Factors Affecting Density of Airborne *Gibberella zeae* Inoculum*

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ABSTRACT

Fusarium head blight (FHB) is a disease of increasing concern in the production of wheat (*Triticum aestivum*). This work studied some of the factors affecting the density of airborne *Gibberella zeae* inoculum. Spore samplers were placed at the edge of a field in order to observe spore deposition over a period of 45 days and nights in September and October, the period that coincides with wheat flowering. *Gibberella zeae* colonies were counted for each period and values transformed to relative density. A stepwise regression procedure was used to identify weather variables helpful in predicting spore cloud density. In general, a predominant night-time spore deposition was observed. Precipitation and daily mean relative humidity over 90% were the factors most hightly associated with peak events of spores in the air. Models for predicting spore cloud density simulated reasonably well with the fluctuation of airborne propagules during both night and day, with potential to be integrated into an FHB risk model framework.

Additional keywords: *Fusarium graminearum*, aerobiology, spore dispersal, epidemiology.

RESUMO

Fatores que influenciam a densidade de inóculo aéreo de *Gibberella zeae*

A giberela, causada por *Gibberella zeae,* é uma das mais importantes doenças do trigo (*Triticum aestivum*). O presente trabalho objetivou estudar alguns aspectos que influenciam a densidade de inóculo aéreo. Armadilhas caça-esporos foram instaladas ao lado de parcelas experimentais de trigo com coletas realizadas durante o período da noite e do dia, por 45 dias nos meses de setembro a outubro, coincidindo com o florescimento do trigo. O número de colônias de *G. zeae*, avaliadas em cada período, foi transformado para valores relativos e correlacionado com variáveis ambientais. Uma análise de regressão foi utilizada para se identificar as variáveis climáticas úteis para a predição da densidade da nuvem de esporos. Foi observada uma predominância de deposição de inóculo aéreo no período da noite. Ocorrência de chuva e umidade relativa média diária superior a 90% estiveram fortemente associados com picos de concentração de inóculo aéreo. Os modelos simularam os incrementos na densidade da nuvem de esporos, apresentando potencial para serem integrados em um modelo de simulação do risco de giberela.

Palavras-chave adicionais: *Fusarium graminearum*, aerobiolobia, dispersão de esporos, epidemiologia.

INTRODUÇÃO

Fusarium head blight (FHB) of wheat (*Triticum aestivum* L.), caused mainly by *Gibberella zeae* (Schwein.) Petch. (anamorph *Fusarium graminearum* Schwabe), is a disease of increasing concern in the production of wheat (Mcmullen *et al.*, 1997). Contamination of the grain by mycotoxins such as deoxynivalenol (DON) (Jones & Mirocha, 1999) has caused economic losses due to decreased quality and yield of the grain. Severe epidemics have been reported in Brazil in recent years which have resulted in considerable yield losses (Panisson *et al.*, 2003). Typically, FHB is a floral disease infecting wheat during extrusion of anthers, although infections are also likely to occur from flowering to grain filling stages (Sutton, 1982; McMullen *et al.*, 1997). Ascospores and macroconidia of the pathogen are produced on over-wintered residues, and either propagule may infect wheat under favorable weather conditions. Ascospores are considered the most important spore type in terms of dispersal potential (Parry *et al.*, 1995; Paulitz, 1999) although macroconidia may play a role in the epidemics especially in regions where other *Fusarium* spp., which do not form a sexual stage, are important FHB pathogens (Rossi *et al.*, 2002). Several empirical weather-based models have been developed for predicting disease incidence, epidemic risk, or DON levels (Moschini & Fortugno, 1996; Hooker *et al.*, 2002; De Wolf *et al.*, 2003). A realistic approach for modeling FHB risk should take into account the effect of inoculum density and host developments as well weather variables (Fernandes & Pavan, 2002). Studies on the

temporal and spatial aspects of *G. zeae* airborne inoculum aimed at clarifying the processes and mechanisms favoring dispersal of propagules over either short or long distance are found in the literature (Ayers *et al.*, 1975; Reis, 1990; Paulitz, 1996; De Luna *et al.*, 2002). The present study investigates some weather variables that affect the fluctuation of airborne *G. zeae* inoculum in a wheat field. Identification of relevant weather variables can be useful for predicting the density of a *G. zeae* spore cloud to be incorporated into a FHB risk model framework.

MATERIAL AND METHODS

This study was carried out from September 1st to October 15th, 2003 at Embrapa Trigo, Passo Fundo, RS. Airborne inoculum of *G. zeae* was detected on selective media for *Fusarium* species (Nash & Snyder, 1962) deposited in plastic Petri dishes (9 cm diameter) mounted on two spore sample types. The first was a wind-driven sampler previously used by Reis (1988) and the second, called platform, was an adaptation of the sampler used by Schmale *et al.* (2002) for detection of *G. zeae* spores by gravitational settling. Plates containing selective media were placed at 1.3 m above the soil surface. Samplers were located 1 m apart on the border of experimental wheat plots, in an area surrounded by wheat and other cereal crops cultivated under the no-till system, which exhibited high levels of residue on the soil surface. Two daily samplings were performed at 9:00 and 21:00 h. 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 ºC and 12 h of darkness) in order to promote fungi growth. The number of *G. zeae* colonies was recorded for each plate as CFU (colony forming units) / 63 cm^2. Prior to evaluation, a sample of 20 confirmed true *G. zeae* colonies (according to Nelson *et al.*, 1983) were plated on the medium for comparisons during the evaluations. Other *Fusarium* species were observed but not identified at the species level. Weather information was acquired at Passo Fundo weather station (latitude -28,25º, longitude -54,4º, altitude 684 m) located 10 m from the wheat plots.

The SAS statistical package (SAS Institute, Inc., Cary, NC) was used to explore data, calculate summary statistics and calculate Pearson's correlation between pairs of variables. To study the weather effect on spore cloud density, a set of weather variables was created based on biological criteria and previous studies. The CFU number was transformed into relative density by dividing the number of colonies in one day by the maximum number of CFU detected during the sampling period. Those values were square-root transformed to normalize data and used as dependent variables. A stepwise regression (0.1 significance level for entry into the model) was applied to select the smallest set of independent variables related to the dependent one. Results of the regression analysis were evaluated on the basis of the standard error of model parameters, residual distribution, coefficient of determination (R^2), and adjusted R^2. The analysis

was applied to both a combination of night- and day-time observations and to only night-time observations. *G. zeae* colonies and weather information from October 16th to October 23rd was used to evaluate the model's performance.

RESULTS

In both sampler types and sampling periods, a total of 1,543 CFU of *G. zeae* colonies was detected during 45 days of collection. A higher proportion of colonies (70%) were detected during the night than in the day-time. In the platform sampler, 76% of 1,031 colonies were detected at night, while in the wind-driven sampler, 56% of 512 colonies, were detected at night (Figure 1). Regression studies were done with the total added values of colonies in both samplers (CFU/ 127cm^2).

Gibberella zeae airborne inoculum was present in most of the sampling days. In 13 out of the 89 day and night periods, inoculum density was equal or less than 3 CFU/127cm^2 (six day-time and seven night-time). In six day-time periods with an absence or only trace airborne inoculum, the mean relative humidity during collecting hours (RH12) ranged from 30 to 47%. In seven events of low inoculum durint night-time samplings, spores were not detected whenever RH12 was consistently below 70%, following a day-time with RH12<50%. Peaks in colony counts (>40 CFU/127cm^2) were observed several times, mainly during night-time. In the day-time collections, three peak events occurred on days with RH12>80%, associated with rain either during collection or during the previous day. At night-time, peaks of colony counts were observed in 11 cases, mostly occurring when RH12 was over 90% on either rainy or non rainy days in the period. Relative humidity variables showed the strongest correlations with airborne inoculum density, mainly during the 12 collecting hours. Weather variables from previously collected 12 hours were weakly or not at all correlated with spore cloud density (data not shown). The fluctuations in spore cloud density tended to follow relative humidity, especially during non-rainy days. In general, spore cloud density increased at night whenever RH12 was over 80% and decreased during the day (Figure 2). Although spore cloud density increased during a sequence of rainy days, a steep decrease was observed after a sequence of over three consecutive rainy days. Based on this, dummy variables for consecutive rainy days (CRD) were empirically created with the following values: 1st rainy day, CRD=1; 2nd rainy day, CRD=2; 3rd rainy day, CRD=2,5; 4th or more rainy days, CRD=0,3. A non rainy day after two consecutive rainy days, CRD=0,3; Non rainy days isolated or following one rainy day, CRD=0. Those dummy variables were used in the regression studies.

After some preliminary analysis, the stepwise regression applied to values observed only at night-time or at both day- and night-time, resulting in the selection of two equations for predicting spore cloud density. Those equations were developed with data from night-time collections given that most spore deposition events were observed at night-time and better correlations were observed:
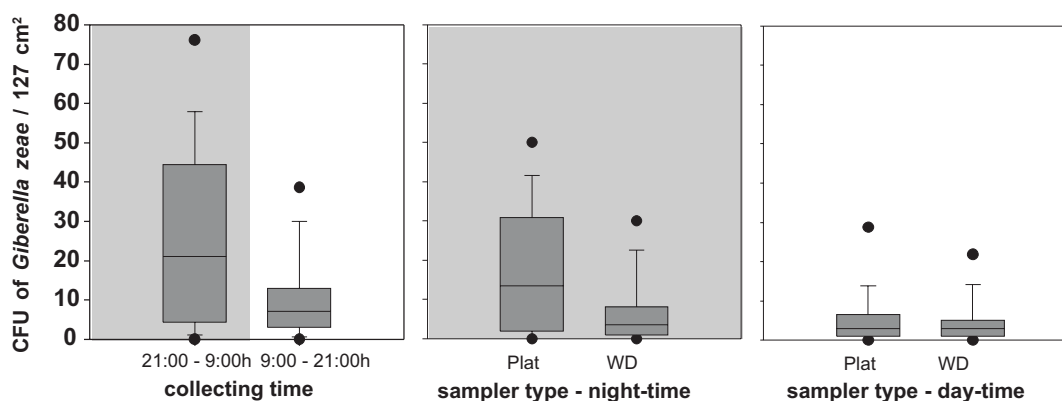
FIG. 1 - *Gibberella zeae* colony forming units (CFU) collected during night- (21:00 – 9:00 h) and day-time (9:00 – 21:00 h) in selective media deposited in Petri dishes mounted on two sampler types (Plat = platform and WD= wind-driven) installed close to wheat (*Triticum aestivum*) plots at a site in Passo Fundo, RS, Brazil, from September 1st to October 15th. Dots represent 5% and 95%; whiskers represent 25% and 75%; box represents 50%; horizontal line inside the box is the median.

$$RDGZ1 = (-0.6306 + 0.0152 \cdot RH24 + 0.1076 \cdot CRD)^2$$
$$RDGZ2 = (0.2553 + 0.0352 \cdot RH90 + 0.1128 \cdot CRD)^2$$

Where, RDGZ is the relative density of *G. zeae* spore cloud density at night-time; RH24 is the daily mean relative humidity; RH90 is the number of hours of relative humidity >90% at night-time; CRD is a dummy variable for positioning a rain in a sequence of rainy days.

Both equations accounted for 70%, and 71% of the experimental variance, respectively and standard errors of the model parameters were small (Table 1). The dummy variable for rain occurrence was selected in the models and contributed to explaining 10% of experimental variance. Relative humidity variables explained over 60% of experimental variance in both models. Other models that selected more than one RH variable (e.g. mean relative humidity and hours of relative humidity > 90%) accounted for over 70% of experimental variance but were not used because variables were collinear. In the validation with data not used to construct the model, the models simulated the peaks of colony counts associated with rain and relative humidity events reasonably well (data not shown).

Gibberella zeae colonies were detected during most of collecting periods, with a distinct increase in colony counts in October, when rain was more frequent and relative humidity higher. Reis (1988) using samplers placed over a glass plot to evaluate the seasonal pattern of *G. zeae* airborne inoculum in Passo Fundo, RS, observed peaks in monthly summaries of colony counts in October 1985 and from August to September 1986, coinciding with wheat flowering. The platform sampler detected a higher number of airborne propagules than the wind-driven sampler, especially during night-time. Even when raining, when one might suppose that spores could be washed off by rain drops, they remained attached to the media. Thus, the use of two sampler types may have effectively sampled a more realistic representation of viable *G. zeae* airborne propagules levels upon the effect of wind, rain and gravitational settling.

Reis (1988), collecting airborne *G. zeae* with the wind-driven sampler, observed that in a three-month average (September to November, 1984) 40% more *G. zeae* spores were collected at night compared to the day. In the present study, only 18% more propagules were collected at night, with the same sampler used by Reis. However, Reis' monthly summaries reveal the most evident differences between day and night colony counts in October, while values detected in November were similar. Intriguingly, in the same study by Reis (1988) spore samplers placed over a clover plot near corn (*Zea mays* L.), detected a consistently higher number of airborne propagules during day-time hours over a period of ten months, and the difference was most evident from September to April. The inconsistent pattern of different crops observed in these reports and compared with the present study using a wind-driven sampler, makes it unclear if spore density is higher at day or night and further studies should be carried out on the effect of wind and distance to inoculum sources. It may be possible that spores detected over clover drops during the day-time, may originate from distant inoculum sources, mainly dispersed by wind especially during the day. Maldonado-Ramirez & Bergstrom (2000) in Central New York observed a higher proportion of ascospores in the air during the day-time using a Burkard sampler and hypothesized that spores can potentially be elevated by turbulence to the low atmosphere, which is higher during the day-time, as was also suggested in a previous study with *Venturia inaequalis* (Cook.) Wint. airborne inoculum (Aylor, 1998). Francl *et al.* (1999) detected *G. zeae* spores kilometers distant from wheat fields, thus confirming that *G. zeae* ascospores can travel long distances from inoculum sources.

The predominant night-time spore deposition, observed in the platform sampler, is in partial agreement with studies by Schmale *et al.* (2002) in Central New York State, USA, using the same sampler type. The authors reported that 94% of the total viable *G. zeae* propagules were collected during night-time (8 h of exposure) in two winter wheat fields, concluding
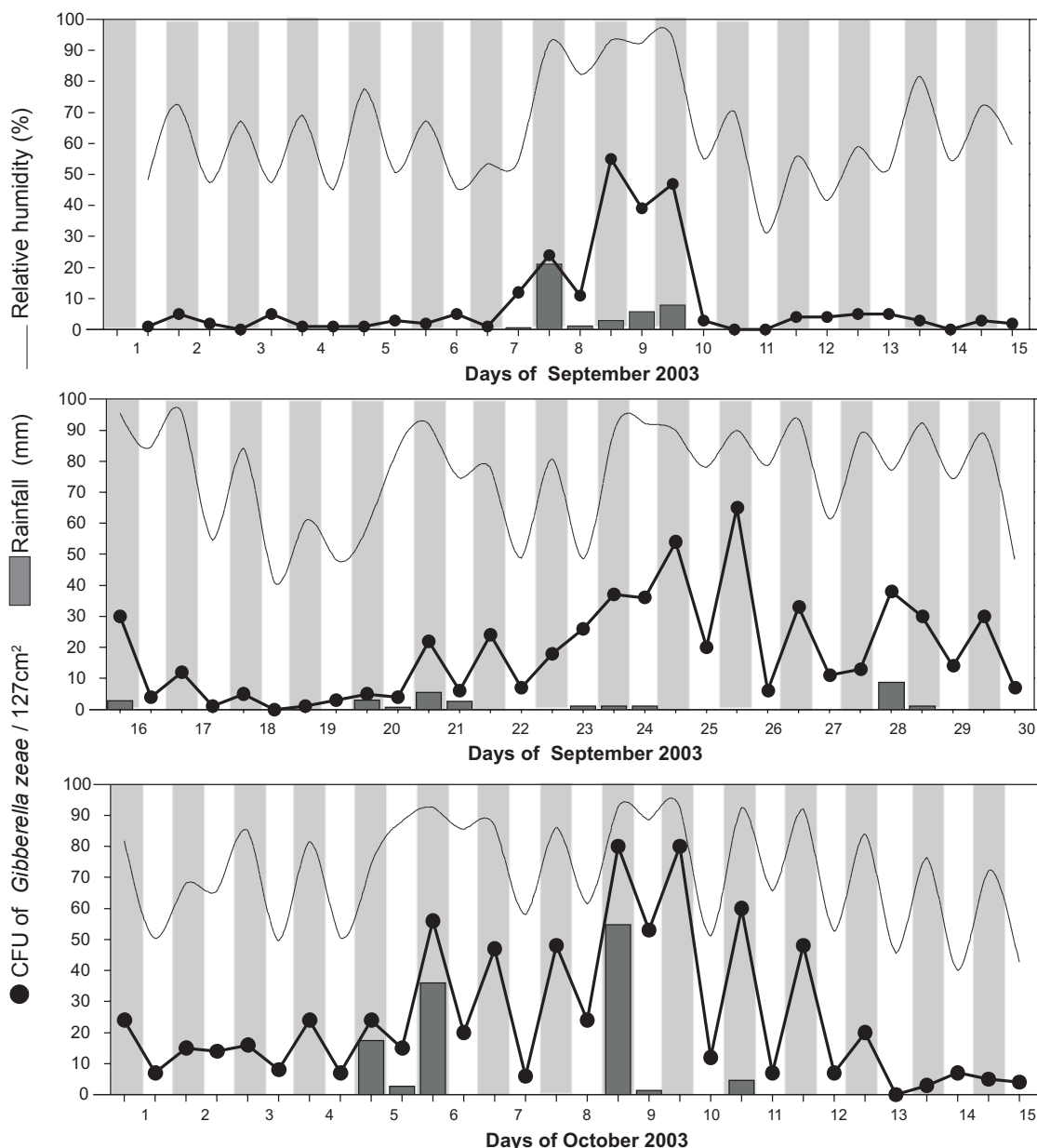
FIG. 2 - Daily and seasonal pattern of *Gibberella zeae* airborne inoculum, as number of colony forming units (CFU) detected in selective media mounted on spore samplers installed from September 1st to October 15th close to wheat (*Triticum aestivum*) experimental plots in Passo Fundo, RS, Brazil. Dotted lines represent CFU; solid bars represent precipitation (mm); solid lines represent mean relative humidity (%). Shaded vertical sections of the graphs' background represent night- time (21:00-9:00 h).

that major deposition events occurred at night. As opposed to day-time, it is suggested that an absence of wind or low wind speeds associated with an inverse layer at night can make spores gravitate over the wheat canopy, preventing escape.

Several studies have correlated the release and dispersal of *G. zeae* spores with the presence of water and high relative humidity (Ayers *et al.*, 1975; Reis, 1990; Paulitz, 1996). This may explain why spore cloud density is higher at night, when relative humidity is usually higher. In a study by Paulitz (1996) on the daily pattern of *G. zeae* spores over wheat plots artificially inoculated with infected corn residues, spores were collected

from 16:00 to 18:00, with density increasing linearly to 23:00 h. Paulitz (1996) suggested that wetness promoted by rain was necessary for perithecia formation, but not for spore release, once spores were collected during non rainy days. Yet, Paulitz (1996) observed the maximum spore concentration in the air occurred from one to four days after a rain event (>5 mm) or whenever RH was consistently over 80%. In the same study, sequential light rainy days temporarily removed spores from the air, while heavy rains promoted the same effect for a longer time. This pattern was also evident in the data presented by Reis (1990) where a steep decrease in colony counts was

TABLE 1 - Parameters and statistics of regression models adjusted to the relation between *Gibberella zeae* spore cloud density at night-time and weather variables

Model and variable[1]	Parameter	SE[2] of parameters	Partial R^2	*P* value	R^2	Adjusted R^2
RDGZ1						
RH24	0.0152	0.002	0.63	<0.0001	0.70	0.68
CRD	0.1075	0.034	0.07	0.0029		
RDGZ						
RH90	0.0351	0.005	0.62	<0.0001	0.71	0.69
CRD	0.1127	0.033	0.08	0.0354		

[1]RDGZ= Relative density of *G. zeae* spore cloud at night-time; RH90= Number of hours of relative humidity > 90% from 21:00 to 9:00 (night-time); RH24= Daily mean relative humidity (%) (day and night-time); CRD= dummy variable for a position of a rainy day in sequence of rainy days (0 to 2.5)
[2]Standard error

observed after four consecutive rainy days. Another study on the fluctuation of airborne macroconidia of *Fusarium* spp. revealed this same pattern (Rossi *et al,* 2002).

The present results agree with previous studies carried out in Passo Fundo during the 80's (Reis, 1988; Reis, 1990) and in the 2000 growing season (Panisson *et al.*, 2002). In those studies, peaks in colony counts were associated with rain events and high relative humidity. However, Panisson *et al.* (2002) observed that the number of propagules detected was 4.5 times higher than previously observed (Reis, 1988). The authors suggested that the higher concentration of residue in the region, due to an increased no-till cropping system, could have contributed to a higher inoculum pressure than found in previous decades (Panisson *et al.*, 2002). The values observed here were most similar to data observed by Reis (1988). Panisson *et al.* (2002) hypothesized that the proximity of the samplers to the wheat heads could have facilitated detection of splashed spores, apparently macroconidia. We also hypothesize a yearly effect, since a higher frequency of rain events from wheat heading to grain filling was observed in the 2000 season, which may have contributed to an increase in spore production and high FHB epidemic levels observed that year (Panisson *et al.*, 2003). The year of 2003 was non-epidemic with FHB ranging from low to moderate levels (data not shown) which may also be correlated with the lower airborne *G. zeae* inoculum levels observed this year, compared to the 2000 season, which was an epidemic year with precipitation above normal throughout the season. However, in the present study, *G. zeae* spores were consistently sampled even in dry periods, confirming that inoculum is always present, as was suggested by Reis (1988).

The simple models obtained aim at predicting overall estimations of relative spore cloud density in a wheat field in the Passo Fundo wheat growing area. Both models are influenced by simple weather variables and might be useful for an FHB forecast model. Validation with data not used to construct the model showed that peaks in airborne inoculum density were well predicted by the models, using only precipitation occurrence and relative humidity from either 12 or 24 h. However, caution needs to be taken when evaluating the models at different locations given the differences in the amount and type of crop residue in the field. It seems clear that an area intensively no-tilled comprises a higher deposit of *G. zeae* inoculum that affects the spore cloud density. Also, some evidence exists that growing wheat following wheat, and specially following corn poses a higher risk of epidemics (Dill-Macky & Jones, 2000), and that this may highly contribute to within-field inoculum. The integration of such models into an FHB modeling framework can more realistically represent the risk and better quantify an infection event, taking into account the density of a *G. zeae* spore cloud potentially depositing on the spikes.

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