ARTIGOS

Determination of the most susceptible phenological stage of rice panicles to infection by species of *Fusarium graminearum*

Bruno Tabarelli Scheidt[1*], Ricardo Trezzi Casa[2], Otávio Ajala Fiorentin[3], Flávio Chupel Martins[4], Paulo Kuhnem[5], Amanda de Lima[4], Mônica Farias[6]

[1]Doutorando do Programa de Pós-Graduação em Produção Vegetal (PPGPV), Universidade do Estado de Santa Catarina (UDESC), Centro de Ciência Agroveterinárias - CAV / Av. Luiz de Camões, 2090 - Conta Dinheiro - Lages - SC - Brasil, CEP: 88.520-000; [2]Professor do PPGPV-UDESC, Centro de Ciência Agroveterinárias - CAV / Av. Luiz de Camões, 2090 - Conta Dinheiro - Lages - SC - Brasil, CEP: 88.520-000; [3]Professor de Fitopatologia Agrícola na Universidade do Oeste de Santa Catarina - UNOESC, Xanxerê e Região - SC. Rua Dirceu Giordani, 696 - Jardim Taruma. CEP: 89820-000 - Santa Catarina- SC - Brasil; [4]Mestrandos do PPGPV, Centro de Ciência Agroveterinárias - CAV / Av. Luiz de Camões, 2090 - Conta Dinheiro - Lages - SC - Brasil, CEP: 88.520-000; [5]Fitopatologista/pesquisador da Biotrigo Genética, Estrada do trigo, 1000. Bairro São José - CEP 99052-160, Caixa postal 3100, Passo Fundo - RS - Brasil; [6]Acadêmica do Curso de Agronomia, UDESC, Centro de Ciência Agroveterinárias - CAV / Av. Luiz de Camões, 2090 - Conta Dinheiro - Lages - SC - Brasil, CEP: 88.520-000. *Parte da dissertação de mestrado do primeiro autor.

Autor para correspondência: Bruno Tabarelli Scheidt (brunotabarelli.s@hotmail.com)

Data de chegada: 06/03/2018. Aceito para publicação em: 05/02/2019.

10.1590/0100-5405/192513

ABSTRACT

Scheidt, B.T; Casa, R.T.; Fiorentin, O.A.; Martins, F.C.; Kuhnem, P.; Lima, A.; Farias, M. Determination of the most susceptible phenological stage of rice panicles to infection by species of *Fusarium graminearum*. ***Summa Phytopathologica,*** v.45, n.3, p.243-246, 2019.

Occurrence of rice seeds infected by *Fusarium graminearum* has shown the importance of identifying the most favorable phenological stage of panicles to grain infection. The experiments were conducted in two crop seasons under greenhouse conditions, using the rice hybrid INOV CL. The phenological stages during inoculation were complete booting, full heading and flowering. All plots were inoculated using two isolates of *Fusarium graminearium* species complex 15A (*F. graminearium* - 15-ADON) and FmNiv (*F. meridionale* - Nivalenol). Disease severity was estimated at weekly intervals and was used to calculate the area under the disease progress curve (AUDPC), while panicles were collected to determine the percentage of spotted grains and *Fusarium* incidence. Percentage of spotted grains and incidence of *F. graminearum* and *F. meridionale* were greater when inoculation was made during flowering stage, significantly differing from heading and booting stages. Rice flowering stage is more susceptible to infection by *F. graminearum* and *F. meridionale*, inducing higher disease severity and incidence of spotted grains, as well as presence of fungi in the grains. Flowering was the most susceptible stage in the two crop seasons for both isolates, and the complete booting stage presented the lowest values of AUDPC.

Keywords: *Gibberella zeae*, *Oryzae sativa*, inoculation, fungus.

RESUMO

Scheidt, B.T; Casa, R.T.; Fiorentin, O.A.; Martins, F.C.; Kuhnem, P.; Lima, A.; Farias, M. Determinação do estádio fenológico mais suscetível a infecção de espécies de *Fusarium graminearum* em panículas de arroz. ***Summa Phytopathologica,*** v.45, n.3, p.243-246, 2019.

A ocorrência de sementes de arroz infectadas por *Fusarium graminearum* mostra a importância de identificar o estágio fenológico da panícula mais favorável à infecção dos grãos. Os experimentos foram realizados em duas safras em condições de casa de vegetação, utilizando o híbrido de arroz INOV CL. Os estádios fenológicos durante a inoculação foram, emborrachamento completo, espigamento pleno e florescimento. Todas as parcelas foram inoculadas com dois isolados do complexo de espécies de *Fusarium graminearium* 15A (*F. graminearium* - 15-ADON) e FmNiv (*F. meridionale* - Nivalenol). A severidade da doença foi estimada em intervalos semanais e utilizada para calcular a área abaixo da curva de progresso da doença (AACPD) e as panículas foram coletadas para determinar a porcentagem de grãos manchados e a incidência de *Fusarium*. A porcentagem de grãos manchados e a incidência de *F. graminearum* e *F. meridionale* foram maiores quando a inoculação foi feita durante o estágio de florescimento, diferindo significativamente dos estágios de emborrachamento e espigamento pleno. O estágio de florescimento do arroz é mais suscetível à infecção de *F. graminearum* e *F. meridionale*, induzindo maior severidade da doença e incidência de grãos manchados e presença dos fungos detectados nos grãos. O estágio de florescimento foi mais suscetível nas duas safras para ambos os isolados, e o estágio de emborrachamento completo apresentou os valores mais baixos de AACPD.

Palavras-chave: *Gibberella zeae*, *Oryzae sativa*, inoculação, fungo.

Rice (*Oryza sativa* L.) is the basic food for a large portion of the world's population and constitutes the basis of the food chain in several countries. It is the second most produced cereal in the world; approximately 168 million hectares cultivated in the 2016/17 season produced 748 million tons of rice (8). The world's largest rice producers are China, India, Indonesia, Bangladesh, Vietnam, Thailand, Myanmar,

Philippines, Brazil and Japan (15).

Brazil stands out as the largest rice producer in the Mercosul bloc, showing an approximate area of two billion hectares and production of 12.32 million tons in the 2016/17 season, basically destined for human consumption (5). The rice areas are located predominantly in Santa Catarina and Rio Grande do Sul States. In Santa Catarina State, rice production is concentrated in the coastal area; in 2016/17 season, its total cultivated area was of approximately 148 thousand hectares and total production was 1.176 million tons (3).

The fungus *Fusarium graminearum* (Schwabe) was one of the first pathogens described infecting rice crops (11). It causes lesions or discoloration in glumes, turning the color of the affected area into brown and, subsequently, salmon due to production of sporodochia and masses of fungal conidia. The infected grains are light and can be brittle (11).

This fungus has been detected in seed pathology tests in irrigated rice produced in the south of the country. In Rio Grande do Sul State, reports of fungi associated with rice seeds have rarely identified *Fusarium* to species level, although *F. graminearum* has been frequently reported in infection levels ranging from 0.25% to around 10% (7, 9, 10, 13). In Brazil, there is no report of *F. graminearum* in the panicle of irrigated or upland rice.

Presence of *F. graminearum* species complex (FGSC) in a seed indicates presence of infection in the field; however, there is no information about the most susceptible phenological stage to infection by this pathogen. According to Lee (11), rice is more susceptible to infection in the flowering stage and less susceptible in the milk grain stage. This information is probably based on wheat, in which the target site for fungal infection is related to the presence of anthers (16). In barley, the infection can occur in cultivars with open or closed flowering, demonstrating a weaker relation to anthesis (anther extrusion) (19).

Infection by FGSC in rice grains indicates the importance of identifying the most susceptible phenological stage to infection by this pathogen in rice panicles, which is crucial to provide information and tools to help in the process of fungal inoculation in panicles with the aim of characterizing resistance in rice genotypes and defining the best moment to apply fungicides in specific infection sites.

This study aims to determine the phenological stage that is most susceptible to infection by *F. graminearum* and *F. meridionale* in rice panicles and grains.

MATERIAL AND METHODS

The study was conducted in Lages Municipality, Santa Catarina State, Southern Brazil, during 2015/16 and 2016/17 growing seasons. The experiments were conducted in a greenhouse, where temperature and relative humidity were partially controlled through an automatic system, maintaining an average temperature of 27 ºC during the day and 22 ºC during the night.

Experimental units consisted of 8.0-kg plastic pots containing substrate made of a mixture of soil, sand and organic matter at 2:1:1 proportion. Hand sowing of the rice hybrid INOV CL was performed on November 23rd, 2015 and 2016, using ten seeds per pot. After seedling emergence, thinning and roguing were performed to maintain only five plants in each pot. Plants were manually irrigated once or twice a day, keeping the pot plate full of water and the soil wet, according to the recommendations for the culture. Weed control was obtained by roguing whenever necessary.

Experimental design was in factorial scheme, testing three moments of inoculation and two isolates of the fungus, distributed in a totally casual arrangement, with four replicates resulting in 20 plants per treatment.

Three growth stages were tested: i) complete booting (R2); ii) full heading (R3) and iii) flowering (R4), according to the phenological scale of rice crop proposed by Couce (4). In each phenological stage, two isolates of FGSC, previously characterized regarding phylogenetics and genotype, were inoculated: Fg15A (*F. graminearum* - 15-ADON) and FmNiv (*F. meridionale* - Nivalenol) (6). For each isolate, 20 panicles were inoculated.

The inoculum was produced in the laboratory according to a methodology similar to that described by Reid et al. (14). The two isolates *F. graminearum* and *F. meridionale* were multiplied in a PDA culture medium (potato-dextrose-agar) contained in 80mm-diameter Petri dishes. To measure mycelial growth and spore production of fungal isolates, the dishes were kept in a growth chamber for ten days at 25ºC and 12-hour photoperiod (12 hours dark and 12 hours light). An ultraviolet light was used in the growth chamber to stimulate spore production. Sterile water was added in each Petri dish and fungal colonies were scraped. A spore suspension was obtained and, through filtering, diluted and counted in a hemocytometer. Concentration of 2 x 10^5 macroconidium mL^{-1} was obtained.

Rice plants were inoculated by using the spore aspersion method for pre-determined phenological stages. Immediately after inoculation, the plants were individually covered with transparent plastic bags and kept in the greenhouse for 48 hours in order to promote the necessary conditions for the pathogen infection. For each inoculation stage, a non-treated control was treated with water. Then, the plastic bags were removed and the plants were kept in the greenhouse.

At 7, 14, 21 and 28 days after inoculation, disease severity was quantified by a single observer, who analyzed the percentage of stained grains in the panicle; the same data were used to evaluate the AUDPC.

At the end of the crop reproductive stage, all panicles were manually collected and stored in paper bags. After drying, spotted and non-spotted grains were separated from each panicle to determine their incidence. The separated grains were subjected to a health test in order to determine the presence of *F. graminearum* and *F. meridionale*. Two hundred grains from each treatment were analyzed, including four replicates of 50 grains. The grains were disinfested in a sodium hypochlorite solution (1%) and distributed in Petri dishes with PDA + culture medium (potato-dextrose-agar + antibiotics 200 mg L^{-1} streptomycin sulfate). The material was incubated in a growth chamber at 25ºC and 12-hour photoperiod for seven days. The presence of the fungi *F. graminearum* and *F. meridionale* was identified and quantified under a microscope.

Data related to disease severity in the panicles of rice hybrid INOV CL obtained in the two years indicated homoscedasticity among the analyzed variables (infection stage, fungal isolates and days of evaluation) but no significant difference ($p<0.05$). Thus, the disease severity data were analyzed all together.

The disease severity data residuals were not normally distributed and were transformed before an ANOVA analysis was performed. The test for comparison of means was employed when significance was reached, according to Tukey's test ($p<0.05$). SAS software, version 9.1 (Cary, NC), was adopted for data analysis.

RESULTS AND DISCUSSION

Mean disease severity in rice panicles was 4.05% for inoculation during complete booting, 42.84% during full heading and 52.29% during flowering, indicating a significant difference among the moments of infection (Table 1). The heading and flowering stages

presented higher disease intensity. According to Lee (11), rice is more susceptible to infection by *F. graminearum* in the flowering stage and less susceptible from the milk grain stage. However, that author did not mention any specific study to support this statement. These data are probably based on wheat, in which *F. graminearum* mainly infects the anthers during the flowering of this winter cereal (12, 16, 17). For barley, there is a description of infections by *F. graminearum* in cultivars with open and closed flowering, demonstrating a weaker relation with anthesis (anther extrusion) (19). For corn, infection by this pathogen occurs through the style stigma and through injuries in the grains (1, 14, 18).

The absolute values of FHB severity evidence that the exterior anthers present in the heading and flowering of rice are related to the infection process by *F. graminearum* in the panicle and grains (Table 1).

Disease severity related to days of evaluation after fungal inoculation proportionally increased with time (Table 1). The lowest severity mean was recorded on the seventh day (12.9%), while the highest value was recorded on the 28th day (54.9%), demonstrating a significant difference between days after inoculation and subsequent fungal colonization in the panicles.

Comparing the mean FHB severity specifically to each moment of evaluation, considering each moment of fungal inoculation, no significant difference was verified for the non-treated plants only on the 7th and 14th days and for inoculated plants only during the booting stage (Table 1). Disease quantification on the 7th, 14th, 21st and 28th days after inoculation indicated a significant difference among all moments, and severity values varied from 0% to 22.2% on the 7th day, from 0.8% to 39.8% on the 14th day, from 4.7% to 67.2% on the 21st day, and from 10.6% to 79.9% on the 28th day of evaluation (Table 1).

According to the area under the disease progress curve (AUDPC), in 2015/16 and 2016/17 growing seasons, both isolates showed significant differences for the phenological growth stages. Flowering was the most susceptible stage in the two crop seasons for both isolates. Complete booting, in both years, presented the lowest values of AUDPC (Table 2).

There was a deficiency in the grain formation and filling and in the percentage of spotted grains originated from panicles inoculated with the fungi in the three development stages. In the two experiments, the pathogens colonized the rice grains, and the amount of spotted grains varied according to the moment of inoculation. There was a growing incidence of spotted grains (Table 3), similar to the severity values detected in the panicle (Table 1), more frequently obtained when the fungi were inoculated during booting and flowering (Table 3). In these two stages, the grains were in formation and glumelles were exposed, which might have favored fungal infection, differently from the complete booting stage, when the panicle was surrounded by the flag leaf, probably hindering infection. No spotted grains were detected in the control.

The symptoms of colonization by *F. graminearum* and *F. meridionale* in the glumes of the rice panicle appeared at 8, 4 and 3 days after inoculation during complete booting, full heading and flowering stages, respectively, as small light-brown spots which became larger and dark brown over time, completely darkening the glume in several cases. The grains infected by *F. graminearum* and *F. meridionale* were dry, brittle and many of them presented deformations. According to Lee (11), rice grains infected by *F. graminearum* may present a brown affected area which may become salmon due to the production of sporodochia and conidial mass; they are described as light but can also be brittle. In this study, production of sporodochia was found in some grains after storage.

F. graminearum and *F. meridionale* were detected in spotted and non-spotted grains in the three moments of inoculation and for the two isolates of the pathogen (Table 3). There was no significant difference between

Table 1. Severity of Fusarium Head Blight in panicles of the rice hybrid INOV CL weekly evaluated after inoculation of *Fusarium graminearum* (15-ADON) and *Fusarium meridionale* (NIV) in three development stages of the plants. Lages, Santa Catarina State, 2017.

	Days/ Severity (%)				
Stage	**7**	**14**	**21**	**28**	**Mean**
Complete booting	0.0 Aa	0.8 Aa	4.7 Aab	10.6 Ab	4.02
Full heading	16.6 Ba	31.0 Bb	49.6 Bc	74.1 Bd	42.82
Flowering	22.2 Ba	39.8 Cb	67.2 Cc	79.9 Cd	52.27
Mean*	12.9 a	23.9 b	40.5 c	54.9 d	
C.V. (%)		35.61			

*Means followed by the same lowercase letter on the line and uppercase letter in the column do not differ, according to Tukey's test ($p<0.05$)

Table 2. Area under the disease progress curve (AUDPC) in panicles of the rice hybrid INOV CL for *Fusarium graminearum* (15-ADON) and *Fusarium meridionale* (NIV) in three development stages of the plants. Lages, Santa Catarina State, 2017.

	AACPD			
Stage	**15ADON**		**NIV**	
	2015/2016	**2016/2017**	**2015/2016**	**2016/2017**
Complete booting	73.1 c	84.3 c	66.1 c	68.7 c
Full heading	990.1 b	912.9 b	945.8 b	912.2 b
Flowering	1192.8 a	1218.1 a	1151.8 a	1174.4 a
Mean	752.0	738.5	721.2	718.4
C.V. (%)	22.4	25.7	31.0	24.7

* Means followed by the same letter do not differ, according to Tukey's test ($p<0.05$).

Table 3. Incidence of spotted grains of the rice hybrid INOV CL and mean incidence of *Fusarium graminearum* (15-ADON) and *Fusarium meridionale* (NIV) in spotted and non-spotted rice grains in relation to inoculation in the three development stages of the plants. Lages, Santa Catarina State, 2017.

Stage	Incidence of spotted grain (%)	Incidence of fungi (%)	
		Spotted grain	Non-spotted grain
Complete booting	10.35 c	78.0 Ca	9.5 Cb
Full heading	70.65 b	83.5 Ba	16.5 Bb
Flowering	79.02 a	89.2 Aa	21.5 Ab
C.V. (%)	19.12	13.15	

*Means followed by the same uppercase letter in the column and lowercase letter on the line do not differ, according to Tukey's test ($p \leq 0.05$).

isolates, demonstrating that they presented similar infection levels.

Considering the culture medium, the colonized grains had reddish pink colonies with aerial brown mycelium. According to Booth (2), *F. graminearum* colonies present variable coloration (grey, pink or brown) in a culture medium but are reddish pink with yellowish brown aerial mycelium in PDA medium.

The recovery rate of fungi in the spotted grains was high, varying from 77.5% to 90.0% for the isolate 15-ADON and from 78.5% to 88.5% for the isolate NIV, which demonstrated a correlation between the presence of the fungi and the symptoms in the grains. However, the fungi were also recovered from non-spotted grains, although at a lower incidence, varying from 10.0% to 22.5% (isolate 15-ADON) and from 9.0% to 20.5% (isolate NIV), which demonstrated that the pathogens can infect grains not showing symptoms of its colonization in the caryopsis. In any case, there is a significant difference in the incidence of fungi in spotted and non-spotted grains, considering the mean of the three inoculation moments, for the isolate 15-ADON (83.5% in spotted and 16.7% in non-spotted grains) and for the isolate NIV (83.5% in spotted and 15.0% in non-spotted grains) (Table 3).

There was a higher recovery of *F. graminearum* and *F. meridionale* from spotted and non-spotted grains for the two isolates of the fungus when the inoculation occurred during the flowering period (Table 3). Greater severity of FHB (Table 1) and larger number of spotted grains (Table 3) were also obtained with inoculation during flowering.

In conclusion, heading and flowering are the most susceptible stages of the rice plant to infection by the fungi *F. graminearum* and *F. meridionale*, inducing higher disease severity and higher incidence of spotted grains and fungi in the grains.

ACKNOWLEDGMENTS

This study was financially supported by Santa Catarina State University (CAV/UDESC), CNPq (The National Council for Scientific and Technological Development) and CAPES (Coordination for the Improvement of Higher Level - or Education - Personnel).

REFERENCES

1. Andriolli, C.F.; Casa, R.T.; Kuhnen, P.R.; Bogo, A.; Zancan, L.; Reis, E.M. Timing of fungicide application for the control of Gibberella ear rot of maize. **Tropical Plant Pathology**, Viçosa, v.41, p.264-269, 2016.
2. Booth, C. **The genus fusarium**. Kew: Commonwealth Mycological Institute, 1971. 237p.
3. CEPA/EPAGRI. Grãos Arroz. **Boletim Agropecuário**, Florianópolis, n.51, ago. 2017. Available at: <http://docweb.epagri.sc.gov.br/website_cepa/Boletim_agropecuario/boletim_agropecuario_n51.pdf>. Access in: 23 set. 2017.
4. Counce, P.; Keisling T.C.; Mitchell, A.J. A uniform, objective, and adaptive system for expressing rice development. **Crop Science**, Madison, v.40, n.2, p.436-443, 2000.
5. CONAB. Estimativa de produção. **Acompanhamento da safra brasileira de grãos,** Brasília, v.4, n.12, set. 2017. Available at: <file:///C:/Users/Seven/Downloads/Boletim_Graos_setembro_2017.pdf>. Access in: 25 oct. 2017.
6. Del Ponte, M.E.; Spolti, P.; Ward, T.J.; Gomes, L.B.; Nicolli, C.P.; Kuhnem, P.R.; Silva, C.N.; Tessmann, D.J. Regional and Field-Specific Factors Affect the Composition of Fusarium Head Blight Pathogens in Subtropical No-Till Wheat Agroecosystem of Brazil. **Phytopathology**, St. Paul, v.105, p.246-254, 2015.
7. Farias, J.R.C.; Afonso, S.P.A.; Branção, F.M. Incidência de fungos associados a sementes de arroz em seis regiões produtoras do Rio Grande do Sul. **Revista Brasileira de Agrociência**, Pelotas, v.14, n.4, p.487-490, 2007.
8. Food and Agriculture Organization. **Seguimento del Mercado del Arroz de la FAO**. 4.ed. Roma, 2016. v.19.
9. Franco, D.F.; Ribeiro, A.S.; Nunes, C.D. Fungos associados a sementes de arroz irrigado no Rio Grande do Sul. **Revista Brasileira de Agrociência**, Pelotas, v.7, n.3, p.235-236, 2001.
10. Gomes, L.B.; Ward, T.J.; Badiale-Furlong, E.; Del Ponte, E.M. Species composition, toxigenic potential and pathogenicity of *Fusarium graminearum* species complex isolates from southern Brazilian rice. **Plant Pathology**, London, v.64, p.980-987, 2015.
11. Lee, F.N. Scab. In: Webster, R.K.; Gunnell, P.S. (Ed.). **Compendium of rice diseases**. St. Paul: APS Press, 1992. 30p.
12. Nerbass, F.R.; Casa, R.T.; Kuhnem, P.R.; Bogo, A.; Sangoi, L.; Fingtag, M.; Vieira Junior, J.A.L.; Stoltz, J.C. Evaluation of *Fusarium graminearum* inoculation methods in maize ears and hybrid reaction to Gibberella ear rot under southern Brazilian environmental conditions. **European Journal Plant Pathology,** Dordrecht, v.144, p.45-53, 2016.
13. Nunes, I.L.; Magagnin, G.; Bertolin, T.E.; Furlong, E.B. Arroz comercializado na região Sul do Brasil: aspectos micotoxicológicos e microscópicos. **Ciência e Tecnologia de Alimentos**, Campinas, v.2, p.190-194, 2003.
14. Reid, L.M.; Hamilton, R.I. Effects of inoculation position, timing, macroconidial concentration, and irrigation on resistance of maize to *Fusarium graminearum* infection through kernels. **Canadian Journal Plant Pathology,** Ontario, v.18, p.279-285, 1996.
15. Sosbai. Arroz irrigado: recomendações técnicas da pesquisa para o Sul do Brasil. In: Reunião Técnica da Cultura do Arroz Irrigado, 31., 2016, Bento Gonçalves. **Anais**. Pelotas: Sociedade Sul-Brasileira de Arroz Irrigado, 2016. p.10-197.
16. Strange, R.N.; Smith, H. A fungal growth stimulant in anthers which predisposes wheat to attack by *Fusarium graminearum*. **Plant Pathology**, London, v.1, p.141-150, 1971.
17. Suty, A.; Mauler-Machnik, A. Fusarium head blight on wheat – new findings on the epidemiology and control of *Gibberella zeae* the teleomorph of *Fusarium graminearum* with Folicur**. Pflanzenschutz-Nachrichten Bayer**, Leverkusen, v.49, n.1, p.55-70, 1996.
18. Sutton, J.C. Epidemiology of wheat head blight and maize ear rot caused by Fusarium graminearum. **Canadian Journal of Plant Pathology**, Ontario, v.4, p.195-209, 1982.
19. Yoshida, M.; Kawada, N.; Nakajima, T. Effect of infection timing on Fusarium Head Blight and mycotoxin accumulation in open- and closed-flowering barley. **Phytopathology**, Lowa, v.97, p.1054-1062, 2007.