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ORIGINAL ARTICLE

Interactions between temperature and wheat head wetting duration on fusarium head blight intensity

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ABSTRACT

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In experiments conducted in growth chambers with the susceptible wheat cultivar BR 23, interactions between five temperatures (10, 15, 20, 25 and 30°C) and eleven wetting periods were assessed for fusarium head blight (FHB) intensity. Each temperature consisted of one experiment and the wetting hours corresponded to the treatments. The disease occurred even at 10ºC, the minimum tested temperature, and the maximum incidence was at 25ºC, both after 50h wetting. Variations in wheat FHB intensity with temperature were explained by the Beta generalized model, and spike wetting duration by the Gompertz model. Disease intensity was modeled according to temperature and wetness duration. The resulting equation represents a description of the response of FHB spikelet incidence to the combined effects of temperature and wetness duration. Since the infection requires a long wetness period, its origin may not be dew but rain, which suggests fungicide application before the occurrence of predicted rain during the wheat predisposition period.

Keywords: Decision support, climate, *Fusarium graminearum*, fusarium head-blight, *Gibberella zeae, Triticum aestivum*.

RESUMO

Reis, E.M.; Zoldan, S.M.; Zanatta, M. Interações entre temperatura e duração da molhagem das espigas de trigo na intensidade da giberela. ***Summa Phytopathologica,*** v.49, p.1-5, 2023.

Em experimentos conduzidos em câmaras de crescimento com trigo suscetível cultivar BR 23, foram avaliadas as interações entre cinco temperaturas (10, 15, 20, 25 e 30°C) e onze períodos de molhamento na intensidade da giberela (FHB). Cada temperatura constou um experimento e as horas de molhamento os tratamentos. A doença ocorreu mesmo a 10ºC, a temperatura mínima testada, e a incidência máxima a 25ºC ambas com 50h de molhamento. As variações na intensidade de FHB do trigo em função da temperatura foram explicadas pelo modelo Beta generalizado, e a duração do molhamento das espigas pelo modelo de Gompertz. A intensidade da doença foi modelada em função da temperatura e da duração dos picos de molhamento. A equação resultante representa uma descrição da resposta da incidência de espiguetas de FHB aos efeitos combinados de temperatura e duração do molhamento. Como a infecção requer um longo período de molhamento, sua origem não deve ser o orvalho e sim a chuva, sugere-se que antes da ocorrência de uma chuva prevista, durante o período de predisposição do trigo, seja feita a aplicação de fungicidas.

Palavras chave: Apoio à decisão, clima, *Fusarium graminearum*, giberela, *Gibberella zeae, Triticum aestivum*.

Wheat fusarium head blight (FHB), or scab, is frequently a destructive fungal disease caused by several *Fusarium* species, but *F. graminearum* Schwabe [teleomorph *Gibberella zeae* (Schwein) Petch] is the principal causal agent. This disease occurs all over the world in regions where winter cereals are grown under hot, humid and semi-humid climates with frequent rainfall resulting in a long anther wetting duration (1).

FHB has been known for a long time in Brazil and little progress has been made for its control. This disease was probably noted for the first time in 1942, in Veranópolis, Rio Grande do Sul (RS) (5).

Up to 39.8% damage was reported by Casa & Kunen Junior (3).

The major inocula for infection are ascospores saprofitically produced in perithecia on numerous native grasses that are senesced or killed by frost (18). This mechanism ensures the presence of the inoculum in the air every day of the year (15, 17, 19). In Southern Brazil, where wheat is grown, corn is cultivated in a reduced area and, therefore, may not be the major inoculum source as previously reported (11, 23); thus, corn residue is not necessary for the maintenance and production of FHB inoculum.

FHB is a floral infection highly dependent on rainfall during or after flowering (7, 16, 23).

The wheat predisposition period extends from the presence of anthers, since their release, to the wheat maturation, i.e., presence of partially exerted anthers (6, 20, 23). Thus, as long as green spikes are

present, the infection can occur post-anthesis (absence of loose anthers) (4). This is a clear indication that a potent fungicide should be deposited on the anthers to protect heads from infection, as well as on the partially exposed anthers present after flowering.

It has been accepted that FHB is related to long periods of rain and mild temperatures after the crop is heading. The disease onset depends on a minimum head wetness duration during the predisposition period (1, 23, 24). Occurrence of FHB in Brazil is limited to the southern states, where rainfall is frequent during and after wheat flowering. The last recorded epidemic was in 2017 growing season (OR seeds communication).

FHB has been recognized as a disease of difficult control because the shape of heads is similar to a vertical cylinder, which make them difficult targets for fungicide deposition. Since the infection depends on a long wetness period of infection sites, decision making regarding the application of fungicides to protect the anthers can be based on the first event of rain prediction during the predisposition period.

The relationship between the disease and the environment can be improved using the following methods: a) fundamental: developed based on data obtained experimentally under controlled conditions, in which the effect of temperature and wetness duration on the infection is evaluated by describing one or more aspects of the pathogen-host-environment interaction, and b) empirical: developed from the collection and analysis of historical data on disease records and environmental conditions in a given location (13, 26).

The continuous effect of wetting period (WP) and temperature, required for infection to occur, greatly vary among pathosystems. Andersen (1) carried out a pioneer study of temperature and relative humidity effects on FHB development in a controlled environment.

The hypothesis formulated here is that the infection of wheat ears requires long periods of wetness together with high temperatures. Defining the interactions between temperature and ear wetness duration will also be useful in the artificial inoculation of wheat cultivars/lines, aiming at selection for resistance under similar field conditions.

The objective of the present study was to conduct new investigation to better understand the interactions between temperature and wetting duration, under controlled conditions, on FHB intensity in wheat, and how this piece of information may help in decision making for fungicide spraying.

MATERIAL AND METHODS

The study was carried out in the laboratory and in climatized chambers equipped with temperature and leaf wetness control system at University of Passo Fundo – RS.

Plant cultivation

Seeds of wheat cultivar BR 23 treated with the fungicide triadimenol 40 g a.i. 100 kg^{-1} and the insecticide imidacloprid 36 g a.i. 100 kg^{-1}, according to Technical Information for the 2020 season (9), were grown in plastic buckets containing vegetable garden soil; 10 seeds per pot were sown. The pots were kept in a greenhouse during the vegetative stage of plants. At the boot phenological stage, they were transferred to a climatized chamber at 25ºC and 12h photoperiod.

Inoculum production

The inoculum was produced from a pure colony of a *Fusarium graminearum* isolate obtained and used by Telles Neto (27) and deposited in the mycoteca of "Faculdade de Agronomia e Medicina Veterinária". From the original colony, the fungus was subcultured to Petri dishes containing the culture medium ¼ BSA (50 g potato, 5 g sucrose and 15 g agar) for 1000 mL distilled water plus antibiotic (streptomycin sulfate 0.2 g in 50 ml sterile-distilled water). The plates were incubated at 25°C ± 2°C and 12h photoperiod. From the colonies, a macroconidial suspension was prepared in distilled water with two drops L^{-1} polyoxyethylene sorbitan (Tween 20), adding distilled water on the colony while brushing it to release the propagules. The suspension was strained and the inoculum density was determined by counting the conidia in 0.01 µL, poured onto a slide, and by scanning examination under a microscope. From this concentration, dilution was used to obtain the desired concentration for inoculation with 40,000 spores mL^{-1}, according to Telles Neto (27).

Inoculation was carried out by depositing the inoculum suspension with a manual sprayer on the wheat ears until runoff, at 7-8 days after the beginning of anther extrusion. Only plants that showed uniform development and flowering had the ears inoculated.

After inoculation, the plants were kept in a chamber at programmed temperatures and times (10, 15, 20, 25 and 30°C and 12 h photoperiod). Plants were protected by individual plastic shelters for each treatment (0, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 hours wetness). Each shelter contained a sprinkler on the top for continuous wetting of ears during pre-established periods; an electronic timer activated the electric motor of the water pump pressure at three-hour intervals. The maximum temperature for incubation was 30ºC, since higher temperatures for several hours impair the development of wheat plants, which constitute a winter crop.

At the end of each wetting period, plants were transferred to another climatized chamber, at constant 25ºC, 12h photoperiod and relative humidity below 70%.

On the tenth day after inoculation, individual wheat ears were evaluated by disease intensity quantification based on the number of infected spikelets or spikelet incidence (%).

A completely randomized design was adopted with five replicates. Each experiment (temperature) consisted of eleven treatments, which correspond to the different hours of wetness, totaling six temperatures and eleven wetting periods. The experimental units consisted of six pots with five plants each, and each treatment totaled 30 ears.

All experiments were repeated twice since they showed similar trends, and the data from the experiment with less variation were used for analysis.

Temperature effect on FHB intensity in wheat ears

To adjust the temperature data, SAS statistical program (21) was used in the non-linear procedure. The data were adjusted by means of nonlinear regression, using the Beta function cited by Jesus Junior et al. (10), which explains the effect of temperature on the development of plant diseases, where:

$$Y=B1((X-B2)^{B4})((B3-X)^{B5})$$

Parameters B2 and B3 represent the minimum and maximum temperatures, respectively; Y, the disease intensity, and X, the tested temperatures; B1, B4 and B5 are model parameters and have no biological significance.

Effect of ear wetness duration on wheat FHB intensity

The temperature of 25ºC, established as the optimal temperature for FHB occurrence, was used to adjust the ear wetness data. Data were adjusted using nonlinear regression, testing Gompertz, Logistic and Monomolecular (2). The best model was chosen by considering the highest adjusted coefficient of determination (R^2); the value of the mean square of deviations; the smallest distribution of residuals, and the shape of the curve of the observed result versus the predicted result

for each model (2, 10).

Non-linear regression analysis was conducted, the equation was generated and an Excel 6.0 spreadsheet was completed with the desired FHB intensity in the cells, expressed as disease intensity (0 to 100%), and the temperature from 10 to 35ºC; the wetness duration (hm) was regarded as unknown and calculated to obtain desired probabilities of disease intensities, called daily values of probability of infection (DPI).

RESULTS AND DISCUSSION

The temperature range between 20°C and 30°C, based on Andersen (1), resulted in the highest disease intensity, peaking at 25°C (90.96 ± 3.9%) in interaction with the longest wetting period (50 h) (Table 1). The effect of temperature on FHB spikelet incidence during the maximum wetness period (50 h) can also be seen in Figure 1. The disease occurred even at the minimum studied temperature (10ºC), in the absence of ear wetness; this may have happened because relative humidity in the incubation chamber varied at certain periods, which may have provided favorable conditions for infection and colonization. Since it is a closed environment, although not measured, the relative humidity that favored infection may have been kept high.

Differently from Andersen (1), who obtained 1.6% disease at 15ºC (48 h), in the present study at this same temperature approximately 43% infected spikelets were recorded at maximum wetness (50 h), while disease levels close to 5% were observed at 10ºC, the minimum

Table 1. Interactions between temperatures and wetting duration for wheat ears inoculated at flowering on FHB incidence in wheat spikelets.

Head wetting duration (h)	Temperature (ºC)					
	10	15	20	25	30	35
	Spikelet incidence (%)[y]					
0	0.66 ± 0.402[z]	6.78 ± 1.562	6.63 ± 0.76	0.49 ± 0.3032	3.03 ± 1.223	0.00
5	1.1 ± 0.45	3.8 ± 0.453	6.64 ± 2.938	2.52 ± 1.816	2.80 ± 0.79	0.00
10	1.82 ± 0.048	3.27 ± 0.976	6.62 ± 2.011	10.67 ± 1.5616	6.10 ± 1.116	0.00
15	0.7 ± 0.714	3.92 ± 1.107	12.48 ± 1.62	11.04 ± 1.483	7.63 ± 2.625	0.00
20	1.7 ± 0.53	8.65 ± 2.091	11.62 ± 2.58	16.34 ± 3.418	12.12 ± 1.738	0.00
25	0.3 ± 0.33	10.75 ± 2.925	15.0 ± 0.4	27.04 ± 3.4787	28.76 ± 5.031	0.00
30	1.14 ± 0.47	10.42 ± 1.632	21.76 ± 4.993	33.21 ± 3.3735	59.43 ± 1.49	0.00
35	4.9 ± 2.4	11.14 ± 1.848	33.8 ± 5.426	68.4 ± 3.9123	67.53 ± 5.87	0.00
40	3.52 ± 1.44	15.66 ± 2.795	51.56 ± 2.669	53.76 ± 2.0682	86.69 ± 2.48	0.00
45	3.6 ± 1.67	22.52 ± 3.734	64.8 ± 3.55	80.12 ± 8.7663	86.41 ± 1.54	0.00
50	5.4 ± 0.91	42.86 ± 6.163	77.1 ± 3.887	90.96 ± 3.8876	83.46 ± 2.243	0.00

[y] Percentage of infected spikelets 10 days after inoculation.[z] Mean standard error.

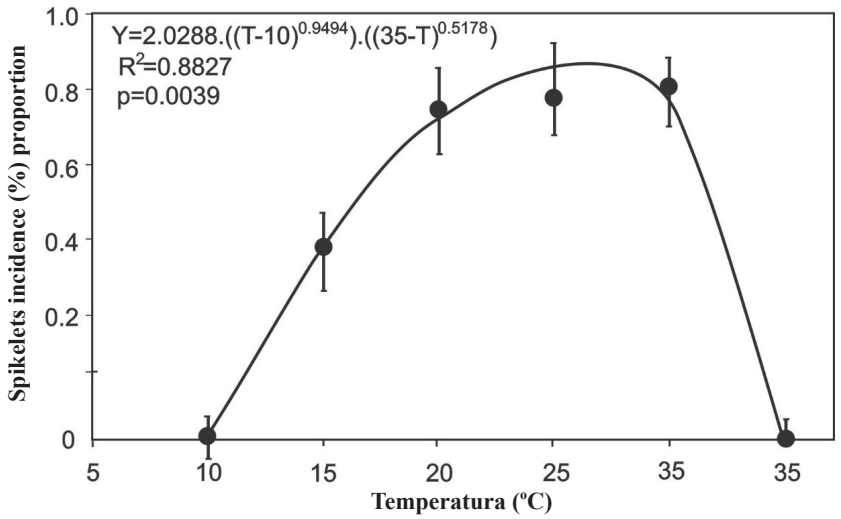


Figure 1. Incidence of *Gibberella zeae* in spikelets of wheat cv. BR 23 (Y) at different temperatures (T) and 50 hours of ear wetting. Bars represent the standard error of the mean and the bold line represents the curve obtained by fitting the Beta - Generalized model.

studied temperature. At 30ºC, the disease intensity began to decrease, especially in wetness periods shorter than 25 h.

Concerning the isolated effect of ear wetness periods on FHB intensity, it may be concluded that the data best fit to Gompertz model:

$$Y= EXP(-(-Ln(yo)).EXP(-r.hm))$$

Where Y represents the disease intensity; 'yo', the minimum wetness necessary for the disease to occur; 'r', the progress rate for Gompertz model, and 'hm', the ear wetness period (Fig. 2).

Wetness periods shorter than 10h showed an infection percentage close to or lower than 6%, except at 25ºC, at which this percentage exceeded 10%.

FHB is highly dependent on wetness, which can be seen in Figure 2. The longer the wetness period, the greater the number of infected spikelets. A 30-h wetting period and temperature equal to or greater than 20ºC resulted in 25% infected spikelets, while at 25ºC this intensity was reached with 25 h wetness. Wetting lasting longer than 40 h, at a temperature above 20ºC, results in more than 50% infected spikelets.

The interaction between temperature and wetness on FHB intensity was obtained by combining the equations found for the two factors, where:

$$Y=2.0288.((T-10)^{0.9594}).((35-T)^{0.5178})).(EXP(-(-Ln(0.00243)).EXP(-0.060839.hm))$$

This equation gave rise to the response surface graph (Fig. 3). For better visualization of the numerous interactions, a table was elaborated with 5ºC interval.

Under field conditions, the effects of the interactions between temperature and wetness hours on the infection are influenced by numerous factors and may differ from those obtained under controlled

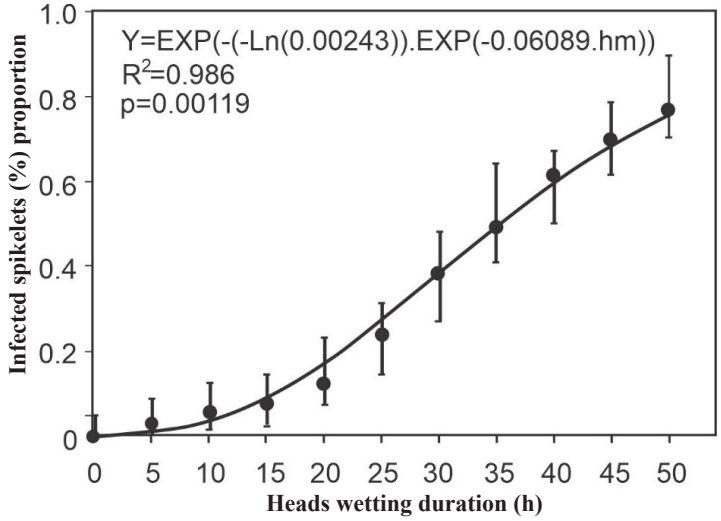
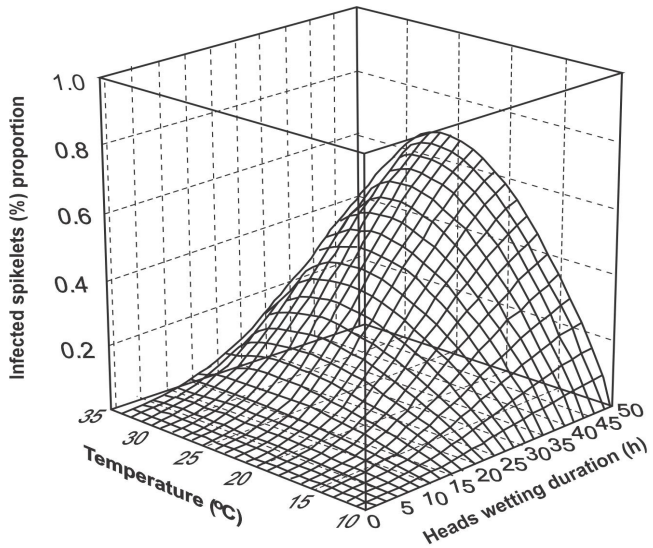


Figure 2. Incidence of *Gibberella zeae* in spikelets of wheat cv. BR 23 (Y) in different ear wetting periods (hm) at 25ºC. Bars represent the standard error of the mean and the bold line represents the curve obtained by fitting the Gompertz



$$Y=(2.0288.((T-10)^{0.9594}).((35-T^{0.9594}).(EXP(-(-Ln(0.00243)).EXP(-0.06839.hm))$$

Figure 3. Surface response of the interaction between ear wetness duration (hm) and temperature (T) on the incidence of *Gibberellla zeae* in spikelets of wheat cv. BR 23.

conditions. Some of these factors cited by Sutton (21) are: variation in climate conditions, availability of produced inoculum (density), host predisposition (age of susceptible tissues/organs), presence of nutrients and pesticides in the phylloplane, and biological antagonistic activity of residents on the phylloplane. Therefore, precise disease intensity under natural cultivation conditions is difficult to achieve.

The data obtained under controlled conditions suggest that wetness longer than 35 h can cause approximately 68% infected spikelets when the temperature is close to 25ºC. Disagreeing with the data reported by Andersen (1), the present study suggests that the fungus causes greater infection in shorter wetness periods and adapts to lower temperatures, developing at up to 10°C, although maintaining the infection and development at 25ºC.

The present study confirms the potential risk of FHB, especially due to the temperatures in southern Brazil, where the largest wheat cultivation area is concentrated. Most wheat crops bloom in October, which has 167 mm normal mean rainfall, 10 event frequency per month and 17.7 ºC average temperature (CNPtrigo Embrapa).

It can be inferred that FHB is a disease that requires long wetness periods, which must be satisfied by rain and not by dew. Therefore, if there is no rain, the disease will probably not occur, and rain forecast can be used as a decision-making strategy in its control. When the wheat crop is at predisposition stage (from the onset of anthesis, while head are still green and PEA is present), the most efficient fungicide and the best spraying technology should be preventively used before the predicted rain onset (20).

According to Moschini & Fortugno (10), FHB requires two consecutive days of rain: > 0.2 mm on the first day, RH > 81, and > 78 on the second day. This combination was called the critical period. No rain means no wetting period for wheat head infection.

Not all types of rain can result in head wetness > 48h and, on the other hand, heavy rain is not related to the duration of wheat head wetting. Thus, whenever there is rain, regardless of its volume, resulting in different wetness periods, there will be infection. Therefore, the amount of rain required for wetting wheat spikes can be a useful tool to forecast future FHB occurrence. The proposal presented here is based on rain forecast, which has the advantage of its simplicity to obtain information and improved accuracy in indicating the time for fungicide application. Rain has been forecasted 48h to 72h beforehand by CPTEC/INPE (www.cptec.inpe.br/).

Therefore, at the predisposition period and before the predicted rain occurs, wheat should be sprayed with efficient fungicide, considering that growers have access to accurate rain forecasts but the wetting duration is still unknown.

The present proposal may be more accurate and feasible than other forecasting system attempts described in the literature (8, 12, 14, 22, 26).

REFERENCES

1. Andersen, A.L. The development of *Gibberella zeae* head blight of wheat. **Phytopathology**, St. Paul, v.38, p.595-611, 1948.
2. Brustolin, R.; Zoldan, S.M.; Reis, E.M.; Zanatta, T.; Carmona, M. Weather requirements and rain forecast to time fungicide application for Fusarium head blight control in wheat. **Summa Phytopathologica**, Botuctu, v.39, n.4, p.248-251, 2013.
3. Campbell, C.L.; Madden, L.V. **Introduction to Plant Disease Epidemiology**. New York: John Wiley & Sons Inc., 1990. 532p.
4. Casa, R.T.; Kuhnen Junior, P.R. Danos causados nos hospedeiros. In: Reis, E.M. (org.). **Seminário 18 sobre giberela em cereais de inverno:** coletânea de trabalhos. Passo Fundo: Berthier, 2011. p. 73-86.
5. Cowger, C.; Patton-Özcurt, J.; Brown-Guedira, G.F.; Perugini, L. Post--anthesis moisture increased Fusarium head blight and deoxinevalenol levels in North Carolina winter wheat. **Phytopathology**, St. Paul, v.99, p. 320-327, 2008.
6. Costa Neto, J.P. **Parasitas de plantas cultivada no Rio Grande do Sul**. Porto Alegre: Secretaria de Estado dos Negócios da Agricultura, Indústria e Comércio, 1947. 21p.
7. Del Ponte, E.M.; Fernandes, J.M.C.; Pierobom, C.R.; Bergstrom, G.C. Giberela do trigo – aspectos epidemiológicos e modelos de previsão. **Fitopatologia Brasileira**, Brasília, DF, v.29, p.587-605, 2004
8. De Vries, A.P.H. Flowering biology of wheat, particularly in view of hybrid seed production - A review. **Euphytica**, Wageningen, v.20, p.152-170, 1971.
9. Engle, J.S.; Lipps, P.E.; Grahan, T.L.; Boehm, M.J. Effects of choline, betaine, and wheat floral extracts on growth of *Fusarium graminearum*. **Plant Disease**, St. Paul, v.88, p.175-180, 2004.
10. Giroux, M.E.; Bourgeois, G.; Dion, Y.; Rioux, S.; Pageau, D.; Zoghlami, S.; Parent, C.; Vachon E.; Vanasse, A. Evaluation of forecasting models for Fusarium head blight of wheat under growing conditions of Quebec, Canada. **Plant Disease**, St. Paul, v.100, p.1192-1201, 2016.
11. Informações técnicas para trigo e triticale: safra 2020: 13ª Reunião da Comissão Sul Brasileira de Pesquisa de Trigo e Triticale. Passo Fundo: Biotrigo Genética, 2020. Disponível em: <https://www.embrapa.br/busca--de-publicacoes/-/publicacao/1123960/informacoes-tecnicas-para-trigo-e--triticale-safra-2020>. Acesso em: 4 out. 2022.
12. Jesus Junior, W.C.; Pozza, E.A.; Vale, F.X.R.; Anguilera, G.M. Análise temporal de epidemias. In: Vale F.X.R.; Zambolim L. **Epidemiologia aplicada ao manejo de doenças de plantas**. Belo Horizonte: Editora Perfil, 2004. p.126-191.
13. Khonga, E.B.; Sutton, J.C. Inoculum production and survival of Gibberella zeae in maize and wheat residues. **Canadian Journal of Plant Pathology**, Ontario, v.10, p.232-239, 1988
14. Moschini, R.C.; Fortugno, C. Predicting wheat head blight incidence using models based on meteorological factors in Pergamino, Argentina. **European Journal of Plant Pathology**, Wagenigen, Cidade, v.102, p.211-218, 1996.
15. Moschini, R.C.; Pioli, R.; Carmona, M.A.; Sacchi, O. Empirical predictions of wheat head blight in the northern Argentinian Pampas regions. **Crop Science**, Cidade, v.41, p.1541-1545, 2001.
16. Nicolau, M.; Fernades, J.M. A predictive model for daily inoculum levels of Gibberella zeae in Passo Fundo, Brazil. **International Journal of Agronomy**, London, v.2012, 7 p., 2012. DOI:10.1155/2012/795162.
17. Panisson, E.; Reis, E.M.; Boller, W. Quantification of *Gibberella zea* propagules in the air and head blight infection in wheat anthers. **Fitopatologia Brasileira**, Brasília, DF, v.27, p.489-494, 2002.
18. Parry, D.W.; Jenkinson, P.; McLeod, L. Fusarium ear blight (scab) in small grain cereals – a review. **Plant Pathology**, London, v.44, p.7-238, 1995.
19. Reis, E.M. Quantificação de propágulos de *Gibberella zea*e no ar através de armadilhas de esporos. **Fitopatologia Brasileira**, Brasília, DF, v.13, n.4, p.324-327, 1988.
20. Reis, E.M. Perithecial formation of *Gibberella zeae* on senescent stems of grasses under natural conditions. **Fitopatologia Brasileira**, Brasília, DF, v.15, p.52-53, 1990.
21. Reis, E.M.; Wordell Filho, J.A. Princípios de epidemiologia. In: Reis, E.M. (org.). **Previsão de doenças de plantas**. Passo Fundo: Editora UPF, 2004. P.49-64.
22. Reis, E.M.; Boareto, C.; Danelli, A.L.D.; Zoldan, S.M. Anthesis, the infectious process and disease progress curves for fusarium head blight in wheat. **Summa Phytopathologica**, Botucatu, v.42, n.2, p.134-139, 2016.
23. SAS Institute Inc. SAS®. Leaninig Edition 2.0. Cary, SAS Institute, 2004.
24. Shah, D.A.; De Wolf, E.D.; Paul, P.A.; Madden, L.V. Predicting Fusarium head blight epidemics with boosted regression trees. **Phytopathology**, St. Paul, v.104, p.702-714, 2014.
25. Sutton, J.C. Epidemiology of wheat head blight and maize era rot caused by *Fusarium graminearum*. **Canadian Journal of Plant Pathology**, Ottawa, v.4, n.2, p.195-209, 1982.
26. Sutton, J.C. Predictive value of weather variables in the epidemiology and management of foliar diseases. **Fitopatologia Brasileira**, Brasília, DF, v.12, p.305-312, 1988.
27. Telles Neto, F. **Transmissão e controle de *Fusarium graminearum* em sementes e danos causados pela giberela em trigo.** Dissertação (Mestrado em nome do programa de PG) – FAMV, Universidade de Passo Fundo, Passo Fundo, 2004.

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