Eur J Plant Pathol (2019) 153:327–339
https://doi.org/10.1007/s10658-018-1578-5

MAJOR REVIEWS

Saprotrophic survival of *Magnaporthe oryzae* in infested wheat residues

Carlos Augusto Pizolotto · João Leodato Nunes Maciel ·
José Maurício Cunha Fernandes · Walter Boller

Accepted: 19 August 2018 / Published online: 19 September 2018

Abstract Wheat blast caused by *Magnaporthe oryzae* is a relatively new disease that has caused considerable losses in wheat fields of several South American countries, including Brazil. The 2016 report of wheat blast occurrence in Bangladesh raised concern in South Asia where wheat represents a significant crop. The sources of primary inoculum and survival from season to season of the fungus remain largely unknown. The effect of wheat residues on the onset of blast epidemics and the potential for survival of *M. oryzae* in the residues were studied under subtropical climatic conditions, in the South of Brazil. The objective of this study was to monitor the saprotrophic development of *M. oryzae* on wheat debris and explore the relative importance of crop residues as a source of inoculum. The wheat cultivars BRS 229 and Anahuac 75, moderately and highly susceptible to the disease, respectively, were inoculated with a spore suspension of 10^{-5} conidia mL^{-1} using an aggressive (*Py* 12.1.209) and a less aggressive (*Py* 12.1.132) isolate. At maturity, a portion of leaves, stems and spikes were detached from plants, and a group of ten lesions were randomly selected and marked on each type of plant organ. The air-dried plant organs were placed separately inside bags and exposed outside. The experiment was conducted over three different time intervals. Each 14 days, samples were taken from the field and tested for sporulation. The survival of the blast fungus decreased rapidly on the rachis when compared to stems and leaves. Sporulation of the fungus was observed on the wheat residues for up to five months. Based on the results of this study, the possibility that the causal agent of wheat blast survives under Brazilian conditions from one crop to another in wheat residues is very low. The management of crop residues is not a key point to control the development of wheat blast. A strong emphasis should be placed on the presence of other hosts.

Keywords Wheat blast · *Triticum aestivum* · Primary inoculum · Wheat straw · Disease cycle

Introduction

Wheat (*Triticum aestivum* L.) is an important crop in Brazil responsible for approximately half of the domestic needs. The wheat-growing regions are divided into three main zones: the Central, South-Central and South. The latter, constitutes about 90% of the growing area but, yield and quality are frequently reduced by

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C. A. Pizolotto (✉)
CAPES/Embrapa Scholarship in the Programa de Pós-Graduação em Agronomia da Universidade de Passo Fundo (PPGAgro/UPF), Av. Brasil Leste, 285 - São José, Passo Fundo, RS 99052-900, Brazil
e-mail: carlos.pizolotto@yahoo.com.br

J. L. N. Maciel · J. M. C. Fernandes
Centro Nacional de Pesquisa de Trigo (Embrapa Trigo), Rodovia BR 285, Km 294, s/n - Zona Rural
Passo Fundo, RS 99050-970, Brazil

W. Boller
Programa de Pós-Graduação em Agronomia da Universidade de Passo Fundo (PPGAgro/UPF), Av. Brasil Leste, 285 - São José
Passo Fundo, RS 99052-900, Brazil

excessive rain and fungal diseases (Munaro et al. 2014). As a result, wheat production has been gradually moving to the Central region known as Cerrado under irrigation or rainfed conditions (Galindo et al. 2017). In Central and South-Central mega-environments, wheat blast outbreaks occur every 4 to 5 years. Fernandes et al. (2017) reported that increased humidity and warm temperatures were associated with wheat blast outbreaks. Importantly, wheat blast represents a considerable impediment to wheat production in the Cerrado region which represents a potential of 5 million hectares for expansion (Ceresini et al. 2018; Bornhofen et al. 2018).

Wheat blast is caused by the ascomycetous fungus *Magnaporthe oryzae* (Catt.) B.C. Couch 2002 (anamorph *Pyricularia oryzae* Cavara 1892) (Couch and Kohn 2002; Zhang et al. 2016). The emergence of the wheat blast-causing pathogen is relatively recent and dates back to 1985 when the pathogen was first reported in the state of Paraná in southern Brazil (Igarashi et al. 1986). The fungus *M. oryzae* is subdivided into multiple lineages with limited host range, i.e., *Oryzae*, *Triticum*, *Lolium*, *Setaria*, among others (Gladieux et al. 2018). Cruz and Valent (2017) provide an extensive and updated review of the importance, emergence and spread of wheat blast in South America and, more recently, in Bangladesh, Asia.

The economic importance of this disease arises from the fact that the fungus can reduce wheat yield and quality (Goulart 2005). Highest yield losses occur when spike infections start during flowering or early grain formation (Goulart et al. 2007). The disease can attack all above-ground parts of the plant, but severe spike infection can be observed with a minimal infection on the leaves or culms. Yield losses up to 100% have been reported in susceptible cultivars (Kohli et al. 2011). Fungicides are ineffective under high disease pressure and partially effective under moderate-to-low pressure. A 2NS translocation segment from *Aegilops ventricosa* was reported to confer moderate resistance to wheat blast in some genotypes (Cruz et al. 2016). However, in Bolivia, during the 2015 epidemic, the best resistance available was insufficient for controlling blast (Vales et al. 2018).

The reasons for the erratic occurrence of wheat blast epidemics are unknown, but one possible explanation is that outbreaks are a consequence of specific weather conditions favoring inoculum build-up before the reproductive stage of wheat (Fernandes et al. 2017). In Central and South-Central regions, wheat is cultivated in sequence with summer crops such as soybean and corn. Typically, wheat cultivars have short cycles of 110–120 days and yield is around 2–3 tons ha^{-1}. In theory, a wheat crop can return to the same plot at 9-month intervals. In the landscape, areas with native grasses or cultivated with crops considered as potential hosts to *M. oryzae* are common and could contribute to inoculum load for wheat (Marangoni et al. 2013; Chávez and Kohli 2015; Castroagudin et al. 2016).The prevalence of rain and warm temperatures warrant pathogen survival during summer and autumn months on various plant materials. Inoculum build-up potentially involves dispersal of multiple fungal structures such as conidia and mycelia from over summer refugia to crop locations.

The *M. oryzae* fungus is a hemibiotrophic pathogen that has an initial biotrophic phase followed by a necrotrophic phase (Horbach et al. 2011). *Magnaporthe oryzae* can survive in living and dead tissues of many species of Poaceae (Maciel et al. 2014), in addition to surviving on infected seeds (Dias Martins 2004; Reis et al. 1995). The widespread adoption of no-tillage practices in wheat growing regions in Brazil may contribute to extending the survival period. Therefore, crop debris on the soil surface may provide an adequate substrate for survival and multiplication of inoculum.

The importance of agricultural residues as potential sources of inoculum may depend on several factors. These include disease intensity in the previous crop, crop sequence, residue decomposition rate, pathogen competitiveness and sporulation potential, and climatic conditions (Fernandez et al. 1993; Li et al. 2014). Harmon and Latin (2005), in the state of Indiana, USA, showed that *M. oryzae* could overwinter in crop residues of previously infected ryegrass (*Lolium multiflorum* Lam.). However, with a temperate climate, the magnitude of survival of the pathogen may be insufficient for these populations to serve as a source of primary inoculum to result in a blast outbreak during the summer. In contrast, under upland conditions in Madagascar, sporulation of the fungus was observed on rice stems left on the mulch for up to 18 months. It was concluded that under field conditions, the presence of infected rice residues could initiate an epidemic of blast (Raveloson et al. 2018).

It is widely accepted that pathogen inoculum on straw at the soil surface gives a higher risk for disease at conditions favorable for disease development, but the knowledge about factors influencing pathogen survival on straw is limited. In Brazil, with a subtropical climate, the relative importance of wheat residues as a reservoir of primary inoculum of *M. oryzae* for the next wheat crop is unknown. Therefore, the objective of this study was to investigate the persistence of *M. oryzae*, on decaying straw under varying conditions. The survival assessment of *M. oryzae* on rotting wheat residues should provide a better understanding of the role of residues as an inoculum source.

Materials and methods

The work was carried out in two parts, the first in the greenhouse and the second in the field, both at the experimental station of Embrapa Wheat, Passo Fundo, RS, Brazil (28^{o}15' S, 52^{o}24' W). Local climate is type Cfa (temperate rainy climate) according to the classification of Köppen, and the soil is a typical dystrophic Red Latosol (Streck et al. 2008).

The experimental design was a completely randomized block design with three factors: plant organs (spike, stem and leaf), wheat cultivars (Anahuac 75 and BRS 229) and *M. oryzae* isolates (*Py* 12.1.132 and *Py* 12.1.209). The experiment was performed three times during different periods: August 2015 to February 2016 (experiment A); June 2016 to November 2016 (experiment B); December 2016 to March 2017 (experiment C).

Wheat plants

The choice of the wheat cultivars used in this study was based on previous studies indicating that Anahuac 75 was more susceptible to wheat blast than BRS 229 (data not shown). Anahuac 75 is a cultivar developed by CIMMYT (International Maize and Wheat Improvement Center), released for commercial cultivation in Mexico in 1975 (CIMMYT 1977) and introduced in Brazil in the late 1970's. BRS 229 is a cultivar developed by Embrapa and commercially released in 2004 (Brunetta et al. 2006). Five seeds from each cultivar were planted in ten plastic pots measuring 22 cm in diameter and containing eight liters of soil. A total of 20 pots were placed in a glass greenhouse with 24 °C day / 19.5 °C night temperature regime. During the period that the plants remained in the greenhouse, the typical light regime/day length for this region was approximately 13 h.

The chemical composition from plant tissues was measured in both wheat cultivars. The observed chemical composition was analyzed by the Near Infrared (NIR) Spectroscopy. The principle of NIR analysis consists of the application of different wavelengths, based on the different characteristics of absorption and dispersion of light, it evaluates, quantitatively and qualitatively, the molecular components of biological tissues.

The samples were dried at a temperature of 60 °C under air circulation for 48 h. After drying, the samples were milled (1 mm) in a Wiley type mill and submitted to NIR. Subsequently, the samples were analyzed by the physicochemical method for the following parameters: DM = dry matter; ADF = acid detergent fiber; CP = crude protein; DMS = dry matter digestibility; NDF = neutral detergent fiber; NDT = total digestible nutrients.

Fungal isolates

The isolates of *M. oryzae* used in this study were selected from the collection of isolates archived at Embrapa Wheat. This collection assembled in 2012 contains isolates from different regions of Brazil. Intentionally, it was selected an aggressive (*Py* 12.1.132) and a less aggressive (*Py* 12.1.209) isolate. The aggressiveness of each isolate was based on susceptible reaction of wheat cultivars at seedling and head stage of wheat (Danelli 2015).

Both isolates were obtained from symptomatic wheat plants; *Py* 12.1.132 from plants collected in the municipality of Amambai, MS (19°02' S, 55°16' W), and the isolate *Py* 12.1.209, from plants collected in the municipality of São Borja, RS (28^{o}41' S, 55^{o}16' W). The isolates were grown on agar plates containing oatmeal agar (oatmeal, 60 g/l). For the inoculum preparation, the plates were flooded with distilled water plus Tween 80® (0.01%) and a brush was used to dislodge conidia. The conidia suspension which was adjusted to 10^5 spores mL^{-1}.

Infected wheat residues

Both wheat cultivars were inoculated at growth stage 61 of the Zadoks et al. (1974) scale using a handheld plastic atomizer. After inoculation, the plants were maintained in a growth chamber for 24 h in the dark at 24 °C and under RH > 90% (Fig. 1a). After 24 h, the photoperiod was adjusted to 12 h and the RH to approximately 60–70%. The plants remained under these conditions for

10 days coinciding with the observation of wheat blast symptoms.

At maturity, spikes, stems and leaves were removed from symptomatic wheat blast infected plants of both wheat cultivars and for each isolate. On the stems and leaves, blast lesions were marked with a non-toxic red ink (Fig. 1b).

Stems, leaves and spikes were packed separately into nylon bags (0.5 × 0.5 m); the combination of the isolate, cultivar and type of residue resulted in a total of 12 bags. These bags were tagged and distributed in the field in an area of land covered with grasses including ryegrass (*Lolium multiflorum* Lam.) (Fig. 1c).

Field monitoring of wheat residues

Samples from each bag were returned to the laboratory at 14-day intervals. Five randomly selected sub-samples with two marked lesions were removed from each bag with stems and leaves. These parts were cut into 5 cm length segments (Fig. 1d) and washed under running water. Then, the segments were soaked in 0.5% sodium hypochlorite solution for 1 min and then rinsed under running water for 1 min.

A group of five spikes were randomly selected from bags containing spikes. The spikelets were removed from the spike, leaving the rachis with the infection points. Finally, the rachises and the 5 cm segments of stems and leaves were placed on top of moistened blotting paper inside transparent plastic boxes (35 mm high × 110 mm wide × 110 mm long). A group of 10 lesions were selected randomly on each organ of the plant (Fig. 1e).

The plastic boxes were transferred to a growth chamber with a photoperiod of 12 h and a temperature of 25 ± 2 °C for 3 days to promote *M. oryzae* sporulation. After 3 days, the marked lesions were examined under a dissecting microscope for the presence/absence of conidiophores bearing *M. oryzae* conidia (Fig. 1f). A score scale of 0 to 1 (0 = absence of sporulation and 1 = presence of sporulation) was established.

Weather data

Weather data were obtained from the INMET (National Institute of Meteorology) automated weather station network. The automated weather station was located at approximately 700 m from the experimental site. Hourly temperature (°C), relative humidity (%), and rainfall (mm/h) readings were available.

Statistical analyses

All statistical analyses were performed using the R statistical package (R DEVELOPMENT CORE TEAM 2017). Initially, a descriptive analysis was performed to determine absolute and relative frequencies and compare cultivars, isolates, plant organs and periods of exposure wheat residues in the field. The data associated with the survival of *M. oryzae* during the experimental periods was submitted to survival analysis using the package survival (Therneau and Grambsch 2000; Modeling Survival Data: Extending the Cox Model. Springer, New York, ISBN 0–387–98,784-3). Kaplan–Meier analysis of survival was performed, with a cumulative probability of survival based on sporulating estimated days, according to each variable of interest and period of exposure of wheat residues to the environment..By convention, the Kaplan-Meier plots are represented with steps to indicate the time in which the terminal event (sporulation ceases) occurs and signs (+) to indicate censored observations (Goel et al. 2010). Statistical significance was assessed by the log rank test, which evaluated the null hypothesis that there was no difference between the survival curves of each treatment, i.e., the probability that a lesion ceased sporulation at any point in time was the same in all cultivars, plant organs, isolates or periods of exposure of wheat residues in a field.

A Cox regression or proportional hazards model was used to calculate the risk or hazard ratio (HR) in survival analysis, with a confidence interval (CI) of 95%. Univariate analysis was followed by multivariate analysis. Associations were considered statistically significant with a significance level of less than 5%.

Results

There were statistical differences ($p < 0.0001$) among the three experiments (Fig. 2). Comparing the survival curves among experiments, a significant difference in survival rate was observed. The duration of survival time for *M. oryzae* on wheat residues was approximately 140, 160, 80 in experiments A, B and C, respectively (Fig. 2). In experiment C, the sporulation period of the pathogen was less (Fig. 2), although during this

Fig. 1 tiff. Chronological sequence used in experiments **a**, **b** and **c**. Plants in the growth chamber after inoculation (1A); Blast lesions on wheat residues circled with red ink using an atomic brush (1B); Nylon bags with the inoculated wheat residues distributed in the field in an area with ryegrass (1C); Samples of the wheat residues with a mean size of 5 cm, with the lesions previously encircled in red (1D); Five specimens of each vegetative organ with a group of ten lesions were selected randomly and placed in transparent plastic boxes (1E); Lesions marked in red were examined under a dissecting microscope for conidiophores carrying *P. oryzae* conidia (1F)

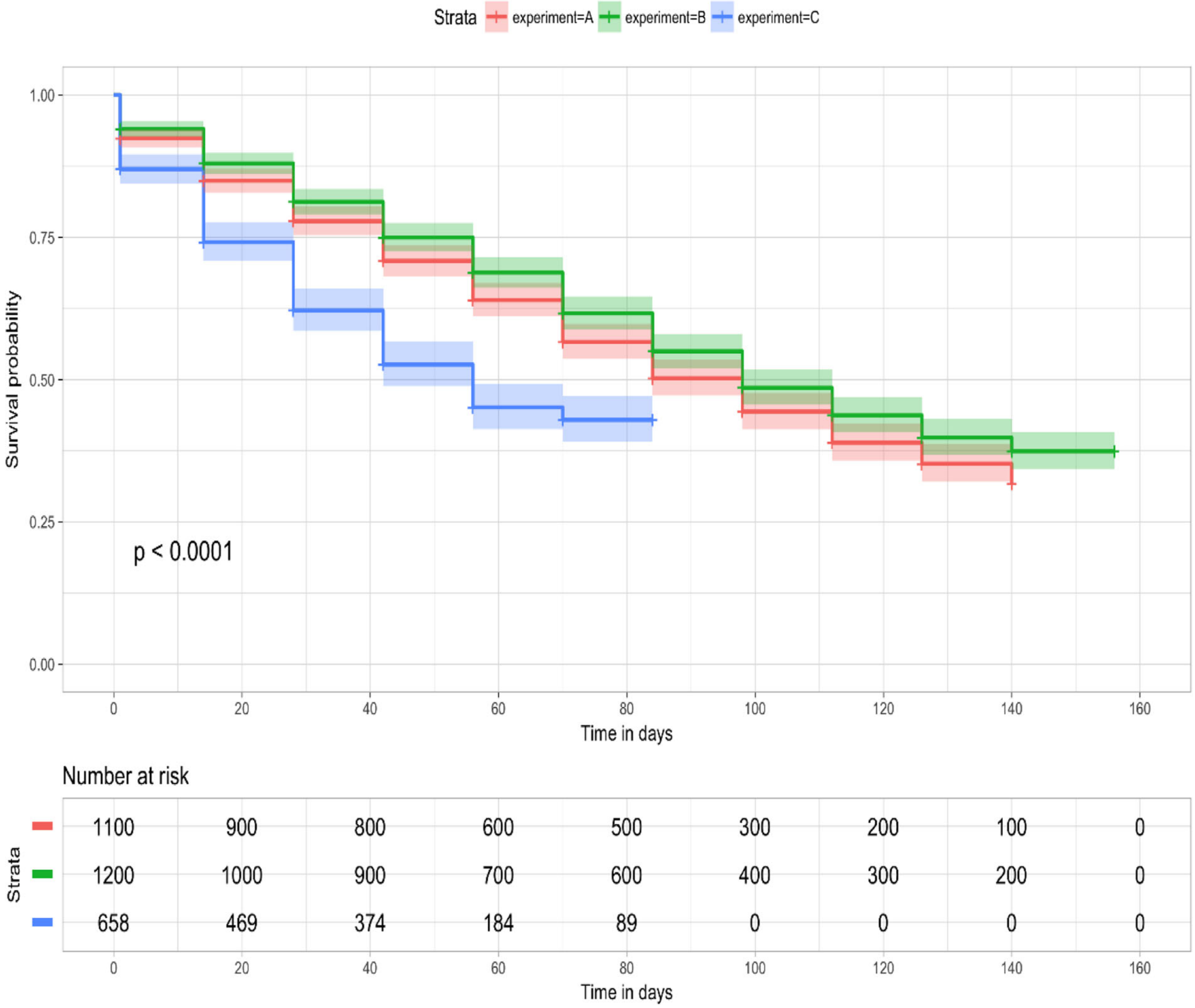
experiment the lowest temperature was never negative, differently from experiments A and B, where was observed a reduction in sporulation rate of the pathogen when exposed to negative temperatures. Additionally, the cumulative precipitation was 537.6 mm in experiment C, whereas in experiments A and B, cumulative rainfall was 1218.8 and 886.4 mm, respectively. To summarize, the number of sporulating lesions was reduced drastically at each observation in experiment C, whereas in experiments A and B, the reduction occurred more gradually.

The results of the univariate Cox analysis are presented in Table 1. Among the experiments A, B and C, the sporulation rate on wheat residues in experiment C was lower, reducing the survival period of *M. oryzae* (HR = 1.6, Se = 0.069). In the comparison of the two isolates, the survival rate of isolate *Py* 12.1.209 was higher (HR = 1.1, Se = 0.050); however, the rate was not significantly different from that of *Py* 12.1.132. The longest survival period of the fungus isolates was observed in cultivar BRS 229 (HR = 0.9, Se = 0.050); although compared with cultivar Anahuac 75, the difference was not significant. For the three organs, the lowest probability of survival of *M. oryzae* over time was on the rachises (HR = 1.8, Se = 0.063).

The final multivariate Cox regression model is presented in Table 2. The differences and similarities among the survival characteristics determined in the univariate Cox analysis were the same as those determined in the Cox multivariate analysis. The predictive variables for the reduction in survival time of *M. oryzae* in wheat residues were as follow: experiment C (HR = 1.6, CI 1.42–1.86), the cultivar BRS 229 (HR = 0.9, CI 0.80–0.97), the isolate *Py* 12.1.209 (HR = 1.1, CI 0.99–1.21), and the rachis (HR = 1.8, CI 1.59–2.04).

During the three experiments, variations occurred in the temperature gradient, which indicated that the *M. oryzae* fungus could survive in the crop residues even with large thermal oscillations. The thermal amplitude in experiment A was 33.1 °C, in experiment B 34.0 °C, and in experiment C 20.7 °C. However, a reduction in sporulation rate of the pathogen was observed when exposed to negative temperatures in experiments A and B. In experiment B, the sporulation rate on wheat crop residues was lower than that in experiments A and C, demonstrating a strong influence of temperature on crop residue decomposition.

In experiment A, carried out from August 2015 to February 2016, rainfall occurred in 73 days, and the average temperature on days when rainfall was recorded



Strata	0	20	40	60	80	100	120	140	160
🟥	1100	900	800	600	500	300	200	100	0
🟩	1200	1000	900	700	600	400	300	200	0
🟦	658	469	374	184	89	0	0	0	0

Fig. 2 tiff. Kaplan-Meier curves calculated for each experiment over cultivars Anahuac 75 and BRS 229 and both *M. oryzae* isolates (*Py* 12.1.132 and *Py* 12.1.209) ($N = 360$). Short vertical lines represent censored data, and shaded colours represent 95% confidence intervals. Numbers at risk are indicated

was 19.7 °C. The duration of time for *M. oryzae* survival on wheat residues was approximately 140 days (Fig. 2) and the accumulated rainfall during the whole period was 1218.8 mm (Fig. 3a), which on average represented 8.75 mm of rain/day of experiment. The number of days it rained at least 1 mm was 63. On average, this means that it rained at least 1 mm every 2.22 days. The minimum temperature of −0.3 °C was observed on September 12 and the maximum of 32.8 °C was observed on January 24. The average temperature throughout the experiment was 25.5 °C (Fig. 3a).

In experiment B, carried out from June 2016 to November 2016, rainfall was recorded in 62 days, and the average temperature on rainy days was 14.5 °C. The duration of time for *M. oryzae* survived in wheat residues was approximately 160 days (Fig. 2) and the accumulated rainfall during the whole period was 886.4 mm (Fig. 3b), which, on average, represents 5.54 mm of rain/day of experiment. The number of days it rained at least 1 mm was 44. On average, this means that it rained at least 1 mm every 3.64 days. The minimum temperature of −0.3 °C was observed on September 12 and the maximum temperature of 32.8 °C was observed on January 24. The average temperature throughout the experiment was 25.5 °C (Fig. 3b).

In experiment C, carried out from December 2016 to March 2017, rainfall was recorded in 52 days and the mean temperature during rainy days was 22.8 °C. The

Table 1 Univariate Cox model analysis of predictive variables of the number of *M. oryzae* lesions. Passo Fundo, Brazil, 2015-2017

Survival character		*N* = 360		*p* value
		HR	Se	
Experiment	A	–	–	–
	B	0.9	0.057	0.009
	C	1.6	0.069	<0.006
Isolate	*Py* 12.1.132	–	–	–
	Py 12.1.209	1.1	0.050	0.160
Cultivar	Anahuac 75	–	–	–
	BRS 229	0.9	0.050	0.04
Organ	Leaf	–	–	–
	Stem	1.1	0.059	0.320
	Rachis	1.8	0.063	<0.002

HR hazard ratio, *Se* standard error

lowest temperature of 11.9 °C, was observed on February 7 and the highest of 32.6 °C was observed on December 26. The duration of time for *M. oryzae* survived on wheat residues was approximately 80 days (Fig. 2) and the accumulated rainfall during the whole period was 537.6 mm (Fig. 3c), which, on average, represents 6.72 mm of rain/day of experiment. The number of days that rained at least 1 mm was 40. On average, this means that it rained at least 1 mm every 2.0 days. The average temperature throughout the experiment was 23.0 °C (Fig. 3c).

During the five months of experiment A, the average temperature during rainy days was close to 20.0 °C, and the cumulative rainfall volume was approximately two-thirds of the total forecast for the entire year. With the highest volume of rain accumulated in experiment A among the three experiments, the sporulation rate on wheat crop residues in A was higher than that in experiment B; however, the rate was lower than that in experiment C, because the temperature on the days with rainfall was higher in experiment C.

Survival analysis revealed no significant differences ($p < 0.16$) in the survival curves between the isolates *Py* 12.1.132 and *Py* 12.1.209 of *M. oryzae* over time in all three experiments (Fig. 4). The total number of sporulating *M. oryzae* lesions in the wheat residues in all three experiments varied according to the pathogen survival period. At time zero, the sum of sporulating lesions found in rachises, leaves and stems was higher for isolate *Py* 12.1.132 at 1497 than that for isolate *Py* 12.1.209 at 1461 (Fig. 4), even both isolates were statistically equal at that time. From time zero to time 140, the number of sporulating lesions of both isolates gradually declined. At time 140, the number of sporulating lesions was very similar for both isolates, with 152 lesions for isolate *Py* 12.1.132 and 148 lesions for isolate *Py* 12.1.209. At time 154, no sporulation lesions were observed in the wheat residues for either of the isolates.

The survival of the two *M. oryzae* isolates in the wheat residues of the two cultivars over the three experiments was significantly different (Fig. 5). Comparing the total number of lesions in the three experiments, the number of sporulating lesions was higher in BRS 229

Table 2 Multivariate Cox model analysis of predictive variables of the number of *M. oryzae* lesions. Passo Fundo, Brazil, 2015-2017

Survival character		Total number of lesions	Number of sporulate lesions	HR	95% CI	*p* value
Experiment	A	1.200	1.100	–	–	–
	B	1.320	1.200	0.9	0.78–0.97	0.010
	C	840	658	1.6	1.42–1.86	<0.001
Isolate	*Py* 12.1.132	1.680	1.497	–	–	–
	Py 12.1.209	1.680	1.461	1.1	0.99–1.21	0.071
Cultivar	Anahuac 75	1.680	1.460	–	–	–
	BRS 229	1.680	1.498	0.9	0.80–0.97	0.012
Organ	Leaf	1.200	1.194	–	–	–
	Stem	1.320	1.200	1.1	0.94–1.19	0.330
	Rachis	840	564	1.8	1.59–2.04	<0.002

HR hazard ratio, *CI* confidence interval

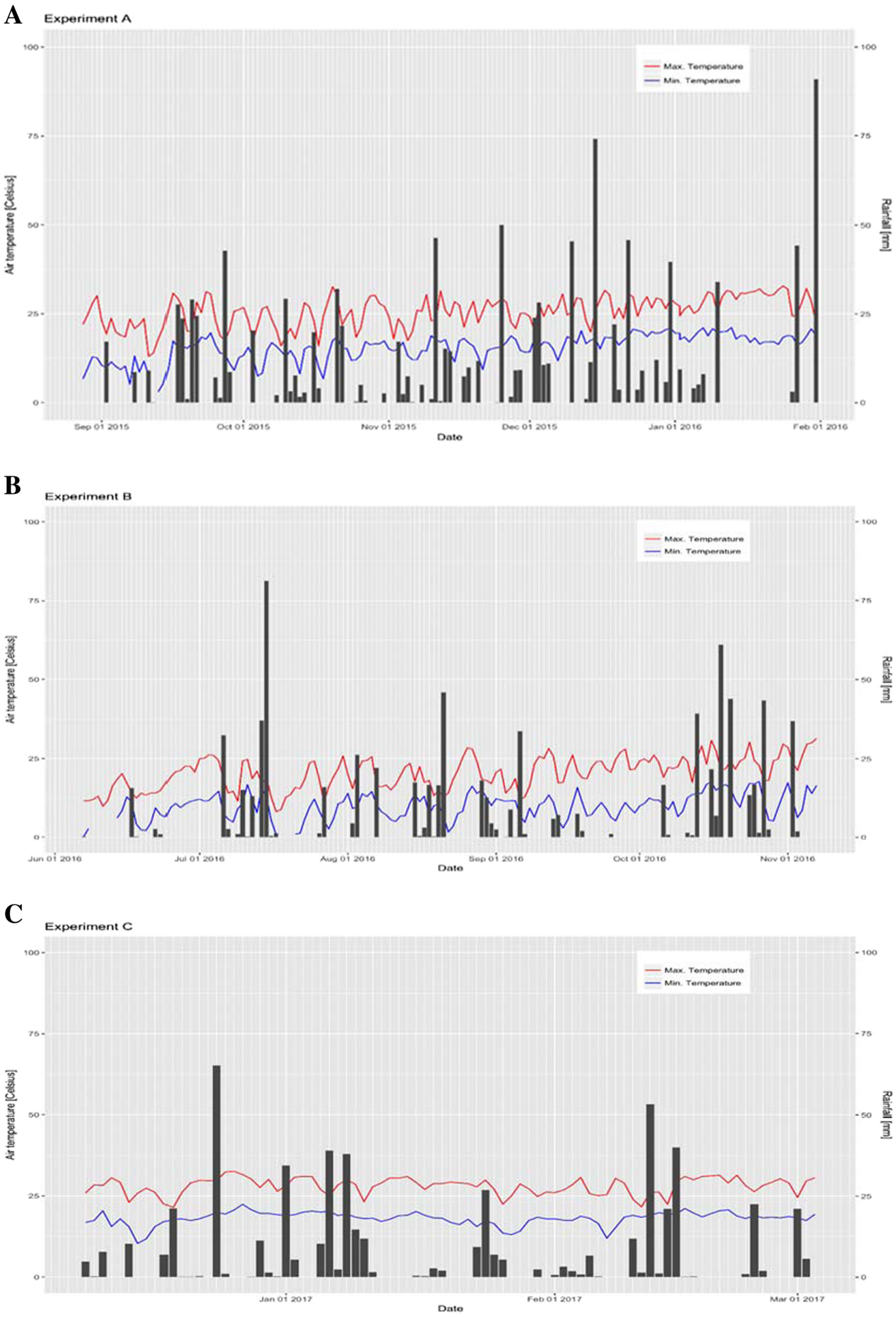


Fig. 3 tiff. Characterization of climatic conditions in Passo Fundo, RS, Brazil, during experiments **a**, **b** and **c**

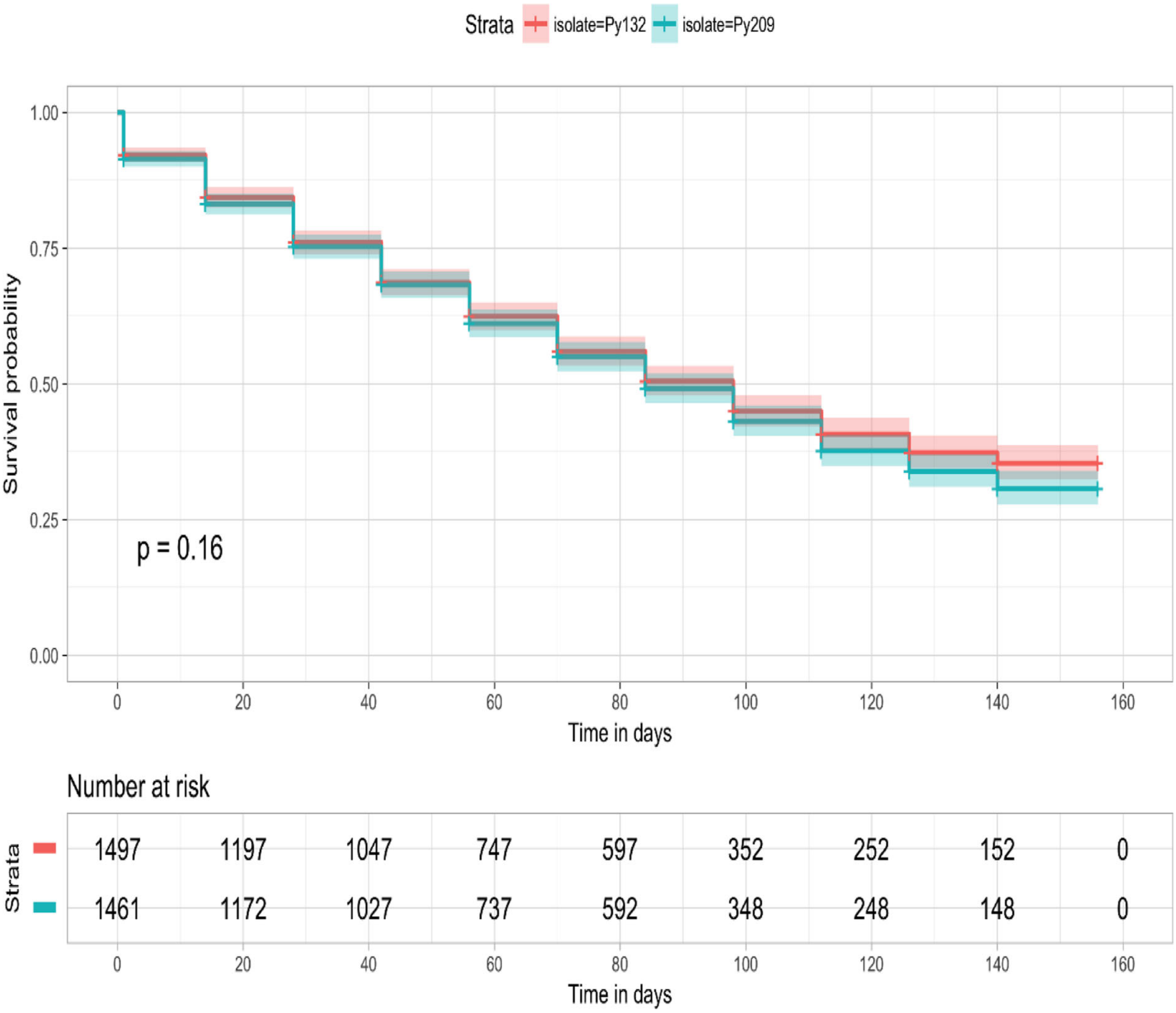


Fig. 4 tiff. Kaplan-Meier curves calculated for isolates *Py* 12.1.132 and *Py* 12.1.209 of *M. oryzae* colonizing Anahuac 75 and BRS 229 during all three experiments ($N = 120$). Short vertical lines represent censored data, and shaded colours represent 95% confidence intervals. Numbers at risk are indicated

than in Anahuac 75 residues. However, the difference was only observed from time zero to time 84 after which the number of sporulating lesions in both cultivars was the same. Despite this difference observed between the two cultivars at the initial evaluation phase of the sporulation capacity, the NIRS method (Table 3) showed a great similarity between the two wheat genotypes evaluated in relation to their chemical composition.

The results showed that the number of sporulating lesions on leaves, spikes and stems diminished with time (Fig. 6). Sporulation was not observed after 154 days of exposure of wheat residues in a field. A shorter survival curve was observed on the spikes than that on leaves and stems. The survival curves for the isolates were similar. In experiments A, B and C, the shortest period of survival of the pathogen in the wheat residues occurred on the rachises, and the number of sporulating lesions declined from the beginning of the laboratory evaluations.

Discussion

This study showed that climatic variables directly interfered with the time of decomposition of plant residues and, most likely, with the rate and period of active

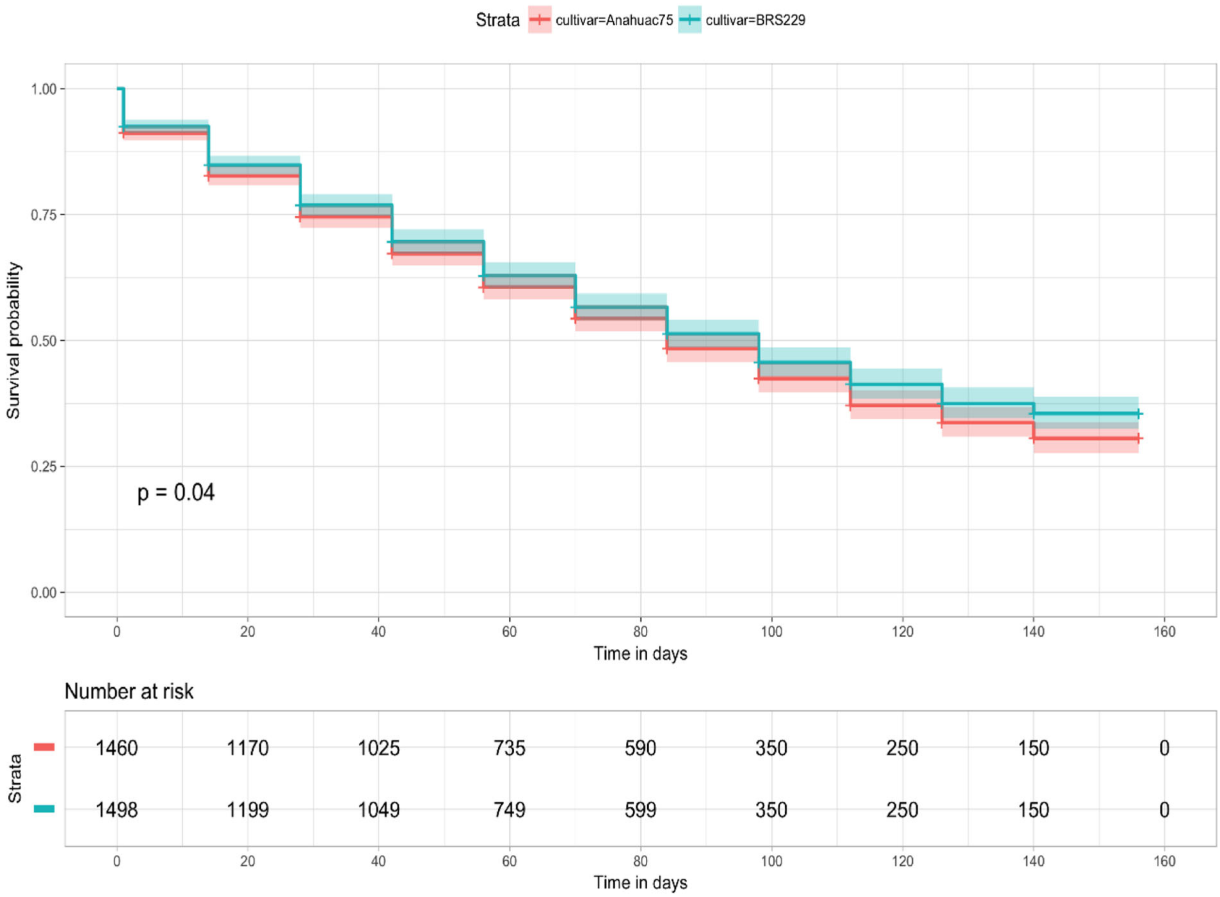


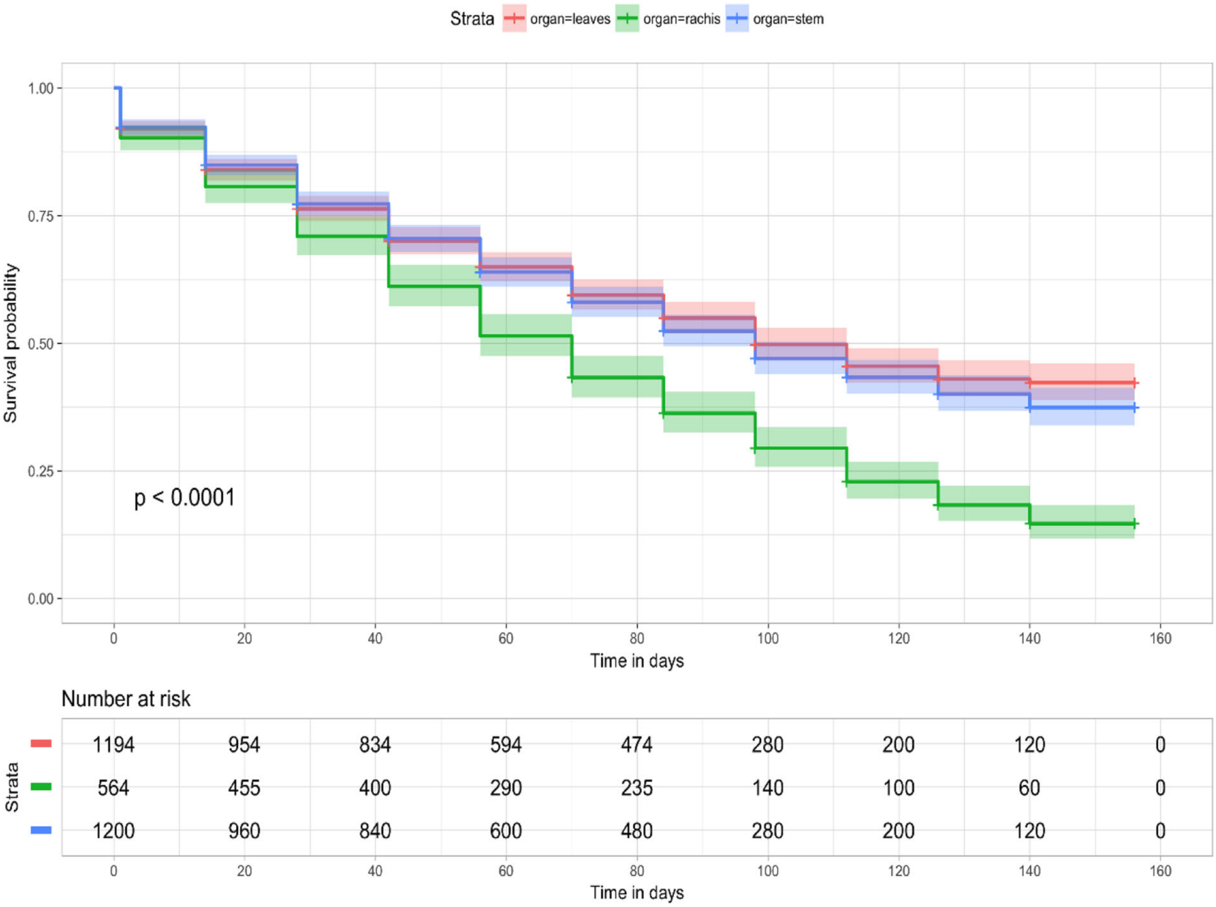
Fig. 5 tiff. Kaplan-Meier curves calculated for cultivars Anahuac 75 and BRS 229 for both *M. oryzae* isolates (*Py* 12.1.132 and *Py* 12.1.209) and all three experiments ($N = 120$). Short vertical lines represent censored data, and shaded colours represent 95% confidence intervals. Numbers at risk are indicated

sporulation of the pathogen. In this context, the most notable result of this study was the variation of the period of sporulation of *M. oryzae* in wheat plants residues, which varied from 140 and 160 days in August 2015 to February 2016 and June 2016 to November 2016, respectively, to 80 days in December 2016 to March 2017 (Fig. 2). Among the environmental factors affecting exposed residues, we concluded that temperature had the most important effect in the sporulation activity of *M. oryzae*. Notably, the lower mean temperatures observed during August 2015 to February 2016 and June 2016 to November 2016 were critical for *M. oryzae* to keep sporulating for a longer period than in December 2016 to March 2017. It is plausible to speculate that interaction among factors such as rainfall amount, rainfall frequency, and temperature that occurred throughout the experiments may have had some influence in the difference observed among the three experiments. However, nothing appears to have been more determining and important than the lower mean

Table 3 Chemical composition of Anahuac 75 and BRS 229 wheat cultivars based on the Near Infrared (NIR) Spectroscopy

Bromatological composition	Cultivar	
	Anahuac 75	BRS 229
DM (%)	16.01	15.25
Composition with DM (%)		
ADF	23.56	22.17
CP	18.04	20.00
DMS	70.54	71.63
NDF	54.72	51.56
NDT	71.34	72.32

DM dry matter, *ADF* acid detergent fibre, *CP* crude protein, *DMS* dry matter digestibility, *NDF* neutral detergent fibre, *NDT* total digestible nutrients



Strata	0	20	40	60	80	100	120	140	160
🟥	1194	954	834	594	474	280	200	120	0
🟩	564	455	400	290	235	140	100	60	0
🟦	1200	960	840	600	480	280	200	120	0

Fig. 6 tiff. Kaplan-Meier curves calculated for organs of Anahuac 75 and BRS 229 over both *M. oryzae* isolates (*Py* 12.1.132 and *Py* 12.1.209) and all three experiments ($N = 360$). Short vertical lines represent censored data, and shaded colours represent 95% confidence intervals. Numbers at risk are indicated

temperatures during August 2015 to February 2016 and June 2016 to November 2016.

Furthermore, it was evident that the decline in the proportion of sporulation lesions was much more distinct in December 2016 to March 2017 than in August 2015 to February 2016 and June 2016 to November 2016. For instance, in the first 20 days after the first evaluation, this great difference among the experiments was evident (Fig. 2). At that period, there were reductions of 18.18%, 16.67 and 28.72% in the percentage of sporulating lesions in August 2015 to February 2016, June 2016 to November 2016 and December 2016 to March 2017, that is, the number of sporulating lesions ranged from 1100 to 900, from 1200 to 1000, and from 658 to 469, respectively.

Even though the total number of days with sporulation activity in August 2015 to February 2016 and June 2016 to November 2016 (140 and 160 days, respectively) were relatively similar, they were statistically different. This difference was higher or lesser in each one of the 11 evaluations that were conducted in both experiments (Fig. 2). It is possible to list some factors which may have favored the variation between the two experiments. The temperature in the days with rainfall was higher in August 2015 to February 2016 (19.7 °C) than in June 2016 to November 2016 (14.5 °C), the total volume of rainfall was higher in August 2015 to February 2016 (1218.8 mm) than in June 2016 to November 2016 (886.4 mm), or the interval of time without rainfall was lower August 2015 to February 2016 (2.2 days) than in June 2016 to November 2016 (3.64 days). However, what seems to be the most likely is that the combination of these factors was the actual factor that provided a more favorable condition for the sporulation activity period in

June 2016 to November 2016 to be longer than in August 2015 to February 2016.

The two isolates of *M. oryzae* used in the experiments were obtained from places very far from each other, separated by more than 700 km of distance north-south and 10 degrees of latitude; *Py* 12.1.132 (19°02' S, 55°16' W) e *Py* 12.1.209 (28°41' S, 55°57' W). Another important point in the difference between the two places is the following. *Py* 12.1.132 is from a region where the occurrence of wheat blast is quite common and is also one of the reasons why growers in the region are abandoning wheat cultivation (Callaway 2016). In contrast, *Py* 12.1.209, was obtained from a place where the wheat blast is of no economic importance and the climatic conditions are not the most favorable for it. Despite the climatic differences between the sites to which the isolates were obtained, no difference was observed in survival between them (Fig. 4). The results also point out that the differences in virulence presented by the two isolates (data not shown), which was the criterion adopted for their choice to be used in the study, had no influence on their survival capacity.

Our study suggests, it is possible to speculate that the main factor responsible for the difference observed between Anahuac 75 and BRS 229 in relation to the survival capacity of *M. oryzae* (Fig. 5) may be related to the different level of genetic resistance to blast present in the wheat cultivars. This condition has already been demonstrated in several studies that showed that BRS 229 is more resistant to wheat blast than Anahuac 75 (Maciel et al. 2014). Besides, due its great susceptibility to blast, the cultivar Anahuac 75 has been used as control in experiments conducted in the field and under controlled conditions (de Asis Reges et al. 2016). According to Inoue et al. (2017), the cultivar Anahuac 75 does not carry the avirulence gene *Pwt*3 whose products elicit defence responses in wheat cultivars containing the corresponding resistance gene *Rwt*3; therefore, the highest rate of decomposition could be associated with greater aggressiveness of the pathogen in the cultivar Anahuac 75 than BRS 229.

Differences in chemical components of wheat residues such as cellulose, hemicellulose and lignin might be associated with the lower survival rate of *M. oryzae* on the rachises in each of the three experiments (Fig. 6). Results obtained in similar evaluations in the pathosystem *Triticum aestivum-Gibberella zeae* also showed similar results. In this sense, Khonga and Sutton (1988) determined that the saprophytic survival of *Gibberella zeae* in wheat residues is organ dependent. According to these authors, stems, leaves, and rachises differ in biochemical composition and therefore in the survival and production of inoculum of the fungus in its saprophytic stage.

Based on the results of this study, the possibility that the causal agent of wheat blast survives under Brazilian conditions from one wheat crop to another in wheat residues is very low. Maximum survival was five months, making it unlikely that wheat residues infested with *M. oryzae* serve as a source of inoculum for wheat blast in the next wheat crop. Raveloson et al. (2018) showed that,in Madagascar, *M. oryzae* survival in rice stems (*Oryza sativa*) over three years varied from 5 to 18 months, with the highest survival period of the fungus observed in the cold season and the lowest survival in the hot and rainy season.

The development of wheat blast in host residues under subtropical climatic conditions demonstrates that the management of crop residues is not a key point for the control of this pathogen. Therefore, we suggest that future studies strongly focus on the presence of other hosts as the source of primary inoculum, for example, weeds of Poaceae.

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