Tropical Plant Pathology (2019) 44:258–267
https://doi.org/10.1007/s40858-019-00290-3

ORIGINAL ARTICLE

Nitrogen fertilization effects on wheat blast epidemics under varying field environmental conditions

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Received: 31 July 2018 / Accepted: 22 April 2019 / Published online: 15 May 2019

Abstract

Wheat blast, caused by the fungus *Magnaporthe oryzae* Triticum (MoT) pathotype, is one of the most important yield-reducing diseases of wheat, mainly in the tropical wheat-growing regions of South America. In this study, we assessed the effects of nitrogen (N) fertilization on wheat blast epidemics. Factorial experiments were carried out in Londrina (2015 and 2016) and Floresta (two sowings in 2017) with N rates (0, 40, 80 and 120 kg/ha for the first experiment, and 0, 60 and 120 kg/ha for the second) and wheat genotypes (three and six cultivars, for the first and second experiments, respectively) as factors. Wheat blast on spikes occurred only in two out of four experiments: 2015 in Londrina and first sowing of 2017 in Floresta. Nitrogen increased blast severity in the first crop of 2017 in Floresta, where blast severity on spikes ranged from 10 to 94% and yield ranged from 47 to 2823 kg/ha, depending on N rate and genotype. Nitrogen did not affect wheat blast (except for genotype S1) in the 2015 Londrina trial, where blast severity on spikes ranged from 16.9 to 36.8%. Differences in blast severity among genotypes were likely due to different crop heading periods. Our results suggest that N fertilization and sowing time should be considered in a management strategy for reducing the risk of wheat blast epidemics.

Keywords *Magnaporthe oryzae* · *Pyricularia oryzae* · *Triticum aestivum* · Plant nutrition

Introduction

Wheat blast, caused by the hemibiotrophic fungus *Magnaporthe oryzae Triticum* (MoT) pathotype (anamorph: *Pyricularia oryzae*), is one of the most important yield-reducing diseases of wheat (*Triticum aestivum*) in the tropical, and eventually in subtropical wheat-growing regions of Brazil, Bolivia, Paraguay and Argentina (Kohli et al. 2011; Cruz and Valent 2017; Rodrigues et al. 2017). This disease has limited wheat expansion to several regions that are suitable for wheat cropping in the tropics. Recently, wheat blast emerged for the first time in Asia (Bangladesh), where it has been considered a major threat to wheat production in Asia (Callaway 2016; Sadat and Choi 2017).

The fungus infects the plant, after wheat heads are emerged, through the rachis of the spikes. The disease develops to severe levels at warmer temperatures (18 to 28 °C), high relative humidity (> 90%), extended periods of dew or rain (> 15 h) and cloudy days (Lima 2004; Cruz et al. 2009; Kohli et al. 2011). Severe epidemics may lead to yield losses of up to 74% (Goulart et al. 2007) due to damage in the rachis that affects the translocation of nutrients and photosynthates to grains and may cause premature death of spike above the infection site (Cruz and Valent 2017; Rodrigues et al. 2017).

Currently, commercial wheat cultivars do not posses satisfactory levels of genetic resistance to wheat blast, especially during disease-inducing weather conditions. Because fungicides provide only modest and inconsistent results (Goulart et al. 2007; Cruz and Valent 2017) there is great uncertainty on how to effectively control the disease. According to the literature, the effect of nitrogen (N) fertilization on plant disease severity can be either positive or negative varying with the pathosystem. In a meta-analysis study of 57 scientific articles to summarize the effect of mineral fertilization on diseases,

Section Editor: Leandro J. Dallagnol

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authors found that N fertilization tends to increase susceptibility of plants to hemibiotrophic fungal pathogens (Veresoglou et al. 2013). The amount of available N for the plant may have a pronounced and contradictory effect on disease expression (Huber and Thompson 2007). For instance, while severity of cereal rust (*Puccinia graminis*), wheat mildew (*Erysiphe graminis*) and rice blast (*Magnaporthe oryzae*) was greater with the increase of N rates, wheat tan spot (*Pyrenophora tritici-repentis*), wheat stagonospora nodorum blotch (*Stagonospora nodorum*) and cereal take-all (*Gaeumannomyces graminis*) have been consistently reduced in response to increasing N rates. Moreover, plant susceptibility to these diseases is associated with increases in certain free amino acids available for pathogen nutrition in infected tissues. However, increasing host resistance can be due to decrease of peptidase activity and reduced availability of some amino acids needed for pathogen nutrition (Huber and Thompson 2007). According to Mur et al. (2016) the use of NO_3^- or NH_4^+ fertilizers affects the outcome of plant-pathogen interaction, being the effects associated with host resistance and susceptibility, respectively. Metabolically, NO_3^- increases production of polyamines (*e.g.* spermine and spermidine), which are established defense signals, and NH_4^+ augments γ-aminobutyric acid (GABA) levels, which may be a nutrient source for the pathogen.

Information on the effect of N fertilization on wheat blast epidemics in the field, particularly its interaction with wheat genotypes, is not available. Since N is widely used in great amount by wheat growers to maximize yield, such knowledge is key for improving wheat blast management. The objective of this study was to evaluate the interaction effect of N fertilization rates and wheat genotypes on blast severity on spikes under varying field environmental conditions across years, locations and sowing dates.

Material and methods

Experimental setting 1, Londrina trials

The first experimental setting was conducted in Londrina (23°11′37" S, 51°11′03" W; altitude of 628 m), Paraná State, southern Brazil, one in 2015 and the other in 2016 crop seasons. The climate of the region is humid subtropical, Cfa according to the Köppen classification (Köppen 1931). Daily meteorological variables (precipitation, temperature and relative humidity; Fig. 1) were recorded in an automatic weather station (equipped with: datalogger Campbell Scientific®, model 21X; tipping bucket rain gage Texas Eletronics®, model TE525; temperature and relative humidity probe Vaisala®, model HMP35C) located 550 m away from the field trial.

The treatments were arranged in a 3 × 4 factorial: three wheat (*Triticum aestivum*) genotypes (S1, S2 and MR1, commercial cultivars BRS Gralha-Azul, BRS Sabiá and BRS Pardela, respectively) and four N rates (0, 40, 80 and 120 kg/ha). The experimental design was randomized complete blocks, with four replicates, and both factors – genotypes and N rates – were randomly allocated within each block. Genotypes S1 and MR1 are classified as medium-maturing (115 to 130 days), while genotype S2 is early-maturing (< 110 days) (Bassoi et al. 2016). The levels of resistance to blast disease, following Goulart et al. (1991), are defined based on the percentage of wheat spikes with blast symptoms at the end of the cycle: resistant (R), 1 to 5%; moderately resistant (MR), 6 to 25%; moderately susceptible (MS), 26 to 50%; susceptible (S), 51 to 75%; and highly susceptible (HS), 76 to 100%. Two genotypes, S1 and S2, are classified as susceptible, and genotype MR1 is classified as moderately resistant to wheat blast on spikes (Bassoi et al. 2016).

The N source was ammonium nitrate, and it was applied in top dressing during wheat tillering (Z25 stage; Zadoks et al. 1974). The rates of N were chosen based on wheat crop fertilization in the main Brazilian wheat-producing regions, which have ranged from 20 to 140 kg/ha N (Cunha and Caierão 2014). The plots consisted of nine rows, 6.0 m in length, spaced 0.2 m apart (10.8 m^2), and the seeding density was regulated to obtain about 300 plants per m^2 for all genotypes. The sowing dates of the trials were April 16th and April 14th, in 2015 and 2016 crop seasons, respectively. Basal fertilization consisted of 22.4 kg/ha N, 42 kg/ha P_2O_5 and 60 kg/ha K_2O, applied during wheat sowing.

In each plot, wheat severity on spikes with typical disease symptoms (bleaching noticed above dark lesions formed in the rachis) was estimated visually aided by a diagrammatic scale containing 10 grades of severity ranging from 0 to 100% (0, 3.5, 7.5, 21.5, 30.5, 44, 57.5, 68, 86 and 100%) (Maciel et al. 2013). A destructive sampling method was used to collect at least 60 spikes from two central wheat rows 0.5 m long, selected at random inside each plot, when the plants were on average in dough development (Z80 stage; Zadoks et al. 1974). Plot severity was recorded as the average proportion of diseased spikelets per spike, divided by the total number of spikes sampled, including those with zero severity (Paul et al. 2005). In addition, 50 healthy spikes (which were free from the disease) were sampled at random inside each plot and thrashed; the rachises were dried (65 °C for 48 h) and submitted to nutritional analyses (N, P, K, Ca, Mg, S, Zn, Mn, Fe, Cu and B) (Silva 2009). The additional aim of these analyses was to verify a possible correlation between rachis nutritional status and disease severity on spikes. Yield was determined by harvesting wheat plants at maturity (5 m in length) from seven center rows from each plot and recording the grain weight after adjusting the moisture content to 13%.

Experimental data for each crop season (2015 and 2016), individually, were analyzed by the GENES® statistical package (Cruz 2013). Analyses of models assumptions were

performed: Lilliefors' test for normality, and Bartlett's test for homogeneity of variance. Skewness and kurtosis coefficients were also evaluated. According to these tests, no data transformation was needed. Since all assumptions required for analysis of variance (ANOVA) were met, the F-test was performed. When ANOVA was significant ($P \leq 0.05$), the means of yield and blast severity were adjusted by linear and quadratic models of linear regression, using the SAEG® statistical package (Euclydes 1997). Coefficients of adjusted models were assessed by F-test ($P < 0.001$, $P < 0.01$ and $P < 0.05$) considering the mean square error of ANOVA. Finally, between two models with significant coefficients, the one with higher simple or multiple coefficient of determination (r^2 or R^2, respectively) was chosen. The Tukey's test ($P \geq 0.05$) was also used for multiple comparisons, considering yield and blast severity. Additionally, Pearson's correlation coefficient (r) was calculated (at significance levels of $P < 0.001$, $P < 0.01$ and $P < 0.05$, individually for each genotype) between wheat blast severity and grain yield or the concentration of each nutrient (N, P, K, Ca, Mg, S, Zn, Mn, Fe, Cu and B) in the rachis.

Experimental setting 2, Floresta trials

Another experimental setting, in a third year, was established in Floresta (23°35′26" S, 52°04′22" W; altitude of 390 m), Paraná State, southern Brazil, and it was replicated during the 2017 crop season for two sowing dates: March 29th and April 18th. The climate of the region is humid subtropical, Cfa according to the Köppen classification (Köppen 1931). Daily meteorological variables (precipitation, temperature and relative humidity; Fig. 2) were recorded in an automatic weather station (equipped with: datalogger Campbell Scientific®, model CR100; tipping bucket rain gage Campbell Scientific®, model TB4; temperature and relative humidity probe Vaisala®, model HMP35C) located 430 m away from the experiment.

The treatments were arranged in a 6 × 3 factorial: six wheat (*T. aestivum*) genotypes [S1, S2 and MR1 (all the same as the first experiment); MR2, MS1 and MS2, commercial cultivars BRS Graúna, BRS Tangará and BRS Sanhaço, respectively] and three N rates (0, 60 and 120 kg/ha). The experimental design was randomized complete blocks, with three replicates, and both factors – genotypes and N rates – were randomly assigned to each block. In relation to the reaction to blast disease, genotypes S1 and S2 are classified as susceptible, MR1 and MR2 as moderately resistant, and MS1 and MS2 as moderately susceptible (Bassoi et al. 2016). With respect to phenology, genotypes S1, MR1, MS1 and MS2 are classified as medium-maturing (115 to 130 days), while genotypes S2 and MR2 are early-maturing (< 110 days) (Bassoi et al. 2016). The N source was urea, and it was applied in top dressing at the beginning of wheat tillering (Z22 stage; Zadoks et al. 1974).

The plots consisted of 13 rows, 4.0 m in length, spaced 0.17 m apart (8.84 m^2), and the seeding density was regulated to obtain about 300 plants per m^2 for all genotypes. Basal fertilization consisted of 60 kg/ha P_2O_5 and 60 kg/ha K_2O, applied during wheat sowing. Wheat blast on spikes was evaluated in the same way as described for the first experiment. Yield was determined by harvesting wheat plants at maturity (3 m in length) from 11 center rows from each plot and recording the grain weight after adjusting the moisture content to 13%. Experimental data for each wheat crop (first and second), individually, were statistically analyzed, and the methods were the same as described for the experiment 1, with the exception of regression analysis (which was not performed).

In both experiments (Londrina and Floresta environments), controls of weeds, insects and diseases were performed in accordance with regional technical recommendations for wheat crop (Cunha and Caierão 2014). However, wheat blast on spikes was not controlled by means of fungicide or other techniques. The presence or absence of blast on leaves was not evaluated or observed in the experiments. In addition, MoT strains occurring in these two regions of Paraná State are considered equivalent to each other, according to previous sampling and phenotyping performed by Castroagudín et al. (2015).

Results

Londrina trials

Wheat blast was not recorded during the 2016 crop season. In 2015, a significant interaction between N rate and genotype factors for blast severity was found (Table 1). Nitrogen fertilization increased blast severity on spikes of wheat genotype S1 (Fig. 3a). \The greatest effect was observed with the rate of 80 kg/ha N, which increased blast severity by 44.9% compared with the control treatment (0 kg/ha N).

Genotype MR1 was more resistant to wheat blast than S2 – blast severity was 37.2, 39.6 and 26.9% lower in the N rates of 0, 40 and 120 kg/ha, respectively (Fig. 3a). In turn, blast severity in MR1 was 37.4 and 45.7% lower than in S1 in the N rates of 40 and 80 kg/ha, respectively. Finally, genotypes S1 and S2 did not differ in blast severity (except for N rate of 80 kg/ha).

Nitrogen rate and genotype interaction was significant for grain yield in both seasons (Table 1). In most cases in 2015, yield was highest in S2 genotype than in S1 and MRI genotypes (Fig. 3b). Neither the MR1 nor S2 genotypes responded to N fertilization, but S1 genotype showed a decrease in yield by 24% for the treatment with 80 kg/ha N compared with the control treatment (0 kg/ha N) (Fig. 3b). The correlation

Table 1 Summary of the analysis of variance of grain yield and blast severity for three wheat genotypes (S1, S2 and MR1) in the crop seasons of 2015 and 2016 in Londrina, Paraná (Brazil), in response to four nitrogen (N) rates (0, 40, 80 and 120 kg/ha)

Source of variation	df[a]	Mean squares			
		Grain yield		Blast severity	
		2015	2016	2015	2016[b]
Block	3	52319	231896	117.3	–
Genotype (G)	2	8667293***	1289475***	481.2***	–
N rate (N)	3	307445*	126244^{ns}	77.6*	–
G × N	6	208170^{o}	369725**	53.5*	–
Error	33	104373	96826	23.9	–

Significance by F-test of main effects (genotype and N rate) and their interaction: ^{ns}, ^{o}, *, ** and *** = no significant, and significant at $P \leq 0.1$, 0.05, 0.01 and 0.001, respectively

[a] df, degrees of freedom; [b] There was no wheat blast in the 2016 crop season

between yield and blast severity in genotype S1 was negative and highly significant ($r = -0.90^{***}$).

Considering all genotypes together, N rates did not affect grain yield in the 2016 crop season (Table 1), even in the absence of blast disease for all treatments. However, analyzing each genotype separately (Fig. 3c), the rates of 40 and 80 kg/ha N increased MR1 yield by 13.6 and 15.2%, respectively, when compared with the control treatment (0 kg/ha N). On the other hand, the rate of 120 kg/ha N decreased S2 yield by 16%. Finally, genotype S1 was not responsive to N fertilization.

In 2016, genotype MR1 had the highest yields in the N rates of 40 and 80 kg/ha (5009 and 5078 kg/ha, respectively); and lowest yields were verified in the N rate of 120 kg/ha for S2 (3821 kg/ha) and in the rates of 40 and 80 kg/ha for S1 and S2 (averages of 4297 and 4369 kg/ha, respectively) (Fig. 3c).

The average yield from the 2015 crop season was 26% lower than that obtained for 2016 (Fig. 3b and c), which can be partially attributed to the blast disease, which took place only in 2015 (average of 26.3%). There was a significant effect of N rates on nutrient concentration in the rachis only for Ca and Mg (Tables 2 and 3). Nitrogen rate increased Ca and Mg concentrations by 22% (average of the treatments with 40, 80 and 120 kg/ha N) and 29% (treatment with 80 kg/ha N), respectively, when compared with the control treatment (0 kg/ha N). Genotype MR1 had the highest concentrations of N, P, Mg, Zn (together with S1), Mn, Fe (together with S1) and Cu. In turn, the highest concentrations of K, Ca (together with S1) and S were obtained by genotype S2. In turn, lower concentrations of N, P, Mg and Mn were found for the genotypes S1 and S2; K and S for MR1; and Zn and Cu for S2. There was no difference among genotypes for B concentration in the rachis.

Pearson's correlation coefficients between blast severity on spikes and the concentration of each nutrient (N, P, K, Ca, Mg, S, Zn, Mn, Fe, Cu and B) in the rachis was statistically significant only for N in genotype S2 ($r = 0.59^{*}$).

Floresta trials

Blast severity did not occur on wheat genotypes in the 2017 second-crop. In turn, N rate and genotype interaction was significant for blast severity during the 2017 first-crop (Table 4). In this crop, wheat blast severity was very high, averaging 51% overall (Figs. 4a). The decreasing order of average blast severity in the six wheat genotypes was: MR2 (80%) > S2 (75%) > S1 (57%) > MS2 (52%) > MS1 (32%) > MR1 (12%). Genotype MR1 was the most resistant to wheat blast in the N rates of 60 and 120 kg/ha, with blast severities of 11 and 13%, respectively (Fig. 4a). In turn, in the N rate of 0 kg/ha, MR1 and MS1 were more resistant, with an average of blast severity of 11.5%. In most of genotype *versus* N rate combinations, MR2 and S2 were the most susceptible to wheat blast, since their blast severities were very high, that is, 59 and 48%, 91 and 84%, and 90 and 93% for the N rates of 0, 60 and 120 kg/ha, respectively, and in most situations they were higher than those verified in the other genotypes (S1, MR1, MS1 and MS2).

The effect of N rates on blast severity on spikes was highly significant ($P \leq 0.001$) for five out of six genotypes (except for MR1) in the 2017 first-crop (Table 4 and Fig. 4a). In most cases (except for genotype S1), there was no difference between 60 and 120 kg/ha N on increasing blast severity. On average, the rates of 60 and 120 kg/ha N increased blast severity by 84, 53, 330 and 137% for genotypes S2, MR2, MS1 and MS2, respectively, when compared with the control treatment (0 kg/ha N) (Fig. 4a).

The blast disease strongly impaired wheat yield (Fig. 3a and b). There were high negative correlations between blast severity and grain yield ($r = -0.85^{**}$, -0.84^{**}, -0.93^{***}, -0.74^{*} and -0.75^{*} for genotypes S1, S2, MR2, MS1 and MS2, respectively). The average yields for the 2017 first-crop were 2198, 1037 and 725 kg/ha for average blast severities of 30, 56 and 68%, respectively (for the treatments with 0, 60 and 120 kg/ha N, respectively).

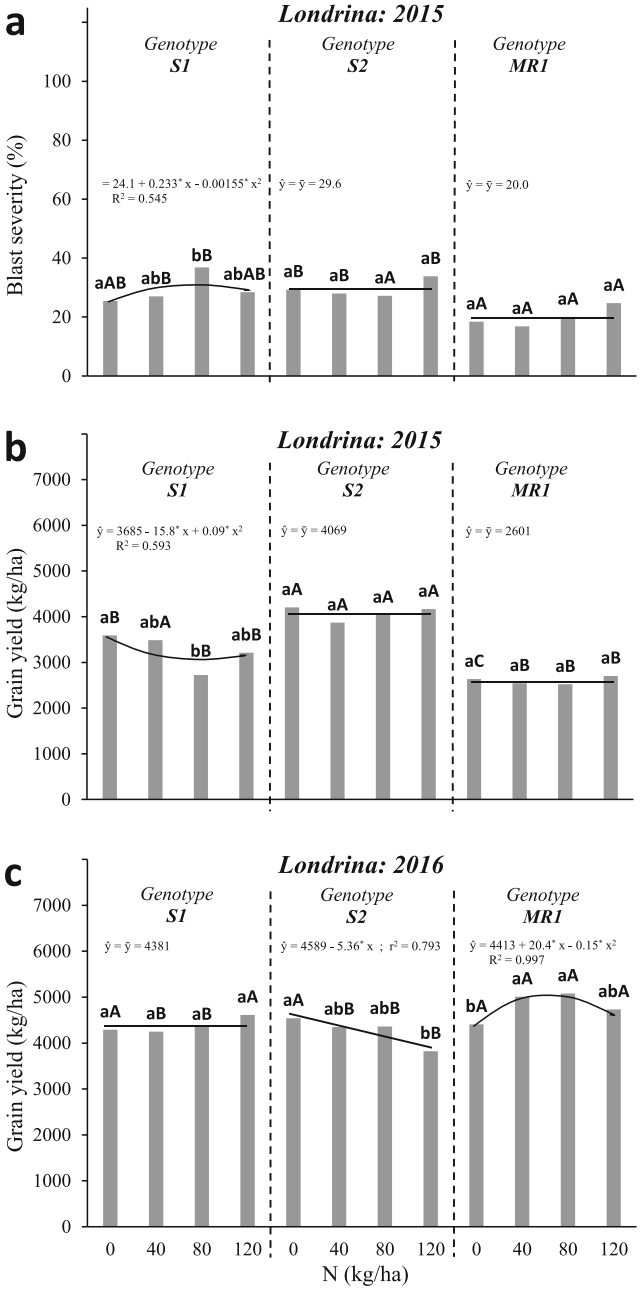

Fig. 1 The weather conditions [precipitation (P), relative humidity (RH) and average temperature (T) of the air] during the crop seasons of 2015 (April 16th to August 20th = 127 days) and 2016 (April 14th to September 8th = 148 days) are shown in **a** and **b**, respectively

There was an interaction between N rate and genotype factors for grain yield during the 2017 first-crop (Table 4). Analyzing each genotype separately, the S1, MS1 and MS2 yields were lower at 120 kg/ha N than at 60 kg/ha N (Fig. 4b). On the other hand, the rate of 120 kg/ha N increased MR1 yield by 31% compared with the treatment with 60 kg/ha N. In turn, there was no difference in grain yield of genotypes S2 and MR2 between treatments with 60 and 120 kg/ha N, which were the least productive (average of 111 kg/ha). Finally, the N rate of 0 kg/ha resulted in higher wheat yields for all genotypes, with an overall average of 2198 kg/ha.

In the 2017 first-crop, the highest grain yields in the N rates of 60 and 120 kg/ha N were obtained by the genotypes MS1 (2467 kg/ha) and MR1 (2066 kg/ha), respectively (Fig. 4b). In turn, in the control treatment (0 kg/ha N) the most productive genotypes were S1, MR1 and MS1, with an average of 2700 kg/ha. In general, the genotypes S2 and MR2 were the least productive, with average grain yields of 1490, 130 and 92 kg/ha in the N rates of 0, 60 and 120 kg/ha, respectively. In addition, it was not possible to verify the effect of N rate on attainable grain yield, due to the confounding effect from the strong blast severity in this 2017 first-crop (Fig. 4a and b).

Blast severity on spikes did not occur in the 2017 second-crop (Table 4). On average, the grain yield from this crop was almost three times higher than that from the first one (Fig. 4b and c), despite the former not receiving any rain after the heading phenological stage (Fig. 2a and b).

In the 2017 second-crop, there was also a significant interaction between N rate and genotype factors for grain yield (Table 4). Nitrogen fertilization increased grain yield only in the genotypes MR1 and MR2 (except for 120 kg/ha N) by 10.8 and 11.6%, respectively (Fig. 4c). Considering each N rate individually, genotype MR1 was the most productive in the rates of 60 and 120 kg/ha N, attaining 4104 and 4151 kg/ha, respectively, and S1 and MR1 had the higher yields at N rate of 0 kg/ha N (average of 3684 kg/ha). The lower grain yields were obtained by MR2 in the N rates of 0 and 120 kg/ha (3061 and 3041 kg/ha, respectively), and by MR2 and MS2 at N rate of 60 kg/ha (average of 3430 kg/ha).

Discussion

Results from our study, at least for one trial, suggest that wheat blast severity tends to increase with N fertilization under field conditions. To our knowledge, this is the first report from field studies about the effect of N fertilization on wheat blast on spikes. However, because out of two independent experiments where the disease occurred, a significant effect was observed in only one of them, a confirmation of a positive effect of N rate on wheat blast needs to be obtained in further studies in order to provide more conclusive information about this N–wheat blast interaction.

Studies on rice blast showed that the application of N above the recommended rate for any given cultivar significantly increased blast disease (caused by *M. oryzae*) on rice leaves, while N treatments did not affect the blast incidence on collar rot or neck (Long et al. 2000). Kurschner et al. (1992) reported that while N fertilizer was essential for increasing rice grain yield, the blast severity on panicle also increased with the rate of N application.

Table 2 Summary of the analysis of variance and multiple comparisons of macronutrient (N, P, K, Ca, Mg and S) concentration in the rachis for three wheat genotypes (S1, S2 and MR1) in the crop season of 2015 in Londrina, Paraná (Brazil), in response to four nitrogen (N) rates (0, 40, 80 and 120 kg/ha)

Source of variation	df[a]	Mean squares					
		N	P	K	Ca	Mg	S
Block	3	3.71	0.050	2.66	0.0028	0.0086	0.116
Genotype (G)	2	47.68***	1.071***	166.54***	0.0164**	0.0585***	0.459***
N rate (N)	3	2.51^{ns}	0.059^{ns}	3.63^{ns}	0.0405***	0.0266**	0.039^{ns}
G × N	6	3.31^{ns}	0.048^{ns}	2.15^{ns}	0.0020^{ns}	0.0051^{ns}	0.025^{ns}
Error	33	3.60	0.049	2.76	0.0029	0.0060	0.024
Factor	Treatment	Macronutrient concentration (g/kg)					
		N	P	K	Ca	Mg	S
Genotype (G)	S1	8.8 b	0.93 b	13.6 b	0.55 ab	0.41 b	1.01 b
	S2	8.6 b	0.77 b	17.0 a	0.59 a	0.41 b	1.18 a
	MR1	11.7 a	1.28 a	10.5 c	0.53 b	0.51 a	0.84 c
N rate (N)	0	9.1 a	0.90 a	13.0 a	0.48 b	0.38 b	0.93 a
	40	9.8 a	1.05 a	13.8 a	0.55 a	0.46 ab	1.04 a
	80	10.1 a	1.04 a	13.6 a	0.60 a	0.49 a	1.03 a
	120	10.0 a	0.98 a	14.4 a	0.61 a	0.45 ab	1.05 a

Significance by F-test of main effects (genotype and N rate) and their interaction: ^{ns}, *, ** and *** = no significant, and significant at $P \leq 0.05$, 0.01 and 0.001, respectively. Within a factor (genotype or N rate), means followed by the same letter are not significantly different at $P > 0.05$ according to Tukey's test

[a] df, degrees of freedom

The physiological mechanism of N effect on blast disease is not well understood. Ballini et al. (2013) showed that a phenomenon called nitrogen-induced susceptibility (NIS) affects the resistance of rice and wheat to blast fungus (*M. oryzae*) infection on leaves. This finding reveals that N partially breaks down resistance triggered by the *Pi1* gene,

Table 3 Summary of the analysis of variance and multiple comparisons of micronutrient (Zn, Mn, Fe, Cu and B) concentration in the rachis for three wheat genotypes (S1, S2 and MR1) in the crop season of 2015 in Londrina, Paraná (Brazil), in response to four nitrogen (N) rates (0, 40, 80 and 120 kg/ha)

Source of variation	df[a]	Mean squares				
		Zn	Mn	Fe	Cu	B
Block	3	206	267	1064	0.72	24.5
Genotype (G)	2	1814***	1668***	1566***	34.44***	11.6^{ns}
N rate (N)	3	90^{ns}	22^{ns}	687^{ns}	0.73^{ns}	16.4^{ns}
G × N	6	22^{ns}	43^{ns}	546^{ns}	0.78^{ns}	5.5^{ns}
Error	33	75	64	273	0.76	7.6
Factor	Treatment	Micronutrient concentration (mg/kg)				
		Zn	Mn	Fe	Cu	B
Genotype (G)	S1	38.5 a	52.1 b	113 ab	2.37 b	3.73 a
	S2	24.0 b	48.7 b	106 b	1.30 c	5.33 a
	MR1	44.8 a	67.8 a	126 a	4.20 a	4.03 a
N rate (N)	0	34.0 a	56.9 a	109 a	2.27 a	3.04 a
	40	38.3 a	57.7 a	125 a	2.64 a	4.19 a
	80	32.9 a	54.8 a	116 a	2.79 a	4.36 a
	120	37.9 a	55.3 a	110 a	2.80 a	5.89 a

Significance by F-test of main effects (genotype and N rate) and their interaction: ^{ns}, *, ** and *** = no significant, and significant at $P \leq 0.05$, 0.01 and 0.001, respectively. Within a factor (genotype or N rate), means followed by the same letter are not significantly different at $P > 0.05$ according to Tukey's test

[a] df, degrees of freedom

Table 4 Summary of the analysis of variance of grain yield and blast severity for six wheat genotypes (S1, S2, MR1, MR2, MS1 and MS2) from two crops (first sowing date: March 29th; second sowing date: April 18th) in the 2017 season in Floresta, Paraná (Brazil), in response to three nitrogen (N) rates (0, 60 and 120 kg/ha)

Source of variation	df[a]	Mean squares			
		Grain yield		Blast severity	
		2017 first-crop	2017 second-crop	2017 first-crop	2017 second-crop[b]
Block	2	415568	3002067	207.2	–
Genotype (G)	5	4795282***	817599***	5916.0***	–
N rate (N)	2	10845920***	289030***	6712.6***	–
G × N	10	476021***	32432***	475.0**	–
Error	34	13104	1284	151.8	–

Significance by F-test of main effects (genotype and N rate) and their interaction: *, ** and *** = significant at $P \leq$ 0.05, 0.01 and 0.001, respectively

[a] df, degrees of freedom; [b] There was no wheat blast in the 2017 second-crop

and cytological analysis indicates that high N does not affect fungus penetration, whereas it does increase its growth inside the plant. Recently, Debona et al. (2012) and Rodrigues et al. (2017) reported that wheat resistance to blast disease was associated with control of the production of reactive oxygen species (ROS), favoring host defense mechanisms against MoT infection on leaves and rachis of wheat. According to these authors, a more efficient antioxidative system (that removes the excess ROS generated during the infection process prevents the cellular damage caused by MoT. According to Gupta et al. (2011), the pathway hydroxylamine-mediated nitric oxide (NO) production is involved in the regulation of the levels of ROS. These authors reported that, upon pathogen infection NO is likely to act as a signal that is required to induce plant defense mechanisms. In turn, Espunya et al. (2012) mentioned that S-Nitrosoglutathione is a mobile reservoir of NO and an important player in defense responses to pathogen attack in plants, acting synergistically with classic hormones involved in plant defense (particularly salicylic acid and jasmonic acid) to activate gene response at local and systemic sites. Considering that nitrate reductase (NR) is the main production pathway for NO in plants, and this enzyme requires nitrite

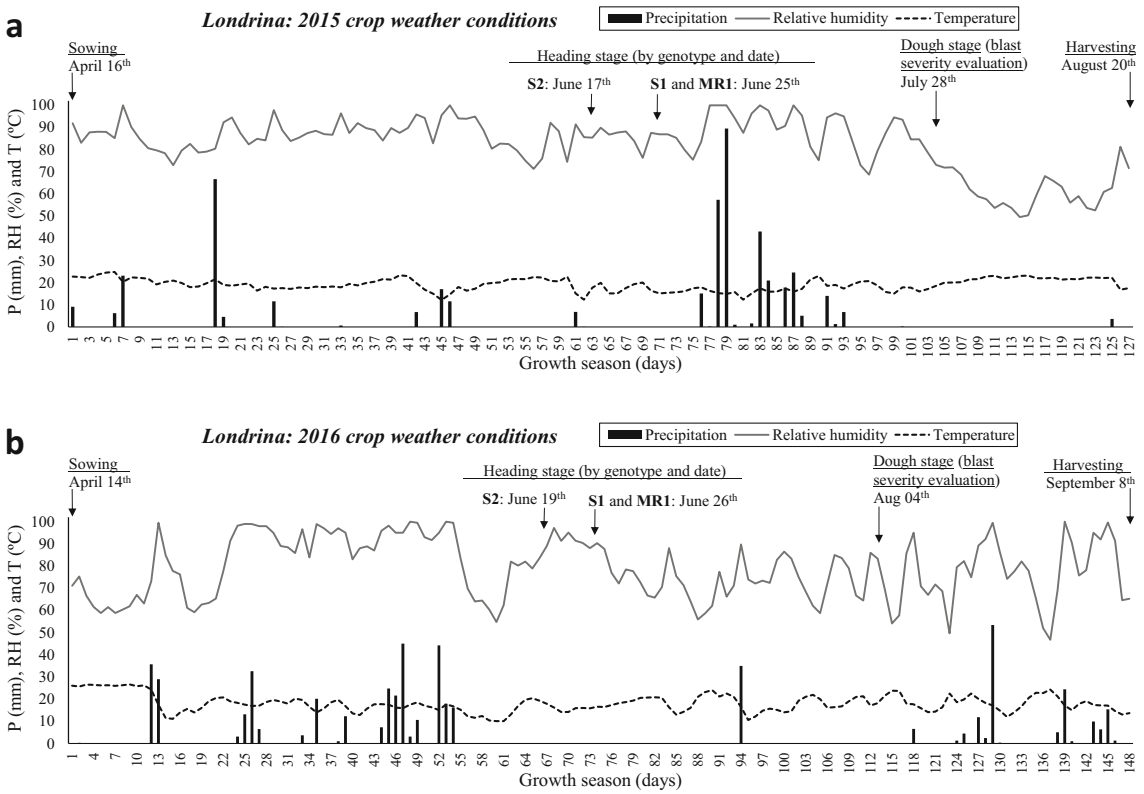

Fig. 2 The weather conditions [precipitation (P), relative humidity (RH) and average temperature (T) of the air] during the first (March 29th to August 1st = 126 days) and second (April 18th to August 25th = 130 days) crop seasons are shown in **a** and **b**, respectively

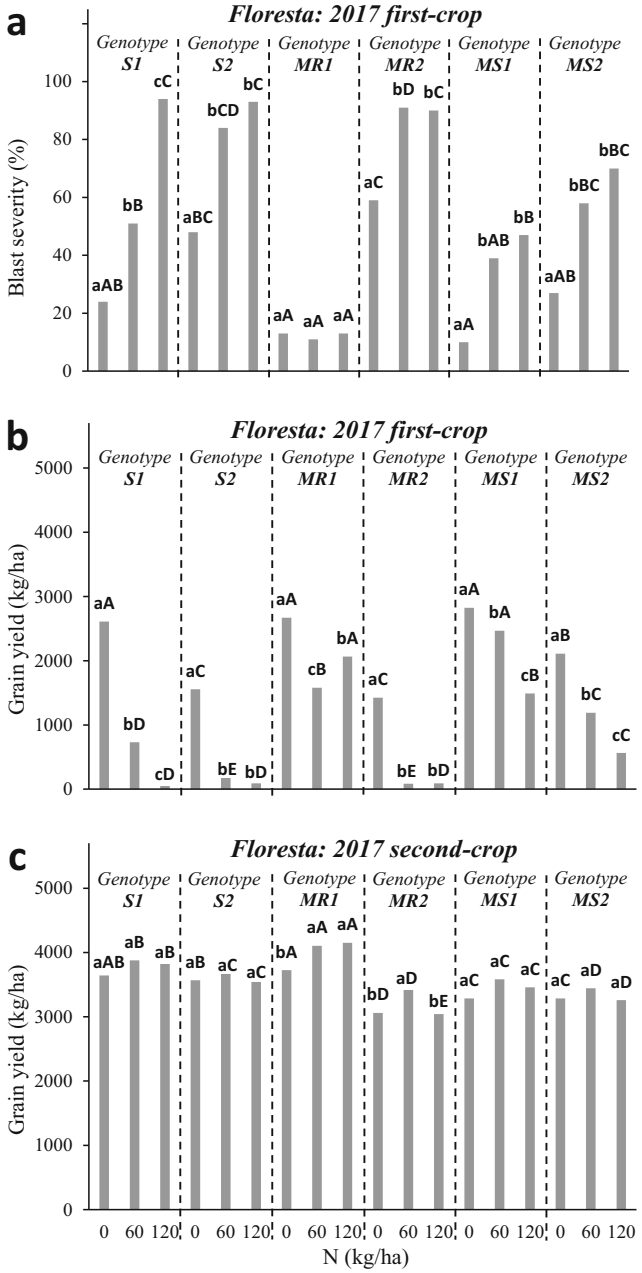

Fig. 3 Blast severity on spikes and grain yield from three wheat genotypes (S1, S2 and MR1) fertilized with four rates of N (0, 40, 80 and 120 kg/ha), in the crop seasons of 2015 (**a** and **b**) and 2016 (**c**) in Londrina, Paraná (Brazil). Individually for each crop season and each wheat genotype, columns comparing N rates followed by the same lowercase letter do not differ significantly (Tukey's test, $P \geq 0.05$). Individually for each crop season and each N rate, columns comparing wheat genotypes followed by the same capital letter do not differ significantly. Above the histograms, there are regression equations adjusted as a function of N rates, individually for each wheat genotype. Note: there was no blast disease in the crop season of 2016

levels to be in excess of the substrate nitrate (Procházková et al. 2014), N fertilization has an indirect effect on this plant defense mechanism, being able to inhibit it, because N fertilizers applied to the soil are microbiologically transformed generating nitrate and/or ammonium, which are the N-ions uptaken by the plants.

Another possibility for increased blast severity in response to N rates is the indirect effect of N on the disease *via* further development of the plant canopy. Hence, large canopies with high shoot density may be more favorable to spore transfer and pathogen infection than sparse canopies. For example, N has been shown to increase the severity of *Fusarium* head blight in wheat spikes, and it has been attributed to the result of an N-induced increase in canopy size, leading to an altered microclimate (which remains humid over a longer period of time after rain or after dew periods), that is more conducive to infectious process (Lemmens et al. 2004). This change in microclimate, when plants grow larger and denser, can decrease air circulation and increase water holding capacity, both of which being effective to increase wetness duration, that is a favorable condition to MoT sporulation and infection. Additionally, this higher humidity within the canopy can also delay crop ripening, and the rachis may remain receptive to fungal infection for a longer period of time.

The weather conditions were key for blast disease development in the 2015 crop season in Londrina, where there was an extended period of rainfall (76th to 93rd days at the growth season; total of 298.4 mm during 18 days) after the heading stage, associated with an average temperature of 17 °C (ranging from 12.4 to 23.1 °C) (Fig. 1a), resulting in medium levels of blast severity. On the other hand, in the 2016 crop season, the absence of wheat blast disease can be partially explained by the insufficient amount of rain (total of 35.3 mm during 40 days) from the last heading to dough stage (74th to 113th days at the growth season, respectively; Fig. 1b), although the average temperature was similar to that in the previous winter crop season.

Wheat blast disease was null during 2017 second-crop in Floresta environment, because there was no rain after the heading stage (Fig. 2b). In other words, the absence of free water on the rachis did not allow infection of MoT in this plant tissue, preventing the effects of N rate and genotype treatments on blast disease. In turn, in 2017 first-crop there was a significant amount of rain (total of 89.2 mm during 36 days; Fig. 2a) from the first heading to dough stage (64th to 99th days at the growth season, respectively), and the wheat crop was highly infected by the disease, which can be considered a blast epidemic crop, since the wheat yield was highly impaired.

Differential responses to blast disease among wheat genotypes are attributed to different associations between the post-heading period (from heading to dough stage, when the rachis tissue is more vulnerable to MoT infection) and the respective weather conditions, resulting in the erratic nature of blast epidemics (Cardoso et al. 2008; Cruz et al. 2016; Cruz and Valent 2017). For example, in 2017 first-crop experiment, the wheat heading dates of genotypes S2 (May 31st), S1 and MR2 (June 3rd) were followed by a rainy period (27.4, 19.6

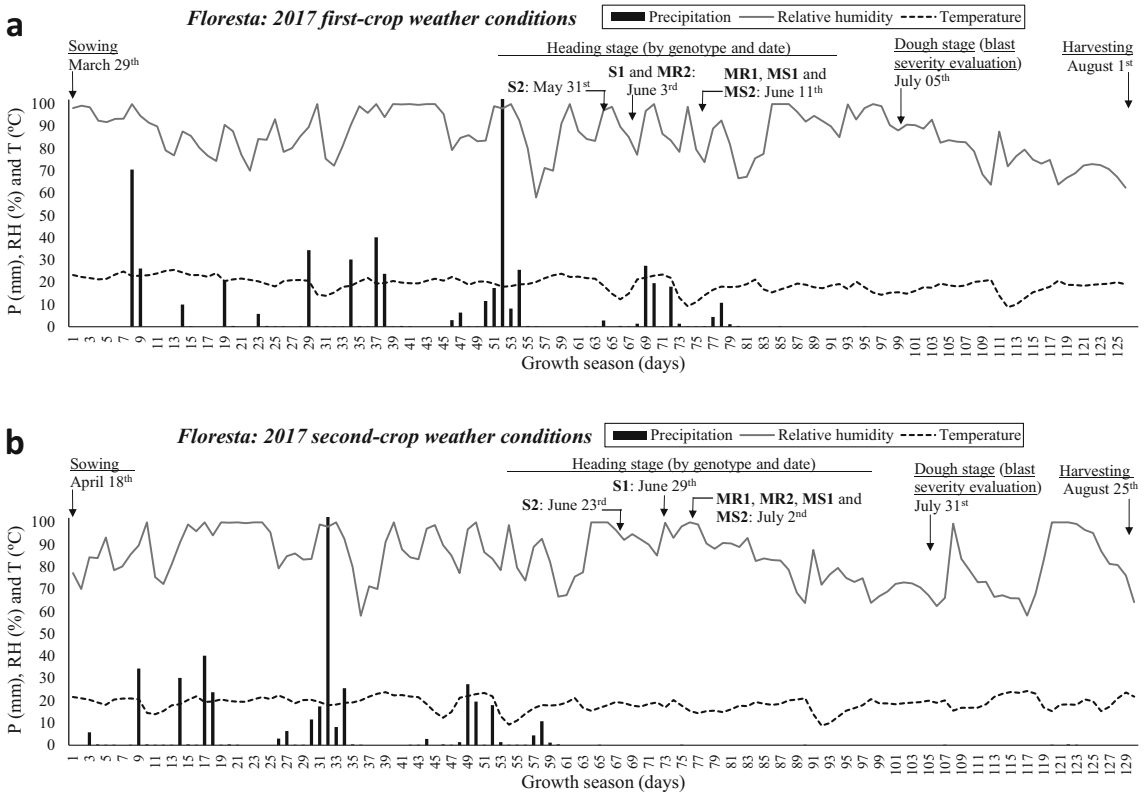

Fig. 4 Blast severity on spikes and grain yield from six wheat genotypes (S1, S2, MR1, MR2, MS1 and MS2) fertilized with three rates of N (0, 60 and 120 kg/ha), in two crops in 2017 (First: **a** and **b**; Second: **c**) in Floresta, Paraná (Brazil). Individually for each crop and each wheat genotype, columns comparing N rates followed by the same lowercase letter do not differ significantly (Tukey's test, $P \geq 0.05$). Individually for each crop and each N rate, columns comparing wheat genotypes followed by the same capital letter do not differ significantly. Note: there was no blast disease in the second crop of the 2017 season

and 18 mm on June 5th, 6th and 8th, respectively) associated with an average temperature of 22.6 °C (ranging from 21.9 to 23.4 °C; Fig. 2a), which resulted in high blast severity and low grain yield. The second group of genotypes (MR1, MS1 and MS2) had its heading stage around June 11th, followed by a shorter period of rain (4.4, 10.8 and 1.2 mm on June 13th, 14th and 15th, respectively; Fig. 2a), which resulted in lower blast severity (approximately half that observed in the first group of genotypes) and higher grain yield (2.5 times higher than the first group).

In the first experiment (Londrina environment), genotypes S1 and S2 with the highest blast severity also had the highest yield in the 2015 crop season (Fig. 3a and b). This is an unexpected result. We hypothesize that, in spite of the lower blast severity, MoT restricted grain filling more severely in genotype MR1, likely due to greater obstruction of phloem vessels into rachises, through which photosynthates and nutrients are translocated to the grains. Another hypothesis is related to the differences among the genotypes in relation to the grain-filling phenological stage (from anthesis to grain ripening). If blast infection occurs later at this stage, the loss of yield will be lower (Cruz and Valent 2017), which may have been the case for genotypes S1 and S2.

To date, prescription of N fertilization for wheat has not considered the N–wheat blast interaction in tropical and subtropical agricultural regions where this disease is endemic. Since N can modify blast severity, a cost-benefit ratio should be considered before deciding the N fertilizer rate to be recommended to wheat crop. Therefore, the findings from these field studies have applied significance for farmers, since N fertilization is widely used in most wheat crop systems.

In conclusion, N fertilization can increase wheat blast severity when weather conditions are favorable for MoT infection in the rachis. For improving the management of this disease, N fertilization technique should be improved, and suitable sowing date chosen, in order to reduce the likelihood of weather conditions favorable for wheat blast development during the post-heading phenological stage. However, its implementation requires accurate information on the probability of disease infection based on historical records and predictive models.

Acknowledgements This research was supported by Brazilian Agricultural Research Corporation (Embrapa), Agricultural Research Institute of Paraná (IAPAR), Fundação Meridional de Apoio à Pesquisa Agropecuária, and COCAMAR Cooperativa Agroindustrial. We thank Fernando Portugal, Danilo Nassar, Caio Mazeto, Leandro Borsato, and Márcio Nunes for their technical assistance. Finally, the authors are

grateful to Dr. Leandro Dallagnol who handled our submission and the three anonymous reviewers for their valuable questions, comments and suggestions, which improved the final quality of this work.

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