Journal of Cereal Science 80 (2018) 119–127

Contents lists available at ScienceDirect

Journal of Cereal Science

journal homepage: www.elsevier.com/locate/jcs

How leaf rust disease and its control with fungicides affect dough properties, gluten quality and loaf volume under different N rates in wheat

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

[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] INTA EEA Bordenave, Bordenave, 8187, Buenos Aires, Argentina
[d] CIC, La Plata, 1900, Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 6 November 2017
Received in revised form
13 January 2018
Accepted 9 February 2018
Available online 10 February 2018

Keywords:
Leaf rust
Fungicides
Yield
Breadmaking quality
Nitrogen fertilization
Wheat

ABSTRACT

The unique viscoelastic properties of gluten make bread wheat (*Triticum aestivum* L.) a staple food. The specific balance between gluten fractions, together with grain protein content (GPC), defines nutritional and end-use properties. The leaf rust (*Puccinia triticina* Eriks.) and its control using succinate dehydrogenase inhibitors (SDHIs) fungicides together with nitrogen (N) fertilization could alter the GPC and its composition during the grain-filling period, modifying dough properties. The aim of this study was to investigate the effects of leaf rust, its control using fluxapyroxad (SDHI) and the interaction with N fertilization on breadmaking quality of 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 subplots using a susceptible cultivar. Leaf rust reduced GPC and modified its composition. The disease shortened the grain-filling period and reduced gluten content. Gluten tenacity (Alv*P*) was more affected than gluten extensibility (Alv*L*) resulting in doughs with lower Alv*P*/*L* ratio, minor gluten strength and inferior loaf volume. The treatment containing fluxapyroxad showed better levels of disease control and it also evidenced additional beneficial effects in most of the evaluated parameters compared to the treatment without this active ingredient.

1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most important sources of food all over the world. The success of this crop relies on its adaptability, high yield potential and especially in their unique viscoelastic properties conferred by gluten, which is responsible for the universal use of wheat for a wide range of products. Gluten is mainly composed by gliadins, which confer dough viscosity and extensibility, and glutenins, which are responsible for dough strength and elasticity (Wieser, 2007). The specific balance between these gluten fractions, together with total protein content, defines nutritional and end-use properties of dough mixing and rheological characteristics including gluten tenacity, gluten extensibility, gluten strength, dough development time, dough stability and loaf volume. Particularly for loaf volume, an equilibrated glutenin/gliadin ratio is important to ensure higher volumes, since the dough needs to be sufficiently extensible to respond to gas pressure generated during fermentation process but also, strong enough to resist collapse.

Gluten fractions accumulate from shortly after anthesis to the end of grain-filling. However, it has been reported that gliadins accumulate earlier in grain-filling period than glutenins (Panozzo et al., 2001). Therefore, any factor that shortens grain-filling or affects differentially the grain filling phases may reduce the total protein content and altered protein composition, modifying the dough functional properties (Jamieson et al., 2001). In this sense, several studies have reported the effect of post-anthesis environmental conditions such as water availability, temperature and atmospheric CO_2 concentration on wheat quality (Blumenthal et al.,

* Corresponding author. Cerealicultura, Departamento de Tecnología Agropecuaria y Forestal, Facultad de Ciencias Agrarias y Forestales, UNLP, 60 y 119, CC31, La Plata, 1900, Argentina.
E-mail address: mcfleitas@agro.unlp.edu.ar (M.C. Fleitas).

https://doi.org/10.1016/j.jcs.2018.02.003
0733-5210/

1993, 1995).

On the other hand, it is well known that application of nitrogen (N) fertilizer typically increases the total protein content and the quantity of gliadin and glutenin storage proteins (Triboi et al., 2000). However, while considerable research effort has been done to increase the understanding of abiotic stresses and single management factors such as N nutrition on wheat quality, there is a general lack of information about the effect of foliar diseases and their interaction with fungicides and N fertilizer applications on dough rheological properties and gluten quality.

Leaf rust caused by *Puccina triticina* Eriks. is a major biotic threat in many wheat-growing regions of the world. The pathogen reduces both, N and carbon accumulation in the grain, but on balance, N concentration is more severely affected (Dimmock and Gooding, 2002; Schierenbeck et al., 2014). Although grain protein content (GPC) decreases have been widely reported, little is known about the effect of leaf rust on protein composition, gluten content, loaf volume and rheological properties determined by alveogram and farinogram parameters. One hypothesis is that the premature leaf senescence and shortening of the grain-filling period caused by the disease could alter protein composition and rheological properties. Disease control using foliar fungicides maintains the canopy green-leaf-area duration (GLAD) and may prevent this alteration. Furthermore, recently released fungicides have been found showing additional beneficial physiological effects, not mediated through pathogen control (Smith et al., 2013; Ajigboye et al., 2014). Thus, succinate dehydrogenase inhibitors (SDHIs) fungicides were reported to exert physiological changes in the plant such as an extension of GLAD, reduction of leaf surface temperature and increasing the maximum photochemical efficiency of PSII (Berdugo et al., 2012; Smith et al., 2013; Ajigboye et al., 2014). As a consequence of these changes, treatment with SDHIs fungicides showed higher yields compared to untreated controls, while potential effects on wheat quality so far have not been investigated.

On the other hand, N application can lead to significantly greater disease incidence and lesion area under infections of biotrophic pathogens like mildews and rusts (Gerard et al., 2015). Therefore, the direct and positive effect on quality of N nutrition could be counteracted by the negative effects associated with a greater severity of leaf rust promoted. N fertilization may also affect the effectiveness of foliar fungicide applications (Varga et al., 2005). In this sense, several studies have documented the positive impact of N fertilization on the total leaf area index (TLAI) explained by its effect on tillering and leaf expansion, which in turn, determine the size of the canopy (above-ground biomass) (Hawkesford, 2014). This might dilute the amount of fungicide applied per leaf area which could cause an increase of some foliar diseases severity with increasing biomass levels under the same fungicide dose as it was recently reported by Jensen and Jørgensen (2016).

Wheat quality is a complex trait influenced by genetical, environmental and management factors. In recent years, it has been determined the possible effects associated with some of them; however, very few studies have addressed the effects of leaf rust and its interaction with management practices including N fertilization and fungicide applications on dough rheological properties. In addition, most of the research was done under natural infections where is difficult to ascertain the effect of foliar diseases caused by pathogens with different nutritional habit, which have a different impact in the N/starch balance (Dimmock and Gooding, 2002). Moreover, the evaluation of the disease was carried out only through assessing disease severity and the area under disease progress curve (AUDPC) which involved substantial inaccuracies since they assume that (i) the area of healthy leaves does not vary between genotypes, between environments, within the plant, or through time and (ii) the total leaf area is not affected by disease (Parker et al., 2004; Paveley et al., 2005). Finally, the literature available focuses mainly on the effect of foliar diseases and fungicides on grain yield and GPC without considering other important breadmaking traits or was done applying conventional active ingredients such as triazoles and strobilurins, therefore, the effect of new fungicide active ingredient such as carboxamides are unknown. In view of the insufficiency information in the existing literature, the aim of this study was to investigate the effects of the leaf rust disease, its control with a triple-mixture fungicide containing the SDHI fluxapyroxad and the interaction of both factors with N fertilization on wheat breadmaking quality including GPC, wet gluten content, loaf volume and dough rheological properties (alveogram and farinogram parameters).

2. Materials and methods

2.1. Field trials and experimental design

Two field experiments were conducted during 2014 and 2015 under artificial *P. triticina* infections at the Estación Experimental Julio Hirschhorn (EEJH), Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, Buenos Aires, Argentina. The trials were sown on July 28 and June 16 respectively under conventional tillage, after a rotation of fallow-wheat-fallow. 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. The EEJH has a silt loam soil, classified as Typic Argiudoll. Analysis of the soil samples indicated the following mean values by weight in each year. In the top-0.20 m, organic matter was 3.59%, total N 0.20%, NO_3-N 10.4 μg/g, P 28.3 μg/g, and pH 5.8. At 0.20–0.40 m depth, NO_3-N was 5.1 μg/g, and pH 6.0.

The experimental design was a split-split-plot with three replications. Within every year, the main plots were the fungicide treatments applied at tillering (GS23) (Zadoks et al., 1974) and at flag leaf (GS39) (for calendar dates of every growth stage see Supplementary material 1): (*i*) nil fungicide (untreated control); (*ii*) Opera®, a product with a combination of a triazole (epoxiconazole, 50 g L^{-1}) and a strobilurin (pyraclostrobin, 133 g L^{-1}) with a total dose of 1 L ha^{-1} (TS) and using an application rate of 140 L ha^{-1}; (*iii*) Orquesta Ultra®, a product with a combination of a triazole (epoxiconazole, 50 g L^{-1}), a strobilurin (pyraclostrobin, 133 g L^{-1}) and a carboxamide (fluxapyroxad, 50 g L^{-1}) with a total dose of 1.2 L ha^{-1} (TSC) using the same application rate of the TS treatment. The subplots were the N treatments: nil N (0N) and 70 and 140 kg N ha^{-1} (70N, 140N) applied as granulated urea, half at sowing and the other half at growth stage GS33. The genotype used in the experiments was Baguette Premium 11 (Nidera), moderately susceptible to leaf rust (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 (>8 h). The genotype was sown in a 7.7 m^2 plot (5.5 m long by 1.4 m wide) containing seven rows, with a distance of 0.2 m between rows. The entire experiment was fertilized with 50 kg P_2O_5 ha^{-1} as calcium triple superphosphate at sowing. Weed control was made by Glyphosate (2 L ha^{-1}) 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 (GS13).

2.2. Inoculum multiplication and inoculations

Leaf rust disease was artificially promoted using a mixture of virulent races of *P. triticina* on Baguette P11 provided by INTA EEA Bordenave (Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Bordenave, Buenos Aires, Argentina). Uredospores of *P. triticina* (from now on called spores) were multiplied onto seedlings of highly susceptible wheat cultivars Nidera Baguette 21 and Nidera Baguette 30. Five to ten seeds were sown in pots and ten days after sowing, seedlings with two leaves were inoculated with 0.5 mg of fresh spores per seedling with talc powder. The inoculated seedlings were incubated in a dark dew chamber for overnight at 18–20 °C temperature with 90% humidity, then moved to a greenhouse and maintained at 18–25 °C with a photoperiod of 10–14 h. After incubation (12–15 days post-inoculation), spores were collected and stored in a refrigerator till further field use.

Two border rows (0.2 m apart) surrounding main plots (42 linear m) of susceptible cultivars (Baguette 21 and Baguette 30) were dusted with 0.5 mg spores per plant with talc as a vehicle. Inoculations were done at the beginning of tillering (GS21) and at the beginning of shoot development (GS31). After inoculations, plants were kept moist by spraying with water several times a day with sprinklers during a period of two days. Oat plots (*Avena sativa* L.) of the same area as the susceptible cultivar Baguette P11 (7.7 m^2) were sown between main plots to reduce the spread of inoculum between treated and untreated plots.

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

Leaf rust severity was estimated by visual estimation of the percentage of leaf area affected by *P. triticina* pustules using the modified Cobb scale (Peterson et al., 1948) on the upper four leaves at GS39 and at anthesis (GS60); and on the upper two leaves (flag leaf and the leaf below flag leaf) at early dough stage (GS82) on seven to ten plants in each plot. The AUDPC of every treatment was calculated to summarize the progress of the disease through the 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 TLAI (total leaf m^2/soil m^2) was estimated in all stages 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, 1990) of seven tillers. In the statistical analysis, only the TLAI at anthesis was considered, given that this was the stage in which the highest TLAI was observed. Discounting the disease severity to the TLAI, the green-leaf-area index (GLAI, green leaf m^2/soil m^2) was calculated at GS39, GS60 and GS82 and the GLAD (days) was estimated in the same way as AUDPC but using GLAI instead of disease severity (Waggoner and Berger, 1987). Green-flag-leaf area duration (GFLAD, days) was calculated with the same method as GLAD but using flag leaf GLAI.

2.4. Grain yield and breadmaking parameters determinations

Plants along 5 m 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 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. Wet gluten content (%) was determined in accordance with the IRAM (Instituto Argentino de Normalización y Certificación) 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 2200® (Perten instruments, Perkin Elmer Company). Bread loaf volume (cm^3) 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 by using a Chopin Alveograph® (Chopin Technologies, Villeneuve-la-Garenne, France) and a Brabender Farinograph® (Brabender, Duisburg, Germany) in accordance with the IRAM standard methods 15857:1995 and 15855:2000, respectively. The alveograph parameters were measured with a 250-g sample of flour. Gluten tenacity (Alv*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 gluten extensibility (Alv*L*, mm). The area under the curve is proportional to the energy required to cause the dough bubble to break, named gluten strength (Alv*W*, as J × 10^{-4}). The *P*/*L* ratio (Alv*P*/*L*) is the balance between gluten tenacity and gluten extensibility.

Farinograph parameters were measured with a 50-g sample of flour. Water absorption (Far*A*, mL) is the quantity of water necessary to produce dough with a peak development of 500 Brabender units (BU). Dough development time (Far*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. Dough stability (Far*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 (Far*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. Mean values were compared with LSD test ($p<0.05$). Variables AUDPC, GFLAD, GPC, Far*B* and Far*D* (log transformed) and TLAI (exponential transformed) were transformed to meet the assumptions of the ANOVA. In addition, regressions between GLAD and grain yield for every fungicide treatment were performed in order to determine yield gains per additional GLAD day (i.e. GLAD extension). The fall rate of Alv*P* and Alv*L* parameters per day of leaf life decline were estimated through regressions between the overall values of GLAD and Alv*P* means, and the overall values of GLAD and Alv*L* means respectively. Finally, breadmaking parameters were entered into a stepwise multiple regression using loaf volume as the dependent variable and the other breadmaking parameters as the independent.

3. Results

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

The sum of rainfall during the crop cycle was considerably

higher in 2014 (755 mm) than in 2015 (543 mm). The higher amount of precipitation in 2014 was concentrated in October and November, whilst in 2015, was in August and November (data not shown). Differences in temperature and relative moisture values over the two growing seasons were relatively modest (mean 15.1 °C and 14.3 °C, and 71.7% and 69.4% for 2014 and 2015 respectively). Leaf rust was particularly higher in the wetter year (2014), at the three growth stages compared to 2015 (Supplementary material 2). In addition, in 2014 also tan spot (*Pyrenophora tritici-repentis* (Died.) Drechs, anamorph *Drechslera tritici-repentis*) (Died.) Shoem) was present, but at low levels (less than 5%).

Statistical analysis and mean values of AUDPC are presented in Tables 1 and 2, respectively. There were significant differences for *Year*, *Fungicide* and the *Fungicide* × *N* interaction (Table 1). The AUDPC value was significantly higher in 2014 (45.1%) in relation to 2015 (Table 2). The significant *Fungicide* × *N* interaction was due to a greater N effect in the untreated plots compared to the protected ones. Thus, increments in N rates significantly increased AUDPC values in the untreated plots, while under fungicide treatments, no differences were detected (Fig. 1a). Averaged over all treatments, AUDPC was significantly lower with the TSC treatment which averaged 399, followed by TS (mean 471) and the control treatment (mean 784) (Table 2).

The TLAI evidenced significant differences for *Fungicide* and *N* main effects and its interaction (Table 1). The *Fungicide* × *N* interaction arose because TLAI values increased to a greater extent following fungicide applications across rising N rates compared to rusted plots (Fig. 1b). Thus, in the TSC treatment, TLAI increase +85.7% when comparing 0N with 140N rate, a higher increment compared to the TS (+75.0%) and the untreated plot (+72.7%) (Fig. 1b).

There were significant differences of GLAD for *Year*, *Fungicide* and *N* main effects, and the *Fungicide* × *N* interaction (Table 1). In the wettest year (2014), GLAD was significantly higher (+4.5%) than in 2015 (Table 2). The *Fungicide* × *N* interaction arose due to a greater GLAD response to N fertilization under fungicide applications (Fig. 1b). Such response was higher with the TSC fungicide (+55.6%) compared to the TS treatment (+42.0%). Conversely, in the untreated plots values only increased at 140N (+28.0%) (Fig. 1b). Similarly, the GFLAD also showed a significant *Fungicide* × *N* interaction (Table 1). Mean values increased from 0N to 140N, to a much greater extent with the triple-mixture TSC (+80.8%) compared to the double-mixture TS (70.5%) (Fig. 1c). In the untreated control, values showed a slight tendency, although not significant, to decrease (−6.42%) (Fig. 1c). Shorter GLAD and GFLAD evidenced in the untreated plots compared to the fungicide protected, was a result of both, lower TLAI at GS60 (Fig. 1b) and premature leaf senescence (shorter grain-filling period) caused by the disease.

Table 2
Mean values, standard errors of differences (s.e.d.) and least significant differences (l.s.d.) of the area under disease progress curve (AUDPC), total leaf area index (TLAI) at anthesis, green-leaf-area duration (GLAD), green-flag-leaf area duration (GFLAD) and grain yield (GY) of wheat under three fungicide treatments and three nitrogen rates in two years (TS: triazole + strobilurin; TSC: triazole + strobilurin + carboxamide).

Main factors	AUDPC	TLAI	GLAD (days)	GFLAD (days)	GY (kg ha^{-1})
Year					
2014	653	4,3	101	26.9	6169
2015	450	4.0	96.6	25.8	6058
s.e.d.	9.0E-03	0.99	0.41	1.4E-02	314
l.s.d.	70.1	0.70	1.75	2.24	1351
Fungicide					
Untreated	784	2.9	71.1	18.3	4506
TS	471	4.5	108	28.0	6453
TSC	399	5.1	117	32.8	7233
s.e.d.	1.2E-02	2.50	2.54	3.6E-02	240
l.s.d.	39.1	0.61	8.17	4.62	555
Nitrogen rate					
0 kg N ha^{-1}	526	3.0	81.5	20.6	5341
70 kg N ha^{-1}	563	4.2	98.6	27.4	6145
140 kg N ha^{-1}	566	5.3	116	31.1	6706
s.e.d.	1.4E-02	2.00	2.44	1.7E-02	170
l.s.d.	47.1	0.57	5.04	1.65	351

3.2. Grain yield, grain protein content and wet gluten content

Analysis of variance and averages values of grain yield are presented in Tables 1 and 2, respectively. Grain yield significantly varied with fungicide and N treatments (Table 1). Moreover, a significant *Fungicide* × *N* interaction occurred (Table 1) because yield responses at the three N fertilization rates varied across fungicide treatments. Following TSC treatments, grain yield ranged from 5910 kg ha^{-1} (0N) to 8358 kg ha^{-1} (140N), showing a higher increment (+41.4%) compared to the TS treatment (+36.3%), where values fluctuated from 5400 kg ha^{-1} (0N) to 7363 kg ha^{-1} (140N) (Fig. 1d). In contrast, means tended to decline in the untreated plots, although such differences were not significant, with values that rose from 4712 kg ha^{-1} (0N) to 4396 kg ha^{-1} (140N) showing a slight reduction of −6.70%. Grain yield increased per day of extra leaf life (i.e. the extension of GLAD) under fungicide treatment containing the SDHI fluxapyroxad (TSC) was 46.7 kg ha^{-1} day^{-1} (R^2=*0.339**), followed by the TS treatment with 38.7 kg ha^{-1} day^{-1} (R^2=*0.577***) and the untreated with 3.19 kg ha^{-1} day^{-1} (R^2=*0.10*).

There were significant differences in GPC for *Fungicide*, *N* and

Table 1
Mean squares of the area under disease progress curve (AUDPC), total leaf area index (TLAI) at anthesis, green-leaf-area duration (GLAD), green-flag-leaf area duration (GFLAD) and grain yield (GY) of wheat under three fungicide treatments and three nitrogen rates in two years.

Source of variation	Df	AUDPC	TLAI	GLAD	GFLAD	GY
Year (Y)	1	3.20E-01**	119	264*	2.44E-03	1331019
Error a	2	1.18E-03	13.3	2.24	2.52E-03	1649
Fungicide (Fu)	2	4.05E-01***	1483***	10695**	2.83E-01***	35482257***
Y × Fu	2	3.26E-03	40.7	18.8	1.49E-03	966273
Error b	8	1.37E-03	55.3	113	1.18E-02	520611
Nitrogen (N)	2	2.50E-03	237**	5452***	1.20E-01***	8478641***
Y × N	2	6.50E-03*	8.35	5.51	1.86E-03	1097622*
Fu × N	4	6.82E-03*	224**	553**	4.05E-02***	3376192**
Y × Fu × N	4	3.91E-03	25.9	8.31	1.42E-03	409355
Error c	24	1.65E-03	36.0	53.6	2.67E-03	260250
Total	53					

Significant at: **p*<*0.05*, ***p*<*0.01* and ****p*<*0.001*.

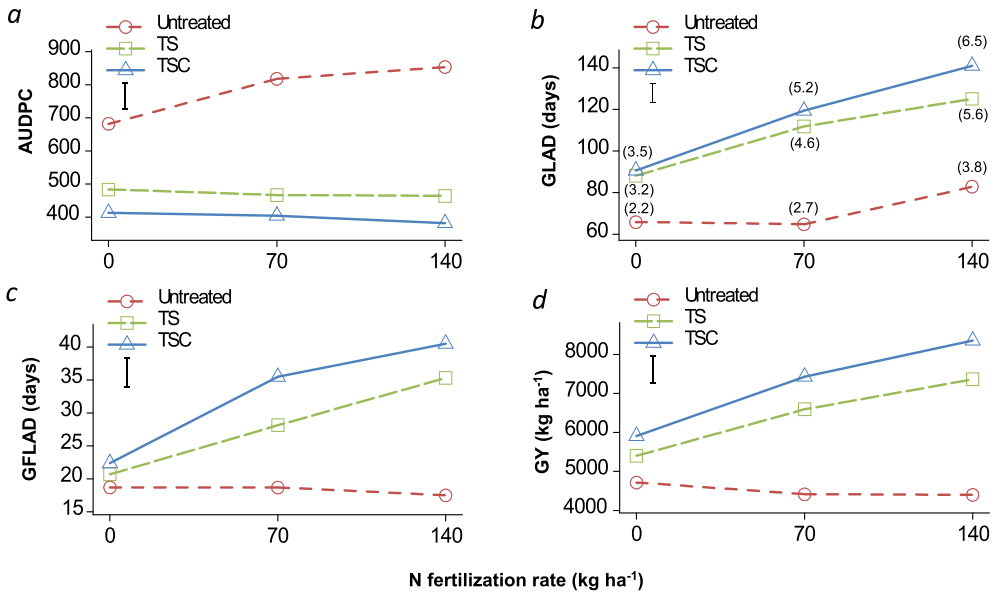

Fig. 1. Mean values of (a) area under disease progress curve (AUDPC), (b) green-leaf-area duration (GLAD) and total leaf area index (TLAI) at anthesis (between parenthesis), (c) green-flag-leaf area duration (GFLAD) and (d) grain yield (GY) in the *Fungicide* × *N* interaction. TS: triazole + strobilurin fungicide, TSC: triazole + strobilurin + carboxamide fungicide). Vertical bars indicate least significant differences (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.)

Table 3
Mean squares of grain protein content (GPC), wet gluten content (WGC), gluten tenacity (Alv*P*), gluten extensibility (Alv*L*), gluten tenacity/gluten extensibility (Alv*P*/*L*), gluten strength (Alv*W*), flour water absorption (Far*A*), dough development time (Far*B*), dough stability (Far*D*), dough softening degree (Far*E*) and bread loaf volume of wheat under three fungicide treatments and three nitrogen rates in two years.

Source of variation	Df	GPC	WGC	Alveogram parameters				Farinogram parameters				Loaf volume
				Alv*P*	Alv*L*	Alv*P*/*L*	Alv*W*	Far*A*	Far*B*	Far*D*	Far*E*	
Year (Y)	1	1.63E-4	3.52	183	19.4	3.26E-3	817	15.3	9.36E-3	4.00E-5	31.1	29043
Error a	2	9.39E-5	12.4	65.8	80.7	3.41E-2	304	113	3.21E-3	2.01E-2	25.4	4970
Fungicide (Fu)	2	8.88E-3*	92.5**	11400***	1989**	3.60E-1**	29361**	291	6.15E-3	1.18E-1*	136	6786*
Y × Fu	2	7.06E-4	4.12	106	3.70	1.14E-2	5.60	20.9	1.30E-3	4.64E-2	153	1738
Error b	8	1.77E-3	7.20	58.3	20.2	1.05E-2	823	71.6	1.60E-2	1.86E-2	663	2683
Nitrogen (N)	2	2.81E-2**	86.3***	4476***	3353***	5.60E-3	21395***	250***	1.02E-1***	4.86E-1***	643	175339***
Y × N	2	2.79E-4	1.79	0.7	0.5	6.60E-4	622	3.59	6.23E-3	7.57E-3	145	427
Fu × N	4	2.20E-3	14.5**	1468**	195*	4.25E-2	4311**	1.57	1.67E-3	7.98E-3	418	1464
Y × Fu × N	4	6.26E-4	2.10	34.9	1.90	3.29E-3	719	6.06	1.31E-3	4.64E-3	38.7	563
Error c	24	6.73E-4	4.20	318	176	3.09E-2	773	23.7	6.43E-3	1.85E-2	407	1379
Total	53											

Significant at: *$p<0.05$, **$p<0.01$ and ***$p<0.001$.

the *Fungicide* × *N* interaction (Table 3). Under the nil N rate (0N), all treatments showed similar GPC values (Fig. 2a). However, means significantly increased from 0N to 140N, particularly following fungicide applications, to a much greater extent under TSC treatments (+29.0%) compared to TS applications (+22.6%). Contrary, in the untreated plots, values of GPC ranged from 9.25% (0N) to 10.1% (140N), a relatively lower but not significant increment (+9.20%) regarding the protected plots (Fig. 2a).

Analysis of variance and averages values of wet gluten content are given in Tables 3 and 4, respectively. Wet gluten content was significantly influenced by the fungicide applications and the N rate, as well as by the *Fungicide* × *N* interaction (Table 3). The *Fungicide* × *N* interaction arose due to a greater response to N fertilization under fungicide applications. Following the TSC treatment, wet gluten content ranged from 22.9% (0N) to 29.6% (140N) increasing a +29.2%, whilst under the TS treatment, the increment was +23.4%, with values that fluctuated from 22.6% (0N) to 27.9% (140N) (Fig. 2b). In contrast, values in the untreated plots rose from 21.0% (0N) to 22.0% (140N) showing a slight (but not significant) increase compared to the protected plots (+4.76%).

3.3. Breadmaking parameters and loaf volume

Alveogram parameters Alv*P*, Alv*L*, and Alv*W* were influenced by *Fungicide*, *N* and the *Fungicide* × *N* interaction (Table 3), mainly explained by different responses across the three N fertilization rates under fungicide treatments. Dough rheological parameters Alv*P* and Alv*W* significantly increased with N fertilization to a much higher magnitude when sprayed with TSC than with TS fungicide (Fig. 2c and e, respectively). Conversely, no differences were observed in the untreated plots across N fertilization rates. Parameter Alv*L* significantly improved with rising N rates independently of the fungicide treatment. However, the magnitude of the increment from 0 to 140N was higher under the TSC treatment, followed by the TS fungicide and the untreated control (Fig. 2d). The fall rate of Alv*P* parameter per day of leaf life decline, was

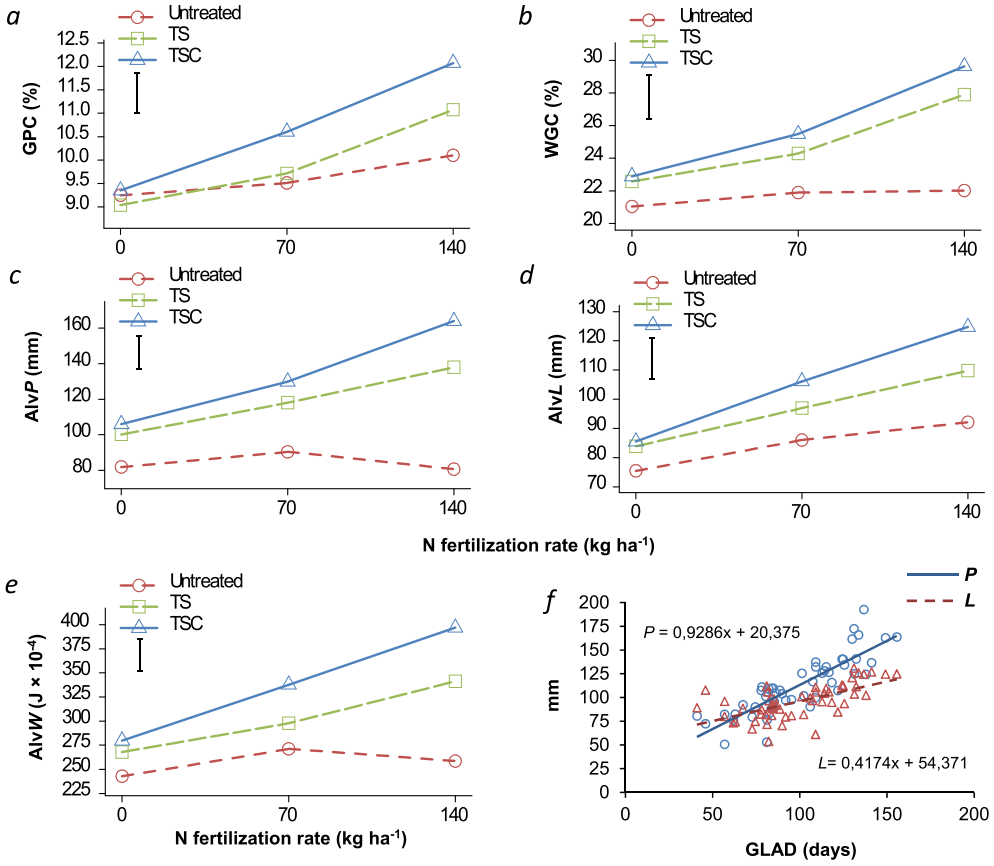

Fig. 2. Mean values of (a) grain protein content (GPC), (b) wet gluten content (WGC), (c) gluten tenacity (Alv*P*), (d) gluten extensibility (Alv*L*) and (e) gluten strength (Alv*W*) in the *Fungicide* × *N* interaction. TS: triazole + strobilurin fungicide, TSC: triazole + strobilurin + carboxamide fungicide). Vertical bars indicate least significant differences (LSD $p<0.05$). (f) Regressions between green-leaf-area duration (GLAD) and gluten tenacity (Alv*P*, circles) or gluten extensibility (Alv*L*, triangles). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

higher than for Alv*L*, being 0.93 mm day^{-1} (R^2=*0.719**) and 0.42 mm day^{-1} R^2=*0.405***) respectively (Fig. 2f). Thus, the Alv*P*/*L* significantly improved under fungicide applications (Table 3) compared to the untreated plots (1.00), which reached the highest values with TSC treatment (1.27), followed by TS (1.23) (Table 4). Moreover, Alv*P*/*L* showed a slight tendency, although not significant, to decrease with rising N rates that rose from 1.18 (0N) to 1.15 (140N) (Table 4). Regressions between Alv*P* and Alv*W*, and Alv*L* and Alv*W*, evidenced that Alv*W* was mainly explained by Alv*P* (R^2=*0.552****).

Statistical analysis of farinograph parameters is presented in Table 3. Highly significant differences in mean Far*A* and Far*B* parameters values were detected among N rates. Thus, values increased +13.8% and +38.6% when N was added from 0 to 140 kg ha^{-1} (Table 4). Parameter Far*D* was influenced by the N rate, but also, by fungicide applications (Table 3). Under fungicide treatment containing the SDHI fluxapyroxad (TSC), Far*D* values increased to a much greater extent (+49.3%) when compared with the untreated control, whilst the double-mixture fungicide (TS) did not statistically differ (Table 4). In addition, Far*D* parameter significantly increased with rising N rates, with values that rose from 2.21 min (0N) to 5.64 min (140N) indicating a +155% increase (Table 4). Loaf volume was influenced by the fungicide treatments and the N rates (Table 3). Values only significantly increased following the TSC fungicide application (457 cm^3) compared to the untreated plots (417 cm^3) (Table 4). Furthermore, loaf volume means ranged from 335 cm^3 (0N) to 533 cm^3 (140N), implying an increase of +59.1% (Table 4). Finally, loaf volume was mainly explained (R^2=*60.1*; *Cp*=*2.89*) by GPC (p=*0.003*), wet gluten content (p=*0.031*) and Alv*W* (p=*0.037*).

4. Discussion

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

The variation in rainfall caused fluctuations in AUDPC and GLAD between years. The 2014 growing season was wetter than 2015 and more conducive to the development of *P. triticina*, therefore, AUDPC was consistently higher in 2014. Similar results were reported by Schierenbeck et al. (2016), who found that wetter conditions in one growing season favored more severe leaf rust infections than in a drier growing season. Moreover, higher mean temperatures after inoculations done at GS21 (September) and GS31 (October) (data not shown) also might have promoted a higher disease development after inoculations. Despite weather conditions in 2014 promoted higher levels of AUDPC, they also increased GLAD compared to 2015, given the higher TLAI evidenced in the wettest year (Table 2).

The AUDPC was significantly higher in the rusted plots than in the fungicide-protected. Mean values increased when N was applied in the rusted plots as previously found in Gerard et al. (2015), while the fungicide protected plots did not show significant differences across the rising N rates. Increased susceptibility of obligate parasites at high N status has been attributed to the various anatomical and biochemical changes caused by the nutrient, together with the increase in the content of the low-molecular-weight organic N compounds which are used as substrates for

Table 4
Mean values, standard errors of differences (s.e.d.) and least significant differences (l.s.d.) of grain protein content (GPC), wet gluten content (WGC), gluten tenacity (Alv*P*), gluten extensibility (Alv*L*), gluten tenacity/gluten extensibility (Alv*P*/*L*), gluten strength (Alv*W*), flour water absorption (Far*A*), dough development time (Far*B*), dough stability (Far*D*), dough softening degree (Far*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	GPC (%)	WGC (%)	Alveogram parameters				Farinogram parameters				Loaf volume (cm3)
			Alv*P* (mm)	Alv*L* (mm)	Alv*P*/*L*	Alv*W* (J × 10^{-4})	Far*A* (mL)	Far*B* (min)	Far*D* (min)	Far*E* (BU)	
Year											
2014	10.1	24.5	110	95.0	1.16	295	58.1	2.17	4.42	55.8	459
2015	10.0	23.9	114	96.2	1.18	303	57.0	2.21	4.21	54.3	413
s.e.d.	3.0E-03	0.96	2.21	2.44	5.0E-02	4.70	2.90	1.5E-02	3.9E-02	1.37	19.2
l.s.d.	0.22	412	9.50	10.5	0.217	20.4	12.5	0.256	1.40	5.90	82.6
Fungicide											
Untreated	9.62	21.7	84.3	84.5	1.00	258	53.1	2.10	3.55	53.6	417
TS	9.94	24.9	119	96.9	1.23	302	58.7	2.17	4.09	58.2	435
TSC	10.7	26.0	133	105	1.27	338	60.9	2.25	5.30	53.3	457
s.e.d.	1.4E-02	0.89	2.55	1.50	3.4E-02	9.60	2.82	4.2E-02	4.6E-02	8.69	17.3
l.s.d.	0.76	2.06	5.87	3.46	0.079	22.0	6.51	0.509	1.10	19.8	39.8
Nitrogen rate											
0 kg N ha^{-1}	9.21	22.2	96.0	81.6	1.18	264	53.6	1.76	2.21	61.5	335
70 kg N ha^{-1}	9.94	23.9	113	96.4	1.17	302	58.0	2.31	4.59	53.9	440
140 kg N ha^{-1}	11.1	26.5	125	109	1.15	332	61.0	2.44	5.64	49.7	533
s.e.d.	9.0E-03	0.68	5.94	4.43	5.9E-02	9.30	1.62	2.7E-02	4.5E-02	6.73	12.4
l.s.d.	0.42	1.41	12.3	9.13	0.121	19.1	3.35	0.304	0.891	13.9	25.5

parasites (Dordas, 2008) such as *P. triticina*. In addition, the triple-mixture (TSC) showed significantly lower values of AUDPC in relation to the double-mixture (TS) treatment. This may be ascribed to a better level of control or, by a lower possibility of reinfection given a more durable residual efficacy. Our findings are in line with Fleitas et al. (2015), who also verified that a TSC treatment was more effective in controlling foliar diseases as rusts than a TS fungicide, even thirty days after applications in a highly susceptible cultivar.

The GLAD tended to remain stable between 0N and 70N in the rusted plots, and it only increased with the maximum N rate (140N). Contrary, in the fungicide protected plots, GLAD increased to a much greater extent across the N rates, with higher values in those plots treated with the triple-mixture (TSC) treatment. The GLAD stability showed in the range of 0N and 70N in rusted plots could be attributed to the fact that N fertilization promotes leaf rust increase but also, TLAI is favored, thus, final GLAD values are compensated. However, at the maximum N status (140N), GLAD increased because the N fertilization had more impact on TLAI, through larger shoot number and leaf area (Fig. 1b) than on leaf rust severity. In this sense, it has been suggested that in the wheat-leaf rust pathosystem, there is a threshold effect of leaf N content on spore production, with an increase in spore production and lesion size in plants receiving medium N compared with those at low N, but little effect on plants receiving medium N and those at high N status (Robert et al., 2004). On the other hand, the differences in the control levels of the triple mixture respect to the double mixture remained constant across N levels (Fig. 1a). However, an increase in the GLAD difference between both treatments was observed across rising N rates. These differences were 15.9 days at the maximum N rate (140N) and only 2.5 days at 0N (Fig. 1b). The same was observed when grain yield was analyzed (Fig. 1d), however, in this trait, the differences between the triple and double mixture through rising N rates occurred to a lesser extent (995 kg ha^{-1} at 140N and 510 kg ha^{-1} at 0N rate). These results would be indicating additional effects to the disease control and are in line with those reported by Smith et al. (2013) who verified that healthy canopy size and duration was increased by the fungicide containing fluxapyroxad in the winter wheat-stripe rust (*Puccinia striiformis* f. Sp. *tritici*) pathosystem. Such results were ascribed to positive physiological effects on the host (not mediated through pathogen control) that involved increased leaf greening, delayed senescence, reduced cell damage, reduced stomatal conductance, improved photosynthetic rate and increased water use efficiency (Berdugo et al., 2012; Ajigboye et al., 2014). In the present study, although control differences were constant across all N rates, higher differences in GLAD between TSC treatment and TS treatment, was observed at higher N rates. This might be indicating additional physiological effects of the triple mixture containing the SDHI fungicide (TSC) positively interacting with N fertilization.

4.2. Grain yield, grain protein content and wet gluten content

In this study, the relationship between disease symptoms and yield loss was properly quantified since the canopy size, which is subject to considerable variation across sites and seasons, was taken into consideration (Parker et al., 2004; Paveley et al., 2005; Schierenbeck et al., 2016). The differential effects of leaf rust *vs.* its control using a foliar fungicide on GLAD were consistently reflected in grain yield. Thus, TSC improved ca.~2700 kg ha^{-1}, whilst TS gained nearly 1900 kg ha^{-1} in grain yield compared to rusted plots. Leaf rust reduced grain per square meter (−19.2%) but also affected 1000-kernel weight (−12.5%) in relation to the TSC treatment (data not shown). Our findings are in line with those observed by Fleitas et al. (2015) who found that fluxapyroxad, in mixture with triazole-strobilurins, consistently increased grain yield through increases in number of kernel per spikes and 1000-kernel weight compared to double mixtures (triazole-strobilurins) under artificial inoculations with *P. triticina* in a highly susceptible cultivar (Baguette 13). In rusted plots, grain yield remained stable across rising N rates, following the same tendency of GFLAD (Fig. 1c) probably ascribed to higher N levels that prompt higher leaf rust severity, AUDPC and therefore, less GFLAD.

Some evidence in the literature suggested that infections with *Puccinia* spp. can be more detrimental to N accumulation in the grain than to dry matter accumulation (Dimmock and Gooding, 2002; Schierenbeck et al., 2014). In this study, GPC improved as the N rate increased to a much greater extent following fungicide application than rusted plots, confirming the deleterious effect of leaf rust to the N accumulation and/or partitioning within plants. This is not to say that carbon accumulation is not also affected by rust infections. In fact, pustules of *P. triticina* on leaves also reduce

the amount of green area available for radiation interception and the assimilated carbon is utilized in fungal structures, but in a balance, GPC is more severely affected.

The GPC values in rusted plots did not show a great variation until the 70N rate, with a tendency to increase with the maximum N rate (140N). Our findings might be suggesting that GPC response, at rising N levels where leaf rust prevails, could have three response zones (Fig. 2a). Thus, under lower N levels (0N), the N availability to meet crop demand for quality is not enough and the values of rust severity are low, hence GPC is low (9.25%). Plants receiving medium N fertilization (70N) have more N availability to translocate to the grain but also, are more susceptible and leaf rust is more severe, thus GPC remains stable (9.51%). Finally, at higher N rates (140N), the N demand of the pathogen is broadly satisfied. Therefore, at these N availabilities, there is a N surplus that can be taken by the plant and derived to the grain, which translates into greater GPG (10.1%). The N threshold observed in this study, from which the N fertilization effect is more important on GPC than on leaf rust severity, was also found by other authors (Robert et al., 2004). Comparing fungicides treatments, the triple-mixture containing the SDHI fungicide (TSC) significantly increased GPC across the rising N rates compared to the TS treatment ascribed to higher levels of disease control and longer values of GLAD and GFLAD. In this sense, the longevity of the flag leaf was closely associated with accumulation of proteins in the grain in line with other authors (Pepler et al., 2005).

Regarding wet gluten content, mean values showed the same tendency of GPC. However, leaf rust effects in the untreated plots were more important on gluten content than on GPC compared to the fungicide protected treatments, particularly, under higher N status (140N). Thus, leaf rust significantly altered the GPC composition through greater reductions in the proportion of insoluble protein fractions. Similarly, to the GPC, the TSC treatment showed higher means of wet gluten content in relation to the TS fungicide, but under both fungicide treatments, the proportion of insoluble protein fractions compared to the total GPC, values remained relatively stable across the rising N rates.

4.3. Breadmaking parameters and loaf volume

Most of the research concerning the effect of foliar diseases on wheat quality focus on specific (test, hectoliter or bushel) weights and/or GPC, but the evidence of the effect of foliar diseases, particularly leaf rust, on breadmaking quality is scarce. In this sense, it is widely accepted that wheat quality, in addition to GPC, is determined by several additional parameters. Thus, when two flours of the same protein content perform differently under the same optimized baking conditions, the reason is that they are of differing protein quality (Carson and Edwards, 2009) and other quality-related parameters.

In the present study, we investigated the effects of the leaf rust disease, its control with a triple-mixture fungicide containing the SDHI fluxapyroxad and the interaction of both factors with N fertilization on several breadmaking parameters. Rusted plots showed significantly lower levels of Alv*P* and Alv*L* in relation to the fungicide protected plots. However, the effect of leaf rust was more important to the Alv*P*, causing reductions in the Alv*P*/*L*, which fall from 1.27 in the TSC treatment to 1.00 in the untreated plots. Even dough both parameters tended to decrease, the fall rate of Alv*P* was twice as big than Alv*L* (Fig. 2f). Although the Alv*P* and Alv*L* are not only related to the content of glutenin and gliadin, respectively (could depend overall in the ratio between glutenin fractions with different molecular weight), our findings could be indirectly associated with the differential deposition of gluten fractions along the filling period (Panozzo et al., 2001). This association, but in the opposite direction, was done by Fullington and Nityagopal (1986), who found that the leaf rust decreased high molecular weights glutenins and hypothesized that consequent possible effects could have been weakening of dough mixing resistance and an increase of dough extensibility. In this way, the shortening of the grain-filling period, derived from premature leaf senescence caused by leaf rust, affected to a more greater extent Alv*P* than Alv*L* (Fig. 2f). Magallanes-López et al. (2017), using a collection of 46 commercial durum wheat (*Triticum turgidum* L. ssp. *durum*) varieties, verified that under drought stress conditions genotypes also displayed more Alv*L* and less Alv*P*, which resulted in lower Alv*P*/*L*, less tenacious doughs favoring higher loaf volumes. On the other hand, when we performed the same analysis but considering only the N effect, rising N rates reduced the Alv*P*/*L* ratio (1.18 at 0N to 1.15 at 140N rate), evidencing a greater effect on the Alv*L* parameter (Table 4) in line with previous reports (Panozzo and Eagles, 2000; Kindred et al., 2008; Godfrey et al., 2010). If we consider that the three N rates represent different environmental conditions, Alv*L* was more affected by environment condition than Alv*P*, being the latter under more genetic control, although this response may differ between cultivars as previously indicated by Godfrey et al. (2010).

Parameter Alv*W* tended to increase with the medium N rate (70N) but the reverse occurred with the maximum rate (140N) in the rusted plots. It is plausible that fluctuations of Alv*P* were accountable for this behavior since this was the main parameter in explaining the Alv*W*. In our study, fungicide applications significantly increased Alv*W* across N rates to a much greater extent when the TSC treatment was applied, following the same pattern of Alv*P* and Alv*L* behavior. In this sense, Blandino and Reyneri (2009) verified that Alv*W* significantly increased under N fertilization after fungicide applications of a triazole-only and TS fungicides. In the same study, there was a tendency (although not significant) to increase the Alv*P*/*L* in the fungicide protected treatments in relation to the control treatment.

Regarding farinograph parameters, only Far*A* and Far*D* were negatively affected by leaf rust which tended to decrease, probably associated with the lower levels of GPC and wet gluten content in the rusted plots. In the same way, N fertilization significantly improved the farinograph parameters except for Far*E*. Finally, loaf volume significantly increased with rising N rates and fungicide applications, clearly ascribed to the higher amounts of GPC, wet gluten content and Alv*W*, which were the best explanatory variables.

In conclusion, our findings showed that leaf rust not only causes reductions in the total protein content but also, an alteration of its composition. The reduction of the GLAD and the filling period due to the premature leaf senescence produced by the pathogen caused a greater reduction of gluten content than GPC and a minor Alv*P* in relation to Alv*L* resulting in lower Alv*P*/*L*, inferior Alv*W* and hence, lower loaf volumes. The control of leaf rust with fungicides not only increased grain yield but also, it improved breadmaking quality. For this reason, when thresholds of leaf rust are fixed for the application of fungicides, not only the effect on grain yield but also, the effect on wheat quality, should be considered. Finally, the TSC treatment containing the SDHI fluxapyroxad, besides showing better levels of disease control with respect to the TS treatment, also evidenced additional beneficial effects on most of the breadmaking traits here evaluated.

Acknowledgments

We are grateful to Nidera Argentina for providing the seeds for the experiment. We thank the assistance of the technical and field staff from the Estación Experimental Julio Hirschhorn, Facultad de

Ciencias Agrarias y Forestales, Universidad Nacional de La Plata (UNLP), Argentina. This study was supported by ANPCyT (PICT 2181/10), CONICET (PIP 0819CO) and UNLP (A 227 and A 300).

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jcs.2018.02.003.

References

Ajigboye, O.O., Murchie, E., Ray, R.V., 2014. Foliar application of isopyrazam and epoxiconazole improves photosystem II efficiency, biomass and yield in winter wheat. Pestic. Biochem. Physiol. 114, 52–60.
Berdugo, C.A., Mahlein, A.K., Steiner, U., Dehne, H.W., Oerke, E.C., 2012. Sensors and imaging techniques for the assessment of the delay of wheat senescence induced by fungicides. Funct. Plant Biol. 40, 677–689.
Blandino, M., Reyneri, A., 2009. Effect of fungicide and foliar fertilizer application to winter wheat at anthesis on flag leaf senescence, grain yield, flour breadmaking quality and DON contamination. Eur. J. Agron. 30, 275–282.
Blumenthal, C.S., Barlow, E.W.R., Wrigley, C.W., 1993. Growth environment and wheat quality: the effect of heat stress on dough properties and gluten proteins. J. Cereal. Sci. 18, 3–21.
Blumenthal, C.S., Bekes, F., Gras, P.W., Barlow, E.W.R., Wrigley, C.W., 1995. Identification of wheat genotypes tolerant to the effects of heat stress on grain quality. Cereal Chem. 72, 539–544.
Carson, G.R., Edwards, N.M., 2009. Criteria of wheat and flour quality. In: Khan, K., Shewry, P.R. (Eds.), Wheat Chemistry and Technology. AACC International Inc, St. Paul MN, USA, pp. 97–118.
Dimmock, J.P.R.E., Gooding, M.J., 2002. The influence of foliar diseases, and their control by fungicides, on the protein concentration in wheat grain: a review. J. Agric. Sci. Camb. 138, 349–366.
Dordas, C., 2008. Role of nutrients in controlling plant diseases in sustainable agriculture. A review. Agron. Sustain. Dev. 28, 33–46.
Fleitas, M.C., Gerard, G.S., Simón, M.R., 2015. Eficacia residual de fungicidas sobre la roya de la hoja del trigo y su efecto sobre componentes del rendimiento y porcentaje de proteínas en grano. Revista FAVE. Sección Ciencias Agrarias 14, 69–84.
Fullington, J.G., Nityagopal, A., 1986. Effect of rust infection on the protein components of wheat. Phytochemistry 25, 1289–1292.
Gerard, G.S., Fleitas, M.C., Schierenbeck, M., Simón, M.R., 2015. Eficacia de control y residualidad de terápicos de semillas sobre enfermedades foliares de trigo en distintas condiciones de fertilización nitrogenada. Revista FAVE. Sección Ciencias Agrarias 14, 85–102.
Godfrey, D., Hawkesford, M.J., Powers, S.J., Millar, S., Shewry, P.R., 2010. Effects of crop nutrition on wheat grain composition and end use quality. J. Agric. Food Chem. 58, 3012–3021.
Hawkesford, M.J., 2014. Reducing the reliance on nitrogen fertilizer for wheat production. J. Cereal Sci. 59, 276–283.
IRAM 15855, 2000. Harina de trigo. Características físicas de las masas. Determinación de la absorción de agua y de las propiedades reológicas utilizando un farinógrafo.
IRAM 15857, 1995. Harina de trigo. Características físicas de las masas. Determinación de las propiedades reológicas. Método del aveógrafo.
IRAM 15858–1, 1996. Cereales. Ensayo de panificación experimental.
IRAM 15864, 2007. Trigo y harina de trigo. Determinación de gluten húmedo, de gluten seco y de índice de gluten. Método de lavado automático.
Jamieson, P.D., Stone, P.J., Semenov, M.A., 2001. Towards modeling quality in wheat: from grain nitrogen concentration to protein composition. Aspect Appl. Biol. 64, 111–126.
Jensen, P.K., Jørgensen, L.N., 2016. Interactions between crop biomass and development of foliar diseases in winter wheat and the potential to graduate the fungicide dose according to crop biomass. Crop Protect. 81, 92–98.
Kindred, D.R., Verhoeven, T.M.O., Weightman, R.M., Swanston, J.S., Agu, R.C., Brosnan, J.M., Sylvester-Bradley, R., 2008. Effects of variety and fertiliser nitrogen on alcohol yield, grain yield, starch and protein content, and protein composition of winter wheat. J. Cereal. Sci. 48, 46–57.
Magallanes-López, A.M., Ammar, K., Morales-Dorantes, A., González-Santoyo, H., Crossa, J., Guzmán, C., 2017. Grain quality traits of commercial durum wheat varieties and their relationships with drought stress and glutenins composition. J. Cereal. Sci. 75, 1–9.
Miralles, D.J., Slafer, G.A., 1990. Estimación del área foliar en trigo: generación y validación de un modelo. In: Proceedings of II Congreso Nacional de Trigo, pp. 76–85. Pergamino.
Panozzo, J.F., Eagles, H.A., 2000. Cultivar and environmental effects on quality characters in wheat. II. Protein. Aust. J. Agric. Res. 51, 629–636.
Panozzo, J.F., Eagles, H.A., Wootton, M., 2001. Changes in protein composition during grain development in wheat. Aust. J. Agric. Res. 52, 485–493.
Parker, S.R., Welham, S., Paveley, N.D., Foulkes, J., Scott, R.K., 2004. Tolerance of Septoria leaf blotch in winter wheat. Plant Pathol. 53, 1–10.
Paveley, N., Foulkes, J., Sylvester-Bradley, R., Parker, S., Lovell, D., Snape, J., Farrar, J., Neumann, S., Nason, J., Ellerbrook, C., 2005. Maximising Disease Escape, Resistance and Tolerance in Wheat through Genetic Analysis and Agronomy. Project report N° 358, 24pp.
Pepler, S., Gooding, M.J., Ford, K.E., Ellis, R.H., Jones, S.A., 2005. Delaying senescence of wheat with fungicides has interacting effects with cultivar on grain sulphur concentration but not with sulphur yield on nitrogen: sulphur ratios. Eur. J. Agron. 22, 405–416.
Peterson, R.F., Champbell, A.B., Hannah, A.E., 1948. A diagrammatic scale for estimating rust intensity of leaves and stem of cereals. Can. J. Res. 26, 496–500.
Robert, C., Bancal, M.O., Lannou, C., 2004. Wheat leaf rust uredospore production on adult plants: influence of leaf nitrogen content and Septoria tritici blotch. Phytopathology 94, 712–721.
Schierenbeck, M., Fleitas, M.C., Cortese, F., Golik, S.I., Simón, M.R., 2014. Enfermedades foliares del trigo afectan diferencialmente la absorción y removilización del nitrógeno hacia los granos. RANA (Rev. Agron. Noroeste Argent.) 34, 242–246.
Schierenbeck, M., Fleitas, M.C., Miralles, D.J., Simón, M.R., 2016. Does radiation interception or radiation use efficiency limit the growth of wheat inoculated with tan spot or leaf rust? Field Crop. Res. 199, 65–76.
Shaner, G., Finney, R.E., 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. Phytopathology 67, 1051–1056.
Smith, J., Grimmer, M., Waterhouse, S., Paveley, N., 2013. Quantifying the non-fungicidal effects of foliar applications of fluxapyroxad (Xemium) on stomatal conductance, water use efficiency and yield in winter wheat. Commun. Agric. Appl. Biol. Sci. 78, 523–535.
Triboi, E., Abad, A., Michelena, A., Lloveras, J., Ollier, J.L., Daniel, C., 2000. Environmental effects on the quality of two wheat genotypes: 1. Quantitative and qualitative variation of storage proteins. Eur. J. Agron. 13, 47–64.
Varga, B., Svecnjak, Z., Macesic, D., Uher, D., 2005. Winter wheat cultivar responses to fungicide application are affected by nitrogen fertilization rate. J. Agron. Crop Sci. 191, 130–137.
Waggoner, P.E., Berger, R., 1987. Defoliation, disease and growth. Phytopathology 77, 393–398.
Wieser, H., 2007. Chemistry of gluten proteins. Food Microbiol. 24, 115–119.
Zadoks, J.C., Chang, T.T., Konzak, C.F., 1974. A decimal code for the growth stages of cereals. Weed Res. 14, 415–421.