Analytical Methods

Deoxynivalenol and nivalenol in commercial wheat grain related to Fusarium head blight epidemics in southern Brazil

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Abstract

A three-year (2006–2008) survey on commercial wheat grain was conducted aimed at quantifying the intensity of Fusarium head blight epidemics related to kernel quality and levels of deoxynivalenol (DON) and nivalenol (NIV). Grain samples, obtained from 38 municipalities throughout the state of Rio Grande do Sul, Brazil, were assessed visually for Fusarium-damaged kernels (FDK) and chemically using liquid chromatography–mass spectrometry (LC–MS/MS). Overall FDK mean levels were 15.5%, not differing among the years. Co-contamination was predominant (59/66) across samples and overall mean levels of DON and NIV were 540 and 337 µg/kg, respectively. When the levels of both mycotoxins were added together (DON + NIV), a higher correlation with FDK was found (R = 0.36, P < 0.01), compared to single toxin data. For the first time, the presence of NIV in levels comparable to DON is reported from a multi-year regional epidemiological survey in the country which should be of concern to the small grains industry.

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1. Introduction

Fusarium head blight (FHB) is a fungal disease of increasing significance for small-grain crops worldwide (McMullen, Jones, & Gallemberg, 1997). It is mainly caused by members of the Fusarium graminearum species complex (Fg complex) (teleomorph: Gibberella zeae) (Goswami & Kistler, 2004). FHB inoculum survives in crop debris and infects wheat crops from flowering to grain filling stages when weather conditions are favourable (McMullen et al., 1997). Even though yield losses are associated with reduced kernel plumpness and weight, the fungus produces mycotoxins that may accumulate to unacceptable levels, making harvested grain and their by-products unsuitable for human and animal consumption (Creppy, 2002). Current integrated management practices include crop rotations, resistant wheat varieties and fungicide applications that help to prevent and/or reduce fungal infection and subsequent mycotoxin production (Edwards, 2004).

Increasing awareness of Fusarium mycotoxins, especially those from the trichothecene group, such as deoxynivalenol (DON), occurred in recent years with the resurgence and consideration of FHB as a major threat to food security (Goswami & Kistler, 2004; van Egmond, Schotborst, & Jonker, 2007). The World Health Organization (WHO) regards DON as teratogen, neurotoxin, and immunosuppressant and trichothecenes in general have been associated with chronic and fatal intoxication of humans and animals through consumption of contaminated food and feed (Rotter, Prelusky, & Pestka, 1996). Hence, urgent measures such as continuous monitoring and regulation of maximum mycotoxin levels in food products and commodities have been set in several countries (van Egmond et al., 2007).

Surveys on Fusarium mycotoxins in small-grain cereals and their by-products are frequently conducted in the major production regions of the world such as North America and Europe (Creppy, 2002; van Egmond et al., 2007). Conversely, information in South America is relatively scarce and previous evidence had placed DON as the main Fusarium toxin detected in wheat and by-products in Argentina (Dalcer, Torres, Etcheverry, Chulze, & Varsavsky, 1997; Lori, Sisterna, Haidukowski, & Rizzo, 2003) and Uruguay (Piñeiro, Dawson, & Costarrica, 1996). In Brazil, current information also places DON as the main target toxin in analyses of commercial wheat grain, flour and by-products (Calori-Domingues et al., 2007; Furlong, Soares, Lasca, & Kohara, 1995; Malmann, Dilkin, Mürman, Dilkin, & Almeida, 2003). Although risk factors related to environment and host genotype are known for playing a role in determining mycotoxin accumulation in grains, genetic profile of the regional populations, especially the type of toxin produced by the fungus (fungal chemotype), is critical for assessing the regional risk of Fusarium mycotoxins in the food chain (Goswami & Kistler, 2004; Ward et al., 2008). Strains of the Fg complex affecting wheat produce trichothecenes in larger quantities among the range of mycotoxins produced, especially the type-B trichothecenes such as nivalenol (NIV) and deoxynivalenol...
2. Materials and methods

2.1. Survey area and sampling

Commercial wheat grain samples (500 g) from several crop varieties commonly grown by farmers in the region were obtained after harvesting operations. Information on wheat varieties, cropping practices, fungicide use and other agronomic factors was not available; only the municipality of origin for each sample was available. Surveyed fields were chosen randomly by a network of collaborators located across the major production regions in the northern portion of the state of Rio Grande do Sul where wheat is mostly grown. The survey was conducted during the 2006–2008 period and a total number of 66 samples were received and originated from 38 municipalities across the state. Grain samples received were identified and stored in the freezer (−5 °C) until analysis.

In the majority (28/38) of the municipalities where samples were obtained in the three-year survey period (2006–2008) at least one sample per location was analyzed in one or another year. For the other 10 municipalities, the number of analyzed samples ranged from two to four, distributed in different years of the survey. An exception was one municipality that had 14 samples analyzed across all years (data not shown).

2.2. Laboratory analyses

Both visual assessment and chemical analyses were performed in a sub-sample of kernels to assess the impact of FHB on wheat grain quality. Fusarium-damaged kernel (FDK) is a commonly at-harvest measure used as an indicator of disease intensity and, in some cases, predictive of DON in the harvested kernels (Beyer, Klik, & Verreet, 2007). FDK, determined by inspecting a sub-sample in some cases, predictive of DON in the harvested kernels (Beyer, 2007). FDK, determined by inspecting a sub-sample in some cases, predictive of DON in the harvested kernels (Beyer, at-harvest measure used as an indicator of disease intensity and, Fusarium grain quality.

In a sub-sample of kernels to assess the impact of FHB on wheat commercial wheat grain from a major production region in southern Brazil was hypothesized in this study considering the number of Fg strains from different years, locations and hosts (Astolfi, dos Santos, et al., 2011; Pereyra, Vero, Gardeminda, Cabrera, & Pianzolla, 2006; Pinto, Termiello, Basilio, & Ritieni, 2008; Ramirez, Reynoso, Farnochi, & Chulze, 2006; Scoz et al., 2009). Around the world, NIV genotypes of the Fg complex are most commonly found, and eventually in higher prevalence than DON, in Asia (Suga, Karugia, Ward, & Gale, 2008; Zhang et al., 2007).

The co-occurrence of NIV and DON mycotoxins in commercial wheat produced in southern Brazil was hypothesized in this study based on our previous identification of NIV genotypes in a considerable number of Fg strains from different years, locations and hosts (Astolfi, Reynoso, et al., 2011; Scoz et al., 2009). The objectives of this study were: (1) to conduct a large-scale sampling of commercial wheat grain from a major production region in southern Brazil, and (2) to quantify the prevalence and intensity of FHB epidemics related to kernel quality and DON and NIV concentrations in commercial wheat grain.

2.3. Statistical analysis

Exploratory and descriptive statistics were used to summarize and map the occurrence, concentration levels and spatial distribution of the mycotoxins across the geographic region. Non-parametric tests (Kruskal–Wallis and Wilcoxon) were used to compare toxin concentration levels among years and between toxin types. Spearman rank correlation coefficient analysis, a non-parametric measure of statistical dependence between two variables, was performed to assess the association between the FHB-related variables assessed under the condition of non-normality of distribution of the data.
2007 and 2008 compared to 2006 growing season. In 2007, maximum DON level was 2740 \( \mu \text{g/kg} \) and the highest within-year variation was observed. For NIV, similar concentration levels were found across the years (Fig. 2).

As to the impact of FHB epidemics related to kernel damage, the overall FDK mean was 15.5\%. A slight variation was observed across the years following a similar pattern to toxin levels, especially for DON, that is, a larger spread of FDK values was also observed in samples of year 2007 (Fig. 3). Correlation analysis showed that DON levels were low but positively correlated \((R = 0.27, P = 0.02)\) to FDK levels. On the other hand, NIV was not significantly correlated to FDK \((R = 0.20, P = 0.14)\). When levels of both toxins were combined, a more significant correlation was found between FDK and DON + NIV \((R = 0.36, P < 0.01)\) (Fig. 3).

4. Discussion

Our results constitute the first detailed report of the co-occurrence, concentrations and spatial distribution of two trichothecenes of major concern and their association with FHB damage in commercial grain samples from a major wheat-growing area in Brazil. The FDK levels found in this work are relatively high compared to levels found in other countries for the same range of toxin levels found (Beyer et al., 2007). FDK is a subjective and qualitative assessment in a single kernel, so toxin content can vary significantly across single “damaged” kernels. In our assessment several samples that showed minimal damage such as discoloration and mycelium growth were counted as damaged. A meta-analysis of 163 studies reporting FDK and DON in the United States has shown that 53% of the variation of DON was explained by FDK in field trials, suggesting that unknown or unmeasurable factors in typical field environments influence the relationship between DON and disease (Paul, Lipps, & Madden, 2005). Conversely, in a German study, over 90% of the DON content in wheat kernels was explained by FDK levels when producing artificial lots by combining damaged and healthy kernels in increasing proportions – the limit of 1250 \( \mu \text{g/kg} \) would be reached with a FDK level of 4.27\% in that work (Beyer et al., 2007). Further refinements of the visual measurement will be required to verify if FDK can be used as screening to trichothecene levels in Brazil, which remain unclear.

In spite of the increasing risk and concern about FHB, monitoring and reporting of the occurrence of Fusarium toxins in commercial wheat grain and by-products in Brazil were limited.
conditions (McMullen et al., 1997). Our results update the current because of different flowering dates, local inocula and weather intensity and mycotoxin levels is expected on a regional basis

to a few research studies or non-public industrial quality assessments. Previous studies in the country placed DON as the main target toxin, together with zearalenone, diacetoxyscirpenol, and T-2 toxins (Calori-Domingues et al., 2007; Furlong et al., 1995; Malmann et al., 2003). In those, information varied from qualitative and quantitative due to a variety of analytical methods which differ in accuracy and detection limits. Interestingly, the mean DON levels (540 µg/kg) found in our survey were within the range of mean concentration levels reported in previous findings for the region. However, our results differed from other surveys because DON was found in all but one sample and was much less prevalent in previous reports. For example, DON levels, found in 55% of 38 samples of commercial wheat grain produced in Brazil, ranged from 400 to 590 µg/kg (Furlong et al., 1995). A large analytical survey conducted between the years 2000 and 2003 showed that approximately 25% of 297 samples of commercial wheat from southern Brazil were contaminated with DON with mean and maximum levels of 603 and 8504 µg/kg, respectively (Malmann et al., 2003). A recent analysis of both national and imported wheat grains showed 94% (mean DON = 332 µg/kg), and 88% (mean = 90 µg/kg) of the samples contaminated with DON, respectively. Although in significantly higher levels, only two national samples showed DON levels exceeding 1250 µg/kg (Calori-Domingues et al., 2007).

The apparently higher prevalence of DON in studies conducted in the current decade, including results of this work, may relate partially to the higher risk of FHB epidemics along the years and also to its increasing awareness. In the last decades, FHB epidemics became more frequent likely due to the predominance of no-till cropping and climate decadal variability in the subtropical environment of southern Brazilian growing regions (Del Ponte, Fernandes, Pavan, & Baethgen, 2009). Infection by FHB pathogens is extremely dependent on specific environmental conditions that occur in a relatively narrow susceptible phase of the host development and so a non-homogeneous pattern of epidemic intensity and mycotoxin levels is expected on a regional basis because of different flowering dates, local inocula and weather conditions (McMullen et al., 1997). Our results update the current status by showing the predominance of DON from both a multi-year and geographical perspective, which was not available in most analytical-oriented studies using random sampling of commercial grain obtained from storage without knowing the specific location where wheat was produced in the country.

Another noteworthy contribution is the first report of the widespread occurrence of NIV in commercial wheat grain in southern Brazil. Previous report of NIV in Brazilian wheat was limited to 20 samples from a one-field experiment carried out in 1990, with two wheat varieties in the state of São Paulo (Furlong et al., 1995). While in that study NIV was detected in three samples (160–400 µg/kg), and DON in four samples (470–590 µg/kg), F. graminearum was not identified among the Fusarium species isolated from the samples.

Natural occurrence of NIV in South America was reported in wheat grain samples from fields grown in southwestern Buenos Aires province of Argentina, during 2001 and 2002 growing seasons. In that work, contrastingly, only two out of 19 samples contained NIV in relatively lower concentrations compared to DON (Pinto et al., 2008). NIV is a common toxin found in other production regions of the world, especially in Asia, where NIV genotypes are present and/or predominate over DON types (Suga et al., 2008; Zhang et al., 2007). In Japan, there have been many reports of DON and NIV co-contamination in domestic wheat and barley by-products (Tanaka et al., 2010; Yoshizawa & Jin, 1995) and both toxins are targets in FHB control studies (Nakajima, 2007). Our findings place Brazil in a similar situation as in Asia and other regions, especially because of the toxigenic potential of the regional fungal populations. The NIV levels found in our work, although not exceeding 1000 µg/kg, are of great toxicological significance given the higher toxicity of NIV compared to DON. The lack of detection of NIV in surveys conducted in Brazil may relate to a number of factors including non-consideration of NIV as a target toxin, lower frequency of NIV genotypes or lack of specific methodology for its detection.

The finding of NIV in commercial grain links to results of our molecular surveillance on Fg populations in southern Brazil where potential NIV-producers (NIV genotypes) were detected together with the most predominant DON-type (15ADON genotype) (Astolfi, dos Santos, et al., 2011; Astolfi, Reynoso, et al., 2011; Scoz et al., 2009). The relatively high NIV levels found in samples of this survey compared to a proportionally lower number of NIV-type strains relative to DON-type, in wheat, suggest that other factors, such as host genotype, environment, and field populations may play a significant role in the production of NIV in the field, which deserves further investigation. In Argentina, although 15ADON genotypes are most prevalent, a recent molecular survey revealed the occurrence of NIV genotype with a distinct phylogenetic species profile (Sampietro et al., 2010), suggesting the need to increase vigilance to detect movement and changes in the chemotype distribution, especially because of the proximity to production regions in Brazil. Further studies will be needed to
identify agronomic and biological factors related to the variability in DON and NIV levels in the region.

On a regional basis, the promulgation of regulations and mycotoxin limits should be based on a number of factors that include the knowledge of the distribution of mycotoxin levels within commodities or products and legislation in other countries with which trade contacts exist (van Egmond et al., 2007). Therefore, besides providing epidemiological information for risk assessment and disease management including chemical and genetic control oriented to minimize both DON and NIV production from FHB epidemics in Brazil, our results provide critical information for the eventual promulgation of regulations for trichothecene limits in cereal grain, as well as alert the small-grains industry of southern Brazil to the widespread occurrence of NIV, a mycotoxin of higher toxicity than DON.

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