Toxigenic Capacity and Trichothecene Production by *Fusarium graminearum* Isolates from Argentina and Their Relationship with Aggressiveness and Fungal Expansion in the Wheat Spike

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ABSTRACT

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At least 20 epidemics of Fusarium head blight (FHB) of wheat have been registered in the last 50 years in Argentina, with variable intensity. Damage induced by the disease is further aggravated by the presence of mycotoxins in affected grains that may cause health problems to humans and animals. The trichothecene chemotype was analyzed for 112 isolates of *Fusarium graminearum* from Argentina by polymerase chain reaction and two field trials were conducted to study the aggressiveness of a subsample of 14 representative isolates and to analyze deoxynivalenol (DON) production in planta and in vitro. All isolates belonged to the 15-acetyl-DON chemotype. Significant differences were observed in both the symptom severity induced in wheat spikes and the in vivo DON production, and a close correlation was found between these two variables. However, in vitro toxigenic potential was not correlated with the capacity of *F. graminearum* isolates to produce DON under natural conditions. The progress of infection in the rachis of inoculated wheat spikes was analyzed and the pathogen presence verified in both symptomatic and symptomless spikes. Even isolates with a limited capacity to induce symptoms were able to colonize the vascular tissue and to produce considerable amounts of DON in planta.

Fusarium head blight (FHB) of wheat, caused by *Fusarium graminearum* (Schwabe), is a destructive disease that frequently causes epidemics in many wheat-cropping areas worldwide (15,38). In Argentina, at least 20 epidemics have been registered in the last 50 years with variable intensity, the more severe occurring in 1945–46, 1976, 1978, 1985, 1993, and 2001 (19,28,43).

The disease affects several major crops, including maize, wheat, barley, rye, and triticale, reducing seed and grain quality as well as yield, leading to poor germination and reduction in flour baking properties. The damages induced by the disease are further aggravated by the frequent presence of mycotoxins in affected grains. These persistent, thermostabile metabolites, produced in association with food and feeds, may cause health problems to humans and animals even in low doses (27).

The trichothecenes deoxynivalenol (DON) and its derivatives 3-acetyl-DON (3-ADON) and 15-acetyl-DON (15-ADON), and nivalenol (NIV) and its acetylate derivative Fusarenone X (FUS-X), are the major mycotoxins produced by *F. graminearum* (36). DON, the most important mycotoxin in terms of human exposition (42), has been found as a contaminant of wheat, maize, and barley, three crops that account for two-thirds of the worldwide production of cereals.

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Trichothecenes are synthesized by a complex metabolic route that involves a series of oxygenations, isomerizations, and esterifications (3). According to the pattern of trichothecene production, isolates of *F. graminearum* can be organized into three different chemotypes: *Ia*, producing DON and 3-ADON; *Ib*, producing DON and 15-ADON; and *II*, producing NIV and FUS-X (24,44). Most of the genes involved in the biosynthesis of trichothecenes and, thus, in its variations, are localized in a cluster of at least 10 genes (8) that encode regulatory proteins as well as most of the enzymes required for trichothecene synthesis (3). Among them, *Tri13* and *Tri7* are responsible for the production of DON- or NIV-like trichothecenes (30), while differences in the sequence of *Tri8* have been identified as the basis for the production of either 3-ADON or 15-ADON (2). The identification of these genes provided the basis for the development of valuable polymerase chain reaction (PCR) tools for fast chemotype determination in *F. graminearum* (58,63).

Differences in the geographic distribution of *F. graminearum* chemotypes have been reported. In Chinese populations of the pathogen, DON-like chemotypes prevail, with similar frequencies of 15-ADON and 3-ADON strains (65); whereas, in Japan, the prevalent chemotype is NIV (59). In Europe, 15-ADON is the most frequent chemotype, followed by 3-ADON and only a few representatives of NIV strains (26,47,51). In the United States and Canada, where 15-ADON was previously identified as the predominant chemotype, increasing frequencies of 3-ADON and NIV strains have been recently reported (52,58). In Brazil and Uruguay, 15-ADON is the most commonly found trichothecene chemotype, with only sporadic recovery of strains of NIV chemotype (7,46).

Controversy exists regarding the chemotype composition of *F. graminearum* populations from Argentina. Several authors reported the prevalence of DON chemotypes in the wheat-cropping area (4,10,16,54), while others also reported the presence of NIV chemotypes (18,33,55,57). These antecedents suggest that, even when DON seems to be the predominant chemotype in *F. graminearum* isolates from Argentina, the potential exists for the occurrence of grain contamination with NIV.

Qualitative and quantitative differences in the synthesis of enzymes and mycotoxins might be responsible for the great variability in aggressiveness observed within *F. graminearum* populations (41). The phytotoxic activity of trichothecenes, and particularly of DON, has been widely studied. On plant tissue, these compounds inhibit protein synthesis and mitochondrial function and affect cell division and membranes (56). Reduced production of trichothecenes does not influence the capacity of *F. graminearum* to infect wheat or maize but affects the progression of the infection due to a decrease in aggressiveness (49,50). However, the mechanism by which these metabolites induce the development of FHB remains largely unclear. It has been proposed that, during disease spread, trichothecenes could be translocated in the plant before the growth of the pathogen, reducing protein synthesis without inducing the activation of defense mechanisms (45,48). Furthermore, trichothecenes have been found to inhibit the development of defense mechanisms in the wheat rachis node that would otherwise prevent the movement of the fungus into the internode (25). On the other hand, evidence has been found that DON may activate a range of plant defense responses and programmed cell death in wheat, simultaneously favoring necrotrophic growth of the pathogen and stimulating antimicrobial defense responses in the host (14).

A correlation between aggressiveness and DON production has been proposed for *F. graminearum* and *F. culmorum* (13,62). McCormick (36) suggested that the role of trichothecenes as aggressiveness factors highlights the possibility of developing wheat cultivars resistant to the accumulation of mycotoxins that could improve plant response to the disease. However, experiments on wheat have yielded controversial results and several authors have either failed to find a significant correlation between FHB and production of trichothecenes or found inconsistent results (1,6,32,61).

In Argentina, a high level of variability in the aggressiveness of *F. graminearum* was found within isolates from wheat (5,35). Alvarez et al. (5) carried out specific assays but were unable to establish the relationship between FHB symptoms on wheat spikes, toxigenic capacity of *F. graminearum* isolates, and progression of the pathogen on wheat tissue.

Therefore, the clarification of chemotype distribution and of the relationship between mycotoxin accumulation and aggressiveness of isolates of *F. graminearum* from Argentina remains an issue of great importance, because it could greatly help the development of wheat genotypes tolerant or resistant to FHB. With this in mind, the objectives of the present work were to (i) identify the mycotoxin chemotypes of *F. graminearum* isolates from the Buenos Aires Province in Argentina, (ii) quantify their toxigenic capacity, and (iii) correlate the accumulation of trichothecenes in wheat grains with aggressiveness of the pathogen and its capacity to colonize the spike.

MATERIALS AND METHODS

***F. graminearum* chemotype analysis.** Chemotype analysis were carried out on 112 *F. graminearum* isolates obtained from grain samples of common wheat (*Triticum aestivum* L.) collected from 28 different localities distributed within Buenos Aires Province, Argentina (35). Isolate chemotypes were determined by means of the two multiplex PCRs developed by Starkey et al. (58) using the chemotype-specific primers previously validated by Ward et al. (63). The sequence of the primers used, target genes, and amplified products are detailed in Table 1.

Both multiplex-PCRs were carried out in a 25-µl volume containing 5 to 10 ng of genomic DNA, 1 U of T-plus DNA polymerase (Highway Molecular Biology-InBio-UNICEN, Tandil, Argentina), 0.5 µM each primer (FAGOS/Ruralex, Buenos Aires, Argentina), 200 µM each deoxynucleoside triphosphate, and 1.5 mM $MgCl_2$ in 1× reaction buffer (500 mM KCl, 100 mM Tris-Cl [pH 9.0], and 1% Triton X-100 without Mg^{++}). Both PCR cycles consisted of an initial denaturation step of 3 min at 94°C; followed by 35 cycles of 30 s at 94°C, 30 s at 53°C, and 1 min at 72°C; and a final extension step of 10 min at 72°C. Amplification products were resolved on 1.5% (wt/vol) agarose gels containing ethidium bromide at 0.2 mg ml^{-1} in Tris-borate-EDTA buffer. Gels were visualized under UV using a GeneGenius (Syngene, Frederick, USA) image analyzer. Photos were taken with GeneSnaps software and the size of the resulting fragments was estimated using GeneTools and GeneDirectory software by comparison with the bands generated by a 1-kb DNA ladder (Highway Molecular Biology-InBio-UNICEN). In each of the amplification reactions, a negative control without DNA and positives controls for each chemotype were included.

Evaluation of the aggressiveness of *F. graminearum* isolates. Two field trials were conducted in consecutive years to evaluate aggressiveness of the 112 isolates of *F. graminearum* using common wheat 'Klein Chajá' (35). In both trials, 20 field-grown wheat spikes were point inoculated with each isolate at anthesis (Zadoks growth stage 65) (64) in a completely randomized block design with four replicates, each consisting of five spikes.

Spikes were visually rated for disease severity 21 days post-inoculation (dpi), considering symptomatic spikelets for each spike above and below the point of inoculation (PI). Disease severity, thousand-kernel weight (TKW), and area under the disease progress curve (AUDPC) differences between isolates, as well as the correlations found between these parameters, are reported elsewhere (35). The spikes inoculated with 14 isolates were selected to further evaluate infection progress along the rachis and mycotoxin production. These isolates were selected because they reflected the variability present among the aggressiveness groups constructed with the results obtained from the field tests (35).

TABLE 1. Primers used for determination of the chemotype of 112 isolates of *Fusarium graminearum* from the Buenos Aires Province in Argentina by multiplex polymerase chain reaction

Primer	Sequence (5′–3′)	Chemotype[z]	Target gene	Amplified product (bp)
3NA	GTG CAC AGA ATA TAC GAG C	NIV	*TRI3*	840
3D15A	ACT GAC CCA AGC TGC CAT C	15-ADON	…	610
3D3A	CGC ATT GGC TAA CAC ATG	3-ADON	…	243
3CON	TGG CAA AGA CTG GTT CAC	…	…	…
12NF	TCT CCT CGT TGT ATC TGG	NIV	*TRI12*	840
12-15F	TAC AGC GGT CGC AAC TTC	15-ADON	…	670
12-3F	CTT TGG CAA GCC CGT GCA	3-ADON	…	410
12CON	CAT GAG CAT GGT GAT GTC	…	…	…

[z] NIV = nivalenol and 15- and 3-ADON = 15- and 3-acetyl-deoxynivalenol, respectively.

Analysis of the wheat spike colonization. At maturity, the wheat spikes inoculated with each of the 14 selected *F. graminearum* isolates in each of the two field tests were hand threshed. The grain was saved for TKW and mycotoxin determinations while the resulting rachis were superficially disinfected by dipping in 70° ethanol for 1 min and in a 5% commercial NaClO solution (Cl at 55 g liter^{-1}) for 1 min, followed by rinsing in distilled water for 5 min. Surface-sterilized rachis were cut, so that each of the resulting fragments consisted of a rachis node and the adjacent internode, and plated on 2% (wt/vol) potato dextrose agar medium (PDA) supplemented with chloramphenicol at 250 mg liter^{-1} and pentachloronitrobenzene (75% wettable powder) at 600 mg liter^{-1}. Fragments were numbered with positive or negative successive numbers according to their relative position from the PI and the disposition of the fragments of the rachis in the spike was taken into consideration when plating (Fig. 1). The rachis of the control treatments of the field tests were used as a control.

After 5 days of incubation at 25 ± 2°C, expansion in the rachis was evaluated as the percentage of rachis fragments colonized by *F. graminearum*. To avoid false positives, infection of the fragments by *F. graminearum* was confirmed by two complementary approaches: microscopic observation of the colonies obtained on PDA and of the macroconidia obtained in carnation leaves agar or PCR analysis by using primers FG16N F and FG16N R, as previously reported (35).

In vivo and in vitro DON production. In vivo and in vitro DON productions were evaluated for the 14 isolates of *F. graminearum* tested for pathogen expansion along the rachis. Toxin was quantified by means of the RIDASCREEN FAST DON (R-Biopharm, Germany) enzyme-linked immunosorbent assay (ELISA) kit, according to the manufacturer's instructions (22,23). For in vivo DON production analysis, the grains obtained from the field-test-inoculated spikes were ground, weighed, and used for quantification. The grains obtained from field test control spikes were considered to be negative controls.

For in vitro DON quantification, the 14 *F. graminearum* isolates were cultivated in triplicate in 250-ml Erlenmeyer flasks containing 25 g of polished rice and 20 g of distilled water (80% humidity). Previously autoclaved substrate was inoculated with a 5-mm-diameter plug of a 7-day-old *F. graminearum* colony grown on PDA. As a negative control, three Erlenmeyer flasks containing the rice substrate were inoculated with a 5-mm-diameter plug of autoclaved PDA. Flasks were cultivated for 28 days at 25 ± 2°C in the dark, dried in a stove with forced-air circulation until constant weight, ground, and weighed.

For DON quantification, the full amount of the ground *F. graminearum*-contaminated grain from each inoculated block of both field trials was used for in vivo production while all the rice substrate from each Erlenmeyer flask was used for in vitro production. Ground material was diluted 1:20 (wt/vol) in distilled water and homogenized in a magnetic stirrer for 3 min, the extract obtained was filtered through Whatman number 1 filter paper, and a 50-µl aliquot was pipetted into each well of the ELISA plate for the analysis. Absorbance was measured in a Beckman Coulter dtx-800 multimode detector (Beckman Coulter, Brea, USA) at 450 nm and data were converted to DON concentration by means of the RIDASOFT Win software using the standard curve obtained from the DON standards provided in the kit.

Statistical analysis. Data taken as percentage were arcsine-transformed prior to analysis. Aggressiveness, trichothecene production, and expansion of *F. graminearum* along the spike were analyzed by analysis of variance and means were compared using Tukey's test ($P \leq 0.05$). Correlations were carried out for all combinations of parameters at $\alpha = 0.05$. For in vivo DON production, TKW, and premature bleaching of the spike, data were collected using blocks as experimental units. All the analyses were performed using Statistix v.8.

A combined analysis model (37) was applied to the disease severity at 21 dpi, phenotypic variance components were estimated from the expected mean squares, and broad-sense heritability was calculated using the formula by Fehr (17), as previously reported (35).

RESULTS

Chemotype analysis of *F. graminearum* isolates. All 112 *F. graminearum* isolates obtained from 28 localities in Argentina belonged to the 15-ADON chemotype according to the size of the fragments amplified by both multiplex PCR protocols (58).

Selection of isolates for the evaluation of the expansion of *F. graminearum* in the spike, in vivo, and in vitro DON production. The 14 isolates selected were tested to check that this subsample reflected the results previously obtained (35). To do so, the analysis of severity 21 dpi, TKW reduction, and heritability according to Fehr (17) carried out for the greater group of isolates were repeated.

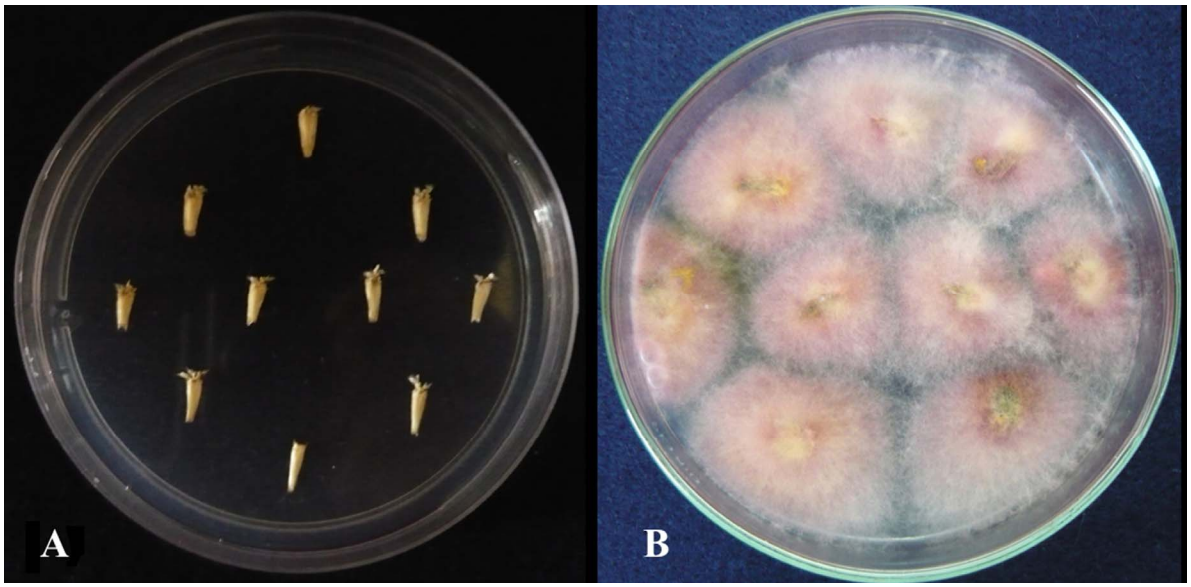

Fig. 1. A, Plating on potato dextrose agar culture medium of the upper rachis portion fragments of a wheat spike inoculated with an isolate of *Fusarium graminearum* and **B,** development of colonies of *F. graminearum* from the plated rachis fragments of the lower portion of another spike.

The variability found in the severity of the symptoms induced in wheat spikes ($F = 7.34$, $P < 0.01$) and in the TKW relative to the uninoculated control ($F = 5.31$, $P < 0.01$) among the 14 isolates reflected the differences previously found for 112 isolates, as did the percentage of inoculated spikes showing symptoms of premature bleaching (30%). Similarly, a close correlation was found between FHB severity and the relative TKW ($r = –0.900$, $P < 0.01$).

The heritability of symptom severity for the subsample was $H^2 = 0.96$; hence, very similar to the value found for the full sample. This result suggests that the greatest part of the variability found was the result of genotypic differences between the isolates rather than a consequence of the effect of the year of testing.

The results obtained in both years of field tests for severity, reduction of TKW, mycotoxin production, and colonization of the rachis were pooled and values are presented in Table 2.

Progression of *F. graminearum* in wheat spikes from the point of inoculation. In >90% of the analyzed rachis, the presence of *F. graminearum* was found colonizing the fragments of the rachis that supported the inoculated spikelets in the field tests (+0 and –0) (Figs. 2 and 3). Isolates differed significantly in their ability to colonize these fragments ($F = 7.22$, $P < 0.01$), with values that ranged from 48 to 86% of the total fragments for isolate MR18 and isolates BA14 and SP1, respectively (Table 2).

For all the treatments and all the spikelet/supporting fragment pairs, the percentage of the presence of the pathogen in the rachis was higher than the percentage of symptomatic spikes in field tests (Fig. 2). The percentage of colonization that resulted was even higher than the percentage of spikes showing symptoms of premature bleaching for all of the spikelet-rachis fragment pairs, except for the most distal position (+6).

Even when no inoculated spike showed FHB symptoms above the +3 spikelet, *F. graminearum* was found colonizing the apical fragment of the rachis in >25% of the considered spikes.

The tendency of the distribution of FHB symptoms prevalently downward from the PI observed in the field tests was also verified when the expansion of *F. graminearum* was analyzed (Fig. 3). The higher percentages of colonization were found for the portions corresponding to the inoculated spikelets, disregarding their relative position (–0 and +0). No significant differences were found between these portions and those located immediately above (+1 and +2) and below (–1 and –2) them. The downward distribution of the pathogen was verified from the +3 and –3 fragments onward, because the fall of the percentage of rachis fragments colonized was much more pronounced upward than downward the PI, with final values of >75 and 25% for the –6 and +6 fragments, respectively (Fig. 3).

The percentage of colonization of the rachis fragments by *F. graminearum* was significantly correlated with the severity of FHB symptoms ($r = 0.710$, $P < 0.01$) and, to a lesser extent, with the relative TKW ($r = –0.641$, $P < 0.05$).

In vivo and in vitro DON production. Isolates differed in their toxigenic potential (Table 2). Significant differences existed for in vivo DON production when the grains collected from the spikes tested in the field were analyzed by means of ELISA ($F = 5.91$, $P < 0.01$). Toxin production ranged from 4.5 ppm for the isolate MR18 to 31.3 ppm for isolate SP1. Significant differences were also found between isolates for in vitro DON production on sterile rice at 80% relative humidity ($F = 4.33$, $P < 0.01$). In this case, mycotoxin accumulation values ranged from 126 ppm for isolate MR25 to 373 ppm for isolate MR46 (Table 2).

A close correlation was found between the severity of the symptoms induced by the isolates in the field tests and in vivo DON production ($r = 0.907$, $P < 0.01$), as well as between pathogen colonization of the rachis and DON accumulation in the grains ($r = 0.808$, $P < 0.01$). In vitro DON production and severity, on the other hand, were not significantly correlated ($r = 0.313$), and neither were colonization of the rachis and DON production in rice substrate ($r = 0.483$) or toxin accumulation in vivo and in vitro ($r = 0.144$). The regression analysis showed a linear and positive relation between in vivo DON production and severity of FHB symptoms ($R^2 = 0.57$) (Fig. 4).

DISCUSSION

Differences in toxicity between DON and NIV (11,12) make the accurate determination of the chemotypes of *F. graminearum* found in different cropping areas of the world an important factor for the prediction of the risks related to the occurrence of mycotoxin contamination in food and feed.

In Argentina, controversy exists regarding the prevalence of *F. graminearum* chemotypes. Most of the work carried out with Argentinian isolates of the pathogen suggests that the predominant trichothecene is DON (4,10,34,53,55); nonetheless, potential for NIV contamination could be present (18,21,33).

Here, we analyzed 112 isolates of *F. graminearum* obtained from 28 localities of the Buenos Aires Province in Argentina (35) to determine their chemotype. All tested isolates were characterized as 15-ADON producers based on the amplification of specific fragments of two trichothecene core genes using multiplex PCR (58,63). Our finding agrees with previous reports on the prevalence of the DON chemotype in populations of the pathogen from wheat in Argentina (4,10,34,53,55). In maize, on the other hand, Sampietro et al. (57) reported an important presence of isolates belonging to the NIV chemotype, which the authors

TABLE 2. Severity of Fusarium head blight induced on field grown spikes of 'Klein Chajá' wheat, mycotoxin production, and expansion in the rachis of 14 isolates of *Fusarium graminearum* from Buenos Aires province, Argentina[z]

Isolate	Severity 21 dpi (%)	TKW (% of control)	DON in vivo (ppm)	DON in vitro (ppm)	Spike infection (%)
SP1	50 a	65 c	31.3 a	227.8 abc	86 a
LF2	35 b	70 abc	16.2 bc	221.9 abc	69 abcd
BA15	28 b	79 ab	19.1 ab	316.9 abc	83 ab
BA14	27 b	82 ab	21.2 ab	238.2 abc	86 a
LH11	26 b	75 abc	21.4 ab	366.7 a	78 abc
30A3	25 bc	91 ab	14.3 bc	328.3 abc	69 abcd
LF1	25 bc	86 ab	16.2 bc	316.9 abc	77 abc
MR46	23 bc	80 ab	14.8 bc	373.0 a	81 abc
LH7	22 bcd	84 ab	14.7 bc	311.0 abc	80 abc
GA1	14 bcd	90 ab	13.0 bc	140.6 bc	60 bcd
MR40	14 bcd	87 ab	11.9 bc	357.9 ab	72 abcd
MR25	11 bcd	92 ab	12.7 bc	126.5 c	71 abcd
MR4	10 cd	91 ab	9.4 bc	244.7 abc	57 cd
MR18	6 d	94 a	4.5 c	177.2 abc	48 d
Control	0 d	100 a	0.1 c	0.0 c	3 e

[z] TKW = thousand-kernel weight and DON = deoxynivalenol. Different letters correspond to significant differences between treatments ($\alpha = 0.05$).

attributed to potential differences in the preference for the different hosts. These results suggest that the toxigenic potential present in *F. graminearum* populations from Argentina is complex and that there is a chance for significant variation in the range of mycotoxins that could potentially contaminate food and feeds.

In a previous work, we reported the aggressiveness variation present in 112 isolates of *F. graminearum* obtained from 28 localities of Buenos Aires Province in Argentina following inoculation on field-grown wheat spikes (35). To check the representativeness of a subsample of 14 of these isolates, the same parameters previously analyzed for the complete collection of isolates were taken into consideration. The results obtained indicate that the findings previously reported are also valid for these 14 isolates, and that the subsample chosen is representative of the behavior shown by the complete collection (35).

The present work is the first attempt to analyze and relate both the capacity of *F. graminearum* isolates from Argentina to induce symptoms in field-grown wheat spikes and DON production under both in vitro and in vivo culture conditions. Isolates differed in their ability to produce mycotoxins both in the grains obtained from field-grown wheat spikes (in vivo) and under laboratory conditions on a rice substrate (in vitro). Under field conditions, the amount of DON produced surpassed, in all cases, the 2 ppm tolerance limits for commercialization imposed by the European Union standards (60). In vitro DON production was several-fold greater than in vivo production for all isolates but no correlation was found between these two parameters. Similar results were obtained by Walker et al. (61) when testing DON production under field and laboratory conditions. These results suggest that the potential for mycotoxin production shown by *F. graminearum* under controlled conditions has no correlation with its capacity to produce them in planta.

Unlike previous results obtained with *F. graminearum* isolates from Argentina (5), a close correlation between the severity of the symptoms induced by the isolates in field tests and in vivo DON production is reported here. Furthermore, the regression analysis performed showed a positive linear relation between the severity of FHB and DON accumulation in the grain. These results confirm that the trichothecene contamination of wheat grains is a consequence of the severity of FHB symptoms and agree with those obtained by Mesterházy et al. (40) and Mesterházy (39).

On the other hand, neither the aggressiveness of the isolates nor the relative TKW were correlated with in vitro toxin accumulation. Among the resistance mechanisms present in wheat genotypes toward FHB, types III (resistance to infection of the grain) and IV (tolerance to infection) could be related to the capacity of the host to degrade or tolerate DON (29). Recently, it has been demonstrated that some wheat lines possess the ability to detoxify DON by its glucosylation and that this ability is linked to the quantitative trait locus *Qfhs.ndsu-3BS* (31). The presence of these or other detoxification mechanisms in the host and their interaction with aggressiveness and toxigenic capacity of *F. graminearum* isolates could be responsible for the absence of a relationship between in vitro and in vivo DON production.

The colonization of the rachis by *F. graminearum* isolates in inoculated wheat spikes is a variable trait. For all the analyzed treatments and spikelet–rachis portion pairs, the percentage of colonization was higher than the visible FHB symptoms. Furthermore, the tendency observed in the distribution of FHB symptoms in the spike from the PI downward (35) was verified when the colonization of the rachis was considered. These results confirm

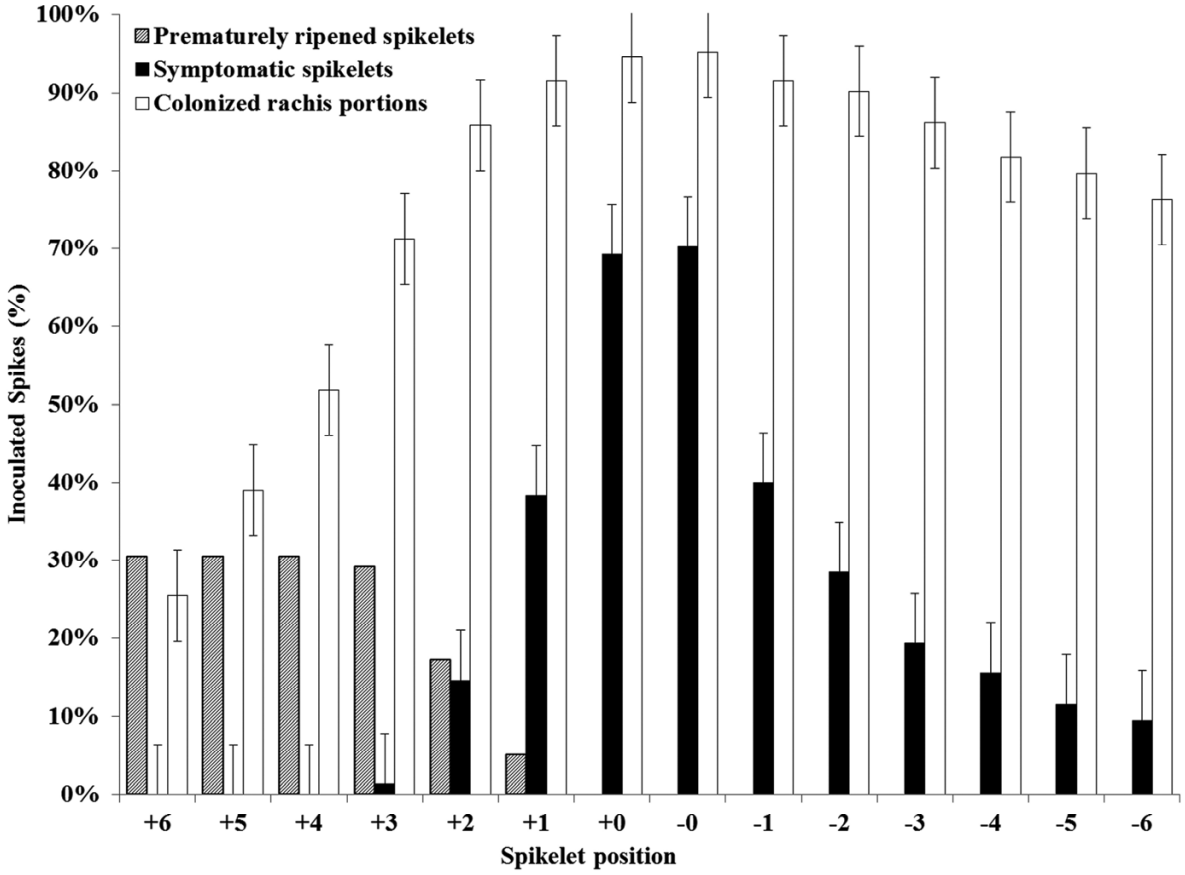

Fig. 2. Percentage of field-grown wheat spikes point-inoculated with each of 14 isolates of *Fusarium graminearum* showing symptoms of Fusarium head blight, symptoms of premature bleaching, and colonization of rachis fragments by the fungus above and below the point of inoculation (PI). Positive numbers represent the spikelets located above the PI while negative numbers indicate those located below it (i.e., +0 and –0 are the inoculated spikelets and +1 and –1 the spikelets immediately above and below them, respectively).

that the progress of *F. graminearum* invasion along the spike mainly follows a basipetal route along the vascular tissues and agree with recent reports (9). Differences in connection between the vascular bundle and the rachis of point-inoculated wheat spikelets above and below the PI have been suggested as the cause of the slower acropetal movement of the pathogen in the spike (9).

Rachis colonization was closely correlated with the severity of FHB symptoms and, to a lesser extent, with the relative TKW. Furthermore, colonization was highly correlated with the amount of DON present in wheat grains but not with DON production in vitro. These results agree with those reported by Gilbert et al. (20), who found no correlation between severity of FHB, fungal biomass, and in vitro DON production.

In >90% of the total evaluated spikes, the presence of the pathogen could be verified in the rachis portions that corresponded to the inoculated spikelets of the field-grown spikes. Such percentage exceeded the 70% incidence observed when visible symptoms of the disease were analyzed in the field (35). According to these results, in a high proportion of inoculated spikes, the simple presence of *F. graminearum* infecting the rachis was not sufficient for the development of FHB symptoms. In this regard, Brown et al. (9) found evidence of a macroscopically asymptomatic stage in wheat spikes where FHB symptoms remained confined to point-inoculated spikelets though, at the cellular level, internal infection by *F. graminearum* was clearly visible.

The colonization of the rachis of asymptomatic spikes by the pathogen could explain the fact that, for isolates that induced the expression of FHB symptoms in extremely low severity values, DON levels detected in the harvested grain were often higher than those tolerated by commercialization standards. These results imply that even isolates with a limited capacity of induction of visible symptoms of FHB and reduction of TKW may be able to

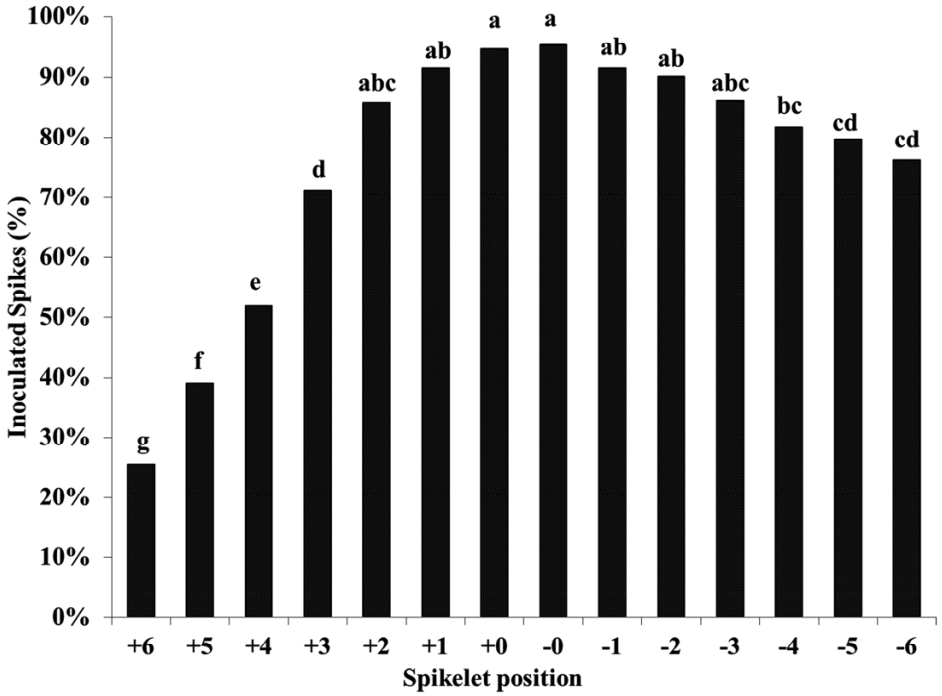

Fig. 3. Percentage of field-grown wheat spikes point inoculated with 14 isolates of *Fusarium graminearum* with rachis portions above and below the point of inoculation (PI) showing colonization by the fungus. Positive numbers represent the spikelets located above the PI while negative numbers indicate those located below it (i.e., +0 and –0 are the inoculated spikelets and +1 and –1 the spikelets immediately above and below them, respectively). Different letters indicate the existence of significant differences between treatments ($\alpha = 0.05$).

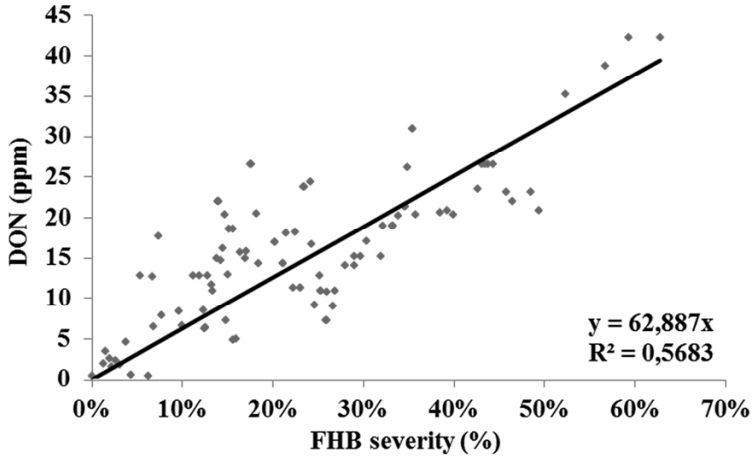

Fig. 4. Regression analysis between the severity of symptoms of Fusarium head blight (FHB) induced by 14 isolates of *Fusarium graminearum* from Argentina point inoculated on field-grown wheat spikes and deoxynivalenol (DON) concentration in the resulting wheat kernels.

colonize the rachis of wheat spikes, producing detectable amounts of DON.

The search for wheat genotypes tolerant or resistant to FHB, which could aid in lowering the impact of mycotoxin contamination of food and feed, constitutes a fundamental objective for breeders. The results obtained in our work allow hypothesizing that limiting the exposition of wheat genotypes to DON as well as increasing the tolerance of the plant to trichothecenes could reduce the development of *F. graminearum* and, as a consequence, diminish the severity of FHB attacks. Recently, we reported that the presence of high levels of variability among the populations of *F. graminearum* prompted the need to include mixtures of isolates when screening for disease-resistant genotypes (35). According to the results reported in the present work, the fact that even isolates with limited aggressiveness present an important risk of DON contamination emphasizes the need to use combinations of isolates that reflect not only the variable levels of aggressiveness but also the variability in toxigenic potential present in the populations of the pathogen.

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