Research Article

A Predictive Model for Daily Inoculum Levels of *Gibberella zeae* in Passo Fundo, Brazil

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The deposition of spores of *Gibberella zeae*, the causal agent of *Fusarium* head blight of wheat, was monitored during 2008–2011, in Passo Fundo, RS, Brazil. The sampling was carried out in a 31-day period around wheat flowering. The numbers of colonies formed were related to meteorological variables. In this study, a hierarchical autoregressive binary data model was used. The model relates a binary response variable to potential covariates while accounting for dependence over discrete time points. This paper proposes an approach for both model parameter inference and prediction at future time points using the Markov chain Monte Carlo (MCMC). The developed model appeared to have a high degree of accuracy and may have implications in the disease control and risk-management planning.

1. Introduction

Wheat (*Triticum aestivum* L.) is an important crop in Brazil especially in the South: 90% of the growing area is established in the states of Rio Grande do Sul, Santa Catarina, and Paraná. In this subtropical region, weather conditions during the growing season favor the occurrence of foliar and flowering diseases [1]. Usually, two to three fungicide applications may be needed to control these diseases, thus increasing production costs [2].

Among wheat diseases, *Fusarium* head blight (FHB) has increased its pressure on crops in many production regions. Apart from losses in grain yield and reductions in baking and seed quality, the major peril due to FHB is the contamination of grain with toxic fungal secondary metabolites known as mycotoxins. The most prevalent myco-toxins are trichothecenes such as deoxynivalenol (DON) and nivalenol (NIV). To protect consumers from mycotoxicosis, many countries, including Brazil, have established maximum allowed levels for the most prevalent *Fusarium* mycotoxins in cereals and cereal products [3].

The main causal agent of the disease is *Gibberella zeae* (Schwein.) Petch (anamorph *Fusarium graminearum* Schwabe) [4], a homothallic fungus that survives in host debris on the soil. Inoculum is made up of ascospores and macroconidia that are dispersed by rain splash and wind, landing on wheat heads and infecting the plant during flowering and grain-filling stages [5]. FHB has worldwide distribution although the severities of the outbreaks are influenced by local weather conditions [6]. The wider adoption of minimum and no tillage, short rotations with maize and global climate variability and change are central in the debate on the causes for the re-emergence and expansion of the disease worldwide [7].

In Brazil, similar to other parts of the world, an increasing frequency of severe FHB outbreaks has been reported over the last two decades (especially after 1990) resulting in severe yield losses [1, 8, 9]. No wheat varieties are immune to FHB and resistance is generally controlled by several genes of moderate/weak effect and they are defined genetically as quantitative trait loci (QTL). In addition to these, mycotox-ins affect production throughout the world, the ability to predict FHB and DON and other mycotoxin contamination is important to reduce the year-to-year risk for producers. Owing to these dangerous consequences of reducing wheat yield and quality around the world, computer models, based

on weather variables (temperature, rainfall, and moisture level), have been developed to predict the likelihood of occurrence of FHB and DON contamination in wheat [10].

Inoculum quantification is an important step in process-based model development [11]. It has been shown that weather factors such as precipitation and temperature are highly related to inoculum density in the atmosphere [12–15]. Statistical models for this purpose have been made using techniques based on linear regression or other generalizations. When the response of the models is binary data, such as inoculum incidence, data fitting with generalized linear models based on logit link function [16] has proven to be the most appropriated. However, when the data are collected at successive time points such as daily or hourly, it may be correlated and under these circumstances an autoregressive structure, specially AR(1), can be used to solve the correlation in the data. Examples of this approach were proposed by [17–19].

This study examines the potential impact of climate variability on daily deposition levels of *G. zeae* propagules using hierarchical logistic model techniques. Our goal is to establish a statistical model of spore deposition that can be used to calculate probabilities of FHB infection as the wheat phenology advances from heading to soft dough stage. Within this framework, we aim, in the future, to relate the risk of FHB infection to the amount of inoculum within wheat fields, host phenology, susceptibility, and weather factors.

2. Materials and Methods

2.1. Study Area. Passo Fundo is located at the Planalto Médio Region, northern Rio Grande do Sul State, Brazil (latitude $28°15'00''$S, longitude $52°25'12''$W, altitude 684 m) (Figure 1). The region is one of the major wheat production areas in Brazil.

2.2. Data Collection. Patterns of spore deposition were monitored during 2008 through 2011. Each sample period is referred to as a wheat growing season environment. Consecutive sample periods covered the interval of 31 days starting from September 15th. Petri dishes (90 mm in diameter; surface area = 283 mm^2) containing *Fusarium* selective media (FSM) were used to sample viable spores of *G. zeae* from air. The FSM consisted of a modified Nash-Snyder formulation, prepared as described by [20]. The plates were mounted on a wind-driven sampler previously used by [12]. Two daily samplings performed at 9:00 and 21:00 h. were used with days deemed to begin and end at 09:00 hours GMT for consistency with the meteorological data. Plates were exposed in two periods of 12 h each, called night- and day-time sampling. After exposure to the environment, the plates were transported to the laboratory and incubated in a growth chamber (25°C and 12 h of darkness) in order to promote fungal growth. Colonies of *G. zeae* were identified according to color and morphology. Doubtful cases were transferred to Petri dishes containing PDA (potato dextrose agar) for comparison with confirmed true *G. zeae* colonies. The number of *G. zeae* colonies was
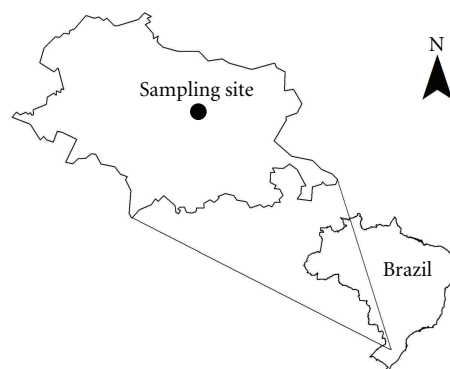

Figure 1: Location of the sampling site in Passo Fundo, Brazil.

recorded for each plate as CFU (colony forming units). Other *Fusarium* species were observed but not identified at the species level. Plates were placed, in the local weather station site, at 120 cm above a grass-covered ground.

Daily weather data comprised of maximum and minimum temperature (°C), total precipitation (mm), sunshine hours (h), and mean relative humidity (%). The data were provided by the National Institute of Meteorology (INMET).

2.3. Data Analysis. Records from the spore sampler were used as daily values (sum of two 12-hour periods), expressed as observed proportion of spores per day. The maximum colony count per Petri dish was fixed to 60 due to operation limitations (visual accuracy during colony identification) in this way the maximum count in each day was 120. The dataset consists of 93 observations where the variable of interest is a binary indicator $y_{[t]}$ with values in range 0-1 at time t. For the climate variables, each observation was centered on climatological normal representing the prevailing set of weather conditions calculated over a period of 30 years (1961–1990) in Passo Fundo this preprocessing step improved the simulation stability and accounted for strong serial correlation intrinsic to environmental data sequentially registered. For example in a given day the total precipitation observed is 25 mm, so the adjusted value is calculated like $25 − 6.2317 = 18.7683$ where the value 6.2317 corresponded to the precipitation mean observed in the months of September and October from the climatological normal in Passo Fundo.

We fitted an Hierarchical Autoregressive Binary Data Model (HARBDM) to the data. Model development was based on a combined approach from [19, 21, 22] using the free available software WinBUGS [23]. The statistical analysis and graphs were done in R [24] using the package R2WinBUGS [25]. We ran the simulation with 10000 interactions, in 3 chains, discarding the first 5000. The convergence of the chains was tested using Gelman-Rubin method [26]. We then took percentiles 5, 50, and 95% from the simulation results to get parameter estimates and credibility intervals. The density probability for the median spore incidence, by year, was fitted to a beta distribution.

MCMC (Markov chain Monte Carlo) methodology [27] is adopted to simulate from the full posterior distribution.

Updates were obtained by using the Gibbs sampler [28, 29]. The Gibbs sampler split the state vector into a number of components and updated each in turn by a series of Gibbs transitions. Posterior probability estimates for the incidence of spores in a given day were obtained in the context of the group (year).

The data of 2008–2010 was used to construct the model and the data of 2011 to validate the model.

2.3.1. Data Model. The functional form of the model is shown below:

$$
\begin{aligned}
\text{logit} \left\{ \Pr(Y_{[1,i]} = 1) \right\} &= \pi_{[1,i]}, \\
\pi_{[1,i]} &= \beta_{0[i]} + \sum_{k=1}^{4} \beta_{k[i]} X_{k[1,i]} \\
&\quad + \varepsilon_{[1,i]}, \quad \text{when } t = 1, \\
\text{logit} \left\{ \Pr(Y_{[t,i]} = 1) \right\} &= \pi_{[t,i]}, \\
\pi_{[t,i]} &= \beta_{0[i]} + \sum_{k=1}^{4} \beta_{k[i]} X_{k[t,i]} + \phi_{[i]} \pi_{[t-1,i]} \\
&\quad + \varepsilon_{[t,i]}, \quad \text{when } t \geq 2.
\end{aligned}
\tag{1}
$$

In (1) we used terms i for years (2008 to 2010) and t for days (1 to 31) after 15th of September.

3. Results and Discussion

A total of 93 sampling days was included in the study. During the sampling time 2076 *G. zeae* colonies were accumulated. The lowest number of colonies (343) was recorded in 2010. Spores were present in 86 out of 93 days. Summary statistics for each dependent and independent variable are shown in Table 1. The number of rainy days by year were, respectively, 14, 15, and 13.

Visual observations in Figure 2 revealed that climate variability and the number of *G. zeae* spores present in the air appeared to be associated. Both relative humidity and rain were associated positively with spore incidence while sunshine hours were associated negatively. Temperature amplitude appeared to be weakly related to spore incidence.

The mean and median values of *G. zeae* incidence were very similar in 2008 and 2009 but contrasted to those observed in 2010 (Table 1).

The monitoring of deposition of *G. zeae* spores by means of Petri dishes containing selective media provided estimates of inoculum levels in the air of Passo Fundo area. Moderate-to-severe *Fusarium* head blight epidemics occurred during the study period. Thus, the strategy of monitoring spores of *G. zeae* through different wheat growing seasons was successful in obtaining data from *Fusarium* head blight epidemic and nonepidemic years. During each sampling period, *Fusarium* head blight incidence ranged from traces to about 100% of spikes affected. The wheat seasons of 2008, 2009, and 2010 in Passo Fundo area were categorized, respectively, as epidemic, highly epidemic, and nonepidemic.

TABLE 1: Summary statistics from raw data observed from September 15th to October 15th, during three years, in Passo Fundo, RS, Brazil.

	GZ	RH	SH	TA	RAIN
2008					
Mean	0.23	0.73	0.55	10.59	4.58
SD	0.28	0.13	0.38	4.20	9.62
MED	0.11	0.70	0.61	11.70	0.00
IQR	0.27	0.24	0.68	7.35	4.00
2009					
Mean	0.23	0.78	0.47	10.19	10.78
SD	0.32	0.11	0.37	4.12	22.98
MED	0.08	0.76	0.52	9.20	0.00
IQR	0.17	0.20	0.72	6.95	8.25
2010					
Mean	0.09	0.74	0.48	10.66	8.72
SD	0.12	0.12	0.35	4.23	25.28
MED	0.03	0.75	0.53	11.50	0.00
IQR	0.10	0.18	0.69	6.10	3.15

GZ: *Gibberella zeae*, RH: relative humidity, SH: sunshine hours, TA: temperature amplitude, RAIN: rainfall. SD: standard deviation, MED: median, IQR: interquartile range.

Coincidently, the ENSO phases in each period corresponded to "neutral," "warm," and "cold," respectively. This is in agreement with reports [9] that FHB epidemics are likely to be more severe in "neutral" and "warm" than in the "cold" phase, in this part of the world.

The model constructed with the data between September 15 and October 15 in 2008, 2009, and 2010, respectively, was used to predict the density of *G. zeae* spores in the air of Passo Fundo. In Table 2, the estimated parameters by group factor (year) are deviations from the climatological normal. In this context we can see that, for a day with no deviation from normal, the probability of incidence of FHB, expressed by $e^{\beta_0}/(1 + e^{\beta_0})$ for each day, corresponds, by year, to 0.18, 0.20, and 0.06, respectively. The correlation index (ϕ) (Table 2) between days in 2008 and 2009 were negative and in 2010, positive.

In Table 3, the scale parameter β can be used to estimate the daily inoculum level for a specific year. For example in Passo Fundo, on years with β below a cutpoint (7.0), an alert for moderate-severe status could be set in a monitoring disease system. Otherwise, these parameters (α, β) could be used as priory information in Bayesian model framework.

Another measure of interest is the odds ratio (Table 4) that represents the increase in the incidence by each change in unit deviation from variables from the model.

The adjusted model is showed in (Figure 3) and was then validated by the actual observations (Figure 4). The validation analysis indicates that the model had reasonable accuracy over the predictive period, even though in day 9 the predicted spike was well behind that of the actual peak.

Mechanisms of spore deposition are gravity and scrubbing by rain drops which contribute in a random manner

Table 2: Estimated posterior medians and 95% credibility interval for the autoregressive model.

	2008			2009			2010		
	2.5%	50%	97.5%	2.5%	50%	97.5%	2.5%	50%	97.5%
β_0	−1.699	−1.511	−1.305	−1.586	−1.364	−1.186	−3.027	−2.826	−2.649
β_{TA}	−0.124	−0.064	−0.004	0.105	0.145	0.187	0.020	0.087	0.153
β_{RAIN}	0.028	0.043	0.057	0.014	0.017	0.021	0.005	0.009	0.013
β_{RH}	5.023	6.683	8.269	−0.927	1.180	3.561	−1.240	1.546	4.176
β_{SH}	−0.034	0.671	1.373	−3.427	−2.609	−1.626	−3.705	−2.691	−1.600
ϕ	−0.913	−0.429	0.141	−0.996	−0.880	−0.469	0.329	0.873	0.995

β_0: intercept, TA: iemperature implitude, RAIN: rainfall, RH: relative humidity, SH: sunshine hours, ϕ: correlation index [AR(1)].
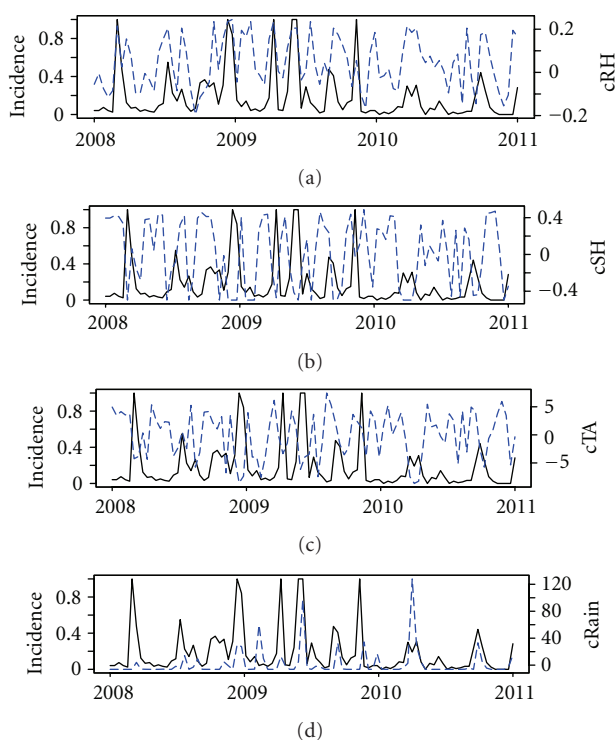

Figure 2: Relative daily incidence of *Gibberella zeae* spores (continuous line) and meteorological variables (dashed lines top to bottom graphs: relative humidity, sunshine hours, temperature amplitude, and rainfall), centered on climatological normal in Passo Fundo during 31-day period starting on September 15th of 2008, 2009, and 2010, respectively.

Table 3: Adjusted parameters for the beta distribution.

	2008	2009	2010
α	1.20	1.86	1.49
β	3.63	5.99	14.58

to spore deposition [30]. Wet deposition becomes relatively more important as the distance from a source of spores increases, because dry deposition tends to be limited to the removal of spores near the ground, whereas wet deposition can sweep spores from the entire depth of the spore cloud. For example, a significant portion of ascospores of

Venturia inaequalis was collected during hours that rainfall rate was less than 0.25 mm h-1 [31].

Therefore, it is likely that the error range in predicting spore deposition, in our work, is due to the fact that we used total daily rainfall in the model in lieu of actual time courses of rainfall.

Another possible explanation would pertain to packets of air (a localized region of low air density or a descending air current) that settled those days containing higher spore populations due to an earlier massive spore release of some origin in an upwind direction, perhaps at a considerable distance.

The model we developed in this paper describes the deposition probability of airborne spores according to weather factors. In this study, a HARBD model was used in this attempt to develop a *G. zeae* spore density forecasting system for improving our capacity to predict FHB outbreaks. The developed model appeared to have a high degree of accuracy and may have implications in the disease control and risk-management planning.

The weaknesses of this study must be acknowledged. First, this is a broad assessment of the relationship between climate variability and the incidence of spores of *G. zeae* at one location. More detailed risk assessment at regional and farm levels may also be required if a comprehensive and systematic risk assessment is to be made. Inclusion of other information (e.g., crop management, stubble characteristics, and other fungal-relevant environmental information) may further improve the model. Second, the model may only be applicable to Passo Fundo and areas with a similar climate background, since only local data were used in the construction of the model.

4. Conclusions

The autoregressive model is a useful tool for interpreting and applying to local plant disease control measures. Once a satisfactory model has been obtained, it can be used to forecast expected numbers of cases for a given number of future time intervals. Since predictions from HARBD model have the capacity to forecast when an outbreak is likely to occur, it therefore has great potential to be used as a decision-support tool for both tactical and strategic recommendations for FHB management.

TABLE 4: Odds ratio and 95% interval for the autoregressive model.

	2008			2009			2010		
	2.5%	50%	97.5%	2.5%	50%	97.5%	2.5%	50%	97.5%
β_0	0.18	0.22	0.27	0.20	0.26	0.31	0.05	0.06	0.07
β_{TA}	0.88	0.94	1.00	1.11	1.16	1.21	1.02	1.09	1.17
β_{RAIN}	1.03	1.04	1.06	1.01	1.02	1.02	1.01	1.01	1.01
β_{RH}	151.87	798.71	3901.05	0.40	3.25	35.20	0.29	4.69	65.10
β_{SH}	0.97	1.96	3.95	0.03	0.07	0.20	0.02	0.07	0.20

β_0: intercept, TA: temperature amplitude, RAIN: rainfall, RH: relative humidity, SH: sunshine hours.
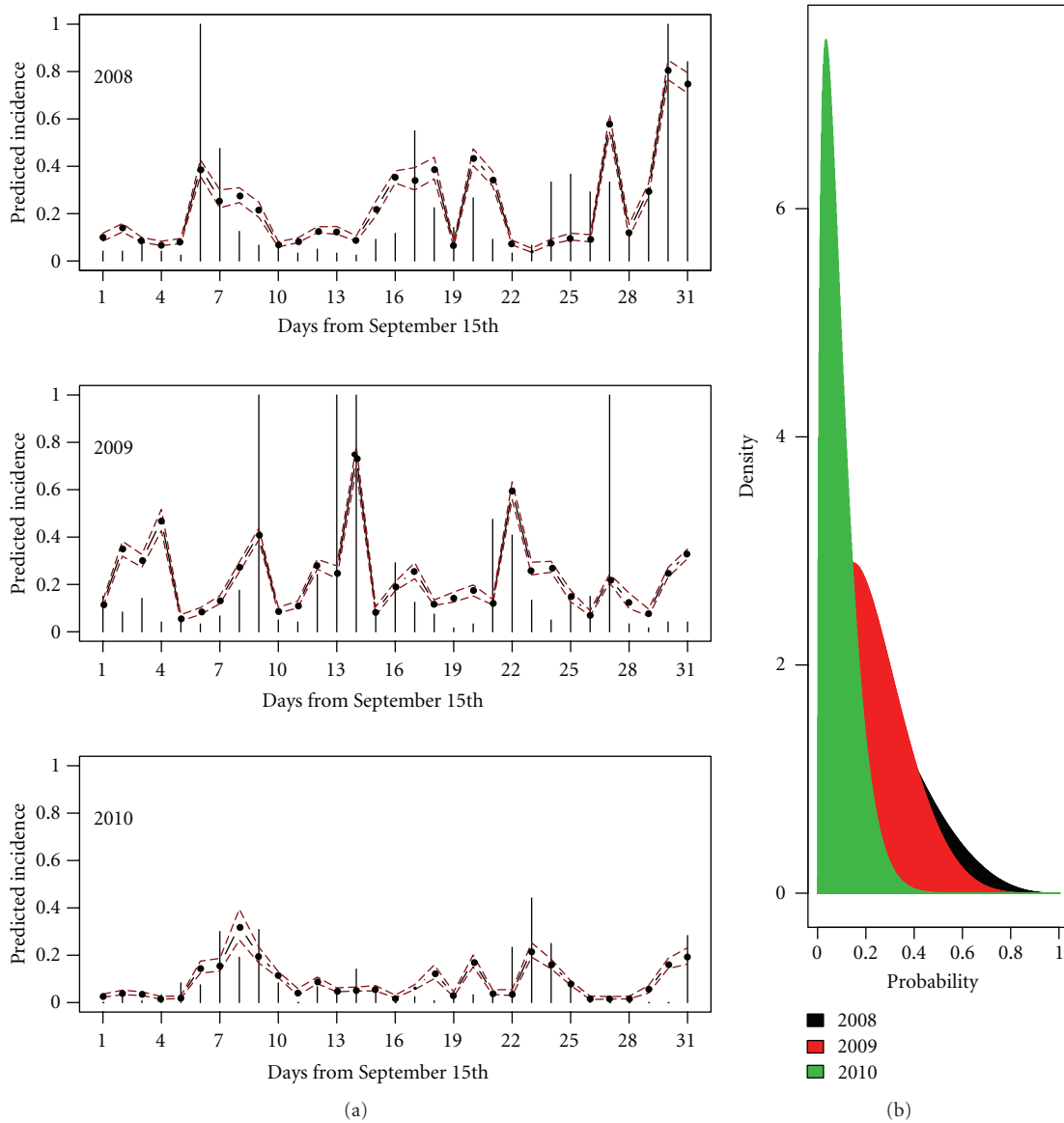

FIGURE 3: Predicted incidence of spores in a given day by year represented by the continuous line and simulated interval (5, 95 percents) dashed line. The vertical lines represent observed values (a). Estimated density for the beta probability distribution function in the different years (b).
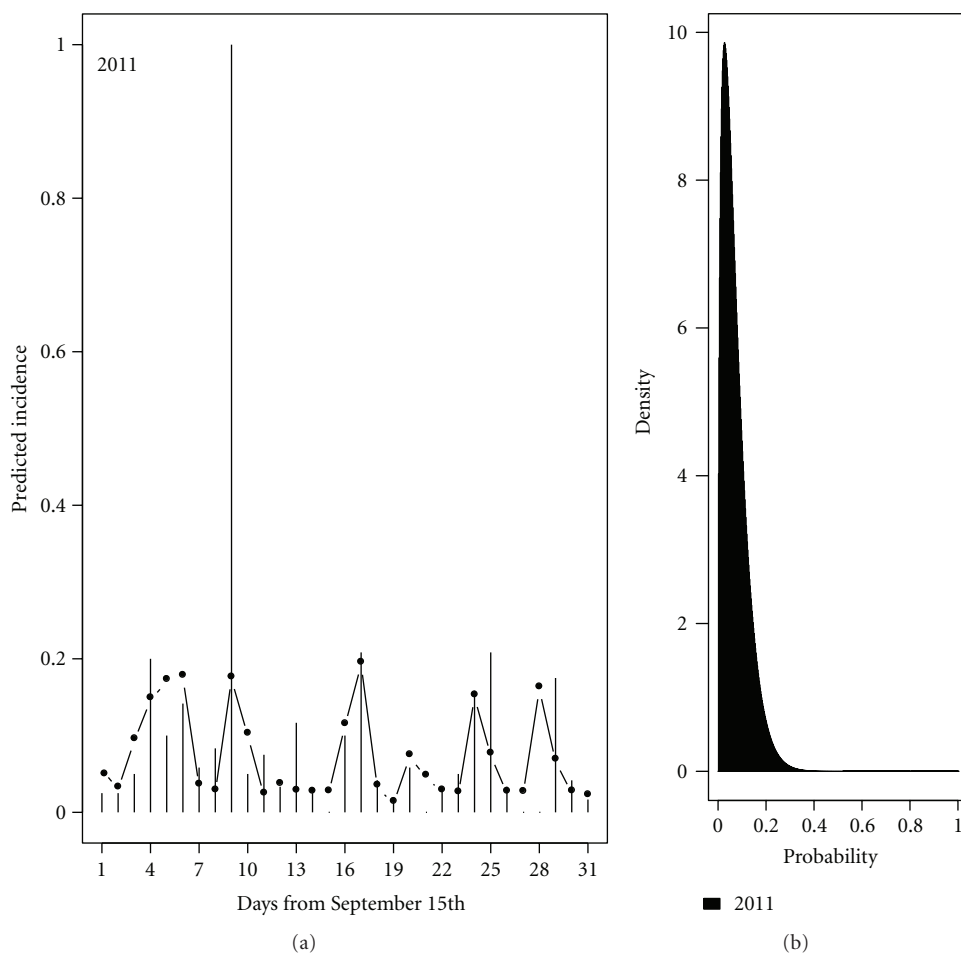
(a)

(b)

FIGURE 4: Validated model of spores incidence using climate variation in Passo Fundo, Brazil. The vertical lines represent the observed values and the continuous line the predicted values (a). Estimated density for the beta probability distribution function (b). The validation period was from September 15 to October 15, 2011.

Based on information from the model, we can establish a lower threshold of FHB probability incidence at 0.20.

In the future, combination of this model with an infection process model may result in a complex but more complete model. The combined model may be useful to quantify the impact of FHB epidemics on wheat yield and quality. The development of reliable epidemic forecasting systems should play an important role in FHB management, especially, if associated with expected advances in weather forecasting. Should an outbreak of FHB occur, a farm-scale intervention is usually required. Early warning based on forecasts from the model can assist in improving FHB control. Increasing fungicide spraying during high-risk periods and decreasing it during low-risk periods will improve cost effectiveness of operations. Crop advisers, if anticipating a higher FHB risk of occurrence, can increase vigilance, for example, by alerting farmers, planning for fungicide spraying and preparing for dealing with problem areas. These attempts, if successful, may have significant implications in wheat decision-making and practices, and may help farmers use resources more effectively and efficiently.

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