MAÍRA RODRIGUES DUFFECK

SURVEY, EXPERIMENTAL AND SIMULATION-BASED APPROACHES FOR UNDERSTANDING THE RISKS OF YIELD LOSSES AND MYCOTOXIN CONTAMINATION TO IMPROVE REGIONAL MANAGEMENT OF FUSARIUM HEAD BLIGHT IN WHEAT

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Fitopatologia, para obtenção do título de *Doctor Scientiae*.

Orientador: Emerson Medeiros Del Ponte

VIÇOSA - MINAS GERAIS
2019

Ficha catalográfica elaborada pela Biblioteca Central da Universidade Federal de Viçosa - Campus Viçosa

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Duffeck, Maíra Rodrigues, 1992-

D856s
2020

Survey, experimental and simulation-based approaches for understanding the risks of yield losses and mycotoxin contamination to improve regional management of Fusarium head blight in wheat / Maíra Rodrigues Duffeck. – Viçosa, MG, 2020.

87 f. : il. (algumas color.) ; 29 cm.

Orientador: Emerson Medeiros Del Ponte.

Tese (doutorado) - Universidade Federal de Viçosa.

Inclui bibliografia.

1. Plantas - Doenças e pragas. 2. Micotoxinas. 3. Fungos fitopatogênicos. 4. *Triticum aestivum*. 5. *Fusarium graminearum*. 6. Giberela. I. Universidade Federal de Viçosa. Departamento de Fitopatologia. Programa de Pós-Graduação em Fitopatologia. II. Título.

CDD 22. ed. 632.4

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APROVADA: 23 de setembro de 2020.

Assentimento:

Maíra Rodrigues Duffeck

Maíra Rodrigues Duffeck
Autora

Emerson Medeiros Del Ponte
Orientador

Aos meus amados pais, Anália e Edemilcio (in memorian)
À minha querida irmã, Maite
Aos meus familiares e amigos que sempre torceram por mim
dedico*.*

ACKNOWLEDGMENTS

Gostaria de agradecer em primeiro lugar a Deus, por me dar forças para lutar pelos meus objetivos todos os dias, e por acreditar em mim, mesmo até quando eu não acreditei mais nele.

A minha amada mãe Anália, por seu amor incondicional e por abrir mão dos seus sonhos para que os meus pudessem ser realizados. Ao meu pai Edemilcio, por me apoiar e me incentivar mesmo em meio a tantas dificuldades. A minha irmã Maite, por ser meu raio de luz em dias de escuridão, e razão pela qual eu continuo lutando todos os dias.

A toda a minha família pelo apoio e suporte emocional durante todos esses anos de trajetória acadêmica.

Aos professores, funcionários, e amigos da Escola de Educação Básica Valentin Gonçalves Ribeiro, Monte Castelo, SC, pela educação e pelos valores morais repassados durante os 10 anos em que estive presente na instituição.

A todos os meus amigos e também profissionais de Monte Castelo, por acreditar em mim e por me ajudar a me inserir em um curso de graduação, em especial a minha amada amiga Patrícia.

Aos professores, funcionários, e amigos do Instituto Federal Catarinense, Campus Rio do Sul, SC, por me ajudar a crescer como profissional e como cidadã, e em especial ao Prof. Leandro Luiz Marcuzzo, pela mentoria e incentivo durante os 4 anos em que trabalhamos juntos nos projetos de iniciação científica, e por guiar os passos consecutivos da minha trajetória acadêmica.

A Universidade Federal de Viçosa (UFV), ao Departamento de Fitopatologia e ao Programa de Pós-graduação em Fitopatologia por proporcionarem condições de realizar este trabalho.

Aos professores do Departamento de Fitopatologia da Universidade Federal de Viçosa, pelos ensinamentos. A todos os funcionários da UFV, o meu muito obrigada.

A Comissão de Aperfeiçoamento de Pessoal do Nível Superior (CAPES), pelo apoio financeiro.

Ao Prof. Emerson Medeiros Del Ponte pela orientação, paciência, apoio, amizade, e por sempre acreditar no meu potencial e continuar me incentivando a ser uma profissional melhor.

Aos amigos do Laboratório de Epidemiologia pelo companheirismo, pela ótima convivência, e pela ajuda nos experimentos e análises. Obrigada por terem sido a minha família durante os anos de Mestrado e Doutorado na UFV.

Aos colegas de mestrado e doutorado, e aos amigos de Viçosa pela amizade e apoio, tornando os momentos difíceis mais leves e descontraídos.

Ao grupo do Instagram Epidemio Raiz pela amizade, pelas risadas, críticas, sugestões e pela compreensão durante todos esses anos.

Ao Prof. Paul David Esker, por me proporcionar a oportunidade de realizar o Doutorado Sanduíche, que sem dúvidas foi a experiência mais enriquecedora da minha vida, e por ser exemplo de liderança e mentoria.

Aos colegas do Esker Lab, especialmente a Dilooshi, Ananda and Tyler pela ajuda na condução do trabalho e Karen pelos momentos de descontração.

A Penn State University (PSU) e ao Department of Plant Pathology and Environmental Microbiology (PPEM) por me acolher durante o período do Doutorado Sanduíche.

Aos colegas de State College por terem me acolhido tão bem quando cheguei nos EUA, especialmente aos amigos dos grupos Cafofo e Desmantelo dos Zunidos.

Ao meu melhor amigo de State College e meu companheiro de todas as horas Pedro Henrique Vilela Carvalho, por ser meu maior exemplo de liderança e “Lovecat-ismo”.

A todas as pessoas que de maneira direta ou indireta me deram apoio e incentivo, meus sinceros agradecimentos.

BIOGRAPHY

MAÍRA RODRIGUES DUFFECK, filha de Anália Grein Bueno Duffeck e Edemilcio José Rodrigues Duffeck, nasceu em Mafra, Santa Catarina, em 21 de março de 1992.

Em fevereiro de 2006, ingressou no Ensino Médio Técnico em Horticultura oferecido pela Escola de Educação Básica Valentin Gonçalves Ribeiro, Monte Castelo, SC.

Em fevereiro de 2010, ingressou no curso de Agronomia no Instituto Federal Catarinense - IFC, Campus Rio do Sul, Rio do Sul, SC, onde lhe foi conferido o título de Engenheira Agrônoma.

Em fevereiro de 2015, iniciou no Programa de Pós-graduação a nível de Mestrado em Fitopatologia na Universidade Federal de Viçosa, sob orientação do Prof. Emerson Medeiros Del Ponte. Após 18 meses, realizou a passagem direta para o doutorado sem defesa de dissertação.

Em agosto de 2016, iniciou no Programa de Pós-graduação a nível de Doutorado em Fitopatologia na Universidade Federal de Viçosa, sob orientação do Prof. Emerson Medeiros Del Ponte. No período de dezembro de 2018 a agosto de 2019 participou de um programa de doutorado sanduíche no Departamento de Fitopatologia na Pennsylvania State University sob orientação do Prof. Paul David Esker, submetendo-se à defesa de tese em setembro de 2020.

ABSTRACT

DUFFECK, Maíra Rodrigues, D.Sc., Universidade Federal de Viçosa, September, 2020. **Survey, experimental and simulation-based approaches for understanding the risks of yield losses and mycotoxin contamination to improve regional management of Fusarium head blight in wheat.** Adviser: Emerson Medeiros Del Ponte.

Fusarium head blight (FHB) is a major disease of wheat, barley and other small grain cereals due to contamination of grain with mycotoxins produced by the pathogenic fungus. The first chapter focused on quantifying and assessing the spatial variability of mycotoxins presented in commercial wheat grain samples in Brazil, as well as evaluating the accuracy and precision of immunoassay kits compared with the reference chromatographic method. The mycotoxins deoxynivalenol (DON), zearalenone (ZEA) and ochratoxin A (OTA) were quantified in wheat grain samples collected in 2015 across 78 locations in the states of Paraná, Rio Grande do Sul, and São Paulo. The OTA mycotoxin was not found in the samples by both methods. The overall mean levels of DON and ZEA quantified by the reference method was 795.2 μg kg^{-1} and 79.78 μg kg^{-1}, respectively. The ZEA levels estimated by the immunoassay agreed with the reference method, while DON levels were overestimated. In the second chapter, I employed a model-based approach to estimate the relative wheat yield losses due to FHB, and predict economic scenarios for fungicides application. First, a review of the literature on fungicide efficacy evaluated in Brazilian field trials was conducted to obtain FHB-yield data and explore their relationship following a meta-analytic method. Based on the median of maximum yields across trials, the studies were grouped into low ($Yl \leq$ 3,631 kg ha^{-1}) or high ($Yh >$ 3,631 kg ha^{-1}) baseline yields. Population-average intercepts, but not the slopes, differed between Yl (2,883.6 kg ha^{-1}) and Yh (4,419.5 kg ha^{-1}) baselines yields and the damage coefficients were 1.60%$^{-1}$ and 1.05%$^{-1}$, respectively. Our modeling of yield losses due to FHB in a 28-year period (1980 to 2007) showed that the magnitudes and trends obtained in this study were in general agreement with literature reports. The profitability of fungicide spray tended to increase in general after the 1990s, when a single fungicide spray (but not often two sprays) was more likely to pay off. In the third chapter, 421 *Fusarium* isolates from naturally FHB-infected spikes of different small grain hosts obtained across Pennsylvania (PA) in 2018 and 2019 were identified by molecular methods, using a FGSC-specific primer. The toxigenic potential of this isolates, as well as an additional set of 77 *F. graminearum* isolates from overwintering crop residues during the Winter 2012, were determined with a multiplex PCR assay that amplified portions of the *TRI3* and *TRI12* genes. A subsample of 31 *F. graminearum* isolates, 16 of the 3ADON and 15 of the 15ADON type, were assessed for their reproductive fitness and sensitivity to triazole-based fungicides. Results showed that *F. graminearum* accounted for 97% of the FHB pathogen profile in PA, wherein 95.7%, 3.7%, and 0.6% of these isolates possessed the 15ADON, 3ADON and NIV trichothecene genotype. The frequency of the toxin types did not differ among sampled hosts nor years. The two genotypes could not be differentiated for most of the saprophytic traits based on multivariate analysis. All isolates were sensitive to tebuconazole and metconazole fungicides *in vitro*. The results obtained in these studies contribute to advance knowledge of an important disease to both countries and may serve as a basis to improve regional management strategies.

Keywords: *Triticum aestivum*. *Fusarium graminearum*. Trichothecenes. Deoxynivalenol. meta-analysis.

RESUMO

DUFFECK, Maíra Rodrigues, D.Sc., Universidade Federal de Viçosa, setembro de 2020. **Utilização de abordagens experimentais e de simulação na compreensão dos riscos de perdas de produtividade e contaminação por micotoxinas visando melhorar o manejo regional da giberela em trigo.** Orientador: Emerson Medeiros Del Ponte.

A giberela é uma das principais doenças do trigo, cevada e outros cereais de inverno devido à contaminação dos grãos com micotoxinas produzidas pelo patógeno. O primeiro capítulo teve como objetivo quantificar e avaliar a variabilidade espacial das micotoxinas apresentadas em amostras comerciais de grãos de trigo no Brasil, bem como avaliar a acurácia e precisão dos kits de imunoensaio em comparação com o método cromatográfico de referência. As micotoxinas deoxinivalenol (DON), zearalenona (ZEA) e ocratoxina A (OTA) foram quantificadas em amostras de grãos de trigo coletadas em 2015 em 78 cidades nos estados do Paraná, Rio Grande do Sul, e São Paulo. OTA não foi encontrada nas amostras por ambos os métodos. Valores médios de DON e ZEA quantificados pelo método de referência foram 795,2 μg kg^{-1} e 79,78 μg kg^{-1}, respectivamente. Níveis de ZEA estimados pelo imunoensaio concordaram com o método de referência, enquanto os níveis de DON foram superestimados. No segundo capítulo, uma abordagem de modelagem foi utilizada para estimar as perdas de produtividade do trigo devido à giberela e prever cenários econômicos para aplicação de fungicidas. Para isso, dados de produtividade de trigo e severidade da doença foram obtidos através de uma revisão sistemática, e os estudos foram classificados em grupos de baixa ($Yl \leq$ 3.631 kg ha^{-1}) ou altas ($Yh >$ 3.631 kg ha^{-1}) produtividades com base na mediana dos rendimentos máximos entre os ensaios. Um modelo meta-analítico foi utilizado para avaliar a heterogeneidade da relação entre doença e produtividade. Os interceptos médios estimados, mas não os slopes, diferiram entre os grupos Yl (2.883,6 kg ha^{-1}) e Yh (4.419,5 kg ha^{-1}), com coeficientes de dano médio estimados de $1{,}60\%^{-1}$ e $1{,}05\%^{-1}$, respectivamente. Simulações de perdas de produtividade do trigo devido à giberela em um período de 28 anos (1980 to 2007) obtidas neste estudo concordaram com os relatos da literatura. De maneira geral, a lucratividade da aplicação de fungicida aumentou após a década de 1990, quando uma única aplicação de fungicida apresentou uma probabilidade maior de lucro em relação a duas aplicações. No terceiro capítulo, 421 isolados de *Fusarium* obtidos a partir de espigas de diferentes hospedeiros no estado da Pensilvânia (PA) em 2018 e 2019, foram identificados por métodos moleculares, utilizando de um par de primer específico para FGSC (complexo de espécies *Fusarium graminearum*). O potencial toxigênico destes isolados, bem como um conjunto adicional de 77 isolados de *F. graminearum* obtidos de restos culturais durante o inverno de 2012, foram determinados através de um ensaio de PCR multiplex que amplificou porções dos genes *TRI3* e *TRI12*. Uma subamostra de 31 isolados de *F. graminearum*, 16 do tipo 3ADON e 15 do tipo 15ADON, foram avaliados quanto à sua aptidão reprodutiva e sensibilidade a fungicidas à base de triazóis. Os resultados mostraram que 97% dos isolados foram identificados como *F. graminearum*, sendo que 95,7%, 3,7%, e 0,6% destes isolados possuem o genótipo tricoteceno 15ADON, 3ADON e NIV, respectivamente. Os isolados dos dois genótipos não puderam ser diferenciados para a maioria das características saprófitas com base na análise multivariada. Todos os isolados foram sensíveis aos fungicidas tebuconazol e metconazol *in vitro*. Os resultados obtidos nesses estudos contribuem para o avanço do conhecimento de uma doença importante para os dois países e podem servir de base para o aprimoramento das estratégias de manejo regional.

Palavras-chave: *Triticum aestivum*. *Fusarium graminearum*. Tricotecenos. Deoxynivalenol. meta-análise.

SUMMARY

GENERAL INTRODUCTION

Fusarium head blight (FHB) is a major flowering disease of wheat, barley, and other small-grain cereals caused by *Fusarium* species. Since its resurgence during the early 1990s, FHB epidemics have contributed to significant crop losses in small grains, the fungus causes physical damage to kernels that are reduced in size and weight. In addition, infected grains that are not eliminated during harvest are contaminated with mycotoxins produced by the fungus during host infection and colonization. The lack of more effective pre-harvest management strategies to reduce mycotoxin contamination, which depend on host genetic resistance and chemical control, have led many countries or regions to establish maximum tolerated limits to protect consumers.

Species of the *Fusarium graminearum* species complex (FGSC) are the main causal agent of FHB worldwide. One of them, *F. graminearum* sensu stricto, is the most dominant globally. *F. graminearum* isolates are capable of producing several mycotoxins but special attention has been given to zearalenone (ZEA) and type B-trichothecenes, including deoxynivalenol (DON) and nivalenol (NIV) that are detected at leves considered unsafe for both for human and animal consumption. Therefore, FHB pathogens represent both a food security and a food safety threat.

In Brazil, decadal variability in the climatic patterns in the main wheat-growing region in the South has been linked to variations in FHB risk over several dozen years according to simulation modeling. The increase in the frequency of more severe FHB epidemics after the 1990s raised concerns about the magnitude of yield losses that were estimated, in small plots experiments, to increase from 5.4% during the period 1984 to 1994 to 21.6% after the 2000s. Additionally, surveys on *Fusarium* mycotoxins showed the increased levels of mycotoxin contamination in small-grain cereals and their by-products in Brazil. In this context, DON has been the prevalent mycotoxin associated with grain samples in studies conducted in the current decade, with 30% of them showing mycotoxin levels above the maximum tolerated limits that have been promulgated in Brazil. NIV and ZEA have also been found to be common grain contaminants. However, considerable variation has been found across sampled years and locations, which may be explained partially due to a variety of analytical methods used to quantify the toxins which differ in accuracy and detection limits. Thus, monitoring and reporting of the occurrence of *Fusarium* toxins in commercial wheat grain and by-products in Brazil, as well as the comparisons among different analytical methods methods used to quantity toxin levels is warranted.

In the context of crop losses due to diseases, knowledge of the diversity and composition of the pathogen population is fundamental to adjust disease management especially when the population can be highly variable and prone to genetic shifts that further shape pathogenic and toxigenic traits. In this context, continuous surveillance is important to rapidly detect variations in FHB pathogen populations, which may have practical implications for the durability of the implemented FHB control tactics since individuals may be selected for or against depending on the tactic employed. Reports of shifts in FHB pathogen populations from Canada and the Upper Midwest region of the U.S. motivated the research conducted in the third study of this thesis, regarding the current distribution of *Fusarium* species causing FHB in all wheat-growing regions of Pennsylvania.

This dissertation is structured as a series of three studies. The first study used a survey-based approach to tested the hypotheses of co-occurrence of DON and ZEA mycotoxins in commercial wheat in Brazil, and that the immunoassay kits may present similar accuracy and precision as chromatographic methods regarding mycotoxin levels detection. In the second study, a model-based approach was used to test the hypothesis that yield losses in Brazilian spring wheat due to FHB was greater after the 1990s with more frequency of profitable scenarios for fungicide application. In a third study, conducted during my Visiting Scholar period at The Pennsylvania State University, USA, field survey and experimental data were used to determine species diversity and toxigenic profile of FHB pathogens in Pennsylvania, as well as characterized the populations based on reproductive and triazole sensitivity profiles.

CHAPTER 1

Survey of mycotoxins in Southern Brazilian wheat and evaluation of immunoassay methods

This chapter is published: Duffeck, M. R., Tibola, C. S., Guarienti, E. M., and Del Ponte, E. M. 2017. Survey of mycotoxins in Southern Brazilian wheat and evaluation of immunoassay methods. Scientia Agricola. DOI:10.1590/1678-992x-2016-0263.

ABSTRACT

One hundred commercial wheat grain samples were collected during the 2015 season across 78 municipalities in the states of Paraná (PR), Rio Grande do Sul (RS), and São Paulo (SP), Brazil. Separate subsamples were analyzed for the concentration of deoxynivalenol (DON), zearalenone (ZEA) and ochratoxin A (OTA) mycotoxins using two methods: UHPLC-MS/MS (reference method) and a commercial enzyme-linked immunosorbent assay (ELISA) (AgraQuant®). The OTA mycotoxin was not found in the samples by both methods. DON and ZEA were detected in 55% and 39% of the samples by the reference method, with overall mean levels of 795.2 μg kg^{-1} and 79.78 μg kg^{-1}, respectively. There was a significant and positive correlation (Spearman rank) between DON and ZEA estimates by the reference method ($r = 0.77$, $P < 0.001$). The DON levels estimated by the immunoassay agreed poorly with the reference, being largely overestimated. Based on a cut-off level of 1000 μg kg^{-1}, the immunoassay correctly classified 57 samples as true negatives and 15 as true positives. Only 28 were classified as false positives. For ZEA, the levels estimated by the two methods were in better agreement than for DON. Using the cut-off level of 200 μg kg^{-1}, 96% of the samples were classified correctly as true positives and only one sample was classified as false positive. The levels for both mycotoxins were mostly acceptable for human consumption. Further studies should focus on multi-toxin methods compared with immunoassays to understand the reasons of overestimation and the role of immunoassays as a cost-effective solution for fast screening of mycotoxins in the food chain.

Keywords: *Triticum aestivum*, UHPLC, ELISA, trichothecenes, ochratoxin

INTRODUCTION

Fusarium head blight (FHB) is a fungal disease of major concern to wheat production. In Brazil, the disease is caused by members of the *Fusarium graminearum* species complex (FGSC), which infect wheat florets and contaminate grains with dangerous mycotoxins (Del Ponte et al. 2015). These fungi are able to synthesize a range of mycotoxins such as trichothecenes, mainly deoxynivalenol (DON), and zearalenone (ZEA) (Bryden 2012). Ochratoxin A (OTA) is not a *Fusarium* mycotoxin, but it is found in grain stored under inadequate conditions and infected by *Aspergillus* and *Penicillium* species (Ghali et al. 2008).

In order to mitigate the harmful effects of mycotoxins on public health, maximum limits have been established to reduce *Fusarium* mycotoxins around the world (Cheli et al. 2014), including Brazil (ANVISA 2011). Surveys on mycotoxins in commercial Brazilian wheat grain and byproducts provide critical information for growers to assess the impact of control measures as well as to inform consumers and policy makers on the mycotoxin risks (Furlong et al. 1995, Oliveira et al. 2002, Calori-Domingues et al. 2007, Del Ponte et al. 2012, Tralamazza et al. 2016). Among the quantitative methods for mycotoxin analysis, the most specific and sensitive ones include the ultra-high performance liquid chromatography-tandem with triple quadrupole mass spectrometry (UHPLC-MS/MS), liquid chromatography coupled to mass spectrometry (LC-MS) and gas chromatography coupled to mass spectrometry (GC-MS) (Shephard et al. 2011). However, high costs are limiting factors for their use in routine for processing large number of samples (Lattanzio et al. 2009, Xu et al. 2010). Commercial kits based on enzyme-linked immunosorbent assays (ELISAs) are widely used for screening commodities and foods, given their low cost and easy operation (Lattanzio et al. 2009). Although these assays allow high throughput, disadvantages related to antibody cross-reactivity can lead to overestimation of the targeted mycotoxin compared to a reference (chromatographic) method (Berthiller et al. 2013, Liu et al. 2012). The main objective of this study was to quantify and assess the spatial variability of DON, ZEA and OTA in commercial grain harvested in southern Brazil, in the 2015 season, using UHPLC-MS/MS. A secondary objective was to evaluate commercial immunoassay kits targeting the three mycotoxins based on the accuracy, precision and validity of the estimates compared to the reference method.

MATERIALS AND METHODS

Survey area and sampling

Grain samples were collected from the harvest of commercial wheat fields in wheat-growing regions in southern Brazil where FHB occurs frequently. The samples (10 kg) were collected in 78 municipalities randomly chosen in the main producing regions of Paraná (PR, 60% of the samples), Rio Grande do Sul (RS, 35%) and São Paulo (SP, 5%) States (Fig. 1B). In most cases, one sample was obtained per municipality, but in eight municipalities, two samples were collected. Municipalities that provided more than two samples were Carambei ($n = 4$), Imbau ($n = 6$) and Tibagi ($n = 7$), all in Paraná State. Cooperatives of local growers and private industry provided the samples, which were collected at the storage facilities. The sampling procedures followed the protocols adopted by the provider. The moisture content across the samples ranged from 12% to 14%.

UHPLC and immunoassay mycotoxin quantification

Two subsamples (300 g) were obtained for the analyses of DON, ZEA and OTA using two different methods. One subsample was sent for chemical analysis by a commercial and certified laboratory (Santa Maria, RS) where the three mycotoxins were analyzed by UHPLC-MS/MS with an automated extraction, clarification and derivation, as described in Varga et al. (2012). The method has the following limits of quantification and recovery ratio: 200 μg kg^{-1} and 80% for DON, 20 μg kg^{-1} and 85% for ZEA and 2 μg kg^{-1} and 80% for OTA. Another subsample was analyzed for the same three mycotoxins using a commercial direct competitive enzyme-like immunosorbent assay (ELISA) kit (AgraQuant®). The limit of detection (LOD) and the limit of quantification (LOQ) in this test were 200 μg kg^{-1} and 250-5000 μg kg^{-1}, respectively. Extraction procedure, calibration and reading were performed according to manufacturer instructions. Details were described by Zheng et al. (2004).

Statistical analysis

Descriptive statistics were used to summarize frequency and central tendency measures of the mycotoxin data from all samples. The frequency of the number of positive detections (above the detection limit) was compared between samples from RS and PR and SP combined using the χ^2 test ($P < 0.05$). Geographic maps were made to show the origin and the mean concentration levels of each mycotoxin aggregated by municipality. The correlation between levels of two mycotoxins was assessed using the Spearman rank correlation coefficient because the data normality could not be assumed. The accuracy (systematic and constant bias) and precision of estimates by immunoassay, compared to those by UHPLC, used as reference, were determined by the components of Lin's concordance correlation analysis (LCC) (Lin 1989). Absolute errors of the estimates by the immunoassay were also calculated.

In addition, the estimates by the two methods were categorized into two classes based on a cut-off level of 1000 µg kg^{-1} and 200 µg kg^{-1} for DON and ZEA respectively, both determined based on the current legislation for each mycotoxin in wheat (ANVISA 2011). The number of cases in each category according to the cut-off, determined by each method, was related to one another and the validity (sensitivity and specificity) of the immunoassay compared to the reference method (UHPLC) was calculated based on the false positive rate, false negative, true positive and true negatives. Non-parametric tests were used to compare the mean DON and ZEA levels between the two states (Mann-Whitney test) and between the two methods (Wilcoxon test) ($P < 0.05$) because normality could not be assumed.

RESULTS

UHPLC analysis of mycotoxins

OTA mycotoxin was not found in the samples. DON was detected in 55% of the samples, with levels ranging from 200 (detection limit) to 2,743 µg kg^{-1} in the positive samples, with a mean (median) of 795.2 and 682.8 µg kg^{-1} in these positive samples. When all samples were considered in the calculation (including negatives), the mean and median values were 437.4 and 210.4 µg kg^{-1}, respectively.

The frequency of positive DON detections varied between samples from RS (33 in 35) and PR+SP (22 in 65) States (Fig. 1B). The mean (median) DON levels in the positive samples were 685.1 (557.5) µg kg^{-1} in RS (n = 33) and 960.3 (854.0) µg kg^{-1} in PR+SP States (n = 22), respectively. When all samples were used in the calculation, the mean (median) of DON levels were 646.1 (479.4) µg kg^{-1} in RS (n = 35) and 325.0 (0) µg kg^{-1} in PR+SP States (n = 65), respectively (Fig. 1A).

In only 15 samples, five from RS and ten from PR, DON levels were > 1000 µg kg^{-1} (Fig. 1A). In RS, higher DON levels were found in locations from the Planalto region, while in PR State, higher contamination was found in the southeastern region at levels similar to those found in RS. Most samples from western PR and SP State were not contaminated with DON (Fig. 1B).

ZEA was detected in 39% of the samples, with an overall mean (median) of 79.78 (35.90) µg kg^{-1} in the positive samples. The relative frequency of positive ZEA detections was also higher in RS (25 in 35) than in PR (14 in 65). Mean (median) ZEA levels were 60.94 (34.4) µg kg^{-1} in the 25 positive samples of RS and 113.4 (94.8) µg kg^{-1} in the 14 positive samples of PR (Fig. 2A). ZEA was not detected in SP State. Only three samples (2 from PR and 1 from RS) showed levels of ZEA > 200 µg kg^{-1} (Fig. 2A). The spatial variation of the

ZEA levels followed that of DON levels (Fig. 2B). Overall, there was a significant and positive correlation (Spearman rank) between DON and ZEA determined using UHPLC (r = 0.77, S = 36728, P < 0.0001).

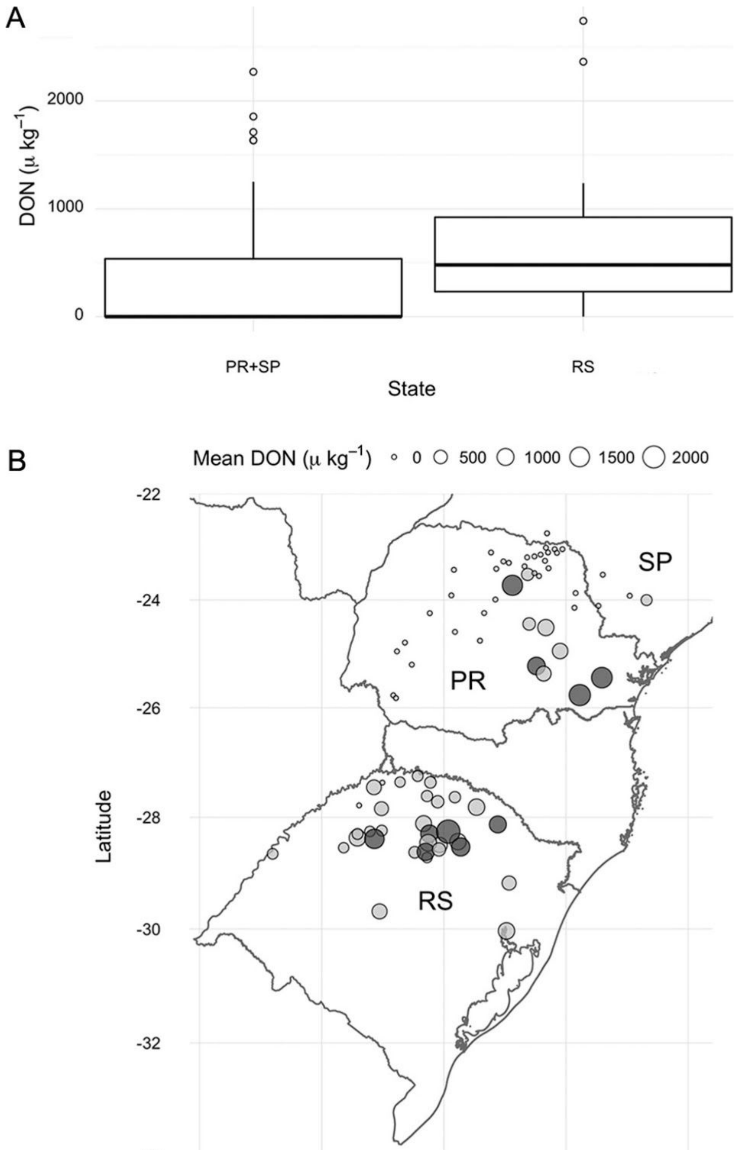

Figure 1. A, Boxplot for the distribution of deoxynivalenol (DON) levels (μg kg^{-1}) estimated by UHPLC method in commercial wheat samples from Paraná and São Paulo (PR + SP) (north) States and Rio Grande do Sul (RS, south) State. **B**, The circle represents the geographic location of each 78 municipalities and its size is proportional to the mean DON levels for the location. The darker grey circle represents locations with mean DON above the 1,000 μg kg^{-1} threshold.

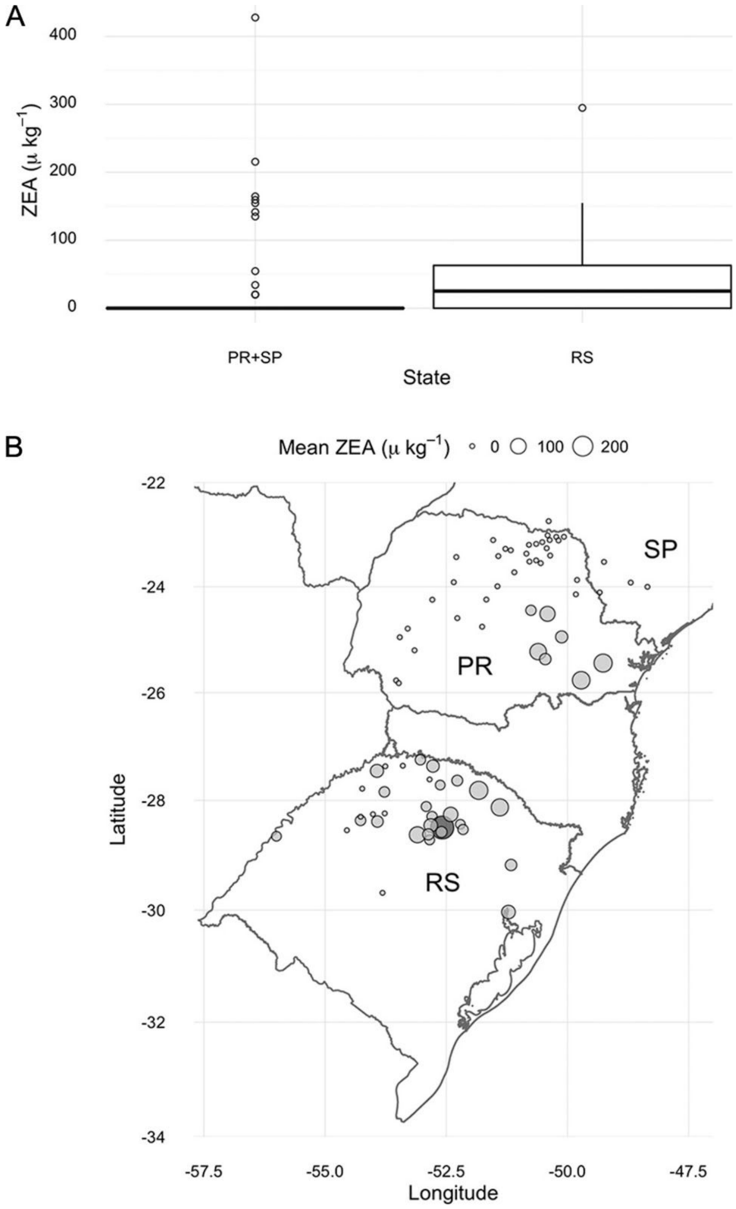

Figure 2. A, Boxplot for the distribution of zearalenona (ZEA) levels (μg kg^{-1}) estimated by the UHPLC method in commercial wheat samples from Paraná and São Paulo (PR + SP) (north) States and Rio Grande do Sul (RS, south) State. **B,** The circle represents the geographic location of each 78 municipalities and its size is proportional to the mean ZEA levels for the location. The darker circle represents locations with mean ZEA above the 200 μg kg^{-1} threshold (B).

Immunoassay analysis of mycotoxins

OTA was not found with the direct competitive immunoassay, agreeing with results of the UHPLC analysis. Conversely, DON was detected in all samples when the immunoassay was used, differing from UHPLC. There was only one sample in which DON was not detected by both methods. In 44 samples where DON levels were below the detection limit based on

UHPLC analysis, DON levels determined by immunoassay averaged 690 μg kg^{-1} (190 to 1490 μg kg^{-1}); eight samples showed DON levels > 1000 μg kg^{-1}.

The mean (median) of DON estimated by the immunoassay for all samples was 1274.5 (800) μg kg^{-1}, which was significantly higher than the mean (including all samples) levels measured by UHPLC (437.3 μg kg^{-1}) (Fig. 3A). In 43% of the samples, DON was greater than 1000 μg kg^{-1}. There was no difference ($P > 0.05$) in the mean DON levels in the samples from RS (1792.4 μg kg^{-1}) and PR+SP States (1824.54 μg kg^{-1}).

ZEA levels were similar between the two methods, although the frequency of positive detection was higher using UHPLC (39%) when compared with the immunoassay (27%). The mean estimated ZEA levels were 31.11 and 35.94 μg kg^{-1} for UHPLC and the immunoassay, respectively (Fig. 3B), and did not differ statistically ($P > 0.05$).

Accuracy and validity of immunoassay

The estimates of DON levels by immunoassay showed good precision ($r = 0.87$). However, there were large positive deviations from the UHPLC estimates in 54% of the samples, which resulted in low accuracy (C_b = 0.57) and moderate overall concordance between the two methods (ρc = 0.5; 95% CI: 0.42 - 0.58) (Fig. 3C).

For samples classified as accepted or rejected based on the cut-off level of 1000 μg kg^{-1}, immunoassay correctly classified 57 samples as true negative (correctly accepted) and 15 as true positives (correctly rejected). There were 28 false positives (incorrectly rejected) and no false negative (incorrectly accepted) (Fig. 3C). The immunoassay test showed 72% of accuracy, 35% of sensitivity and 100% of specificity.

ZEA levels estimated by immunoassay were less precise than those estimated for DON ($r = 0.79$), but they were more accurate (C_b= 0.91), resulting in a higher overall concordance among results by the two methods compared to DON (ρc = 0.72; 95% CI: 0.67 - 0.77) (Fig. 3D). Based on a 200 μg kg^{-1} threshold, 96% of the samples were classified correctly as true positive and only one sample was classified as false positive (Fig. 3D). False negative values were not found either. The immunoassay presented 99% of accuracy, 75% of sensitivity and 100% of specificity. The errors of the estimated DON by immunoassay and UHPLC were mostly positives in high magnitude > 3000 μg kg^{-1} (Fig. 3E). On the other hand, ZEA errors by immunoassay were both negative and positive < 150 μg kg^{-1} (Fig. 3F).

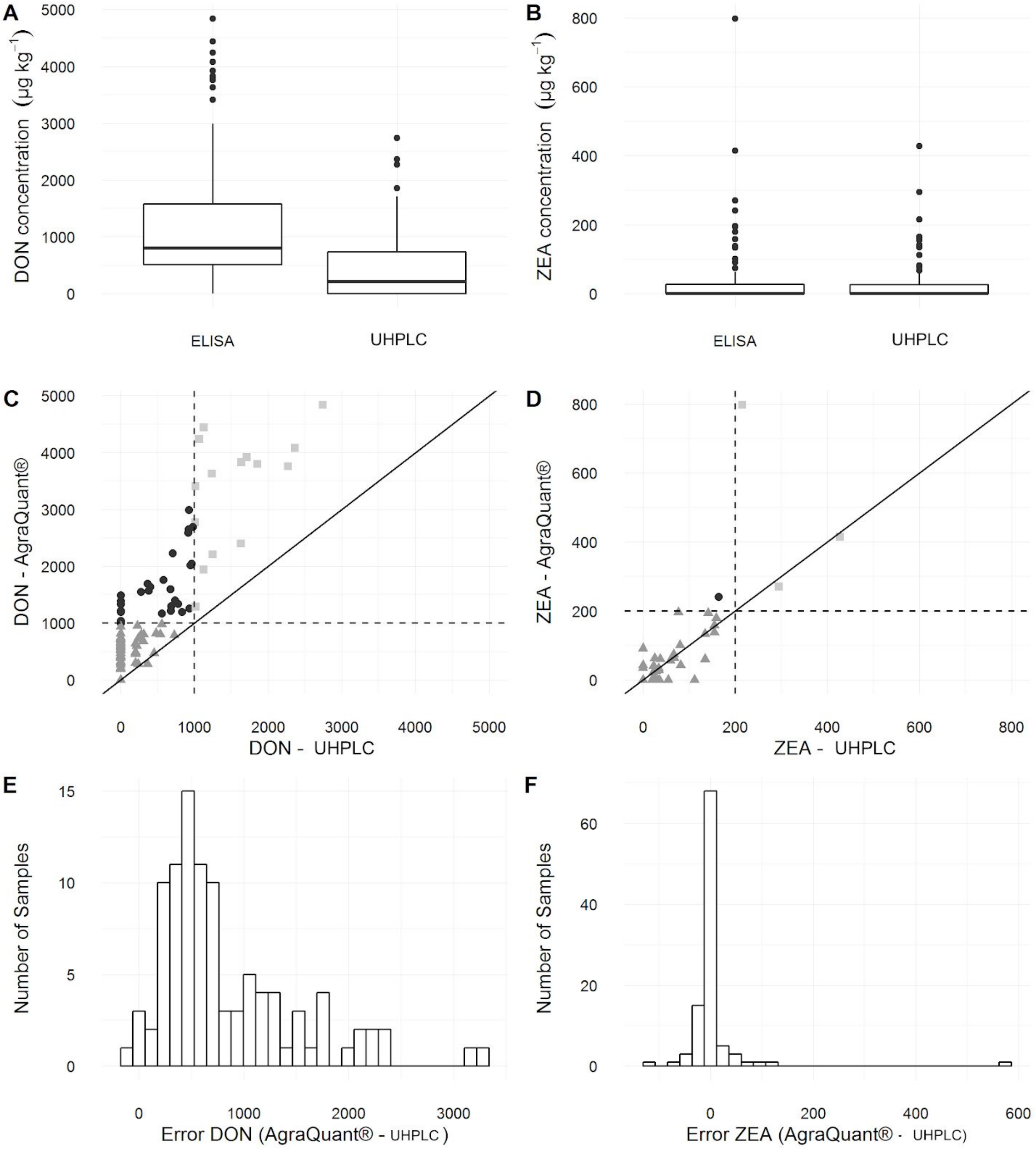

Fig. 3. A, Boxplot for the distribution of deoxynivalenol (DON), and **B,** zearalenone (ZEA) levels (µg kg^{-1}) obtained by the reference method (UHPLC) and commercial immunoassay (ELISA) methods (AgraQuant®). Relationship between concentration of **C,** DON and **D,** ZEA estimated by the UHPLC and immunoassay methods. In **C** and **D**, the samples were classified as true negative (triangle), true positives (square) and false positives (circle) considering the threshold of 1000 µg kg^{-1} for DON and 200 µg kg^{-1} for ZEA. Frequency of samples by ranges of absolute errors (µg kg^{-1}) of the estimates to **E,** DON and **F,** ZEA by immunoassay in relation to the concentration determined by the reference method.

DISCUSSION

This study updated critical data on the occurrence and spatial distribution of two important *Fusarium* mycotoxins in commercial wheat grain produced in the main growing regions in Brazil. Results suggested that the DON and ZEA levels in commercial wheat were generally safe. Only a relatively small percentage showed contamination levels above the threshold, considering the reference (UHPLC) method. The DON levels determined in our study are in agreement with previous reports in the country, using chromatography methods. However, year-to-year (or decades) and region-to-region variations are expected, given the strong dependence on seasonal weather conditions as well as management practices that contribute to suppress the disease and mycotoxin levels (Del Ponte et al. 2009). For example, Furlong et al. (1995), two decades earlier, analyzed 38 samples of commercial wheat grain and found DON in 55% of the samples, but at lower mean and range (400 to 590 μg kg^{-1}) than those found in our study. The analyses of 50 Brazilian and 50 imported wheat samples showed mean DON levels of 332 μg kg^{-1} and 90 μg kg^{-1}, in 94% and 88% of the samples, respectively (Calori-Domingues et al. 2007). More recently, using LC-MS/MS, 12 in 64 commercial wheat samples showed DON > 1000 μg kg^{-1} and an average of 540 μg kg^{-1} was found across three growing seasons (2009 to 2011) (Del Ponte et al. 2012). These results were similar to the median value (437.3 μg kg^{-1}) found in this study, including all samples. We found that DON was significantly higher in samples from RS compared to those from PR, when all samples are taken into account. This is in agreement with a previous study that reported mean DON ranging from 426 to 453 μg kg^{-1} in samples from PR and SP States, and 1200 μg kg^{-1} in samples from RS, all from the 2012 season (Tralamazza et al. 2016).

We found ZEA at lower levels than DON levels and only in samples where DON was also present, being 14 samples from PR and 25 samples from RS. The co-occurrence of both toxins has been reported previously in the country (Tralamazza et al. 2016). Although ZEA is an important mycotoxin produced by members of the FGSC, few studies report its occurrence in Brazilian wheat (Tibola et al. 2015, Tibola et al. 2016). It is known that ZEA co-occur with DON especially when produced by FGSC isolates (Tangni et al. 2010), which is indeed the main pathogen in Brazil and a known ZEA producer (Geraldo et al. 2006). The ZEA levels found in our study are similar to those reported in previous studies on commercial wheat from Brazil (RS, 70.9 μg kg^{-1} and PR, 57.9 μg kg^{-1}) (Tralamazza et al. 2016).

OTA is produced by species of *Aspergillus* and *Penicillium*, which commonly grow during grain storage (Duarte et al. 2010). The absence of OTA in our study may be due to short storage time and good conditions, which may not have favored growth of these fungi. OTA is an important target due to its frequent report in cereal food and feed products such as rice and wheat (González et al. 2006, Ghali et al. 2008). In Brazil, OTA has been reported in corn and rice samples (Machinski et al. 2001).

The DON levels estimated by immunoassay were generally higher than those estimated by chromatography, which would result in considerable increase in the rejections based on the current regulation. Overestimation of *Fusarium* mycotoxins by immunoassays was known from previous studies targeting DON in other countries (Meneely et al. 2011, Lattanzio et al. 2009). An essential requirement for an immunoassay is the antibody specificity. Many studies described the successful production of monoclonal or polyclonal antibodies against the trichothecenes, but often the cross-reactivity profile is not ideal and can lead to overestimation and increased uncertainty in the measurements (Meneely et al. 2011). This is true for DON where most literature highlights that antibodies raised against deoxynivalenol show strong cross-reactivity to 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol or both (Meneely et al. 2011). The antibodies designed for DON may also cross-react against DON-3-β-glucoside (DON-3G) and monoacetylated derivatives (3ADON) (Tangni et al. 2010). The presence of acetylated DON derivatives (15ADON and 3ADON) has been generally reported together with DON, especially in cereals, although at relatively lower levels than DON levels (Berthiller et al. 2013).

Using an immunoassay in Brazil, Santos et al. (2013) quantified DON levels in wheat samples from Paraná State and reported 66% of positive samples at levels ranging from 206.3 to 4732.3 μg kg^{-1} (mean 1894.9 μg kg^{-1}). These levels are similar to those found in our study using immunoassay, but no comparison was made with a reference method in that study. In southern Brazil, FHB is caused by *F. graminearum* strains that potentially produce 15ADON, but also *F. meridionale* that produces nivalenol, and a few other species that produce 3ADON (Del Ponte et al. 2015, Nicolli et al. 2015). Further studies should focus on the analysis of acetylates, glucosilates and other masked forms, which are known to be related to mechanisms of plant resistance. Considering that acetylates and conjugates are also toxicologically important, immunoassay results are useful for fast screening of DON in commodities. On the other hand, the ZEA levels estimated by immunoassay were comparable to those determined by UHPLC, agreeing with previous findings (Shephard et al. 2011).

In summary, the results of this study suggested that Brazilian wheat is mostly acceptable for human consumption. However, it is important to increase vigilance of mycotoxins in wheat because of yearly fluctuations due to climate variability and crop management practices. Future studies should focus on multi-toxin methods combined with immunoassays to improve the accuracy of the screening methods in routine analyses.

ACKNOWLEDGMENTS

The authors would like to thank Embrapa (Brazilian Agricultural Research Corporation) (Embrapa management system - SEG 02.14.01.012.00.00) for funding. Furthermore, we are grateful to the grain companies that provided the samples. The first and last authors are grateful to FAPEMIG (Minas Gerais State Foundation for Research Support) and CNPq (Brazilian National Council for Scientific and Technological Development) for the research grant and student scholarship, respectively.

REFERENCES

Agência Nacional de Vigilância Sanitária [ANVISA]. 2011. Resolução RDC 7. Dispõe sobre limites máximos tolerados (LMT) para micotoxinas em alimentos, constante do Anexo desta Resolução. Diário Oficial da União; Poder Executivo, de 26 de fevereiro de 2011.

Berthiller, F., Crews, C., Dall'Asta, C., Saeger, S.D., Haesaert, G., Karlovsky, P., Oswald, I.P., Seefelder, W., Speijers, G., Stroka, J. 2013. Masked mycotoxins: A review. Molecular Nutrition & Food Research. 57:165–186.

Bryden, W. L. 2012. Mycotoxin contamination of the feed supply chain : Implications for animal productivity and feed security. Animal Feed Science and Technology. 173:134–158.

Calori-Domingues, M. A., Almeida, R. R.;, Tomiwaka, M. M., Gallo, C. R., Gloria, E. M., Dias, C. T. S. 2007. Occurrence of deoxynivalenol in national and imported wheat used in Brazil. Science and Technology Food. 27:181–185.

Cheli, F., Battaglia, D., Gallo, R., Dell´Orto, V. 2014. EU legislation on cereal safety: an update with a focus on mycotoxins. Food Control. 37:315–325.

Del Ponte, E. M., Fernandes, J. M., Pavan, W., Baethgen, W. E. 2009. A model-based assessment of the impacts of climate variability on Fusarium head blight seasonal risk in Southern Brazil. Journal of Phytopathology. 157:675–681.

Del Ponte, E. M., Garda-Buffon, J., Badiale-Furlong, E. 2012. Deoxynivalenol and nivalenol in commercial wheat grain related to Fusarium head blight epidemics in Southern Brazil. Food Chemistry. 132:1087–1091.

Del Ponte, E. M., Spolti, P., Ward, T. J., Gomes, L. B., Nicolli, C. P., Kuhnem, P. R., Silva, C. N., Tessmann, D. J. 2015. Regional and field-specific factors affect the composition of Fusarium head blight pathogens in subtropical no-till wheat agroecosystem of Brazil. Phytopathology. 105:246–254.

Furlong, E. B., Soares, L. M. V., Lasca, C. C., Kohara, E. Y. 1995. Mycotoxins and fungi in wheat harvested during 1990 in test plots in the state of São Paulo, Brazil. Mycopathologia. 131:185–190.

Geraldo, M. R. F., Tessmann, D. J., Kemmelmeier, C. 2006. Production of mycotoxins by *Fusarium graminearum* isolated from small cereals (wheat, triticale and barley) affected with scab disease in Southern Brazil. Brazilian Journal of Microbiology. 37:58–63.

Ghali, R., Hmaissia-khlifa, K., Ghorbel, H., Maaroufi, K., Hedili, A. 2008. Incidence of aflatoxins , ochratoxin A and zearalenone in Tunisian foods. Food Control. 19:921–924.

González, L., Juan, C., Soriano, J. M., Moltó, J. C., Mañes, J. 2006. Occurrence and daily intake of ochratoxin A of organic and non-organic rice and rice products. International Journal of Food Microbiology. 107:223–227.

Lattanzio, V. M. T., Pascale, M., Visconti, A. 2009. Current analytical methods for trichothecene mycotoxins in cereals. Trends in Analytical Chemistry. 28:758–768.

Lin, L. I. K. 1989. A concordance correlation coefficient to evaluate reproducibility. Biometrics 45: 255–268.

Liu, J., Zanardi, S., Powers, S., Suman, M. 2012. Development and practical application in the cereal food industry of a rapid and quantitative lateral flow immunoassay for deoxynivalenol. Food Control. 26:88–91.

Machinski, M., Soares, L. M. V., Sawazaki, E., Bolonhezi, D., Castro, J. L., Bortolleto, N. 2001. Aflatoxins, ochratoxin A and zearalenone in Brazilian corn cultivars. Journal of the Science of Food and Agriculture. 81:1001–1007.

Meneely, J. P., Ricci, F., Egmond, H. P. V., Elliott, C.T. 2011. Current methods of analysis for the determination of trichothecene mycotoxins in food. Trends in Analytical Chemistry. 30:192–203.

Nicolli, C. P., Spolti, P., Tibola, C. S., Fernandes, J. M. C., Del Ponte, E. M. 2015. Fusarium head blight and trichothecene production in wheat by *Fusarium graminearum* and *F. meridionale* applied alone or in mixture at post-flowering. Tropical Plant Pathology. 40:134–140.

Oliveira, M. S., Prado, G., Abrantes, F. M., Santos, L. G., Veloso, T. 2002. Incidence of aflatoxins, deoxynivalenol and zearalenone in products commercialized in the cities of Minas Gerais state in 1998-2000. Revista do Instituto Adolfo Lutz 61:1–6.

Santos, J. S., Souza, T. M., Ono, E. Y. S., Hashimoto, E. H., Bassoi, M. C., Miranda, M. Z., Itano, E. N., Kawamura, O., Hirooka, E. Y. 2013. Natural occurrence of deoxynivalenol in wheat from Paraná State, Brazil and estimated daily intake by wheat products. Food Chemistry. 138:90–95.

Shephard, G. S., Berthiller, F., Burdaspal, F., Crews, C., Jonker, M. A., Krska, R., MacDonald, S., Malone, B., Maragos, C., Sabino, M., Solfrizzo, M., van Egmond, H. P., Whitaker, T. B. 2011. Developments in mycotoxin analysis: an update for 2009-2010. World Mycotoxin Journal. 4:3–28.

Tangni, E. K., Motte, J. C., Callebaut, A., Pussemier, L. 2010. Cross-reactivity of antibodies in some commercial deoxynivalenol test kits against some fusariotoxins. Journal of Agricultural and Food Chemistry. 58:12625–12633.

Tibola, C. T., Fernandes, J. M. C., Guarienti, E. M., Nicolau, M. 2015. Distribution of *Fusarium* mycotoxins in wheat milling process. Food Control. 53:91–95.

Tibola, C. T., Fernandes, J. M. C., Guarienti, E. M. 2016. Effect of cleaning, sorting and milling processes in wheat mycotoxin content. Food Control. 60:174–179.

Tralamazza, S. M., Bemvenuti, R. H., Zorzete, P., Garcia, F. S. 2016. Fungal diversity and natural occurrence of deoxynivalenol and zearalenone in freshly harvested wheat grains from Brazil. Food Chemistry. 196:445–450.

Varga, E., Glauner, T., Köppen, R., Mayer, K., Sulyok, M., Schuhmacher, R., Krska, R., Berthiller, F. 2012. Stable isotope dilution assay for the accurate determination of mycotoxins in maize by UHPLC-MS/MS. Analytical and Bioanalytical Chemistry. 402:2675–2686.

Xu, Y., Huang, Z-B., He, Q-H., Dengc, S-Z., Li, L-S., Li, Y-P. 2010. Development of an immunochromatographic strip test for the rapid detection of deoxynivalenol in wheat and maize. Food Chemistry 119: 834-839.

CHAPTER 2

Modeling of yield losses and risk analysis of fungicide profitability for managing Fusarium head blight in brazilian spring wheat

This chapter is published: Duffeck, M. R., Alves, K. S., Machado, F. J., Esker, P. D., and Del Ponte, E. M. 2019. Modeling yield losses and fungicide profitability for managing Fusarium head blight in brazilian spring wheat. Phytopathology. DOI:10.1094/PHYTO-04-19-0122-R.

ABSTRACT

Fusarium head blight (FHB) and wheat yield data were gathered from fungicide trials to explore their relationship. Thirty-seven studies over 9 years and 11 locations met the criteria for inclusion in the analysis: FHB index in the untreated check ≥ 5% and the range of index in a trial ≥ 4 percent points. These studies were grouped into two baseline yields, low ($Yl \leq$ 3,631 kg ha^{-1}) or high ($Yh >$ 3,631 kg ha^{-1}), defined based on the median of maximum yields across trials. Attainable (disease-free) yields and FHB index were predicted using a wheat crop and a disease model, respectively, in 280 simulated trials (10 planting dates in a 28-year period, 1980 to 2007) for the Passo Fundo location. The damage coefficient was then used to calculate FHB-induced yield loss (penalizing attainable yield) for each experiment. Losses were compared between periods defined as before and after FHB resurgence during the early 1990s. Disease reduction from the use of one or two sprays of a triazole fungicide (tebuconazole) was also simulated, based on previous meta-analytic estimates, and the response in yield was used in a profitability analysis. Population-average intercepts, but not the slopes, differed significantly between Yl (2,883.6 kg ha^{-1}) and Yh (4,419.5 kg ha^{-1}) baseline yields and the damage coefficients were 1.60%$^{-1}$ and 1.05%$^{-1}$, respectively. The magnitudes and trends of simulated yield losses were in general agreement with literature reports. The risk of not offsetting the costs of one or two fungicide sprays was generally higher (> 0.75) prior to FHB resurgence, but fungicide profitability tended to increase in recent years, depending on the year. Our simulations allowed us to reproduce trends in historical losses, and may be further adjusted to test the effect and profitability of different control measures (host resistance, other fungicides, etc.) on quality parameters such as test weight and mycotoxin contamination, should the information become available.

Keywords: crop losses, ecology and epidemiology, meta-analysis, *Triticum aestivum*, wheat scab

INTRODUCTION

Fusarium head blight (FHB) is a damaging disease of wheat and barley that affects yield and contaminates grains with mycotoxins produced by the fungus (Goswami and Kistler 2004, McMullen et al. 1997). FHB has been considered a resurgent problem worldwide since the early 1990s, with economic losses estimated around $2.7 billion in the United States over a two-decade period (McMullen et al. 2012). Similar patterns have been reported in wheat and barley regions in South America, where the frequency of FHB epidemics increased during the 1990s compared with the previous two decades (Del Ponte et al. 2009, Fernandes 1997, Moschini et al. 2013). Two hypotheses for the increase in FHB importance include the wider adoption of conservation tillage practices, as well as the increased use of corn-wheat rotations in North America and Europe (Landschoot et al. 2013, Schaafsma et al. 2005). In Brazil, modeling work also showed a significant contribution of climate variability to the resurgence of FHB based on analysis of a 50-year weather dataset for a location in southern Brazil (Del Ponte et al. 2009).

The occurrence and intensity of FHB epidemics are driven by the amount of airborne inoculum (primarily ascospores) dispersed from both within and outside the field, combined with humid conditions around anthesis (McMullen et al. 2012). Disease control is best achieved through the use of integrated practices, including cultural measures aimed at reducing local inoculum, the use of moderately resistant cultivars, and fungicide sprays (Wegulo et al. 2011). Although chemical fungicides, mainly of the triazole chemistry, have been recommended for controlling FHB, the efficacy levels are generally modest and influenced by the type of active ingredient, wheat cultivar, number of sprays, and application technology (Edwards and Godley 2010, Machado et al. 2017, Paul et al. 2008, Willyerd et al. 2012).

Coinciding with the resurgence of FHB, research conducted since the mid-1990s has focused on evaluating a range of control methods, including host resistance and fungicide sprays, all with the explicit goal to reduce mycotoxin contamination and protect wheat yields. Thus, these efforts have generated an increased amount of epidemiological data (McMullen et al. 2012, Machado et al. 2017, Shah et al. 2018). These large data sets have enabled the use of meta-analytic approaches to study the effects of fungicides on disease control, yield increase and mycotoxin reduction, as well as to explore empirical relationships between FHB index and wheat yield or mycotoxin levels (Madden and Paul 2009, Salgado et al. 2011, 2014, 2015). Meta-analysis has become standard for studying empirical relationships between disease and yield data for other diseases such as soybean rust (Dalla Lana et al. 2015), soybean white mold (Lehner et al. 2017) and soybean target spot (Edwards Molina et al. 2018).

The majority of studies on the relationship between FHB intensity and other variables, such as yield, test weight, and mycotoxin contamination have been conducted in the temperate regions in North American cropping conditions (Madden and Paul 2009, Salgado et al. 2011), where several abiotic and biotic conditions may differ from the subtropics of South America, including management practices (Del Ponte et al. 2015, Spolti et al. 2014). In North America, the relationship between FHB index and yield has been studied using field trial data obtained from multiple location-years to estimate damage coefficients (*Dc*) (percent reduction in yield per percent point increase in FHB index), which varied for winter wheat (Madden and Paul 2009, Salgado et al. 2015) and spring wheat (Madden and Paul 2009).

In Brazil, previous efforts to estimate yield losses due to FHB used a destructive random sampling of wheat heads in replicated nontreated plots of field experiments to estimate attainable yield (weight of disease-free spikes per sample weight) and actual yield (sample weight), with losses estimated in both absolute and relative terms. Using this method, mean yield losses for the period 1999 to 2010 were estimated at higher levels compared with 1984 to 1994 (Panisson et al. 2003, Reis et al. 1996, Reis and Carmona 2013). Since the early 2010s, significant yield reductions due to FHB have been reported in nontreated plots from a network of uniform fungicide trials (UFTs) (Santana et al. 2012, 2014, 2016a,b,c). A recent meta-analysis of the effect of fungicides on FHB control and yield using data from several sources, including peer-reviewed articles published since the year 2000 and the UFTs data from Brazil, explored yield responses and economic benefits from using fungicides. Results of that study suggested that a spray of a triazole fungicide could be profitable, depending on disease risk, but only taking yield responses, not wheat quality parameters, into account (Machado et al. 2017).

Although several studies have highlighted the impact of FHB on wheat in Brazil, the heterogeneity of the functional relationship between FHB intensity and wheat yield has not been explored for our conditions. Although knowledge is available for studies conducted in North America, it is not known whether the *Dc* are valid for our conditions of high and low baseline yields in spring wheat, a hypothesis that we tested in this study. Once the relationship is established, *Dc* could be useful as a non-destructive method for predicting FHB index and improving the risk assessment of economic losses due to FHB.

The primary objective of this study was to model the relationship between a single (critical-point) assessment of FHB index and wheat yield data obtained from independent studies in order to obtain estimates of *Dc* in a manner similar to other previously published studies (Edwards Molina et al. 2018, Lehner et al. 2017, Madden and Paul 2009, Salgado et al. 2015). A secondary objective was to use the *Dc* to reconstruct yield losses curves in a 28-year time series by coupling wheat yield and disease model, and further calculating the profitability of fungicide applications to reduce predicted yield loss, not the quality parameters, for a range of wheat prices and fungicide costs for each year.

MATERIALS AND METHODS

Data source, description, and criteria for inclusion

A systematic review of peer- and non-peer-reviewed articles and reports published after the year 2000 was conducted to identify studies reporting wheat yield and FHB intensity data in Brazil. In total, 29 publications reported results from trials conducted at 23 locations in southern Brazil spanning a 16-year period (2000 to 2016). Altogether, these locations comprise 90% of the total wheat production in Brazil (CONAB 2018). A portion of these data has been used in a previous study on the effect of fungicides on disease and yield (Machado et al. 2017). In brief, the studies were conducted following a standard protocol and regional recommendations of agronomic practices. FHB disease development was natural in all trials. The number of treatments evaluated in each trial ranged from 5 to 13 (average of 8.5 treatments/trial). All trials included a nontreated control. Fungicides were typically applied once at flowering (Zadok's scale 60 to 64) or twice, the first at flowering and the second 10 days later. The specific fungicide treatments and respective application rates varied among trials (Machado et al. 2017).

The disease response variable used in our study was FHB index (%), which represents the proportion of diseased spikelets in the sample of spikes (Paul et al. 2005, 2007). This index was assessed during the soft dough stage (Zadoks scale 85), prior to senescence. Plots were harvested and the mean yield (kilograms per hectare) was adjusted to 13% grain moisture. Data from all trials were inspected closely for both FHB index and yield, from which two criteria were defined to select trials for inclusion in the analysis: (i) FHB index in the untreated check of the trial should be at least 5% (usually the maximum FHB index in the trial) and (ii) the range of FHB index from the largest to smallest value was at least 4%. The latter criterion was adapted from a previous study that used 2% as the minimum range (Madden and Paul 2009). Twenty-seven trials did not meet these minimum criteria (FHB index values in the untreated check were mostly <5%) and were excluded from subsequent analyses. Influence analysis was also performed and five influential studies, or those that exert a very high influence on the overall results and distort the pooled effect (Viechtbauer and Cheung 2010), were identified and excluded (data not shown). Therefore, data were available for 37 trials reported in 18 studies (Supplementary Table 1).

Effect sizes and meta-analytic modeling

Intercepts (β_0) and slopes (β_1) (using ordinary least square regression modeling) were estimated for the relationship between FHB index and wheat yield at the trial level (Dalla Lana et al. 2015, Madden and Paul 2009). The interdecile (ID) range summarized the estimates of linear coefficients for each trial (Lehner et al. 2017, Madden and Paul 2009).

A multilevel (random coefficients) model was fitted to the yield-FHB index relationship, and the population-average intercept and slope were estimated as described elsewhere, assuming a linear relationship between FHB index and yield (Lehner et al. 2017, Madden and Paul 2009). The *lmer* function of the *lme4* package of R was used to estimate the mean effect based on the between-study variance and within-study variance and to predict the study-specific intercept (β_0) and slope (β_1) coefficients, also termed as best linear unbiased predictions (Lehner et al. 2017). A categorical baseline yield was created to represent trials with high yield (*Yh*) or low yield (*Yl*) based on the maximum yield achieved in the trial. We used the median of the maximum yield values across trials (3,631 kg ha^{-1}) as a threshold of baseline yield. Baseline yield was tested as a moderator variable in order to account for the heterogeneity in the intercept or slope of the yield-FHB index relationship. Wald-type tests were performed to determine whether the inclusion of the covariate in the model significantly affected the model coefficients. We obtained a *Dc* (percent reduction in yield in our study) to compare with coefficients determined in other studies. The *Dc* for the *Yl* and *Yh* production situations was calculated by dividing the estimated population-average slope ($\widehat{\beta}_1$) (kilograms per hectare per percentage) by the estimated population-average intercept ($\widehat{\beta}_0$) (kilograms per hectare) x 100 (Lehner et al. 2017, Madden and Paul 2009).

Simulation of yield losses

The *Dc* obtained from the meta-analysis was used to calculate actual yield, representing a reduction in attainable yield based on the predicted FHB index obtained by a disease simulation model for the same experiment (Del Ponte et al. 2005, Del Ponte et al. 2009). Percent yield loss was defined as the relative reduction of the attainable yield due to FHB. This was done over the entire time series, encompassing two periods defined as before (pre-1990) and after FHB resurgence in Brazil.

To simulate attainable yield, or the yield constrained only by factors like radiation, temperature, crop phenology, and physiology, as well as water and soil nutrients (Savary et al. 2018), we used a crop simulation model (cropsim-wheat) calibrated with the parameter for a spring wheat cultivar and soil profiles for the conditions of Passo Fundo (28°15’00”S and 52º25’12”W) (Lazzaretti et al. 2015). In total, 280 virtual experiments were simulated, combining 10 planting dates in a 28-year period (1980 to 2007). The 10 yearly runs were performed using historical daily weather data (minimum and maximum temperature, solar radiation, and rainfall) for a series of sowing dates from 1 to 30 June (spaced 3 days apart), which is the recommended sowing period for Passo Fundo. Starting on the heading date simulated by the wheat model, the FHB model predicted daily infection risks using daily records of rainfall, air relative humidity, and air temperature (Del Ponte et al. 2015). The daily risks were accumulated during the flowering period and further used to predict FHB severity index based on a linear regression model (Del Ponte et al. 2005). The approach was similar to a previous study that simulated FHB index (30 planting dates after June 1st) for a longer (50-year) dataset (Del Ponte et al. 2009). The attainable yields predicted by the crop model were penalized by the respective *Dc*, depending on the value of attainable yield, using the predicted value FHB index for each experiment. For example, for the sowing date of 6 June 1990, simulated yield and FHB severity were 3,645.38 kg ha^{-1} and 19.5%, respectively. For this situation, using the *Dc* for high yield, FHB reduced yield by 20.5% or an absolute reduction of 747.17 kg ha^{-1}.

To test for significance of the trends in yield loss predictions in the time series, as well as to test the effect of two sowing periods (sowing dates before or after June 15th), a generalized additive model (GAM) (Wood 2017) was fitted to the data using the *gam* function of *mgcv* R package (Wood 2017). The smooth terms estimated for each sowing period were compared at 5% significance.

Risk analysis and fungicide profitability

We were also interested in evaluating and comparing trends of fungicide profitability over the decades, especially before and after FHB resurgence. This analysis was conducted in several steps, with the values of the probability of not offsetting the costs of the fungicide sprays being obtained through a simulation. First, we used available meta-analytic estimates, both the mean and the uncertainty values, of control efficacy (C) for one or two sprays tebuconazole fungicide used in FHB management in Brazil (Machado et al. 2017). We assumed that C follows a uniform distribution given by:

$$C_{ij} = Uniform\,(a,b)_{ij} \tag{1}$$

where i the planting date and j is the year, totaling 280 simulations of control efficacy (10 planting dates in June) for one (1x) and two (2x) fungicide applications; a and b represent the lower (CI_L) and upper (CI_U) limits of the 95% confidence interval (CI) around C. The CIs ranged from 46.9 to 67.5% for one (1x) fungicide application, and from 44.3 and 60.7% for two (2x) fungicide applications (Machado et al. 2017).

A simulated C value was then used to update both the FHB index and yield in the fungicide-protected ($Yield_f$) plots. The mean yield difference (D) for one spray was given by the difference in yield between fungicide-protected ($Yield_f$) and no fungicide ($Yield_{nf}$) plots (Machado et al. 2017, Madden et al. 2016). The D from using two sprays was calculated by adding a simulated (normally distributed) mean yield gain from using a second spray of tebuconazole, as determined in the previous meta-analysis study (Machado et al. 2017) (Equation 2).

$$D_{ij} = \left(Yield_{f\,(ij)} - Yield_{nf\,(ij)}\right) + N(\mu,\ \sigma^2)_{ij} \tag{2}$$

where i the planting date and j the year; μ is 101.6 kg ha^{-1}, representing the mean yield difference between the 1x and 2x fungicide applications (Machado et al. 2017); and σ is 43.8, which was calculated based on the mean of the lower (CI_L) and upper (CI_U) limits of the 95% CI around D for one spray. Finally, the between-year variance σ^2_{year} was estimated based on the 10 values of D within each year, or across planting dates.

The estimates of D and σ^2_{year} were used to calculate the risk (probability) of not offsetting fungicide application cost (P_{loss}), taking both fungicide application cost (F_c = fungicide plus application costs) and the average wheat price (W_p) by year. This probability is the cumulative standard-normal function given by:

$$P_{loss} = 1 - \Phi\left(\frac{D - \frac{F_c}{W_p}}{\sqrt{\sigma^2_{year}}}\right) \tag{3}$$

where Φ is the cumulative standard-normal function (Machado et al. 2017, Paul et al. 2011).

Five F_c values (\$10 to \$30 ha^{-1}) and five W_p values (\$125 to \$225 ha^{-1}) were created to simulate 25 benefit-cost scenarios. The minimum and maximum F_c were defined based on previous studies in the region (Machado et al. 2017, Panisson et al. 2003, Reis et al. 1996). Likewise, the range of prices for W_p was defined based on published statistics for southern Brazil (Brum et al. 2005, CEPEA 2018). A summary value of P_{loss} was calculated for each year by taking the mean (and respective 95% CI) of all combinations of benefit-cost scenarios and planting dates (n=250). Line and time series plots were created to visualize the magnitude of the probabilities for each benefit-cost scenario as well as probabilities over time considering one and two sprays. A simplified diagram depicting the steps of our modeling approach is presented in Figure 1.

Data availability and reproducibility

All data gathered for this study as well as the fully annotated computational codes, prepared in R Markdown, were organized as a research compendium publicly available at https://osf.io/3h9ye/. A website was generated to facilitate visualization of the commented scripts (https://emdelponte.github.io/paper-FHB-yield-loss/). All analyses were conducted in R statistical software (R Core Team 2013).

RESULTS

Yield-severity relationship

Simple linear regression model. Across all studies, FHB index in the nontreated plots ranged from 5 to 89.3% (mean = 23.6%). In 19 trials (51.3%), FHB index was >10%, which is considered a threshold for epidemics of concern (Shah et al. 2013). Maximum yield ranged from 1,460 to 5,692 kg ha^{-1} (mean = 3,676 kg ha^{-1}).

Intercepts and slopes varied considerably within each baseline yield category. For Yl, intercepts ranged from 1,708 to 4,299 kg ha^{-1}, with an overall mean of 2,977 kg ha^{-1}, and slopes ranged from 43.5 to –381.9 kg ha^{-1}, with an overall mean of –71.8 kg ha^{-1} (Fig. 2A). For the Yh crop situation, intercepts ranged from 3,000 to 6,097 kg ha^{-1}, with an overall mean of 4,473 kg ha^{-1} (Supplementary Table 2), and slopes ranged from 52.9 to –220.2 kg ha^{-1}, with an overall mean of –60.9 kg ha^{-1} (Fig. 2A). All but two slopes (β_1) were negative, one in each yield group, indicating that yield generally decreased as the FHB index increased.

Random coefficients model. The multilevel model that considers the random effect of individual trials on both the intercept and slope provided a better fit to the data, compared with slope- or intercept-only models (data not shown), across the 37 trials. Mixed model-derived intercept and slope values varied among individual studies (Supplementary Table 2). The study-specific intercepts ($\widehat{\beta}_0$) ranged from 1,620 to 5,786 kg ha^{-1} (ID = 2,470.6 kg ha^{-1}) and study-specific slope ($\widehat{\beta}_1$) values ranged from 4.5 to 112.5 kg ha^{-1} %$^{-1}$ (ID = 76.8). The estimates of population-average of the intercept and slope were $\widehat{\beta}_0$ = 3,645.3 kg ha^{-1} (standard error [SE] = 164.6) and $\widehat{\beta}_1$ = 49.1 kg ha^{-1} %$^{-1}$ (SE = 7.4), respectively. Both parameters were statistically different from 0 ($P < 0.001$).

The best model, defined based on likelihood ratio test ($P < 0.001$) and lowest Akaike information criterion (4,595), included baseline yield as a covariate. A Wald-type test showed that effect of baseline yield on the slope (ς) did not differ from 0 ($P > 0.80$), suggesting that the slope was not affected by baseline yield. However, as expected, effect of baseline yield on the intercept (ξ) differed from zero ($P < 0.01$). The study-specific parameters estimated were $\widehat{\beta}_0$ = 4,419.5 kg ha^{-1} (SE = 148.3), $\widehat{\beta}_1$ = 46.3 kg ha^{-1} %$^{-1}$ (SE = 9.6), and $\widehat{\xi}_{low}$= –1,535.9 kg ha^{-1} (SE = 209.4). Population-average predictions were then split into two regression equations to account for the effects of the covariate on the intercept. In addition, ξ was a dummy variable of value of zero for high yield (i.e., $\widehat{\xi}_{high}$= 0). Because of the negative value for $\widehat{\xi}_{low}$, wheat yield was, on average, 1,535.9 kg ha^{-1} lower than in the high-yield trials. The population-average predictions of yield are given by:

$$Yield = \widehat{\beta}_0 + \widehat{\xi} - \widehat{\beta}_1 \ x \ FHB_{index} \tag{4}$$

which gives the following equations for high and low wheat yield:

$$Yh = 4,419.5 + 0 - 46.3 \ x \ FHB_{index} \tag{5}$$

$$Yl = 4,419.5 + (-1,535.9) - 46.3 \ x \ FHB_{index} \tag{6}$$

The population-average (and respective 95% CI_s) predictions for the inclusion of the covariate baseline yield in the model are shown in Figure 2B. Because the estimated slope did not differ between low- and high-yield class, a reduction of 1,000 kg ha^{-1} would occur at an

FHB index of 21.6% for both yield classes. The calculated *Dc* for high-yield and low-yield baselines were 1.05%$^{-1}$ and 1.6%$^{-1}$, respectively.

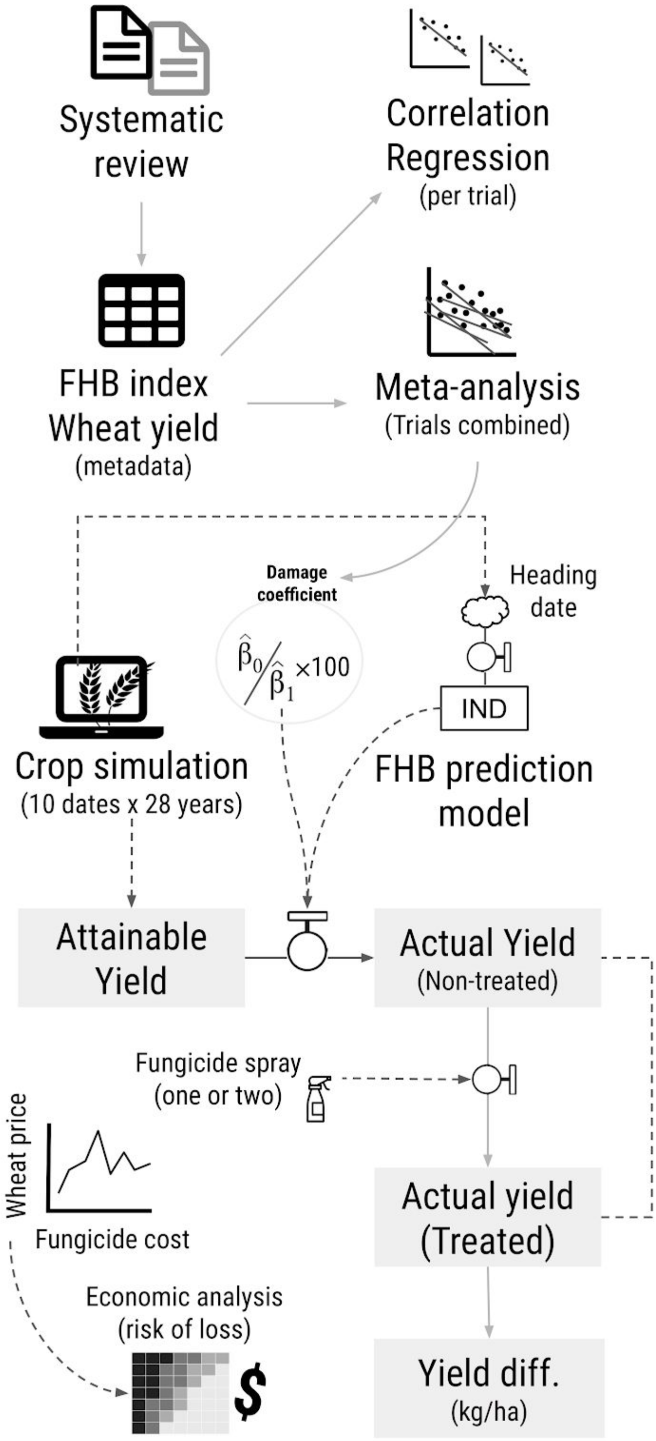

Fig. 1. Simplified diagram depicting the steps to model yield losses caused by Fusarium head blight in spring wheat in Brazil and assess the economic impact of fungicide application for preventing the losses

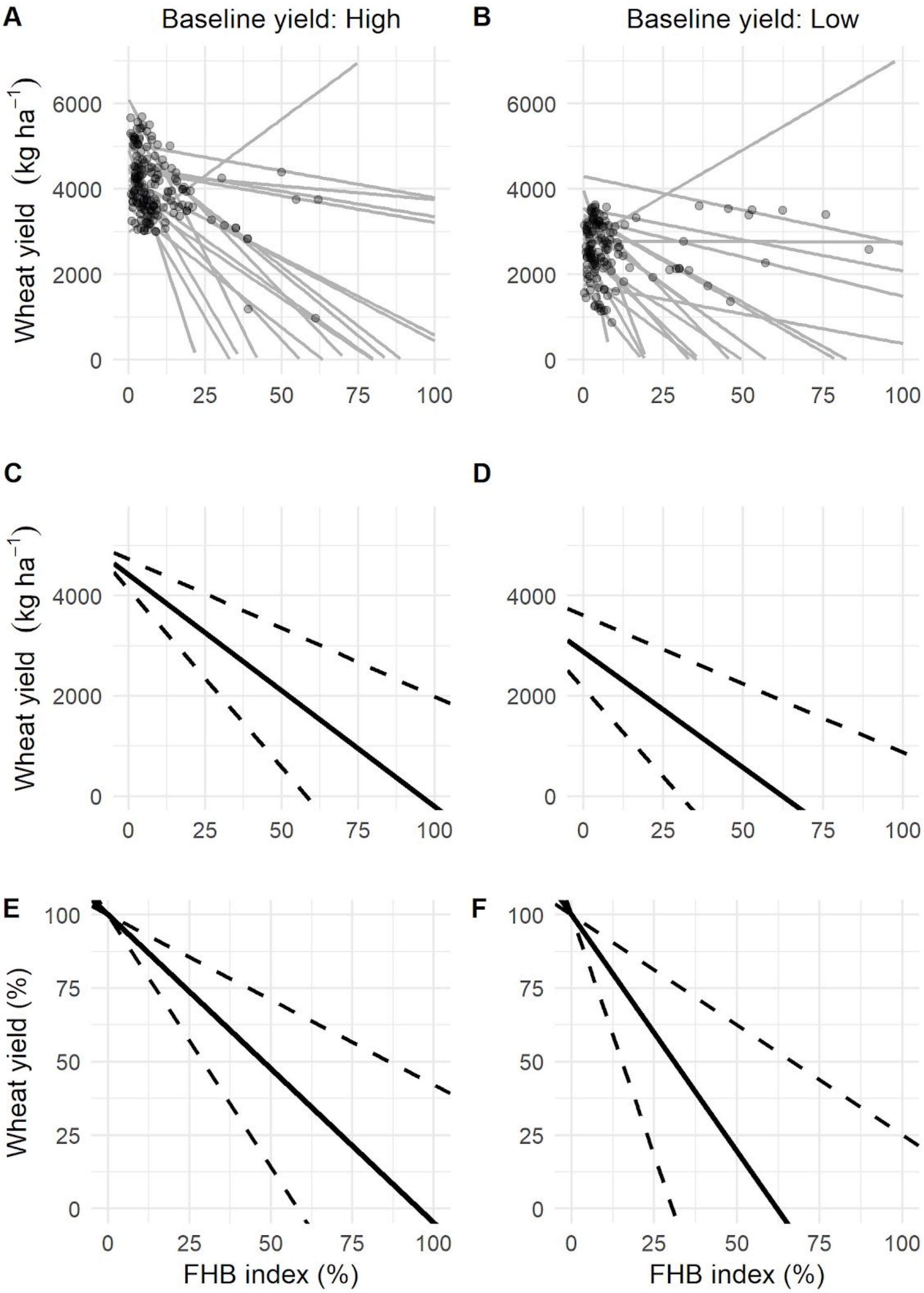

Fig. 2. Results for the fit of a simple linear model to wheat yield (kilograms per hectare) and FHB index (percent) for both crop situations of **A**, high yield or **B**, low yield. Results for the fit of a random-coefficient model to wheat yield (kilograms per hectare) and FHB index (percent) with the population-average predictions (thick solid black) of absolute (kilograms per hectare) and relative (percent) yield and respective 95% confidence interval (thick dashed black) for **C** and **E**, high yield or **D** and **F**, low yield.

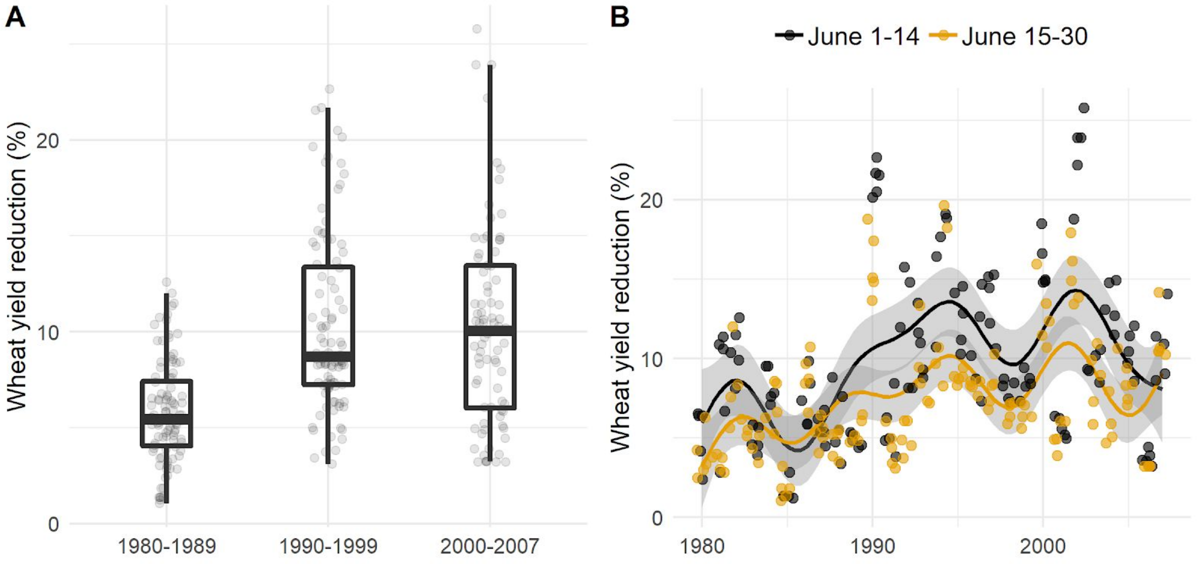

Fig. 3. Relative yield loss estimated by a phenology-based model in a 28-year period (1980 to 2007) for Passo Fundo, RS, Brazil. **A**, Box plots represent the variability of the relative losses within three time periods. **B**, Relative yield loss represents the estimated values encompassing the sowing dates before and after 15 June, with the fitted smoothing lines of the generalized additive model for both sowing periods.

Model-predicted yield losses

All simulated values of attainable yields were greater than our threshold used to define the category of baseline yield (3,631 kg ha^{-1}). Hence, Dc for high-yield conditions (Dc_h = 1.05%$^{-1}$) to obtain actual yields (FHB-induced loss). Yield losses varied both intra- and inter annually but especially when comparing the decadal periods. Prior to 1990, yield losses from 1.0% to 12.6% (mean = 5.8%). For the two subsequent periods, 1990 to 1999 and 2000 to 2007, losses ranged from 3.1% to 22.6% (mean = 10.3%) and from 3.2% to 25.8% (mean = 10.2%), respectively (Fig. 3A). A significant upward trend in yield loss was detected in the time series ($P < 0.01$). The differences between the smooth terms of the model fitted using two planting date periods as covariates, representing the first and second half of June, were not significant ($P > 0.05$), although numerically, the reductions appeared higher for the first plantings of June (Fig. 3B).

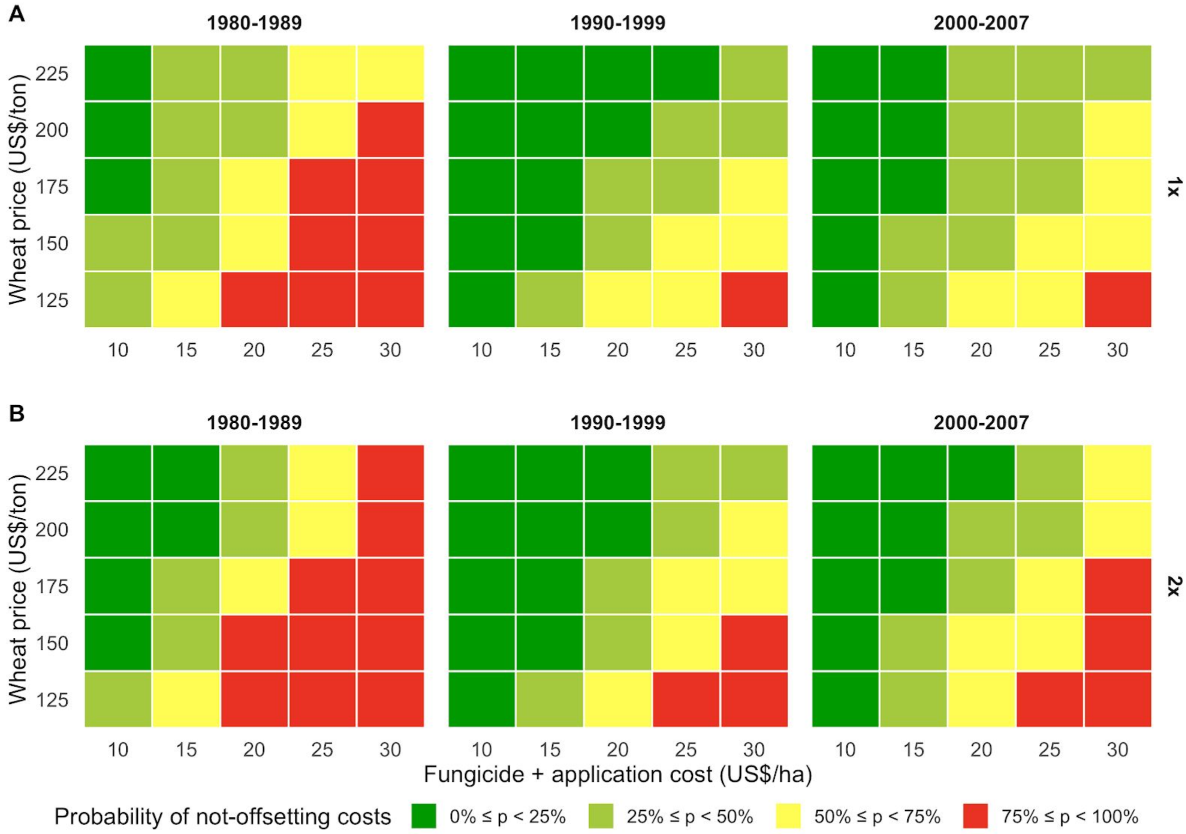

Fig. 4. Probability categories of not offsetting on fungicide investment for different scenarios of wheat prices and fungicide costs (product price + operational costs) for **A**, one or **B**, two sprays (first spray at early to mid flowering and a second 7 to 10 days later) for Fusarium head blight (FHB) control. Probability for each time period, encompassing the periods prior to (1980 to 1989) and after (1990 to 1999 and 2000 to 2007) FHB resurgence, was calculated using the estimates of the mean difference (*D*), and respective between-year standard deviation obtained from the yield estimates with and without fungicide application for 28 growing seasons in Brazil.

Fungicide profitability

The probability of loss (P_{loss}) was generally greater prior to 1990 compared with the other years (Fig. 4A). For this period, $P_{loss} > 75$ and 50% were calculated for 8 and 14 benefit-cost scenarios, respectively. After 1989, these same probabilities were reduced to only one and six scenarios (Fig. 4). The number of sprays appeared to have little effect on P_{loss}. For example, for an average benefit-cost scenario (W_p = U.S.$175/ton and F_c = U.S.$20/ha), P_{loss} was 59, 26 and 35% for 1980 to 1989, 1990 to 1999, and 2000 to 2007, respectively. When using two sprays, the P_{loss} values for the same scenarios were 59, 33 and 34%. The lack of a clear benefit from two sprays compared with one spray is apparent in the series of the yearly mean and uncertainty of P_{loss} (Fig. 5). Prior to 1990, the mean P_{loss} was > 50% in 7 of 10 years, decreasing considerably afterward. After 1989, P_{loss} > 60% was estimated for only 3 of 18 years. For this same period, P_{loss} was mostly higher for two sprays compared with one.

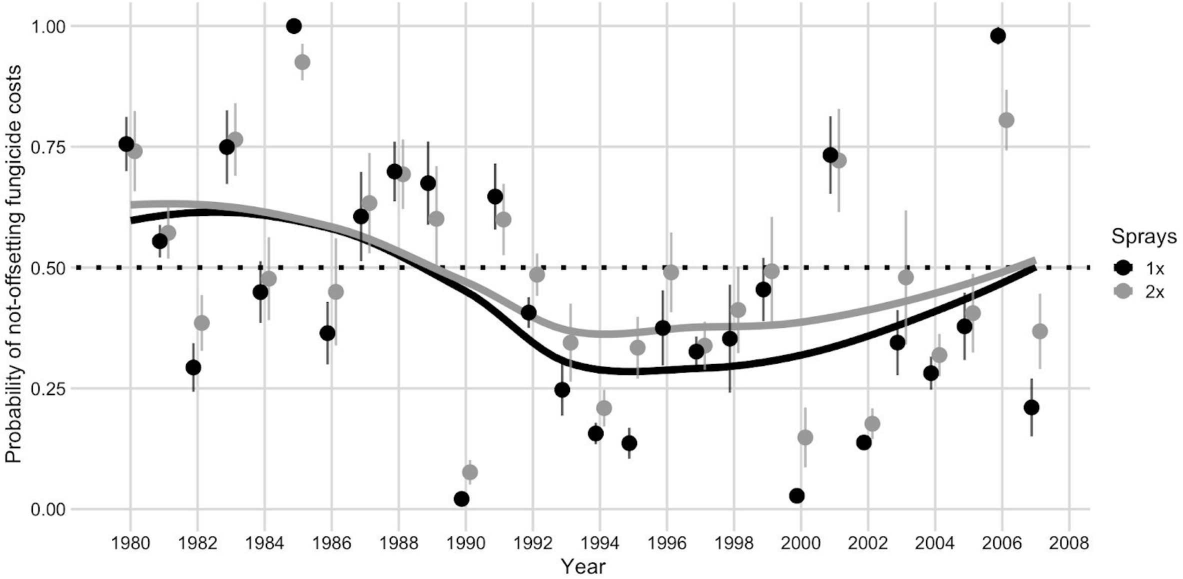

Fig. 5. Temporal series of the mean probability, and respective 95% confidence interval, of not offsetting the costs (probability of loss) for benefit-cost ratios (wheat price/fungicide cost in U.S. dollars) ranging from 5 to 15. Probabilities were calculated for both one and two fungicide applications.

DISCUSSION

Our modeling of yield losses confirms that FHB is an important yield-limiting disease of spring wheat in the subtropics, especially after the 1990s, when damaging epidemics became more frequent (Casa et al. 2004, Panisson et al. 2003, Del Ponte et al. 2009, Spolti et al. 2015). This study also provided numerical estimates of yield loss trends due to FHB in a temporal series of 28 years by linking the meta-analytical estimate parameters to a crop and a disease model that estimate yield and disease, respectively.

Our meta-analytic estimate of yield (population-average intercept) was similar to the estimates for the same wheat type (spring) in the United States using a similar statistical approach (Madden and Paul 2009). The variance in the parameter estimates of meta-regression models may be reduced by inclusion of additional covariates in the model (Madden and Paul 2009). Although baseline yield did not affect the population-average slope in our study, intercept values differed between trials representing a low or a high baseline yield, which was not an unexpected result. In fact, a similar pattern was found in the U.S.

study (Madden and Paul 2009), where population-average slopes did not differ between spring and winter wheat, which typically represent low- and high-yielding wheat, respectively. Given that intercept values varied among the studies, the magnitude of the *Dc*, which depends on both coefficients, differed between baseline yields but was rather similar between countries for an equivalent baseline yield. For example, for our high-yielding spring wheat, the *Dc* calculated in our study ($1.05\%^{-1}$) was very close to an overall (unconditioned) *Dc* calculated for spring wheat in the U.S. ($0.99\%^{-1}$) (Madden and Paul 2009). In a further U.S. study using data for soft red winter wheat, the *Dc* due to FHB was slightly greater ($1.17\%^{-1}$) and neither wheat cultivar nor the presence of foliar disease Stagonospora leaf blotch (SLB) significantly affected the slopes (Salgado et al. 2015).

The increased *Dc* for subtropical spring wheat in the low-yielding situation ($1.60\%^{-1}$) may be due to the additional effect of unobserved biotic and abiotic stresses that weaken the plant and its defenses against FHB (Al-Khatib and Paulsen 1999, Asseng et al. 2015, Buck et al. 2007, Sharma et al. 2007). In addition, given that we used data from fungicide trials, we cannot rule out the potential effect of fungicides, especially demethylation inhibitor + quinone outside inhibitor mixtures applied during flowering, that control flag-leaf diseases such as tan spot in Brazil. This can affect the *Dc* due to reduction of the impact of foliar disease, thus protecting yield (Blandino et al. 2006, 2011, Wegulo et al. 2011).

Using a model-based approach that linked disease and attainable yield predictions with actual weather information as an input for each model and estimating disease-induced losses based on the *Dc*, yield loss trends were quantified at magnitudes that matched earlier literature reports. For example, a quantitative review of yield losses, measured using a destructive sampling approach in fungicide trials spanning 21 years (1984 to 2010) in southern Brazil, reported that average losses due to FHB shifted from 5.4% during the period 1984 to 1994 to 21.6% for the period 2000 to 2010 (Reis et al. 1996, Reis and Carmona 2013). Our simulations confirmed this shift by showing that FHB changed from a secondary disease to an economically damaging disease of wheat after the 1990s. Several reasons explain the reemergence of FHB worldwide, including the wider adoption of conservation tillage practices and increase of wheat-corn rotations, which have been proposed as important risk factors (Dill-Macky and Jones 2000, Schaafsma et al. 2001, McMullen et al. 1997). Although cultural practices aimed at reducing local inoculum are recommended in those studies, current evidence supports the claim that potential effects of local residue management may be overshadowed by other risk factors in Brazil that include (i) overwintering of several grasses that harbor the pathogen (Reis 1990); (ii) the presence of airborne inoculum throughout the year (Reis 1988, Fernandes 1997); and (iii) non-influence of the presence of within-field maize residues on FHB risk (Spolti et al. 2015). Collectively, results from our simulations, which are based only on seasonal weather and not taking into account changes in agronomic practices (that promote inoculum build-up), confirm that the re-emergence of FHB in Brazil has been more likely due to variation in seasonal weather patterns (Del Ponte et al. 2009) rather than cultural practices (Fernandes 1997). The information is critical for breeders, who should intensify efforts to incorporate genetic resistance, which has proven more effective to control the disease when combined with fungicides, a practice that has been widely adopted not only to protect yield but mainly to reduce mycotoxin contamination (McMullen et al. 2012, Wegulo et al. 2015).

Prior to the early 1990s, the profitability of a single fungicide spray during flowering for FHB control was questioned due the sporadic nature and mostly mild FHB epidemics in the preceding decades (Picinini and Fernandes 1998, Reis et al. 1996). However, accurate yield loss estimates, using a destructive sampling approach during the early 2000s, contradicted the previous recommendation and showed economic benefits from using one fungicide spray at full flowering (Panisson et al. 2003). Our model-based estimates of yield losses and fungicide profitability showed that the risk of not offsetting a single fungicide spray was predominantly high prior to the resurgence (pre-1990s), as shown by the low frequency of more profitable conditions, which is agreement with current recommendation for the period (Reis et al. 1996). The profitability tended to increase in general after the 1990s, when a single fungicide spray (but not often two sprays) was more likely to pay off. Caution is needed in interpretation because, due to current lack of knowledge, we are not taking into account the effect of fungicide sprays on other traits affected by FHB such as test weight and mycotoxin content, which downgrade grain value (Salgado et al. 2014, 2015).

We found that trends in yield loss were not affected by planting period, although it is expected that there may be annual variation and reports of increasing risk of FHB for later planting dates (after June 15th) compared to prior dates (Lima et al. 2005, 2006). Therefore, it is difficult to ascertain any general recommendation on planting dates for managing FHB, though it is important to mention that our disease model does not take into account an increase in airborne inoculum levels, which tend to peak later in the season (Del Ponte et al. 2009). Given the dependence of FHB epidemics on weather conditions during a relatively short period (flowering) (Del Ponte et al. 2005), diversification of planting dates has been proposed as a way to minimize risk through an escape mechanism (Reis and Carmona 2013).

Indeed, our simulations showed significant within-year variations in yield losses, varying in magnitude across the years.

The framework developed in this study proved useful to quantify the impact and highlight the importance of FHB as a yield-limiting disease. The model parameters and predicted responses matched quite well results from other countries with regards the impact of the disease on yield as well as historical information on the impact of FHB in Brazil. As long as information on the impact of FHB on quality parameters that downgrade wheat and the effectiveness of control methods targeting them are available for the region, further economic analysis may provide even more useful estimates. However, considering only losses in the yield, our simulations confirm that one spray of tebuconazole is generally profitable in the current scenario, except during years that are not conducive for FHB, which are less likely in the case of Brazil, as shown by our FHB predictions. Although it may be argued that the use of chemicals that are more effective than tebuconazole may provide better response in yield, they are generally more expensive, and thus, not dramatically affect profitability. Under the simulated scenarios that used the available meta-analytic estimate for the mean increment in yield from using a second spray of tebuconazole, we found that the profitability was not affected. Situations at which a second fungicide spray is worth the cost should be better explored by considering different chemicals and cultivar resistance and their effect on quality parameters that downgrade grain quality such as test weight and mycotoxin contamination.

ACKNOWLEDGMENTS

We thank A. Lazzaretti and J. M. Fernandes for providing simulated yield data from the AgroWEB system. Research conducted by Paul Esker was also supported by the USDA National Institute of Food and Federal Appropriations under Project PEN04660 and Accession number 1016474. First and Last authors are thankful to CAPES and CNPq for the scholarship and research fellow support, respectively.

REFERENCES

Asseng, S., Ewert, F., Martre, P., Rötter, R. P., Lobell, D. B., Cammarano, D., et al. 2015. Rising temperatures reduce global wheat production. Nat. Clim. Change. 5:143–147.

Al-Khatib, K., and Paulsen, G. M. 1999. High-temperature effects on photosynthetic processes in temperate and tropical cereals. Crop Sci. 39:119.

Blandino, M., Minelli, L., and Reyneri, A. 2006. Strategies for the chemical control of Fusarium head blight: Effect on yield, alveographic parameters and deoxynivalenol contamination in winter wheat grain. Eur. J. Agron. 25:193–201.

Blandino, M., Pascale, M., Haidukowski, M., and Reyneri, A. 2011. Influence of agronomic conditions on the efficacy of different fungicides applied to wheat at heading: effect on flag leaf senescence, Fusarium head blight attack, grain yield and deoxynivalenol contamination. Ital. J. Agron. 6:32.

Brum, A. L.; Heck, C. R. 2005. A economia do trigo no Rio Grande do Sul: breve histórico do cereal na economia do estado. Revista Análise. 16:29–44.

Buck, H. T., Nisi, J. E., Salomón, N. 2007. Wheat production in stressed environments. Springer, Dordrecht, NL.

Casa, R. T., Bogo, A., Moreira, É. N., and Kuhnem Junior, P. R. 2007. Época de aplicação e desempenho de fungicidas no controle da giberela em trigo. Ciênc. Rural. 37:1558–1563.

Casa, R. T., Reis, E. M., Blum, M. M. C., Bogo, A., Scheer, O., and Zanata, T. 2004. Danos causados pela infecção de *Gibberella zeae* em trigo. Fitopatol. Bras. 29:289–293.

CEPEA. 2018. Centro de Estudos Avançados em Economia Aplicada. Preço médio do trigo CEPEA/ESALQ. Piracicaba, SP. https://www.cepea.esalq.usp.br/br/indicador/trigo.aspx.

CONAB, 2018. Companhia Nacional de Abastecimento. Acompanhamento de safra brasileira de grão. Brasília, DF. https://www.conab.gov.br/info-agro/safras

Dalla Lana, F., Ziegelmann, P. K., Maia, A. de H. N., Godoy, C. V., and Del Ponte, E. M. 2015. Meta-analysis of the relationship between crop yield and soybean rust severity. Phytopathology. 105:307–315.

Del Ponte, E. M., Fernandes, J. M. C., and Pavan, W. 2005. A risk infection simulation model for Fusarium head blight of wheat. Fitopatol. Bras. 30:634–642.

Del Ponte, E. M., Fernandes, J. M. C., Pavan, W., and Baethgen, W. E. 2009. A model-based assessment of the impacts of climate variability on Fusarium head blight seasonal risk in southern Brazil. J. Phytopathol. 157:675–681.

Del Ponte, E. M., Spolti, P., Ward, T. J., Gomes, L. B., Nicolli, C. P., Kuhnem, P. R., et al. 2015. Regional and field-specific factors affect the composition of Fusarium head blight pathogens in subtropical no-till wheat agroecosystem of Brazil. Phytopathology. 105:246–254.

Deuner, C. C. 2009. Eficácia agronômica de fungicidas no controle de giberela na cultura do trigo. Pages 269-274 in: Controle de doenças em plantas. FUNDACEP 1993-2008. Resultados de pesquisa. Acervo histórico. Divulgação técnica N° 3. Cruz Alta.

Dill-Macky, R., and Jones, R. K. 2000. The effect of previous crop residues and tillage on Fusarium head blight of wheat. Plant Dis. 84:71–76.

Edwards, S. G., and Godley, N. P. 2010. Reduction of Fusarium head blight and deoxynivalenol in wheat with early fungicide applications of prothioconazole. Food Addit. Contam. Part A. 27:629–635.

Edwards Molina, J. P., Paul, P. A., Amorim, L., da Silva, L. H. C. P., Siqueri, F. V., Borges, E. P., Campos, H. D., Venancio, W. S., Meyer, M. C., Martins, M. C., Balardin, R. S., Carlin, V. J., Grigolli, J. F. J., Belufin, L. M. de R., Godoy, C. V. 2018. Effect of target spot on soybean yield and factors affecting this relationship. Plant Pathol. 68:94–106.

Feksa, H. R., Gardiano, C. G., Duhatschek, B., Proença, C., and Tessmann, D. J. 2014a. Ensaio manejo químico de giberela na cultura do trigo cultivar BRS Guamirim. In: Proceedings of Reunião da comissão brasileira de pesquisa de trigo e triticale (CD-ROM).

Feksa, H. R., Gardiano, C. G., Duhatschek, B., Proença, C., and Tessmann, D. J. 2014b. Ensaio manejo químico de giberela na cultura do trigo cultivar CD 105. In: Proceedings of Reunião da comissão brasileira de pesquisa de trigo e triticale (CD-ROM). Embrapa Trigo, Passo Fundo, Brazil.

Feldmann, N.A., Mühl, F.R., Hahn, L., Hoffmann, J.T.R., Klein, R., Zambiazi, M.P., Martini, A., Jantsch, M., Lima, A., 2014. Avaliação de fungicidas no controle de giberela do trigo. Anais eletrônicos da VIII Reunião da Comissão Brasileira de Pesquisa de Trigo e Triticale. Canela, 2014. CD - ROM.

Fernandes, J. M. C. 1997. As doenças das plantas e o sistema de plantio direto. Revisão Anual de Patologia de Plantas. 5:317–352.

Goswami, R. S., and Kistler, H. C. 2004. Heading for disaster: *Fusarium graminearum* on cereal crops. Mol. Plant Pathol. 5:515–525.

Guterres, C. W., Bruinsma, J. da S., Seidel, G. 2015. Efficacy of fungicides for FHB control and reduction of deoxynivalenol in wheat. In: Proceedings of 5th International symposium on Fusarium head blight. Florianópolis, 2015. CD-ROM.

Landschoot, S., Audenaert, K., Waegeman, W., De Baets, B., and Haesaert, G. 2013. Influence of maize–wheat rotation systems on Fusarium head blight infection and deoxynivalenol content in wheat under low versus high disease pressure. Crop Prot. 52:14–21.

Lazzaretti, A. T., Fernandes, J. M., Pavan, W. 2015. Calibração do cropsim-wheat para simulação do desenvolvimento e rendimento de grão de trigo no Sul do Brasil. Rev. Bras. Ciênc. Agrár. - Braz. J. Agric. Sci. 10:356–364.

Lehner, M. S., Pethybridge, S. J., Meyer, M. C., and Del Ponte, E. M. 2017. Meta-analytic modelling of the incidence-yield and incidence-sclerotial production relationships in soybean white mould epidemics. Plant Pathol. 66:460–468.

Lima, M. I. P. M., Só e Silva, M., Schereen, P. L., Del Duca, I. J. A., Pires, J. L., Nascimento, A., Jr. 2005. Avaliação de giberela em genótipos de trigo do ensaio estadual de cultivares, na região de Passo Fundo, em 2004. Documento online 52. Passo Fundo, Embrapa Trigo, 11p. http://www.cnpt.embrapa.br/biblio/do/p_do52.htm.

Lima, M. I. P. M., Só e Silva, M.; Caierão, E., Schereen, P. L., Del Duca, I. J. A., Pires, J. L., 2006. Avaliação de giberela em genótipos de trigo do ensaio estadual de cultivares, na região de Passo Fundo, em 2005. Documento online 66. Passo Fundo, Embrapa Trigo, 7p. http://www.cnpt.embrapa.br/biblio/do/p_do66.htm.

Machado, F. J., Santana, F. M., Lau, D., and Del Ponte, E. M. 2017. Quantitative review of the effects of triazole and benzimidazole fungicides on Fusarium head blight and wheat yield in Brazil. Plant Dis. 101:1633–1641.

Madden, L. V., and Paul, P. A. 2009. Assessing heterogeneity in the relationship between wheat yield and Fusarium head blight intensity using random-coefficient mixed models. Phytopathology. 99:850–860.

Madden, L. V., Piepho, H.-P., and Paul, P. A. 2016. Statistical models and methods for network meta-analysis. Phytopathology. 106:792–806.

Maffini, F. S., Arbugeri, F. E., Canova, E., Dahmer, J., Pinto, F. F., Ebone, A., Uebel, J. D., Balardin, R. S. 2012. Programas de controle químico de giberela (*Fusarium graminearum*) na cultura do trigo. In: XVI Simpósio de Ensino, Pesquisa e Extensão. 2012. (CD-ROM).

McMullen, M., Bergstrom, G., De Wolf, E., Dill-Macky, R., Hershman, D., Shaner, G., Van Sanford, D. 2012. A unified effort to fight an enemy of wheat and barley: Fusarium head blight. Plant Dis. 96:1712–1728.

McMullen, M., Jones, R., and Gallenberg, D. 1997. Scab of wheat and barley: a re-emerging disease of devastating impact. Plant Dis. 81:1340–1348.

Moschini R. C., Martínez, M. I. and Sepulcri M.G. 2013. Modeling and forecasting systems for Fusarium head blight and deoxynivalenol content in wheat in Argentina. In: Alconada Magliano, T. M. and Chulze, S. N. (Eds.). Fusarium head blight in Latin America. Springer, The Netherlands pp. 205–227.

Panisson, E., Reis, E. M., and Boller, W. 2002. Efeito da época, do número de aplicações e de doses de fungicida no controle da giberela em trigo. Fitopatol. Bras. 27:489–494.

Panisson, E., Reis, E. M., and Boller, W. 2003. Quantificação de danos causados pela giberela em cereais de inverno, na safra 2000, em Passo Fundo, RS. Fitopatol. Bras. 28:189–192.

Paul, P. A., Lipps, P. E., Hershman, D. E., McMullen, M. P., Draper, M. A., and Madden, L. V. 2008. Efficacy of triazole-based fungicides for Fusarium head blight and deoxynivalenol control in wheat: a multivariate meta-analysis. Phytopathology. 98:999–1011.

Paul, P. A., Lipps, P. E., and Madden, L. V. 2005. Relationship between visual estimates of Fusarium head blight intensity and deoxynivalenol accumulation in harvested wheat grain: a meta-analysis. Phytopathology. 95:1225–1236.

Paul, P. A., Lipps, P. E., Hershman, D. E., McMullen, M. P., Draper, M. A., and Madden, L. V. 2007. A quantitative review of tebuconazole effect on Fusarium head blight and deoxynivalenol content in wheat. Phytopathology 97:211-220.

Paul, P. A., Madden, L. V., Bradley, C. A., Robertson, A. E., Munkvold, G. P., Shaner, G., et al. 2011. Meta-analysis of yield response of hybrid field corn to foliar fungicides in the U.S. Corn Belt. Phytopathology. 101:1122–1132.

Picinini, E. C., and Fernandes, J. M. C. 1998. Avaliação de fungicidas no controle de giberela em trigo. Fitopatol Bras. 23:270.

Pizolotto, C. A., and Boller, W. 2015. Spray nozzles and frequency of fungicides applications to the control of Fusarium head blight in wheat. In: Proceedings of 5th International symposium on Fusarium head blight. Florianópolis, 2015. CD-ROM.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Reis, E. M. 1988. Quantificação de propágulos de *Gibberella zeae* no ar através de armadilhas de esporos. Fitopatol Bras 13:324–327.

Reis E. M. 1990. Perithecial formation of *Gibberella zeae* on senescent stems of grasses under natural conditions. Fitopatol Bras 15:52-54.

Reis, E. M., and Carmona, M. A. 2013. Integrated disease management of Fusarium head blight. Pages 159–173. In: Fusarium head blight in Latin America. Magliano, T. M. A., Chulze, S. N, eds. Springer, Dordrecht, NL.

Reis, E. M., Blum, M. M. C., Casa, R., Medeiros, C. A., 1996. Grain losses caused by the infection of wheat heads by *Gibberella zeae* in southern Brazil, from 1984 to 1994. Summa Phytopathol. 22:134-137.

Salgado, J. D., Madden, L. V., and Paul, P. A. 2014. Efficacy and economics of integrating in-field and harvesting strategies to manage Fusarium head blight of wheat. Plant Dis. 98:1407–1421.

Salgado, J. D., Madden, L. V., and Paul, P. A. 2015. Quantifying the effects of Fusarium head blight on grain yield and test weight in soft red winter wheat. Phytopathology. 105:295–306.

Salgado, J. D., Wallhead, M., Madden, L. V., and Paul, P. A. 2011. Grain harvesting strategies to minimize grain quality losses due to Fusarium head blight in wheat. Plant Dis. 95:1448–1457.

Santana, F. M., Lau, D., Aguilera, J. G., Sbalcheiro, C. C., Feksa, H., Floss, L. G., and Guterres, C. W. 2016a. Eficiência de fungicidas para controle de *Gibberella zeae* em trigo: resultados dos Ensaios Cooperativos - Safra 2013. Comunicado Técnico 362. Embrapa Trigo, Passo Fundo, Brazil.

Santana, F. M., Lau, D., Cargnin, A., Seixas, C. D. S., Schipanski, C. A., Feksa, H. R., Wesp, C., Blum, M., and Bassoi, M. C. 2014. Eficiência de fungicidas para controle de giberela em trigo: resultados dos ensaios cooperativos - safra 2012. Comunicado Técnico 336. Embrapa Trigo, Passo Fundo, Brazil.

Santana, F. M., Lau, D., Maciel, J. L. N., Cargnin, A., Seixas, C. D. S., Bassoi, M. C., Schipanski, C. A., Feksa, H., Casa, R. T., Wesp, C., Navarini, L., and Blum, M. 2012. Eficiência de fungicidas para controle de giberela em trigo: resultados dos ensaios cooperativos - safra 2011. Comunicado Técnico 23. Embrapa Trigo, Passo Fundo, Brazil.

Santana, F. M., Lau, D., Sbalcheiro, C. C., Feksa, H., Guterres, C. W., and Venâncio, W. S. 2016b. Eficiência de fungicidas para controle de *Gibberella zeae* em trigo: resultados dos Ensaios Cooperativos - Safra 2015. Comunicado Técnico 368. Embrapa Trigo, Passo Fundo, Brazil.

Santana, F. M., Lau, D., Sbalcheiro, C. C., Schipanski, C. A., Seixas, C. D. S., Feksa, H., Floss, L. G., Guterres, C. W., and Venâncio, W. S. 2016c. Eficiência de fungicidas para controle de *Gibberella zeae* em trigo: resultados dos Ensaios Cooperativos - Safra 2014. Comunicado Técnico 364. Embrapa Trigo, Passo Fundo, Brazil.

Savary, S., Nelson, A. D., Djurle, A., Esker, P. D., Sparks, A., Amorim, L., Bergamin Filho, A., Caffi, T., Castilla, N., Garrett, K., McRoberts, N. Rossi, V., Yuen, J., Willocquet, L. 2018. Concepts, approaches, and avenues for modelling crop health and crop losses. Eur. J. Agron. 100:4–18.

Schaafsma, A. W., Ilinic, L. T.-, Miller, J. D., and Hooker, D. C. 2001. Agronomic considerations for reducing deoxynivalenol in wheat grain. Can. J. Plant Pathol. 23:279–285.

Schaafsma, A. W., Tamburic-Ilincic, L., and Hooker, D. C. 2005. Effect of previous crop, tillage, field size, adjacent crop, and sampling direction on airborne propagules of *Gibberella zeae*/*Fusarium graminearum*, Fusarium head blight severity, and deoxynivalenol accumulation in winter wheat. Can. J. Plant Pathol. 27:217–224.

Shah, D. A., Molineros, J. E., Paul, P. A., Willyerd, K. T., Madden, L. V., and De Wolf, E. D. 2013. Predicting Fusarium head blight epidemics with weather-driven pre- and post-anthesis logistic regression models. Phytopathology. 103:906–919.

Shah, L., Ali, A., Yahya, M., Zhu, Y., Wang, S., Si, H., et al. 2018. Integrated control of Fusarium head blight and deoxynivalenol mycotoxin in wheat. Plant Pathol. 67:532–548.

Sharma, R. C., Duveiller, E., and Ortiz-Ferrara, G. 2007. Progress and challenge towards reducing wheat spot blotch threat in the Eastern Gangetic Plains of South Asia: Is climate change already taking its toll? Field Crops Res. 103:109–118.

Spolti, P., Del Ponte, E. M., Dong, Y., Cummings, J. A., and Bergstrom, G. C. 2014. Triazole sensitivity in a contemporary population of *Fusarium graminearum* from New York wheat and competitiveness of a tebuconazole-resistant isolate. Plant Dis. 98:607–613.

Spolti, P., Guerra, D. S., Badiale-Furlong, E., and Del Ponte, E. M. 2013. Single and sequential applications of metconazole alone or in mixture with pyraclostrobin to improve Fusarium head blight control and wheat yield in Brazil. Trop. Plant Pathol. 38:85–96.

Spolti, P., Shah, D. A., Fernandes, J. M. C., Bergstrom, G. C., and Del Ponte, E. M. 2015. Disease risk, spatial patterns, and incidence-severity relationships of Fusarium head blight in no-till spring wheat following maize or soybean. Plant Dis. 99:1360–1366.

Venancio, W.S., Santos, T., Boratto, V.N.M., Skodowski, L.H. 2014. Manejo de doenças do trigo com fungicidas, alternando a mistura pronta de isoftalonitrila + triazol. Anais eletrônicos da VIII Reunião da Comissão Brasileira de Pesquisa de Trigo e Triticale. Canela, 2014. CD-ROM.

Viana, C. 2013. Giberela em trigo: sobrevivência, reação de cultivares e controle químico. 111 f. Dissertação (Mestrado em Agronomia) – Faculdade de Agronomia e Medicina Veterinária da Universidade de Passo Fundo, Passo Fundo, 2013.

Viechtbauer, W., and Cheung, M.W.L. 2010. Outlier and influence diagnostics for meta-analysis. Res. Synth. Methods 1: 112–25.

Wegulo, S. N., Baenziger, P. S., Hernandez Nopsa, J., Bockus, W. W., and Hallen-Adams, H. 2015. Management of Fusarium head blight of wheat and barley. Crop Prot. 73:100–107.

Wegulo, S. N., Bockus, W. W., Nopsa, J. H., De Wolf, E. D., Eskridge, K. M., Peiris, K. H. S., et al. 2011. Effects of integrating cultivar resistance and fungicide application on Fusarium head blight and deoxynivalenol in winter wheat. Plant Dis. 95:554–560.

Willyerd, K. T., Li, C., Madden, L. V., Bradley, C. A., Bergstrom, G. C., Sweets, L. E., et al. 2012. Efficacy and stability of integrating fungicide and cultivar resistance to manage Fusarium head blight and deoxynivalenol in wheat. Plant Dis. 96:957–967.

Wood, S. N. 2017. Generalized Additive Models: An Introduction with R, 2nd ed. Chapman and Hall CRC, New York, NY, U.S.A.

SUPPLEMENTARY MATERIAL

Table S1. Summary information for 37 fungicide trials conducted in Brazil for evaluating the effect of fungicides on Fusarium head blight index and wheat yield.

S[a]	T[b]	Location-State[c]	Year[d]	Cultivar[e]	Range[f]	
					FHB (%)	Wheat yield
1	01	Passo Fundo, RS	2000	BRS 23	3.5 – 38.8	2838.0 – 4312.0
1	02	Passo Fundo, RS	2000	BRS 23	3.1 – 34.9	3085.0 – 5055.0
2	03	Passo Fundo, RS	2000	BRS 23	3.5 – 38.8	2838.0 – 4312.0
2	04	Passo Fundo, RS	2000	BRS 23	3.1 – 34.9	3085.0 – 5055.0
3	05	Muitos Capões, RS	2004	BRS Louro	0.7 – 5.9	4984.0 – 5666.0
3	06	Lages, SC	2005	BRS Louro	3.4 – 12.7	1835.0 – 2379.0
4	07	Cruz Alta, RS	2009	Campo Real	2.1 – 7.3	2567.0 – 3631.0
4	08	Passo Fundo, RS	2009	BRS Guamirim	0.9 – 5.0	1283.0 – 2935.0
4	09	Lagoa Vermelha, RS	2009	Raízes	0.4 – 10.1	1253.0 – 2379.0
4	10	Muitos Capões, RS	2009	Supera	2.4 – 31.3	2573.0 – 2919.0
5	11	Itaara, RS	2011	Fundacep 52	1.0 – 39.0	1191.6 – 3779.3
6	12	Cruz Alta, RS	2011	BRS 208	0.4 – 7.7	2718.0 – 3208.0
6	13	Guarapuava, PR	2011	BRS 208	2.8 – 10.0	4198.0 – 5692.0
7	14	Passo Fundo, RS	2012	Mirante	3.8 – 8.9	878.0 – 1460.3
8	15	Cruz Alta, RS	2012	BRS 208	4.2 – 8.7	3015 – 3681.0
8	16	Guarapuava, PR	2012	BRS 208	2.8 – 16.5	3331.0 – 3575.0
9	17	Passo Fundo, RS	2013	BRS 208	1.9 – 6.3	3591.0 – 4240.0
9	18	Passo Fundo, RS	2013	BRS 208	0.8 – 7.2	3478.0 – 3927.0

9	19	Guarapuava, PR	2013	CD 105	2.0 – 54.7	3755.8 – 4773.5
10	20	Guarapuava, PR	2013	BRS Guamirim	3.5 – 50.0	4392.5 – 5210.0
11	21	Guarapuava, PR	2013	CD 105	7.0 – 62.0	3755.7 – 4773.5
12	22	Cruz Alta, RS	2015	Marfim	5.0 – 9.0	3002.0 – 4035.0
12	23	Guarapuava, PR	2015	ORS 25	5.0 – 57.0	2269.0 – 3256.0
12	24	Passo Fundo, RS	2015	BRS 208	27.0 – 46.0	1368.0 – 2141.0
13	25	Água Santa, RS	2014	Sinuelo	2.1 – 6.6	3159.4 – 4667.3
13	26	Cruz Alta, RS	2014	TEC 10	2.7 – 8.8	2077.0 – 2575.0
13	27	Guarapuava, PR	2014	Campeiro	1.8 – 30.4	4101.0 – 4586.0
13	28	Palmeira, PR	2014	Marfim	2.6 – 8.4	2972.0 – 3546.0
13	29	Passo Fundo, RS	2014	BRS 208	2.7– 9.3	2983.0 – 3480.0
13	30	Passo Fundo, RS	2014	BRS 208	6.8 – 21.8	1925.0 – 2794.0
14	31	Cruz Alta, RS	2007	Fundacep 52	2.3 – 7.7	1165.0 – 3576.0
15	32	Cruz Alta , RS	2014	TEC 10	1.1 – 6.6	1626.5 – 2104
15	33	Cruz Alta, RS	2014	Linhagem 01/14	0.9 – 6.2	2148.9 – 2526.3
15	34	Santo Augusto, RS	2014	TBIO Iguaçú	1.1 – 7.6	1499.9 – 2392.0
16	35	Passo Fundo, RS	2014	TBIO Pioneiro	36.2 – 89.4	2579.0 – 3608.0
17	36	Itapiranga, SC	2013	BRS 208	3.9 – 9.5	3028.7 – 3649.0
18	37	Palmeira, PR	2013	Quartzo	4.7 – 61.0	972.6 – 4122.4

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[a] Study number (S): 1 = Panisson et al. (2002), 2 = Panisson et al. (2003), 3 = Casa et al. (2007), 4 = Spolti et al. 2013, 5 = Maffini et al. (2012), 6 = Santana et al. (2012), 7 = Viana (2013), 8 = Santana et al. (2014), 9 = Santana et al. (2016a), 10 = Feksa et al. (2014a), 11 = Feksa et al. (2014b), 12 = Santana et al. (2016b), 13 = Santana et al. (2016c), 14 = Deuner (2009), 15 = Guterres et al. (2015), 16 = Pizolotto and Boller (2015), 17 = Feldmann et al. (2014), 18 = Venancio et al. (2014).

[b] Trial number (T).

[c] States: RS = Rio Grande do Sul, SC = Santa Catarina, and PR = Paraná.
[d] Harvest year (HY).
[e] Wheat cultivar (CV).
[f] Mean percentage of disease spikelets per spike (IND) and minimum and maximum (YLD).

Table S2. Coefficients estimated by a simple linear regression model fitted to data at the study level; and estimated coefficients (best linear unbiased prediction (EBLUP) by a random-coefficients model fitted to data on the relationship between wheat yield (kg ha^{-1}) and Fusarium head blight index (%) in 37 field trials conducted in Brazil with variable number of observations (*N*). Summary information for 37 fungicide trials conducted in Brazil for evaluating the effect of fungicides on Fusarium head blight index and wheat yield.

Study code	*N*	Linear regression model coefficients (estimates)		Random coefficient model predictions (EBLUPs)	
		β_0	β_1	$\widehat{\beta_0}$	$\widehat{\beta_1}$
1	9	4209.05	-36.29	4204.29	-36.23
2	9	5026.71	-59.56	4991.38	-57.79
3	8	4250.78	-38.09	4242.51	-37.88
4	8	4967.05	-55.66	4918.16	-53.32
5	13	5339.96	-75.35	5292.09	-56.04
6	6	2463.24	-49.64	2469.94	-49.33
7	7	2752.43	43.47	2981.76	-13.90
8	7	3317.41	-381.87	2689.21	-104.12
9	7	1768.87	-13.85	1820.43	-27.46
10	7	2777.91	-0.19	2816.52	-3.90
11	13	3561.56	-56.12	3558.42	-55.74
12	10	3157.51	-55.08	3149.81	-50.05
13	10	6097.41	-144.79	5809.06	-92.30
14	7	1707.88	-92.73	1557.25	-63.27
15	9	4024.71	-121.88	3699.86	-63.17
16	6	3545.49	-14.64	3596.23	-22.79
17	10	4236.45	-75.42	4158.75	-53.18
18	10	3842.97	-48.15	3838.89	-47.02
19	11	4470.76	-12.55	4470.85	-13.08
20	7	5063.22	-12.67	5058.38	-13.12
21	11	4542.70	-11.88	4543.67	-12.26
22	11	4480.69	-122.38	4160.09	-72.38
23	8	3324.86	-18.37	3334.12	-18.98
24	7	3384.62	-42.67	3418.01	-43.65
25	8	4905.19	-220.18	4486.32	-90.82

26	8	2861.55	-86.39	2717.59	-61.46
27	8	4395.90	-6.43	4407.43	-8.83
28	8	3570.72	-77.83	3474.92	-56.20
29	9	3398.29	-41.09	3411.46	-44.13
30	9	3102.08	-54.02	3093.85	-53.14
31	7	3962.25	-201.55	3432.06	-109.24
32	7	2005.79	-56.52	1996.29	-50.31
33	7	2576.25	-70.53	2540.84	-53.23
34	8	2582.42	-134.08	2408.91	-85.99
35	7	4298.95	-15.83	4271.66	-15.46
36	12	3000.12	52.88	3403.71	-5.10
37	8	4096.11	-51.03	4090.10	-50.83

CHAPTER 3

Fusarium head blight pathogens of small-grain cereals in Pennsylvania: species diversity and toxigenic, reproductive and triazole sensitivity profiles

ABSTRACT

Fusarium head blight (FHB) is caused mainly by members of the *Fusarium graminearum* species complex (FGSC). This disease may lead to significant yield reductions in small-grain cereals worldwide, with additional losses due to the contamination of grain with harmful mycotoxins. Recent changes in the North American FHB pathogen composition have shown the need for continued surveillance at a regional scale, especially in poorly sampled regions such as Pennsylvania (PA). In this study, 421 single-spore *Fusarium* isolates were collected from naturally FHB-infected spikes of different small grain hosts (wheat, spelt, barley, and rye) during the Summer 2018 and 2019 and identified using a FGSC-specific primer, while *EF-1α* sequences were obtained for the non-FGSC isolates. The trichothecene (toxin) types for those isolates, as well as for an additional set of 77 *F. graminearum* isolates from overwintering crop residues during the Winter 2012, were determined with a multiplex PCR assay that amplified portions of the trichothecene 15-*O*-acetyltransferase (*TRI3*) and trichothecene efflux pump (*TRI12*) genes. A subsample of 31 *F. graminearum* isolates, 16 of the 3-acetyldeoxynivalenol (3ADON) and 15 of the 15-acetyldeoxynivalenol (15ADON) type, were assessed for their reproductive fitness and sensitivity to triazole-based fungicides. Results showed that *F. graminearum* accounted for 97% of the isolates. Three other non-FGSC species were found at very low frequency: *F. avenaceum* (*n* = 6); *F. sporotrichioides* (*n* = 5) and *F. temperatum* (*n* = 1). The FGSC isolates were primarily represented by the 15ADON (95.7%), followed by 3ADON (3.7%) and NIV (0.6%) toxin types. The frequency of the toxin types did not differ among sampled hosts nor years. However, the similar frequency of 15ADON and 3ADON genotypes in Northern PA (Potter County) suggest that population dynamics in this part of the state are likely shaped by ecological and environmental factors not yet identified. The 3ADON or 15ADON types could not be differentiated for most of the saprophytic traits based on multivariate analysis. All isolates were sensitive to tebuconazole and metconazole fungicides *in vitro*. Information from this study increases knowledge of the diversity of FHB pathogens and their trichothecene type in PA, which will be useful to assess the sustainability of disease management practices and provide a baseline for future FHB surveys.

Keywords: *Fusarium graminearum*, scab, deoxynivalenol, small grains, saprophytic traits, fungicide sensitivity.

INTRODUCTION

Fusarium head blight (FHB) is one of the most destructive and widespread diseases of wheat, barley, and other small grain cereals worldwide, causing significant losses in both the yield and grain quality (McMullen et al. 2012). During the 1990s, the disease caused a tremendous impact on North American agriculture with economic losses due to FHB estimated at approximately $3 billion in the U.S. (Windels 2000). Recently, FHB was considered as the second most important pest and pathogen in wheat globally (Savary et al. 2019). Direct losses due to FHB occur from failed kernel development, or grains that become shriveled and discolored and with low test weights (Osborne and Stein 2007). In addition, infected grains are usually contaminated mainly with type-B trichothecenes mycotoxins, particularly, deoxynivalenol (DON), and its acetylated forms 3-acetyl-deoxynivalenol (3ADON) and 15-acetyl-deoxynivalenol (15ADON), as well as nivalenol (NIV) and its acetylated derivative 4-acetyl nivalenol (4ANIV) (Miller et al. 1991). While in North America DON is the predominant mycotoxin contaminating small grains (McMullen et al. 1997), both DON and NIV are common in some parts of Asia, Europe and South America, likely due to the presence of other toxigenic species (Ichinoe et al. 1983; Del Ponte et al. 2015). These mycotoxins may accumulate in the grains at levels considered unsafe for both human and livestock consumption and pose a serious threat to food security and crop production (Pestka 2010, Rocha et al. 2005).

In North America, an endemic population of the *F. graminearum* sensu stricto (hereafter *F. graminearum*) of the 15ADON toxin type has been historically responsible for FHB (Zeller et al. 2003, 2004). However, a displacement of this dominant population by a newly introduced and highly virulent 3ADON population has been documented in the Upper Midwest of the U.S. and Western Canada (Gale et al. 2007, Puri and Zhong 2010, Ward et al. 2008). Similarly, 3ADON genotypes have been reported in surveys conducted from southeastern (North Carolina) to the northeastern (New York) areas of the U.S. (Schmale et al. 2011). The NIV toxin type of the FGSC is absent or found at very low frequencies in North America (Campbell et al. 2002, Starkey et al. 2007), especially occuring in small wheat growing areas of typical rice-growing areas such as those in Louisiana (Gale et al. 2011).

Reports of shifts in toxin profile of FHB populations in the U.S. raise concerns about the current distribution of these populations, especially in poorly sampled regions such as many areas of Pennsylvania (PA). Previously, Schmale et al. (2011) reported 15ADON as the predominant type (92%) in almost a thousand *F. graminearum* isolates obtained from six eastern U.S. states. In that study, 62 isolates were obtained from four commercial winter wheat fields during 2006 in Pennsylvania (PA), of which five were of the 3ADON genotype. Comparisons among the trichothecene genotypes of *F. graminearum* isolates obtained from differents fungal habitats in NY, including wheat, corn, aerial populations, and wild spikes and stems, have shown an increased frequency of the 3ADON genotype, with a general frequency of 15%, 18% and 22% observed by Schmale et al. (2011), Kuhnem et al. (2015) and Fulcher et al. (2019), respectively. In some of those studies, the 3ADON genotype comprised more than 40% of isolates in some individual fields.

While the reasons associated with significant changes in FHB populations and trichothecene genotype frequency are not entirely clear, several factors have been suggested including migration, host preference, and ecological and environmental conditions (Backhouse 2014, Boutigny et al. 2011, Lee et al. 2009). Ward et al. (2002) data previously suggested that trichothecene genotype diversity has been maintained in FHB pathogen populations by balancing selection, indicating that chemotype differences among *F. graminearum* populations may have fitness consequences. Indeed, some studies showed that 3ADON isolates were more fertile (sexual and asexual) (Ward et al. 2008), more aggressive, and produced more toxin than 15ADON isolates (Foroud et al. 2012, Puri and Zhong 2010, Ward et al. 2008). Conversely, Spolti et al. (2014a) compared 14 different attributes of saprophytic and pathogenic fitness and could not find differences in the 15ADON and 3ADON populations from NY. The reasons for those inconsistencies are not entirely clear but may be likely due to population or isolate-specific profiles that are encountered in some regions.

Continuous surveillance of plant pathogens is critical for assessing the risk and defining management strategies, especially in PA. The state currently ranks first in the U.S. for craft beer production, which coincides with an increased demand for locally sourced barley and wheat, further raising concerns about the risk and impact of FHB epidemics and mycotoxin contamination (Schwartz and Horsley 2019). Using a relatively large regional collection of several hundreds of *F. graminearum* isolates, the main objectives of this study were 1) to determine the composition and frequency of FHB-causing species and their mycotoxin profile isolated from spikes of wheat and other small-grain cereals as well as overwintering crop residues, and 2) to compare the trichothecene types of FGSC with regards to reproductive (sexual and asexual) traits and triazole sensitivity.

MATERIALS AND METHODS

Study area and sampling

During Summer 2018 and 2019, naturally FHB-infected spikes of different small grain hosts (wheat, barley, spelt, rye) were collected from the major cereal-producing regions of PA (herewith defined as South, Central, and North). Samples were collected along ten arbitrary transects through each field where ten spikes were collected, totaling 100 spikes per field. Twelve fields (10 wheat and 2 spelt) located in seven counties were surveyed in 2018 and 11 fields (7 wheat, 2 barley, 1 spelt, and 1 rye) in six counties were surveyed in 2019 (Table 1). FHB severity (%), defined as the mean percentage of diseased spikelets per spike, was recorded in 11 and 10 fields of 2018 and 2019 seasons, respectively. Previous crop information was also obtained from 11 and 7 fields each year (Table S1; S2).

Fusarium spp. isolate collections

In the laboratory, FHB-symptomatic kernels were surface sterilized in 70% ethanol for 1 min, immersed in 5% sodium hypochlorite for 2 min, and rinsed three times in sterile distilled (Qu et al. 2008). After samples dried, kernels were transferred to potato dextrose agar (PDA) media supplemented with streptomycin at 10μg ml^{-1}. Plates were incubated for four days at 25 ºC ± 2 ºC under continuous darkness. Fragments of mycelia from typical *Fusarium* spp. colonies were transferred to new PDA plates. Colonies identified both macro- and microscopically as *Fusarium* spp. (Leslie and Summerell 2006) were subcultured on specific nutrient-poor agar (SNA) to obtain pure, single spore isolates. Isolates were frozen at -80°C in 15% glycerol for long-term storage.

DNA extraction and PCR assays

Before DNA extraction, isolates of *Fusarium* spp. were grown in potato dextrose broth (PDB) medium and incubated on an orbital shaker (150 rpm) for approximately 4 days at 25 ºC ± 2 ºC. Mycelia were filtered through two layers of sterilized cheesecloth, dried on filter paper, and stored at -80 °C. DNA was extracted using MasterPure™ Yeast DNA Purification Kit (Lucigen®) protocol. The DNA from all isolates was then amplified by PCR using the Fg16F/Fg16R primers, which produced polymorphic products (~450 bp) with DNA from members of the *F. graminearum* species complex (FGSC) (Nicholson et al. 1998). Isolates that could not be identified by FGSC-specific primers were analyzed using partial sequences of the translation elongation factor 1α (*EF-1α*) gene as previously described (Cerón-Bustamante et al. 2018). Sequence similarity searches were performed with BLAST network service of the Fusarium ID database (http://isolate.fusariumdb.org/blast.php) (Geiser et al. 2004). For isolates identified as FGSC members, the trichothecene genotype (3ADON, 15ADON, or NIV) was determined through multiplex PCR assays targeting portions of the *TRI3* and *TRI12* genes (Starkey et al. 2007). Four separate primers were used to amplify each gene: 3CON, 3NA, 3D15A, 3D3A for *Tri3* and 12CON, 12NF, 12-15F, 12-3F for *Tri12* (Ward et al. 2002). Both multiplex reactions were performed in 25 uL volumes containing 1x PCR buffer, 2mM $MgCl_2$, 2U Taq DNA polymerase, 0.2mM of each deoxynucleoside triphosphate, 0.2 uM concentrations of each primer, and 25 ng of genomic DNA. Cycling conditions consisted of an initial denaturation step at 94 ºC for 2 min, followed by 25 cycles of 30 s at 94 ºC, 30 s at 52 ºC, and 1 min at 72 ºC (Starkey et al. 2007). The resulting PCR products were resolved on 2% (wt/vol) agarose gels and visualized under UV illumination. The *TRI3* multiplex produced amplicons of approximately 840 bp, 610 bp, and 243 bp with isolates that had NIV, 15ADON, and 3ADON chemotypes, respectively. The *TRI12* multiplex produced amplicons of approximately 840 bp, 670 bp, and 410 bp with isolates that had NIV, 15ADON, and 3ADON chemotypes, respectively. The reference isolate PH-1 was used throughout all the PCR assays as a positive control for *F. graminearum* species possessing 15ADON genotype (Cuomo et al. 2007).

Trichothecene genotypes from overwintering residues

The trichothecene genotype (3ADON, 15ADON, or NIV) of a collection of 77 *F. graminearum* isolates obtained from overwintering residues was also determined. Samples were obtained during Winter 2012 from the primary corn-producing regions of PA, with a total of 40 fields sampled across 16 locations (Table S3). Isolates were identified based on partial DNA sequencing of the translation elongation factor-1α (EF-1α) gene (O'Donnell et al. 1998). Sequence similarity searches were performed with BLAST network service of the Fusarium ID database (http://isolate.fusariumdb.org/blast.php) (Geiser et al. 2004). Multiplex PCR assays targeting portions of the *Tri3* and *Tri12* genes were conducted as previously described.

Saprophytic and fitness traits

Thirty-one *F. graminearum* isolates were selected from the isolate collection obtained during Summer 2018. Fifteen isolates of *F. graminearum* possessing the 15ADON genotype and 16 possessing the 3ADON genotype were selected to represent the geographic distribution of these two populations across PA. The isolates were assigned to species and trichothecene genotypes according to molecular identification described previously. Detailed information for these isolates is shown in Supplementary Table 04.

Mycelial growth

Isolates were grown on potato dextrose agar (PDA) media for 7 days at 25°C under continuous darkness. Mycelium plugs (6 mm in diameter) of each isolate were then excised from the margins of the colonies and individually placed upside down in the center of PDA plates (90 mm in diameter). Cultures were incubated in growth chambers at 23°C under continuous darkness. To estimate the average mycelial growth rate (cm^2), plates were photographed on days three and four by opening plates and taking a photo in a laminar flow cabinet using an iPhone XR camera (12MP) at a standard distance between the mobile device and the colonies. Photographs were analyzed in ImageJ (imagej.nih.gov/ij/) where the perimeter of the colony was drawn "by hand" on photographs within the ImageJ software and the enclosed area was recorded in cm^2 (Newbery et al. 2020). The mycelial growth rate of each isolate was calculated as the difference in the mycelium growth on the fourth and third day, and the area of the agar plug (0.283 cm^2) was subtracted from this value. Two replicates (plates) were used per treatment. The experiment was performed twice.

Macroconidia production

The asexual reproduction capacity of FHB isolates was determined based on macroconidia production culture medium. Isolates were grown on synthetic nutrient agar media (SNA) for 7 days under a cycle of 12 h of light and 12 h of darkness at 25°C (Leslie and Summerell 2006). After this period, three discs (6 mm in diameter) were removed from the edges of the developing colonies and immersed in 5 ml of sterile water containing 0.1 ml of Tween 0.001% in a test tube and shaken for 20 s (Nicolli et al. 2018). The spore concentration was quantified by using a hemocytometer and expressed as the number of macroconidia per ml (spore/ml). Two replicates (plates) were used per treatment. The experiment was performed twice.

Perithecia production on carrot agar

This experiment was conducted following a standard protocol (Cavinder et al. 2012) with some adaptations. Briefly, FHB isolates were grown on PDA media for 7 days under continuous darkness at 25°C. Mycelium plugs (6 mm in diameter) of each isolate were then excised from the margins of the colonies and individually placed upside down in the center of carrot agar plates (60 mm in diameter). The inoculated plates were incubated in growth chambers under bright fluorescent lights for 7 days at 25°C. After this period, the aerial mycelia was gently removed with a sterile toothpick and an aliquot of 1 ml of 2.5% Tween 20 solution was added to the surface. The plates were then returned to the growth chamber. The formation of perithecia was checked at three and six days after removal of the aerial mycelium when photographs of the plates were taken. For photography, the plates were opened and photographed in a laminar flow cabinet using an iPhone XR camera (12MP) at a standard distance between the mobile device and the plates. Photographs were analyzed in ImageJ (imagej.nih.gov/ij/) where the area occupied by perithecia in one square centimeter (cm^2) of the culture medium was quantified and expressed as the percentage of perithecia (%) (Steward et al. 2016). Two replicates (plates) were used per treatment. The experiment was performed once.

Ascospore production on carrot agar

This experiment was conducted as previously described for perithecia production. The production of ascospores was evaluated from the sixth day of removal of the aerial mycelia. Three plugs (1 cm diameter) from each plate were cut out of the medium with a cork borer. Each plug was sliced in half and the half-plugs were placed on a glass microscope slide with 3 plugs per slide (Cavinder et al. 2012). Slides were placed in a humidity chamber overnight under lights and the accumulated spores washed off from the slide with water and quantified using a Neubauer chamber. There were two replicated humid chambers with 31 slides each (one slide per isolate). Two replicates (plates) were used per treatment. The experiment was performed twice.

Fungicide sensitivity assay

The sensitivity of selected FHB isolates to tebuconazole and metconazole was assessed by measuring mycelial growth on PDA media amended with increasing concentrations of both fungicides (Becher et al. 2010, Spolti et al. 2014b). A stock solution (100 µg of active ingredient [a.i]/ml) was adjusted by dilution of the commercially formulated

Folicur 3.6F (38.7% a.i, Bayer CropScience) and Caramba® 90 (90% a.i., Basf Corporation). Concentrations tested for both fungicides were 0 (non-amended agar - PDA), 0.01, 0.1, 0.5, 1, 10 μg/ml. Mycelial agar plug (6 mm of diameter) from the edge of a culture of each isolate was placed in the center of a petri dish (90 mm diameter). The plates were incubated under continuous darkness at 25°C. The colony diameter was measured on the fourth and fifth day from two perpendicular directions using a digital caliper, and the agar plug diameter (6 mm) was subtracted. The mycelium growth of each isolate was calculated by the difference in the diameter colonies on the fourth and third day. For each combination of isolate-dose-fungicide, two replicates (plates) were used. The experiment was performed twice.

Data analysis

All data processing and analyses, as well as graphical work, were performed running R version 3.6.0 (2019-04-26) (R Core Team, 2019). Descriptive statistics summarized the frequency of trichothecene genotypes among hosts, years, and locations. Fisher's exact test (for small sample size) was used to evaluate whether there was a significant association between the trichothecene genotype composition (3ADON, 15ADON, NIV) of the isolates obtained from naturally FHB-infect spikes among years, locations, and host of origin. The same test was also used to compare the frequency of the trichothecene genotypes from the saprophytic and pathogenic phase of the fungus life cycle. All the fitness trait experiments were conducted as a completely randomized design with two replicates, and data from assays conducted two times were combined for analysis. The Student's t-test was used to compare means of the 15ADON and 3ADON populations regarding their fitness traits. Statistical significance was considered at the $P < 0.05$ level. The '*drm*' function of the '*dcr*' package of R (Ritz et al. 2015) was used to estimate the effective concentration leading to a 50% reduction of mycelial growth (EC_{50}). To compare the distribution of the EC_{50} values for tebuconazole and metconazole fungicides among 15ADON and 3ADON genotypes, a nonparametric Kolmogorov-Smirnov test ($P = 0.05$) was used. A multivariate analysis of variance (MANOVA) was performed in order to compare the overall saprophytic fitness of the two trichothecene genotypes groups. In addition, a principal component analysis (PCA) was performed using the mean values of the variables for each isolate, averaged over replicates and experiments. The contribution of each fitness trait to each principal component was also estimated. A correlogram was made using the overall means for each pair of variables using the package 'corrplot' (Wei and Simko 2017). The packages 'FactoMineR' (Lê et al. 2008) and 'factoextra' (Kassambara and Mundt 2017) were used for the PCA.

RESULTS

Species and trichothecene genotypes

Of the 421 total isolates, 317 and 104 were obtained from 2018 and 2019, respectively. The number of isolates per field ranged from 1 to 139 (mean = 18 and median = 8). Most of the isolates (78%) were obtained from wheat, followed by spelt (10.5%), barley (9.5%) and rye (1.4%).

The large majority of the isolates (97%) were molecularly identified as *F. graminearum* sensu lato; all amplified a ~450 bp fragment with the Fg16 primer. Twelve isolates (3%) that failed in PCR were identified as *F. avenaceum* (*n* = 6 isolates), *F. sporotrichioides* (*n* = 5 isolates) and *F. temperatum* (*n* = 1 isolate) based on based on *EF-1α* gene.

The *F. graminearum* isolates were mainly of 15ADON type (94.9%; 388/409), followed by the 3ADON (4.4%; 18/409) and NIV (0.7%; 3/409) type, respectively (Table 1). Fisher's exact test showed that the frequency of toxin types did not depend on the year (2018 and 2019) ($P = 0.337$). In 2018, the overall frequency was 93.8% (288/307) for the 15ADON genotype, 5.2% (16/307) for the 3ADON genotype, and 1.0% (3/307) for the NIV genotype. In 2019, 98.0% (100/102) of the isolates were characterized as the 15ADON genotype and 2.0% (2/102) as the 3ADON genotype. When genotype frequencies were compared across locations within each year, differences were only observed in 2018 (Fisher's exact test, $P = 0.000$). With the exception of samples obtained from Potter County in 2018, where the proportion of 15ADON and 3ADON genotypes were similar (46.7% and 40.0%, respectively), 15ADON was the predominant genotype in all other locations in 2018 (96.2%; 381/292) (Fig. 2, Table 1). In 2019, there were no differences in the frequency of the genotypes across locations ($P = 0.616$). The NIV genotype was detected only in 2018 (0.7%; 3/409). No difference in the trichothecene genotype frequency was found among sampled hosts ($P = 0.480$), where the 15ADON represented the majority of the isolates collected from wheat (94.7%; 304/321), followed by 3ADON (4.4%; 14/321), and NIV (0.9%; 3/321). Only the 15ADON genotype was recovered from spelt, barley, and rye hosts.

Among the 77 isolates obtained from overwintering residues, 72 were collected from maize stubble, two from wheat residue, and three from Johnsongrass (*Sorghum halepense*). All 77 *F. graminearum* isolates possessed the 15ADON type (Table S3). The frequency of the toxin types did not depend on the source (spikes or residues) ($P = 0.152$).

Asexual and sexual fertility

The 3ADON isolates grew significantly faster *in vitro* and produced more macroconidia than 15ADON isolates (Table 2, Fig. 2A,B). Mean perithecia production did not differ between the two genotypes when compared at 3 ($P = 0.378$) and 6 days ($P = 0.598$), but there was a difference between the two evaluation days ($P = 0.000$) (Fig 2D). Similarly, no statistical difference was found for mean ascospore production between the two trichothecene genotypes (Table 2, Fig 2C).

Table 1. Trichothecene genotype frequency of *Fusarium graminearum* isolates collected from naturally FHB-infected spikes of different small grain hosts (wheat, barley, spelt, rye) across Pennsylvania in 2018 and 2019 years

F[a]	Year	Location	Region	Host	Number of isolates (%)[b]			
					15ADON	3ADON	NIV	*N*[c]
1	2018	Lancaster	South	Wheat	34 (100.0)	0 (0.0)	0 (0.0)	34
2	2018	York	South	Spelt	34 (100.0)	0 (0.0)	0 (0.0)	34
3	2018	Armstrong	Central	Wheat	24 (88.9)	2 (7.4)	1 (3.7)	27
4	2018	Centre	Central	Wheat	136 (97.8)	3 (2.2)	0 (0.0)	139
5	2018	Centre	Central	Wheat	19 (90.5)	2 (9.5)	0 (0.0)	21
6	2018	Lebanon	South	Wheat	2 (100.0)	0 (0.0)	0 (0.0)	2
7	2018	Potter	North	Wheat	3 (100.0)	0 (0.0)	0 (0.0)	3
8	2018	Potter	North	Spelt	0 (0.0)	4 (100.0)	0 (0.0)	4
9	2018	Potter	North	Wheat	4 (50.0)	2 (25.0)	2 (25.0)	8
10	2018	Tioga	North	Wheat	26 (92.9)	2 (7.1)	0 (0.0)	28
11	2018	Tioga	North	Wheat	2 (66.7)	1 (33.3)	0 (0.0)	3
12	2018	Tioga	North	Wheat	4 (100.0)	0 (0.0)	0 (0.0)	4
13	2019	Lancaster	South	Barley	25 (100.0)	0 (0.0)	0 (0.0)	25
14	2019	Lancaster	South	Rye	6 (100.0)	0 (0.0)	0 (0.0)	6
15	2019	Lancaster	South	Spelt	6 (100.0)	0 (0.0)	0 (0.0)	6
16	2019	Lancaster	South	Wheat	1 (100.0)	0 (0.0)	0 (0.0)	1

17	2019	Centre	Central	Barley	13 (100.0)	0 (0.0)	0 (0.0)	13
18	2019	Potter	North	Wheat	5 (100.0)	0 (0.0)	0 (0.0)	5
19	2019	Erie	North	Wheat	11 (100.0)	0 (0.0)	0 (0.0)	11
20	2019	Crawford	North	Wheat	3 (100.0)	0 (0.0)	0 (0.0)	3
21	2019	Lebanon	South	Wheat	12 (100.0)	0 (0.0)	0 (0.0)	12
22	2019	Lebanon	South	Wheat	3 (100.0)	0 (0.0)	0 (0.0)	3
23	2019	Lebanon	South	Wheat	15 (88.2)	2 (11.8)	0 (0.0)	17

[a] Individual fields sampled in each location (F);

[b] Genotypes 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON) and nivalenol (NIV).

[c] *N* = total number of isolates.

Fungicide sensitivity

EC_{50} values ranged from 0.01 to 0.41 µg/ml (0.13 ± 0.12) for tebuconazole and from 0.00 to 0.13 µg/ml (0.02 ± 0.03) for metconazole. EC_{50} estimates for tebuconazole were greater ($P = 0.000$) than metconazole (Fig. 3A). However, no statistical differences were observed between the two trichothecene genotypes for the EC_{50} estimates for tebuconazole ($P = 0.847$) and metconazole ($P = 0.498$) (Table 2).

Multivariate analysis

Based on MANOVA, there was no difference between the 3ADON and 15ADON trichothecene genotypes based on examining the combined dependent variables ($P = 0.239$). The correlation analysis for each pairwise comparison ($n = 21$) for all 7 variables used to characterize fitness of the two trichothecene groups (Table 2) indicated that there were 7 significant ($P < 0.05$) correlations (Fig. S1). Overall, mycelial growth rate was positively correlated with macroconidia production ($P = 0.01$). However, the mycelial growth rate and macroconidia production were negatively associated with the EC_{50} estimates for tebuconazole and metconazole. As expected, perithecia production on day 3 was highly correlated with perithecia production on day 6, as well as the EC_{50} estimates for tebuconazole and metconazole.

The PCA suggested that EC_{50} estimates for tebuconazole, mycelial growth rate, and EC_{50} estimates for metconazole contributed the most for the PC1: 22.7%, 21.4%, and 18.5%

respectively of the total, with 34.4% of all variation explained by this first PC (Fig. 3B). The second PC explained 26.9% of the variation, with perithecia production on days 3 and 6 contributing to 33.0% and 31.9%, respectively. Thus, the first two PCs explained 61.3% of the variation among the isolates. The third PC explained 15.1% of the variation among isolates, with a contribution of 58.0% from the variable ascospore production.

Table 2. Summary information of saprophytic traits of 31 *Fusarium graminearum* isolates obtained from naturally FHB-infected spikes of small-grain cereals from 2018 possessing either a 3-acetyl-deoxynivalenol (3-ADON) or a 15-ADON trichothecene genotype.

	Trichothecene genotype[a]				
Variable	*n*	15ADON	*n*	3ADON	*P*-value[b]
Mycelial growth[c]	60	6.87 ± 4.23	64	9.00 ± 4.61	0.008
Spore production[d]	60	26.58 ± 16.53	64	41.07 ± 18.89	0.000
Ascospore production[e]	60	38.42 ± 31.77	64	42.89 ± 33.76	0.449
Perithecia production[f]					
3 days	30	5.11 ± 6.79	32	6.95 ± 9.40	0.378
6 days	30	11.49 ± 10.04	32	12.98 ± 12.00	0.598
EC_{50} tebuconazole[g]	15	0.14 ± 0.14	16	0.11 ± 0.11	0.847
EC_{50} metconazole[h]	15	0.02 ± 0.03	16	0.02 ± 0.02	0.498

[a] Isolates were identified to trichothecene genotype using polymerase chain reaction assays targeting *Tri3* and *Tri12* genes (Starkey et al. 2007). In total 31 *Fusarium graminearum* isolates ($n_{15ADON} = 15$; $n_{3ADON} = 16$) were used in this study. Data shown are means ± standard deviation.

[b] *P*-value for comparisons of trichothecene genotypes based on *Student*'s t-test (mycelial growth, spore production, ascospore production, perithecia production) and Kruskal-Wallis test (EC_{50} tebuconazole, EC_{50} metconazole).

[c] Mycelial growth (cm^2) were determined on potato dextrose agar (PDA) at 25 ºC incubated for four days under continuous darkness.

[d] Macroconidia production (× 10^4 macroconidia/ml) on synthetic nutrient agar media (SNA) for 7 days under a cycle of 12 h of light and 12 h of darkness at 25°C.

[e] Ascospores (× 10^4 ascospore/ml) produced from carrot agar (CA) and discharged and harvested from a glass microscope slide.

[f] Perithecia production (%) in one cm^2 of carrot agar (CA) after 3 and 6 days of incubation at 25°C under constant lights.

[g,h] Effective concentration of tebuconazole and metconazole fungicides (µg/ml) that reduces 50% of the mycelial growth of each of the isolates.

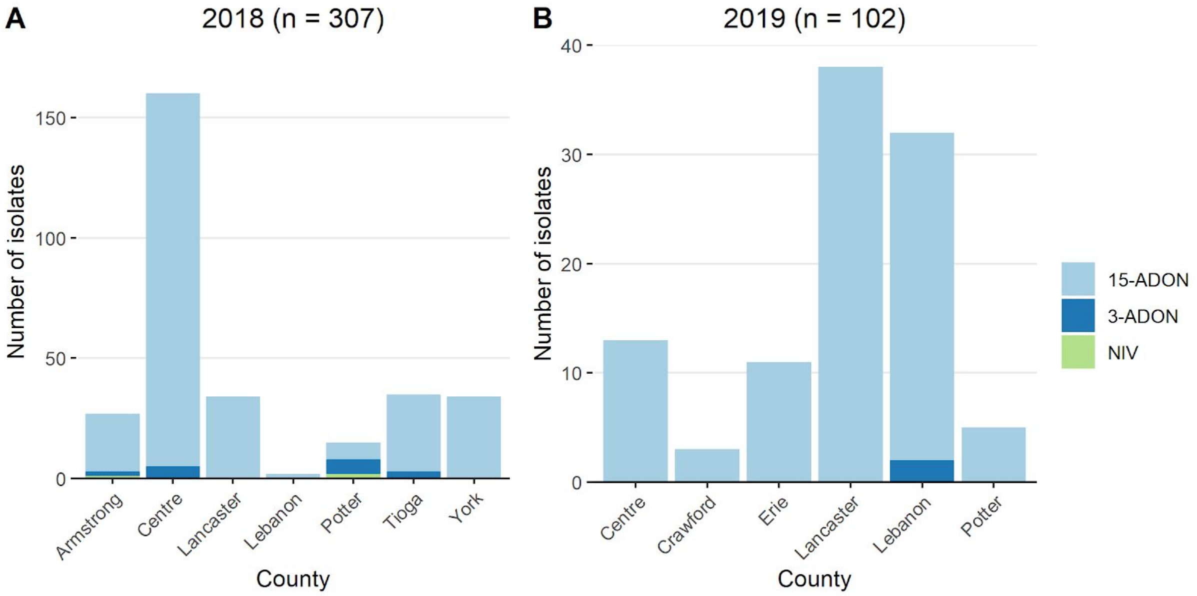

Fig. 1. Number of *Fusarium graminearum* isolates collected from naturally FHB-infected spikes of small-grain cereals during **(A)** 2018 and **(B)** 2019 growing season by county in Pennsylvania, possessing 3-acetyldeoxynivalenol (3ADON), 15-acetyldeoxynivalenol (15ADON), and nivalenol (NIV) genotypes.

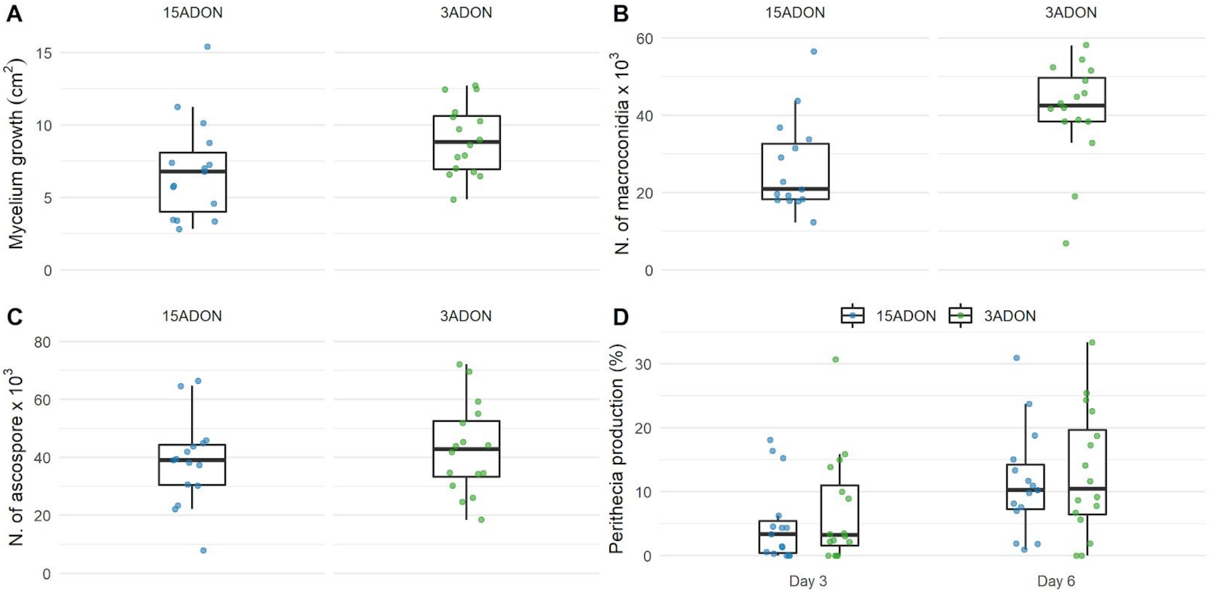

Fig. 2. (A) Average mycelial growth per day on PDA medium, **(B)** macroconidia production on SNA medium, **(C)** ascospore production on carrot agar medium, and **(D)** percentage of

perithecia in 1 cm^2 of carrot agar medium on 3 and 6 days for a sample of 31 *Fusarium graminearum* isolates (n_{15ADON} = 15; n_{3ADON} = 16) obtained from naturally FHB-infected spikes of small-grain cereals from 2018 across Pennsylvania. Data points for each isolate averaged over replicates and experiments.

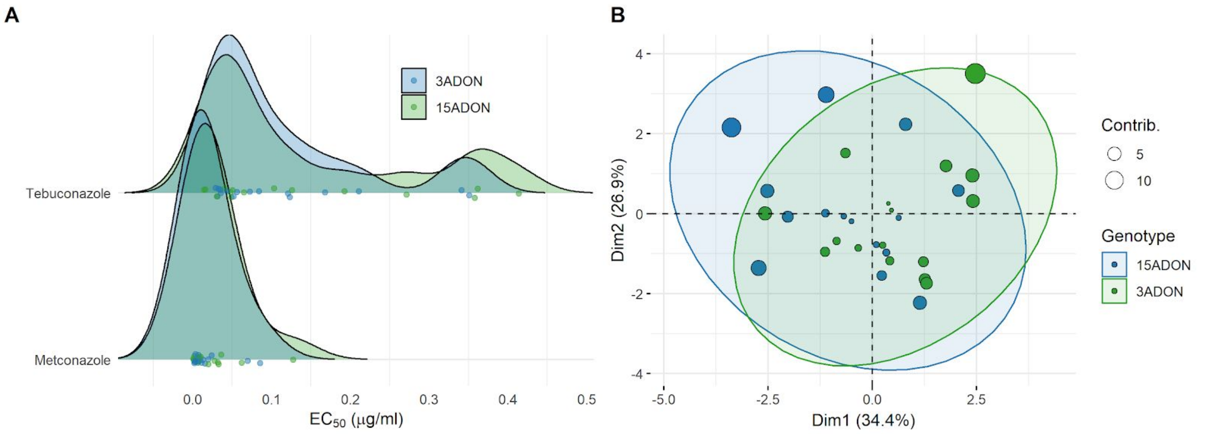

Fig. 3. **(A)** Density plots of the effective concentration of tebuconazole and metconazole that reduces 50% of the mycelial growth (EC_{50}), and **(B)** Scatterplot from the Principal Components Analysis (PCA) of a sample of 31 *F. graminearum* isolates (n_{15ADON} = 15; n_{3ADON} = 16) obtained from naturally FHB-infected spikes of small-grain cereals from the 2018 season across Pennsylvania. PCA was performed using data from a correlation matrix, and the estimated eigenvectors and eigenvalues were obtained for each principal component (PC). The first and second PC explained 34.4% and 26.9% of all the variation among the isolates. Contrib. = contribution (%) of each individual isolate in explaining all variation among them.

DISCUSSION

This is the first comprehensive study conducted in Pennsylvania on the composition and spatial distribution for a collection of FHB pathogens obtained from small grain (wheat, barley, rye, and spelt) and overwintering residues. Our results corroborate previous findings of the large dominance of the *F. graminearum* of the 15ADON genotype in the Eastern US, which was consistent in samples from both symptomatic spikes and overwintering residues (Cowger et al. 2020, Kuhnem et al. 2015, Schmale et al. 2011). These isolates may be part of a genetically divergent North American population of *F. graminearum* that cause FHB and typically produces the 15ADON mycotoxin (Gale et al. 2007, Kelly and Ward 2018).

The non-FGSC isolates were in very low frequency (3%), but they are known to produce different types of mycotoxins, which could be a concern should they frequency increase in the future. For example, *F. avenaceum* is the most important FHB-causing species within the *F. tricinctum* species complex (FTSC), and has the ability to produce moniliformin, enniatins analogues, and beauvericin (Beccari et al. 2018). Previous studies also demonstrated that *F. temperatum*, which is typically a maize pathogen and a member of the *F. fujikuroi* species complex (FFSC), was able to accumulate the same mycotoxin types as *F. avenaceum* in infected maize kernels. However, *F. sporotrichioides*, which is classified in the broader *F. sambucinum* species complex (FSAMSC) (O'Donnell et al., 2013), is able to produce type-A trichothecenes mycotoxins, including T-2 and HT-2, and these are considered to be more toxic to humans and animals than type-B trichothecenes (Logrieco et al. 1990, Krska et al. 2001). Recently, Cowger et al. (2020) showed that FTSC species account for 11.3% of the *Fusarium* isolates obtained from symptomatic wheat spikes in the 2013-14 growing seasons in North Carolina. In that case, members of the FTSC, specially *F. avenaceum*, were frequent or even dominant in some fields. Clearly, data on non-FGSC pathogens causing FHB in wheat in the US is lacking. The recent reports reinforces the need for continued monitoring of regional FHB pathogen populations in PA, especially to characterize possible trends in pathogen diversity that may result from changes in cropping systems and agricultural practices across the state.

The frequency of the trichothecene genotypes reported in our study is in agreement with previous reports in the Northeastern U.S. where 15ADON was the predominant genotype, followed by 3ADON and NIV (Cowger et al. 2020, Kuhnem et al. 2015, Fulcher et al. 2019, Schmale et al. 2011). Schmale et al. (2011) reported 15ADON as the dominant genotype across the Eastern U.S. states (92%). Interestingly, the frequency of the 15ADON (91.9%) and 3ADON (8.1%) types of 62 isolates collected from four commercial winter wheat fields at four regions in PA, more than a decade ago in the Schmale et al. (2011) study, was similar to that in our study. Moreover, we also did not find the 3ADON type found in the Southern fields. These consistencies in the results may be an indication that FHB pathogen populations in PA are following a unique evolutionary trajectory. Nonetheless, both reports from PA contrast with Western Canada and the Upper Midwest of the U.S., where an *F. graminearum* population with a 15ADON genotype has been replaced by an introduced 3ADON population (Ward et al. 2008, Gale et al. 2007, Liang et al. 2014, Puri and Zhong 2010). The low recovery of NIV genotype isolates in our study is in agreement with previous findings for the Northeastern U.S., where a north-south cline of increasing frequency of NIV genotype was observed, with a large population of NIV producers observed in Louisiana (Fulcher et al. 2019, Gale et al. 2011, Schmale et al. 2011). Recent data from North Carolina for a collection of 2197 isolates also showed the low frequency of the NIV producing isolates (2.2%), with no more than >12% observed in a single field (Cowger et al. 2020).

It has been suggested that the distribution of trichothecene genotypes and FGSC species in certain geographic regions may be shaped by differences in host and/or ecological preferences and cropping system (Lee et al. 2009, Yang et al. 2018, Dill-Macky and Jones 2000). In South America, *F. meridionale* and *F. cortaderiae* species, both possessing the NIV genotype, were more frequently obtained from maize, and *F. graminearum* with the 15ADON or 3ADON genotypes from wheat (Del Ponte et al. 2015, Sampietro et al. 2011, Kuhnem et al. 2016). The indication of host preference was also reported by Boutigny et al. (2011) in South Africa, where *F. boothii* was the exclusive species associated with maize, and *F. graminearum* accounted for more than 85% of the FGSC isolates obtained from wheat and barley. Conversely, our results showed similar frequencies of the 15ADON genotype obtained from maize stubble and symptomatic spikes. While in the NY study (Kuhnem et al. 2015), the 3ADON genotype was also recovered from maize stubble, only the 15ADON genotype was found in the stubble-borne isolates from PA.

In this study the isolates were collected from locations with unique climate conditions and cultural practices. For example, Lancaster, Lebanon, and York counties are in a warmer region of southern PA, where traditional corn-wheat-soybean rotations are very common. In this area, only two *F. graminearum* with the 3ADON genotype were recovered. In contrast, Potter County is located in a colder region and at higher altitudes in Northern PA where different crop species are grown in the rotations compared with the other regions. We found the greatest frequency of the 3ADON genotype in Potter county. The ecological factors driving dynamics of FHB populations in PA are still not clear. However, it has been suggested that the geographic distribution and population dynamics among North American *F. graminearum* populations are influenced by a complex ecological and adaptive landscape (Kelly et al. 2015). In addition, inherent regional differences in the landscape also seem to have influenced the composition of the trichothecene genotypes in NY (Kuhnem et al. 2015).

In general, the *F. graminearum* isolates of the two toxin types could not be differentiated for the majority of measures of saprophytic fitness in this study. However, mycelial growth rate was faster for 3ADON which also produced more macroconidia *in vitro* than 15ADON isolates. Ward et al. (2008) suggested that the greater fecundity and growth rate of the 3ADON population that supposedly displaced the 15ADON population in Western Canada, were indicative of an adaptive fitness advantage if the same aggressiveness pattern were displayed *in vivo*. However, Milgroom (2015) argues that *in vitro* assays estimates are often poor predictors of fitness in natural field environments. Contradictory results were reported by Spolti et al. (2014a) who found that the mycelial growth rate did not differ at any tested temperature ranging from 15 to 30°C between the 3ADON and 15ADON populations from NY. In our study, the two toxin types were similar with regards to sexual fertility, as suggested by measures of perithecia formation and ascospore production on carrot agar. Spolti et al. (2014a) also observed similar ascospore production on corn stalk among both genotypes, but this was not true for ascospore production on carrot agar, where 3ADON isolates were more fertile.

Although the differential sensitivity to fungicides could be one of the factors related to possible adaptive fitness advantages of 3ADON isolates compared with 15ADON isolates (Spolti et al. 2014a), we found no statistical differences in EC_{50} estimates for tebuconazole and metconazole between the two toxy types, although a greater variability in the EC_{50} was found for the sensitivity levels to tebuconazole. It is not clear whether the extended use of tebuconazole against *F. graminearum* is a factor leading to lower sensitivity or the populations are naturally variable (Spolti et al. 2012b; Spolti et al. 2014b; Becher et al., 2010). Regardless of the individual effect of each saprophytic trait and sensitivity of triazole-based fungicides to the 3ADON and 15ADON genotypes, results from the multivariate analysis showed that there was no clear distinction in fitness attributes between isolates of the 3ADON and 15ADON genotypes. These results agree with those by Spolti et al. (2014a) who compared 14 different attributes of saprophytic and pathogenic fitness and reported no differences between populations of *F. graminearum* with 15ADON and 3ADON genotypes from New York.

We provide the first full report on the distribution and composition of the trichothecene genotypes of *F. graminearum* isolates obtained from two fungal habitats in Pennsylvania, including naturally FHB-infected spikes of different small grain hosts (wheat, barley, spelt, rye), and overwintering residues, especially maize stubble. Results indicated that the regional population of *F. graminearum* is composed mainly of the 15ADON genotype in frequency that agrees with reports from more than a decade ago, suggesting no shift in the population causing FHB in PA. Whether the north-south cline of increasing frequency of 3ADON genotype in PA depends on cropping system and/or environmental factors merits further investigation. It would be instructive to further investigate the presence of the recently described NX2 chemotype among North American *F. graminearum* populations (Liang et al. 2014, Varga et al. 2015). Our findings expand knowledge of the trichothecene diversity in Pennsylvania, highlight the need to further explore the genetic diversity and population structure analysis in future studies in order to assist in answering questions about the sustainability of resistant cultivars and fungicides used to manage FHB in Pennsylvania.

ACKNOWLEDGEMENT

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-9-064. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture. Partial support for research conducted in this study was from the USDA National Institute of Food and Federal Appropriations under Project PEN04660 and Accession number 1016474 (P.D. Esker). Further support was provided to G.A. Kuldau by the Pennsylvania Department of Agriculture under the Award number 44123722. E. M. Del Ponte is thankful to CNPq for the research fellow's support, respectively. We are also thankful to the members of the Penn State Field and Forage Crops Extension Team for collecting small grains samples across Pennsylvania.

REFERENCES

Beccari, G., Colasante, V., Tini, F., Senatore, M. T., Prodi, A., Sulyok, M., et al. 2018. Causal agents of Fusarium head blight of durum wheat (*Triticum durum* Desf.) in central Italy and their in vitro biosynthesis of secondary metabolites. Food Microbiol. 70:17–27.

Backhouse, D. 2014. Global distribution of *Fusarium graminearum*, *F. asiaticum* and *F. boothii* from wheat in relation to climate. Eur. J. Plant Pathol. 139:161–173.

Becher, R., Hettwer, U., Karlovsky, P., Deising, H. B., and Wirsel, S. G. R. 2010. Adaptation of *Fusarium graminearum* to tebuconazole yielded descendants diverging for levels of fitness, fungicide resistance, virulence, and mycotoxin production. Phytopathology. 100:444–453.

Boutigny, A.-L., Ward, T. J., Van Coller, G. J., Flett, B., Lamprecht, S. C., O'Donnell, K., et al. 2011. Analysis of the *Fusarium graminearum* species complex from wheat, barley and maize in South Africa provides evidence of species-specific differences in host preference. Fungal Genet. Biol. 48:914–920.

Campbell, H., Choo, T. M., Vigier, B., and Underhill, L. 2002. Comparison of mycotoxin profiles among cereal samples from eastern Canada. Can. J. Bot. 80:526–532.

Cavinder, B., Sikhakolli, U., Fellows, K. M., and Trail, F. 2012. Sexual development and ascospore discharge in *Fusarium graminearum*. J. Vis. Exp. :3895. Online publication. doi:10.3791/3895

Cowger, C., Ward, T. J., Nilsson, K., Arellano, C., McCormick, S. P., and Busman, M. 2020. Regional and field-specific differences in *Fusarium* species and mycotoxins associated with blighted North Carolina wheat. Int. J. Food Microbiol. 323:108594.

Cuomo, C. A., Güldener, U., Xu, J. R., Trail, F., Turgeon, B. G., Di Pietro, A., Walton, J. D., Ma, L. J., Baker, S. E., Rep, M. and Adam, G. 2007. The *Fusarium graminearum* genome reveals a link between localized polymorphism and pathogen specialization. Science 317:1400-1402.

Del Ponte, E. M., Spolti, P., Ward, T. J., Gomes, L. B., Nicolli, C. P., Kuhnem, P. R., et al. 2015. Regional and field-specific factors affect the composition of Fusarium head blight pathogens in subtropical no-till wheat agroecosystem of Brazil. Phytopathology. 105:246–254.

Dill-Macky, R., and Jones, R. K. 2000. The effect of previous crop residues and tillage on Fusarium head blight of wheat. Plant Dis. 84:71–76.

Foroud, N. A., McCormick, S. P., MacMillan, T., Badea, A., Kendra, D. F., Ellis, B. E., and Eudes, F. 2012. Greenhouse studies reveal increased aggressiveness of emergent Canadian *Fusarium graminearum* chemotypes in wheat. Plant Dis. 96:1271–1279.

Fulcher, M. R., Winans, J. B., Quan, M., Oladipo, E. D., and Bergstrom, G. C. 2019. Population genetics of *Fusarium graminearum* at the interface of wheat and wild grass communities in New York. Phytopathology. 109:2124–2131.

Gale, L. R., Harrison, S. A., Ward, T. J., O'Donnell, K., Milus, E. A., Gale, S. W., et al. 2011. Nivalenol-type populations of *Fusarium graminearum* and *F. asiaticum* are prevalent on wheat in southern Louisiana. Phytopathology. 101:124–134.

Gale, L. R., Ward, T. J., Balmas, V., and Kistler, H. C. 2007. Population Subdivision of *Fusarium graminearum* sensu stricto in the Upper Midwestern United States. Phytopathology. 97:1434–1439.

Geiser, D. M., Jiménez-Gasco, M., Kang, S., Makalowska, I., Veeraraghavan, N., et al. 2004. FUSARIUM-ID v.1.0: A DNA sequence database for identifying *Fusarium*. Eur. J. Plant Pathol.110:473–479.

Ichinoe, M., Kurata, H., Sugiura, Y., and Ueno, Y. 1983. Chemotaxonomy of *Gibberella zeae* with special reference to production of trichothecenes and zearalenone. Appl. Environ. Microbiol. 46:1364–1369.

Kassambara, A., and Mundt, F. 2017. *factoextra: extract and visualize the results of multivariate data analyses*. Available at: https://CRAN.Rproject.org/package=factoextra.

Kelly, A. C., Clear, R. M., O'Donnell, K., McCormick, S., Turkington, T. K., Tekauz, A., et al. 2015. Diversity of Fusarium head blight populations and trichothecene toxin types reveals regional differences in pathogen composition and temporal dynamics. Fungal Genet. Biol. 82:22–31.

Kelly, A. C., and Ward, T. J. 2018. Population genomics of *Fusarium graminearum* reveals signatures of divergent evolution within a major cereal pathogen ed. Sung-Hwan Yun. PLOS ONE. 13:e0194616.

Krska, R., Baumgartner, S., and Josephs, R. 2001. The state-of-the-art in the analysis of type-A and -B trichothecene mycotoxins in cereals. Fresenius J. Anal. Chem. 371:285–299.

Kuhnem, P. R., Spolti, P., Del Ponte, E. M., Cummings, J. A., and Bergstrom, G. C. 2015. Trichothecene genotype composition of *Fusarium graminearum* not differentiated among isolates from maize stubble, maize ears, wheat spikes, and the atmosphere in New York. Phytopathology. 105:695–699.

Kuhnem, P. R., Ward, T. J., Silva, C. N., Spolti, P., Ciliato, M. L., Tessmann, D. J., et al. 2016. Composition and toxigenic potential of the *Fusarium graminearum* species complex from maize ears, stalks and stubble in Brazil. Plant Pathol. 65:1185–1191.

Lee, J., Chang, I.-Y., Kim, H., Yun, S.-H., Leslie, J. F., and Lee, Y.-W. 2009. Genetic diversity and fitness of *Fusarium graminearum* populations from rice in Korea. Appl. Environ. Microbiol. 75:3289–3295.

Lê, S., Josse, J., and Husson, F. 2008. *FactoMineR: A Package for Multivariate Analysis*. J. Stat. Softw. 25:1–18.

Leslie, J. F., and Summerell, B. A. 2006. The Fusarium Laboratory Manual. Wiley Online Library, Ames, IA.

Liang, J. M., Xayamongkhon, H., Broz, K., Dong, Y., McCormick, S. P., Abramova, S., et al. 2014. Temporal dynamics and population genetic structure of *Fusarium graminearum* in the upper Midwestern United States. Fungal Genet. Biol. 73:83–92.

Logrieco, A., Chelkowski, J., Bottalico, A., and Visconti, A. 1990. Further data on specific trichothecene production by *Fusarium* sect. Sporotrichiella strains. Mycol. Res. 94:587–589.

McMullen, M., Bergstrom, G., De Wolf, E., Dill-Macky, R., Hershman, D., Shaner, G., et al. 2012. A unified effort to fight an enemy of wheat and barley: Fusarium head blight. Plant Dis. 96:1712–1728.

McMullen, M., Jones, R., and Gallenberg, D. 1997. Scab of wheat and barley: a re-emerging disease of devastating impact. Plant Dis. 81:1340–1348.

Miller, J. D., Greenhalgh, R., Wang, Y., and Lu, M. 1991. Trichothecene chemotypes of three *Fusarium* species. Mycologia 83:121–130.

Newbery, F., Ritchie, F., Gladders, P., Fitt, B. D. L., and Shaw, M. W. 2020. Inter‐individual genetic variation in the temperature response of *Leptosphaeria* species pathogenic on oilseed rape. Plant Pathol. 00:1–13.

Nicholson, P., Simpson, D. R., Weston, G., Rezanoor, H. N., Lees, A. K., Parry, D. W., et al. 1998. Detection and quantification of *Fusarium culmorum* and *Fusarium graminearum* in cereals using PCR assays. Physiol. Mol. Plant Pathol. 53:17–37.

Nicolli, C. P., Machado, F. J., Spolti, P., and Del Ponte, E. M. 2018. Fitness traits of deoxynivalenol and nivalenol-producing *Fusarium graminearum* species complex strains from wheat. Plant Dis. 102:1341–1347.

O'Donnell, K., Rooney, A. P., Proctor, R. H., Brown, D. W., McCormick, S. P., Ward, T. J., et al. 2013. Phylogenetic analyses of *RPB1* and *RPB2* support a middle Cretaceous origin for a clade comprising all agriculturally and medically important fusaria. Fungal Genet. Biol. 52:20–31.

O'Donnell, K., Kistler, H. C., Cigelnik, E., and Ploetz, R. C. 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: Concordant evidence from nuclear and mitochondrial gene genealogies. Proc. Natl. Acad. Sci. 95:2044–2049.

Osborne, L. E., and Stein, J. M. 2007. Epidemiology of Fusarium head blight on small-grain cereals. Int. J. Food Microbiol. 119:103-108.

Paul, P. A., Lipps, P. E., Hershman, D. E., McMullen, M. P., Draper, M. A., and Madden, L. V. 2007. A quantitative review of tebuconazole effect on Fusarium head blight and deoxynivalenol content in wheat. Phytopathology. 97:211–220.

Pestka, J. J. 2010. Deoxynivalenol: mechanisms of action, human exposure, and toxicological relevance. Arch. Toxicol. 84:663–679.

Puri, K. D., and Zhong, S. 2010. The 3ADON population of *Fusarium graminearum* found in North Dakota is more aggressive and produces a higher level of DON than the prevalent 15ADON population in spring wheat. Phytopathology. 100:1007–1014.

Qu, B., Li, H. P., Zhang, J. B., Huang, T., Carter, J., Liao, Y. C., et al. 2008. Comparison of genetic diversity and pathogenicity of Fusarium head blight pathogens from China and Europe by SSCP and seedling assays on wheat. Plant Pathol. 57:642–651.

Ritz, C., Baty, F., Streibig, J. C., and Gerhard, D. 2015. Dose-response analysis using R ed. Yinglin Xia. PLOS ONE. 10:e0146021.

Rocha, O., Ansari, K., and Doohan, F. M. 2005. Effects of trichothecene mycotoxins on eukaryotic cells: A review. Food Addit. Contam. 22:369–378.

Sampietro, D. A., Díaz, C. G., Gonzalez, V., Vattuone, M. A., Ploper, L. D., Catalan, C. A. N., et al. 2011. Species diversity and toxigenic potential of *Fusarium graminearum* complex isolates from maize fields in northwest Argentina. Int. J. Food Microbiol. 145:359–364.

Savary, S., Willocquet, L., Pethybridge, S. J., Esker, P., McRoberts, N., and Nelson, A. 2019. The global burden of pathogens and pests on major food crops. Nat. Ecol. Evol. 3:430–439.

Schmale, D. G., Wood-Jones, A. K., Cowger, C., Bergstrom, G. C., and Arellano, C. 2011. Trichothecene genotypes of *Gibberella* zeae from winter wheat fields in the eastern USA. Plant Pathol. 60:909–917.

Schwarz, P. and Horsley, R. 2019. Branching out with barley: the Eastern spring barley nursery. In: *The New Brewer*. vol. 36, n°6, November/December. page:107-116.

Spolti, P., Del Ponte, E. M., Cummings, J. A., Dong, Y., and Bergstrom, G. C. 2014a. Fitness attributes of *Fusarium graminearum* isolates from wheat in New York possessing a 3-ADON or 15-ADON trichothecene genotype. Phytopathology. 104:513–519.

Spolti, P., Del Ponte, E. M., Dong, Y., Cummings, J. A., and Bergstrom, G. C. 2014b. Triazole sensitivity in a contemporary population of *Fusarium graminearum* from New York wheat and competitiveness of a tebuconazole-resistant isolate. Plant Dis. 98:607–613.

Starkey, D. E., Ward, T. J., Aoki, T., Gale, L. R., Kistler, H. C., Geiser, D. M., et al. 2007. Global molecular surveillance reveals novel Fusarium head blight species and trichothecene toxin diversity. Fungal Genet. Biol. 44:1191–1204.

Stewart, E. L., Hagerty, C. H., Mikaberidze, A., Mundt, C. C., Zhong, Z., and McDonald, B. A. 2016. An improved method for measuring quantitative resistance to the wheat pathogen *Zymoseptoria tritici* using high-throughput automated image analysis. Phytopathology. 106:782–788.

Yang, M., Zhang, H., Kong, X., van der Lee, T., Waalwijk, C., van Diepeningen, A., et al. 2018. Host and cropping system shape the *Fusarium* population: 3ADON-producers are ubiquitous in wheat whereas NIV-producers are more prevalent in rice. Toxins. 10:115.

Ward, T. J., Bielawski, J. P., Kistler, H. C., Sullivan, E., and O'Donnell, K. 2002. Ancestral polymorphism and adaptive evolution in the trichothecene mycotoxin gene cluster of phytopathogenic *Fusarium*. Proc. Natl. Acad. Sci. 99:9278–9283.

Ward, T. J., Clear, R. M., Rooney, A. P., O'Donnell, K., Gaba, D., Patrick, S., et al. 2008. An adaptive evolutionary shift in Fusarium head blight pathogen populations is driving the rapid spread of more toxigenic *Fusarium graminearum* in North America. Fungal Genet. Biol. 45:473–484.

Wei, T., and Simko, V. 2017. *R package "corrplot": Visualization of a Correlation Matrix.* Available at: https://github.com/taiyun/corrplot.

Windels, C. E. 2000. Economic and social impacts of Fusarium head blight: Changing farm and rural communities in the northern Great Plains. Phytopathology 90:17–21.

Zeller, K. A., Bowden, R. L., and Leslie, J. F. 2003. Diversity of epidemic populations of *Gibberella zeae* from small quadrats in Kansas and North Dakota. Phytopathology. 93:874–880.

Zeller, K. A., Bowden, R. L., and Leslie, J. F. 2004. Population differentiation and recombination in wheat scab populations of Gibberella zeae from the United States. Mol. Ecol. 13:563–571.

SUPPLEMENTARY MATERIAL

Table S1. Summary information of sampled small-grain cereals fields in 2018 growing season in Pennsylvania.

F[a]	County	Host	FHB (%)[b]	Previous crop		
				2015	2016	2017
1	Lancaster	Wheat	68.6	Corn	Barley	Corn
2	York	Spelt	49.1	Corn	Wheat	Corn
3	Armstrong	Wheat	16.6	Wheat	Corn	Soybean
4	Centre	Wheat	28.6	Soybean	Corn	Oats
5	Centre	Wheat	48.6	Corn	Soybean	Cucumber
6	Potter	Wheat	20.0	Corn	Corn	Green beans
7	Potter	Spelt	10.0	Wheat	Hay	Green beans
8	Potter	Wheat	7.8	Corn	Corn	Barley
9	Tioga	Wheat	11.0	Soybean	Soybean	Soybean
10	Tioga	Wheat	18.9	Hay	Corn	Corn
11	Tioga	Wheat	10.0	Soybean	Corn	Green beans

[a] Field number (F).

[b] Mean of Fusarium head blight severity (FHB, %) per field.

Table S2. Summary information of sampled small-grain cereals fields in 2019 growing season in Pennsylvania.

F[a]	Location	Host	FHB (%)[b]	Previous crop		
				2016	2017	2018
1	Lancaster	Barley	25.9	Soybean	Wheat	Corn
2	Lancaster	Rye	43.3	--	--	--
3	Lancaster	Spelt	35.9	--	--	--
4	Lancaster	Wheat	76.5	--	--	--
5	Centre	Barley	34.5	Soybean	Corn	Oats
6	Potter	Barley	22.8	Corn	Corn	Corn
7	Potter	Wheat	38.3	--	Green beans	Soybean
8	Erie	Wheat	22.0	Wheat	Soybean	Soybean
9	Erie	Wheat	14.4	Soybean	Soybean	Oats
10	Crawford	Wheat	14.1	Wheat	Soybean	Corn

[a] Field number (F).

[b] Mean of Fusarium head blight severity (FHB, %) per field.

Table S3. Summary information of sampled overwintering residues fields during the Winter 2012 in Pennsylvania.

Code	County	Host	Species	Genotype
AD-DS-W-2	Adams	Wheat residue	*F. graminearum*	15ADON
AD-DS-W-3	Adams	Wheat residue	*F. graminearum*	15ADON
AD-BC2	Adams	Corn stubble	*F. graminearum*	15ADON
AD-KH5	Adams	Corn stubble	*F. graminearum*	15ADON
AR-TG5	Armstrong	Corn stubble	*F. graminearum*	15ADON
BK-WW5	Berks	Corn stubble	*F. graminearum*	15ADON
BK-DZ1	Berks	Corn stubble	*F. graminearum*	15ADON
BK-EM-1	Berks	Corn stubble	*F. graminearum*	15ADON
BK-EM-2	Berks	Corn stubble	*F. graminearum*	15ADON
BK-EM-4	Berks	Corn stubble	*F. graminearum*	15ADON
BK-MB-2	Berks	Corn stubble	*F. graminearum*	15ADON
BK-MB-4	Berks	Corn stubble	*F. graminearum*	15ADON
BT-RW4	Butler	Corn stubble	*F. graminearum*	15ADON
CB-MR2	Columbia	Corn stubble	*F. graminearum*	15ADON
CB-DA1	Columbia	Corn stubble	*F. graminearum*	15ADON
CB-DA2	Columbia	Corn stubble	*F. graminearum*	15ADON
CB-DA3	Columbia	Corn stubble	*F. graminearum*	15ADON
CB-DA4	Columbia	Corn stubble	*F. graminearum*	15ADON
CB-DA-5	Columbia	Corn stubble	*F. graminearum*	15ADON
CB-DA-JG-2	Columbia	Johnson grass	*F. graminearum*	15ADON
CB-DA-JG-3	Columbia	Johnson grass	*F. graminearum*	15ADON
CB-CS3	Columbia	Corn stubble	*F. graminearum*	15ADON
CB-CS5	Columbia	Corn stubble	*F. graminearum*	15ADON
DN-KB-1	Dauphin	Corn stubble	*F. graminearum*	15ADON
FN-SM-B2	Franklin	Corn stubble	*F. graminearum*	15ADON

FN-SM-B3	Franklin	Corn stubble	*F. graminearum*	15ADON
FN-SM-B4	Franklin	Corn stubble	*F. graminearum*	15ADON
FN-RS1	Franklin	Corn stubble	*F. graminearum*	15ADON
FN-RS2	Franklin	Corn stubble	*F. graminearum*	15ADON
FN-RS3	Franklin	Corn stubble	*F. graminearum*	15ADON
FN-SB1	Franklin	Corn stubble	*F. graminearum*	15ADON
FN-SB2	Franklin	Corn stubble	*F. graminearum*	15ADON
FN-SB3	Franklin	Corn stubble	*F. graminearum*	15ADON
FN-F1	Franklin	Corn stubble	*F. graminearum*	15ADON
FN-F2	Franklin	Corn stubble	*F. graminearum*	15ADON
FN-F4	Franklin	Corn stubble	*F. graminearum*	15ADON
FN F JG 1	Franklin	Johnson grass	*F. graminearum*	15ADON
FN-WB3	Franklin	Corn stubble	*F. graminearum*	15ADON
FN-WB4	Franklin	Corn stubble	*F. graminearum*	15ADON
LN-JF-3	Lancaster	Corn stubble	*F. graminearum*	15ADON
LN-BS4	Lancaster	Corn stubble	*F. graminearum*	15ADON
LN-EM-1	Lancaster	Corn stubble	*F. graminearum*	15ADON
LN-EM-2	Lancaster	Corn stubble	*F. graminearum*	15ADON
LN-EM-4	Lancaster	Corn stubble	*F. graminearum*	15ADON
LN-DH-1	Lancaster	Corn stubble	*F. graminearum*	15ADON
LN-DH-3	Lancaster	Corn stubble	*F. graminearum*	15ADON
LN-DH-4	Lancaster	Corn stubble	*F. graminearum*	15ADON
LN-MG-3	Lancaster	Corn stubble	*F. graminearum*	15ADON
LN-MG5	Lancaster	Corn stubble	*F. graminearum*	15ADON
LW-BW-2	Lawrence	Corn stubble	*F. graminearum*	15ADON
LB-GK-1	Lebanon	Corn stubble	*F. graminearum*	15ADON
LB-GK-5	Lebanon	Corn stubble	*F. graminearum*	15ADON

LH-TB-3	Lehigh	Corn stubble	*F. graminearum*	15ADON
LH-TB-4	Lehigh	Corn stubble	*F. graminearum*	15ADON
NU-DJ-10YR-2	Northumberland	Corn stubble	*F. graminearum*	15ADON
NU-DJ-10YR-4	Northumberland	Corn stubble	*F. graminearum*	15ADON
NU-DM3	Northumberland	Corn stubble	*F. graminearum*	15ADON
PT1-2	Potter	Corn stubble	*F. graminearum*	15ADON
PT3-2	Potter	Corn stubble	*F. graminearum*	15ADON
SKRK2	Schuylkill	Corn stubble	*F. graminearum*	15ADON
SK-RK5	Schuylkill	Corn stubble	*F. graminearum*	15ADON
SK-RSII-2	Schuylkill	Corn stubble	*F. graminearum*	15ADON
SK-RSII-4	Schuylkill	Corn stubble	*F. graminearum*	15ADON
SK-JH-2B	Schuylkill	Corn stubble	*F. graminearum*	15ADON
SK-JHII-5	Schuylkill	Corn stubble	*F. graminearum*	15ADON
TG-DC-3	Tioga	Corn stubble	*F. graminearum*	15ADON
TG-CW-1	Tioga	Corn stubble	*F. graminearum*	15ADON
TG-CW-2	Tioga	Corn stubble	*F. graminearum*	15ADON
TG-CW-4	Tioga	Corn stubble	*F. graminearum*	15ADON
TG-MF-2	Tioga	Corn stubble	*F. graminearum*	15ADON
TG-MF-3	Tioga	Corn stubble	*F. graminearum*	15ADON
TG-TW-1	Tioga	Corn stubble	*F. graminearum*	15ADON
YK-AFI-4	York	Corn stubble	*F. graminearum*	15ADON
YK-AFII-2	York	Corn stubble	*F. graminearum*	15ADON
YK-JR-1	York	Corn stubble	*F. graminearum*	15ADON
YK-JR-3	York	Corn stubble	*F. graminearum*	15ADON
YK-JR-4	York	Corn stubble	*F. graminearum*	15ADON

Table S4. Summary information for a collection of 31 *Fusarium graminearum* (n_{15ADON} = 15; n_{3ADON} = 16) isolates obtained from naturally FHB-infected spikes of small-grain cereals from 2018 across Pennsylvania, which were used in five different experiments in this study.

Number	Code	Location	Region	Host	Genotype
01	18SG20iii	Armstrong	Central	Wheat	3ADON
02	18SG20iv	Armstrong	Central	Wheat	3ADON
03	18SG74i	Centre	Central	Wheat	3ADON
04	18SG74iii	Centre	Central	Wheat	3ADON
05	18SG84i	Centre	Central	Wheat	3ADON
06	18SG94i	Centre	Central	Wheat	3ADON
07	18SG94iii	Centre	Central	Wheat	3ADON
08	18SG140i	Tioga	North	Wheat	3ADON
09	18SG140ii	Tioga	North	Wheat	3ADON
10	18SG145i	Tioga	North	Wheat	3ADON
11	18SG168i	Potter	North	Spelt	3ADON
12	18SG168iii	Potter	North	Spelt	3ADON
13	18SG168iv	Potter	North	Spelt	3ADON
14	18SG168v	Potter	North	Spelt	3ADON
15	18SG178ii	Potter	North	Wheat	3ADON
16	18SG178iii	Potter	North	Wheat	3ADON
17	18SG01ii	Lancaster	South	Wheat	15ADON
18	18SG09i	Lancaster	South	Wheat	15ADON
19	18SG11iv	York	South	Spelt	15ADON
20	18SG12i	York	South	Spelt	15ADON
21	18SG25iii	Armstrong	Central	Wheat	15ADON
22	18SG29i	Armstrong	Central	Wheat	15ADON
23	18SG34i	Centre	Central	Wheat	15ADON
24	18SG67i	Centre	Central	Wheat	15ADON
25	18SG76iii	Centre	Central	Wheat	15ADON

26	18SG119i	Potter	North	Wheat	15ADON
27	18SG120i	Potter	North	Wheat	15ADON
28	18SG135ii	Tioga	North	Wheat	15ADON
29	18SG161i	Tioga	North	Wheat	15ADON
30	18SG180ii	Potter	North	Wheat	15ADON
31	18SG182i	Centre	Central	Wheat	15ADON

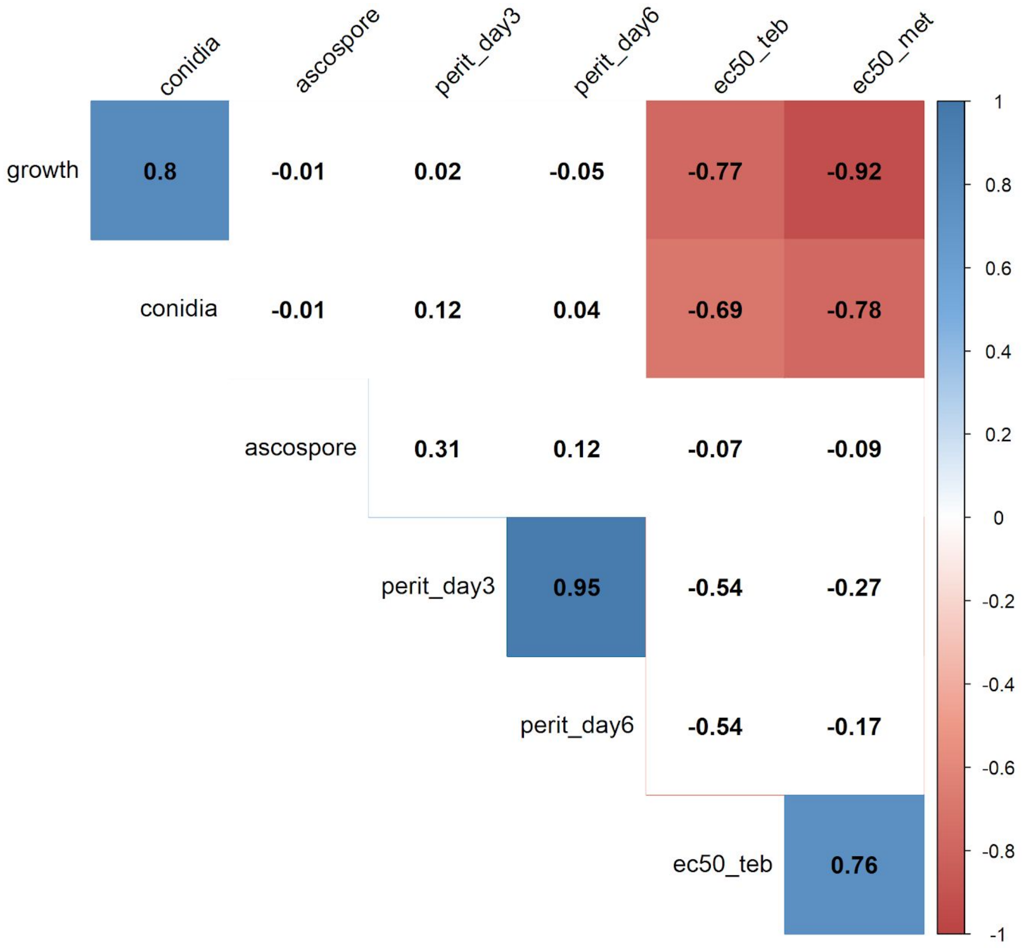

Figure S1. Pearson's correlation coefficients for all pairwise comparisons among fitness traits. Correlation coefficient was calculated using the average each trait per isolate. Negative correlation values are shown in red and positive values in blue. Lighter the color closer the correlation values to zero. Blanks values are not significant at 95% of confidence.

GENERAL CONCLUSIONS

Chapter 1:

DON was detected in 55% and 39% of the samples by the reference method.

The OTA mycotoxin was not found in the samples by both methods.

ZEA was only detected in the samples where DON was also present, indicating the co-occurrence of both toxins in wheat grain samples in Brazil.

The DON levels estimated by immunoassay were generally higher than those estimated by chromatography.

The levels estimated by the two methods for ZEA were in better agreement than for DON.

Chapter 2:

FHB is an important yield-limiting disease of spring wheat in the subtropics.

Our simulations results confirm the trend of increase in yield loss verified in Brazil after FHB resurgence, with no major changes afterward.

One fungicide application proved to be a more profitable production practice for the period after FHB resurgence than for the before period.

Chapter 3:

F. graminearum of the 15ADON genotype is the dominant species associated with FHB in the Eastern U.S., regardless of fungal habitat.

The 3ADON and NIV toxin types were found in lower frequencies than DON.

F. graminearum isolates possessing 3ADON or 15ADON types could not be differentiated for most of the saprophytic traits based on multivariate analysis.

All isolates were sensitive to tebuconazole and metconazole fungicides *in vitro*.