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Anthesis in small grains and *Fusarium* head blight infection

Erlei Melo Reis[1,2], Andrea Camargo Reis[2], Marcelo Carmona[1]

[1]Cátedra de fitopatologia, Facultad de Agronomía, Universidad de Buenos Aires, C1417DSE, Buenos Aires, Argentina;
[2]Instituto Agris, Instituto Agris, Rua Miguel Vargas, 291, CEP 99025-380, Passo Fundo, RS, Brasil
Corresponding authors: Erlei Melo Reis (erleireis@upf.br)
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ABSTRACT

Reis, E.M.; Reis, A.C.; Carmona, M.A. **Anthesis in small grains and *fusarium* head blight infection**. ***Summa Phytopathologica***, v.49, p.1-5, 2023.

Attempts to control *Fusarium* head blight (FHB) in wheat and barley ha ve mainly consisted of using resistant cultivars and/or spraying fungicides. The efficacy of chemical control depends on the potency of fungicides, time of application, and deposition on the infection sites. The objective of this study was to determine the relationship between presence of anthers and FHB. The e xperiment was conducted in the field in two sowing periods to assess anthesis duration, anther size, and infection in partially and fully exserted anthers . Incidence of FHB in spikes was not explained based only on the presence of partially exserted anthers in infected spikelets. Pampeano and CEP 00-59 were the least susceptible wheat cultivars, while CD 114 showed the highest incidence in spikes. Occurrence of FHB was, but not exclusively, related to anthesis duration, anther size, and presence of partially exserted anthers. All these factors may be involved in the infection of spikes.

Keywords: *Fusarium graminearum, Gibberella zeae, Hordeum vulgare, Secale cereale, Triticum aestivum, Triticum secalotricum.*

RESUMO

Reis, E.M.; Reis, A.C.; Carmona, M.A. **Antese em cereais de inverno e infecção da giberela**. ***Summa Phytopathologica***, v.49, p.1-5, 2023.

As tentativas de controle da giberela em trigo e cevada têm sido principalmente através da criação de cultivares resistentes e/ou pela aplicação de fungicidas. A eficiência do controle químico depende da potência do fungicida, do momento da aplicação e da deposição nos sítios infecção. O objetivo do trabalho foi determinar a relação da presença das anteras na infecção da giberela. O trabalho foi conduzido no campo em duas épocas de semeadura avaliando-se a duração da antese, o tamanho das anteras e a infecção em anteras parcialmente expostas e completamente expostas. A incidência da giberela em espigas não foi explicada unicamente pela presença de anteras parcialmente expostas em espiguetas infectadas. As cultivares de trigo Pampeano e CEP 00-59 foram as menos suscetíveis, enquanto a CD 114 apresentou a maior incidência em espigas. A ocorrência da giberela relacionou-se, mas não exclusivamente, com a duração da antese, tamanho de anteras e presença de anteras parcialmente expostas. Todos esses fatores podem estar envolvidos com a infecção das espigas.

Palavras-chave: *Fusarium graminearum, Gibberella zeae, Hordeum vulgare, Secale cereale, Triticum aestivum, Triticum secalotricum.*

During the 2021/22 growing season, wheat was grown in approximately 2.74 million hectares in Brazil, generating mean yields of 2.9 t/ha and production of 8.351 million tons. Brazilian annual consumption is estimated at 11.0 million tons (7).

Fusarium head blight (FHB) or scab usually occurs at wheat growing regions in hot, humid and semi-humid environments, especially where rainfall is frequent, resulting in increased head wetness duration longer than 48 hours (1, 5, 16, 18). Its occurrence in Brazil is limited to southern states, where rainfall is frequent during and after wheat flowering (5, 11, 19).

Under prevailing weather in the southern part of the country, where wheat is grown, the principal diseases are powdery mildew, leaf rust, leaf blight and FHB (5, 15).

The major pathogen associated with FHB in winter small grains in Brazil is *Gibberella zeae* (Schw.) Petch (anamorph = *Fusarium graminearum* Schwb.). As a floral infectious disease (2, 15) , initial FHB infection in wheat has been reported to occur via the anthers (2, 16). In the 1970's, the relationship between FHB and anthesis was explained by the presence of high concentrations of the molecule stimulants betaine and choline. However, more recently, Engle et al. (10) reported that *F. graminearum* spore germination was not significantly affected by choline and betaine and that radial hyphal growth was not consistently affected either; hence, the se endogenous compounds may not be associated with *F. graminearum* infection.

The pathogen has a wide host range and saprophytically colonizes and survives on grass plant debris of different plant species (14). The practice of maintaining crop residues on the soil surface for erosion control, with no till, has contributed to inoculum increase and pathogen survival between growing seasons, thus ensuring abundant inoculum all year around (12, 13, 14).

Wheat grain yield loss due to FHB, identified in southern Brazil from 1984 to 1994, averaged 5.41% (6). From the 1990's, with increased no till acreage in large areas, FHB intensity has raised not only in wheat, but also in oat (*Avena sativa* L.), barley (*Hordeum vulgare* L.) and triticale (*Triticum secalotricum* Meister). More recently, Casa & Kuhnem Junior (6) reported yield damage in wheat reaching up to 39.9%.

Unfortunately, the current resistance/tolerance of wheat cultivars will not prevent economic damage, while chemical control efficiency up to 46.6% is not enough (3, 6, 7).

The wheat flower has three anthers that may remain in three positions after fertilization: (i) those that are not expelled, remaining inside the flower; (ii) those fully exposed, of short duration, suspended by the filet, which detach in a few hours, and when they are visible the wheat plant is said to be at flowering stage; and (iii) those only partially exposed, having just the apical ends visible at the tips of the glumes and remaining there until the end of the wheat cycle (9).

Atanasoff (2), in a pioneer study, observed that, even after anthesis, wheat continued to be susceptible to *G. zeae* infections. Recently (16), FHB disease progress curves were drawn, showing evidence of new infections occurring after anthesis.

The wheat susceptible stage is associated with the presence of infection sites, fully exserted anthers (FEA) during flowering, and partially exserted anthers (PEA) after flowering (16).

In the present study, FHB is hypothesized to be associated with anthesis duration, anther size and presence of partially exserted anthers.

The objective of the current study was to improve knowledge on the role of anthers during *G. zeae* infection in wheat heads.

MATERIAL AND METHODS

The study was conducted in the experimental field of University of Passo Fundo (25º15'46"S, 57º 24' 24"W, 587 m. a.s.l). Wheat BRS 179, CD 114, Pampeano and CEP 00-59 , recommended as the cultivars most resistant to FHB, and the susceptible cultivar BR 23, were used. Other susceptible grass species, like BR 1 rye (*Secale cereale* L.), Minotauro triticale and BRS 195 barley (*Hordeum vulgare* L.), were also tested.

Experimental plots consisted of seven 5m-long lines, spaced at 0.17 m, which were seeded at two different times.

Anthesis duration

Assessment of anthesis duration was performed from the exposure of the first fully exserted anther (FEA) pending externally and hanged by the filament to its complete dehiscence. For each cultivar, six plants per plot were marked at random, including their tillers for daily anthesis duration assessment. Anthesis duration was determined per spike in the main stem, per plant with its tillers and in all plants in a 1.0 m^2 area. In the present paper, anthesis or flowering duration mean s the period from FEA first presence (light yellow, visible, hanged by the fillet) to its dehiscence (absence). After dehiscence, only partially exserted anthers (PEA) were present and persisted up to the ripening stage. Partially exserted anthers were those remaining trapped between the apical ends of the glumes and only the tip was visible, and internal anthers were those remaining within the flower and not externally visible.

Fusarium graminearum incidence in FEA and PEA

After wheat flowering (no visible hanged anthers), spikes were collected from the marked plants (6 plants/plot) and the number of PEA and spikelets per spike were counted, followed by dissection . Partially exserted anthers and FEA, 100 each, were randomly collected and plated onto a semi-selective medium (17). Plated anthers were incubated for seven days at 25 ± 2ºC, under fluorescent daylight (Osram, 40 W, light intensity of 2,150 lux), 12h photoperiod, and room relative humidity. Plates were examined after seven days of incubation under a stereo microscope (Zeiss Stemi 2000C - 40 X) and the formed colonies of *F. graminearum* were evaluated. Data were presented as *F. graminearum* incidence in both anther types.

Anther size

The length of 100 FEA was measured with a digital caliper (Mitutoyo Digimatic Caliper, 0.01-150mm) . For the barley crop, only the number of PEA was considered since the objective was to work with fully exserted anthers.

Relationship between infected spikelets and presence of PEA

Twenty-five spikes of each genotype, with only one infected spikelet, were collected per plot. Assessment of PEA was performed for the two outer spikelets, avoiding the central un fertilized flowers. Data were expressed as the percentage of spikelets with PEA in each genotype. Data from s tatistical analysis of genotypes for the presence of PEA were transformed to Logn.

Disease assessment

The incidence of FHB in spikelets per spike, at the best contrast between healthy and diseased spikelets, was determined. For the disease evaluation, 100 heads were collected and the proportion of healthy and diseased spikelets was determined.

Statis tical analysis of random treatments was performed with four replicates and means compared according to Duncan's test .

RESULTS

Anthesis duration

Thew heat flower has three types of anthers according to their position in the floret: not exserted, partially exserted (PEA) and fully exserted (FEA). It is likely that FHB infection after anthesis (only PEA present) is through the anthers, which remain exposed up to harvest (9).

Anthesis duration (days of anthesis duration - DAD), during presence of FEA, in the plots was 14.3 days for Pampeano and BRS 179 and 17.6 days for CD 114 and rye. The shortest anthesis duration in a plant (ADP ; main stem and tillers) was 5.8 days for BRS 179 and the longest ADP was 10.3 days for CD 114. Anthesis duration per spike (ADS) was shortest (4.3 days) for BRS 179 and longest (6.6 days) for CD 114.

Regarding the number of PEA per head, the lowest value was 4.4 for CD 114 and the highest one was 7.2 for BR 23. Considering the number of spikelets per head, 12.3 was the lowest value, found for CD 114, and 13.8 was the highest number, found for BRS 179 (Table 1).

Table 1. Days of anthesis duration (DAD) in 1.0 m^2, anthesis duration in a plant (ADP), anthesis duration in a spike (ADS), number of partially exserted anthers (PEA) per spike, number of spikelets per spike (SNS), and percentage of PEA per spike (PPEAS).

Cultivar	DAD (days)	ADP (days)	ADS (days)	PEA (no.)	SNS (no.)	PPEAS (%)
W - BR 23	17.3 a	8.3 b	5.4 abc	7.2 cd	13.3 d	54.1
W - BRS 179	14.3 b	5.8 d	4.3 c	11.9 b	13.8 d	86.23
W - CEP 0059	16.6 ab	7.8 bc	4.8 bc	4.9 de	13.7 d	35.8
W - CD114	17.6 a	10.3 a	6.6 a	4.4 e	12.3 d	35.8
W - Pampeano	14.3 b	8.9 b	5.6 ab	4.9 de	12.7 d	38.6
Triticale	17.3 a	7.9 bc	5.3 bc	8.7 c	20.3 c	42.9
Rye	17.6 a	6.5 cd	5.6 ab	5.4 de	31.4 a	17.2
Barley	-	-	-	17.0 a	24.2 b	70.2
C.V.%	7.8	9.2	11.5	16.59	11.7	

W – wheat. Means followed by the same letter in the same column do not differ according to Duncan's test at 5%.

BRS 179 showed the lowest number of days in anthesis (FEA), considering the ear, the plant and the area, but showed the highest number of PEA in relation to the other wheat cultivars, which could help explain its susceptibility .

Relationship between infected spikelets and presence of PEA

The percentage of spikelets with present PEA was highest for CD 114 (69.2%) cultivar and lowest for CEP 0059 (35.4%) (Table 2). The presence of PEA in infected spikelets explains only in part (53.6%) the infection sites.

Table 2. Percentage of heads with partially exserted anthers in infected spikelets.

Cultivar	Mean (%)
BR 23	50.7ab
BRS 179	66.7 a
CEP 0059	35.4 b
CD114	69.2 a
Pampeano	46.0 ab
Mean	53.6

Means followed by the same letter in the column are not significantly different according to Duncan's test at 5%.

Anther size

There were differences in anther size among genotypes, as well as among wheat, triticale and rye. The shortest anther length was found for CD 114 and CEP 0059, 2.7 mm, while the longest one was obtained for rye, 8.6 mm. The longer the FEA, the greater the anther infected by *F . graminearum* (Table 3).

Presence of PEA , longer anthers, larger impacted area and glume opening during anthesis could explain the cultivar's susceptibility.

***Fusarium graminearum* incidence in PEA and FEA**

Partially exserted anthers colonized by *F. graminearum* was 50% in CEP 0059 and BRS 179, and 72% in rye. On the other hand, *F. graminearum* incidence in FEA was zero (Table 3).

Regarding PEA, 58.7% show ed *F. graminearum* incidence, while no infection was detected for FEA. The percentage of PEA infection in triticale was not statistically different from that in rye, BR 23, CD 114 and Pampeano.

DISCUSSION

Relationship between FHB infection and wheat flowering

The first symptoms of FHB are spikelet necrosis showing white discoloration with bristle-like awns. T he anthers have been accepted as the major infection sites of *G. zeae* (2, 11, 15).

Table 3. Fully exserted anther size (FEAS), and *Fusarium graminearum* incidence on partially (PEA) and fully exserted anthers (FEA).

Cultivar/Line	FEAS (mm)	*F. graminearum* incidence (%)	
		PEA (%)	FEA (%)
Wheat BR 23	3.3 c	60.0 abc	0.0
Wheat BRS 179	2.9 de	50.0 c	0.0
Wheat CD114	2.7 de	58.0 bc	0.0
Wheat CEP 00 59	2.7 de	50.0 c	0.0
Wheat Pampeano	2.9 de	55.0 bc	0.0
Triticale	4.5 b	66.0 ab	0.0
Rye	8.6 a	72.0 a	0.0
Mean	3.9	58.7	0.0
C.V.%	3.8	15.3	0.0

Means followed by the same letters in the same column are not significantly different according to Duncan's test at 5%.

Therefore, the short duration of FEA does not explain the occurrence of new infections after anthesis, as noted by some authors (16). Occurrence of new infections (after anthesis) can be explained by the presence of PEA. These anthers remain exposed up to plant ripening and, while the ears remain green, new infections can occur.

Infection of FEA (shorter exposure to the inoculum) is much less frequent , or absent, than th at of PEA (longer exposure time). Based on the se findings, PEA should be protected by fungicides throughout the presence of green ears since they are subject to new infections.

There was no strong evidence that a wheat cultivar with the highest percentage of PEA per head would be the most susceptible cultivar to FHB. Other entry routes , not only the anthers, may be involved as infection sites. It was shown that the higher the infected spikelets, the higher the percentage of PEA , which does not fully explain the susceptibility of the cultivar. To have the susceptibility explained by PEA, the infected spikelets should have 100% present PEA. Thus, other infection sites can be involved.

Fusarium head blight has been noticed to occur when favorable weather is present during or after anthesis. There is increased intensity (incidence in spikes and spikelets) of FHB after each new favorable environment and period after anthesis. Such an increase may be due to new infections via still present PEA or to resumed colonization. Cowger et al. (8) inoculated wheat ears in the middle of anthesis and subjected the heads to wet periods after anthesis in a greenhouse. They showed that the wet periods after anthesis resulted in increased FHB severity. New infections would not develop via the anther (absent inoculum), but severity could have increased due to resumed colonization under a favorable environment. Fungal growth would reach the rachis and kill new spikelets, resulting in increased severity.

Anther size and cultivar/species reaction to FHB

Considering rye, the relationship between disease severity and FEA length can likely explain its high susceptibility to FHB. Rye anthers had the longest length (8.6 mm) and the highest percentage of infection by *F. graminearum* (72%). In a study carried out by Panisson et al. (12), rye was most susceptible among winter small grains, suggesting that this condition may be related to anther size. The longer the anthers, the greater the impact area for ascospore deposition. Characteristics like head type, phenology, PEA, plant height and plant cycle were related to susceptibility to FHB (11). This trait alone is not enough to fully explain susceptibility/resistance among wheat cultivars. On the other hand , rye and triticale are considered highly susceptible (12). Our data support that, for both species, susceptibility can be related to the longest anther size.

Presence of PEA and predisposition to FHB infection

The presence of PEA and infection showed values considered low to conclude that PEA were the most important infection site. The current data evidenced that there was spikelet infection in the absence of PEA. There is still lacking information to fully clarify the infection sites in FHB. Infection could occur through FEA during rainfall, resulting in a long head wet period. According to the data obtained by Panisson et al. (12) , *F. graminearum* infection was 11.8% for FEA and 24.3% for PEA. The infection likely occurs in both FEA and PEA. However, the duration of PEA exposure to inoculum deposition is longer than that of FEA. Hence, PEA is thought to provide an important pathway for mycelium to grow into florets, compared to FEA.

The present study showed that the presence of PEA in infected spikelets alone did not fully explain the major FHB infection sites . Initial FHB infection has been observed since the beginning of flowering (present FEA), continuing up to the beginning of maturation. The infection likely occurs only through the present PEA (16).

***Gibberella zeae* penetration - via anthers or glume opening?**

Some authors argue that *G. zeae* can enter the floret through the glume opening at the time of anther exclusion. During anthesis, the anthers naturally split to release pollen, which provides an opening for the pathogen to enter the floret. During anthesis, florets of wheat head remained open for only 12 to 20 minutes (9). Although glum opening can be a site of penetration , occurrence of new infections after absent FEA , when only PEA is present, indicates that the glume opening is not the only penetration site.

Anthesis in barley

According to Briggs (4), barley flowering differed significantly between cultivars. In closed flowering cultivars, anthers can be pushed to the tip of the spikelet and stand between the palea and the lemma or can be eliminated subsequently by the development of the grain; on the other hand, in open flowering cultivars, anthers can be fully exposed like in wheat. Barley was statistically different from those materials having the largest number of partially exserted anthers per spike.

Although all *G. zeae* infection sites in wheat spikelets were not completely identified, full coverage of the head sides by fungicides is required to improve control efficacy.

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Editor associado para este artigo: Paulo César Ceresini