Fusarium species and fumonisins associated with maize kernels produced in Rio Grande do Sul State for the 2008/09 and 2009/10 growing seasons

R. Stumpf¹, J. dos Santos¹, L.B. Gomes¹, C.N. Silva², D.J. Tessmann², F.D. Ferreira³, M. Machinski Junior³, E.M. Del Ponte¹

¹Departamento de Fitossanidade, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.
²Departamento de Agronomia, Universidade Estadual de Maringá, Maringá, PR, Brazil.
³Departamento de Ciências Básicas da Saúde, Universidade Estadual de Maringá, Maringá, PR, Brazil.

Submitted: August 2, 2011; Approved: July 2, 2012.

Abstract

Ear rots caused by Fusarium spp. are among the main fungal diseases that contribute to poor quality and the contamination of maize grains with mycotoxins. This study aimed to determine the visual incidence of fungal-damaged kernels (FDKs), the incidence of two main Gibberella (a teleomorph of Fusarium) complexes (G. fujikuroi and G. zeae) associated with maize using a seed health blotter test, and the fumonisin levels, using high performance liquid chromatography, in samples of maize grains grown across 23 municipalities during the 2008/09 and 2009/10 growing seasons. Additionally, 104 strains that were representative of all of the analysed samples were identified to species using PCR assays. The mean FDK was seven per cent, and only six of the samples had levels greater than six per cent. Fusarium spp. of the G. fujikuroi complex were present in 96% of the samples, and G. zeae was present in 18% of the samples (5/27). The mean incidence of G. fujikuroi was 58%, and the incidence of G. zeae varied from 2 to 6%. FB₁ was found in 58.6%, FB₂ in 37.9%, and both toxins in 37.9% of the samples. The FB₁ and FB₂ levels were below the quantification limits for 41.3% of the samples, and the mean FB₁ levels (0.66 μg/g) were higher than the mean FB₂ levels (0.42 μg/g). The PCR identification separated the 104 isolates into three of the G. fujikuroi complex: F. verticillioides (76%), F. subglutinans (4%) and F. proliferatum (2%); and G. zeae (anamorph = F. graminearum) (18%). Our results confirmed the dominance of F. verticillioides, similar to other regions of Brazil, but they differed due to the relatively higher incidence of F. graminearum. Total fumonisin levels were below the maximum limit determined by current Brazilian regulations.

Key words: Fusarium graminearum, Fusarium verticillioides, fumonisins, Zea mays L.

Introduction

In Brazil, maize (Zea mays L.) is grown under a diverse range of climate and cropping conditions. In the southernmost subtropical climate, maize is a typical summer crop, succeeding small-grain cereal crops. Several diseases potentially limit maize yields, such as those affecting leaves, stalks and ears, and are caused by several pathogenic fungi (White, 1999; Casa and Reis, 2003). Ear rots caused by various fungi, including Fusarium species, contribute to poor grain quality and contaminate grains with mycotoxins, which represent a threat to both human and animal health (Logrieco et al., 2002; Munkvold, 2003).

Two Fusarium-induced ear rots are commonly found in Brazil, eventually in association: Fusarium ear rot (FER) caused by species within the Gibberella fujikuroi complex, especially its anamorphic species, F. verticillioides, F. subglutinans and F. proliferatum, and Gibberella ear rot (GER) caused by Gibberella zeae, the teleomorphic stage of the Fusarium graminearum species complex (Casa and Reis, 2003; Reis et al., 2004).
Climatic conditions and crop management practices, such as crop rotation, tillage, planting date and fertilisation, influence the occurrence and prevalence of the *Fusarium* species that affect maize (Munkvold, 2003). In some regions where both species are present, GER epidemics are most commonly found during wet years, whereas FER epidemics tend to occur during dry years (Doohan et al., 2003). In Europe, *F. verticillioides* is more prevalent in the southern regions and is found associated with maize grain and by-products in France, Spain and Italy (Bottalico, 1998). In Belgium, however, *F. graminearum* is the most prevalent species associated with *Fusarium*-induced ear rots (Scauflaire et al., 2011); in New Zealand, *F. graminearum* is also the most dominant species among several others found to infect kernels, whereas *F. verticillioides* is rarely found (Hussein et al., 2002). In Africa, several reports indicate *F. verticillioides* as the most prevalent fungus on maize (Fandohan et al., 2003).

In Brazil, empirical evidence shows that *F. verticillioides* is the most prevalent species causing FER in the central-western, tropical maize production regions (Almeida et al., 2002), whereas *F. graminearum* is more commonly found in the southernmost regions of the country, although it is second in prevalence. In the southernmost region, a combination of a wet subtropical environment and a rotational system that includes small grains, such as wheat, barley and oats, favour inoculum accumulation and epidemics of GER and stalk rots caused by *F. graminearum* (Casa and Reis, 2003; Reis et al., 2004).

Surveys for mycotoxins in Brazilian maize grain are sporadic, and most studies have focused on the detection of fumonisins, a group of mycotoxins produced by *F. verticillioides* and other related species of the *G. fujikuroi* complex that affects maize, such as *F. subglutinans* (Marasas, 2001). In a large spatial-scale survey and analysis of 214 maize grain samples from several locations in Brazil, 99% of the samples were contaminated with fumonisin B1 (FB1) in levels ranging between 0.2 and 6.1 μg/g (Vargas et al., 2001). More recently, trichothecene mycotoxins, especially deoxinivalenol (DON) and nivalenol (NIV), a class of mycotoxins mainly produced by members of the *F. graminearum* species complex, have been found in 16 and two samples, respectively, out of 80 samples of Brazilian maize grains (Milanez and Valente-Soares, 2006).

Because the type of mycotoxins found in grain is dependent on the toxigenic profile of the pathogenic populations in the field, knowledge on the prevailing species and mycotoxin levels may help to develop regional strategies aimed at preventing both the ear rot and stalk rot caused by the local populations. Additionally, the promulgation of maximum tolerance levels of fumonisins in Brazilian maize grain and by-products established in 2011 reinforces the need to increase vigilance and define strategies to prevent mycotoxin contamination based on the regional status (Anvisa, 2011).

Therefore, the main objective of this study was to determine the post-harvest incidence of two *Fusarium* species complexes, the fumonisins levels and the incidence of visibly fungal-damaged maize kernels in a sample of maize grains from the major production regions of Rio Grande do Sul State, Brazil, obtained during two consecutive growing seasons across several locations. Additionally, *Fusarium* strains representative of the groups found in all of the grain samples were identified to the species level using PCR assays.

**Materials and Methods**

**Study area and sampling**

A total of 29 maize grain samples were obtained from one to two months after the harvest of maize crops in experimental areas or fields located across 23 municipalities in Rio Grande do Sul State, Brazil. The majority of the municipalities were located in the northern production regions of the state (Figure 1). Sixteen maize samples were from the 2008/09 growing season and thirteen from the 2009/10 season. The grain samples were collected by collaborators in both experimental and commercial fields, and information, such as the hybrid and cropping practices, were not available for most of the samples.

**Incidence of Fusarium and visibly fungal-damaged kernels**

A subsample of 100 kernels, randomly taken from a 500 g field grain sample, was assessed in the laboratory for symptoms and signs of fungal infection, especially shriveled and discoloured kernels. Each kernel was visually inspected to determine whether it had any symptom or sign of fungal infection. The incidence of visibly fungal-damaged kernels was expressed as a percentage.
The incidence of *Fusarium* infection was determined using an adapted protocol of a standard freezing-blotter seed health test (Machado and Langerak, 2002). A subsample of 200 kernels was surface sterilised (1% sodium hypochlorite for 1 min) and rinsed twice in sterile, distilled water (30 seconds). The kernels were plated (25 per recipient) equidistantly on moist blotters and incubated at 25 °C for 10 days. Thereafter, each kernel was inspected with the aid of a stereomicroscope (40x magnification), and the percentage of kernels showing colonies resembling species belonging to either the *Gibberella fujikuroi* or *Gibberella zeae* species complex was determined. All of the ratings were performed by an experienced rater, and, whenever needed, slides of the microscopic structures were prepared to aid the classification to one of the two species complexes.

**Determination of fumonisins by high-performance liquid chromatography (HPLC)**

Fumonisins B₁ (FB₁) and fumonisins B₂ (FB₂) were determined using an established protocol (Camargos et al., 1999), with modifications. The fumonisins standards were obtained from Sigma Chemical Co. (St. Louis, MO, USA). The working standard solutions were prepared in acetonitrile-water (1:1), with concentrations of 5 μg/mL each for FB₁ and FB₂. An aliquot of each sample (50 g) was extracted with 100 mL of methanol-water (3:1) for 5 min in a blender (Waring Co., Torrington, USA). After centrifugation and filtering, the pH of the filtrate was adjusted to 5.8-6.5 with 0.1 N HCl or 0.1 N NaOH. A strong anion exchange cartridge (500 mg, Sep-Pak, Waters), was used to purify the sample, previously conditioned with 6 mL of methanol (Honeywell Burdick & Jackson, Muskegon, USA) and 10 mL of methanol-water (3:1). An aliquot (10 mL) of the sample extract was applied to the cartridge, followed by 10 mL of methanol-water (3:1) and 6 mL of methanol. The FB₁ and FB₂ peaks were eluted with 20 mL of methanol-acetic acid (95:5). The eluate was dried in a sample concentrator at 60 °C (Tecnal, Piracicaba, Brazil) and kept at -20 °C until analysis. The dried extract of methanol-acetic acid (95:5) was dissolved in 1000 μL of acetonitrile-water (1:1). The chromatographic system used was the Finnigan Surveyor Plus model (Thermo Scientific®, San Jose, USA), and post-column derivatisation was performed by Vector PCX (Pickering Laboratories®, Mountain View, USA) with fluorescence detection using a Finnigan Surveyor FL Plus Detector (Thermo Scientific®, San Jose, USA). The eluate (100 μL) was injected in the chromatograph under the following conditions: a mobile phase of methanol-sodium phosphate buffer, pH 3.35 (70:30); a flow rate of 0.5 mL/min; an excitation wavelength of 330 nm and an emission at 465 nm; a C18 Spherisorb® column of 5 μm (250 x 4.6 mm, Waters®, Wexford, Ireland); a total run time of 30 min; and integration by ChromQuest 5.0 Chromatography Data System (Thermo Fisher Scientific®).

The post-column reagent pump flow rate was set at 0.15 mL/min and the reactor temperature at 65 °C. The OPA reagent (Pickering Laboratories®, Mountain View, USA) was used for the derivatisation of the fumonisins. The average recovery and the variation coefficients for FB₁ and FB₂ were 84.6% and 8.1% and 102.9% and 6.2%, respectively. The limit of quantification was 0.078 and 0.043 μg/g for FB₁ and FB₂, respectively.

**Fungal isolation, purification and DNA extraction**

During the assessments of the seed health test, four *Fusarium* isolates from each of the 29 samples were obtained. Each isolate was randomly selected from an individual recipient, and the selection of the colony accounted for the morphological differences, such that the four isolates could represent potentially different groups at the same proportion found in each grain sample. A fragment of mycelia from the selected *Fusarium* colony was transferred to malt agar media (2%) and incubated in a growth chamber (24 ± 1 °C under dark conditions) for seven days. The isolates were purified through monosporic culturing, transferred to SNA medium (Leslie and Summerell, 2006) and stored in microtubes (1.5 mL) under cold conditions (4 °C). Mycelia were produced in liquid potato-dextrose media amended with 50 mg/L of streptomycin sulphate (Sigma-Aldrich®) under shaking conditions for ten days and at ± 25 °C. The DNA was extracted using a modified 2% CTAB (Hexadecyl trimethyl-ammonium bromide) DNA extraction protocol (Doyle and Doyle, 1987) and was stored at -20 °C until analysis.

**PCR-based species identification**

All of the selected and purified isolates were identified to the species level using polymerase chain reaction (PCR) assays found in the literature using primer sets (VER1, VER2, PRO1, PRO2, SUB1 AND SUB2) for the differentiation of three species of the *Fusarium* complex, *F. verticillioides* and *F. proliferatum* (Mulé et al., 2004) and a primer set for *Gibberella zeae* (*Fusarium graminearum* species complex), Fg16F/R (Nicholson et al., 1998). The PCR assays were conducted using 20-30 ng of fungal DNA in a total volume of 25 μL containing 1.5 mM MgCl₂, 2 U Taq DNA polymerase, 20 μM dNTPs, and 1 μM of each primer. The PCR products were separated by gel electrophoresis, stained with SYBR® Safe DNA gel and visualised under UV light.

**Results and Discussion**

Visibly fungal-damaged kernels (FDKs) were found in 16 out of the 29 samples. The mean FDK incidence was seven per cent, and six samples had levels greater than six per cent, which is considered the maximum tolerated level for the corn trade in Brazil (Pinto et al., 2007); three samples had incidence levels > 25%. This result parallels previ-
ous reports in the country in which the differences in the FDK levels were attributed to the cultivars or incidence of fungal species other than *Fusarium* (Pinto et al., 2007).

All but one (Santa Maria, 2009/10) of the maize samples was infected with one or two species of the *Gibberella* complexes (Table 1). The two species complexes co-occurred in the samples, but a distinct frequency of these two complexes was found: *G. fujikuroi* was present in 96% of the samples and *G. zeae* in 18% (5/27 samples). Overall, the mean incidence of *G. fujikuroi* was 58% (51% in 2008/09 and 67% in 2009/10), and the mean incidence of *G. zeae* varied from 2 to 6% and was mostly limited to samples from the 2008/09 growing season (Table 2). With regards to the presence of fumonisins, 58.6% of the samples were contaminated with FB1, 37.9% with FB2, and 37.9% with both fumonisins (Table 1). The FB1 and FB2 levels were below the detection limit for 41.3% of the samples. The overall mean FB1 level (0.66 μg/g) was higher than the mean FB2 levels (0.42 μg/g).

Compared to previous studies, the fumonisin levels were, for the majority of the samples, lower than those reported in 109 maize grain samples from the state of Paraná, Brazil (Ono et al., 2006). In that study, FB1 and FB2 were

| Year       | Location                  | FDK (%) | GF (%) | GZ (%) | FB1 (μg/g) | FB2 (μg/g) |
|------------|---------------------------|---------|--------|--------|------------|------------|
| 2008/09    | Boa Vista das Missões      | 13      | 36     | 0      | 1.04       | 0.81       |
|            | Boa Vista das Missões      | 4       | 39     | 0      | 1.63       | 0.71       |
|            | Casca                     | 0       | 58     | 0      | 0.29       | 0.33       |
|            | Caxias do Sul             | 0       | 44     | 0      | 0.23       | ND         |
|            | Coixilha                  | 61      | 39     | 3      | 1.18       | ND         |
|            | Júlio de Castilhos        | 5       | 29     | 0      | ND         | ND         |
|            | Lagoa Vermelha            | 0       | 73     | 0      | ND         | ND         |
|            | Passo Fundo               | 5       | 71     | 2      | ND         | ND         |
|            | Passo Fundo               | 0       | 9      | 0      | 0.73       | ND         |
|            | Portão                    | 3       | 64     | 6      | 0.30       | 0.18       |
|            | Santa Maria               | 29      | 0      | 0      | ND         | ND         |
|            | São João da Urtiga        | 1       | 52     | 0      | ND         | ND         |
|            | Vacaria                   | 7       | 69     | 0      | ND         | ND         |
|            | Vacaria                   | 4       | 87     | 0      | ND         | ND         |
|            | Vacaria                   | 0       | 96     | 4      | ND         | ND         |
|            | Vacaria                   | 0       | NA     | NA     | ND         | ND         |
| Year mean  |                           | 8.25    | 51.0   | 1      | 0.77       | 0.51       |
| 2009/10    | Boa Vista do Cadeado       | 0       | 61     | 0      | ND         | ND         |
|            | Boa Vista do Cadeado       | 0       | 100    | 0      | ND         | ND         |
|            | Casca                     | 15      | 79     | 4      | 0.75       | 0.16       |
|            | Casca                     | 0       | NA     | NA     | 0.35       | 0.15       |
|            | Cruz Alta                 | 2       | 66     | 0      | ND         | ND         |
|            | Eldorado do Sul           | 4       | 94     | 0      | 0.10       | ND         |
|            | Encantado                 | 4       | 78     | 0      | 2.03       | 0.81       |
|            | Encantado                 | 0       | 75     | 0      | 0.32       | 0.27       |
|            | Machadinho                | 0       | 28     | 0      | 0.60       | 0.13       |
|            | Santa Bárbara             | 41      | 70     | 0      | 0.29       | 0.21       |
|            | Três Passos               | 5       | 37     | 0      | 1.01       | 0.84       |
|            | Tupanciretã               | 0       | 20     | 0      | 0.07       | ND         |
|            | Veranópolis               | 0       | 97     | 0      | 0.09       | ND         |
| Year mean  |                           | 5.46    | 67.08  | 0.33   | 0.56       | 0.37       |
| Total mean |                           | 7       | 58.1   | 0.70   | 0.66       | 0.42       |

NA = Data not available; ND = Values lower than the quantification limit (FB1: -0.078 μg.g⁻¹ e FB2: -0.043 μg.g⁻¹).
determined separately for symptomatic and asymptomatic kernels, and the mycotoxin levels varied between 0.57 to 20.38 μg/g for the asymptomatic kernels and from 68.98 to 336.38 μg/g for the symptomatic kernels. In our study, FB₁ and FB₂ were also detected in asymptomatic samples, which is also in agreement with a previous report (Ottoni, 2008) in which relatively low fumonisin levels were found, varying from non-detected to 2.1 μg/g in 15 asymptomatic maize grain samples from the Brazilian states of Minas Gerais, Goiás, Mato Grosso, Mato Grosso do Sul and Pará.

In our study, the three samples showing extreme FDK values did not show the highest fungal incidence or fumonisin levels. Conversely, a relatively high incidence of *Fusarium* species of the *G. fujikuroi* complex was found in the thirteen samples with 100% of the asymptomatic kernels. In fact, it is well known that *F. verticilloides* is able to colonise the plant systemically and invade grains without causing symptoms, which can explain the lack of correlation between symptoms and *Fusarium* incidence or mycotoxin levels (Munkvold, 2003).

Differences in the mycotoxin levels across studies are expected, even for the same region, as they are under the influence of many biological, abiotic and agronomic factors, such as the toxigenic potential of the prevailing fungal species, host genetics, management practices and environmental conditions (Munkvold, 2003). Hence, continuous surveillance is appropriate, especially when linking analytical and epidemiological survey data aiming to understand risk factors related to mycotoxin contamination. In our study, the dominance of *F. verticilloides* over the other species confirms previous survey studies with maize grain from other regions of Brazil (Ono et al., 1999; Buiate et al., 2008). The presence of *F. graminearum sensu lato* at relatively lower prevalence and incidence levels in maize kernels is in agreement with a previous study in the Rio Grande do Sul State, where it was found to be the second species in prevalence in a monoculture vs. crop rotation study (Treto et al., 2011). Collectively, these results suggest that maize kernels from the southernmost region of Brazil may also be contaminated with trichothecene mycotoxins because of the presence of *F. graminearum sensu lato*, as has been previously shown in a few maize samples from other Brazilian regions (Milanez and Valente-Soares, 2006).

Of the 104 monosporic isolates obtained from grains representative of all of the maize samples, the PCR-based identification showed the presence of four anamorphic species of the two Gibberella complexes: *F. verticilloides* was the dominant species (76% of the isolates), followed by *F. graminearum* (18%), *F. subglutinans* (4%) and *F. proliferatum* (2%) (Table 2). Molecular tools have been used to identify *Fusarium* species from maize in Brazil only very recently (Ottoni, 2008; Querales, 2010), and their use can be advantageous given the accuracy of the results, especially for the differentiation of species of the *G. fujikuroi*, which demands several steps and experience in the identification based on morphological traits. Our findings of the three anamorphic species within the *G. fujikuroi* complex, especially the presence of *F. proliferatum* and *F. subglutinans* in relatively low prevalence (< 5%), is in agreement with previous studies conducted in Brazil using PCR assays. Ottoni (2008) has analysed 197 isolates from maize grains obtained in several growing regions of Brazil and demonstrated the prevalence of *F. verticilloides* (82%) and *F. subglutinans* (3%); none of the isolates were identified as *F. proliferatum*. Moreover, using the same protocol as Ottoni (2008), Querales (2010) analysed 100 isolates from maize kernels produced in the states of São Paulo, Minas Gerais, Bahia, Paraná, Rio Grande do Sul and Mato Grosso do Sul during 2001 to 2006 and identified *F. verticilloides* (93% of the isolates), *F. proliferatum* (4%) and *F. subglutinans* (3%).

Our results add to the current knowledge and confirm the dominance of *F. verticilloides* within the species of the *G. fujikuroi* complex, across the production regions of Rio Grande do Sul State, in prevalence levels similar to those observed in other regions of Brazil. Additionally, we found *F. graminearum sensu lato* to be the second in prevalence in the PCR-based identification. All of the isolates taken from the *G. zeae*-like colonies were identified as *F. graminearum sensu lato* using the Fg16F/R primer set, which demonstrated that the separation of the two Gibberella/Fusarium complexes based on morphology was accurate during the evaluation of the seed health test by an experienced rater. Conversely, the distinction of the three *Fusarium* species of the *G. fujikuroi* complex was not accurate based on the colony morphology, when compared to the results of the PCR assays (data not shown).

As found in this study, the higher prevalence of *G. zeae* in Southern Brazil compared to other regions of Brazil may be due to cooler climatic conditions and the year-round presence of inocula from epidemics caused by *F. graminearum* in small-grained cereals, mainly wheat and barley, and the survival of the sexual stage of the fungus (perithecia) in crop residues. This pattern parallels the situation found in Argentina, where species of the *G. fujikuroi*, mainly *F. verticilloides*, and *F. graminearum* determined using PCR assays, associated with samples of maize kernels from 23 municipalities in Rio Grande do Sul State, Brazil, 2008/09 and 2009/10 growing seasons.

Table 2 - Number (and total %) of isolates for each *Fusarium* species and growing season, determined using PCR assays, associated with samples of maize kernels from 23 municipalities in Rio Grande do Sul State, Brazil, 2008/09 and 2009/10 growing seasons.

| Fusarium species                                      | 2008/09 | 2009/10 | Total    |
|-------------------------------------------------------|---------|---------|----------|
| *Fusarium verticilloides*                             | 41      | 38      | 79 (75%) |
| *Fusarium subglutinans*                               | 4       | 0       | 4 (4%)   |
| *Fusarium proliferatum*                               | 0       | 2       | 2 (2%)   |
| *Fusarium graminearum sensu lato*                     | 15      | 4       | 19 (19%) |
| Total                                                 | 60 (57%)| 44 (43%)| 104 (100%)|
sensu lato, have been frequently isolated from maize kernels, especially in the northwestern region of that country for the latter species (Chulze et al., 1996; Sampietro et al., 2011).

A higher diversity of *Fusarium* species was found associated with maize kernels produced in the southernmost maize-growing regions of Brazil compared to previous reports from other northern regions. Thus, a range of *Fusarium* mycotoxins other than fumonisins can be produced by the regional populations, especially the trichothecenes, deoxynivalenol and nivalenol, which are produced by the *F. graminearum sensu lato* populations that affect wheat and barley grown in the same region (Scoz et al., 2009; Astolfi et al., 2011). The fumonisins in our study were found at levels that are considered safe for consumption because none presented a level greater than the maximum limit (FB$_1$ + FB$_2$) of 5 µg/g determined by the current Brazilian regulation for mycotoxins (Anvisa, 2011). Future studies shall be directed at elucidating the toxigenic potential of the representatives of these populations and the analysis of the presence of other *Fusarium* mycotoxins as an important step towards the definition of strategies to prevent and minimise the risk of maize contamination in Southern Brazil.

Acknowledgments

This work was supported by the following grants from the National Council for Scientific and Technological Development (CNPq): MAPA/SDA 064/2008, a postdoctoral fellowship to the second author, and a research fellow for the fifth and the corresponding author. This paper was part of M.Sc. thesis work presented by the first author to the Programa de Pós-graduação em Fitotecnia, Universidade Federal do Rio Grande do Sul funded by grants offered by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

References

Almeida AP, Fonseca H, Fancelli AL, Direito GM, Ortega EM, Corrêa B (2002) Mycoflora and fumonisin contamination in Brazilian corn from sowing to harvest. J Agric Food Chem 50:3877-3882.

Anvisa (2011) Resolução RDC 7. Dispõe sobre limites máximos tolerados (LMT) para micotoxinas em alimentos. Available at http://www.brasilsus.com.br/legislacoes/anvisa/107378-7.html. Accessed February 26, 2011.

Astolfi P, Santos J, Schneider L, Gomes LB, Silva CN, Tessmann DJ, Del Ponte EM (2011) Molecular survey of trichothecene genotypes of *Fusarium graminearum* species complex from barley in Southern Brazil. Int J Food Microbiol 148:197-201.

Bottalico A (1998) *Fusarium* diseases of cereals: Species complex and related mycotoxin profiles, in Europe. J Plant Pathol 80:85-103.

Buiate EAS, Brito CH, Batistella RA, Brandão AM (2008) Reação de híbridos de milho e levantamento dos principais fungos associados ao complexo de patógenos causadores de “grão ardido” em Minas Gerais. Horizonte Científico, 2(1). http://www.seer.ufu.br/index.php/horizontecientifico/issue/view/309. Accessed 21 Jul 2011.

Camargos SM, Machinski Jr M, Valente Soares LM (1999) Avaliação de métodos para determinação de fumonisinas B1 e B2 em milho. Rev Inst Adolfo Lutz 58:71-79.

Casa RT, Reis EM (2003) Doencas na cultura do milho. In: Fancelli AL, Dourado Neto D (eds) Milho: Estratégias de Manejo e Alta Produtividade. Escola Superior da Agricultura “Luiz de Queiroz”, Piracicaba, pp 1-18.

Chulze SN, Ramirez ML, Farmochi MC, Pascale M, Visconti A, March G (1996) *Fusarium* and fumonisin occurrence in Argentinean corn at different ear maturity stages. J Agric Food Chem 44:2797-2801.

Doohan FM, Brennan J, Cooke BM (2003) Influence of climatic factors on *Fusarium* species pathogenic to cereals. Eur J Plant Pathol 109:755-768.

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19:11-15.

Fandohan P, Hell K, Marasas WFO, Wingfield MJ (2003) Infection of maize by *Fusarium* species and contamination with fumonisin in Africa. Afr J Biotechnol 2:570-579.

Hussein HM, Christensen MJ, Baxter M (2002) Occurrence and distribution of *Fusarium* species in maize fields in New Zealand. Mycopathologia 156:25-30.

Leslie JF, Summerell BA (2006) The *Fusarium* Laboratory Manual. Blackwell Professional, Ames, 388 pp.

Logrieco A, Mulé G, Moretti A, Bottalico A (2002) Toxigenic *Fusarium* species and mycotoxins associated with maize ear rot in Europe. Eur J Plant Pathol 108:597-609.

Machado JC, Langerak CJ (2002) General incubation methods for routine seed health analysis. In: Machado JC, Langerak CJ, Jaccound-Filho DS (eds) Seed-Borne Fungi: A Contribution to Routine Seed Health Analysis. International Seed Testing association, Bassersdorf, pp 48-59.

Marasas WFO (2001) Discovery and occurrence of the fumonisins: A historical perspective. Environ Health Perspect 109:239-243.

Milanetz TV, Valente-Soares LMB (2006) Gas Chromatography - mass spectrometry determination of trichothecene mycotoxins commercial corn harvested in the state of São Paulo, Brazil. J Braz Chem Soc 17:412-416.

Mulé G, Susca A, Stea G, Moretti A (2004) Species-specific PCR assay based on the calmodulin partial gene for identification of *Fusarium verticillioides*, *F. proliferatum* and *F. subglutinans*. Eur J Plant Pathol 110:495-502.

Munkvold GP (2003) Epidemiology of *Fusarium* diseases and their mycotoxins in maize ears. Eur J Plant Pathol 109:705-713.

Nicholson PP, Simpson DR, Weston G, Rezanoor HN, Lees AK, Parry DW, Joyce D (1998) Detection and quantification of *Fusarium culmorum* and *Fusarium graminearum* in cereals using PCR assays. Physiol Mol Plant Pathol 53:17-37.

Ono EYS, Sugiura Y, Homechin M, Kamogae M, Vizzoni É, Ueno Y, Hirooka EY (1999) Effect of climatic conditions on natural mycoflora and fumonisins in freshly harvested corn of the State of Paraná, Brazil. Mycopathologia 147:139-148.
Ono EYS, Biazon L, Silva M, Vizzoni É, Sugiura Y, Ueno Y, Hirooka EY (2006) Fumonisins in Corn: Correlation with Fusarium sp. count, damaged kernels, protein and lipid content. Braz Arch Biol Technol 49:63-71.

Ottoni RJ (2008) Análise da Incidência de Fusarium spp. Toxicógeno e de Níveis de Fumonisinas em Grãos Ardidos de Milho Híbrido. M.Sc. Dissertation, Escola Superior de Agricultura “Luiz de Queiroz”, Piracicaba, 54 pp.

Pinto NFJA, Vargas EA, Preis RA (2007) Qualidade sanitária e produção de fumonisina B1 em grãos de milho na fase de pré-colheita. Summa Phytopathol 33:304-306.

Querales P (2010) Caracterização Morfológica e Genética de Fusarium spp. Isolados de Sementes e Associados à Podridão do Colmo do Milho (Zea mays L.). Ph.D. Thesis, Escola Superior de Agricultura “Luiz de Queiroz”, Piracicaba, 77 pp.

Reis EM, Casa RT, Bresolin ACR (2004) Manual de Diagnose e Controle de Doenças do Milho. Graphel, Lages, 144 pp.

Sampietro DA, Díaz CG, Gonzalez V, Vattuone MA, Ploper LD, Catalan CAN, Ward TJ (2011) Species diversity and toxigenic potential of Fusarium graminearum complex isolates from maize fields in northwest Argentina. Int J Food Microbiol 145:359-64.

Scauflaire J, Mahieu O, Louvieaux J, Foucart G, Renard F, Munaut F (2011) Biodiversity of Fusarium species in ears and stalks of maize plants in Belgium. Eur J Plant Pathol 131:59-66.

Scoz LB, Astolfi P, Reartes DS, Schmale III DG, Moraes MG, Del Ponte EM (2009) Trichothecene mycotoxin genotypes of Fusarium graminearum sensu stricto and Fusarium meridionale in wheat from southern Brazil. Plant Pathol 58:344-351.

Trento SM, Irgang HH, Reis EM (2002) Efeito da rotação de culturas, da monocultura, e da densidade de plantas na incidência de grãos ardidos em milho. Fitopatol Bras 27:609-613.

Vargas EA, Castro PL, Silva CMG (2001) Co-occurrence of aflatoxins B1, B2, G1, G2, zearalenona and fumonisin B1 in Brazilian corn. Food Addit Contam 18:981-986.

White DG (ed) (1999) Compendium of Corn Diseases. 3.edition. APS Press, St Paul, 128 pp.

All the content of the journal, except where otherwise noted, is licensed under a Creative Commons License CC BY-NC.