fungicide*, *N* and *Cultivar* as fixed effects and blocks as random effects. Mean values were compared with l.s.d. test at $P = 0.05$.

Results

Weather conditions, area under disease-progress curve and green-leaf-area duration

Environmental conditions over the two years are presented in Table 1, as well as historical climatic values (1969–2008) for monthly rainfall and temperature. Rainfall varied considerably between years. Rainfall during the crop cycle was 631 mm in 2009 and 344 mm in 2010, whereas the historical rainfall value for the region is 461 mm (Table 1). Differences in temperature values over the two growing seasons were relatively modest compared with the historic values.

Statistical analysis of AUDPC and GLAD are presented in Table 2. Mean AUDPC values were significantly higher in 2009 (+90.2%) than in 2010 because of the higher precipitation (Table 3). Fungicide applications decreased AUDPC compared with the control, without significant differences between fungicide types. There were differences among wheat cultivars for AUDPC (Table 2); Escorpion was the most affected cultivar (mean 2949) and Baguette 10 the least affected (mean 1872) (Table 3). The year × N interaction was significant; in 2009, the higher the N rate, the lower the AUDPC (Fig. 1*a*), whereas in 2010, there were no differences among N rates. The significant fungicide × N interaction ($P = 0.083$, Table 2) was due to a greater N effect when fungicides were applied than in the untreated plots (Fig. 1*b*).

For GLAD, significant differences occurred with respect to the main factors and some interaction effects (Table 2). Mean values of GLAD were significantly lower (–34.0%) in the drier year (Table 3). Fungicide treatments showed an increase in GLAD compared with untreated plots, although no differences were found between fungicide types. Increasing the N rate significantly prolonged GLAD (Table 3). In addition, Escorpion had the highest values of GLAD (115 days),

Table 1. Data for monthly mean, maximum and minimum temperature, mean relative moisture, and precipitation for the experimental site during the field trial

Historical (1969–2008) data for mean monthly temperature and precipitation are also presented

	Temperature (°C)							Mean rel. moist. (%)			Precipitation (mm)		
	2009			2010			Hist.	2009	2010		2009	2010	Hist.
	Mean	Min.	Max.	Mean	Min.	Max.							
July	8.4	3.6	13.9	9.4	5.0	13.7	9.8	70.0	79.0		106	86.8	61.0
Aug.	12.9	7.4	18.8	9.8	4.6	15.2	11.1	70.0	76.0		39.0	26.8	60.4
Sep.	11.5	6.9	16.3	13.1	8.5	18.2	12.9	76.0	78.0		97.4	111	67.1
Oct.	15.2	9.5	20.6	14.8	9.1	20.3	15.8	69.0	73.0		122	40.4	95.5
Nov.	19.0	13.9	24.1	18.0	11.7	24.7	18.6	74.0	70.0		161	28.0	97.0
Dec.	20.3	15.3	25.2	22.6	15.7	29.2	18.6	73.0	58.0		106	51.0	80.0
Mean	14.5	9.4	19.8	14.6	9.1	20.2	14.5	72.0	72.3	Total	631	344	461

Table 2. Probabilities values of the area under disease-progress curve (AUDPC), green-leaf-area duration (GLAD), grain yield (GY), grain protein concentration (GPC), tenacity (*P*), extensibility (*L*), *P* : *L* ratio, dough strength (*W*), water absorption (*A*), dough development time (*B*), stability (*D*) and softening degree (*E*) of three wheat cultivars under three fungicide treatments and three nitrogen rates in two years

Source of variation	d.f.	AUDPC	GLAD (days)	GY (kg/ha)	GPC (%)	*P* (mm)	*L* (mm)	*P* : *L* ratio	*W* ($J \times 10^{-4}$)	*A* (mL)	*B* (min)	*D* (min)	*E* (BU)[A]
Year (Y)	1	0.001	0.013	0.127	0.107	0.381	0.028	0.686	0.085	0.400	0.044	0.023	0.007
Error a	2												
Fungicide (Fu)	2	0.003	0.002	0.004	<0.001	0.123	0.197	0.477	0.525	0.292	0.503	0.318	0.026
Y × Fu	2	0.320	0.950	0.293	0.254	0.004	0.354	0.164	0.037	0.026	0.662	0.270	0.039
Error b	8												
Nitrogen (N)	2	<0.001	<0.001	<0.001	<0.001	0.629	<0.001	0.080	0.006	0.494	0.672	0.326	0.836
Y × N	2	<0.001	0.219	<0.001	0.211	0.786	0.025	0.463	0.081	0.320	0.300	0.111	0.034
Fu × N	4	0.083	0.390	0.421	0.129	0.036	0.259	0.009	0.684	0.424	0.001	0.006	0.016
Y × Fu × N	4	0.654	0.246	0.566	0.904	0.060	0.846	0.435	0.321	0.033	0.004	0.041	0.048
Error c	24												
Cultivar (Cv)	2	<0.001	<0.001	0.004	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.031	0.805
Y × Cv	2	0.070	0.001	0.542	0.709	0.740	<0.001	<0.001	0.003	0.196	0.388	0.675	0.133
Fu × Cv	4	0.352	0.381	0.487	0.504	0.041	0.054	0.307	0.724	0.382	0.258	0.107	0.058
N × Cv	4	0.122	<0.001	0.858	0.707	0.046	0.339	0.185	0.315	0.053	0.229	0.632	0.310
Y × Fu × Cv	4	0.840	0.500	0.374	0.849	0.336	0.120	0.874	0.778	0.276	0.089	0.040	0.025
Y × N × Cv	4	0.699	0.130	0.145	0.855	0.662	0.132	0.811	0.930	0.137	0.565	0.004	0.115
Fu × N × Cv	8	0.856	0.003	0.690	0.810	0.243	0.190	0.483	0.950	0.357	0.015	0.021	0.429
Y × Fu × N × Cv	8	0.964	0.928	0.890	0.987	0.708	0.466	0.429	0.983	0.327	0.210	0.286	0.666
Error d	72												

[A]BU, Brabender units.

followed by Guapo (108 days) and Baguette 10 (101 days) (Table 3). The interaction year × cultivar was significant (Table 2) due to differences in GLAD among cultivars in the drier year (2010) and no differences in the wetter year (2009) (Fig. 2). The N × cultivar and fungicide × N × cultivar interactions were mainly due to a higher response of Escorpion to an increase in N rate (0N to 70N, +62.3%) in the untreated and Folicur treatments than Guapo and Baguette 10 (+24.0% and +53.8%, respectively).

Grain yield and grain protein concentration

Responses of GY to fungicide type, N rate and cultivar are shown in Table 2. There was a GY increase in plots treated with fungicides compared with untreated plots (Table 3); however, no differences were found between fungicide types. Significant differences in GY among cultivars were detected (Table 2). Baguette 10 had higher GY (6933 kg/ha) than Guapo and Escorpion (6193 and 5909 kg/ha, respectively), which did not differ statistically (Table 3). Year × N interactions occurred (Table 2). In both years, GY increased with rising N rates (Fig. 3); however, in 2009, there were no significant differences between treatments 70N and 140N, whereas in 2010, a significantly higher value was obtained with 140N than 70N, mainly explained by differences in soil conditions between years.

Grain protein concentration was increased by SLB, and was significantly reduced by the fungicide Nativo (Table 3). In addition, GPC tended to increase greatly with dose increment of N applied (Table 3). Although the fungicide × N interaction was not significant, GPC reductions with the fungicide Nativo tended to be lower as the dose of N increased (–6.3%, –4.5% and –4.2% for 0N, 70N and 140N, respectively). Differences were observed among cultivars; GPC was significantly lower in Baguette 10 (11.5%) than Guapo and Escorpion (12.4% and 12.3%, respectively), which did not differ statistically (Table 3). Although the fungicide × cultivar interaction was not significant, cultivar Baguette 10 tended to have the greatest

Table 3. Mean values of the area under disease progress curve (AUDPC), green leaf area duration (GLAD), grain yield (GY), grain protein concentration (GPC), tenacity (*P*), extensibility (*L*), *P*:*L* ratio, dough strength (*W*), water absorption (*A*), dough development time (*B*), stability (*D*) and softening degree (*E*) of three wheat cultivars under three fungicide treatments and three nitrogen rates in two years

For each parameter and factor, means followed by the same letter are not significantly different at $P = 0.05$

Main factors	AUDPC	GLAD (days)	GY (kg/ha)	GPC (%)	*P* (mm)	*L* (mm)	*P*:*L* ratio	*W* ($J \times 10^{-4}$)	*A* (mL)	*B* (min)	*D* (min)	*E* (BU)[A]
						Year						
2009	3231b	130b	7098a	11.9a	95.20a	57.0b	1.9a	223a	57.4a	6.3a	10.1a	68.5b
2010	1699a	85.7a	5592a	12.2a	87.50a	46.8a	2.0a	176a	58.4a	11.8b	20.3b	39.0a
l.s.d. ($P = 0.05$)	230	21.6	2564	0.49	29.9	7.5	0.76	62.1	4.1	5.1	6.8	10.5
						Fungicide						
Nativo	2053a	111b	6805b	11.7a	87.3a	52.1a	1.9a	193a	58.0a	8.2a	15.7a	61.7b
Folicur	2290ab	115b	6957b	12.1b	92.2a	53.1a	1.9a	203a	57.4a	10.0a	17.1a	44.9a
Untreated	3053b	97.4a	5273a	12.3b	94.6a	50.6a	2.0a	203a	58.3a	9.0a	12.7a	54.5ab
l.s.d. ($P = 0.05$)	467	7.9	896	0.24	7.3	2.8	0.20	23.6	1.3	3.4	6.4	11.3
						Nitrogen						
0 kg N/ha	2758c	75.1a	5066a	11.3a	89.4a	49.1a	2.0ab	186a	57.8a	9.2a	14.6a	53.8a
70 kg N/ha	2474b	109b	6777b	11.8b	92.7a	49.3a	2.1b	193a	58.0a	8.7a	14.6a	55.1a
140 kg N/ha	2164a	139c	7193b	13.1c	92.0a	57.3b	1.8a	220b	57.8a	9.3a	16.3a	52.3a
l.s.d. ($P = 0.05$)	241	4.9	447	0.33	7.3	4.0	0.22	20.9	1.2	1.4	2.7	9.6
						Cultivar						
Guapo	2574b	108b	6193a	12.4b	103.6c	51.8b	2.2b	218b	58.9b	11.6c	17.1b	54.5a
Escorpion	2949c	115c	5909a	12.3b	92.3b	62.9c	2.1b	232b	58.5b	8.9b	14.7ab	54.6a
Baguette 10	1872a	101a	6933b	11.5a	78.2a	41.1a	1.6a	148a	56.2a	6.7a	13.7a	52.1a
l.s.d. ($P = 0.05$)	260	4.7	603	0.45	6.7	4.5	0.27	17.5	1.0	1.9	2.6	8.6

[A]BU, Brabender units.

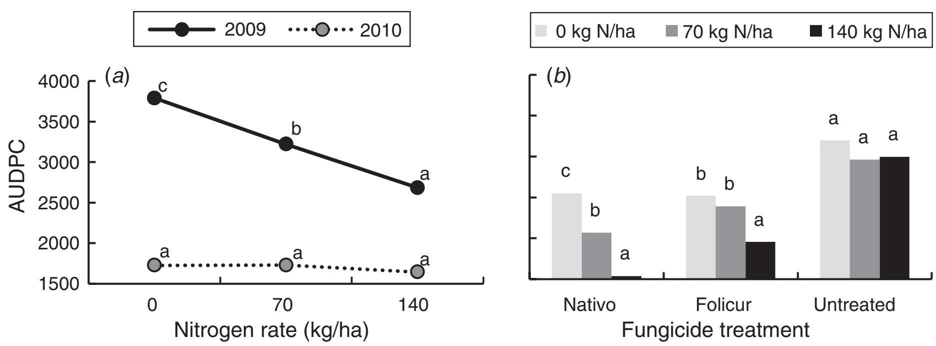

Fig. 1. Mean values of area under disease-progress curve (AUDPC) in (*a*) year × nitrogen (N), and (*b*) fungicide × N interaction. Means with the same letter within the same year are not significantly different (l.s.d. $P = 0.05$); means with the same letter within the same fungicide treatment are not significantly different (l.s.d. $P = 0.10$).

reduction in GPC with both fungicides (–8.5% with Nativo and –3.8% with Folicur) followed by Guapo (–6.1% with Nativo and –2.0% with Folicur) and Escorpion (–0.41% with Nativo and –1.2% with Folicur).

Alveograph parameters

Regarding parameter *P*, significant year × fungicide, fungicide × N, fungicide × cultivar and N × cultivar interaction effects were found (Table 2). SLB increased parameter *P*, whereas only Nativo (triazole + strobilurin mixture) significantly reduced it in the wetter year (2009) (Fig. 4*a*). In addition, SLB increased parameter *P* mainly in the N-fertilised plots; however, in those plots treated with Folicur, parameter *P* decreased with increasing N rate (Fig. 4*b*). Guapo, the cultivar from the best quality group, increased its parameter *P* values under the effect of SLB; thus, fungicide treatments decreased it (Fig. 4*c*). Only Baguette 10, the cultivar from lowest quality group, which showed the lowest parameter *P* values, showed increased values of *P* with higher N rates (Fig. 4*d*).

In the driest year, parameter *L* significantly decreased (–17.8%) (Table 3). The interaction year × N was significant (Table 2); in the wetter year, the highest N rate significantly increased *L* compared with 0N and 70N (Fig. 5*a*). In

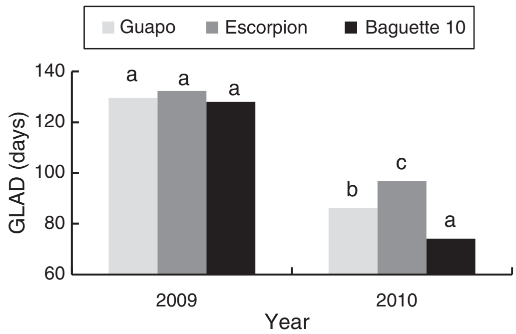

Fig. 2. Mean values of green leaf area duration (GLAD) in the year × cultivar interaction. Means with the same letter within the same year are not significantly different (l.s.d. at $P = 0.05$).

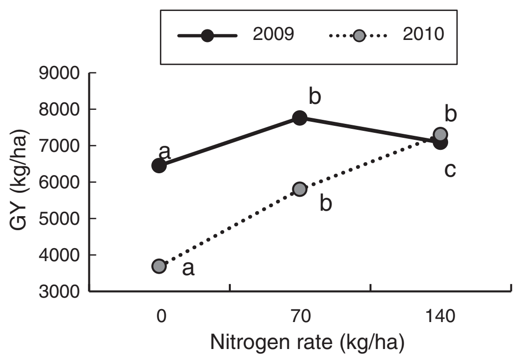

Fig. 3. Mean values of grain yield (GY) in the year × N interaction. Means with the same letter within the same year are not significantly significant (l.s.d. $P = 0.05$).

addition, the year × cultivar interaction was significant; *L* values significantly increased in the wetter year in Guapo and Escorpion (Fig. 5*b*).

Regarding the *P* : *L* ratio, Guapo and Escorpion had the highest values according to their bread-making characteristics (Table 3). Fungicide × N and year × cultivar interaction effects were also significant (Table 2); however, results were not consistent (data not shown).

Significantly higher values of parameter *W* were observed at the highest N rate (140N) and in the cultivars with better bread-making characteristics Guapo and Escorpion (Table 3). SLB increased parameter *W* values; however, only Nativo decreased it in the wetter year (Fig. 6*a*). Furthermore, cultivars Guapo and Escorpion had the highest *W* values in the wetter year (Fig. 6*b*).

Farinograph parameters

Statistical analysis of farinograph parameters is presented in Table 2. Highly significant differences in mean *A* values were detected among cultivars; *A* values were consistently higher in Guapo and Escorpion, with better bread-making characteristics (58.9 and 58.5 mL, respectively) than in Baguette 10 (56.2 mL) (Table 3). The year × fungicide × N interaction was also significant but results were not consistent (data not shown).

In the drier year, parameter *B* was statistically higher (+87.2%) (Table 3). In addition, Guapo showed the highest *B* value (11.6 min) followed by Escorpion and Baguette 10 (8.9 and 6.7 min, respectively) (Table 3). Significant year × fungicide × N and fungicide × N × cultivar interactions effects (Table 2) were not consistent. Parameter *D* values significantly increased in the drier year (+102%) and were statistically higher in Guapo (17.1 min) (Table 3). Finally, *E* values showed

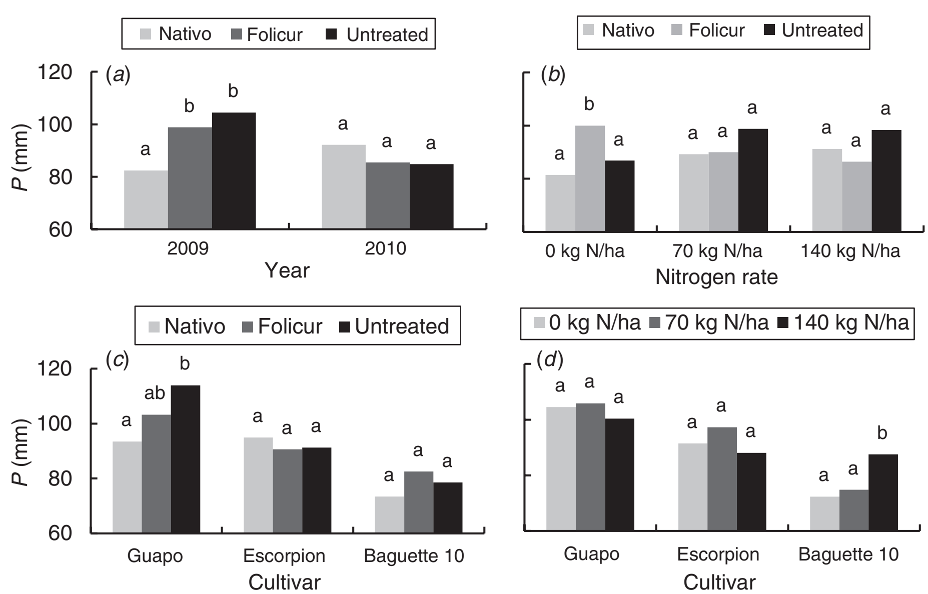

Fig. 4. Mean values of tenacity (*P*) in (*a*) year × fungicide, (*b*) fungicide × N, (*c*) fungicide × cultivar, and (*d*) N × cultivar interaction. Means with the same letter within the same year, N treatment or cultivar are not significantly different (l.s.d. $P = 0.05$).

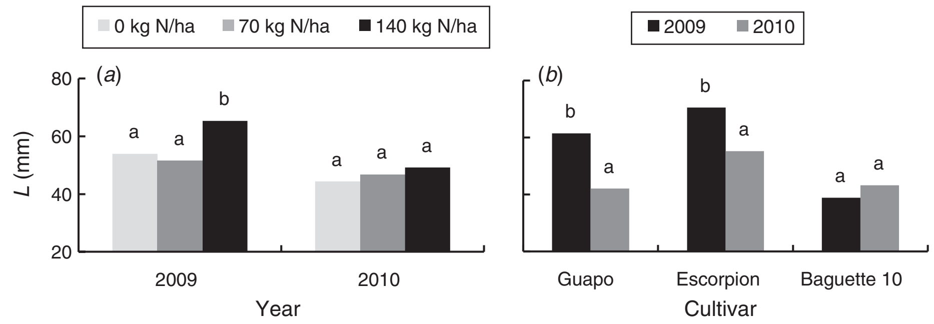

Fig. 5. Mean values of extensibility (L) in (*a*) year × N, and (*b*) year × cultivar interaction. Means with the same letter within the same year or cultivar are not significantly different (l.s.d. P = 0.05).

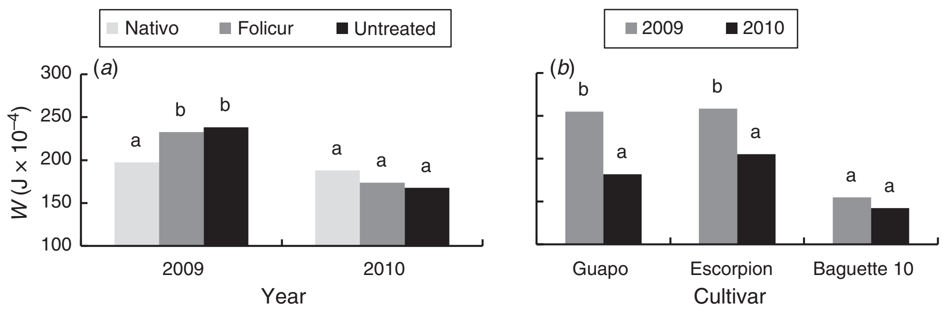

Fig. 6. Mean values of dough strength (W) in (*a*) year × fungicide, and (*b*) year × cultivar interaction. Means with the same letter within the same year or cultivar are not significantly different (l.s.d. P = 0.05).

significant year × fungicide × N and year × fungicide × cultivar interaction effects (Table 2). Thus, in the drier year (2010), the application of Folicur led to significant increases among N rates, but in the wetter year (2009), increases were observed only with Nativo (data not shown).

Discussion

Area under disease progress curve and green leaf area duration

The variation in rainfall was reflected in fluctuations in AUDPC, GLAD, GY and GPC between years. The 2009 growing season was wetter than 2010 and more conducive to the dispersal and development of *M. graminicola.* AUDPC was consistently higher in the wetter year. Similar results were reported by Ruske *et al.* (2003) and Rodrigo *et al.* (2015), who found that wetter conditions in one growing season favoured more severe SLB than in a drier growing season.

Disease severity was significantly higher in the untreated plots than in the fungicide-protected plots; however, the application of Nativo (triazole-strobilurin) did not result in significant differences from Folicur (triazole-only) treatment. Jørgensen *et al.* (1999) and Ruske *et al.* (2003) reported that a fungicide containing strobilurins (azoxystrobin) was more effective in controlling SLB than a triazole-only fungicide. Regarding cultivars, Escorpion presented the highest AUDPC despite its moderate resistance to SLB, possibly because severity can vary according to the isolate of the pathogen. GLAD was significantly higher in 2009 than in 2010, clearly associated with high rainfall during the growing season favouring leaf retention. Fungicide applications significantly increased GLAD compared with the untreated plots, with no significant differences between fungicides. Whereas Bayles (1999) reported that strobilurins are able to prolong the duration of the green flag leaf area much more than triazoles, in this study, differences between fungicides in GLAD improvements were slight. Our findings are in line with those of McCartney *et al.* (2007), who reported that adding a strobilurin did not significantly increase the GLAD compared with a triazole-only fungicide in either year of their study. Likely, the strobilurin molecule type or the number of fungicide applications may influence these results. Three fungicide applications were used in this study to achieve better control of SLB, and taking into account that three applications are usual in the high-input Argentinian wheat-production areas, mainly in non-tillage systems.

Nitrogen fertilisation is necessary to achieve high yields and better grain quality but may also influence the development of SLB. Unlike the findings of Gheorghies (1974), Prew *et al.* (1983), Howard *et al.* (1994), Leitch and Jenkins (1995) Simón *et al.* (2002, 2003), in this study, increasing the N rate reduced AUDPC in the wetter year. Similarly, Johnston *et al.* (1979) reported a decrease in the severity of the disease with increased N in one year of their experiments. Although disease severity seems to be reduced by N applications in these experiments, this could have been caused by a reduction in senescence. This hypothesis can be confirmed by the greater reduction of AUDPC under fungicide application when N was applied than in unfertilised plots. Bockus and Davis (1993) suggested that N fertilisers also appear to reduce the severity of disease caused by the necrotrophic pathogen *Pyrenophora tritici-repentis* (Died.) Drechs. (anamorph *Drechslera tritici-repentis*) (Died.) Shoem), the causal agent of the tan spot; however, its effect may be related to limiting the natural senescence of leaf tissue.

Grain yield and grain protein concentration

The two fungicide treatments significantly improved GY compared with untreated plots, although there were no significant differences between the mixture (Nativo) and the triazole-only fungicide (Folicur), coinciding with results of McCartney *et al*. (2007); Blandino and Reyneri (2009); Wegulo *et al*. (2012). However, other researchers have found that adding at least one strobilurin application to the triazole program resulted in further yield increases (Bayles 1999; Jørgensen *et al*. 1999; Ruske *et al*. 2004). In the wetter year of this study, application of Nativo tended to increase GY more than Folicur, although this difference was not significant. These increases would be associated with maintenance of canopy photosynthesis during the grain-filling period, but also prolonging of the duration of green flag leaf area (Dimmock and Gooding 2002*a*). The response of GY to N rate varied depending on the year. Thus, yield increased in 2010 with rising N rates, whereas in 2009, no differences were detected between 70N and 140N, probably due to differences in the amount and distribution of rainfall and in the soil properties (higher amount of nitrates in 2009). Year × N interactions were also found in the studies of Garrido-Lestache *et al*. (2004) and Varga *et al*. (2005); this indicates that N fertilisation response is highly dependent on annual variations in the amount and distribution of rainfall and on the uptake and efficiency of fertiliser N at various stages in wheat growth.

The effect of foliar diseases on GPC may vary depending on foliar disease type. When biotrophic fungal pathogens such as leaf rust (*Puccinia triticina* Eriks.) affect wheat, the protein concentration usually decreases (i.e. the pathogen causes more damage to the accumulation and partitioning of N in the grain than to the accumulation and partitioning of dry matter) (Dimmock and Gooding 2002*b*). Moreover, when wheat is affected by necrotrophic pathogens as *P. tritici-repentis*, protein concentration increases. Finally, hemibiotrophic pathogens such as *M. graminicola* may cause both effects, depending on the genotype and environmental conditions. Unlike the findings of Arabi *et al*. (2007), this study found increases in protein concentration when the severity of SLB increased. Similarly, prior studies (McKendry *et al*. 1995; Dimmock and Gooding 2002*b*; Gooding 2007) have documented that control of pathogens such as *M. graminicola* with fungicides is associated with reductions in GPC; this might suggest that the necrotroph phase is the important contributor. Dimmock and Gooding (2002*b*) mentioned that cultivars specifically bred for bread making, where high protein concentration is a selection criterion together with high yields, may be able to maintain grain N accumulation more effectively because senescence is delayed and yield increases through the fungicide effect, compared with cultivars suited to biscuit making, where protein concentration is much less important. In this work, decreases in GPC were independent of the cultivars (fungicide × cultivar interaction not significant). However, Guapo and Escorpion, the two cultivars from the best quality group, maintained higher levels of GPC under fungicide applications.

According to Dimmock and Gooding (2002*b*), when *M. graminicola* is the dominant pathogen, the use of fungicides can reduce GPC. However, the same authors pointed out that losses are usually small and can be diminished or eliminated through application of foliar urea during grain filling. In the present work, GPC increased with N fertiliser rate from 0 to 140 kg N/ha. Similar findings were reported by Ayoub *et al*. (1995), who found that GPC increased with increasing levels of N fertiliser. It is recognised that the increase in grain protein under conditions of high N fertilisation (Godfrey *et al*. 2010) or late N supply results from greater synthesis and accumulation of storage proteins.

In our work, there were no significant fungicide × N interactions; however, reductions in GPC under fungicide applications were smaller in the N fertilised treatments.

Alveograph and farinograph parameters

Previously, we hypothesised that SLB severity increases GPC, and consequently, some bread-making parameters are affected. Although GPC showed a significant decrease with fungicide treatments, alveograph and farinograph parameters measured in flour samples did not show consistent results. In this study, parameters *W* and *P* decreased with fungicide applications but only in the wetter year, likely associated with GPC reductions derived from higher yields. Similarly, Cuniberti *et al*. (2004) found that parameter *W* and bread volume decreased with fungicide application, showing a negative effect on industrial quality. Conversely, Blandino and Reyneri (2009) documented consistent benefits in *W* with the use of fungicides containing triazole–strobilurin, but found no significant differences in the $P:L$ variable. Furthermore, Cátedra Cerón and Solís Martel (2003) found no significant differences between the means of the *W* and $P:L$ variables with or without fungicides, and Wang *et al*. (2004) did not find significant effects of fungicide for gluten content and rheology properties. In the present study, we observed that when the N rate increased, rheological parameters such as *L* and *W* improved. Garrido-Lestache *et al*. (2005), Fuertes-Mendizábal *et al*. (2010) and Godfrey *et al*. (2010) stated that there is a favourable effect of N fertilisation on quality by increasing the GPC. In agreement with Fuertes-Mendizábal *et al*. (2010), both, *L* and *W* were more sensitive to N fertilisation management than was parameter *P*; however, unlike those authors, we found that the increase in *L* with respect to *P* did not result in an improvement of the dough $P:L$ ratio when higher N concentration was achieved in the grain. This indicates that N fertiliser not always obtain a more equilibrated dough.

Although protein concentration was slightly less reduced by fungicides when N fertilisation was applied, bread-making quality was not modified. Renzi *et al*. (2007) found that when GPC increased in cultivars with better bread-making characteristics (quality group 1), the *W* increased, whereas in cultivars of lower bread-making characteristics (quality group 3), the *W* values increased in a less steep way. García *et al*. (2001) found that although the percentages of protein and gluten can be estimated reciprocally, these parameters do not showed significant association with *W*. The quality of proteins (gliadin : glutenin ratio) is mainly determined by the genotype. That is why favourable conditions for the accumulation of proteins and increases in the gluten concentration in 'modern' cultivars do not usually correspond to *W* increases or other improvements in bread-making quality, as in this study.

Almost all alveograph and farinograph parameters clearly responded to the genotype and fertiliser N rate. Denčić *et al*. (2011) found that ranges of some quality parameters were significantly higher across cultivars than across environments. Among the cultivars used, Guapo and Escorpion presented the best values of *P*, *L*, *W* and *P* : *L*, in line with their bread-making characteristics. A positive relationship between the addition of N and an increase in *W* was found in Guapo, but the opposite happened in Escorpion.

Regarding farinograph parameters, significant differences among cultivars were found, according to their bread-making characteristics. Guapo presented the best values of farinograph parameters, followed by Escorpion and Baguette 10, indicating that genotype is more important than genotype × environment interaction. In line with these results, some studies concluded that genetic influence is more important than environmental influence (Souza *et al*. 2004; Denčić *et al*. 2011). Denčić *et al*. (2011), who evaluated the effect of 140 cultivars from different countries in different environments, also concluded that the genetic component is mainly responsible for variations in quality variables compared with the environmental effects, which had a major effect on the GPC. Conversely, Peña *et al*. (2007) found changes in farinograph parameters *B* and *D* for the same cultivar in different locations, and Abbate *et al*. (2010) found that the location and year effects on quality traits were the same as or more important than the genotype effect. None of the variables, except *E*, was modified by the fungicide treatments. Wang *et al*. (2004) found no significant differences in dough rheological properties between treatments with and without fungicides from the groups of triazoles and strobilurins.

In conclusion, *M. graminicola* infection increases GPC with less influence on the quality parameters determined by alveograph and farinograph. No differences in GLAD increases were found between fungicides containing triazole–strobilurins and triazole only. Regarding the effect of the N rate on SLB severity, we found a negative association. Nitrogen fertilisation also extended GLAD, which, combined with the lower levels of disease in the fertilised treatments, resulted in higher GY. GPC increased with SLB, and only fungicides containing triazoles and strobilurins decreased it. Reductions of GPC caused by these types of fungicides tended to be lower when the N rate increased. Cultivars with low bread-making characteristic showed a tendency of greater reductions in GPC with both fungicide types. Regarding quality parameters, only *P* and *W* were reduced by Nativo. In addition, *P*, *A* and *B* were higher in the cultivars from the best quality group than the lower groups. Furthermore, alveograph parameters such as *L* and *W* could be improved when N fertiliser was applied.

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