

Occurrence of Different Species of *Fusarium* from Wheat in Relation to Disease Levels Predicted by a Weather-Based Model in Argentina Pampas Region

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Abstract *Fusarium* head blight (FHB) is an important disease throughout many of the world wheat-growing areas that have humid to semi-humid climate. The infection happens mainly during the anthesis of the wheat, when there have been favorable conditions of moisture and temperature. The direct relation of the infection to environmental factors makes possible the formulation of mathematical models that predict the disease. The causal agent of the FHB of the spike of wheat is attributed principally to *Fusarium graminearum*. High economic losses due yield decrease have been recorded in Argentina. In the present work, 67 isolates of *Fusarium* spp. were obtained from samples of wheat grains from Pampas region from 15 locations distributed in Buenos Aires, Entre Ríos, Santa Fe and Córdoba provinces during

2006 and 2007 wheat-growing seasons. The identification of species from monosporic isolates was carried out by morphological characterization and use of species-specific PCR-based assays. Both identification criteria were necessary and complementary for the species determination, since in some cases the molecular identification was not specific. Scanty presence of *F. graminearum* was observed in 2006 wheat-growing season coinciding with the lack of favorable meteorological conditions for producing FHB infection events. High presence of *F. graminearum* isolates was observed in 2007 wheat-growing season, in accordance with moderate incidence of the disease according to spatial distribution of FHB incidence values. The aim of this report was to identify the causal agent of the FHB disease by

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different taxonomic criteria and to relate its occurrence with disease incidence values predicted by a weather-based model in Argentina.

Keywords *Fusarium* head blight incidence · *Fusarium* spp. · Identification criteria · Meteorological conditions · Wheat-growing season

Abbreviations

INTA Instituto Nacional de Tecnología Agropecuaria
 SENASA Servicio Nacional de Sanidad y Calidad Agropecuaria

Introduction

Fusarium head blight (FHB) or scab on small-grain cereals is one of the most devastating diseases. Epidemics are associated with high yield losses and overall reduction in seed quality due to contamination of grains with mycotoxins [1–4]. It is a disease spread in producing areas of wheat (*Triticum aestivum* L. emm. Thell) all over the world when very humid and moderate warm periods occur from flowering to grain filling developmental stages [2]. Several species can cause head blight, although *Fusarium graminearum* is the predominant pathogen in most regions of the world [5]. However, other species can be highly pathogenic or can often be in association with the disease [6]. Most of the species can be found in much of the geographical area affected by FHB, but individual species usually dominate a specific region and *F. graminearum* dominates in most regions, which suggests that it is more broadly adapted to environmental variability than other species [6]. Severe and frequent epidemics have occurred all over the world in the last 25 years, being this disease a growing threat to the world food production. Recent outbreaks were reported in USA, Canada, Europe, Asia, Australia and South America. In North America, this disease resulted in loss of three billion dollars for agriculture market during the 1990s [7, 8]. FHB is an important wheat disease in Pampa region of Argentina, being *F. graminearum* (teleomorph: *Gibberella zeae*) the predominant associated pathogen [9]. Other species of *Fusarium* can be associated with

the disease but less frequently as *F. poae*, *F. equiseti*, *F. semitectum* and *F. moniliforme* [10]. The Argentinean wheat cropping area is very extensive (nearly 6,000,000 ha), distributed in five provinces with different ecological conditions (Pampas region: Buenos Aires, Córdoba, Santa Fe, Entre Ríos and La Pampa). Therefore, no epidemics have ever covered the whole area at the same time. Latitude, temperature and the importance of susceptible grass weeds and rotational crops (particularly *Zea mays* L.) influence the pathogen distribution, while the frequency and timing of spring rainfall appear to regulate disease outbreaks.

For the northeast area of the Pampas region, in the last 37 wheat-growing seasons, severe epidemics of the FHB were registered in 1978, 85, 93 and 2001. The loss of wheat yield was between 10 and 30% in 1978 (moderate epidemics) and between 24 and 50% in 1993 (severe epidemics) [4]. In the southeast wheat-growing region, severe epidemics occurred in 1963, 1976, 1978 and 1985. Epidemics of FHB (like those of many other disease systems) are strongly influenced by local and regional environment: host factors such as physiological state and genetic make-up, and pathogen factors including adaptation and virulence [6, 11].

The sporadic nature of the disease strongly associated with the environmental factor makes possible that the pathosystem can be modeled mathematically. Different meteorological-based models to predict occurrence of *Fusarium* toxins have been elaborated in the world [12, 13]. Predictive models of FHB incidence were developed in Pergamino location (humid Pampas region) [14] and one of those equations was adjusted and validated for locations further north [15] and further south [16] than Pergamino. The climate risk of the Pampas region regarding FHB is maximum in the northeastern, gradually decreasing toward the southwestern [17]. Besides, the capacity of infection of *F. graminearum* is related to the fact that it can survive in host debris on the soil and fulfill part of the cycle in diverse alternative hosts [18]. According to Galich and Galich [4], since Argentina wheat cultivars are susceptible or moderately susceptible, the difference in the variability of the disease is not attributed to the degree of sensitivity of the host to the pathogen. In addition, the spores are light and can be spread by the wind over long distances [19].

The aim of this study was to identify the causal agent of the FHB disease by different taxonomic criteria and to relate its occurrence with disease incidence values predicted by weather-based models in Argentina.

Materials and Methods

Mycological analysis was carried out on wheat grain samples with different probability of infection according to the climatic models of disease incidence in diverse geographical locations.

Biological Material

Fusarium spp. isolates were obtained from wheat samples (consisting of three replications, mechanically harvested) of 26 commercial cultivars using standard production practices, during the wheat-growing seasons of 2006 and 2007. The cultivars used belonged to the national wheat nursery conducted at INTA Marcos Juárez, Pergamino, Oliveros, Paraná, Concepción del Uruguay, Balcarce and Barrow (integrated farm) INTA Experimental Stations. Also samples were taken from concentration silos of SENASA during 2006 wheat-growing season. A total of 69 samples of 26 wheat cultivars from 15 locations were analyzed.

Isolation of *Fusarium* spp.

For isolation of *Fusarium* spp., wheat grains were surface-sterilized in sodium hypochlorite 10% (v/v) in Erlenmeyer flasks with orbital movement for 2 min, and then rinsed with sterile distilled water. Under sterile conditions, 12 grains of each sample in triplicate were placed on Petri dishes containing wetted filter paper with sterile distilled water and incubated under darkness at 25°C for 7 days and daily observed [20]. The mycelium growing on grains was transferred onto potato dextrose agar (PDA) successively until obtaining a seemingly pure culture and identified as *Fusarium* spp. Then they were subcultured on synthetic nutrient agar (SNA), and from these cultures, single-spore isolates were prepared according to Nelson et al. [21] on SNA. These isolates were finally transferred to PDA for cultural characterization and to carnation leaves agar

(CLA) [22, 23] for microscopic characterization. PDA plates were incubated in darkness at 25°C for 7 days, and both SNA and CLA were incubated at 20–25°C under a 12-h fluorescent light and near ultraviolet (NUV) regime for 12 days. For its maintenance, single-spore isolates were kept in tubes with SNA under a layer of mineral oil, at 4°C.

Morphological Characterization of *Fusarium* spp.

From single macroconidial isolates of *Fusarium* spp. obtained from SNA, cultures characterizations were carried out. Cultural characterization of *Fusarium* spp. grown on PDA as growth rate, pigmentation and aerial mycelium aspect were visually assessed [21]. For microscopical characterization, the isolates were grown on CLA. Microscopic observations were carried out at $\times 100$ magnification (Leica 2500, Germany), observing macroconidia morphology, presence/absence of microconidia and perithecium production. The identification was carried out by means of the keys of Gerlach and Nirenberg [24], Burgess et al. [23], Leslie and Summerell [25], and the considerations of Summerell et al. [26].

Molecular Characterization of *Fusarium* spp.

For this characterization, inoculates were prepared from 10-mm plugs cut out from the outer edge of a 5-day-old colony from single-spore isolates grown on Petri dishes (2% potato-agar) at 26°C. Potato dextrose broth medium (PDB) was used to grow the isolates at 26°C in darkness for 4 days on an orbital shaker (100 rpm) in 125-ml Erlenmeyer flask containing 65 ml of medium. Mycelium was harvested by filtration onto filter paper (Whatman # 1). Then, it was rinsed with sterile water, stored at -20°C and lyophilized. About 100 mg of powered mycelia were used for DNA isolation following a modified version of the cetyltrimethylammonium bromide (CTAB) method [27]. In 750 μl of extraction buffer (100 mM Tris-HCl, pH 8, supplemented with 100 mM EDTA, 250 mM NaCl, 2% CTAB) and 15 μl of 2-mercaptoethanol were added the lyophilized mycelia and incubated at 65°C for 30 min. Cellular proteins were precipitated with 300 μl of 3 M potassium acetate (pH: 4.8). After centrifugation at 17,500g in a microcentrifuge (Hermle Labortechnik GmbH, Wejomingen, Germany), the supernatant was transferred to a new tube

and extracted with 500 μ l of phenol/chloroform/isoamyl alcohol (25:24:1) until the interface became transparent. Nucleic acids were precipitated by adding 750 μ l of cold isopropanol followed by incubation at 4°C for 30 min. After centrifugation at 17,500g for 5 min, the pellet was rinsed twice with 500 μ l of 70% (v/v) ethanol, air-dried and dissolved in 100 μ l of Tris–EDTA buffer [10 mM Tris–HCl (pH: 8) and 1 mM EDTA]. PCRs were performed in a thermal cycler (Master Cycler Eppendorf, Germany).

PCR DNA analyses were performed separately using the following species-specific primers Fg16NF/R for *F. graminearum* [28], FP82F/R for *F. poae* [29], FAF/R for *F. acuminatum* [30] and J1AF/R for *F. avenaceum* [31]. PCRs were performed in a Mastercycler gradient (Eppendorf AG, Hamburg Germany) in a final volume of 20 μ l, containing 10 ng of template DNA, 1.5 mM MgCl₂, 0.2 mM each of four dNTPs, 0.5 mM of each primer and 1 unit of Taq polymerase (Invitrogen, Carlsbad, CA) in the corresponding reaction buffer. Thermal cycling conditions for *F. graminearum* involved an initial denaturation step at 95°C for 2 min, 30 cycles of 94°C for 30 s, 62°C for 1 min and 72°C for 5 min, and a final extension at 72°C for 5 min. Annealing temperatures of 56 and 57°C were used for *F. poae* and *F. acuminatum* or *F. avenaceum*, respectively. Fifteen microliters of PCR products were loaded in agarose gel 2%, and were run for 1 h at 120 V, stained with ethidium bromide and photographed by a Fotodyne system (Hartland, WI). Ladder 100 pb (Invitrogen) was used as molecular marker kit.

Predicted FHB Incidence Values (PFHBI %)

In a previous paper [14], meteorological-based predictive wheat FHB incidence equations were developed for Pergamino location (33°56'S, 60°30'W, humid Pampas region). The equation [1] was selected for predicting FHB incidence (PFHBI %) in the central Pampas region around Pergamino:

$$\text{PFHBI \%} = 20.37 + 8.63 \text{ NP} - 0.49 \text{ DD} \quad R^2 = 0.86 \quad (1)$$

where NP: number of two-day periods with precipitation (≥ 0.2 mm) and relative humidity $> 81\%$ in the first day and relative humidity $\geq 78\%$ in the second day; DD represents the daily accumulation of the

residuals resulting from subtracting 9 to the minimum temperature values ($< 9^\circ\text{C}$) and the exceeding amounts of maximum temperatures from 26°C. These variables were processed in a time period beginning 8 days prior to heading date (emergence of first heads) and ending when 530 degree-days (base mean daily temperature = 0°C) were accumulated. This period was regarded as the critical period length (CPL). This equation [1] was adjusted and validated for more northern [15] and southern [16] locations than Pergamino, making only a few changes. Maximum temperature threshold of the variable DD was changed to 30°C, when equation [1] was used for predicting FHB incidence in northern Pampas region [15]. For southern Pampas region disease incidence estimations, maximum and minimum temperature thresholds of the variable DD were increased to 30°C and to 11°C, respectively, and CPL was reduced to 450 degree-days [16].

For 38 stations of the Pampas region with daily meteorological data for the 2006 and 2007 wheat-growing seasons, FHB incidence values were estimated by Equation [1] and their adjustments. Early and mean heading dates were established after accumulating 1,250 and 1,320 degree-days from 1 July (base mean daily temperature = 0°C), respectively [32]. With these information, we obtained the spatial distribution of PFHBI (%) in the Pampas region (early and mean heading date).

Results

Morphological Identification

From the analyzed wheat samples, 67 isolations of *Fusarium* spp. were obtained. The species identification determined four species of *Fusarium* according to cultural and microscopical morphological characteristics (Tables 1, 2).

Fusarium graminearum produced fertile homothallic perithecia on CLA or SNA, whereas other *Fusarium* spp. did not. Conidium formation was considered on CLA (Table 2).

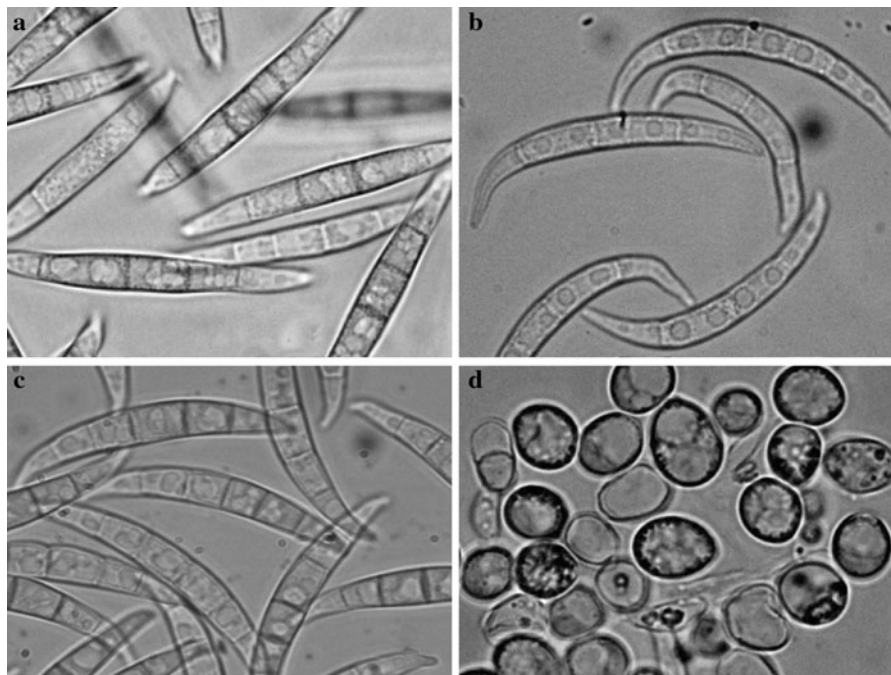
Figure 1 show the macro and microconidial observed in the diverse *Fusarium* spp. The shape of basal and apical cells was also observed for characterizations.

Table 1 *Fusarium* spp. cultural characterization on PDA

	Growth rate	Pigmentation	Aerial mycelium
<i>F. graminearum</i>	Rapid	Purple color in both faces	Cottony
<i>F. poae</i>	Rapid	White color above, clear purple reverse surface	Smoothly
<i>F. acuminatum</i>	Slow	Red color reverse surface	Smoothly
<i>F. avenaceum</i>	Rapid	Purple color in both faces	Cottony

Table 2 *Fusarium* spp. microscopic characterization on CLA

	Macroconidia	Microconidia	Sporodochia
<i>F. graminearum</i>	Straight and medium length	Absent	Some times cream orange
<i>F. poae</i>	Absent	Present	Absent
<i>F. acuminatum</i>	Equal curvature	Absent	Pale orange
<i>F. avenaceum</i>	Long and slender	Absent	Pale orange

**Fig. 1** Variation in macroconidial shape and length of *Fusarium* spp.; *F. graminearum* (a), *F. acuminatum* (b), *F. avenaceum* (c) grown on CLA ($\times 100$). Microconidia of *F. poae* (d) grown on CLA ($\times 100$)

PCR Detection of *Fusarium* spp.

After morphological identification, molecular identification was carried out to corroborate the determinations. *Fusarium graminearum* species-specific PCR Fg 16NF/R primer set amplified the expected 280-bp DNA fragment in all 60 isolates tested. None of the

other species produced a product with this primer set, indicating the specificity of Fg16NF/R primer set.

The three *F. poae* isolates yielded the expected 220-bp DNA fragment with the primer set FP82F/R. PCR performed on the DNA isolates of the other *Fusarium* species did not result in the amplification of DNA fragment, indicating the specificity of the

primer set. For *F. acuminatum* we used FAF/R primer set, chosen by its specificity according to several studies. However, it turned out to be non-specific for our isolates, amplifying the expected 600-bp DNA fragment either in *F. acuminatum* isolates or in *F. graminearum* isolates.

Similar results were obtained for *F. avenaceum*, using J1AF/R primer set, the expected 220-bp product of amplification was obtained for both *F. avenaceum* and *F. graminearum*, therefore resulting non-specific. In both cases, the PCR experimental conditions were tested thoroughly. In this report for *F. graminearum* and *F. poae*, the molecular identification was accurate and fast, coinciding with morphological determinations, but for *F. acuminatum* and *F. avenaceum* it was not decisive.

As it is observed in Tables 3 and 4, out of the 67 isolates obtained, sixty corresponded to *F. graminearum*,

three to *F. poae*, three to *F. acuminatum* and one to *F. avenaceum*.

Relation Between Spatial Distribution of FHB Incidence Values and Occurrence of *Fusarium* spp.

Weather conditions during 2006 wheat-growing season were not conducive for the occurrence of FHB infection events. According to Fig. 2, only very light incidence values were estimated for the central sector of the Pampas region, being coincident with the presence of *F. graminearum* in one sample of wheat grain obtained from 9 de Julio location. Presence of the pathogen in grain samples from the other locations was not detected, in agreement with the extremely low to null FHB incidence values estimated by the meteorological-based Eq. 1. Other *Fusarium*

Table 3 Distribution of *Fusarium* spp. isolates obtained by locality

F. graminearum (1),
F. poae (2), *F. acuminatum*
(3), *F. avenaceum* (4) for
2006 and 2007 wheat-
growing season from INTA

Wheat-growing season	Location	Wheat cultivars n°	Isolates	1	2	3	4
2006	Balcarce	6	0				
	Barrow	6	2			2	
2007	Barrow	6	0				
	Balcarce	6	0				
	C. del Uruguay	4	3	3			
	Oliveros	10	26	24	1	1	
	Paraná	5	14	14			
	Pergamino	5	19	18			1

Table 4 Distribution of *Fusarium* spp. isolates obtained by locality

F. graminearum (1),
F. poae (2), *F. acuminatum*
(3), *F. avenaceum* (4) for
2006 wheat-growing season
of concentration silos from
SENASA

Wheat-growing season	Location	Isolates	1	2	3	4
2006	9 de Julio	1	1			
	Azul					
	Balcarce	1		1		
	C. del Uruguay					
	Córdoba					
	Galeguaychú	1		1		
	Mar del Plata					
	Paraná					
	Rafaela					
	Río Cuarto					
Santa Fe						
Tandil						

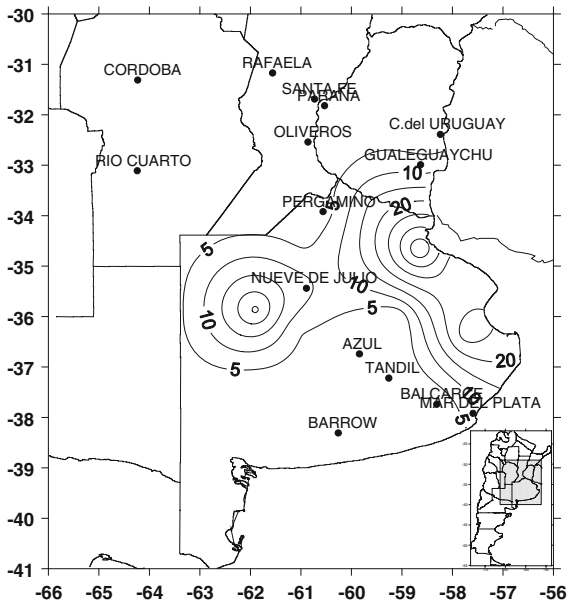


Fig. 2 Spatial distribution of FHB incidence values for 2006 wheat-growing season. Mean heading date. The sites sampled in 2006 and 2007 growing seasons are also indicated

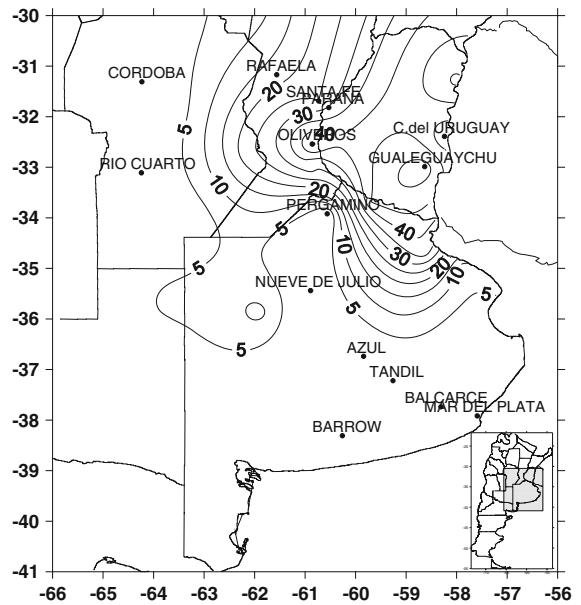


Fig. 3 Spatial distribution of FHB incidence values for 2007 wheat-growing season. Mean heading date. The sites sampled in 2006 and 2007 growing seasons are also indicated

spp. were found in those locations of very low FHB incidence.

In contrast to 2006, weather conditions during 2007 growing season were conducive to disease infections events, especially for those wheat cultivars with early heading. Figure 3 shows the moderate FHB incidence values estimated by Eq. 1 in the north-eastern quadrant of the Pampas region. In wheat grain samples from locations such as Oliveros, Concepción del Uruguay and Paraná, isolates of *F. graminearum* were detected, in agreement with FHB incidence values higher than 30% predicted by Eq. 1, for a mean heading date (1,320 degree-days accumulated after 1 July). For earlier heading date (1,250 degree-days accumulated after 1 July), higher predicted FHB incidence values accounted for the most favorable weather conditions prevailing at the beginning of October, when is the begin of period of flowering of wheat in the region.

The presence of *F. graminearum* isolates in grain samples from Pergamino location was explained by the moderate FHB incidence values estimated by Eq. 1 and by the early heading date (Fig. 4).

Actually, most wheat cultivars escaped from weather conditions conducive to disease outbreaks

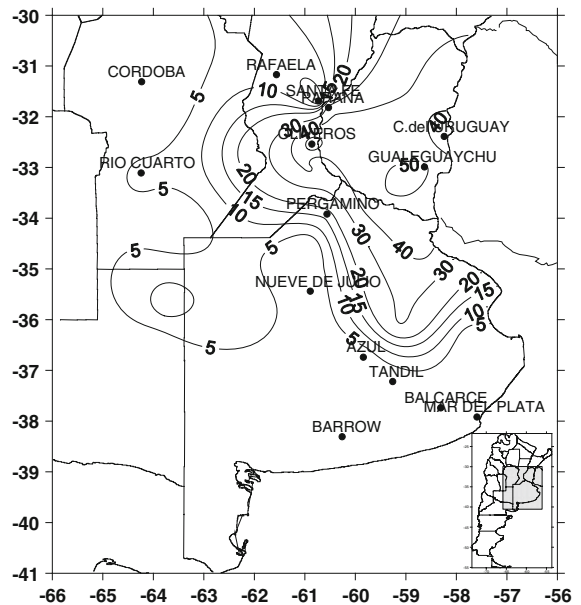


Fig. 4 Spatial distribution of FHB incidence values for 2007 wheat-growing season. Early heading date. The sites sampled in 2006 and 2007 growing seasons are also indicated

at the beginnings of October because of a delayed heading due to below-normal temperatures registered before the reproductive stage (2007 wheat-growing season). During November, when the southern sector

of the Pampas region concentrated heading and anthesis stages, the weather conditions were adverse for producing FHB infection events. This situation could explain the lack of *F. graminearum* isolates from wheat grain samples obtained from southern locations like Barrow and Balcarce locations. Accordingly, no FHB incidence values were estimated by Eq. 1 for the southern Pampas region during 2007 growing season (Fig. 3).

Discussion

In this report, we contribute to the accurate identification of the causal agent of FHB in relation to disease predictions from meteorological-based systems in the main wheat-growing area of Argentina.

Sixty-seven *Fusarium* spp. isolates were obtained from two consecutive wheat-growing seasons, which were identified according to morphological and molecular characterization by specific primers PCR-DNA reaction. For *F. graminearum* and *F. poae*, both characterizations were successful, but for *F. avenaceum* and *F. acuminatum* the molecular characterization was non-specific, showing cross-reaction with *F. graminearum*. Also the ability to produce homothallic perithecia was confirmed for *F. graminearum*.

Since the species identification by PCR primers is considered a sensitive and rapid method it is often used for detection of *Fusarium* spp. in wheat grains instead of the traditional macro and micromorphological identification which is considered tedious and time consuming. There are few previous reports in Argentina that discuss this issue. When *F. graminearum* isolates were identified in wheat in Argentina by Ramírez et al. [33] it was by morphological identification. The same criterion was utilized by Lori et al. [34] in *Fusarium* spp. isolates from Buenos Aires province. Diverse technical reports by the INTA in Argentina have also been based on morphological characterizations. In our research, the morphological characterization was decisive to identify *F. avenaceum* and *F. acuminatum*. We consider that in laboratories where morphological identifications are routinely carried-out, good results are obtained, depending on the practice. Our results revalue the concept about using several criteria for the definition of the species, giving a more reliable identification. Leslie et al. [35] considered that the most robust species definitions are

those in which the three species concepts—morphological, biological and phylogenetic—reach the same identification result. Schilling et al. [36] detected *F. avenaceum* and *F. graminearum* by PCR species-specific primers which revealed no significant cross-reactions for any assays. Demeke et al. [37] observed that primers reported as specific for *F. avenaceum* [38] amplified all isolates of both *F. avenaceum* and *F. acuminatum*. Therefore, they were not useful for distinguishing between these two species. Instead, good results were obtained when using the primers reported by Turner et al. [31]. Turner et al. [31] have determined a high level of reability for primer pair utilized for *F. avenaceum* detection, but have considered that future cross-reaction problems could possibly appear. To interpret these cases of cross-reaction of species-specific primers between related species it is necessary to consider the structure and reproductive behavior of field populations of *Fusarium* spp. [39]. The molecular sequences of gene regions have indicated that different species of *Fusarium* are closely genetically related [40]. Doohan et al. [38] also observed some difficulties using PCR analysis for identifying fungal species present in wheat with FHB. Yli-Mattila et al. [41] concluded that for *Fusarium* species in Finland and northwestern Russia, the results obtained by species-specific primers mostly agreed with the identification results based on morphology. However, in a few cases, contradictory results were obtained. For *Fusarium* it is demonstrated that the genetic variation within the species is very high and influences aggressiveness, toxin spectrum and abundances, host interaction, sexual and asexual reproduction and environmental response [33, 42]. Leslie et al. [35] considered that evolution should continually generate new DNA sequences and intermediates between various species. This might partly explain several cases worldwide analyzed, in which the observed damage changes according to the geographical location of *Fusarium* isolates [43, 44].

In this report, the spatial distribution of FHB incidence values for 2006 wheat-growing season determined the absence of weather conducive to FHB infection events, observing only very low incidence values for the central sector of the Pampas region. From this wheat sampling, very few *Fusarium* spp. isolates were obtained, one of them identified as *F. graminearum* from 9 de Julio, location placed in

the central sector. The remained isolates were identified as other *Fusarium* spp. different from *F. graminearum*, obtained from locations without meteorological conditions for infection.

The spatial distribution of FHB incidence values for 2007 wheat-growing season reflected the most favorable weather conditions for producing FHB infection events. In this sampling, 95% of the obtained *Fusarium* spp. isolates were identified like *F. graminearum*. According to another research in Argentina, 90% of the pathogens isolated from FHB were *F. graminearum* [45]. Our results confirmed the predominance of *F. graminearum* in wheat grain associated with FHB in Argentinian Pampas region, by different criteria of species identification.

Although, several reports refer to the genotypic variability on *F. graminearum* population in different regions of the world such as in USA [11, 46], Canada [47, 48], Europe [43], Korea [49], China [43], there is little available information about this topic in South America in general, particularly in Argentina. Ramírez et al. [33] determined the vegetative compatibility groups (VCGs) and mycotoxin production on populations obtained from wheat in three Argentinian localities. Several studies in Argentina were focused to analyze related aspects between distribution of *F. graminearum* and deoxynivalenol (DON) contamination in Córdoba province [50] and in the south of Buenos Aires province [51]. However, in Argentina, there is no available information about the relationship between disease and environment. Each fungus in the FHB disease system has a different behavior as regards adaptation, virulence, environmental requirements and host factors, as well as physiological and genetic make-up. All this may, in part, explain why the occurrence of these species varies by location [6]. In order to predict FHB, both the pathogen and the environment must be related [19]. In several countries such as Belgium, Canada, Italy and USA, computer models based on weather variables have been developed to predict the occurrence of FHB and DON contamination in wheat [13]. In Argentina, the predictive climatic model has been developed by Moschini and Fortugno [14, 52].

In conclusion, from 2006 wheat-growing season sampling only 5 isolations were obtained being the presence of *Fusarium* spp. scarce coinciding with the scarce incidence of the disease. Only one of them was identified as *F. graminearum*, two as *F. poae* and two

as *F. acuminatum*. From 2007 wheat-growing season sampling, 62 isolates were obtained, coinciding with major FHB incidence values of the disease, being 98% *F. graminearum* and the rest was distributed among *F. poae*, *F. acuminatum* and *F. avenaceum*. Therefore, different distributions of *Fusarium* species and number of isolates in both 2006 and 2007 wheat-growing seasons were associated to the spatial distribution of FHB incidence values estimated by a weather-based model. Beside, in wheat-growing season with weather conditions conducive for the occurrence of FHB infection events, *Fusarium graminearum* was the principal species detected.

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References

1. Monds RD, Cromei MG, Lauren DR, di Menna M, Marshall J. *Fusarium graminearum*, *F. cortaderiae* and *F. pseudograminearum* in New Zealand: molecular phylogenetic analysis, mycotoxin chemotypes and co-existence of species. *Mycol Res*. 2005;109(4):410–20.
2. Bai G-H, Shaner G. Scab of wheat: prospects for control. *Plant Dis*. 1994;78:760–6.
3. Bai G-H, Desjardins AE, Plattner RD. Deoxynivalenol-nonproducing *Fusarium graminearum* causes initial infection, but does not cause disease spread in wheat spikes. *Mycopathologia*. 2001;153:91–8.
4. Galich AN, de Galich MTV. Enfermedades de trigo en el área central norte de la región cerealera Argentina. *Informe Técnico 121*. E.E.A INTA Marcos Juárez, Córdoba, Argentina. 1996 (in Spanish).
5. Dyer RB, Kendra DF, Brown DW. Real-time PCR assay to quantify *Fusarium graminearum* wild-type and recombinant mutant DNA in plant material. *J Microbiol Methods*. 2006;67:534–42.
6. Osborne LE, Stein JM. Epidemiology of Fusarium head blight on small-grain cereals. *Int J Food Microbiol*. 2007;119(1–2):103–8.
7. McMullen M, Jones R, Gallenberg D. Scab of wheat and barley: a re-emerging disease of devastating impact. *Plant Dis*. 1997;81(12):1340–8.
8. Windels C. Economical and social impacts of Fusarium Head Blight changing farms and rural communities in the northern great plains. *Phytopathology*. 2000;90:17–21.
9. Galich MT. Fusarium head blight in Argentina. In: Duvin HJ, Gilchrist R, Reeves J, McNab A, editors. *Fusarium Head Scab: global status and future prospects*. México: Centro Internacional de Mejoramiento de Maíz y Trigo; 1997. p. 19–28.

10. Galich MT. Importancia y difusión de la Fusariosis de trigo en Argentina. In: Kohli MM, editors (Ed CIMMYT). International Maize and Wheat Improvement Center; 1989. p. 7–31 (in Spanish).
11. Zeller K, Bowden R, Leslie J. Diversity of epidemic populations of *Gibberella zeae* from small quadrats in Kansas and North Dakota. *Phytopathology*. 2003;93: 874–80.
12. Schaafsma AW, Hooker DC. Climatic models to predict occurrence of *Fusarium* toxins in wheat and maize. *Int J Food Microbiol*. 2007;119:116–25.
13. Prandini A, Sigolo S, Filippi L, Battilani P, Piva G. Review of predictive models for Fusarium head blight and related mycotoxin contamination in wheat. *Food Chem Toxicol*. 2009;47:927–31.
14. Moschini RC, Fortugno C. Predicting wheat head blight incidence using models based on meteorological factors in Pergamino, Argentina. *Eur J Plant Pathol*. 1996;102:211–8.
15. Moschini RC, Pioli R, Carmona M, Sacchi O. Empirical predictions of wheat head blight in the northern Argentinean pampas region. *Crop Sci*. 2001;41:1541–5.
16. Carranza M, Moschini RC, Kraan G, Bariffi JH. Examination of meteorology-based predictions of Fusarium head blight of wheat grown at two locations in the southern Pampas region of Argentina. *Aust Plant Pathol*. 2007;36:1–4.
17. Moschini RC, Bischoff S. Meteorological-based systems for predicting and managing Fusarium Head Blight epidemics in the wheat growing area of Argentina. In: Proceedings of the 5th Canadian workshop on Fusarium Head Blight 2007. Winnipeg, Canada, November 27th–30th, p. 88–95.
18. Parry DW, Pettitt TR, Jenkinson P, Lees AK. The cereal Fusarium complex. In: Blakerman P, Williamson B, editors. Ecology of plant pathogens. Wallingford: CAB International; 1994. p. 301–20.
19. Parry DW, McLeod L, Jenkinson P. Fusarium head blight (scab) in small grain cereal a review. *Plant Pathol*. 1995;4:207–38.
20. Neergaard P. Seed pathology. London: MacMillan; 1977.
21. Nelson PE, Dignani MC, Anaissie EJ. Taxonomy, biology and clinical aspects of *Fusarium* species. *Clin Microbiol Rev*. 1994;7(4):479–504.
22. Fisher NL, Burgess LW, Tousson TA, Nelson PE. Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. *Phytopathology*. 1982;72:151–3.
23. Burgess LW, Summerell BA, Bullock S, Gott KP, Backhouse D. Laboratory manual for Fusarium research. 3rd ed. Sydney: University of Sydney; 1994.
24. Gerlach W, Nirenberg H. The genus *Fusarium*—a pictorial atlas. *Mitt Biol Bundesanst. Ld – u Forstw*. Berlin- Dahlem 1982;209:1–406.
25. Leslie JF, Summerell BA. The *Fusarium* laboratory manual, 1st edn. Ames: Backwell, 2006;110 4:573–85.
26. Summerell BA, Salleh B, Leslie JF. A utilitarian approach to *Fusarium* identification. *Plant Dis*. 2003;87:117–28.
27. Murray MG, Thompson WF. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res*. 1980; 8:4321–5.
28. Nicholson P, Simpson DR, Weston G, Rezanoor HN, Lees AK, Parry DW, Joyce D. Detection and quantification of *Fusarium culmorum* and *Fusarium graminearum* in cereal using PCR assays. *Physiol Mol Plant Pathol*. 1998;53:17–37.
29. Parry DW, Nicholson P. Development of a PCR assay to detect *Fusarium poae* in wheat. *Plant Pathol*. 1996;45: 383–91.
30. Williams KJ, Dennis JJ, Smyl C, Wallwork H. The application of species-specific assay based on the polymerase chain reaction to analyze *Fusarium* crown rot of durum wheat. *Austr Plant Pathol*. 2002;31:19–129.
31. Turner AS, Lees AK, Rezanoor HN, Nicholson P. Refinement of PCR-detection of *Fusarium avenaceum* and evidence from DNA marker studies for phenetic relatedness to *Fusarium tricinctum*. *Plant Pathol*. 1998;47:278–88.
32. Moschini RC, Bischoff S, Martínez MI. Variabilidad climática y ocurrencia de enfermedades: caso de estudio: Fusariosis de la espiga de trigo. *Horizonte A. Magazine de las Ciencias Agrarias* 2008. Año 5 N°21. Facultad de Agronomía de la Universidad de Buenos Aires, p. 10–5 (in Spanish).
33. Ramírez ML, Reynoso MM, Farnochi MC, Chulze S. Vegetative compatibility and mycotoxin chemotypes among *Fusarium graminearum* (*Gibberella zeae*) isolates from wheat in Argentina. *Eur J Plant Pathol*. 2006;115: 139–48.
34. Lori GA. Género *Fusarium* en Argentina II. Identificación de cultivos en la zona de La Plata. *Rev Arg Microbiol*. 1985;17(2):61–7 (in Spanish).
35. Leslie JF, Anderson LL, Bowden RL, Lee Y-W. Inter- and intra-specific genetic variation in *Fusarium*. *Int J Food Microbiol*. 2007;119(1–2):25–32.
36. Schilling AG, Moller WM, Geiger HH. Polymerase chain reaction-based assays for species-specific detection of *Fusarium culmorum*, *F. graminearum*, and *F. avenaceum*. *Phytopathology*. 1996;85(5):515–22.
37. Demeke T, Clear RM, Patrick SK, Gaba D. Species-specific PCR-based assays for the detection of *Fusarium* species and a comparison with the whole seed agar plate method and trichothecene analysis. *Int J Food Microbiol*. 2005;103:271–84.
38. Doohan FM, Parry DW, Jenkinson P, Nicholson P. The use of species-specific PCR-based assays to analyze Fusarium head blight of wheat. *Plant Pathol*. 1998;4:109–205.
39. Akinsami OA, Backhouse D, Simpfendorfer S, Chakraborty S. Mycelial compatibility reactions of Australian *Fusarium graminearum*, *F. pseudograminearum* isolates compared with AFLP groupings. *Plant Pathol*. 2008;57:251–61.
40. Chung W-H, Ishii H, Nishimura K, Ohshima M, Iwama T, Yoshimatsu H. Genetic analysis and PCR-based identification of major *Fusarium* species causing head blight on wheat in Japan. *J Genet Plant Pathol*. 2008;74:364–74.
41. Yli-Mattila T, Paaavanen-Huhtala S, Parikka R, Konstantinova P, Gagkaena TY. Molecular and morphological diversity of *Fusarium* species in Finland and north-western Russia. *Eur J Plant Pathol*. 2004;110:573–85.
42. Busso C, Nobuyoshi Kaneshima E, de Assis Franco F, Boto Querol C, Alves de Castro-Prado MA. Vegetative compatibility and molecular characterization of *Fusarium graminearum* isolates from the State of Paraná, Brazil. *Ciencia Rural*. 2007;37(6):2–6.

43. Gagkaeva TY, Yli-Mattila T. Genetic diversity of *Fusarium graminearum* in Europe and Asia. *Eur J Plant Pathol.* 2004;110:551–62.
44. Láday M, Juhász Á, Mulé G, Moretti A, Logrieco A. Mitochondrial DNA diversity and lineage determination of European isolates of *Fusarium graminearum* (*Gibberella zae*). *Eur J Plant Pathol.* 2004;110:545–50.
45. de Galich MTV, Galich AN. Enfermedades del trigo en el área sur de Santa Fé y Córdoba. Información para extensión 5. Córdoba, Argentina: EEA-INTA Marcos Juárez; 1994 (in Spanish).
46. Bowden RL, Leslie JF. Diversity of *Gibberella zae* (*Fusarium graminearum*) at small spatial scales. *Phytopathology.* 1994;84:1140 (Abstr.).
47. Gilbert J, Abramson D, McCallum S, Clear R. Comparison of Canadian *Fusarium graminearum* isolates for aggressiveness, vegetative compatibility, and production of ergosterol and mycotoxins. *Mycopathologia.* 2001;153: 209–15.
48. MacCallum BD, Tekauz A, Gilbert J. Vegetative compatibility among *Fusarium graminearum* (*Gibberella zae*) isolates from barley spikes in Southern Manitoba. *Can J Plant Pathol.* 2001;23:83–7.
49. Moon J-H, Lee Y-H, Lee Y-W. Vegetative compatibility groups in *Fusarium graminearum* isolates from corn and barley in Korea. *Plant Pathol J.* 1999;15:53–6.
50. Dalcero A, Torres A, Etcheverry M, Chulze S, Varsavsky E. Occurrence of deoxynivalenol and *Fusarium graminearum* in Argentinean wheat. *Food Addit Contam.* 1997;14(1):11–4.
51. Lori GA, Sisterna MN, Haldukowski M, Rizzo I. *Fusarium graminearum* and deoxynivalenol contamination in the durum wheat area of Argentina. *Microbiol Res.* 2004;158(1):29–35.
52. Moschini RC, Carranza MR, Carmona M. Meteorological-based predictions of wheat head blight epidemic in the southern Argentinean pampas region. *Cereal Res Commun.* 2004;32:45–52.