



Rapid emergency response to yellow rust epidemics caused by newly introduced lineages of *Puccinia striiformis* f. sp. *tritici* in Argentina

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Abstract

Yellow rust (YR), caused by *Puccinia striiformis* f. sp. *tritici*, is one of the most destructive diseases of wheat worldwide. In 2017, YR emerged in Argentina and spread quickly into three million hectares, causing damage at levels only seen during the severe epidemics during the late 1930s. This widespread occurrence coincided with reports of newly introduced exotic races into the country. Therefore, little was known about actual impact of the disease on yield, reaction of commercial wheat cultivars, efficacy and best timing for fungicide applications. This study addressed these fundamental questions to provide a quick response to the re-emergence of YR in Argentina. Thirty wheat cultivars (short and long cycle) were evaluated for their response to a *PstS13* lineage of *Puccinia striiformis* f.sp. *tritici*. The efficacy of one or two fungicide applications for controlling YR was also assessed. Disease severity reached about 50% in the untreated plots at early crop growth stages. Disease and yield data analyses showed that one fungicide application provided effective YR control, but two applications further secured a significant relative increase in yield. Grain yield was negatively correlated with disease severity, and losses reached up to 4,700 kg/ha in the untreated control plots for several varieties. We provide new and important information on the control of and potential yield losses by a new exotic race of *Puccinia striiformis* f.sp. *tritici* introduced to South America.

Keywords *Puccinia* · Stripe rust · Epidemiology · Disease control · QoI + DMI · *PstS13*

Yellow rust (YR), also known as stripe rust, is caused by *Puccinia striiformis* Westend. f. sp. *tritici* Eriks (*Pst*). It is one of the most damaging diseases of wheat in regions that experience cool and moist weather (Hovmøller et al. 2011). Recently, the disease has been reported to occur up in relatively warmer

zones (Milus et al. 2009; Beddow et al. 2015). In Argentina, the first severe epidemic of YR occurred between 1928 and 1930 (Lindquist 1982) and caused significant losses, forcing replacement of the wheat cultivars grown at that time. Until recently, occurrence of YR in Argentina had only been sporadic and confined to cooler regions, such as those in the southeast of Buenos Aires province (Germán et al. 2007).

However, during the 2016/2017 and 2017/2018 cropping seasons, the disease was found in regions where it has not been seen before. Severe and yield-damaging epidemics affected more than three million hectares of wheat grown in Santa Fe, Córdoba, Entre Ríos and Buenos Aires provinces. Unusual and early infections of YR were detected at several locations in the core agricultural area of the Argentina Pampas region, regardless of the cultivar. Recently, Beddow et al. (2015) reported that 88% of the world wheat production is currently vulnerable to YR. Molecular identification of rust-infected wheat leaf samples collected in September 2017 from 22 sites, confirmed that a strain of the genetic lineage *PstS13*, which caused severe epidemics on durum and bread wheat in Italy in 2017, was prevalent in most

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surveyed wheat areas of Argentina in 2017 (Hovmøller et al. 2018). A different strain, *PstS14*, first detected in Northern Africa and Europe in 2016, causing severe epidemics on bread wheat in Morocco in 2017, was also detected in 2017 (Hovmøller et al. 2018).

The 2017 YR epidemics in Argentina forced farmers to apply fungicides for suppressing the disease, but the measure did not help to prevent economic losses. Applications were mainly based on premixes of strobilurin-triazole fungicides. With more aggressive races of the pathogen already confirmed in the country, YR epidemics are expected to be more severe in the future. Little is known about the reaction of wheat varieties to YR in Argentina. In addition, best timing, dosages, and number of fungicide applications for effective control of the disease is not available.

The objectives of this study were to assess 1) the response of wheat cultivars to YR; 2) the effect of one or two applications of a strobilurin-triazole fungicide in controlling the disease, and 3) estimate grain yield losses caused by the new YR genetic lineage *PstS13* in naturally-occurring epidemics.

Thirty commercial winter bread wheat varieties were evaluated including 16 long-cycle and 14 short-cycle varieties representative of cultivars grown in the region. Long-cycle varieties were planted on June 13, 2017 and short-cycle varieties were planted on July 3, 2017. The experiment was conducted in Landeta, Santa Fe Province, Argentina. Each variety was planted to a 120-m long, 1.4-m wide strip, which was later divided into nine 12.3-m long plots with 50-cm alleys separation. Wheat rows within plots were spaced at 21 cm. The previous crop was soybean. The entire experimental area was fertilized at sowing with 70 kg N/ha and 55 kg P/ha. Within each variety or strip, two fungicide treatments or an untreated control were tested in a randomized complete block design with three replications. The fungicide treatments were: one application at stem elongation (growth stage Zadoks 33) or two applications: one at stem elongation (Zadoks 33) and other at early ear emergence (growth stage Zadoks 50) (Zadoks et al. 1974). The fungicide was a commercial premix of picoxystrobin + cyproconazole (80 and 32 g a.i./ha, respectively) applied with a carbon dioxide sprayer equipped with four 35-cm apart, hollow cone spray nozzles (Albuz TVI 020®) and providing an overall pattern width of 1.4 m. The sprayer was operated at a pressure of 4.5 kg/cm² and at a water volume rate of 70 L/ha. Given the high number of varieties, space limitations, and operational constraints for the experiment set up, the variety factor in the experiment was non-replicated. However, as aforementioned, the number of replications of the fungicide treatment (untreated, one or two applications) within variety was increased to gain a higher precision in the covariance parameter estimate *Plot*Fungicide treatment*. Intensity of YR was measured as foliar incidence and severity on the leaves (all main tillers) of twenty plants chosen arbitrarily in each plot. Disease incidence was estimated as the number of diseased leaves / total leaves × 100, whereas foliar rust severity was visually estimated as the average percentage of green leaf

area covered by YR pustules and chlorosis. Severity was assessed for each leaf and average of all leaves was computed. Both incidence and severity were assessed at late booting, early ear emergence, and grain milk stages (21, 30 and 41 days after the first fungicide application, daa), respectively, according to the Zadok's wheat growth staging system. With data from those three assessments, the area under the disease progress curve (AUDPC) for incidence and severity was calculated for each treatment (Madden et al. 2007). Linear regression analysis was conducted to characterize the relationship between YR intensity (incidence and severity) and grain yield, discriminating between short and long cycle varieties.

Plots were harvested on 5 December 2017 when kernels had 15% moisture. Grain yield response to fungicide application and net economic return were calculated and expressed as kg/ha and U.S. dollars/ha, respectively. Grain yield response to fungicide applications was calculated as the difference between yield from treated plots minus yield from untreated plots; net economic return (ER) to the fungicide treatment was calculated as ER = [(grain yield advantage)*(average wheat grain price)] - (Average cost of fungicide + application). Average wheat price used was 162 usd/tn and average fungicide and application costs was 25 U.S. dollars/ha. Pricing of the grain, product cost and application cost were obtained from corresponding average prices in the region for the year of study according to the Rosario Board of Trade (Bolsa de Comercio de Rosario, 2018).

The effect of fungicide application (untreated, one, or two applications) on YR AUDPC and grain yield was analyzed with a linear mixed-effect model fitted with PROC MIXED in SAS ver. 9.3 (SAS Institute, Cary, NC). The effect of *variety* was not examined as a main effect because it lacked replications in the experiment. Instead, since *variety* was naturally nested within *variety cycle length*, and the latter did have replications, then the effect *variety (cycle length)* was included in the model as a main effect. Moreover, considering that the fungicide treatment effect could have been affected by *variety* and *variety within cycle length* the interaction between those factors was considered and entered in the model as a random effect. The final model representing all effects and sources of variation was:

$$Y_{ijk} = \mu + Var(C_j)_i + F_k + F.Var(C_j)_{ik} + \varepsilon_{ijk}$$

$$E_{ijk} \sim N(0, \sigma^2)$$

$$F.Var(C_j)_{ik} \sim N(0, \sigma^2)$$

Where:

Y_{ijk} is the AUDPC or the grain yield (kg/ha) of the i th variety ($i = 1, 2, \dots, 14$ or 16) within the j th variety cycle length ($j = \text{short or long}$) receiving the k th number of fungicide applications (F ; $k = 0, 1, 2$); μ is the AUDPC or yield overall mean (=model intercept); $Var(C_j)_i$ is the main effect of the i th variety within the j th cycle length ($j = \text{short or long}$);

Table 1 Response of 30 wheat varieties, naturally infected with yellow rust, to fungicide applications, as assessed by disease incidence and severity and grain yield in field experiments in the Province of Santa Fe, Argentina in 2017

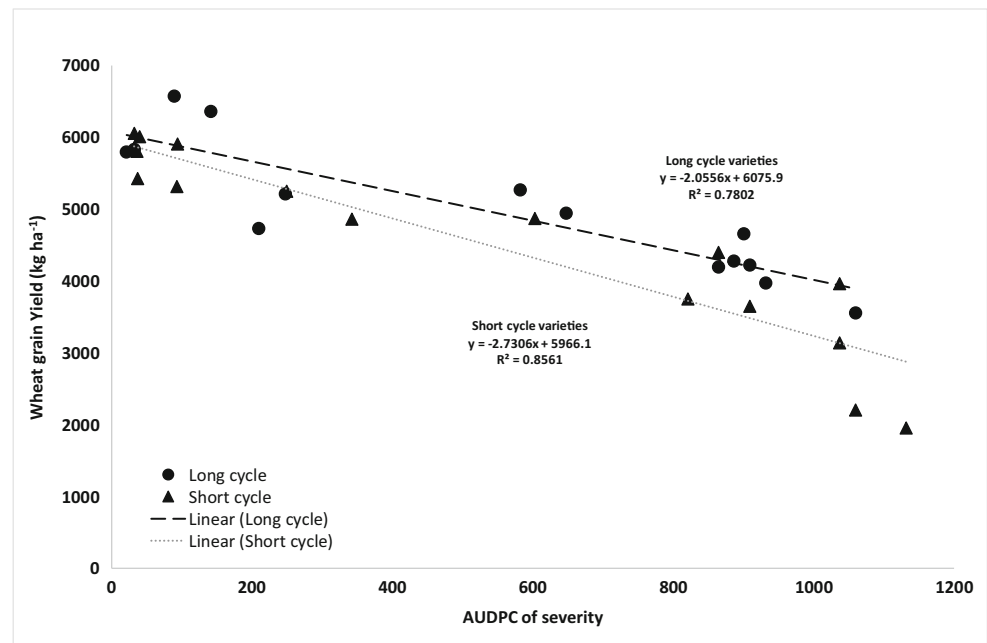
Wheat variety and cycle length type ^a	AUDPC-I ^b			AUDPC-S ^c			Grain yield (t/ha) (relative response) ^d			Net economic return (usd/ha) ^e	
	Non-treated	1 spray	2 sprays	Non-treated	1 spray	2 sprays	Non-treated	1 spray	2 spray	1 spray	2 sprays
	DM Fuste (S)	2000	1083	1039	1133	520	454	1.96	5.05 (158%)	6.70 (242%)	476
DM Algarrobo (L)	1924	473	434	821	116	72	3.72	6.39 (72%)	8.05 (116%)	407	676
Experimental (S)	2000	514	481	1060	111	83	2.26	4.91 (117%)	6.41 (183%)	405	647
Sursem Nogal 90 (S)	2000	490	419	1060	89	67	3.60	5.95 (65%)	7.15 (99%)	356	552
Klein Serpiente (L)	2000	351	295	1038	109	67	3.10	4.58 (48%)	6.21 (100%)	214	479
NIDERA Baguette 680 (L)	1879	105	50	910	58	36	4.17	6.43 (54%)	7.28 (74%)	341	478
DM Ceibo (S)	2000	295	264	887	183	144	4.33	6.88 (59%)	7.39 (71%)	389	471
Klein Huracan (L)	2000	407	372	1038	255	222	4.02	6.05 (50%)	6.95 (73%)	303	450
ACA 910 (S)	1969	429	403	910	410	360	3.73	5.57 (50%)	6.25 (68%)	274	383
Buck Claraz (S)	2000	424	388	865	28	22	4.22	6.02 (43%)	6.58 (56%)	267	358
Bioceres Basilio (L)	717	26	0	343	8	6	4.99	5.90 (20%)	7.25 (48%)	138	357
ACA 303 plus (L)	1602	361	310	932	41	16	3.97	5.57 (40%)	6.11 (54%)	234	322
AG Seed Floripan 300 (L)	2000	198	171	901	105	72	4.64	5.82 (26%)	6.71 (45%)	167	311
ACA Cedro (L)	281	29	0	210	14	6	4.71	5.78 (23%)	6.73 (43%)	149	303
Buck Alumine (L)	1434	89	78	582	27	16	5.20	6.59 (27%)	7.17 (38%)	200	294
Buck SY 300 (S)	610	51	0	248	28	0	5.24	6.45 (23%)	7.06 (35%)	171	270
Buck SY 330 (S)	339	39	0	249	53	0	5.42	6.25 (15%)	7.14 (32%)	110	254
ACA 908 (S)	1771	204	171	865	199	133	4.48	6.30 (40%)	6.20 (38%)	269	253
Macro Seed INTA 815 (S)	218	50	0	603	55	39	4.91	5.66 (15%)	6.10 (24%)	96	168
Sursem Lapacho (L)	117	59	0	142	105	78	6.45	7.04 (9%)	7.46 (16%)	70	138
Buck SY 120 (L)	171	29	0	90	53	17	6.63	6.94 (5%)	7.62 (15%)	26	135
ACA 360 (L)	1299	228	186	93	39	27	5.36	5.65 (5%)	6.15 (5%)	22	103
ACA 909 (S)	110	28	0	37	7	6	5.88	5.96 (1%)	6.66 (13%)	-12	101
Buck SY 211 (L)	18	29	0	32	9	6	6.21	6.53 (5%)	6.96 (12%)	27	97
AG Seed Floripan 100 (S)	1573	110	93	648	101	67	5.06	5.48 (8%)	5.81 (15%)	43	96
Bioceres 1006 (S)	29	26	0	21	26	11	5.91	6.34 (7%)	6.58 (11%)	45	84
Macroseed INTA 415 (L)	220	28	0	94	18	6	5.99	5.99 (0%)	6.61 (10%)	-25	74
Buck Saeta (S)	100	22	0	40	6	0	6.15	6.15 (0%)	6.68 (9%)	-26	61
ACA 602 (L)	20	29	0	38	9	6	5.48	5.51 (0.5%)	5.81 (6%)	-21	28
Klein Minerva (L)	28	6	0	32	7	0	5.80	5.98 (3%)	6.07 (5%)	5	20

^a S = short-cycle varieties and L = long-cycle varieties^b The area under the disease progress curve (AUDPC) of incidence^c The area under the disease progress curve (AUDPC) of severity^d Yield advantage to fungicide is shown in parentheses as percentage increase with respect to the untreated control

Grain yield response to fungicide applications was calculated as yield from treated plots - yield from untreated plots

^e Net economic return from treated plots was calculated as [grain yield response * average wheat price (equal to 162 usd/tm)] - [average fungicide and application costs (equal to 25 usd/ha)]

Fig. 1 Relationship between AUDCP of severity and wheat grain yield in plots without fungicide application, Santa Fe, Argentina, 2017–18



F_k is the main effect of the k th fungicide application ($k = 0, 1, 2$); $F. Var(C_j)_{ik}$ is the random effect associated to the interaction between the k th number of fungicide applications and the i th variety within the j th cycle length, assumed to be a random variable with mean zero and variance σ^2 ; and ε_{ijk} is the experimental error associated to i th variety within the j th cycle length receiving the k th number of fungicide applications, assumed to be a random variable with mean zero and variance σ^2 . Multiple comparisons for the number of fungicide applications were performed using a Tukey test, while those for the *variety within cycle length* effect were performed using Bonferoni's correction to account for the large number of pairwise comparisons, thus to reduce the incidence of Type I error.

All YR strains from the leaf samples collected for the trial were confirmed to belong to the genetic lineage *PstS13* (Hovmøller et al. 2018). Yellow rust was first observed at the tillering stage and severity reached up to 50% at the heading stage in the non-sprayed plots of some varieties, at or about 21 days after the fungicide application. At milk stage (41 daa) severity reached 65% in non-sprayed plots. As expected, grain yield was negatively associated with YR severity (Table 1, Figs. 1 and 2), agreeing with previous reports (Boshoff et al. 2003). The slope and coefficient of determination (R^2) for the linear model of the relationship between for severity AUDPC and grain yield in untreated plots were -2.73 and 0.86 for short cycle varieties and -2.06 and 0.78 for long cycle varieties, respectively (Fig. 1). In other words, for unitary increase in

Fig. 2 Yellow rust incidence AUDPC, severity AUDPC, and wheat grain yield between treatments with one fungicide application (gray bars), two applications (black bars) or no application (white bars). Left bar group corresponds to incidence, middle group to severity and right group to yield. S = significant differences between columns, n.s. = not significant ($P \leq 0.05$). Error bars show 95% confidence intervals

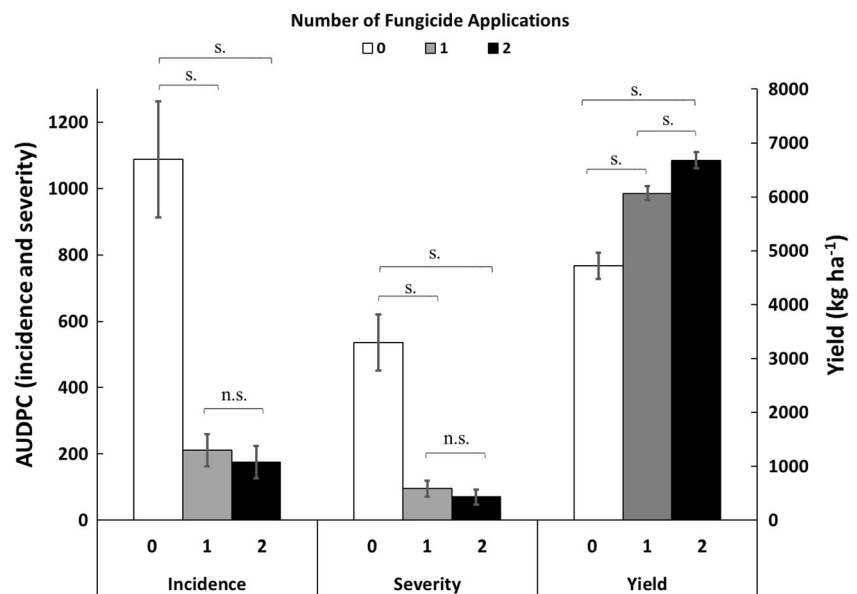


Table 2 Analysis of variance of yellow rust incidence (upper panel), yellow rust severity (middle panel) and grain yield (lower panel) of 30 wheat varieties (short and long cycle) receiving one or two applications of a strobilurin-triazole mix, or left untreated

Effect or Source of Variation	DF	Sum of Squares	Mean Square	F	P value
F^a	2	47,746,781	23,873,391	48.96	<0.0001
$Var(C)^b$	29	45,167,859	1,557,512	3.19	<0.0001
$F*Var(C)^c$	58	28,281,903	487,619	284.28	<0.0001
Residual ^d	180	308,755	1,715.30		
F^a	2	12,190,479	6,095,240	47.28	<0.0001
$Var(C)^b$	29	9,709,120	334,797	2.60	0.0010
$F*Var(C)^c$	58	7,477,566	128,924	142.46	<0.0001
Residual ^d	180	162,891	904.95		
F^a	2	178,446,315	89,223,158	69.40	<0.0001
$Var(C)^b$	29	96,679,596	3,333,779	2.59	0.0010
$F*Var(C)^c$	58	74,562,475	1,285,560	7.56	<0.0001
Residual ^d	180	30,627,955	170,155		

^a Main effect of the *k*th fungicide application (k = 0,1,2)

^b Main effect of the *i*th variety within the *j*th cycle length (j = short or long)

^c Random effect associated to the interaction between the *k*th number of fungicide applications and the *i*th variety within the *j*th cycle length, assumed to be a random variable with mean zero and variance σ^2

^d Experimental error associated to *i*th variety within the *j*th cycle length receiving the *k*th number of fungicide applications, assumed to be a random variable with mean zero and variance σ^2

the YR AUDPC severity, a yield reduction of 2.73 kg/ha and 2.06 kg/ha is expected for short and long cycle varieties, respectively. For the 10 most YR-susceptible varieties, the slope and R^2 for severity (%) estimated at 41 daa and yield (kg/ha) were –54 and 0.75, respectively (data not shown). The susceptible

wheat variety “NIDERA Baguette 680” showed the greatest decrease in AUDPC following the two fungicide applications.

Compared to the untreated control, one or two fungicide applications significantly decreased YR incidence and severity and protected grain yield (Table 2). However, the YR severity

Table 3 Pairwise comparisons of AUDPC for yellow rust incidence (upper panel) and severity (middle panel) and wheat grain yield (lower panel) of varieties of different cycle length (S = short, L = long)

Variety (Cycle length) Comparison	Difference	Standard Error	DF	t Value	Pr > t	Adjustment	Adj P
Bioceres 1006 (S) vs. DM Fuste (S)	–1355.33	329.18	58	–4.12	0.0001	Bonferroni	0.0535
Aca 909 (S) vs. DM Fuste (S)	–1327.67	329.18	58	–4.03	0.0002	Bonferroni	0.0708
Buck Saeta (S) vs. DM Fuste (S)	–1333.22	329.18	58	–4.05	0.0002	Bonferroni	0.0669
DM Fuste (S) vs. Klein Minerva (L)	1362.56	329.18	58	4.14	0.0001	Bonferroni	0.0497
DM Fuste (S) vs. Aca 602 (L)	1357.22	329.18	58	4.12	0.0001	Bonferroni	0.0525
DM Fuste (S) vs. Buck sy 120 (L)	1307.11	329.18	58	3.97	0.0002	Bonferroni	0.0870
DM Fuste (S) vs. Buck Sy 211 (L)	1357.78	329.18	58	4.12	0.0001	Bonferroni	0.0522
Aca 909 (S) vs. DM Fuste (S)	–685.56	169.26	58	–4.05	0.0002	Bonferroni	0.0669
Bioceres 1006 (S) vs. DM Fuste (S)	–682.89	169.26	58	–4.03	0.0002	Bonferroni	0.0705
Buck Saeta (S) vs. DM Fuste (S)	–686.89	169.26	58	–4.06	0.0001	Bonferroni	0.0652
DM Fuste (S) vs. Klein Minerva (L)	689.00	169.26	58	4.07	0.0001	Bonferroni	0.0625
DM Fuste (S) vs. Aca 602 (L)	684.56	169.26	58	4.04	0.0002	Bonferroni	0.0682
Experimental (S) vs. Sursem Lapacho (L)	–2504.22	534.49	58	–4.69	<.0001	Bonferroni	0.0075
Experimental (S) vs. Buck sy 120 (L)	–2550.22	534.49	58	–4.77	<.0001	Bonferroni	0.0056
DM Fuste (S) vs. Sursem Lapacho (L)	–2418.11	534.49	58	–4.52	<.0001	Bonferroni	0.0133
DM Fuste (S) vs. Buck sy 120 (L)	–2464.11	534.49	58	–4.61	<.0001	Bonferroni	0.0098
Klein Serpiente (L) vs. Sursem Lapacho (L)	–2316.00	534.49	58	–4.33	<.0001	Bonferroni	0.0257
Klein Serpiente (L) vs. Buck sy 120 (L)	–2362.00	534.49	58	–4.42	<.0001	Bonferroni	0.0191

Only varieties that showed a significant or marginally significant difference with at least another variety are shown

did not differ between one or two applications, though a significant yield advantage was noticed in the two applications treatment over the single application (Fig. 2). Increased grain yield response to the fungicide treatment was more evident in the varieties with high initial YR severity (Table 1). Yield response to fungicide varied from -5 to 3091 and 277 to 4743 kg/ha for one and two sprays, respectively (Table 1). The estimated yield losses in the non-sprayed compared to the 2 fungicide spray treatment ranged from 300 to 4700 kg/ha (20 to 743 usd/ha) across the 30 varieties. The average losses in the seven most susceptible varieties reached 3700 kg/ha (53%), with a maximum of 4700 kg/ha (70%).

Yield losses that we found were higher than reported in previous studies (Morgounov et al. 2012; Sharma et al. 2016) reporting grain losses of up to 40% due to yellow rust. The high level of YR protection provided by the fungicide application confirms that the observed yield differences among cultivars of short or long cycle were due to an increased control of YR by the foliar fungicide treatment (Table 1). No buffer or border zones were established in the experiment, but small alleys between plots. However, close attention was placed on spraying only the area of each plot. Consistency of levels of disease in untreated plots and in plots receiving one or two applications helped verify that interplot interference, if any, was minimized in our experiments.

The effect of variety within a given cycle length had a significant effect on disease intensity (incidence and severity) and yield (Table 2). However, only a few varieties belonging to short or long cycle showed significant difference in YR intensity and yield, as shown by the pairwise comparisons (Table 3). Since the strain of *Pst* race associated with the epidemics characterized in this study is new to the region, it is likely that most wheat varieties commercially grown in the region are susceptible to the pathogen. Only a few varieties out of the 30 tested were tolerant to the disease and yielded highly. Varieties with the best and significant response (lower disease and higher yield without fungicide application) were Buck SY 120 (L), Sursem Lapacho (L), Buck SY 211 (L), Buck Saeta (S), Bioceres 1006 (S), Aca 909 (S), Klein Minerva (L), Aca 602 (L) (Tables 1 and 3). Likewise, it seems plausible that the *Pst* race may not be resistant to the major fungicides used in the region, suggesting that chemical control will be effective to cope with the problem, at least in the short term. In the trials, a single strobilurin-triazole application at booting protected wheat from YR for approximately one month. The mix picoxystrobin-cyproconazole used for the treatments has protectant and curative mode of action (Oliver and Hewitt 2014), being highly effective against early and initial fungal infections.

In summary, the results of this study provide important baseline information about the potential economic losses, reaction of different wheat varieties to YR, response to

fungicides. We found that one or two applications of picoxystrobin + cyproconazole at the booting and early ear emergence wheat growth stages can significantly decrease YR incidence and severity and protect wheat grain yield, and that response to disease intensity is not directly related with the cycle length alone, but to a specific variety within a cycle length. Hence, a judicious use of the major fungicide mechanisms of action in the region may prolong their lifespan. Likewise, there is an urgent need to breeding for resistance to the new races of *Pst* for an effective management of YR in the long term.

Author contribution Marcelo Carmona planned and designed the experiment. Carlos G Grosso, Lucas Vettorello, Barbarina Milanese, Eduardo Corvi and Gustavo Almada executed the field work. Francisco Sautua and Oscar Pérez-Hernández conducted data analyses. Mogens Støvring Hovmøller confirmed the genetic lineage *PstS13* from yellow rust isolates derived from the leaf samples collected at the trial.

Francisco Sautua, Oscar Pérez-Hernández, Marcelo Carmona and Mogens Støvring Hovmøller wrote the manuscript.

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