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Bipolaris oryzae seed borne inoculum and brown spot epidemics in the subtropical lowland rice-growing region of Brazil

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Abstract The effect of increasing seed borne incidence levels (0, 3, 6, 12, 24 and 48 %) of *Bipolaris oryzae* on brown spot epidemics and crop performance was studied in eleven field trials. These trials were conducted at two sites (Bagé – BA and Cachoeirinha – CA) in the major rice-growing region of Brazil over three seasons (2008 to 2010). Disease variables assessed over time were disease incidence (INC, %) on leaves prior to flowering, and disease severity (SEV, %) on flag leaves after flowering. Kernel infection (KI, %) by *B. oryzae* was assessed after harvest. Crop-related variables such as plant population density (PD) and yield (YLD) were also assessed. In only three trials, all in the 2009/10 season, which had well above-normal rainfall in the early season, was the disease found at vegetative stages. In those same trials, a significant effect of seed borne inoculum was found for the area under the disease progress curve of INC and SEV. Overall mean SEV at CA (1.67 %) was higher than at BA (0.22 %). Seed borne inoculum levels did not affect final SEV and KI, which was not correlated between each other. PD was significantly reduced with the increase of seed borne inoculum levels in seven out of eight trials and at levels as high as 48 % (2009/10 season). The seed borne inoculum levels did not affect YLD, although significantly reducing PD, which may be due to the rice having a low population compensated through tillering. The risk of yield loss by sowing *B. oryzae*-infected seeds seems to be low and the early onset of the disease caused by increased levels of seed borne inoculum was dependent on seasonal weather conditions.

Keywords Generalized linear mixed model · *Oryza sativa* · Seed pathology · Tolerance standards

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Introduction

Brown spot (BS), caused by *Bipolaris oryzae* (Breda de Haan) Shoemaker, is an important fungal disease in the major rice-growing areas worldwide (Ou 1985; Savary et al. 2000). In tropical lowlands and subtropical Asia, the disease is widespread and causes yield losses averaging approximately 10 % of the attainable yield, being among the most important contemporary diseases affecting rice (Savary et al. 2000; Barnwal et al. 2013). Outside Asia, Brazil is the largest rice-producing country and where BS is also economically important due to reductions in both crop yield and grain quality (Prabhu et al. 2006). Despite its historical significance (Padmanabhan 1973) and widespread occurrence, BS still remains a poorly defined disease due to the complexity of factors influencing epidemics (Barnwal et al. 2013).

When seedborne, dormant *B. oryzae* mycelium can be reactivated during seed germination and cause lesions on roots, coleoptile and primary leaves, eventually leading to seedling death, depending on the density of the inoculum (Lee 1992; Van Nghiep and Gaur 2004). Seed-to-seedling transmission rate may reach up to 80 % (Prabhu and Vieira 1989; Toledo et al. 2006; Barnwal et al. 2013). Epidemiological studies on rice brown spot in Brazil are scarce and most focused on the control efficacy of fungicides (Ottoni et al. 2000; Dallagnol et al. 2006) and biological control agents (Ludwig et al. 2009), as well as the effects of silicon on host resistance (Zanão Júnior et al. 2009; Dallagnol et al. 2009) and seed-to-seedling transmission of *B. oryzae* (Dallagnol et al. 2013).

Sporadic surveys of *Bipolaris* spp. associated with rice seeds produced in Brazil showed the dominance of *B. oryzae*, although three other species within the *Bipolaris* complex have been found, usually in very low incidence (Farias et al. 2011; Meneses et al. 2014). The role of seeds as a source of primary inoculum of *B. oryzae* in BS epidemics was recently highlighted as a knowledge gap and a research priority to this disease (Barnwal et al. 2013). According to Mew and Gonzales (2002), the presence of infected seeds in a rice seed lot may affect disease establishment; the more the infected seeds, the higher the probability of having an epidemic from seed borne inoculum. Clearly, disease impact varies according to the rice ecosystem (upland, lowland, rainfed, irrigated, tropical, subtropical, etc.), cultural conditions, and types of crop management and production. However, there is a common sense that the threshold level for a disease should be zero in an area where the pathogen is absent (Mew and Gonzales 2002). A single-trial study (Malavolta et al. 2002) showed that *B. oryzae* seed borne inoculum negatively affected seedling emergence, but no information was available on its effect on BS epidemics and rice yield. Knowledge of the impact of seed borne inoculum on epidemics is key to determining tolerance standards of pathogens in seeds (Baker and Smith 1966; McGee 1995). In Brazil, 5 % incidence of *B. oryzae* on rice seeds was arbitrarily suggested as a tolerance standard (Machado and Pozza 2005) but specific knowledge based on epidemiological information from field studies is lacking. Therefore, the main aim of our study was to assess the effect of incidence levels of *B. oryzae* in the seeds on crop establishment, development of brown spot epidemics and rice yield in a range of environmental conditions over three growing seasons in the major lowland irrigated rice-growing subtropical region of Brazil.

Materials and Methods

Study area, growing seasons and field settings

A total of 11 field trials were conducted over three consecutive growing seasons (2008/09, 2009/10 and 2010/11) at two rice-growing regions in the state of Rio Grande do Sul (RS) as defined by the state rice institute (IRGA - Instituto Rio Grandense do Arroz): At the first location, Cachoeirinha (CA), Planície Costeira Externa region (northeastern RS), field trials were conducted at the IRGA's experimental station (29°56'53"S; 51°07'08"W), which has a long history of rice cultivation (>60 years). At the second location, Bagé (BA), Campanha region (southwestern RS), six field trials were conducted at the experimental station of the Universidade da Região da Campanha (31°17'15"S; 53°58'57"W), in an area where rice had been cultivated for only one year prior to the trials. At each location, two sequential trials at different sowing dates were conducted in the same year, except in the 2010/11 season at CA, where only one trial was established. Sowing dates were determined based on the recommendation for each location (Steinmetz and Braga 2001). Seven of the field trials were sown after the recommended period, whereas, three trials were sown during the recommended period and one prior to the recommended period. The higher number of trials sown later than earlier in the season was due to the empirical evidence that rice foliar diseases are usually more severe at the late sowing dates, when warm weather favors disease epidemics in the irrigated lowland rice fields in southern Brazil (Reunião Técnica da Cultura do Arroz Irrigado 2010).

The field trials were conducted in a randomized complete block design with four replications, each being a plot of 8.5 m^2 (5.5 m×1.7 m) spaced at 0.5 m from each other and with 10 rows spaced at 0.17 m. Seeds were sown using a 10-row seed drill. Crop management was based on regional recommendations (Reunião Técnica da Cultura do Arroz Irrigado 2010) without fungicide applications.

At CA, within-season weather data were collected from a station located at the site of the trial. At BA, weather data were collected by a weather station at Embrapa Pecuária Sul research stations, 20 km distant from the experimental site. Data collected comprised daily maximum and minimum temperatures (°C) and rainfall (mm) from the month prior to sowing to the month of harvest. These data were summarized as mean monthly deviations from the 30-year climate normal (1961–90) obtained for each month in each location.

Inoculum preparation and treatments

Seed lots of the IRGA 424 cv. produced in the preceding season were provided by IRGA. Several seed lots were previously assessed for the incidence of *B. oryzae* using a standard seed health blotter test (Agarwal et al. 1989) and a clean lot (no fungal infection) was selected for the trials. A highly aggressive strain of *B. oryzae* was selected based on pathogenicity assay on previous studies, and was used to inoculate the seeds using the water restriction technique, which usually yields high inoculum density (de Farias et al. 2010). Briefly, seeds were placed on top of 5-day-old actively growing colonies of the fungus in petri plates (90 mm) filled with freshly made PDA (potato-dextrose-agar) media adjusted to 0.6 MPa of osmotic potential by the addition of NaCl. The seeds were maintained on the colonies during four days to allow infection and were then dried at 30 to 35 °C for additional two days. Seeds from all plates were mixed prior to be further mixed with the clean seeds to achieve the incidence level defined for each treatment.

The treatments consisted of six increasing (exponential) levels of *B. oryzae* incidence: 0 (clean lot), 3, 6, 12, 24 and 48 %. The inoculation treatments were obtained by mixing a batch of inoculated seeds with clean seeds in the respective proportions. Thereafter, three random samples of 200 seeds from each batch were further analyzed using the blotter test after surface disinfestation of the seeds. Since all inoculated seeds resulted in colonized seeds, the desired incidence was reached in the seed lots. Seed lots of each treatment were homogenized prior to planting.

Crop and disease assessments

Plant population density (PD) was assessed during V3/V4 stage of the crop (Counce et al. 2000) immediately prior to flooding, or around 30 days after sowing. PD was determined by counting the number of plants in two randomly selected subareas of 0.25 m^2 by plot, which were added together and adjusted to plants/m^2.

After seedling emergence, the plots were monitored weekly to detect brown spot symptoms and determine disease incidence (INC) on leaves until flowering. At each INC assessment, ten tillers were randomly selected per plot and the presence/absence of symptoms was determined on each leaf of the tiller, starting from the lower leaves. INC was expressed as a percentage of symptomatic leaves of the total number of sampled leaves. When BS symptoms were first noticed on flag leaves, disease severity (SEV, percent of diseased leaf area) was visually assessed with the aid of a disease diagram (Schwanck and Del Ponte 2014). Twelve flag leaves, three from plants randomly selected at the four central lines of a plot, were marked and severity was assessed at around a 7 to 14-day interval. Both INC and SEV data were used to calculate the area under the disease progress curve ($AUDPC_{INC}$ and $AUDPC_{SEV}$) based on the trapezoidal method (Madden et al. 2007).

When crops reached maturity, the entire plot at CA was mechanically harvested (Shouguang Longchang Machinery ® model DB200) and for plots at the BA trials, the crops were manually harvested in a 4 m^2 section of the plot. Grain moisture was determined using a universal moisture meter Model Multi-grain (Dickey-john ®). Yield (YLD) was adjusted to 13 % moisture (humid base) and expressed as ton/ha. After harvest, a sample of 200 kernels was randomly taken at each plot and subjected to the blotter seed health test as described previously. Then, the presence of *B. oryzae* was determined with the aid of a stereomicroscope on each kernel and expressed as a percentage of kernel infection (KI).

Number of assessments per variable and data analysis

The crop- and disease-based variables described above were not obtained in all trials. The number of variables and assessments in time for each variable differed across the trials (Table 1). In order to analyze non-normal data that involve random effects (block as random effect), we used a generalized linear mixed modeling (GLMM) framework represented in 1.

Table 1 Information on the field trials for the assessment of the effect of increasing incidence levels of seed borne *Bipolaris oryzae* inoculum on brown spot epidemics and yield of irrigated rice in Southern Brazil

Trial code	Location[a]	Growing season	Sowing date	Harvesting date	Variables[b] and no. of assessments during the season				
					PD	INC	SEV	YLD	KI
BA09-3A	Bagé	2008/09	21 Nov	18 Mar	-[c]	-	3	1	-
BA09-3B			19 Dec	30 Apr	-	-	3	1	-
BA10-2		2009/10	30 Oct	05 Apr	1	2	5	1	1
BA10-3			14 Dec	25 May	1	4	4	1	1
BA11-2		2010/11	28 Oct	28 Apr	1	-	4	1	1
BA11-3			23 Nov	10 May	1	-	3	1	1
CA09-3A	Cachoeirinha	2008/09	21 Nov	16 Apr	1	-	3	1	1
CA09-3B			20 Dec	27 Apr	1	-	3	1	1
CA10-2		2009/10	29 Oct	19 Mar	1	6	3	1	1
CA10-3			08 Dec	03 May	-	-	4	1	1
CA11-1		2010/11	17 Sep	10 Mar	1	-	3	1	1

[a] Municipality where trials were conducted: Bagé (BA) e Cachoeirinha (CA)

[b] Response variables: plant population density (PD), disease incidence (INC), disease severity (SEV), rice yield (YLD) and *B. oryzae* incidence on harvested kernels (KI)

[c] Not evaluated

$$f\left(y_{ijk}\right) = \beta_0 + \beta_1 \mathrm{x} + \gamma_j + \varepsilon_{ij}, \quad (1)$$

where f is the link function, y_{ijk} is the value of the response variable y in the treatment i, block j corresponding to the treatment k; β_0 is the intercept of the model (mean response corresponding to the level of infection zero, in the transformed scale) and β_1 the slope of the linear model, or the response (in the transformed scale) of the variable to unitary variation in the inoculum level, γ_j the random effect of block j and ε_{ij} the experimental error associated with each observation.

The specific distribution and link functions were chosen according to the nature of each response variable. For PD, which comprises count data, we used the Poisson distribution with log link. For $AUDPC_{INC}$, $AUDPC_{SEV}$, YLD and KI, which were normally distributed, gaussian models with identity link were used. SEV_{last} data, in the form or proportion, were analyzed assuming a binomial distribution with logit link. The evidence of inoculum level influence on each response variable was determined by the nominal level of significance (p) of a one-sided t-test on β_1 (H_0: β_1=0), once a priori a negative β_1 was assumed in the cases of PD and YLD, or positive, on $AUDPC_{INC}$, $AUDPC_{SEV}$, SEV_{last} (severity at last assessment) and KI variables.

The adequacy of model assumptions and goodness-of-fit were assessed via graphical analysis of Pearson residual panel and Pearson correlation coefficient between observed and model predicted data, respectively. All analyses were performed using the GLIMMIX procedure of the statistical software SAS/STAT® v12 (SAS Inst., Cary, NC). The relationship between BS severity on the flag leaves and *Bipolaris* spp. incidence on harvested kernels was assessed via correlation analysis.

Results

Seasonal weather conditions

Climatologically, BA and CA have a similar rainfall pattern but average temperature for the September-April period is 1.37 °C lower at BA. The 2009/10 season was exceptionally wet at BA, especially during the crop establishment period (Nov and Dec); rainfall was around 400 mm above the expected rain for the period. During 2008/09 and 2010/11 seasons, the early season was drier than normal. Major deviations (±2 °C) in mean monthly temperatures during the season were uncommon, especially at BA (Table 2).

Table 2 Climate normal and departures for the monthly mean temperature (T) and monthly cumulative precipitation (P) during three growing seasons at Bagé (BA) and Cachoeirinha (CA), where field trials were conducted to evaluate the effect of seed born levels of *Bipolaris oryzae* on brown spot epidemics and crop establishment and yield

		Climatenormal (1961–90)		Deviation (observed – normal)[a]					
				2008/09		2009/10		2010/11	
Location	Month	T (°C)	P (mm)	T (°C)	P (mm)	T (°C)	P (mm)	T (°C)	P (mm)
BA	Sep	15.1	134.1	−1.38	−52.1	−0.33	+87.7	+0.55	−18.5
	Oct	17.5	132.1	+0.25	−45.3	−0.44	−18.7	−1.10	**−131.9**
	Nov	20.0	95.7	+1.44	−36.5	+1.46	+**377.7**	−1.04	**−91.1**
	Dec	22.8	99.1	−0.52	−13.5	−0.73	+21.1	+0.86	−41.7
	Jan	23.9	107.5	−1.14	+16.7	−0.12	+30.9	+1.95	−13.9
	Feb	23.3	113.9	−0.14	+27.7	+0.95	+**67.3**	+0.13	−45.7
	Mar	21.4	105.6	+0.77	−49.0	+0.96	−25	+0.63	**+2.2**
	Apr	17.8	83.3	+1.19	**−80.1**	+0.55	+2.5	+1.17	−25.5
	Sum/mean	*20.2*	*871.3*	*+0.06*	*−232.1*	*+0.28*	***+543.5***	*+0.39*	*−366.1*
CA	Sep	16.8	142.2	−1.27	+46.0	**−2.37**	+**144.4**	+0.57	+66.3
	Oct	19.1	121.3	+0.11	+**111.3**	**−5.65**	+23.7	−1.02	**−73.7**
	Nov	21.2	92.4	+0.56	−26.6	+**4.22**	+**161.2**	+0.20	−0.6
	Dec	23.3	93.4	−0.52	+37.4	+0.12	+45.8	−0.17	−2.3
	Jan	24.6	105.9	−1.55	+105.3	+0.56	+**88.7**	+1.17	−19.6
	Feb	24.6	99.2	−0.43	−32.8	+1.74	+51.2	−0.06	+**198.1**
	Mar	23.1	104.7	−1.26	−36.9	+0.84	−11.5	+0.00	+46.3
	Apr	19.9	77.3	−1.56	−61.7	−0.13	−19.3	+0.42	+**116.6**
	Sum/mean	*21.57*	*836.4*	*−0.74*	*+142*	*−1.14*	***+484.2***	*+0.14*	*+331.1*

[a] Bolded values represent cases of deviations lower or higher than 50 % of the normal rainfall and lower and higher than 2 °C of the normal temperature

Cumulative rainfall was above normal for the three seasons during the Sep-Apr period at CA (Table 2). Similarly to BA, 2009/10 was exceptionally wet, with the largest positive deviations occurring during the early to mid-season. In 2008/09, conditions were wetter during the early season and drier than normal at the late season. In 2010/11, an opposite trend was found; conditions were drier at the early season and wetter than normal during the late season. The largest negative deviation in temperature occurred in Oct 2009 (−5.65 °C) and positive deviations in Nov 2009 (+4.22 °C) (Table 2).

Brown spot incidence, severity and kernel infection

BS symptoms were found in all trials and mostly after flowering in the majority of the trials. However, in three trials the disease was found at the early vegetative stages, and so BS INC data could be recorded. These trials were all conducted in the 2009/10 growing season, two at BA (BA10-2, BA10-3) and one at CA (CA10-2). In these cases, INC was first detected from 68 to 85 days after sowing. The overall mean INC ranged from 17.3 % to 32 % across the assessments in these trials. The mixed modeling analysis of the effect of inoculum levels on $AUDPC_{INC}$ data showed that the β_1 parameter differed significantly from zero ($P<0.05$) in two trials, BA10-2 and BA10-3 (Fig. 1A,B). $AUDPC_{INC}$ was estimated to increase 1.8 % (β_1=0.018) and 3 % (β_1= 0.030), respectively, for a unitary percent increase of *B. oryzae* incidence on seeds.

First BS symptoms on the flag leaves were detected on average at 121 days after sowing, ranging from 98 (BA09-3B) to 152 days (CA10-3). The progress curves of BS severity varied in shape among and within growing seasons (data not shown). Mean BS SEV_{last} assessed on flag leaves of all trials was variable and consistently higher in the CA (0.66 to 5.55 %) than the BA trials (<1.18 %) (Table 3).

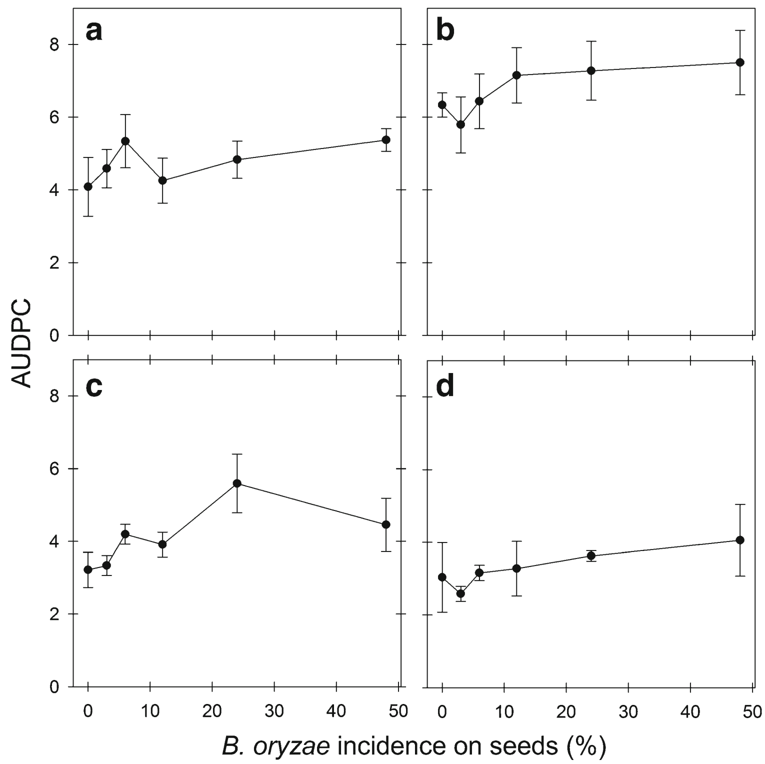

Fig. 1 Area under the disease progress curve (AUDPC) for brown spot incidence (**a** BA10-2; **b** BA10-3) and brown spot severity (**c** BA10-2; **d** BA10-3) in four out of eleven field trials conducted in Bagé (BA) during the the 2009/10 season where there was a significant effect of *Bipolaris oryzae* incidence levels on seeds for this variable. *Error bars* represent the standard error of the estimated mean

Evidence of significant effect ($P<0.05$) of inoculum level on $AUDPC_{SEV}$ data was found in two trials, the same where the disease was recorded prior to flowering and where INC data was available: BA10-2 and BA10-3 (Fig. 1CD). An overall increase of 1.3 % (β_1=0.013) and 1.2 % (β_1=0.012) in $AUDPC_{SEV}$ was estimated for a unitary percent increase of *B. oryzae* incidence on seeds in the BA10-2 and BA10-3 trials, respectively. However, the analysis of SEV_{last} data showed no effect on the inoculum level ($P>0.05$) (Fig. 3). Overall, mean KI was higher at CA (19.8 %) than at BA (6.2 %) (Table 3). No evidence of the effect of *B. oryzae* seed borne inoculum was observed in KI ($P>0.05$), which averaged 13 % in the nine trials where this variable was recorded. Furthermore, there was a weak and non-significant association between final severity (SEV_{last}) and KI considering the whole dataset (r^2=0.16; n=216 plots) or by trial (r^2 varied from 0 to 0.25; n=24 plots).

Table 3 Overall mean (standard error) for disease and crop-related variables in eleven field trials for which there was no effect ($P>0.05$) of treatments ranging in incidence levels (0, 3, 6, 12, 24 and 48 %) of seed borne infection by *Bipolaris oryzae*

Trial code[a]	Disease-related variables		Yield (ton)
	Severity (%)	Kernel infection (%)	
BA09-3A	1.17 (0.06)	-[b]	6.40 (0.20)
BA09-3B	0.58 (0.04)	-	5.59 (0.15)
BA10-2	0.27 (0.02)	5.40 (0.61)	10.01 (0.35)
BA10-3	0.26 (0.03)	5.21 (0.76)	8.31 (0.05)
BA11-2	0.10 (0.01)	6.83 (0.86)	9.74 (0.33)
BA11-3	0.18 (0.03)	7.33 (0.77)	7.56 (0.24)
CA09-3A	5.55 (0.22)	15.29 (1.38)	8.90 (0.10)
CA09-3B	1.55 (0.12)	29.54 (4.09)	7.43 (0.19)
CA10-2	2.98 (0.16)	20.38 (1.47)	11.16 (0.16)
CA10-3	0.66 (0.09)	11.54 (0.89)	10.62 (0.23)
CA11-1	5.13 (0.47)	22.42 (1.95)	9.98 (0.29)

[a] See Table 1 for information on the location, year and sowing dates

[b] data not available

Population density and rice yield

For the PD data assessed in eight trials, mean values were, on average, higher at CA (73 plants/m^2) than at BA (50 plants/m^2) in plots with clean seeds (Fig. 2). The highest PD (>90 plants/m^2) was found in the 2008/09 growing season and the lowest (<30 plants/m^2) in the 2010/11 growing season, both at CA (Fig. 2A,C). There

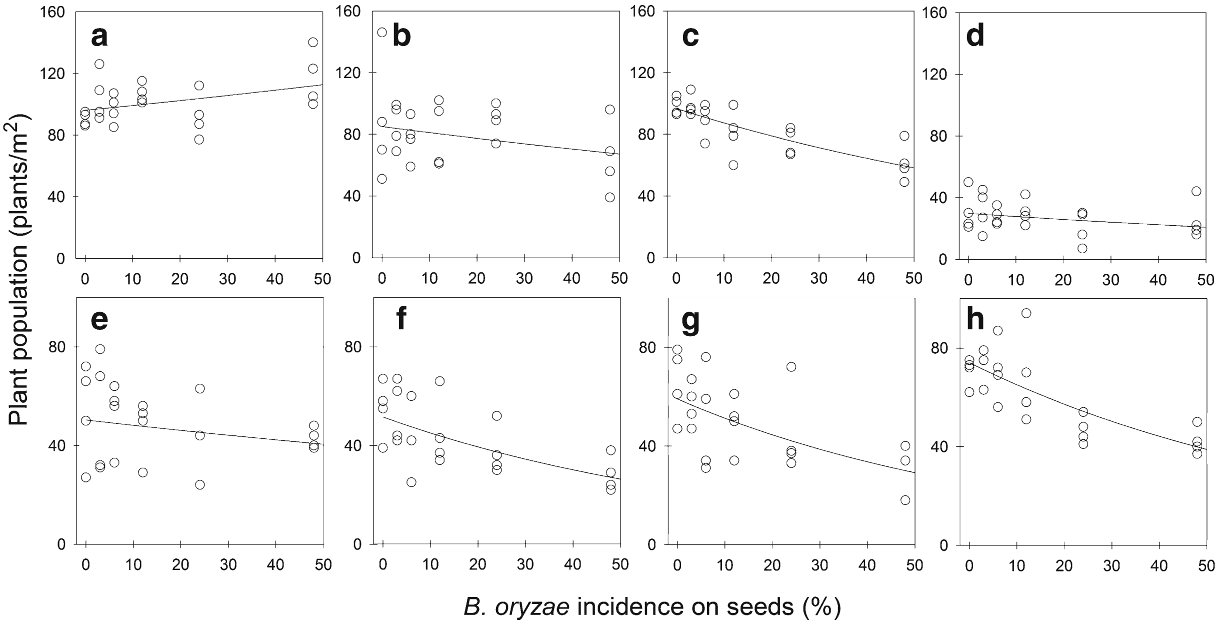

Fig. 2 Plant population density of irrigated rice in V3/V4 stage (Counce et al. 2000) as function of six incremental incidence levels of *Bipolaris oryzae* on seeds for eight field trials: CA09-3A (**a**), CA09-3B (**b**), CA10-2 (**c**), CA11-1 (**d**), BA10-2 (**e**), BA10-3 (**f**), BA11-2 (**g**) and BA11-3 (**h**). Dots represent measurements for each replicated plot and lines represent predicted values via the generalized linear mixed model (values back-transformed to the natural scale, plants/m^2). Model parameters are shown in Table 3

was an overall trend of decreasing PD with the increase of *B. oryzae* inoculum levels in the seeds (Fig. 2). Based on the mixed modeling analysis, PD was significantly affected by the inoculum level in all but one trial (Table 4, Fig. 2A). The slopes of the regression (β_1) for the log-transformed data ranged from 0.003 to −0.014 (Table 4). Overall, PD reductions were higher at BA than at CA; up to 48 % reduction was found in the treatment with the highest inoculum level in one trial (BA11-2, Fig. 2G). Lower relative reductions on PD were most commonly found for rice plots sown later in the season at CA (Fig. 2A,B).

Table 4 Parameters (β_0 and β_1) of a Poisson regression (generalized linear mixed) model with the logarithm link function fitted for plant population density of irrigated rice in V3/V4 stage (Counce et al. 2000) as function of incidence levels of *Bipolaris oryzae* (0, 3, 6, 12, 24 and 48 %) on rice seeds in eleven field trials

Trial code [a]	β_0 (SE[b])	*P*-value [c]	β_1 (SE)	*P*-value [d]
BA10-2	3.920 (0,041)	<0.001	−0,004 (0,002)	0,017
BA10-3	3.944 (0,041)	<0.001	−0,013 (0,002)	<0,001
BA11-2	4.079 (0,039)	<0.001	−0,014 (0,002)	<0,001
BA11-3	4.305 (0,034)	<0.001	−0,013 (0,002)	<0,001
CA09-3A	4.565 (0,028)	<0.001	0,003 (0,001)	0,991
CA09-3B	4.443 (0,031)	<0.001	−0,005 (0,001)	0,003
CA10-2	4.572 (0,030)	<0.001	−0,010 (0,001)	<0,001
CA11-1	3.394 (0,053)	<0.001	−0,007 (0,003)	0,008

[a] See Table 1 for information on the location, year and sowing dates

[b] Standard error

[c] Probability level for type I error of t- test (H_0: β_0=0)

[d] Probability level for type I error of unilateral *t*-test (H_0: β_1=0) to left

No evidence of a significant effect ($P>0.05$) of seed borne inoculum levels on yield was found in any of the trials. The overall mean yield across the eleven trials was 8.69 (±2.4) ton/ha. On average, mean yield was higher at CA (7.4 to 11.66 ton/ha) than at BA (5.6 to 10 ton/ha) (Table 3).

Discussion

In this study, eleven field trials were conducted during three sequential growing seasons at two sites that differed in both seasonal weather and crop history. With one exception, we found that plant population density was reduced to a maximum rate of 48 %, meaning that seed borne infections caused pre-emergence damping-off. The highest reductions in plant population associated with the highest levels of seed borne inoculum were found in the trials conducted at Bagé and at Cachoeirinha during the 2009/10 season. In this season, monthly temperatures were cooler than normal in the early season and very wet in mid-season. This season is classified as a "warm" phase of the El Niño southern oscillation (ENSO), which is associated with above-normal rainfall during spring and early summer in the south of Brazil (Grimm 2011). Increased soil moisture and low temperatures after sowing may reduce germination and emergence rate, thereby extending the period for the fungus to colonize and damage seeds and seedlings (Baker and Smith 1966; McGee 1995).

Accordingly, the 2009/10 season was also the one in which BS symptoms were detected at the early vegetative stages in three trials. In that season, positive departures of temperatures and rainfall were found from January to March, during the assessments of BS incidence on leaves at both sites. Previous studies in controlled environments have shown positive effects of increasing temperature (from 25 to 32 °C) and decreasing levels of photo-irradiance (from 1000 to 15 photons/m^2/s^1) in rice brown spot, both leading to shorter incubation periods and a higher lesion expansion rate (Dallagnol et al. 2011). Photo irradiance was not measured in our study, but cloudiness was likely longer due to the well-distributed rainfall events during January and February at 2009/10 season. Additionally, in a study on wild rice (*Zizania palustris* L.), brown spot infection was favored at 32 °C and 28 h of leaf wetness compared to 25 °C and 16 h (Percich et al. 1997).

The symptoms observed at the early vegetative stage may have resulted from primary seed borne inoculum capable of transmitting and initiating epidemics on the first expanded leaves. The relatively low incidence levels that fluctuated over time were likely due to a "dilution effect" on the incidence of symptomatic leaves due to emission of healthy leaves. Although the fungus is capable of infecting leaves of young seedlings and plants either from inoculated seeds or spray-inoculated plants under high inoculum pressure in controlled environments (Prabhu and Vieira 1989; Malavolta et al. 2002; Van Nghiep and Gaur 2004; de Farias et al. 2007) we found that the presence of symptoms prior to flowering was highly dependent on field conditions that were remarkably favorable in only a few trials, which is in agreement with other reports (Ou 1985; Lee 1992). To produce infected seeds, we used clean rice seeds that were inoculated using the water restriction technique. With this approach, seeds were incubated for a time period that led to high inoculum density in the seeds. However, variation in inoculum density among seeds is expected because one dense layer of seeds is placed on top of the fungal colony that grows and infects the seed during the incubation period. Transmission of seed borne pathogens is highly related to the inoculum level per seed (Colhoun 1983), which was not measured in our study. Nevertheless, infected seed may have carried sufficient amount of inoculum to further colonize the seed during the germination and emergence processes. The lack of symptoms prior to flowering in most trials can also be related to unknown factors inherent to the trials that suppressed disease development prior to flowering. The absence or presence of symptoms at the vegetative stage was observed in all treatments of a trial.

The increasing levels of *B. oryzae* seed borne inoculum significantly affected the progress of BS severity on the flag leaves in two trials, exactly those where BS symptoms were found prior to flowering. Because the experimental site at Bagé has a short history of rice cultivation (only one year) it is likely that epidemics in those trials were initiated and supported by seed borne inoculum; increasing seed borne inoculum levels led to higher brown spot intensity represented by the area under the disease progress curve. It is noteworthy that the seed borne inoculum levels did not affect final severity in all trials, but it is also true that severity was very low in these trials. Overall, mean BS severity on the flag-leaves was less than 1 %, especially at Bagé. In only two trials, both at Cachoeirinha, mean severity was greater than 5 % but no effect of seed borne incidence was found. In fact, BS severity in the field rarely reaches 30 % severity and there is a strong tendency to overestimate severity visually, especially in the case of inexperienced raters (Schwanck and Del Ponte 2014). A large survey conducted in Tropical Asia showed that 75 % of four hundred fields had brown severity <17 % (Savary et al. 2000). The maximum mean BS severity reported in field plots of central regions of Brazil was 17.5 % (Ottoni et al. 2000).

The similar final severity levels among treatments was possible due to secondary spread of inoculum produced during mid to late season, which might have originated from either the same plot or neighboring plots that differed in initial inoculum levels in the seeds. The effect of seed borne inoculum levels observed in two trials for the area under the disease progress curves reflects differences in epidemic patterns, which were not captured when analyzing final severity. No physical barrier was imposed to prevent airborne inoculum from within the experimental or other trials in the station from entry into a plot. This fungus is capable of airborne dispersal (Picco and Rodolfi 2002) and spore samplers placed above the canopy at the Cachoeirinha plots showed that *B. oryzae* conidia were sampled every week during the season (Schwanck and Del Ponte unpublished). At Cachoeirinha, airborne inoculum should be higher than at Bagé due to the long history of rice cropping and several alternative hosts and rice plots planted in the same area of the trial.

It is known that *B. oryzae* inoculum produced during the course of the epidemics can disperse from leaves to the panicle and infect the kernels (Ocfemia 1924). Since severity on flag leaves did not vary across treatments, there was no significant relationship between BS severity and kernel infection. Infections on kernels, under the conditions of the trials, may have been caused by immigrant inoculum (other plots or trials in the area). The kernel infection levels were consistently higher at CA when compared to the BA location, and ranged from 10 to 30 %. This is above the mean incidence (9.5 %) across 197 seed lots of the cultivar used in the trials, IRGA 424, produced in the state during the three years of this study (Meneses et al. 2014).

Although there was a negative impact in plant population, the crop yield did not differ in plots with varying seed infection levels. Studies have shown that rice crops are not too sensitive to planting density with regard to yield in both irrigated rice in southern Brazil (Sousa et al. 1995; Mariot et al. 2003; Carmona et al. 2008) and upland rice (Santos and Costa 1995; Santos et al. 2002). Rice plants are able to tiller and compensate the number of panicles per area to the point of equating final kernel yield (Miller et al. 1991). However, tillering ability may vary according to sowing density, fertilization, variety or hybrid, among other factors (Wu et al. 1998).

In summary, our results show that seed borne inoculum is more harmful to the initial stand by decreasing the plant population due to damping-off, and can initiate epidemics in the early season when weather conditions are favorable. The risk of yield loss was not associated with seed born inoculum, possibly due to the relatively low levels of BS severity and the ability of the rice crop to compensate low population density. Hence, the risk may increase in cropping situations where the crop performance is more sensitive to plant population or production systems in which seeds are exposed to a more favorable environment for the fungus such as growing seedlings raised indoors in seed boxes (Mew and Gonzales 2002).

A tolerance standard could not be derived from our study for the southern Brazilian conditions, but we showed that sowing infected seeds in new areas and during growing seasons more favorable for initial epidemics affects crop establishment. According to Roberts (1999), the term threshold should be avoided because it implies a “magic number” below which there will be no problems. The opposite may be true, which is supported by our results, in that seed lots with infection levels above the threshold will always lead to a problem. The author proposes the use of the term “tolerance standards”, but comments that they have been defined arbitrarily or limited to a few environments. Our results are based on a reasonable number of field trials conducted over three growing seasons. Yet, they may apply to the conditions of lowland irrigated rice systems similar to those of southern Brazil. A recent survey in the region showed that rice seeds produced in the state of Rio Grande do Sul with the current technology have led to improved health quality when compared to previous surveys; mean incidence levels of *B. oryzae* in seed lots varied according to region and cultivar, but were in general at low levels (79 % of 570 seed lots had incidence lower than 5 %) across the state over the three years (Meneses et al. 2014). Collectively, these results indicate that the risk of yield loss associated with

B. oryzae seed borne inoculum in the studied region is relatively low, especially considering that commercial fields are commonly sprayed with fungicides targeting the complex of foliar diseases, including rice blast. It would be instructive to investigate whether these results also apply to cropping situations with more favorable conditions for BS epidemics, such as those encountered in the tropical areas in central areas of Brazil where upland rice is grown during both the wet and warm seasons.

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