University of Nebraska - Lincoln
DigitalCommons@University of Nebraska - Lincoln

Dissertations, Theses, & Student Research in Food Science and Technology | Food Science and Technology Department

2020

Identification, aggressiveness and mycotoxin production of *Fusarium graminearum* and *F. boothii* isolates causing Fusarium head blight of wheat in Nebraska

Esteban Valverde-Bogantes
University of Nebraska - Lincoln, evalverdebogantes2@huskers.unl.edu

Follow this and additional works at: https://digitalcommons.unl.edu/foodscidiss

Part of the Food Science Commons, and the Plant Pathology Commons

Valverde-Bogantes, Esteban, "Identification, aggressiveness and mycotoxin production of *Fusarium graminearum* and *F. boothii* isolates causing Fusarium head blight of wheat in Nebraska" (2020). *Dissertations, Theses, & Student Research in Food Science and Technology*. 108.
https://digitalcommons.unl.edu/foodscidiss/108

This Article is brought to you for free and open access by the Food Science and Technology Department at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Dissertations, Theses, & Student Research in Food Science and Technology by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

IDENTIFICATION, AGGRESSIVENESS AND MYCOTOXIN PRODUCTION OF *FUSARIUM GRAMINEARUM* AND *F. BOOTHII* ISOLATES CAUSING FUSARIUM HEAD BLIGHT OF WHEAT IN NEBRASKA

by

Esteban Valverde Bogantes

A DISSERTATION

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Doctor of Philosophy

Major: Food Science and Technology

Under the Supervision of Professors Heather Hallen-Adams and Stephen N. Wegulo

Lincoln, Nebraska

May, 2020

IDENTIFICATION, AGGRESSIVENESS AND MYCOTOXIN PRODUCTION OF *FUSARIUM GRAMINEARUM* AND *F. BOOTHII* ISOLATES CAUSING FUSARIUM HEAD BLIGHT OF WHEAT IN NEBRASKA

Esteban Valverde Bogantes, Ph.D.

University of Nebraska, 2020

Advisors: Heather E. Hallen-Adams and Stephen N. Wegulo

Fusarium head blight (FHB) is an economically important disease caused by several *Fusarium* species affecting wheat. Besides causing significant yield losses, infected grain may be contaminated with mycotoxins, primarily trichothecenes, that are harmful to both humans and animals. In recent years, reports of shifts in FHB pathogen populations in North America have highlighted the need for increased vigilance. This dissertation describes a four-year survey evaluating the species, trichothecene genotype and phenotypic diversity of FHB pathogens infecting Nebraska wheat. Most of the isolates were identified as *F. graminearum* (n=67). Additional species included *F. boothii* (n=3), *F. poae* (n=2) and *F. acuminatum* (n=1). An *F. graminearum* × *F. boothii* interspecific hybrid was also identified. All *F. graminearum* and *F. boothii* isolates had the 15-ADON genotype, which is the only genotype that has been reported in the state. Since *F. boothii* had not been previously reported from wheat in the U.S., spikes of the susceptible wheat cultivar Wheaton were inoculated with *F. boothii* isolates from Nebraska under greenhouse conditions in order to fulfill Koch's postulates. FHB symptoms developed in plants inoculated with *F. boothii*, and *F. boothii* was re-isolated from symptomatic plants. A larger greenhouse study was performed to compare the aggressiveness and DON production in wheat among 13 *F. graminearum* and all three *F. boothii* isolates from

Nebraska. Isolates of *F. graminearum* were more aggressive and produced more DON than *F. boothii* isolates. Since most FHB pathogens are indistinguishable morphologically and culturally, a simple PCR-based assay was developed to identify FHB pathogens in order to facilitate species surveillance. Primers were designed based on polymorphisms in the trichothecene 3-O-acetyltransferase (*TRI101*) gene. Individual reactions have been developed to differentiate *F. graminearum*, *F. boothii*, *F. asiaticum*, *F. gerlachii*, and *F. culmorum* from related species, as well as a multiplex reaction to target these five species simultaneously. The research described here expands knowledge of the diversity of FHB pathogens in Nebraska, which will be useful for disease management and provide a baseline for future FHB surveys. The PCR assay described here is expected to facilitate pathogen surveillance.

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to my advisors, Dr. Heather Hallen-Adams and Dr. Stephen Wegulo, for their support, advice, and encouragement. Learning from you has been one of the highlights of my academic career and I am very grateful to have had the opportunity to work under your guidance.

I would also like to extend my gratitude to Dr. Andreia Bianchini, Dr. Devin Rose and Dr. Joshua Herr for being part of my supervisory committee. I very much appreciate your feedback and guidance in completing this dissertation.

Thank you to Dr. Andrew Benson and the Benson lab members: Mallory Van Haute, Qinnan Yang, and Nate Korth, for all of their help throughout my program. I would also like to thank Dr. Carlos Bolaños Carriel, Julie Stevens, Russ Canaday, Briana Kouma and Jenifer Velázquez for their guidance and assistance with the greenhouse experiments.

Thank you to our collaborators Dr. Todd Ward (USDA-ARS), Dr. David Schmale III (Virginia Tech) and Niki McMaster (Virginia Tech) for all their help throughout this project.

Last but certainly not least, I sincerely thank my family and friends for their care, love, and support. I could not have been able to do this without you.

GRANT INFORMATION

This work was partially supported by the Nebraska Agricultural Experiment Station with funding from the Hatch Multistate Research capacity funding program (Accession Number 1006715) from the USDA National Institute of Food and Agriculture.

Wheat surveys were funded by the Nebraska Wheat Board.

PREFACE

Crop plant pathogens cause significant economic losses annually and represent an important threat to food security worldwide. The progression of traditional agriculture from small-scale farms to the large-scale and high-density deployment of genetically identical monocultures in modern agriculture, together with the deployment of disease management tactics by humans, has changed the coevolutionary dynamics between plants and pathogens, generating conditions favorable for disease epidemics (McDonald and Stukenbrock 2016).

One of the diseases that has increased in importance over the last 30 years is Fusarium head blight (FHB), which is caused by several *Fusarium* species and affects wheat, barley and other small grain cereals (McMullen et al. 1997). It has been estimated that FHB is globally the second largest cause of crop losses due to pathogens and pests affecting wheat (Savary et al. 2019). Besides causing direct losses in yield, infected grain may be contaminated with mycotoxins that are harmful to both humans and animals, further reducing the marketability of the grains. Therefore, FHB represents both a food security and a food safety threat. Although FHB epidemics tend to be sporadic due to several factors, they can be devastating in terms of primary and secondary economic losses, as well as sociological impacts (McMullen et al. 1997).

In the context of the damage to crop plants caused by plant pathogens, information on the pathogen populations infecting wheat in a particular region is important not only in assessing the risk to the crop, but also in the deployment of effective disease management strategies. Therefore, ongoing surveillance of FHB pathogens is important to monitor their distribution and ecology, as well as to detect

population clines and the introduction of strains with novel phenotypes. Reports of selective sweeps in FHB pathogen populations from Canada and the Upper Midwest region of the United States that were expanding in range due to selective advantages (Ward et al. 2008) and the location of Nebraska in the Midwest motivated the research presented in this dissertation.

This dissertation will begin with a literature review describing recent population changes of FHB pathogens around the world, focusing on drivers and implications of such changes (Chapter 1). This review has been published in the *Canadian Journal of Plant Pathology* (Valverde-Bogantes et al. 2019).

In chapter 2 of this dissertation, the results from a four-year survey of pathogens infecting wheat in Nebraska are presented. It was found that *F. graminearum* remains the main FHB pathogen in the state. This survey also revealed the first association of *F. boothii* with FHB in the U.S.

Following the chapter on the survey of species diversity of FHB pathogens in Nebraska, Chapter 3 describes the fulfillment of Koch's postulates with the newly discovered *F. boothii* isolates in order to formally report *F. boothii* for the first time as an FHB pathogen in the country. The cultural and morphological characteristics of the *F. boothii* isolates obtained in the survey study are described. A summary of the results from this chapter has been published as a Disease Note in *Plant Disease* (Wegulo et al. 2018).

In order to obtain a better understanding of the implications of the presence of *F. boothii* in the state, a greenhouse study was conducted to evaluate the aggressiveness and mycotoxin production in wheat among 13 *F. graminearum* and three *F. boothii* isolates

from Nebraska (Chapter 4). It was observed that the *F. graminearum* isolates were more aggressive and produced more DON than *F. boothii* isolates. This article was submitted for publication in *Plant Health Progress*. (*Valverde-Bogantes E, Bolanos-Carriel C, Hallen-Adams HE, McMaster N, Schmale III DG, and Wegulo SN, "Aggressiveness and deoxynivalenol production of Nebraska isolates of *Fusarium boothii* and *F. graminearum*" (submitted January, 2020).)

The final chapter of this dissertation (Chapter 5) describes a PCR-based method that was developed as an alternative to overcome the difficulties encountered in identifying FHB pathogens to species level due to their overlapping morphological simplicity combined with within-species variability. This method is expected to facilitate rapid pathogen surveillance in a relatively inexpensive and high-throughput way without the need for special equipment.

The research described here expands knowledge of the diversity of FHB pathogens in Nebraska, which will be useful for disease management and provide a baseline for future FHB surveys.

References

McDonald, B. A., and Stukenbrock, E. H. 2016. Rapid emergence of pathogens in agro-ecosystems: Global threats to agricultural sustainability and food security. Philos. Trans. R. Soc. B Biol. Sci. 371.

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.

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.

Valverde-Bogantes, E., Bianchini, A., Herr, J. R., Rose, D. J., Wegulo, S. N., and Hallen-Adams, H. E. 2019. Recent population changes of Fusarium head blight pathogens: drivers and implications. Can. J. Plant Pathol. DOI: 10.1080/07060661.2019.1680442.

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.

Wegulo, S. N., Valverde-Bogantes, E., Bolanos-Carriel, C., Hallen-Adams, H., Bianchini, A., McMaster, N., et al. 2018. First Report of *Fusarium boothii* Causing Head Blight of Wheat in the United States. Plant Dis. 102:2646.

TABLE OF CONTENTS

LIST OF TABLES xiv

LIST OF FIGURES xvi

CHAPTER 1 1

Recent population changes of Fusarium head blight pathogens: drivers and implications 1

Introduction 3

Shifts in populations of FHB pathogens 6

Replacement of *F. culmorum* by *F. graminearum* in Europe 10

Fusarium asiaticum 3-ADON replaces the NIV population in China 13

Fusarium graminearum 3-ADON replacing 15-ADON population in North America 15

Reports of new species/genotypes in a region 21

Fusarium graminearum 'Gulf coast' and 'Southern Louisiana' populations in the USA. 22

Fusarium graminearum NIV in Luxembourg. 24

Fusarium graminearum 15-ADON in Norway. 24

Fusarium graminearum in South Korea. 25

Fusarium boothii in Hungary, France, and the USA. 26

Fusarium vorosii in Hungary. 27

Fusarium cortaderiae in New Zealand, France and Italy. 28

Implications of population changes and introduction of new species/genotypes 29

Concluding remarks 31

References 32

CHAPTER 2 51

A four-year survey of pathogens causing Fusarium head blight of wheat in Nebraska, USA from 2015-2018 51

Abstract 51

Introduction 53

Materials and Methods 55

Samples 55

Isolation of FHB pathogens and DNA extraction 56

Determination of *Fusarium* species 57

Determination of trichothecene genotype 58

Results 59

Species identification 59

Phylogenetic analyses 60

Trichothecene genotypes 61

Discussion 61

Conclusions 63

References 64

CHAPTER 3 88

Fusarium boothii from Nebraska: Fulfillment of Koch's postulates 88

Abstract 88

Introduction 90

Materials and Methods 91

Fusarium isolates 91

Pathogenicity tests in the greenhouse 92

Experiment design 92

Inoculum preparation 93

Plant materials, inoculation and disease rating 94

Mycotoxin quantification and re-isolation of *Fusarium* from symptomatic heads 94

Data analysis 95

Cultural and morphological characteristics of *Fusarium* isolates........ 95

Results........ 96

Pathogenicity tests in the greenhouse 96

Cultural and morphological characteristics of *Fusarium* isolates........ 97

Discussion........ 98

Conclusions........ 100

References........ 101

CHAPTER 4 112

Aggressiveness and deoxynivalenol production of Nebraska isolates of *Fusarium boothii* and *F. graminearum* 112

Abstract........ 112

Greenhouse experiments........ 116

Pathogen isolates........ 116

Inoculum. 116

Plant material and inoculation. 117

Experimental design........ 118

Disease assessment, harvesting, and mycotoxin analysis........ 118

Data analysis. 119

Isolate aggressiveness. 119

Mycotoxin production........ 120

Relationships between AUDPC and mycotoxin concentrations........ 120

Conclusions and Implications for Disease Management........ 121

Acknowledgements........ 124

Literature cited........ 124

CHAPTER 5 ... 136

A novel PCR-based method for the identification of Fusarium head blight pathogens ... 136

Abstract ... 136

Introduction ... 138

Materials and Methods ... 140

Fungal cultures and DNA extraction ... 140

Primer design ... 141

PCR amplification ... 142

Results ... 143

Discussion ... 144

Conclusions ... 148

References ... 149

CHAPTER 6 ... 164

Summary of conclusions and future work ... 164

LIST OF TABLES

Chapter 2

Table 1. Summary of the wheat heads sampled across Nebraska for this study and their location 74

Table 2. Sequence of the primers used for species identification 75

Table 3. Sequence of the primers used to determine trichothecene genotype 75

Table 4. Isolate ID, wheat growing region and species for the *Fusarium* strains obtained from Nebraska wheat in this study 76

Chapter 3

Table 1. Overall means and Fisher's least significant difference (LSD) for FHB severity (percentage of symptomatic spikelets), deoxynivalenol (DON; µg/g), 15-acetyldeoxynivalenol (15-ADON; µg/g) and 3-acetyldeoxynivalenol (3-ADON; µg/g) in spring wheat cultivar Wheaton grown under greenhouse conditions inoculated with *F. boothii* and *F. graminearum* isolates from Nebraska 111

Chapter 4

Table 1. Means and Fisher's least significant difference (LSD) for deoxynivalenol (DON), 15-acetyldeoxynivalenol (15-ADON), Fusarium head blight (FHB) severity, and area under the disease. progress curve (AUDPC) in the susceptible spring wheat cultivar Wheaton grown under greenhouse conditions and inoculated with three *F. boothii* and 13 *F. graminearum* isolates from Nebraska 130

Table 2. Fusarium head blight area under the disease progress curve (AUDPC), deoxynivalenol (DON) and 15-acetyldeoxynivalenol (15-ADON) in spring wheat cultivar Wheaton grown under greenhouse conditions and inoculated with three *Fusarium boothii* and 13 *F. graminearum* isolates from Nebraska 131

Chapter 5

Table 1. *Fusarium* isolates used in this study 155

Table 2. DNA suspensions provided by Dr. Todd Ward (ARS, USDA)........... 155

Table 3. Primers used in this study 156

Table 4. PCR setup for the identification of *F. boothii*, *F. culmorum*, *F. graminearum*, *F. asiaticum*, and *F. gerlachii* 156

LIST OF FIGURES

Chapter 2

Figure 1. Map showing the different wheat growing regions in Nebraska, as well as the sampling sites where wheat grains were obtained 78

Figure 2. Number of isolates by species obtained from Nebraska wheat from 2015-2018 79

Figure 3. Maximum likelihood phylogram for *TEF1* sequences from 74 *Fusarium* isolates collected from Nebraska wheat in relation to reference isolates 80

Figure 4. Data from Figure 3 with *F. graminearum* isolates condensed 81

Figure 5. Maximum likelihood phylogram for *TRI101* sequences from 70 *Fusarium* isolates collected from Nebraska wheat in relation to reference isolates 82

Figure 6. Data from Figure 5 with *F. graminearum* isolates condensed 83

Figure 7. Maximum likelihood phylogram for *RED* sequences from 71 *Fusarium* isolates collected from Nebraska wheat in relation to reference isolates 84

Figure 8. Data from Figure 7 with *F. graminearum* isolates condensed 85

Figure 9. Maximum likelihood phylogram for combined *TEF1*, *TRI101* and *RED* sequences from 70 *Fusarium* isolates collected from Nebraska wheat in relation to reference isolates 86

Figure 10. Data from Figure 9 with *F. graminearum* isolates condensed 87

Chapter 3

Figure 1. Wheaton plants inoculated with *Fusarium* isolates showing premature bleaching characteristic of FHB 106

Figure 2. Premature bleaching of spikelets characteristic of FHB on Wheaton wheat 21 days after inoculation with *F. boothii* isolate NE16-15Fb 106

Figure 3. Premature bleaching of spikelets characteristic of FHB on Wheaton wheat 21 days after inoculation with *F. boothii* isolate NE19-15Fb 107

Figure 4. Front (left) and reverse (right) colony morphology of *F. boothii* isolates NE16-15Fb (A), NE19-15Fb (B), and

F. graminearum isolate NE20-15Fg (C) on PDA incubated at room temperature for 7 days 108

Figure 5. Length and width in µm of 5-septate conidia of *F. boothii* and *F. graminearum* isolates from Nebraska grown on Bilay's media 109

Figure 6. Length and width in µm of mature ascospores of *F. boothii* and *F. graminearum* isolates from Nebraska grown on carrot agar 109

Figure 7. Examples of perithecia and ascospores produced by *F. boothii* isolates NE16-15Fb (A), NE19-15Fb (B), and *F. graminearum* isolate NE20-15Fg (C) on carrot agar 110

Chapter 4

Figure 1. Map of Nebraska showing the geographic origin of the *Fusarium boothii* and *F. graminearum* isolates used in this study. 132

Figure 2. Regressions of deoxynivalenol (DON) concentration in wheat grain on area under the disease progress curve (AUDPC), combined (**A**) and by species (**B**), for three *Fusarium boothii* and 13 *F. graminearum* isolates from Nebraska 133

Supplementary figure S1. Area under the disease progression curve (AUDPC) values of three *F. boothii* and 13 *F. graminearum* isolates from Nebraska 134

Supplementary figure S2. Deoxynivalenol (DON) concentration in grain for three *F. boothii* and 13 *F. graminearum* isolates from Nebraska 135

Chapter 5

Figure 1. Agarose gel electrophoresis showing results obtained after singleplex amplification with the SNAP primer targeting *F. boothii* (FbooSNAP1) and reverse primer (TRI101rev) 157

Figure 2. Agarose gel electrophoresis showing results obtained after singleplex amplification with the SNAP primer targeting *F. culmorum* (FculSNAP1) and reverse primer (TRI101rev) 158

Figure 3. Agarose gel electrophoresis showing results obtained after singleplex amplification with the SNAP primer targeting *F. graminearum* (FgramSNAP1) and reverse primer (TRI101rev) 159

Figure 4. Agarose gel electrophoresis showing results obtained after singleplex amplification with the SNAP primer targeting *F. asiaticum* (FasiSNAP1) and reverse primer (TRI101rev) 160

Figure 5. Agarose gel electrophoresis showing results obtained after singleplex amplification with the SNAP primer targeting *F. gerlachii* (FgerSNAP1) and reverse primer (TRI101rev) 161

Figure 6. Agarose gel electrophoresis showing results obtained after multiplex amplification with SNAP primers 162

Figure 7. Agarose gel electrophoresis showing results obtained after multiplex amplification with SNAP primers 163

CHAPTER 1

Recent population changes of Fusarium head blight pathogens: drivers and implications

This chapter is published: Valverde-Bogantes, E., Bianchini, A., Herr, J. R., Rose, D. J., Wegulo, S. N., and Hallen-Adams, H. E. 2019. Recent population changes of Fusarium head blight pathogens: drivers and implications. Can. J. Plant Pathol. DOI: 10.1080/07060661.2019.1680442.

Abstract

Several *Fusarium* species cause Fusarium head blight (FHB), a devastating disease that affects wheat and other small grain cereals. Besides causing significant yield losses, FHB pathogens reduce the quality of the grain and may produce mycotoxins that are harmful to both humans and animals. The development of effective management strategies for this disease requires an understanding of the composition of the FHB pathogen populations, which is important because different populations could be affected selectively. Recent reports of shifts in populations of FHB pathogens have shown that these populations are dynamic and change continuously, often associated with increased yield losses or changes in the mycotoxins produced in the grain. These population shifts include: replacement of *F. graminearum* by *F. poae* in Italy, hypothesized to be due to variation in environmental conditions; replacement of *F. culmorum* by *F. graminearum*

in Europe, associated with climate change and increased maize production; a more aggressive and toxigenic *F. asiaticum* 3-ADON population replacing the existing NIV population in China; and a highly toxigenic population composed mainly of *F. graminearum* 3-ADON isolates replacing the existing 15-ADON population in North America. Additionally, reports of recent introductions of foreign species or populations into new areas include: *F. asiaticum* outside of Asia, *F. graminearum* NIV isolates in the USA and Luxembourg, *F. graminearum* 15-ADON isolates in Norway, *F. graminearum* in South Korea, *F. boothii* in Europe and USA, *F. vorosii* in Hungary and *F. cortaderiae* in New Zealand and Europe.

Introduction

Fusarium head blight (FHB) is a devastating and economically important disease that affects wheat, barley, oats and other small grain cereals. Even though epidemics of FHB tend to be sporadic, they can be devastating (McMullen et al. 1997). For instance, from 1998 to 2000 FHB epidemics caused losses of almost $3 billion in the USA and Canada alone (Goswami and Kistler 2004), and all of the cereal-growing regions in the world have experienced FHB epidemics (Dean et al. 2012). Furthermore, losses caused by FHB are not limited to grain producers, as they also have an effect on other sectors of the regional economy such as retail trade, finance, insurance and real estate. It has been estimated that secondary impacts of FHB on the economy amount to more than twice the direct losses sustained by grain producers (Nganje et al. 2004).

Fusarium head blight infection causes premature bleaching of spikes that fail to produce seed or produce shrivelled and soft kernels with reduced weight and functional qualities (Trail 2009). Additionally, the grain may be contaminated with mycotoxins, which are harmful to both humans and animals. Grain contaminated with mycotoxins is unsafe for human and animal consumption or for malting (Dean et al. 2012). Mycotoxin contamination further reduces marketability and prices, in many instances forcing farmers to sell at animal feed prices or to be penalized with huge discounts (McMullen et al. 1997). Consequently, grain affected by FHB is difficult to market, export, process, and feed. Low commodity prices coupled with the difficulties inherent in avoiding FHB and the economic losses it represents can make wheat cultivation unattractive to farmers. This situation has forced many US farmers to shift to less risky crops (McMullen et al. 2012; Bianchini et al. 2015).

Fusarium head blight is caused by several *Fusarium* species. Most of the FHB pathogens are grouped within the *Fusarium graminearum* species complex (FGSC), which includes: *F. acaciae-mearnsii*, *F. aethiopicum*, *F. asiaticum*, *F. austroamericanum*, *F. boothii*, *F. brasilicum*, *F. cortaderiae*, *F. gerlachii*, *F. graminearum*, *F. louisianense*, *F. meridionale*, *F. mesoamericanum*, *F. nepalense*, *F. ussurianum* and *F. vorosii* (Aoki et al. 2012). Species within the FGSC show a significant biogeographic structure, which suggests that independent allopatric speciation occurred in different parts of the world (O'Donnell et al. 2004; Aoki et al. 2012). Additionally, some degree of host preference or adaptation has been reported for FGSC species. For example, *F. asiaticum* predominates in rice, while *F. boothii* and *F. meridionale* seem to prefer maize in some areas of the world (Lee et al. 2009; Boutigny et al. 2011; Sampietro et al. 2011). The members of the FGSC are self-fertile (= homothallic) (O'Donnell et al. 2004) and can also cause Gibberella ear rot (GER) and Gibberella stalk rot (GSR) in maize (Gai et al. 2017). Morphological and phenotypic characters are not sufficient to distinguish most of the species within the FGSC due to their morphological simplicity and overlapping conidial features combined with within-species variability (O'Donnell et al. 2004). Hence, molecular methods are required for an accurate identification. In addition, *F. lunulosporum, F. pseudograminearum, F. cerealis* and *F. culmorum* are self-sterile (= heterothallic) FHB pathogens closely related to the FGSC. Three novel species outside of the FGSC that are able to cause FHB, namely *F. dactylidis* (Aoki et al. 2015), *F. praegraminearum* (Gräfenhan et al. 2016) and *F. subtropicale* (Pereira et al. 2018) have recently been described. *Fusarium avenaceum, F. langsethiae, F. poae, F.*

sporotrichioides and the non-toxigenic species *Microdochium nivale* are also commonly associated with the disease in different parts of the world (van der Fels-Klerx et al. 2012).

Fusarium species have the ability to produce an array of harmful mycotoxins. The most common mycotoxins associated with FHB are trichothecenes, but most species can also produce oestrogenic mycotoxins including zearalenone (O'Donnell et al. 2000; Stępień and Chełkowski 2010). The trichothecene mycotoxins are eukaryotic protein synthesis inhibitors and the most important are type A and B trichothecenes (Pestka and Smolinski 2005; Foroud and Eudes 2009). Members of the FGSC usually produce type B trichothecenes, and isolates are classified on their particular toxigenic profile (chemotype). These chemotypes include: (i) 3-ADON, producing deoxynivalenol (DON) and 3-acetyldeoxynivalenol (3-ADON); (ii) 15-ADON, producing DON and 15-acetyldeoxynivalenol (15-ADON); and (iii) NIV, producing nivalenol (NIV) and its acetylated derivatives. PCR methods have been developed to predict the chemotype of isolates (Ward et al. 2002). Symptoms after acute exposure to DON in sensitive animal species can range from vomiting to diarrhoea and anorexia (Pestka and Smolinski 2005). Moreover, DON acts as a virulence factor in wheat, causing necrosis and facilitating the spread of the fungus (Trail 2009). On the other hand, type A trichothecenes are usually produced by non-FGSC species such as *F. poae, F. langsethiae* and *F. sporotrichioides*, and they include the highly toxic T-2 and HT-2 toxins (Stępień and Chełkowski 2010; O'Donnell et al. 2013). Surprisingly, isolates of *F. graminearum* capable of producing a novel type A trichothecene called NX-2 have recently been reported in the northern USA and southern Canada (Varga et al. 2015; Kelly et al. 2016; Kelly and Ward 2018; Lofgren et al. 2018). This observation was unexpected, since *F. graminearum* is part of a diverse

lineage of *Fusarium* species characterized by the production of B type trichothecenes and therefore initially called the B-clade (O'Donnell et al. 2013). Consequently, the B-clade is now referred to as the *Fusarium sambucinum* species complex lineage 1 (FSAMSC-1) in order to avoid assumptions about the type of trichothecene produced by this group (Kelly et al. 2016).

Multiple field surveys have reported changes in the populations of FHB pathogens over time, which have been associated with increased yield losses due to the introduction of more aggressive and toxigenic isolates, or to changes in the mycotoxins accumulated in the grain due to the introduction of isolates with different mycotoxin profiles (van der Lee et al. 2015). Climate change is expected to increase severity of FHB epidemics (Zhang et al. 2014), and global warming will likely create suitable conditions for additional population shifts in the future (van der Lee et al. 2015). Thus, active surveillance is pivotal in early detection of potential population changes. The current review aims to summarize recent changes in populations of FHB pathogens around the world, as well as reports of foreign species or populations introduced in an area, with particular focus on the drivers of change. Moreover, the implications of these population shifts for grain producers and management of the disease are also discussed.

Shifts in populations of FHB pathogens

Changes in populations of FHB pathogens can occur at the species level when a species with a selective advantage replaces another species. Additionally, a different population of the same species could replace the existing population in a region. The

introduction of foreign pathogens could result in a displacement event if the conditions are advantageous for the invading population. The following are recent reports of changes in the composition of FHB pathogens in different regions.

Replacement of *F. graminearum* by *F. poae*

The main causal agents of FHB in Italy have fluctuated throughout the years. The most common pathogens isolated from wheat seed from 1999 to 2002 were *F. graminearum, F. poae* and *M. nivale* (Shah et al. 2005). The incidence of seed infection was higher in the north of the country, with *M. nivale* accounting for most of the infections in that region, followed by *F. graminearum* and *F. poae*. Conversely, the incidence of seed infection was lower in the central and southern regions of Italy, and *F. poae* was the predominant species isolated.

During 2004 to 2006, the most abundant species isolated from organic wheat seed, in decreasing order, were *F. poae, M. nivale, F. verticillioides* and *F. graminearum* (Infantino et al. 2012). Seed infection rates were low (<5% for all species) but *F. poae* was the most commonly isolated species in 2005 and 2006. Moreover, the mean seed infection rate by *F. graminearum* decreased from 0.55% in 2004 to 0.25% in 2006. The predominance of *F. poae* was speculated to be due to the weather in Italy being warmer and drier than other European countries, which favours *F. poae* over other species (Xu et al. 2008).

In another survey study conducted in northern Italy from 1995 to 2007, *F. graminearum* was the most common species causing FHB in durum wheat (Pancaldi et

al. 2010). However, its frequency of isolation from affected heads peaked at 67.5% in 1996, then fluctuated with a gradual downward trend until 2007 when the frequency of isolation was only 15.7%. Conversely, isolation of *F. poae* had an upward trend, with a frequency of 5% in 1999 and 23.2% in 2007. The isolation frequency of *F. poae* was higher in years when *F. graminearum* had a lower frequency and vice versa. *Fusarium poae* was more frequent than *F. graminearum* in 1997, 2005 and 2007. The authors suggested that frequencies varied in response to environmental conditions.

A two-year study conducted in central Italy in 2009 and 2010 revealed that *F. graminearum* was the most commonly isolated species from durum and soft wheat kernels (Covarelli et al. 2015). However, its frequency of isolation, relative to the other species isolated, was lower in 2009 (27% and 16% in durum and soft wheat, respectively) than in 2010 (69% and 60%). In contrast, the isolation frequency of *F. poae* and *F. avenaceum* was higher in 2009 than in 2010. There was lower rainfall and relative humidity, as well as higher temperatures, during anthesis in 2009 than in 2010. The authors indicated that the drier and warmer conditions in 2009 were unfavourable for *F. graminearum*, allowing increased incidence of *F. poae* and *F. avenaceum*.

A similar pattern was reported in Italian barley by many studies. *Fusarium graminearum* was never the predominant species in a study conducted in central Italy from 2011 to 2013, whereas *F. avenaceum*, *F. tricinctum* and *F. poae* were the main species causing FHB in 2011, 2012 and 2013, respectively (Beccari et al. 2017). Another survey from the same region revealed that 63% of the isolates causing FHB of barley in 2013 were identified as *F. avenaceum* (Beccari et al. 2016). However, its prevalence

decreased to 23% in 2014 (Beccari et al. 2018). Additionally, the results from both studies showed that the prevalence of *F. poae* increased from 5% in 2013 to 37% in 2014.

There have been reports of *F. poae* becoming an important pathogen of cereals in other countries as well. *Fusarium poae* has been the main FHB pathogen isolated from wheat in the Czech Republic since 2012, replacing *F. graminearum*, which used to be the predominant species (Sumíková et al. 2017). The authors speculated that dry environmental conditions observed during anthesis in 2012 favoured *F. poae*, which in turn could produce large amounts of inoculum that boosted its frequency in the following years. In addition, a survey in Argentina revealed that *F. poae* was isolated from barley more frequently than *F. graminearum*, 54.7% and 39.5%, respectively (Nogueira et al. 2018).

Disease surveys in Manitoba, Canada have revealed that *F. poae* has been the main FHB pathogen infecting barley in the province in recent years (Tekauz et al. 2013; Banik et al. 2014, 2016; Beyene et al. 2015). However, *F. graminearum* was the predominant pathogen reported in 2016 after excessive moisture during the growing season, which could have favoured *F. graminearum* over *F. poae* (Banik et al. 2017). In 2017, dry conditions predominated in the province, and *F. poae* was once again the predominant pathogen species reported in barley (Banik et al. 2018).

Despite being reported as less aggressive FHB pathogens when compared with the FGSC (Xue et al. 2004; Cerón-Bustamante et al. 2018), the predominance of *F. avenaceum* or *F. poae* could cause a change in the mycotoxins accumulated in the grain. *Fusarium avenaceum* has been reported to produce moniliformin, enniatin analogues and beauvericin (Beccari et al. 2018), while *F. poae* is known to accumulate NIV and type A

trichothecenes such as diacetoxyscirpenol and neosolaniol (Vanheule et al. 2017; O'Donnell et al. 2018). These examples show how changes in climatic conditions continuously shape not only the species composition of FHB pathogens, but also the mycotoxin contamination of the crops as a consequence.

Replacement of *F. culmorum* by *F. graminearum* in Europe

Early reports from many European countries show that FHB was caused predominantly by *F. culmorum* (Parry et al. 1995; Waalwijk et al. 2003). However, *F. graminearum* seems to have increased in importance in Europe. The first indication of *F. graminearum* replacing *F. culmorum* as the main FHB pathogen in Europe was from a two-year study in the Netherlands in the early 2000s, which revealed that *F. graminearum* accounted for 58.5% of the isolates, whereas only 24% were identified as *F. culmorum* (Waalwijk et al. 2003). The long-term nature of this shift was documented by another study in 2009 that found *F. graminearum* in 46.5% of the wheat samples analysed, while *F. culmorum* was detected in only 2.3% of the samples (van der Fels-Klerx et al. 2012).

Similar shifts have been noted in other European countries where *F. graminearum* is now the most abundant FHB pathogen. A five-fold increase in the frequency of *F. graminearum* from 1998 to 2006 in Poland was reported, accompanied by a decline in the frequency of *F. culmorum* (Stepień et al. 2008). More recently, surveys in 2009 and 2012–2014 showed that *F. graminearum* was still the predominant pathogen found in Poland (Chełkowski et al. 2012; Gorczyca et al. 2018). Moreover, the level of *F.*

graminearum has risen in the UK and Ireland at the expense of *F. culmorum* (Jennings et al. 2004; Xu et al. 2005). This finding was unexpected as these countries tend to have cooler summers than the rest of Europe, which would favour *F. culmorum* (Xu et al. 2005).

In Belgium, *F. graminearum* and *F. culmorum* were the most important pathogens isolated from wheat during 2002 to 2005 (Isebaert et al. 2009). Additionally, Isebaert et al. (2009) found that *F. graminearum* was isolated at higher frequencies when wheat was grown after maize or in regions where maize was an important crop. Additional evidence for the decreased prevalence of *F. culmorum* in Belgium was provided by two separate surveys conducted during 2003–2009 and 2007–2008 (Audenaert et al. 2009; Chandelier et al. 2011).

In Denmark, analysis of historical data of wheat samples from 1957 to 2000 showed low levels of *F. graminearum*, while *F. culmorum* was predominant along with *F. avenaceum* and *M. nivale* (Nielsen et al. 2011). However, a rapid increase in the prevalence of *F. graminearum* was noted in samples obtained from 2003 to 2007. *Fusarium graminearum* has also become increasingly important in northern Germany (Klix et al. 2008), as well as in Finland (Hietaniemi et al. 2016) and Sweden (Karlsson et al. 2017).

Even though the underlying causes of the shift from *F. culmorum* to *F. graminearum* have not been unequivocally elucidated, several hypotheses have been proposed. First, *F. graminearum* is regarded as having been recently introduced to Europe along with maize production (Xu et al. 2005). In countries such as Denmark, maize was introduced in the 1980s and the agricultural area dedicated to the crop has

rapidly increased (Nielsen et al. 2011). Maize is often included in crop rotation systems with wheat (Xu et al. 2005), which along with reduced tillage practices favours *F. graminearum* due to its ability to infect maize and overwinter on crop residue (Waalwijk et al. 2003). The fact that *F. graminearum* was more common in wheat grown after maize or from areas where maize was the predominant crop supports this hypothesis (Isebaert et al. 2009). Second, higher mean temperatures caused by climate change could favour *F. graminearum*, as it prefers warmer temperatures than *F. culmorum* (Xu et al. 2005; Nielsen et al. 2011). Finally, certain characteristics give *F. graminearum* increased competitiveness over *F. culmorum. Fusarium graminearum* has usually been regarded as more aggressive than *F. culmorum* in terms of yield loss and mycotoxin production (Jennings et al. 2004). In addition, being a homothallic species, *F. graminearum* can produce ascospores that are very important epidemiologically, unlike *F. culmorum*, which depends on the spread of its macroconidia for dispersal (Audenaert et al. 2009). Additionally, sexual reproduction can be advantageous to *F. graminearum* in terms of generating variability for adaptation to new environments (Xu et al. 2005).

Climate change is expected to bring higher temperatures and more humid conditions, especially to northern Europe, which would favour *F. graminearum* over *F. culmorum* (Parikka et al. 2012). These conditions would also allow for an expansion in maize cultivation to areas where it is currently not grown, providing a suitable substrate for overwintering *F. graminearum* (Parikka et al. 2012; West et al. 2012).

Outside of Europe, a similar shift in *Fusarium* spp. was recently documented in Idaho, USA (Bissonnette et al. 2018). The authors found that *F. graminearum* was causing most of the FHB in the state (87% of the isolates), as opposed to *F. culmorum*

(13%), which had been the prevalent pathogen according to a survey from 1984. Increased maize cultivation in the state, as well as maize-wheat rotation systems, were linked to this change in composition.

Fusarium asiaticum 3-ADON replaces the NIV population in China

The predominant FHB pathogen in China is *F. asiaticum*. Initial studies on the diversity of trichothecene genotypes of *F. asiaticum* isolated from barley in China along the Yangtze river revealed that there were sharp differences in their distribution (Yang et al. 2008). Despite being unable to differentiate between 15-ADON and 3-ADON isolates, the authors noted that NIV isolates were much more common than DON isolates (91% and 8.2%, respectively) in the upper valleys of the river, a region surrounded by large mountain ridges, which might act as a geographic barrier. On the other hand, DON producers had higher frequencies in all other regions surveyed (>70%, middle and lower reaches of the river). Since there was no clear correlation between trichothecene genotype and cropping system or climate variables, it was hypothesized that DON isolates had been recently introduced and were replacing the NIV population, which still existed at higher frequencies in the south-west region because of the mountain barrier.

Subsequently, a study conducted by Zhang et al. (2010) provided greater insight into the population dynamics of FHB pathogens in China. The survey determined that most of the DON isolates in the middle and lower parts of the Yangtze river belonged to the 3-ADON genotype, while a small number of isolates were of the 15-ADON genotype (Zhang et al. 2010). The authors found significantly higher genetic diversity in the upper

reaches, where NIV isolates were dominant. Moreover, pairwise population genetic differentiation (F_{ST}) values showed that NIV and 3-ADON isolates were significantly different and therefore represented separate populations. Finally, isolates with high admixture probabilities revealed that there was a bias in the gene flow from 3-ADON to NIV isolates. Based on these results, the authors concluded that the 3-ADON population was spreading from east to west, replacing the existing NIV population.

In order to determine the cause of the shift in the *F. asiaticum* population, Zhang et al. (2012) carried out another survey and evaluated the fitness and aggressiveness of the isolates from each trichothecene genotype. The results supported the previous hypothesis that the 3-ADON population was increasing in frequency from east to west. In addition, they obtained similar levels of genetic differentiation between the populations, as well as an increase in biased gene flow from 3-ADON to NIV isolates. As far as aggressiveness, the authors found that the 3-ADON isolates caused more disease in moderately resistant and susceptible winter wheat varieties than NIV isolates. *In vitro* tests showed that the 3-ADON producers were not only able to grow faster and produce more and larger conidia, but also accumulated more mycotoxins than the NIV isolates. Additionally, the 3-ADON isolates had a higher level of benzimidazole resistance than NIV isolates. Taken together, these studies show that a population of more aggressive and toxigenic *F. asiaticum* of the 3-ADON genotype and possessing benzimidazole resistance is expanding from east to west, as well as changing the trichothecene genotype composition in China.

Fusarium graminearum **3-ADON replacing 15-ADON population in North America**

Previous studies on the population structure of *F. graminearum* in North America pointed towards the hypothesis that it consisted of a panmictic population with little differentiation, but with high genetic diversity due to frequent recombination events (Zeller et al. 2004). However, this hypothesis was rejected when two distinct populations were discovered in the upper Midwest (Gale et al. 2007). The dominant population (89%) was composed mostly of 15-ADON genotype isolates and it was named MW15ADON (MW = Midwest). The second population found in Minnesota and North Dakota was referred to as UMW3ADON (UMW = upper Midwest) because it contained most of the 3-ADON isolates. The MW15ADON population was more genetically diverse, consistent with the hypothesis that it is the native North American population. The UMW3ADON population not only had low genetic diversity, but also was genetically similar to an Italian population that was included for comparison. Thus, it was speculated that the UMW3ADON isolates were recently introduced into North America from Europe. The MW15ADON and UMW3ADON populations described by Gale et al. (2007) were later renamed as NA1 and NA2, respectively. The reasoning behind this decision was that in addition to the distribution of these populations throughout North America and not only in the Midwest, trichothecene genotype is gradually becoming an unreliable predictor of population due to admixture between individuals of different populations (Liang et al. 2014; Kelly et al. 2015).

Shortly thereafter, a dramatic shift in the FHB pathogen populations of North America was reported (Ward et al. 2008). Analysis of the trichothecene genotype of *F.*

graminearum isolated from wheat in Canada during 1998 to 2004 revealed that 135 (27.4%) of the 492 isolates analysed had the 3-ADON genotype, whereas the rest had the 15-ADON genotype. Remarkably, there was a longitudinal cline in the frequency of 3-ADON isolates, being higher in the eastern provinces (100% in Prince Edward Island) and gradually decreasing in each province from east to west (50% in Quebec, 31% in Manitoba, 10.7% in Saskatchewan and 5.9% in Alberta). It should be noted that no samples from the provinces of Ontario, Nova Scotia or New Brunswick were obtained. Additionally, the 3-ADON isolates had a 14-fold increase in frequency over time during 1998 to 2004 (from 2.6% to 37.6%) in the western provinces of Manitoba, Saskatchewan and Alberta, suggesting that the 3-ADON population was probably introduced recently and is quickly expanding from east to west. There was also significant differentiation between the 3-ADON and 15-ADON populations ($F_{ST} > 0.243$) based on variable number tandem repeat (VNTR) data, which indicated that the two populations had not been in the same geographic area for sufficient time to allow for significant genetic exchange. The 3-ADON population from Canada was not significantly different from the aforementioned UMW3ADON isolates from Minnesota and North Dakota (Gale et al. 2007). Analysis of isolates with a high probability of admixture showed a biased gene flow from the 3-ADON to the 15-ADON population (Ward et al. 2008).

The 3-ADON population in general is able to grow faster and produce more trichothecene, as well as more and larger conidia than the 15-ADON isolates, according to *in vitro* tests (Ward et al. 2008). The authors hypothesized that these characteristics, if expressed under field conditions, could enable the 3-ADON isolates to be more aggressive than the 15-ADON isolates. However, no difference was found in

aggressiveness towards the susceptible wheat cultivar ‘Roblin’ or moderately resistant cultivar ‘5602 HR’. It has been reported that 3-ADON isolates are more resilient to extreme temperatures, and that in response to high or low temperatures they accumulated more DON and zearalenone than the 15-ADON isolates (Vujanovic et al. 2012). Subsequent studies reported that the 3-ADON isolates were not only more aggressive, but also more toxigenic in some wheat lines than their 15-ADON counterparts (Puri and Zhong 2010; Foroud et al. 2012).

Several surveys conducted in barley and wheat in the upper Midwest region of the United States also noted the same population subdivision in *F. graminearum*, with the frequency of 3-ADON isolates rapidly increasing and their distribution expanding. The incidence of 3-ADON isolates in barley from North Dakota and Minnesota increased dramatically from 3.5% in 1997–2000 to 38% in 2008 (Burlakoti et al. 2011). The frequency of isolates with the 3-ADON genotype in North Dakota, South Dakota and Minnesota was reported to gradually decrease from north to south (49% in the northern, 40% in the central and 29% in the southern area sampled), which was consistent with a southward expansion (Liang et al. 2014, 2015). A similar latitudinal cline in the frequency of 3-ADON isolates in winter wheat was reported in the eastern USA from 15.4% in New York in the north, decreasing steadily to 0.5% in North Carolina in the south (Schmale et al. 2011).

Evidence that there were additional regional differences in the distribution of *F. graminearum* populations in North America was initially provided from a study that evaluated several features of *F. graminearum* isolates from New York, USA belonging to both the 15-ADON and 3-ADON genotypes (Spolti et al. 2014). This study investigated

the differences between both genotypes in growth rate, temperature sensitivity, perithecia formation, ascospore discharge, and aggressiveness to the 'Norm' spring wheat cultivar, which is regarded as highly susceptible. There was no difference between genotypes for most of the attributes analysed, which was contradictory to previous studies (Ward et al. 2008; Puri and Zhong 2010; Foroud et al. 2012; Vujanovic et al. 2012).

Expanded sampling in the eastern provinces of Canada between 2005 and 2007 detected other regional differences in the *F. graminearum* populations that were not observed previously, and that these populations had also changed throughout the years depending on the region (Kelly et al. 2015). These regional differences in population dynamics were not reported previously due to small sample sizes from eastern Canadian provinces, as well as the lack of samples from other provinces in prior studies (Ward et al. 2008). Overall, the frequency of 3-ADON isolates grew to 37%, compared with the previous 27.4% reported by Ward et al. (2008). Instead of a large longitudinal cline, there were actually two separate longitudinal clines. The first gradient was between the Maritime provinces (Prince Edward Island, New Brunswick and Nova Scotia) and the eastern provinces (Quebec and Ontario). The frequency of 3-ADON isolates was significantly higher in the Maritime provinces (91%) than in the eastern provinces (22%). The 3-ADON frequencies gradually dropped in these two regions from 98% in Prince Edward Island to 11% in Ontario. The 3-ADON genotype also was dominant in Manitoba (55%), starting the second cline in the western Canadian provinces, which included Manitoba, Saskatchewan and Alberta. Once again, the occurrence of 3-ADON isolates declined from east to west, from 27% in Saskatchewan to 7% in Alberta. The trichothecene genotype composition of the *F. graminearum* population throughout the

study was also variable from year to year depending on the region (Kelly et al. 2015). In the western provinces the 3-ADON frequency increased in the three years that surveys were conducted, except in Alberta where it remained consistently low. On the other hand, the frequency in the Maritimes and in eastern provinces remained stable, which indicated that they had population dynamics different from those in the rest of the country.

As far as population structure using VNTR data, the NA1 and NA2 populations mentioned above were observed in all Canadian regions studied (Kelly et al. 2015). The NA1 population was mainly composed of 15-ADON isolates, while most of the 3-ADON isolates belonged to the NA2 population. Differentiation within each population was low, which was indicative of population homogeneity and that the regional differences observed were not due to significant subdivision of the populations. Kelly et al. (2015) examined isolates with a high probability of genetic admixture and discovered that there were biases in gene flow, yet these also varied depending on the region. Whereas there was a biased gene flow favouring the NA2 over the NA1 population in the Maritimes and western provinces (significantly more isolates with 15-ADON genotypes assigned to the NA2 population, which is mainly composed of 3-ADON isolates), the opposite was observed in Ontario and Quebec where gene flow was biased from NA1 to NA2 (significantly more isolates with 3-ADON genotypes assigned to the NA1 population).

The contrasting results obtained among Canadian regions indicate that there are other factors such as climate and host distribution that may affect the population dynamics of FHB pathogens in Canada, and phenotypic and pathogenicity traits that could be potentially advantageous in the NA2 population (Ward et al. 2008; Puri and Zhong 2010; Foroud et al. 2012). Furthermore, the authors mentioned that it is possible

that those attributes could have been transferred from the NA2 to the NA1 population through recombination, since they were not evaluated in the study (Kelly et al. 2015). Climate differences do not seem to affect the distribution of the populations, since regions with different climates like western and maritime provinces had similar compositions. Lastly, the authors speculated that host distribution could also have an effect, because most of the wheat grown in the western Canadian and Maritime provinces is spring wheat, whereas winter wheat predominates in Ontario. Nevertheless, the drivers of population dynamics across Canada are still unknown.

These results were corroborated by additional survey studies published later that reported a low frequency of 3-ADON isolates in Ontario. In 2010, *F. graminearum* of the 15-ADON genotype represented 97% of the isolates obtained from Ontario wheat samples, while only 3% had the 3-ADON genotype (Tamburic-Ilincic et al. 2015). Similar results were obtained from wheat and maize in Ontario during 2010–2012, with only a small portion of isolates having the 3-ADON genotype (2% for wheat and 4% for maize), and the remaining isolates were of the 15-ADON genotype (Burlakoti et al. 2017).

In 2015, isolates capable of producing a newly discovered type A trichothecene named NX-2 were reported in Minnesota (Varga et al. 2015). Later, they were described as a separate population of *F. graminearum* named NA3 and restricted to the northern USA and southern Canada (Kelly et al. 2016; Kelly and Ward 2018). This further confirms the hypothesis that there are multiple populations of *F. graminearum* in North America and not just one single panmictic population.

Genome wide selection scans on isolates representing the three populations of *F. graminearum* in North America (NA1, NA2 and NA3) have shown that multiple regions in the genome have been the target of selection within each population (Kelly and Ward 2018). These regions contained the trichothecene gene cluster and other genes potentially related to plant infection. In addition, the authors found 121 genes across all three populations that were conserved in one population but absent or rare in the other populations, with predicted functions related to pathogenicity, secondary metabolism, and interactions with other microorganisms. These results showed that each sympatric *F. graminearum* population in North America has unique genomic features that contribute to pathogen specialization and could potentially represent a reservoir of adaptative abilities for this fungus (Kelly and Ward 2018).

Reports of new species/genotypes in a region

Fusarium asiaticum *outside of Asia.*

Fusarium asiaticum is presumed to have originated and evolved in Asia, where it is composed of all type B trichothecene genotypes (3-ADON, 15-ADON and NIV) (O'Donnell et al. 2000, 2004; Aoki et al. 2012). This species is also the main FHB pathogen in Asia, especially in regions where rice is grown (van der Lee et al. 2015), and it is regarded to have rice as its preferred host (Desjardins and Proctor 2011). Indeed, it is well known that *F. asiaticum* is favoured in crop rotation systems that include rice (Qiu et al. 2016; Zhang et al. 2016; Yang et al. 2018). However, no difference was observed in pathogenicity towards rice between *F. graminearum, F. asiaticum* and *F. boothii* isolates

from South Korea, which indicates that the selective advantage *F. asiaticum* has in rice might not be due to pathogenicity (Lee et al. 2009).

Outside of Asia, *F. asiaticum* has been reported in several countries, mostly associated with rice production. When *F. asiaticum* was formally described as a separate species, it was known to be present in oats, rice, ryegrass and wheat from Brazil (O'Donnell et al. 2004; Del Ponte et al. 2013). There have been recent reports of *F. asiaticum* with the NIV genotype in Uruguay (Umpiérrez-Failache et al. 2013) as well as Brazil (Gomes et al. 2015; Castañares et al. 2016), always associated with rice. The fact that *F. asiaticum* was found at low levels in Brazilian regions that are distant from rice-growing fields supports the hypothesis that it has a host-preference for rice (Del Ponte et al. 2015; Gomes et al. 2015).

The first report of *F. asiaticum* in North America was from wheat in southern Louisiana, USA, representing 23.4% of the isolates obtained in that study (Gale et al. 2011). The low genetic diversity found in the isolates and the fact that all of them had the NIV genotype suggested that it was an introduced population. The authors also speculated that this species was probably introduced into the region along with rice, since its distribution overlapped areas where rice is a predominant crop.

Fusarium graminearum *'Gulf coast' and 'Southern Louisiana' populations in the USA.*

In 2007, it was reported for the first time that a *F. graminearum* population in Louisiana and Florida included all three trichothecene genotypes, although the NIV

genotype was the most common (Starkey et al. 2007). Two Florida isolates were obtained from commercially grown leatherleaf fern (*Rumohra adiantiformis*), whereas the six remaining isolates were from Louisiana wheat spikes. These isolates were genetically divergent based on polymorphisms detected in the *MAT* gene and three other loci. The authors named this newly discovered population the 'Gulf coast population' and hypothesized that it was indigenous to the region. Subsequently, after additional sampling and population analyses, 44 more isolates were added to the Gulf coast population (Gale et al. 2011). Even though most of the isolates in the Gulf coast population were from Louisiana, one isolate from Ohio and another from Indiana were also classified as members of this population. The 3-ADON genotype was dominant within this sample (65.9%), followed by NIV (25%) and 15-ADON (9.1%). In the same study, the authors also found a separate population of *F. graminearum* with a high frequency of NIV isolates (93.6%) that was named the 'Southern Louisiana population' because it was found mostly in Louisiana. The F_{ST} values between these two populations were very high (0.5), indicating that they were significantly different from each other. Furthermore, the genetic diversity of these two populations was lower than in the NA1 population, which is widespread in North America, suggesting that they were recently introduced. The origin of both populations is still unknown. As with *F. asiaticum* from the same region, it was conjectured that they could have been introduced with rice or that perhaps the weather in the region could be favourable (Gale et al. 2011).

The introduction of NIV-producing isolates (of both *F. graminearum* and *F. asiaticum*) into the USA represents a food safety risk, since NIV is not currently tested in grain and it is regarded as more toxic to humans than DON (Minervini et al. 2004).

Additionally, NIV is detected poorly by some of the rapid detection methods for mycotoxins approved by the United States Department of Agriculture's Grain Inspection, Packers and Stockyards Administration (Tangni et al. 2010).

Fusarium graminearum *NIV in Luxembourg.*

The first reported observation of *F. graminearum* isolates of the NIV genotype in Luxembourg was in 2009 (Pasquali et al. 2009). The authors reported that the NIV isolates represented 2.5% of the isolates sampled in 2007 and 1% in 2008. Subsequently, Pasquali et al. (2010) went on to show that there was a positive correlation between growing wheat after maize and the presence of the NIV genotype. It was speculated that maize production favoured NIV isolates in Luxembourg, since NIV has been reported to be a virulence factor in maize (Pasquali et al. 2010). In France, Boutigny et al. (2014) reported that isolates of the NIV genotype had a higher frequency in the south of the country (21.2%), where most of the maize in the country is grown, than in the north (4.4%).

Fusarium graminearum *15-ADON in Norway.*

Isolates of *F. graminearum* of the 15-ADON genotype were reported for the first time in Norway at low levels in samples from 2006 and 2007 as part of a study that characterized isolates from 1982–1998 and 2004–2007 (Aamot et al. 2015). Only four of 105 *F. graminearum* isolates were of the 15-ADON genotype (3.8%), while the remaining were the 3-ADON genotype (96.2%). This finding was unexpected, since the

15-ADON genotype, which is more common in western and southern Europe, had not been reported in Norway. The authors linked the introduction of 15-ADON isolates into Norway with the detection of 15-ADON producers in Denmark in 1997, which then could have been carried by the wind or imported with seed. The 15-ADON isolates were more aggressive to wheat than the 3-ADON isolates under greenhouse conditions, but additional studies are required to validate this observation due to the small number of 15-ADON isolates.

Fusarium graminearum *in South Korea.*

Several studies have reported the presence of *F. graminearum* in South Korea, always in regions of the country where maize is an important crop, whereas *F. asiaticum* dominates in rice-growing regions (Lee et al. 2009, 2010, 2012; Shin et al. 2018). In 2002, *F. graminearum* represented 21.5% of the isolates from rice in eastern South Korea, a region where maize predominates, while the remaining isolates were identified as *F. asiaticum* (Lee et al. 2009). The same study found that all the isolates from southern South Korea, where little maize is grown, were identified as *F. asiaticum*. Additional surveys revealed that *F. graminearum* predominates in maize, whereas *F. asiaticum* is the most abundant species in rice (Lee et al. 2010, 2012).

Amplified fragment length polymorphism (AFLP) analyses have shown that the *F. graminearum* population in South Korea has a low genetic diversity (Lee et al. 2009, 2012). A recent introduction of *F. graminearum* to South Korea (possibly with imported maize seed) could explain this observation (Lee et al. 2012). The authors pointed out that

most of the maize seed in South Korea is imported from the USA, where *F. graminearum* predominates, which supports this hypothesis.

Fusarium boothii *in Hungary, France, and the USA.*

Fusarium boothii is believed to have evolved in Central America along with *F. mesoamericanum* (Aoki et al. 2012), but its origins are unclear due to its wide geographic distribution, which includes Mexico and multiple countries in Africa, Asia and South America (Boutigny et al. 2011; Desjardins and Proctor 2011; Sampietro et al. 2011; Malihipour et al. 2012; Duan et al. 2016; Zhang et al. 2016; Cerón-Bustamante et al. 2018). Recent surveys have expanded our knowledge of the distribution of this species. In Hungary, *F. boothii* was isolated from wheat at a low frequency (Láday et al. 2004; Tóth et al. 2005), which probably represents an unintentional introduction. Two *F. boothii* × *F. graminearum* hybrids were isolated from maize in France (Boutigny et al. 2014). Since *F. boothii* was not found in France, this suggests the hybrids were most likely introduced on seed, possibly from South Africa. *Fusarium boothii* is very common in South Africa, especially in maize, and hybrids between *F. graminearum* and *F. boothii* have been reported (Boutigny et al. 2011).

Most studies have isolated *F. boothii* from maize, and host-adaptation of *F. boothii* to maize has been suggested (Boutigny et al. 2011; Zhang et al. 2016; Beukes et al. 2018). *Fusarium boothii* was recently reported to cause FHB of wheat for the first time in Nebraska, USA (Wegulo et al. 2018). *Fusarium boothii* had been previously isolated from maize in Texas (Aoki et al. 2012), but it had never been isolated from

wheat in the USA. This finding could be associated with the fact that maize is one of the main crops in Nebraska and that maize and wheat are often included in crop rotation systems in the state. When compared to *F. graminearum*, *F. boothii* initially seemed to be restricted to warmer regions (Backhouse 2014). However, reports from cooler regions in China show that it can actually withstand −30°C temperatures during winter (Zhang et al. 2016). If the *F. boothii* isolates from Nebraska have a host-preference for maize, as do their South African counterparts (Boutigny et al. 2011), this could represent a threat to maize production in the state. However, comparative aggressiveness and population studies including both wheat and maize isolates will be required to evaluate this hypothesis.

Fusarium vorosii *in Hungary.*

The discovery of an atypical *Fusarium* isolate in Hungary led to the description of *F. vorosii* (Tóth et al. 2005; Starkey et al. 2007). This isolate had unique randomly amplified polymorphic DNA (RAPD) and intergenic spacer region (IGS) restriction fragment length polymorphism (RFLP) haplotypes, as well as a divergent reductase (*RED*) gene sequence, but it was closely related to an *F. asiaticum* isolate (Tóth et al. 2005). Subsequently, Starkey et al. (2007) formally described *F. vorosii* as a novel species using genealogical concordance phylogenetic species recognition (GCPSR) on the Hungarian isolate and two additional isolates from Japan. Two additional isolates of *F. vorosii* were subsequently obtained from the Russian Far East (Yli-Mattila et al. 2009). Multilocus molecular phylogeny using 13 different genes showed that *F. vorosii* clustered with *F. asiaticum* and *F. ussurianum*, indicating very strong bootstrap support for an

Asian clade (Yli-Mattila et al. 2009; Aoki et al. 2012). Thus, it appears that *F. vorosii* originated from Asia and was introduced to Hungary (Yli-Mattila et al. 2009).

Fusarium cortaderiae *in New Zealand, France and Italy.*

The holotype of *F. cortaderiae* was isolated from pampas grass (*Cortaderia selloana*) in New Zealand, hence its name (O'Donnell et al. 2004). However, this species is believed to have originated from South America, along with *F. brasilicum, F. austroamericanum* and *F. meridionale* (O'Donnell et al. 2004; Yli-Mattila et al. 2009; Aoki et al. 2012). Pampas grass is not native to New Zealand, but was imported from South America as an ornamental plant, and at the same time may have introduced *F. cortaderiae*, which has now spread to cereal crops (Monds et al. 2005). Additionally, *F. cortaderiae* is believed to have been introduced to France (Boutigny et al. 2014). The authors reported three *F. cortaderiae* isolates that were obtained from maize in south-western France. The geographic restriction of these isolates and the fact that they were found at low frequency in maize, but not in wheat and barley, suggested that they were recently introduced. This species could have been introduced on seed, as France imports most of the maize seed it needs from South Africa where *F. cortaderiae* has been found (Boutigny et al. 2011, 2014). Survey studies from Italy found *F. cortaderiae* isolates in wheat samples from the Marche region (central Italy) and Sardinia (island in the Mediterranean Sea), which also appear to correspond to recent introductions (Somma et al. 2014; Balmas et al. 2015).

Implications of population changes and introduction of new species/genotypes

Changes in FHB populations in a region could have multiple consequences. New populations with a different chemotype would result in a change in the mycotoxin profile in the grain. It is vital to know what mycotoxins the current pathogen populations produce in order to evaluate the risk that they represent, and to use appropriate toxin detection methods to ensure the safety of the grain supply. Additionally, the introduction of more aggressive isolates can lead to greater economic losses due to reduction in yield and quality that would be worsened by the accumulation of mycotoxins.

The population changes discussed, the reports of recent introductions of exotic populations into new areas, and the fact that *Fusarium* spores can be dispersed long distances in air currents (Schmale et al. 2012; Keller et al. 2014) and through international grain export and germplasm exchange indicates increased effective vigilance and continuous global monitoring of FHB pathogens is needed in order to detect possible population shifts. From this perspective, the international grain trade may represent a risk, and each country should develop phytosanitary regulations to avoid the accidental introduction of more aggressive and toxigenic foreign pathogens. If not prevented, this can result in dramatic changes in the population composition and toxigenic profiles when local conditions are favourable to the introduced population. Phytosanitary regulations should not be limited to grain crops, as the introduction of non-native ornamental plants also represents a risk because they can also harbour FHB pathogens, such as the imported pampas grass hypothesized to introduce *F. cortaderiae* into New Zealand (Monds et al. 2005).

Accurate species and trichothecene genotype identification of isolates for surveillance is therefore critical. This represents a challenge regarding the species within the FGSC, because they are morphologically indistinguishable and the only methods currently available for identifying them are sequencing of informative genes. Currently, these are the translation elongation factor 1-α (*TEF1-α*) or the DNA-directed RNA polymerase II largest (*RPB1*) and second largest subunit (*RPB2*) genes (O'Donnell et al. 2015). Another alternative is the use of the multilocus genotyping (MLGT) assay developed by Ward et al. (2008). The MLGT assay has many advantages, including the ability to simultaneously identify most FHB pathogens and their trichothecene genotype in a high-throughput platform. However, it is not readily available to most labs and there is a need for simpler and more affordable assays, such as species-specific PCR-based methods.

Besides determining the species and trichothecene genotype of the pathogens in a region, it is also important to determine the genetic population structure and the population dynamics over time. This identifies changes at the population level, as well as fitness differences among populations (Ward et al. 2008; Kelly et al. 2015). Moreover, management strategies should be planned by taking into consideration population-level variation due to the fact that different populations could be affected selectively (Kelly et al. 2015). Furthermore, in the context of screening and breeding for FHB resistance, it is important to consider the pathogen populations to which the plants could be exposed, in order to ensure that the varieties developed have broad resistance to all pathogen populations (Ward et al. 2002).

The genomic diversity in *F. graminearum* suggests that this species has a very high potential for adaptation. It was noted that *F. graminearum* isolates have many polymorphisms within a 'two-speed' genome organization (Laurent et al. 2018). In plant pathogens with two-speed genomes, one of the subgenomes tends to evolve more rapidly and have more diversity than the rest of the genome, and genes that are important for infection are usually located in these high-diversity areas (Dong et al. 2015). Together with the ability to undergo sexual recombination, bipartite genome architecture allows for a prompt evolutionary response when faced with selective pressures (Laurent et al. 2017, 2018). This represents a challenge for the control of this economically important disease, because management practices should evolve constantly to keep up with the 'arms race' (Laurent et al. 2017). Attempts have been made to predict changes in populations of FHB pathogens, but this has proven to be challenging due to the complexity and variability of the system and all of the factors that should be considered (Parikka et al. 2012; Vaughan et al. 2016). However, most studies point to the fact that severity and incidence of FHB will increase significantly due to climate change (Madgwick et al. 2011; West et al. 2012; Zhang et al. 2014).

Concluding remarks

The studies reviewed in this article clearly show that populations of FHB pathogens are dynamic and change continuously. The effect of climate and anthropogenic activities on FHB pathogens at the population level is evident; however, most of the causes of population shifts have not been completely elucidated and remain largely unexplored. The effects of climate change and the ability of these populations to travel

long distances underscore the importance of ongoing surveillance and phytosanitary regulations. Understanding the composition of the FHB pathogen populations in an area is critical for developing effective management strategies.

With regard to future challenges, the development of alternative means of identifying FHB pathogens would be valuable in order to effectively monitor populations. Finally, additional studies are necessary to fully understand the drivers of population changes and population differences between regions, which, as was pointed out by Kelly et al. (2015), will result in new management strategies or improvement of the current tools available for the management of FHB.

References

Aamot HU, Ward TJ, Brodal G, Vrålstad T, Larsen GB, Klemsdal SS, Elameen A, Uhlig S, Hofgaard IS. 2015. Genetic and phenotypic diversity within the *Fusarium graminearum* species complex in Norway. Eur J Plant Pathol. 142(3):501–519.

Aoki T, Vaughan MM, McCormick SP, Busman M, Ward TJ, Kelly A, O'Donnell K, Johnston PR, Geiser DM. 2015. *Fusarium dactylidis* sp. nov., a novel nivalenol toxin-producing species sister to *F. pseudograminearum* isolated from orchard grass (*Dactylis glomerata*) in Oregon and New Zealand. Mycologia. 107(2):409–418.

Aoki T, Ward TJ, Kistler HC, O'Donnell K. 2012. Systematics, phylogeny and trichothecene mycotoxin potential of Fusarium head blight cereal pathogens. JSM Mycotoxins. 62(2):91–102.

Audenaert K, Van Broeck R, Bekaert B, De Witte F, Heremans B, Messens K, Höfte M, Haesaert G. 2009. Fusarium head blight (FHB) in Flanders: population diversity, inter-species associations and DON contamination in commercial winter wheat varieties. Eur J Plant Pathol. 125(3):445–458.

Backhouse D. 2014. Global distribution of *Fusarium graminearum, F. asiaticum* and *F. boothii* from wheat in relation to climate. Eur J Plant Pathol. 139(1):161–173.

Balmas V, Scherm B, Marcello A, Beyer M, Hoffmann L, Migheli Q, Pasquali M. 2015. *Fusarium* species and chemotypes associated with Fusarium head blight and Fusarium root rot on wheat in Sardinia. Plant Pathol. 64(4):972–979.

Banik M, Beyene M, Tekauz A. 2014. Fusarium head blight and leaf spot diseases of barley in Manitoba in 2013. Can Plant Dis Surv. 94:107–108. www.phytopath.ca/publication/cpds.

Banik M, Beyene M, Wang X. 2016. Fusarium head blight of barley in Manitoba - 2015. Can Plant Dis Surv. 96:93–94. www.phytopath.ca/publication/cpds.

Banik M, Beyene M, Wang X. 2017. Fusarium head blight of barley in Manitoba - 2016. Can Plant Dis Surv. 97:100–101. www.phytopath.ca/publication/cpds.

Banik M, Beyene M, Wang X. 2018. Fusarium head blight of barley in Manitoba - 2017. Can Plant Dis Surv. 98:95–96. www.phytopath.ca/publication/cpds.

Beccari G, Caproni L, Tini F, Uhlig S, Covarelli L. 2016. Presence of *Fusarium* species and other toxigenic fungi in malting barley and multi-mycotoxin analysis by liquid

chromatography-high-resolution mass spectrometry. J Agric Food Chem. 64(21):4390–4399.

Beccari G, Prodi A, Tini F, Bonciarelli U, Onofri A, Oueslati S, Limayma M, Covarelli L. 2017. Changes in the Fusarium head blight complex of malting barley in a three-year field experiment in Italy. Toxins. 9(4):120.

Beccari G, Senatore MT, Tini F, Sulyok M, Covarelli L. 2018. Fungal community, Fusarium head blight complex and secondary metabolites associated with malting barley grains harvested in Umbria, central Italy. Int J Food Microbiol. 273:33–42.

Beukes I, Rose L, van Coller G, Viljoen A. 2018. Disease development and mycotoxin production by the *Fusarium graminearum* species complex associated with South African maize and wheat. Eur J Plant Pathol. 150(4):893–910.

Beyene M, Banik M, Wang X. 2015. Fusarium head blight of barley in Manitoba – 2014. Can Plant Dis Surv. 95:74–75. www.phytopath.ca/publication/cpds.

Bianchini A, Horsley R, Jack MM, Kobielush B, Ryu D, Tittlemier S, Wilson WW, Abbas HK, Abel S, Harrison G, et al. 2015. DON occurrence in grains: A North American perspective. Cereal Food World. 60(1):32–56.

Bissonnette KM, Wharton P, Chen J, Marshall JM. 2018. Survey of *Fusarium* species associated with Fusarium head blight of spring wheat (*Triticum aestivum*) in Southeastern Idaho. Plant Health Prog. 19:125–127.

Boutigny A, Ward TJ, Ballois N, Iancu G, Ioos R. 2014. Diversity of the *Fusarium graminearum* species complex on French cereals. Eur J Plant Pathol. 138(1):133–148.

Boutigny A, Ward TJ, Van Coller GJ, Flett B, Lamprecht SC, O'Donnell K, Viljoen A. 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(9):914–920.

Burlakoti RR, Neate SM, Adhikari TB, Gyawali S, Salas B, Steffenson BJ, Schwarz PB. 2011. Trichothecene profiling and population genetic analysis of *Gibberella zeae* from barley in North Dakota and Minnesota. Phytopathology. 101(6):687–695.

Burlakoti RR, Tamburic-Ilincic L, Limay-Rios V, Burlakoti P. 2017. Comparative population structure and trichothecene mycotoxin profiling of *Fusarium graminearum* from corn and wheat in Ontario, central Canada. Plant Pathol. 66(1):14–27.

Castañares E, Dinolfo MI, Del Ponte EM, Pan D, Stenglein SA. 2016. Species composition and genetic structure of *Fusarium graminearum* species complex populations affecting the main barley growing regions of South America. Plant Pathol. 65(6):930–939.

Cerón-Bustamante M, Ward TJ, Kelly A, Vaughan MM, McCormick SP, Cowger C, Leyva-Mir SG, Villaseñor-Mir HE, Ayala-Escobar V, Nava-Díaz C. 2018. Regional differences in the composition of Fusarium Head Blight pathogens and mycotoxins associated with wheat in Mexico. Int J Food Microbiol. 273:11–19.

Chandelier A, Nimal C, André F, Planchon V, Oger R. 2011. *Fusarium* species and DON contamination associated with head blight in winter wheat over a 7-year period (2003–2009) in Belgium. Eur J Plant Pathol. 130(3):403–414.

Chełkowski J, Gromadzka K, Ł S, Lenc L, Kostecki M, Berthiller F. 2012. *Fusarium* species, zearalenone and deoxynivalenol content in preharvest scabby wheat heads from Poland. World Mycotoxin J. 5(2):133–141.

Covarelli L, Beccari G, Prodi A, Generotti S, Etruschi F, Juan C, Ferrer E, Mañes J. 2015. *Fusarium* species, chemotype characterisation and trichothecene contamination of durum and soft wheat in an area of central Italy. J Sci Food Agric. 95(3):540–551.

Dean R, Van Kan JA, Pretorius ZA, Hammond-Kosack KE, Di Pietro A, Spanu PD, Rudd JJ, Dickman M, Kahmann R, Ellis J, et al. 2012. The Top 10 fungal pathogens in molecular plant pathology. Mol Plant Pathol. 13(4):414–430.

Del Ponte EM, Spolti P, Ward TJ, Gomes LB, Nicolli CP, Kuhnem PR, Silva CN, Tessmann DJ. 2015. Regional and field-specific factors affect the composition of Fusarium head blight pathogens in subtropical no-till wheat agroecosystem of Brazil. Phytopathology. 105(2):246–254.

Del Ponte EM, Tessmann DJ, Spolti P, Kuhnem PR, Da Silva CN. 2013. Species identification, genetic diversity and phenotypic variation studies on the *Fusarium graminearum* complex populations from Brazil. In: Alconada Magliano TM, Chulze SN, editors. Fusarium head blight in Latin America. Dordrecht: Springer; p. 15–29.

Desjardins AE, Proctor RH. 2011. Genetic diversity and trichothecene chemotypes of the *Fusarium graminearum* clade isolated from maize in Nepal and identification of a putative new lineage. Fungal Biol. 115(1):38–48.

Dong S, Raffaele S, Kamoun S. 2015. The two-speed genomes of filamentous pathogens: waltz with plants. Curr Opin Genet Dev. 35:57–65.

Duan C, Qin Z, Yang Z, Li W, Sun S, Zhu Z, Wang X. 2016. Identification of pathogenic *Fusarium* spp. causing maize ear rot and potential mycotoxin production in China. Toxins. 8(6):186.

Foroud NA, Eudes F. 2009. Trichothecenes in cereal grains. Int J Mol Sci. 10(1):147–173.

Foroud NA, McCormick SP, MacMillan T, Badea A, Kendra DF, Ellis BE, Eudes F. 2012. Greenhouse studies reveal increased aggressiveness of emergent Canadian *Fusarium graminearum* chemotypes in wheat. Plant Dis. 96(9):1271–1279.

Gai XT, Xuan YH, Gao ZG. 2017. Diversity and pathogenicity of *Fusarium graminearum* species complex from maize stalk and ear rot strains in northeast China. Plant Pathol. 66(8):1267–1275.

Gale LR, Harrison SA, Ward TJ, O'Donnell K, Milus EA, Gale SW, Kistler HC. 2011. Nivalenol-type populations of *Fusarium graminearum* and *F. asiaticum* are prevalent on wheat in southern Louisiana. Phytopathology. 101(1):124–134.

Gale LR, Ward TJ, Balmas V, Kistler HC. 2007. Population Subdivision of *Fusarium graminearum sensu stricto* in the Upper Midwestern United States. Phytopathology. 97(11):1434–1439.

Gomes LB, Ward TJ, Badiale-Furlong E, Del Ponte EM. 2015. Species composition, toxigenic potential and pathogenicity of *Fusarium graminearum* species complex isolates from southern Brazilian rice. Plant Pathol. 64(4):980–987.

Gorczyca A, Oleksy A, Gala-Czekaj D, Urbaniak M, Laskowska M, Waśkiewicz A, Stępień Ł. 2018. Fusarium head blight incidence and mycotoxin accumulation in three durum wheat cultivars in relation to sowing date and density. Sci Nat. 105(1):1–11.

Goswami RS, Kistler HC. 2004. Heading for disaster: *Fusarium graminearum* on cereal crops. Mol Plant Pathol. 5(6):515–525.

Gräfenhan T, Johnston PR, Vaughan MM, McCormick SP, Proctor RH, Busman M, Ward TJ, O'Donnell K. 2016. *Fusarium praegraminearum* sp. nov., a novel nivalenol mycotoxin-producing pathogen from New Zealand can induce head blight on wheat. Mycologia. 108(6):1229–1239.

Hietaniemi V, Rämö S, Yli-Mattila T, Jestoi M, Peltonen S, Kartio M, Sieviläinen E, Koivisto T, Parikka P. 2016. Updated survey of *Fusarium* species and toxins in Finnish cereal grains. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 33(5):831–848.

Infantino A, Santori A, Shah D. 2012. Community structure of the *Fusarium* complex on wheat seed in Italy. Eur J Plant Pathol. 132(4):499–510.

Isebaert S, Saeger SD, Devreese R, Verhoeven R, Maene P, Heremans B, Haesaert G. 2009. Mycotoxin-producing *Fusarium* species occurring in winter wheat in Belgium (Flanders) during 2002–2005. J Phytopathol. 157(2):108–116.

Jennings P, Coates ME, Walsh K, Turner JA, Nicholson P. 2004. Determination of deoxynivalenol- and nivalenol-producing chemotypes of *Fusarium graminearum* isolated from wheat crops in England and Wales. Plant Pathol. 53(5):643–652.

Karlsson I, Friberg H, Kolseth A, Steinberg C, Persson P. 2017. Agricultural factors affecting *Fusarium* communities in wheat kernels. Int J Food Microbiol. 252:53–60.

Keller M, Bergstrom G, Shields E. 2014. The aerobiology of *Fusarium graminearum*. Aerobiologia. 30(2):123–136.

Kelly A, Proctor RH, Belzile F, Chulze SN, Clear RM, Cowger C, Elmer W, Lee T, Obanor F, Waalwijk C, et al. 2016. The geographic distribution and complex evolutionary history of the NX-2 trichothecene chemotype from *Fusarium graminearum*. Fungal Genet Biol. 95:39–48.

Kelly AC, Clear RM, O'Donnell K, McCormick S, Turkington TK, Tekauz A, Gilbert J, Kistler HC, Busman M, Ward TJ. 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 AC, Ward TJ. 2018. Population genomics of *Fusarium graminearum* reveals signatures of divergent evolution within a major cereal pathogen. PLoS One. 13(3):e0194616.

Klix MB, Beyer M, Verreet J. 2008. Effects of cultivar, agronomic practices, geographic location, and meteorological conditions on the composition of selected *Fusarium* species on wheat heads. Can J Plant Pathol. 30(1):46–57.

Láday M, Á J, Mulè G, Moretti A, Á S, Logrieco A. 2004. Mitochondrial DNA diversity and lineage determination of European isolates of *Fusarium graminearum* (*Gibberella zeae*). Eur J Plant Pathol. 110:545–550.

Laurent B, Moinard M, Spataro C, Ponts N, Barreau C, Foulongne-Oriol M. 2017. Landscape of genomic diversity and host adaptation in *Fusarium graminearum*. BMC Genomics. 18:203.

Laurent B, Palaiokostas C, Spataro C, Moinard M, Zehraoui E, Houston RD, Foulongne-Oriol M. 2018. High-resolution mapping of the recombination landscape of the phytopathogen *Fusarium graminearum* suggests two-speed genome evolution. Mol Plant Pathol. 19(2):341–354.

Lee J, Chang I, Kim H, Yun S, Leslie JF, Lee Y. 2009. Genetic diversity and fitness of *Fusarium graminearum* populations from rice in Korea. Appl Environ Microbiol. 75(10):3289–3295.

Lee J, Kim H, Jeon J, Kim H, Zeller KA, Carter LLA, Leslie JF, Lee Y. 2012. Population structure of and mycotoxin production by *Fusarium graminearum* from maize in South Korea. Appl Environ Microbiol. 78(7):2161–2167.

Lee S, Lee J, Nam Y, Lee S, Ryu J, Lee T. 2010. Population structure of *Fusarium graminearum* from maize and rice in 2009 in Korea. Plant Pathol J. 26(4):321–327.

Liang J, LL, Ma Z, Ward TJ, Kistler HC. 2015. Population Subdivision of *Fusarium graminearum* from barley and wheat in the Upper Midwestern United States at the turn of the century. Phytopathology. 105(11):1466–1474.

Liang JM, Xayamongkhon H, Broz K, Dong Y, McCormick SP, Abramova S, Ward TJ, Ma ZH, Kistler HC. 2014. Temporal dynamics and population genetic structure of *Fusarium graminearum* in the upper Midwestern United States. Fungal Genet Biol. 73:83–92.

Lofgren L, Riddle J, Dong Y, Kuhnem P, Cummings J, Del Ponte E, Bergstrom G, Kistler HC. 2018. A high proportion of NX-2 genotype strains are found among *Fusarium graminearum* isolates from northeastern New York State. Eur J Plant Pathol. 150(3):791–796.

Madgwick J, West J, White R, Semenov M, Townsend J, Turner J, Fitt B. 2011. Impacts of climate change on wheat anthesis and Fusarium ear blight in the UK. Eur J Plant Pathol. 130(1):117–131.

Malihipour A, Gilbert J, Piercey-Normore M, Cloutier S. 2012. Molecular phylogenetic analysis, trichothecene chemotype patterns, and variation in aggressiveness of *Fusarium* isolates causing head blight in wheat. Plant Dis. 96(7):1016–1025.

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(12):1712–1728.

McMullen M, Jones R, Gallenberg D. 1997. Scab of wheat and barley: A re-emerging disease of devastating impact. Plant Dis. 81(12):1340–1348.

Minervini F, Fornelli F, Flynn KM. 2004. Toxicity and apoptosis induced by the mycotoxins nivalenol, deoxynivalenol and fumonisin B1 in a human erythroleukemia cell line. Toxicol In Vitro. 18(1):21–28.

Monds RD, Cromey MG, Lauren DR, Di Menna M, Marshall J. 2005. *Fusarium graminearum, F. cortaderiae* and *F. pseudograminearum* in New Zealand: molecular phylogenetic analysis, mycotoxin chemotypes and co-existence of species. Mycol Res. 109(Pt4):410–420.

Nganje WE, Bangsund DA, Leistritz FL, Wilson WW, Tiapo NM. 2004. Regional economic impacts of Fusarium head blight in wheat and barley. Appl Econ Perspect Policy. 26(3):332–347.

Nielsen LK, Jensen JD, Nielsen GC, Jensen JE, Spliid NH, Thomsen IK, Justesen AF, Collinge DB, Jørgensen LN. 2011. Fusarium head blight of cereals in Denmark: species complex and related mycotoxins. Phytopathology. 101(8):960–969.

Nogueira MS, Decundo J, Martinez M, Dieguez SN, Moreyra F, Moreno MV, Stenglein SA. 2018. Natural contamination with mycotoxins produced by *Fusarium graminearum* and *Fusarium poae* in malting Barley in Argentina. Toxins. 10(2):78.

O’Donnell K, Kistler HC, Tacke BK, Casper HH. 2000. Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of *Fusarium graminearum*, the fungus causing wheat scab. PNAS. 97(14):7905–7910.

O’Donnell K, McCormick SP, Busman M, Proctor RH, Ward TJ, Doehring G, Geiser DM, Alberts JF, Rheeder JP. 2018. Marasas et al. 1984 “Toxigenic *Fusarium* Species: identity and Mycotoxicology” revisited. Mycologia. 110(6):1058–1080.

O'Donnell K, Rooney AP, Proctor RH, Brown DW, McCormick SP, Ward TJ, Frandsen RJN, Lysøe E, Rehner SA, Aoki T, 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, Ward TJ, Geiser DM, Corby Kistler H, Aoki T. 2004. Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the *Fusarium graminearum* clade. Fungal Genet Biol. 41(6):600–623.

O'Donnell K, Ward TJ, Robert VARG, Crous PW, Geiser DM, Kang S. 2015. DNA sequence-based identification of *Fusarium*: current status and future directions. Phytoparasitica. 43(5):583–595.

Pancaldi D, Tonti S, Prodi A, Salomoni D, Dal Prà M, Nipoti P, Alberti I, Pisi A. 2010. Survey of the main causal agents of Fusarium head blight of durum wheat around Bologna, northern Italy. Phytopathol Mediterr. 49(2):258–266.

Parikka P, Hakala K, Tiilikkala K. 2012. Expected shifts in *Fusarium* species' composition on cereal grain in Northern Europe due to climatic change. Food Addit Contam Part A. 29(10):1543–1555.

Parry DW, Jenkinson P, McLeod L. 1995. Fusarium ear blight (scab) in small grain cereals—a review. Plant Pathol. 44(2):207–238.

Pasquali M, Giraud F, Brochot C, Cocco E, Hoffmann L, Bohn T. 2010. Genetic *Fusarium* chemotyping as a useful tool for predicting nivalenol contamination in winter wheat. Int J Food Microbiol. 137(2–3):246–253.

Pasquali M, Giraud F, Brochot C, Hoffmann L, Bohn T. 2009. First report of the nivalenol chemotype of *Fusarium graminearum* causing head blight of wheat in the Grand Duchy of Luxembourg. Plant Dis. 93(11):1217.

Pereira CB, Ward TJ, Tessmann DJ, Ponte EMD, Laraba I, Vaughan MM, McCormick SP, Busman M, Kelly A, Proctor RH, et al. 2018. *Fusarium subtropicale*, sp. nov., a novel nivalenol mycotoxin–producing species isolated from barley (*Hordeum vulgare*) in Brazil and sister to *F. praegraminearum*. Mycologia. 110(5):860–871.

Pestka JJ, Smolinski AT. 2005. Deoxynivalenol: toxicology and potential effects on humans. J Toxicol Environ Health B. 8(1):39–69.

Puri KD, 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(10):1007–1014.

Qiu J, Dong F, Yu M, Xu J, Shi J. 2016. Effect of preceding crop on *Fusarium* species and mycotoxin contamination of wheat grains. J Sci Food Agric. 96(13):4536–4541.

Sampietro DA, Díaz CG, Gonzalez V, Vattuone MA, Ploper LD, Catalan CAN, Ward TJ. 2011. Species diversity and toxigenic potential of *Fusarium graminearum* complex isolates from maize fields in northwest Argentina. Int J Food Microbiol. 145(1):359–364.

Schmale DG, Ross SD, Fetters TL, Tallapragada P, Wood-Jones AK, Dingus B. 2012. Isolates of *Fusarium graminearum* collected 40–320 meters above ground level

cause Fusarium head blight in wheat and produce trichothecene mycotoxins. Aerobiologia. 28(1):1–11.

Schmale DG, Wood-Jones AK, Cowger C, Bergstrom GC, Arellano C. 2011. Trichothecene genotypes of *Gibberella zeae* from winter wheat fields in the eastern USA. Plant Pathol. 60(5):909–917.

Shah D, Pucci N, Infantino A. 2005. Regional and varietal differences in the risk of wheat seed infection by fungal species associated with Fusarium head blight in Italy. Eur J Plant Pathol. 112(1):13–21.

Shin S, Son J, Park J, Kim K, Yoon Y, Cheong Y, Kim K, Hyun J, Park CS, Dill-Macky R, et al. 2018. Comparative pathogenicity of *Fusarium graminearum* isolates from wheat kernels in Korea. Plant Pathol J. 34(5):347–355.

Somma S, Petruzzella AL, Logrieco AF, Meca G, Cacciola OS, Moretti A. 2014. Phylogenetic analyses of *Fusarium graminearum* strains from cereals in Italy, and characterisation of their molecular and chemical chemotypes. Crop Pasture Sci. 65(1):52–60.

Spolti P, Del Ponte EM, Cummings JA, Dong Y, Bergstrom GC. 2014. Fitness attributes of *Fusarium graminearum* isolates from wheat in New York possessing a 3-ADON or 15-ADON trichothecene genotype. Phytopathology. 104(5):513–519.

Starkey DE, Ward TJ, Aoki T, Gale LR, Kistler HC, Geiser DM, Suga H, Tóth B, Varga J, O'Donnell K. 2007. Global molecular surveillance reveals novel Fusarium head blight species and trichothecene toxin diversity. Fungal Genet Biol. 44(11):1191–1204.

Stępień Ł, Chełkowski J. 2010. Fusarium head blight of wheat: pathogenic species and their mycotoxins. World Mycotoxin J. 3(2):107–119.

Stępień Ł, Popiel D, Koczyk G, Chełkowski J. 2008. Wheat-infecting *Fusarium* species in Poland–their chemotypes and frequencies revealed by PCR assay. J Appl Genet. 49(4):433–441.

Sumíková T, Chrpová J, Džuman Z, Salava J, Štěrbová L, Palicová J, Slavíková P, Stránská-Zachariášová M, Hajšlová J. 2017. Mycotoxins content and its association with changing patterns of *Fusarium* pathogens in wheat in the Czech Republic. World Mycotoxin J. 10(2):143–151.

Tamburic-Ilincic L, Wragg A, Schaafsma A. 2015. Mycotoxin accumulation and *Fusarium graminearum* chemotype diversity in winter wheat grown in southwestern Ontario. Can J Plant Sci. 95(5):931–938.

Tangni EK, Motte J, Callebaut A, Pussemier L. 2010. Cross-Reactivity of antibodies in some commercial deoxynivalenol test kits against some fusariotoxins. J Agric Food Chem. 58(24):12625–12633.

Tekauz A, Stulzer M, Beyene M, Gompf J, Moncomble C. 2013. Fusarium head blight of barley in Manitoba – 2012. Can Plant Dis Surv. 93:91–92.

Tóth B, Á M, Horváth Z, Bartók T, Varga M, Varga J. 2005. Genetic variability of central European isolates of the *Fusarium graminearum* species complex. Eur J Plant Pathol. 113(1):35–45.

Trail F. 2009. For blighted waves of grain: *Fusarium graminearum* in the postgenomics Era. Plant Physiol. 149(1):103–110.

Umpiérrez-Failache M, Garmendia G, Pereyra S, Rodríguez-Haralambides A, Ward TJ, Vero S. 2013. Regional differences in species composition and toxigenic potential among Fusarium head blight isolates from Uruguay indicate a risk of nivalenol contamination in new wheat production areas. Int J Food Microbiol. 166(1):135–140.

van der Fels-Klerx HJ, de Rijk TC, Booij CJH, Goedhart PW, Boers EAM, Zhao C, Waalwijk C, Mol HGJ, van der Lee TAJ. 2012. Occurrence of Fusarium head blight species and *Fusarium* mycotoxins in winter wheat in the Netherlands in 2009. Food Addit Contam Part A. 29(11):1716–1726.

van der Lee T, Zhang H, van Diepeningen A, Waalwijk C. 2015. Biogeography of *Fusarium graminearum* species complex and chemotypes: a review. Food Addit Contam Part A. 32(4):453–460.

Vanheule A, De Boevre M, Moretti A, Scauflaire J, Munaut F, De Saeger S, Bekaert B, Haesaert G, Waalwijk C, Van Der Lee T, et al. 2017. Genetic divergence and chemotype diversity in the Fusarium head blight pathogen *Fusarium poae*. Toxins. 9(9):255.

Varga E, Wiesenberger G, Hametner C, Ward TJ, Dong Y, Schöfbeck D, McCormick S, Broz K, Stückler R, Schuhmacher R, et al. 2015. New tricks of an old enemy: isolates of *Fusarium graminearum* produce a type A trichothecene mycotoxin. Environ Microbiol. 17(8):2588–2600.

Vaughan M, Backhouse D, Del Ponte EM. 2016. Climate change impacts on the ecology of *Fusarium graminearum* species complex and susceptibility of wheat to Fusarium head blight: a review. World Mycotoxin J. 9(5):685–700.

Vujanovic V, Goh YK, Daida P. 2012. Heat- and cold-shock responses in *Fusarium graminearum* 3 acetyl- and 15 acetyl-deoxynivalenol chemotypes. J Microbiol. 50(1):97–102.

Waalwijk C, Kastelein P, Vries I, Kerényi Z, Tvd L, Hesselink T, Köhl J, Kema G. 2003. Major changes in *Fusarium* spp. in wheat in the Netherlands. Eur J Plant Pathol. 109(7):743–754.

Ward TJ, Bielawski JP, Kistler HC, Sullivan E, O'Donnell K. 2002. Ancestral polymorphism and adaptive evolution in the trichothecene mycotoxin gene cluster of phytopathogenic *Fusarium*. PNAS. 99(14):9278–9283.

Ward TJ, Clear RM, Rooney AP, O'Donnell K, Gaba D, Patrick S, Starkey DE, Gilbert J, Geiser DM, Nowicki TW. 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(4):473–484.

Wegulo SN, Valverde-Bogantes E, Bolanos-Carriel C, Hallen-Adams H, Bianchini A, McMaster N, Schmale DG. 2018. First report of *Fusarium boothii* causing head blight of wheat in the United States. Plant Dis. 102(12):2646.

West JS, Holdgate S, Townsend JA, Edwards SG, Jennings P, Fitt BDL. 2012. Impacts of changing climate and agronomic factors on Fusarium ear blight of wheat in the UK. Fungal Ecol. 5(1):53–61.

Xu X, Parry D, Nicholson P, Thomsett M, Simpson D, Edwards S, Cooke B, Doohan F, Brennan J, Moretti A, et al. 2005. Predominance and association of pathogenic fungi causing Fusarium ear blight in wheat in four European countries. Eur J Plant Pathol. 112(2):143–154.

Xu XM, Nicholson P, Thomsett MA, Simpson D, Cooke BM, Doohan FM, Brennan J, Monaghan S, Moretti A, Mule G, et al. 2008. Relationship between the fungal complex causing *Fusarium* head blight of wheat and environmental conditions. Phytopathology. 98(1):69–78.

Xue AG, Armstrong KC, Voldeng HD, Fedak G, Babcock C. 2004. Comparative aggressiveness of isolates of *Fusarium* spp. causing head blight on wheat in Canada. Can J Plant Pathol. 26(1):81–88.

Yang L, van der Lee T, Yang X, Yu D, Waalwijk C. 2008. *Fusarium* populations on Chinese barley show a dramatic gradient in mycotoxin profiles. Phytopathology. 98(6):719–727.

Yang M, Zhang H, Kong X, van der Lee T, Waalwijk C, van Diepeningen A, Xu J, Xu J, Chen W, Feng J. 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(3):115.

Yli-Mattila T, Gagkaeva T, Ward TJ, Aoki T, Kistler HC, O'Donnell K. 2009. A novel Asian clade within the *Fusarium graminearum* species complex includes a newly discovered cereal head blight pathogen from the Russian Far East. Mycologia. 101(6):841–852.

Zeller KA, Bowden RL, Leslie JF. 2004. Population differentiation and recombination in wheat scab populations of *Gibberella zeae* from the United States. Mol Ecol. 13(3):563–571.

Zhang H, Brankovics B, Xu J, Xu JS, Guo C, Guo JG, Jin SJ, Chen WQ, Feng J, van Diepeningen AD, et al. 2016. Crops are a main driver for species diversity and the toxigenic potential of *Fusarium* isolates in maize ears in China. World Mycotoxin J. 9(5):701–715.

Zhang H, van der Lee T, Waalwijk C, Chen W, Xu J, Xu J, Zhang Y, Feng J. 2012. Population analysis of the *Fusarium graminearum* species complex from wheat in China show a shift to more aggressive isolates. PLoS One. 7(2):e31722.

Zhang H, Zhang Z, van der Lee T, Chen WQ, Xu J, Xu JS, Yang L, Yu D, Waalwijk C, Feng J. 2010. Population genetic analyses of *Fusarium asiaticum* populations from barley suggest a recent shift favoring 3ADON producers in southern China. Phytopathology. 100(4):328–336.

Zhang X, Halder J, White RP, Hughes DJ, Ye Z, Wang C, Xu R, Gan B, Fitt B. 2014. Climate change increases risk of fusarium ear blight on wheat in central China. Ann Appl Biol. 164(3):384–395.

CHAPTER 2

A four-year survey of pathogens causing Fusarium head blight of wheat in Nebraska, USA from 2015-2018

Abstract

Fusarium head blight (FHB) is an economically important disease caused by several *Fusarium* species affecting wheat and other small grain cereals. Besides causing significant yield losses, infected grain may be contaminated with mycotoxins, primarily trichothecenes, which are harmful to humans and animals. In recent years, reports of shifts in populations of FHB pathogens around the world have shown that these populations are dynamic and change continuously, often resulting in increased yield losses or changes in the mycotoxins produced in the grain, which highlights the need for increased vigilance. The objective of this research was to identify the species and trichothecene genotype of FHB pathogens in Nebraska in order to monitor their populations and the major toxigenic risks in the state. A total of 42 samples consisting of FHB symptomatic wheat spikes were collected from Nebraska fields during the growing seasons in 2015-2018 and a total of 74 single-spore *Fusarium* isolates were obtained from the samples. Species identities were determined by sequencing portions of the translation elongation factor 1α (*TEF1*) and trichothecene 3-*O*-acetyltransferase (*TRI101*) and reductase (*RED*) genes. A multiplex PCR using two alternative sets of primers based on the trichothecene 15-*O*-acetyltransferase (*TRI3*) and trichothecene efflux pump

(*TRI12*) genes was used to determine trichothecene genotype. Most of the isolates were identified as *F. graminearum* (n=67). Additional species included *F. boothii* (n=3), *F. poae* (n=2) and *F. acuminatum* (n=1). One isolate had *F. graminearum* alleles at *TEF1* and *TRI101*, but a *F. boothii* allele at *RED*, which indicates that it is an interspecific hybrid between these two species. All *F. graminearum* and *F. boothii* isolates had the 15-ADON genotype. This is the first time that *F. boothii* has been isolated from wheat in the USA. Overall, these results show that *F. graminearum* is not the only pathogen causing FHB in Nebraska. Also, this study helps expand knowledge on the worldwide distribution of *F. boothii*. The information obtained from this survey will be useful in developing effective FHB management strategies in Nebraska, since different pathogen populations can cause varying levels of disease intensity and can be selectively sensitive to management tactics.

Introduction

Fusarium head blight (FHB) is one of the most economically significant diseases affecting wheat and other small grain cereals worldwide (Goswami and Kistler 2004). Yield losses caused by FHB are worsened by the production of trichothecene mycotoxins, which cause significant price reductions and make the grain unsafe for human and animal consumption (McMullen et al. 1997). The most important mycotoxin produced by FHB pathogens is deoxynivalenol (DON), also known as vomitoxin, which also acts a virulence factor aiding in spread of the pathogen within spikes in wheat (Bai et al. 2001; Desjardins et al. 1996). Therefore, FHB represents both a food security risk, due to decreased yields and economic impacts; and a food safety risk, due to the production of mycotoxins.

Several *Fusarium* species are the causal agents of FHB. Most of the FHB pathogens are grouped within the *Fusarium graminearum* species complex (FGSC), which is part of the broader *Fusarium sambucinum* species complex lineage 1 (FSAMSC-1). The FGSC includes the following species: *F. acaciae-mearnsii*, *F. aethiopicum*, *F. asiaticum*, *F. austroamericanum*, *F. boothii*, *F. brasilicum*, *F. cortaderiae*, *F. gerlachii*, *F. graminearum*, *F. louisianense*, *F. meridionale*, *F. mesoamericanum*, *F. nepalense*, *F. ussurianum*, and *F. vorosii* (Aoki et al. 2012). *Fusarium avenaceum*, *F. langsethiae*, *F. poae*, *F. sporotrichioides, F. culmorum*, and *F. cerealis* are also commonly associated with FHB in different parts of the world (Laraba et al. 2017; van der Fels-Klerx et al. 2012).

Species within the FSAMSC-1 usually produce type B trichothecenes (O’Donnell et al. 2013), and isolates are classified on their particular toxigenic profile (chemotype).

These chemotypes include: (i) 3-ADON, producing deoxynivalenol (DON) and 3-acetyldeoxynivalenol (3-ADON); (ii) 15-ADON, producing DON and 15-acetyldeoxynivalenol (15-ADON); and (iii) NIV, producing nivalenol (NIV) and its acetylated derivatives. Isolates of *F. graminearum* that produce NX-2 – a type A trichothecene – have been reported at low frequencies in southern Canada and northern U.S. (Kelly et al. 2016). PCR assays have been developed to determine the trichothecene genotype of isolates and predict their chemotype (Ward et al. 2002).

Population studies have shown that there are at least three distinct populations of *F. graminearum* in North America: (i) NA1, composed primarily of 15-ADON producers; (ii) NA2, composed primarily of 3-ADON producers; and (iii) NA3, composed primarily of NX-2 producers (Kelly and Ward 2018). The majority of isolates infecting wheat in North America belong to the NA1 population and have the 15-ADON genotype (Kelly et al. 2015). The geographic distribution of these populations seems to be influenced by many factors, most of them still unknown. However, temporal studies have shown that 3-ADON isolates belonging to the NA2 population have increased in frequency in different parts of Canada and the Upper Midwest of the U.S., replacing the existing NA1 population (Burlakoti et al. 2011; Kelly et al. 2015; Liang et al. 2014). It has been hypothesized that this shift is possibly due to selective advantages in the NA2 population such as faster growth, increased aggressiveness and higher toxigenic potential (Puri and Zhong 2010; Ward et al. 2008). Additional studies have reported the presence of *F. graminearum* isolates with the 3-ADON and NIV chemotype in Louisiana and eastern U.S. (Schmale et al. 2011; Starkey et al. 2007). Besides *F. graminearum*, other species that have been reported causing FHB in North America include: *F. gerlachii*

(Starkey et al. 2007), *F. asiaticum* (Gale et al. 2011), *F. louisianense* (Sarver et al. 2011), *F. culmorum* (Bissonnette et al. 2018), and *F. cerealis* (Amarasinghe et al. 2015; Schmale et al. 2011). These reports from North America, together with reports of shifts in populations of FHB pathogens around the world (Valverde-Bogantes et al. 2019), have shown that these populations are dynamic and change continuously, often resulting in increased yield losses or changes in the mycotoxins produced in the grain, which highlights the need for increased vigilance.

In Nebraska, FHB occurs sporadically due to annual variability in environmental conditions; however, epidemics of FHB have affected the state recently (Wegulo et al. 2008). Previous studies have reported that isolates with the 15-ADON genotype are predominant in Nebraska, and no other genotype has been reported in the state (Nopsa et al. 2014; Panthi et al. 2014). The objective of this study was to obtain information of the species diversity and mycotoxin potential of FHB pathogens infecting wheat in Nebraska from 2015 to 2018 in order to monitor their populations and the major toxigenic risks in the state.

Materials and Methods

Samples

A total of 42 wheat head samples showing FHB symptoms were collected during the 2015, 2016, 2017, and 2018 growing seasons from randomly chosen fields in Nebraska. All samples consisted of hard red winter, which is the most common wheat

class grown in the state. GPS coordinates for each sample were recorded (Fig. 1; Table 1).

Isolation of FHB pathogens and DNA extraction

For isolation of FHB pathogens, the protocol outlined by Panthi et al. (2014) was followed. Wheat kernels from each sample were disinfected in 70% ethanol for 5 min, rinsed twice in sterile distilled water and blotted dry. Subsequently, disinfected kernels were placed on Fusarium Selective Media (FSM) containing pentachloronitrobenzene (Nash and Snyder 1962) and incubated for 5 to 7 days at room temperature.

Typical *Fusarium* colonies were transferred to potato dextrose agar (PDA; Becton Dickinson and Company, MD) or Bilay's media (Hallen-Adams et al. 2011), and incubated for 5 to 7 days at room temperature. One isolate per wheat sample was obtained for the 2015 samples, whereas at least two isolates were obtained for the samples collected thereafter. Single-spore isolation was performed following the protocol outlined by Leslie et al. (2006). Single-spore isolates were stored in 35% glycerol (w/v) at -80°C.

For DNA extraction, single-spore isolates were grown at room temperature for 7 days on PDA or half-strength V8 juice agar (V8 juice, 100 ml; $CaCO_3$, 1.5 g; and agar, 15 g per liter of medium). Approximately 500 mg of mycelium from each isolate was transferred into a 1.5-ml tube using a sterile toothpick or a flame sterilized scalpel without disturbing the agar surface. The mycelia were disrupted inside the tube using a pestle and DNA was extracted using the cetyltrimethylammonium bromide (CTAB)

method followed by a phenol/chloroform extraction as described by Panthi (2012), or using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) per manufacturer's instructions.

Determination of *Fusarium* species

Species identification was performed using partial sequences of the translation elongation factor 1α (*TEF1*), trichothecene 3-*O*-acetyltransferase (*TRI101*) and reductase (*RED*) genes. The sequences for the primer pairs used are in Table 2. Amplification reactions were performed in a total volume of 25 µl prepared by combining 1 µl of template DNA, 2 µl of each primer (10 µM), 7.5 µl of molecular biology grade water and 12.5 µl of DreamTaq Green PCR Master Mix (2X; Thermo Scientific, Vilnius, Lithuania). PCR was carried out in a T100 Thermal Cycler (Bio-rad, Hercules, CA) using the following cycle parameters: 96°C for 2 min for denaturation, followed by 35 cycles at 94°C for 30 s, 53°C for 15 s, and 68°C for 45 s. PCR products were evaluated by agarose electrophoresis and sequenced bidirectionally by Sanger sequencing utilizing an ABI 3730xl platform at Michigan State University's Research Technology Support Facility (East Lansing, MI).

The chromatograms were visually inspected, and forward and reverse sequences were assembled in MEGA7 (Kumar et al. 2016). The consensus sequences were deposited in GenBank under accession numbers MT299342 to MT299556. Basic Local Alignment Search Tool (BLAST) searches were performed against the National Center for Biotechnology Information (NCBI) database for all genes. *TEF1* sequences were

compared with sequences in the Fusarium-ID database (http://isolate.fusariumdb.org/guide.php) (Geiser et al. 2004). The sequences were aligned using the MUSCLE algorithm in MEGA7. Sequences from reference strains downloaded from GenBank were also included in the alignments.

Maximum likelihood (ML) analyses were performed for the nucleotide alignments in IQ-TREE 1.6.12 (Nguyen et al. 2014). Nucleotide substitution models were selected for each phylogeny with ModelFinder (Kalyaanamoorthy et al., 2017) using the Bayesian Information Criterion (BIC). Support for individual branches was estimated with 1000 ultrafast bootstrap (UFboot) replications (Hoang et al. 2017) within IQ-TREE. Phylogenetic analyses were performed for each individual gene, as well as concatenated sequences of the *TEF1*, *TRI101* and *RED* genes. Isolates that yielded no amplicons with the primers used in this study were not included in the respective phylogenies. Phylogenetic trees were visualized using iTOL (Letunic and Bork 2019).

Determination of trichothecene genotype

The multiplex PCR assay outlined by Starkey et al. (2007) was used to determine the trichothecene genotype of isolates within the FSAMSC-1. Two alternative sets of primers were used, which amplify portions of the trichothecene 15-*O*-acetyltransferase (*TRI3*) and trichothecene efflux pump (*TRI12*) genes. The primers based on the *TRI3* gene (TRI3 primers) include 3CON, 3D3A, 3D15A and 3NA; whereas the primers based on the *TRI12* gene (TRI12 primers) are 12CON, 12-3F, 12-15F and 12NF (Table 3). The multiplex reactions consisted of 1 µL of template DNA, 12.5 µL of DreamTaq Green

PCR Master Mix (2X; Thermo Scientific, Vilnius, Lithuania), 0.5 µL of each primer (10 µM) and 9.5 µL of molecular biology grade water. Amplifications were carried out using the following cycle parameters: 95°C for 3 min then 35 cycles of 95°C for 30 s, 52°C for 30 s and 72°C, 1 min, followed by a final extension step of 5 min at 72°C.

The amplification products were separated by electrophoresis in 0.7% agarose gels with 0.5 µg/mL ethidium bromide. Molecular weight markers (GeneRuler 1 kb Plus DNA ladder; Thermo Scientific, Vilnius, Lithuania) were used to estimate the size of the amplicons. The TRI3 primers produce bands of 243, 610 and 840 bp, whereas the TRI12 primers yield bands of 410, 670 and 840 bp for the 3-ADON, 15-ADON and NIV genotypes, respectively.

Results

Species identification

In this survey, a total of 74 isolates were obtained from the 42 wheat samples collected from 2015 to 2018 (Table 4). Partial sequences from *TEF1*, *TRI101* and *RED* genes were used to identify isolates to species level. Based on sequence homologies ranging from 99% to 100%, most of the isolates were identified as *F. graminearum* (91%, n=67). Three isolates of *F. boothii* (4%), two isolates of *F. poae* (3%), and one isolate of *F. acuminatum* (1%) were identified (Figure 2). The *F. poae* and *F. acuminatum* isolates were obtained from 2016 samples from which *F. graminearum* was also isolated. Finally, one isolate (1%) was identified as a hybrid between *F.*

graminearum and *F. boothii*. Sequence similarity analysis revealed that this hybrid isolate had *F. graminearum* alleles at the *TEF1* and *TRI101* genes, and a *F. boothii* allele at the *RED* gene (based on 100% homology with the reference strains in the respective databases).

Phylogenetic analyses

The nucleotide substitution models selected by ModelFinder according to BIC were TIM2e + G4, K2P, K2P, and TNe + I for the *TEF*, *TRI101*, *RED* and concatenated phylogenies, respectively.

In the *TEF1* phylogeny, *F. graminearum* isolates clustered with reference isolate *F. graminearum* NRRL 29169, *F. boothii* isolates clustered with reference isolate *F. boothii* NRRL 29105, *F. poae* isolates clustered with reference isolate *F. poae* NRRL 39674, and the *F. acuminatum* isolate clustered with reference isolate *F. acuminatum* NRRL 36147 (Figures 3 and 4). In the *TRI101*, *RED*, and concatenated sequences phylograms, *F. graminearum* and *F. boothii* isolates clustered with their respective reference isolates (Figures 5-10). The *F. graminearum* × *F. boothii* hybrid isolate clustered with *F. graminearum* isolates in the *TEF1* and *TRI101* phylograms (Figures 3 and 5), and with *F. boothii* isolates in the *RED* phylogeny (Figure 7), confirming that it had *F. graminearum* alleles at *TEF1* and *TRI101*, and a *F. boothii* allele at *RED*. Phylogenetic analysis of concatenated *TEF1*, *TRI101* and *RED* sequences placed the *F. graminearum* × *F. boothii* hybrid isolate in its own branch in about 94% of the bootstrap replications (Figures 9 and 10).

Trichothecene genotypes

The trichothecene genotypes of all *F. graminearum*, *F. boothii* isolates, as well as the F. *graminearum* × *F. boothii* hybrid isolate, were determined using a multiplex PCR targeting portions of the *TRI3* and *TRI12* genes. All of these isolates yielded products consistent with the 15-ADON genotype. No other trichothecene genotype was identified in this study.

Discussion

The results of this study show that *F. graminearum* remains the main causal agent of FHB of wheat in Nebraska. Of the 74 isolates obtained in this study, 67 (91%) were identified as *F. graminearum*. This is consistent with previous studies from Nebraska and North America that show *F. graminearum* as the main FHB pathogen in the region (Bec et al. 2015; Nopsa et al. 2014; Panthi et al. 2014; Ward et al. 2008). All *F. graminearum* isolates obtained in this study had the 15-ADON genotype. Survey studies carried out from 2007 to 2010 by Nopsa et al. (2014) and Panthi et al. (2014) reported that all 77 *Fusarium* isolates obtained from wheat fields and grain elevators in Nebraska had the 15-ADON genotype, which is the only genotype that has been reported in the state. The authors identified those isolates as *F. graminearum*. However, these studies relied on primers that are unable to differentiate species within the FGSC (Hafez et al. 2019; Valverde-Bogantes 2017).

In addition to *F. graminearum*, three isolates (4%) of *F. boothii* were identified in this study from wheat samples collected in 2015. Two of the *F. boothii* isolates were obtained from samples collected in Chase County, in the West Central region of the state; and one isolate was obtained from Box Butte County in the Panhandle (Table 4). This finding represents the first time that *F. boothii* has been isolated causing FHB of wheat in the U.S. All three isolates of *F. boothii* had the 15-ADON genotype, consistent with previous reports for this species (Aoki et al. 2012). The next chapter of this dissertation describes the fulfillment of Koch's postulates for *F. boothii* isolates from Nebraska, which has been published as a Disease Note (Wegulo et al. 2018).

Previous studies have isolated *F. boothii* from corn in Texas (Aoki et al. 2012) and *F. boothii* was one of the species causing root rot of corn in South Dakota in 2015 (Okello et al. 2019), which suggests a larger distribution for *F. boothii* in the U.S., albeit at low levels. This species is believed to have evolved in Mesoamerica (Aoki et al. 2012), and it is, in fact, the predominant pathogen of FHB of wheat in southern Mexico (Cerón-Bustamante et al. 2018). However, *F. boothii* has a very wide geographic distribution, having been reported in Africa, Asia, Europe and South America (Boutigny et al. 2011; Desjardins and Proctor 2011; Duan et al. 2016; Láday et al. 2004; Malihipour et al. 2012; Sampietro et al. 2011; Tóth et al. 2005; Zhang et al. 2016).

An interspecies hybrid between *F. boothii* and *F. graminearum* was isolated in this study from a wheat sample from Saunders County in the southeast region of Nebraska. This *F. boothii* × *F. graminearum* hybrid had *F. graminearum* alleles at the *TEF1* and *TRI101* genes, and a *F. boothii* allele at the *RED* gene. Hybrids between these two species have been previously reported in South Africa, where *F. graminearum* and *F.*

boothii are also sympatric (Boutigny et al. 2011). Two *F. boothii* × *F. graminearum* hybrids were isolated from maize in France (Boutigny et al. 2014), possibly representing recent introductions since *F. boothii* was not found in that survey. Interspecies hybrids between other species within the FGSC have been reported to occur in nature (O'Donnell et al. 2000) and under laboratory conditions (Cumagun et al. 2004; Fuentes-Bueno 2012; Jurgenson et al. 2002). However, fitness of interspecies hybrids and implications for crop production remain largely unexplored.

In this study, two isolates of *F. poae* (3%), and one isolate of *F. acuminatum* (1%) were identified from wheat samples. These species are reported to have a different mycotoxin profile than species within the FGSC. *F. poae* is known to produce NIV and type A trichothecenes, such as diacetoxyscirpenol and neosolaniol (O'Donnell et al. 2018; Vanheule et al. 2017); whereas *F. acuminatum* produces enniatins and moniliformin (Beccari et al. 2019). Both species are regarded as weak FHB pathogens on wheat (Beccari et al. 2019), and they were isolated from samples in this study that also had *F. graminearum*. Under greenhouse conditions, isolates of *F. acuminatum* from Kentucky did not cause any symptoms on the susceptible spring wheat variety 'Norm' (Bec et al. 2015). Therefore, it is possible that these isolates were growing saprophytically, or causing secondary infections.

Conclusions

A survey of FHB pathogens infecting wheat in Nebraska was conducted over the course of four growing seasons. This study provides valuable information on species and

trichothecene genotype diversity of FHB pathogens in the state. It was found that *F. graminearum* is not the only pathogen infecting wheat in Nebraska, since three *F. boothii* isolates and a *F. boothii* × *F. graminearum* hybrid were also identified. The results obtained expand knowledge on the global distribution of *F. boothii* to include Nebraska. The information obtained from this survey will be useful in developing effective FHB management strategies in Nebraska.

This study shows that increased pathogen surveillance efforts and continuous monitoring is needed in order to detect possible changes in the species or toxin genotype distributions in Nebraska. Additional studies should also consider the genetic population structure and the temporal population dynamics of FHB pathogens in the state, because different pathogen populations can cause varying levels of disease intensity and can be selectively sensitive to management tactics.

The following chapters of this dissertation describe experiments aiming to fulfill Koch's postulates for *F. boothii* isolates from Nebraska, as well evaluate the aggressiveness and mycotoxin production of *F. boothii* and *F. graminearum* isolates obtained from this survey.

References

Amarasinghe, C. C., Tittlemier, S. A., and Fernando, W. G. D. 2015. Nivalenol-producing *Fusarium cerealis* associated with fusarium head blight in winter wheat in Manitoba, Canada. Plant Pathol. 64:988–995.

Aoki, T., Ward, T. J., Kistler, H. C., and O'Donnell, K. 2012. Systematics, Phylogeny

and Trichothecene Mycotoxin Potential of Fusarium Head Blight Cereal Pathogens. JSM Mycotoxins. 62:91–102.

Bai, G. H., Desjardins, A. E., and Plattner, R. D. 2001. Deoxynivalenol-nonproducing *Fusarium graminearum* causes initial infection, but does not cause disease spread in wheat spikes. Mycopathologia. 153:91–98.

Bec, S., Ward, T., Farman, M., O'Donnell, K., Hershman, D., Van Sanford, D., et al. 2015. Characterization of *Fusarium* Strains Recovered From Wheat With Symptoms of Head Blight in Kentucky. Plant Dis. 99:1622–1632.

Beccari, G., Arellano, C., Covarelli, L., Tini, F., Sulyok, M., and Cowger, C. 2019. Effect of wheat infection timing on Fusarium head blight causal agents and secondary metabolites in grain. Int. J. Food Microbiol. 290:214–225.

Bissonnette, K. M., Wharton, P., Chen, J., and Marshall, J. M. 2018. Survey of *Fusarium* Species Associated with Fusarium Head Blight of Spring Wheat (*Triticum aestivum*) in Southeastern Idaho. Plant Heal. Prog. 19:125–127.

Boutigny, A.-L., Ward, T., Ballois, N., Iancu, G., and Ioos, R. 2014. Diversity of the *Fusarium graminearum* species complex on French cereals. Eur. J. Plant Pathol. 138:133–148.

Boutigny, A.-L., Ward, T. J., Coller, G. J. Van, 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.

Burlakoti, R. R., Neate, S. M., Adhikari, T. B., Gyawali, S., Salas, B., Steffenson, B. J., et al. 2011. Trichothecene profiling and population genetic analysis of *Gibberella zeae* from barley in North Dakota and Minnesota. Phytopathology. 101:687–695.

Cerón-Bustamante, M., Ward, T. J., Kelly, A., Vaughan, M. M., McCormick, S. P., Cowger, C., et al. 2018. Regional differences in the composition of Fusarium Head Blight pathogens and mycotoxins associated with wheat in Mexico. Int. J. Food Microbiol. 273:11–19.

Cumagun, C. J. R., Bowden, R. L., Jurgenson, J. E., Leslie, J. F., and Miedaner, T. 2004. Genetic mapping of pathogenicity and aggressiveness of *Gibberella zeae* (*Fusarium graminearum*) toward wheat. Phytopathology. 94:520–526.

Desjardins, A. E., and Proctor, R. H. 2011. Genetic diversity and trichothecene chemotypes of the *Fusarium graminearum* clade isolated from maize in Nepal and identification of a putative new lineage. Fungal Biol. 115:38–48.

Desjardins, A. E., Proctor, R. H., Bai, G., McCormick, S. P., Shaner, G., Buechley, G., et al. 1996. Reduced virulence of Trichothecene-nonproducing mutants of *Gibberella zeae* in wheat field tests. Mol. Plant-Microbe Interact. 9:775–781.

Duan, C., Qin, Z., Yang, Z., Li, W., Sun, S., Zhu, Z., et al. 2016. Identification of Pathogenic *Fusarium* spp. Causing Maize Ear Rot and Potential Mycotoxin Production in China. Toxins. 8:186.

Fuentes-Bueno, I. 2012. Dissection of fertility barriers among lineages of *Gibberella zeae*. MS thesis. Kansas State University.

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.

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

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

Hafez, M., Abdelmagid, A., Adam, L. R., and Daayf, F. 2020. Specific Detection and Identification of *Fusarium graminearum* Sensu Stricto Using a PCR-RFLP Tool and Specific Primers Targeting the Translational Elongation Factor 1α Gene. Plant Dis. 104:1076–1086.

Hallen-Adams, H. E., Cavinder, B. L., and Trail, F. 2011. *Fusarium graminearum* from Expression Analysis to Functional Assays. In Fungal Genomics: Methods and Protocols, eds. Jin-Rong Xu and Burton H Bluhm. New York: Humana Press, p. 79–101.

Hoang, D. T., Chernomor, O., von Haeseler, A., Minh, B. Q., and Vinh, L. S. 2017. UFBoot2: Improving the Ultrafast Bootstrap Approximation. Mol. Biol. Evol. 35:518–522.

Jurgenson, J. E., Bowden, R. L., Zeller, K. A., Leslie, J. F., Alexander, N. J., and Plattner, R. D. 2002. A genetic map of *Gibberella zeae* (*Fusarium graminearum*). Genetics. 160:1451–1460.

Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K. F., Von Haeseler, A., and Jermiin, L. S. 2017. ModelFinder: Fast model selection for accurate phylogenetic estimates. Nat. Methods. 14:587–589.

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. PLoS One. 13:e0194616.

Kelly, A., Proctor, R. H., Belzile, F., Chulze, S. N., Clear, R. M., Cowger, C., et al. 2016. The geographic distribution and complex evolutionary history of the NX-2 trichothecene chemotype from *Fusarium graminearum*. Fungal Genet. Biol. 95:39–48.

Kumar, S., Stecher, G., and Tamura, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol. Biol. Evol. 33:1870–1874.

Láday, M., Juhász, Á., Mulè, G., Moretti, A., Szécsi, Á., and Logrieco, A. 2004. Mitochondrial DNA Diversity and Lineage Determination of European Isolates of *Fusarium graminearum* (*Gibberella zeae*). Eur. J. Plant Pathol. 110:545–550.

Laraba, I., Boureghda, H., Abdallah, N., Bouaicha, O., Obanor, F., Moretti, A., et al. 2017. Population genetic structure and mycotoxin potential of the wheat crown rot and head blight pathogen *Fusarium culmorum* in Algeria. Fungal Genet. Biol.

103:34–41.

Leslie, J. F., Summerell, B. A., and Bullock, S. 2006. *The Fusarium Laboratory Manual*. Blackwell Publishing, Ames, IA.

Letunic, I., and Bork, P. 2019. Interactive Tree Of Life (iTOL) v4: recent updates and new developments. Nucleic Acids Res. 47:W256–W259.

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.

Malihipour, A., Gilbert, J., Piercey-Normore, M., and Cloutier, S. 2012. Molecular Phylogenetic Analysis, Trichothecene Chemotype Patterns, and Variation in Aggressiveness of Fusarium Isolates Causing Head Blight in Wheat. Plant Dis. 96:1016–1025.

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.

Nash, S. M., and Snyder, W. C. 1962. Quantitative and estimations by plat counts of propagules of the bean rot *Fusarium* in field soils. Phytopathology. 52:567–572.

Nguyen, L.-T., Schmidt, H. A., von Haeseler, A., and Minh, B. Q. 2014. IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. Mol. Biol. Evol. 32:268–274.

Nopsa, J. F. H., Wegulo, S. N., Panthi, A., Hallen-Adams, H. E., Harris, S. D., and Baenziger, P. S. 2014. Characterization of Nebraska Isolates of Causing Head Blight

of Wheat. Crop Sci. 54:310–317.

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.

O'Donnell, K., Kistler, H. C., Tacke, B. K., and Casper, H. H. 2000. Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of *Fusarium graminearum*, the fungus causing wheat scab. Proc. Natl. Acad. Sci. 97:7905–7910.

O'Donnell, K., McCormick, S. P., Busman, M., Proctor, R. H., Ward, T. J., Doehring, G., et al. 2018. Marasas et al. 1984 "Toxigenic *Fusarium* Species: Identity and Mycotoxicology" revisited. Mycologia. 110:1058–1080.

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.

Okello, P. N., Petrović, K., Kontz, B., and Mathew, F. M. 2019. Eight Species of *Fusarium* Cause Root Rot of Corn (Zea mays) in South Dakota. Plant Heal. Prog. 20:38–43.

Panthi, A. 2012. Characterization of Chemotype and Aggressiveness of Nebraska Isolates of *Fusarium graminearum*. MS thesis. University of Nebraska-Lincoln.

Panthi, A., Hallen-Adams, H., Wegulo, S. N., Nopsa, J. H., and Baenziger, P. S. 2014.

Chemotype and aggressiveness of isolates of *Fusarium graminearum* causing head blight of wheat in Nebraska. Can. J. Plant Pathol. 36:447–455.

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. Phytopathol. 100:1007–1014.

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.

Sarver, B. A. J., Ward, T. J., Gale, L. R., Broz, K., Kistler, H. C., Aoki, T., et al. 2011. Novel Fusarium head blight pathogens from Nepal and Louisiana revealed by multilocus genealogical concordance. Fungal Genet. Biol. 48:1096–1107.

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.

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.

Tóth, B., Mesterházy, Á., Horváth, Z., Bartók, T., Varga, M., and Varga, J. 2005. Genetic Variability of Central European Isolates of the *Fusarium graminearum* Species Complex. Eur. J. Plant Pathol. 113:35–45.

Valverde-Bogantes, E. 2017. Species and Trichothecene Genotypes of Fusarium Head Blight Pathogens in Nebraska, USA in 2015-2016. MS thesis. University of Nebraska-Lincoln.

Valverde-Bogantes, E., Bianchini, A., Herr, J. R., Rose, D. J., Wegulo, S. N., and Hallen-Adams, H. E. 2019. Recent population changes of Fusarium head blight pathogens: drivers and implications. Can. J. Plant Pathol. DOI: 10.1080/07060661.2019.1680442.

van der Fels-Klerx, H. J., de Rijk, T. C., Booij, C. J. H., Goedhart, P. W., Boers, E. A. M., Zhao, C., et al. 2012. Occurrence of Fusarium Head Blight species and *Fusarium* mycotoxins in winter wheat in the Netherlands in 2009. Food Addit. Contam. Part A. 29:1716–1726.

Vanheule, A., Marthe, D. B., Moretti, A., Scauflaire, J., Munaut, F., Sarah, D. S., et al. 2017. Genetic Divergence and Chemotype Diversity in the Fusarium Head Blight Pathogen *Fusarium poae*. Toxins. 9:255.

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. U.S.A. 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.

Wegulo, S. N., Baenziger, P. S., Nelson, L. A., Nopsa, J. H., Millhouse, J. C., Mengistu, N., and Breathnach-Stevens, J. 2008. The 2008 Fusarium head blight epidemic in Nebraska. In: Proc. 2008 National Fusarium Head Blight Forum, eds. S. Canty, A. Clark, E. Walton, D. Ellis, J. Mundell, and D. Van Sanford. Indianapolis, IN. p. 72.

Wegulo SN, Valverde-Bogantes E, Bolanos-Carriel C, Hallen-Adams H, Bianchini A, McMaster N, Schmale DG. 2018. First report of *Fusarium boothii* causing head blight of wheat in the United States. Plant Dis. 102(12):2646.

Zhang, H., Brankovics, B., Xu, J., Xu, J. S., Guo, C., Guo, J. G., et al. 2016. Crops are a main driver for species diversity and the toxigenic potential of *Fusarium* isolates in maize ears in China. World Mycotoxin J. 9:701–715.

Table 1. Summary of the wheat heads sampled across Nebraska for this study and their location. Sample names indicate the year of collection.

Sample ID	GPS coordinates	County	Wheat growing region
NE1-15	40°11'01.8"N 99°53'30.5"W	Furnas	West Central
NE2-15	40°12'22.7"N 100°58'00.2"W	Hitchcock	West Central
NE3-15	40°26'27.9"N 96°57'39.8"W	Saline	Southeast
NE4-15	41°09'56.4"N 96°44'43.8"W	Saunders	Southeast
NE5-15	41°14'04.0"N 96°31'46.1"W	Saunders	Southeast
NE6-15	40°35'32.9"N 96°58'16.3"W	Saline	Southeast
NE7-15	41°08'44.0"N 96°29'47.1"W	Saunders	Southeast
NE8-15	41°01'50.0"N 96°29'32.6"W	Lancaster	Southeast
NE9-15	40°42'36.9"N 98°03'29.4"W	Clay	South Central
NE10-15	40°48'08.3"N 98°75'26.2"W	Kearney	South Central
NE11-15	40°45'23.7"N 99°29'89.1"W	Phelps	South Central
NE12-15	40°30'61.7"N 99°71'57.4"W	Harlan	West Central
NE13-15	40°25'22.1"N 97°35'45.2"W	Fillmore	South Central
NE14-15	40°50'24.2"N 101°41'44.6"W	Perkins	West Central
NE15-15	40°11'34.4"N 100°59'01.8"W	Hitchcock	West Central
NE16-15	40°38'12.6"N 101°37'43.8"W	Chase	West Central
NE17-15	40°49'18.4"N 101°39'57.7"W	Perkins	West Central
NE18-15	40°20'46.3"N 100°95'16.3"W	Hitchcock	West Central
NE19-15	40°27'36.0"N 101°32'01.0"W	Chase	West Central
NE20-15	41°09'06.9"N 103°29'30.3"W	Kimball	Panhandle
NE21-15	42°20'18.6"N 103°04'22.1"W	Box Butte	Panhandle
NE22-15	41°13'56.7"N 103°01'13.0"W	Cheyenne	Panhandle
NE23-15	41°15'52.7"N 97°12'40.9"W	Butler	Southeast
NE1-16	40°16'10.4"N 99°79'17.8"W	Furnas	West Central
NE2-16	40°24'24.5"N 99°25'37.2"W	Phelps	South Central
NE3-16	40°54'46.6"N 98°84'55.4"W	Kearney	South Central
NE4-16	40°57'54.2"N 98°12'85.7"W	Clay	South Central
NE5-16	41°22'86.0"N 96°48'92.0"W	Saunders	Southeast
NE6-16	41°05'23.9"N 96°54'05.1"W	Saunders	Southeast
NE7-16	40°43'11.4"N 96°96'10.0"W	Saline	Southeast
NE8-16	40°24'24.5"N 99°25'37.2"W	Nuckolls	South Central
NE9-16	40°48'03.8"N 97°17'59.5"W	Saline	Southeast
NE10-16	40°32'11.4"N 98°44'25.3"W	Webster	South Central

NE1-17	41°10'49.7"N 96°28'13.8"W	Saunders	Southeast
NE2-17	41°10'54.6"N 96°28'12.2"W	Saunders	Southeast
NE3-17	40°51'24.1"N 96°36'44.7"W	Lancaster	Southeast
NE4-17	40°51'22.9"N 96°36'49.0"W	Lancaster	Southeast
NE1-18	41°03'29.7"N 100°45'03.7"W	Lincoln	West Central
NE2-18	41°03'14.6"N 100°45'06.1"W	Lincoln	West Central
NE3-18	41°02'49.3"N 100°45'02.6"W	Lincoln	West Central
NE4-18	42°14'53.0"N 103°01'11.0"W	Box Butte	Panhandle
NE5-18	41°09'47.2"N 101°59'32.4"W	Keith	West Central

Table 2. Sequence of the primers used for species identification.

Primer	Target gene	Direction	Sequence (5'-to-3')	Reference
EF1	*TEF1*	Forward	ATGGGTAAGGARGACAAGAC	(O'Donnell et al. 1998)
EF2		Reverse	GGARGTACCAGTSATCATGTT	
RED1d	*RED*	Forward	TCTCAGAAAGACGCATATATG	(O'Donnell et al. 2000)
RED2		Reverse	CGTAACTGCGTCATTCGGC	
AT1	*TRI101*	Forward	AAAATGGCTTTCAAGATACAGC	(O'Donnell et al. 2000)
AT2		Reverse	CRTAYTGCGCRTARTTGGTCCA	

Table 3. Sequence of the primers used to determine trichothecene genotype (Starkey et al. 2007).

Primer	Sequence (5'-to-3')
3CON	TGGCAAAGACTGGTTCAC
3D3A	CGCATTGGCTAACACATG
3D15A	ACTGACCCAAGCTGCCATC
3NA	GTGCACAGAATATACGAGC
12CON	CATGAGCATGGTGATGTC
12-3F	CTTTGGCAAGCCCGTGCA
12-15F	TACAGCGGTCGCAACTTC
12NF	TCTCCTCGTTGTATCTGG

Table 4. Isolate ID, wheat growing region and species for the 74 *Fusarium* strains obtained from Nebraska wheat in this study.

Isolate ID	Wheat growing region	Species ID
NE1-15Fg	West Central	*F. graminearum*
NE2-15Fg	West Central	*F. graminearum*
NE3-15Fg	Southeast	*F. graminearum*
NE4-15Fg	Southeast	*F. graminearum*
NE5-15Fg	Southeast	*F. graminearum*
NE6-15Fg	Southeast	*F. graminearum*
NE7-15Fg	Southeast	*F. graminearum*
NE8-15Fg	South Central	*F. graminearum*
NE9-15Fg	South Central	*F. graminearum*
NE10-15Fg	South Central	*F. graminearum*
NE11-15Fg	West Central	*F. graminearum*
NE12-15Fg	South Central	*F. graminearum*
NE13-15Fg	West Central	*F. graminearum*
NE14-15Fg	West Central	*F. graminearum*
NE15-15Fg	West Central	*F. graminearum*
NE16-15Fb	West Central	*F. boothii*
NE17-15Fg	West Central	*F. graminearum*
NE18-15Fg	West Central	*F. graminearum*
NE19-15Fb	West Central	*F. boothii*
NE20-15Fg	Panhandle	*F. graminearum*
NE21-15Fb	Panhandle	*F. boothii*
NE22-15Fg	Panhandle	*F. graminearum*
NE23-15Fg	Southeast	*F. graminearum*
NE1a-16Fg	West Central	*F. graminearum*
NE1b-16Fg	West Central	*F. graminearum*
NE1c-16Fg	West Central	*F. graminearum*
NE2a-16Fg	South Central	*F. graminearum*
NE2c-16Fg	South Central	*F. graminearum*
NE3a-16Fg	South Central	*F. graminearum*
NE3b-16Fg	South Central	*F. graminearum*
NE3c-16Fg	South Central	*F. graminearum*
NE4a-16Fg	South Central	*F. graminearum*
NE5a-16FgxFb	Southeast	*F. graminearum* × *F. boothii* hybrid
NE5b-16Fg	Southeast	*F. graminearum*
NE5c-16Fpoae	Southeast	*F. poae*
NE6a-16Fg	Southeast	*F. graminearum*
NE6b-16Fg	Southeast	*F. graminearum*
NE6c-16Fg	Southeast	*F. graminearum*

NE7a-16Fpoae	Southeast	*F. poae*
NE7b-16Fg	Southeast	*F. graminearum*
NE7c-16Fg	Southeast	*F. graminearum*
NE8a-16Facum	South Central	*F. acuminatum*
NE8b1-16Fg	South Central	*F. graminearum*
NE8b2-16Fg	South Central	*F. graminearum*
NE8c1-16Fg	South Central	*F. graminearum*
NE8c2-16Fg	South Central	*F. graminearum*
NE8d1-16Fg	South Central	*F. graminearum*
NE8d2-16Fg	South Central	*F. graminearum*
NE9a-16Fg	Southeast	*F. graminearum*
NE9b-16Fg	Southeast	*F. graminearum*
NE9c-16Fg	Southeast	*F. graminearum*
NE10a-16Fg	South Central	*F. graminearum*
NE10b-16Fg	South Central	*F. graminearum*
NE1b-17Fg	Southeast	*F. graminearum*
NE1c-17Fg	Southeast	*F. graminearum*
NE3a-17Fg	Southeast	*F. graminearum*
NE3b-17Fg	Southeast	*F. graminearum*
NE4b-17Fg	Southeast	*F. graminearum*
NE6b-17Fg	Southeast	*F. graminearum*
NE1a-18Fg	West Central	*F. graminearum*
NE1b-18Fg	West Central	*F. graminearum*
NE1c-18Fg	West Central	*F. graminearum*
NE2a-18Fg	West Central	*F. graminearum*
NE2b-18Fg	West Central	*F. graminearum*
NE2c-18Fg	West Central	*F. graminearum*
NE3a-18Fg	West Central	*F. graminearum*
NE3b-18Fg	West Central	*F. graminearum*
NE3c-18Fg	West Central	*F. graminearum*
NE4a-18Fg	Panhandle	*F. graminearum*
NE4b-18Fg	Panhandle	*F. graminearum*
NE4c-18Fg	Panhandle	*F. graminearum*
NE5a-18Fg	West Central	*F. graminearum*
NE5b-18Fg	West Central	*F. graminearum*
NE5c-18Fg	West Central	*F. graminearum*

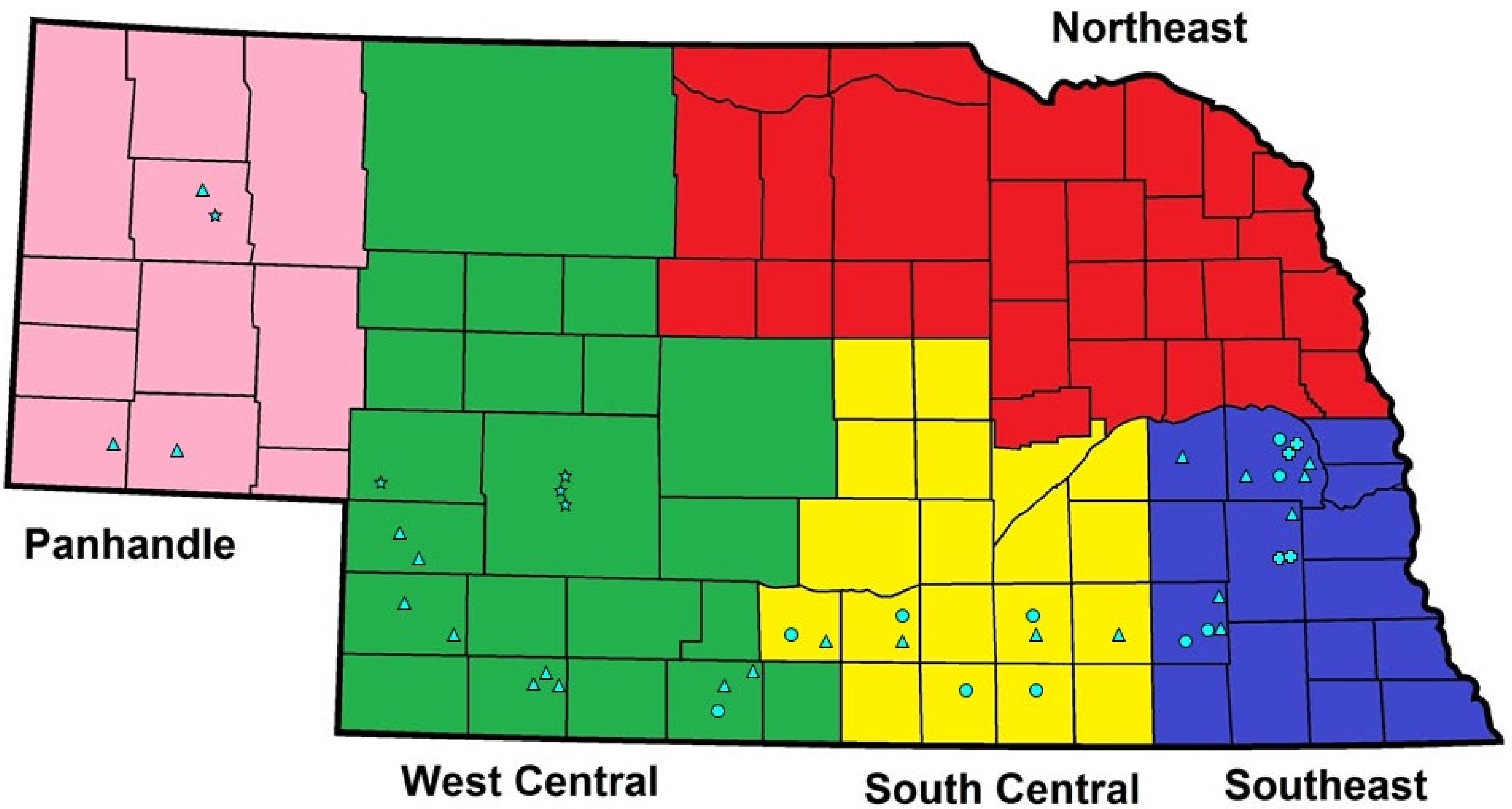


Figure 1. Map showing the different wheat growing regions in Nebraska, as well as the sampling sites where wheat grains were obtained. Each triangle (Δ) represents one sample from 2015, circle (○) from 2016, plus sign (✚) from 2017 and star (☆) from 2018.

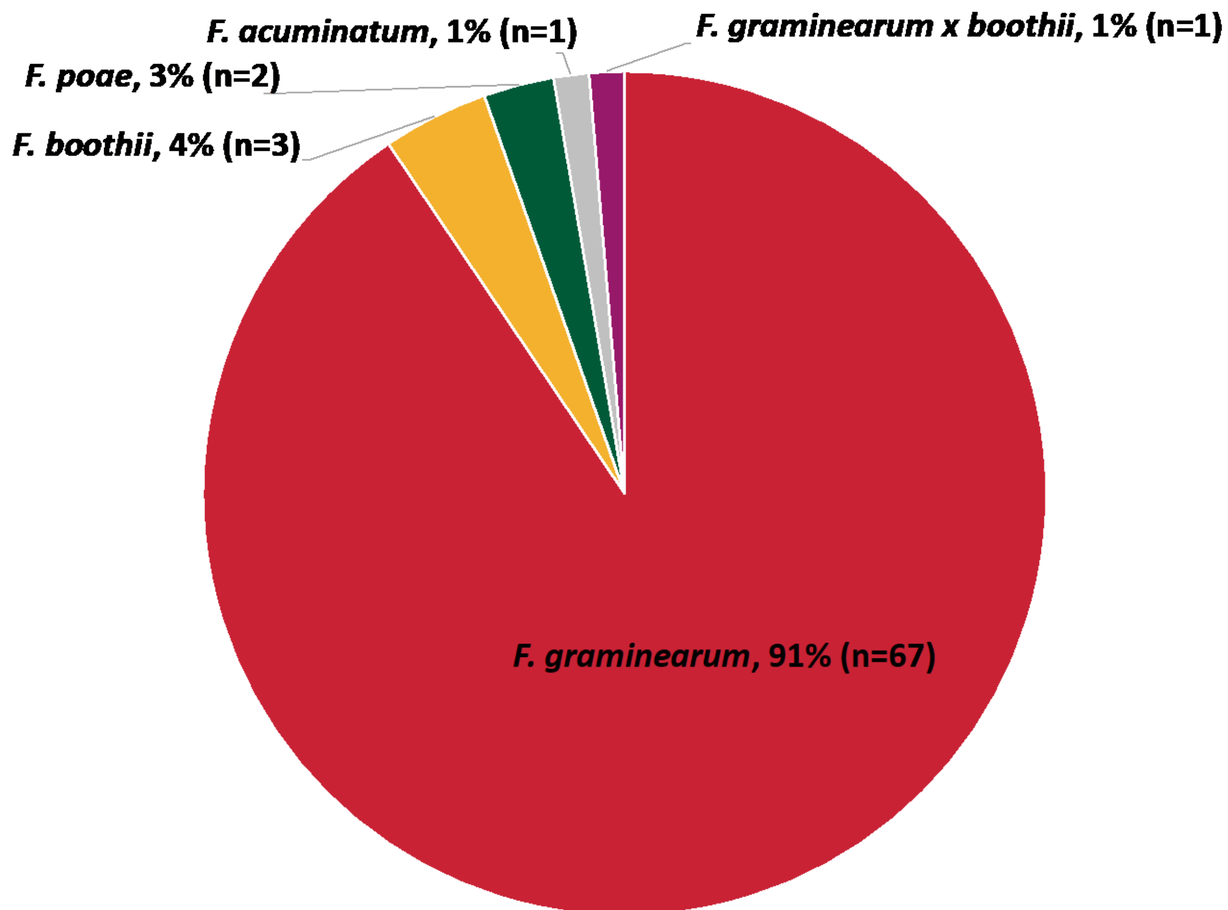


Figure 2. Number of isolates by species obtained from Nebraska wheat from 2015-2018.

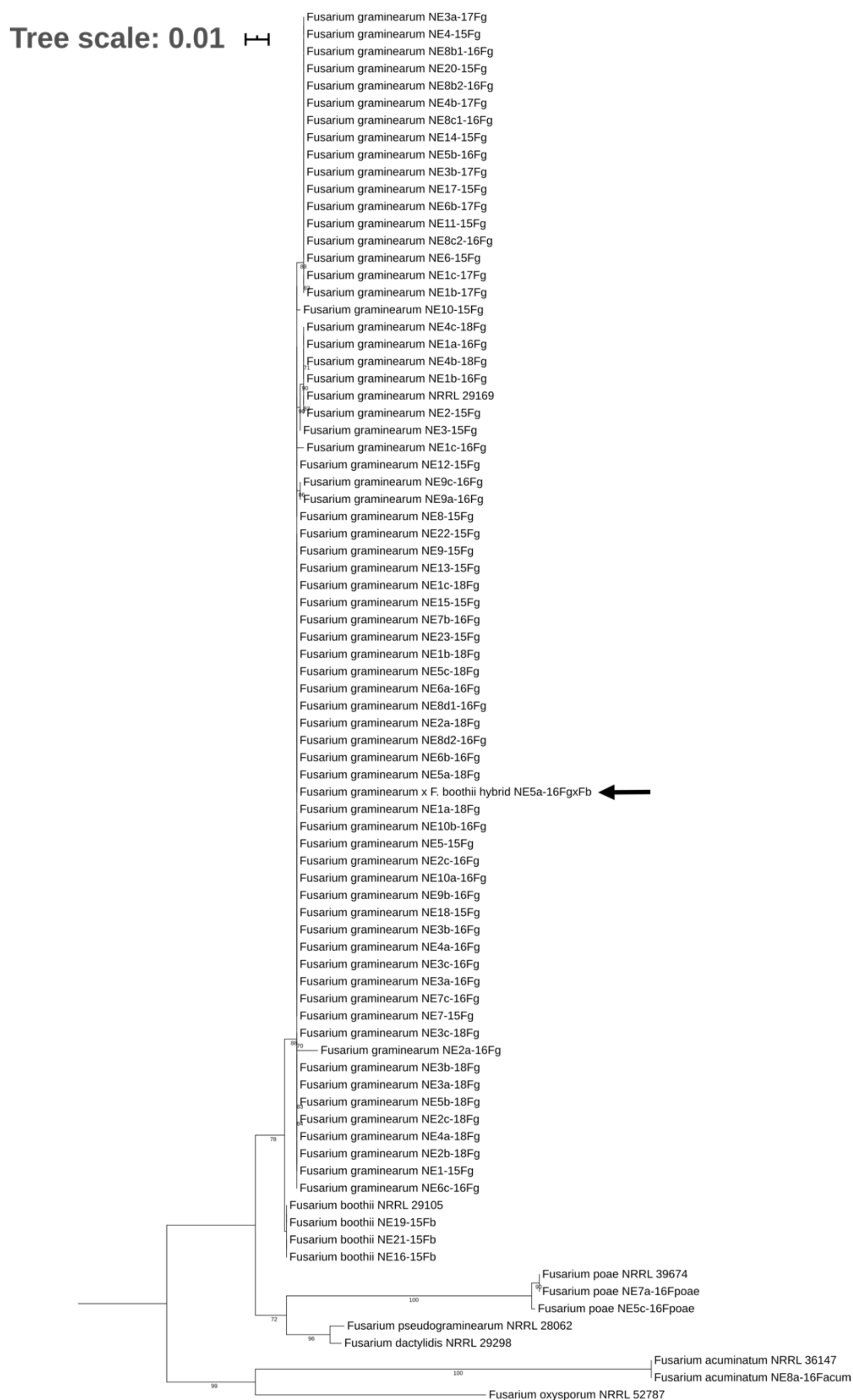


Figure 3. Maximum likelihood phylogram for *TEF1* sequences from 74 *Fusarium* isolates collected from Nebraska wheat in relation to reference isolates (denoted with NRRL accession numbers). Phylogram was built using the TIM2e + G4 model of evolution and numbers on branches indicate bootstrap support from 1000 UFboot replications. There were a total of 733 positions in the final dataset, including 539 invariant, 210 variable and 146 informative sites. The position of the *F. graminearum* × *F. boothii* hybrid isolate is indicated with an arrow.

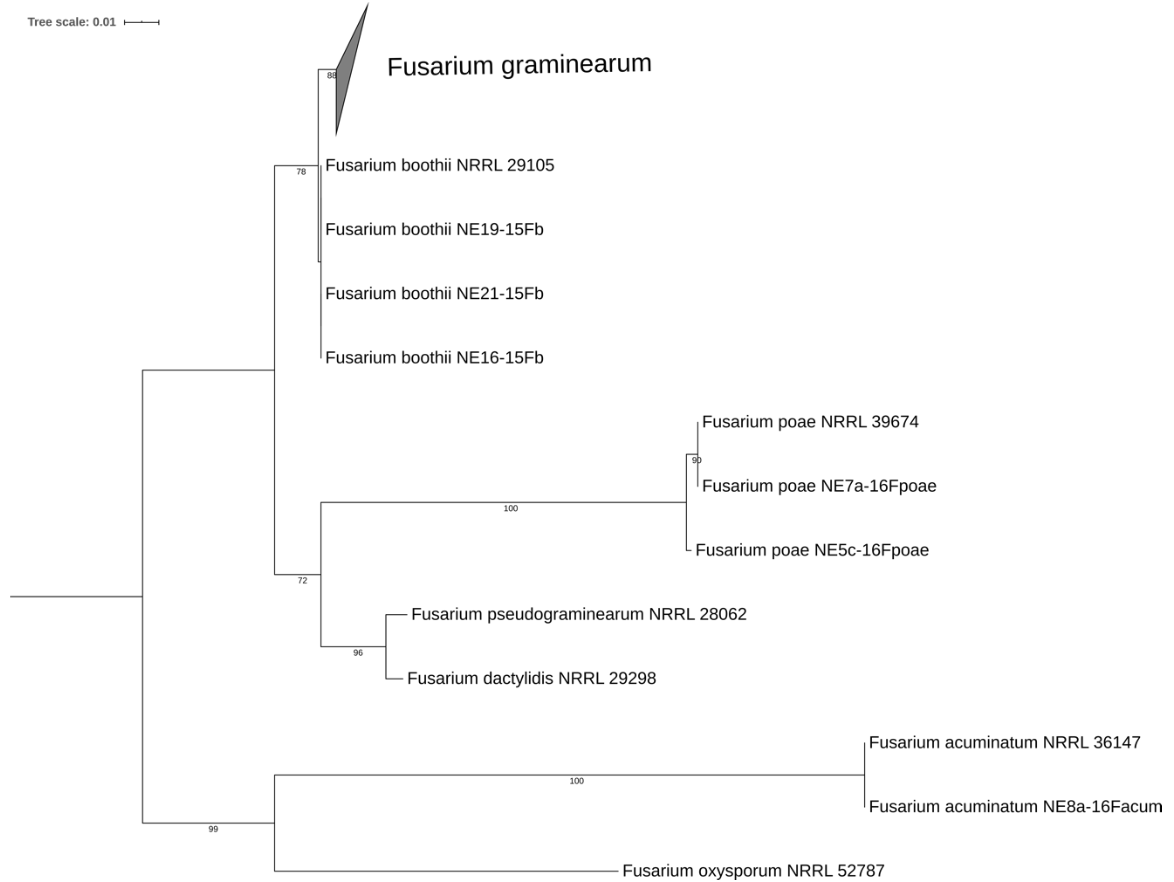


Figure 4. Same as Figure 3. Isolates clustering together with *F. graminearum* NRRL 29169, including the *F. graminearum* × *F. boothii* hybrid, were compressed for better visualization and are represented by a dark triangle.

Figure 5. Maximum likelihood phylogram for *TRI101* sequences from 70 *Fusarium* isolates collected from Nebraska wheat in relation to reference isolates (denoted with NRRL accession numbers). Phylogram was built using the K2P model of evolution and numbers on branches indicate bootstrap support from 1000 UFboot replications. There were a total of 1195 positions in the final dataset, including 1114 invariant, 80 variable and 54 informative sites. The position of the *F. graminearum* × *F. boothii* hybrid isolate is indicated with an arrow.

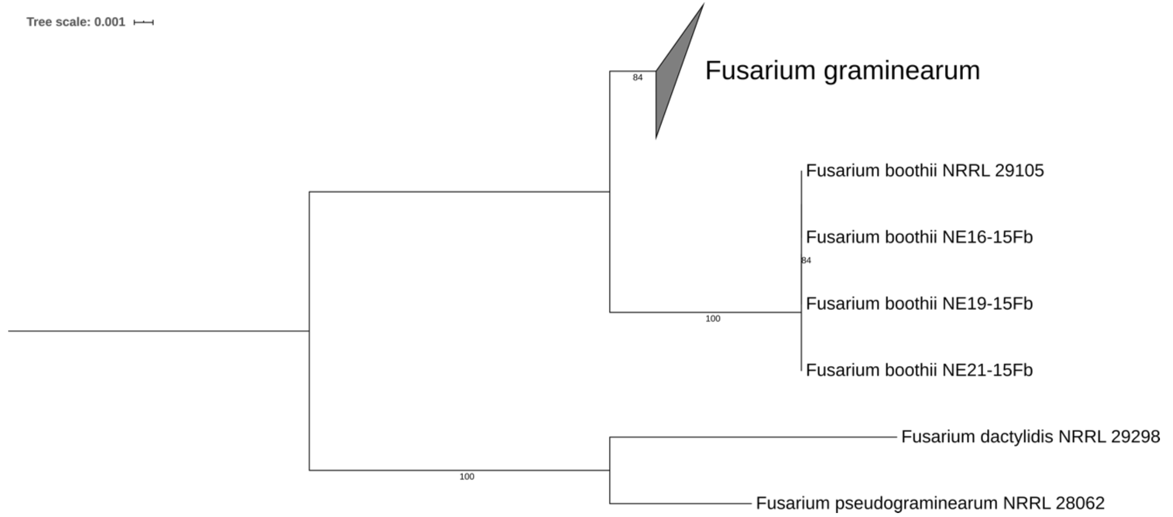


Figure 6. Same as Figure 5. Isolates clustering together with *F. graminearum* NRRL 29169, including the *F. graminearum* × *F. boothii* hybrid, were compressed for better visualization and are represented by a dark triangle.

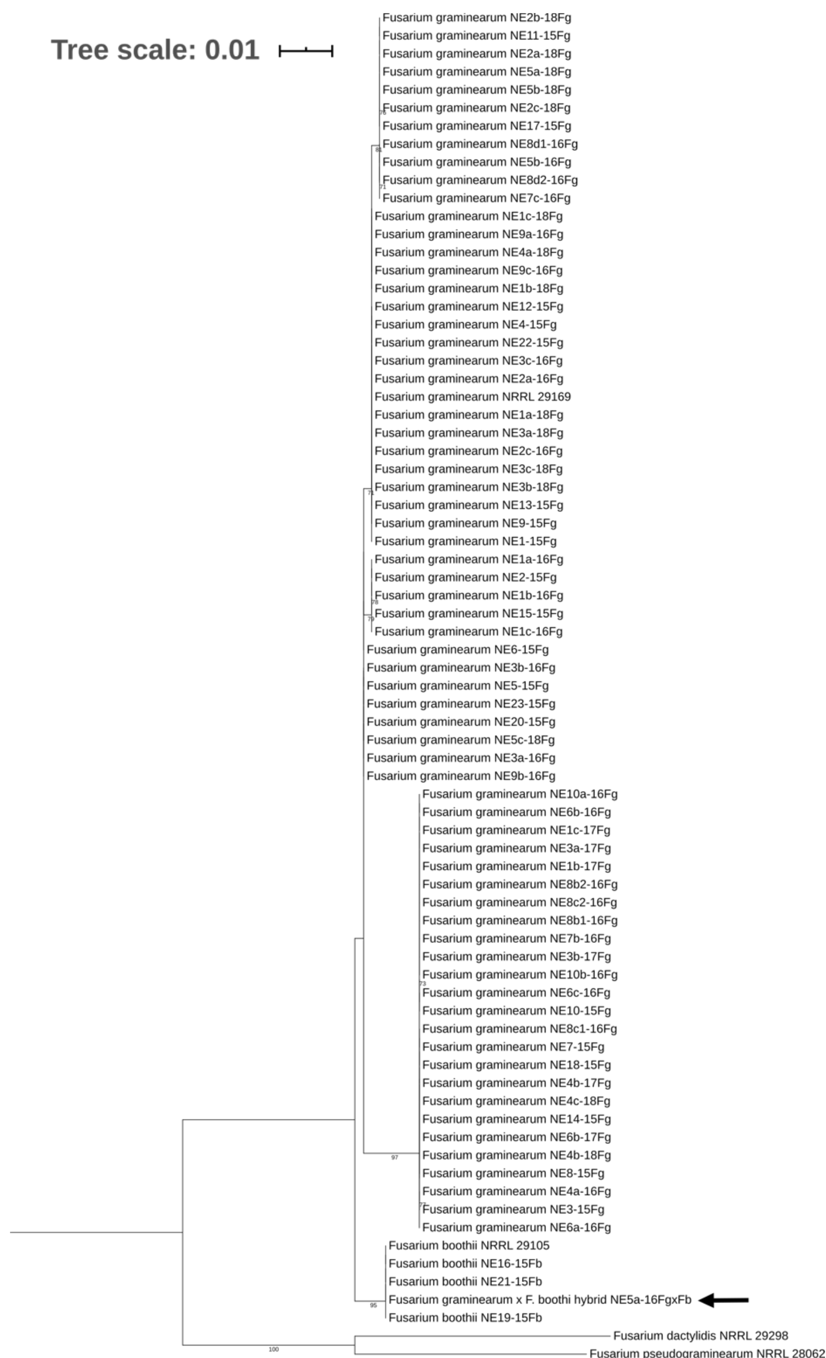


Figure 7. Maximum likelihood phylogram for *RED* sequences from 71 *Fusarium* isolates collected from Nebraska wheat in relation to reference isolates (denoted with NRRL accession numbers). Phylogram was built using the K2P model of evolution and numbers on branches indicate bootstrap support from 1000 UFboot replications. There were a total of 812 positions in the final dataset, including 706 invariant, 79 variable and 51 informative sites. The position of the *F. graminearum* × *F. boothii* hybrid isolate is indicated with an arrow.

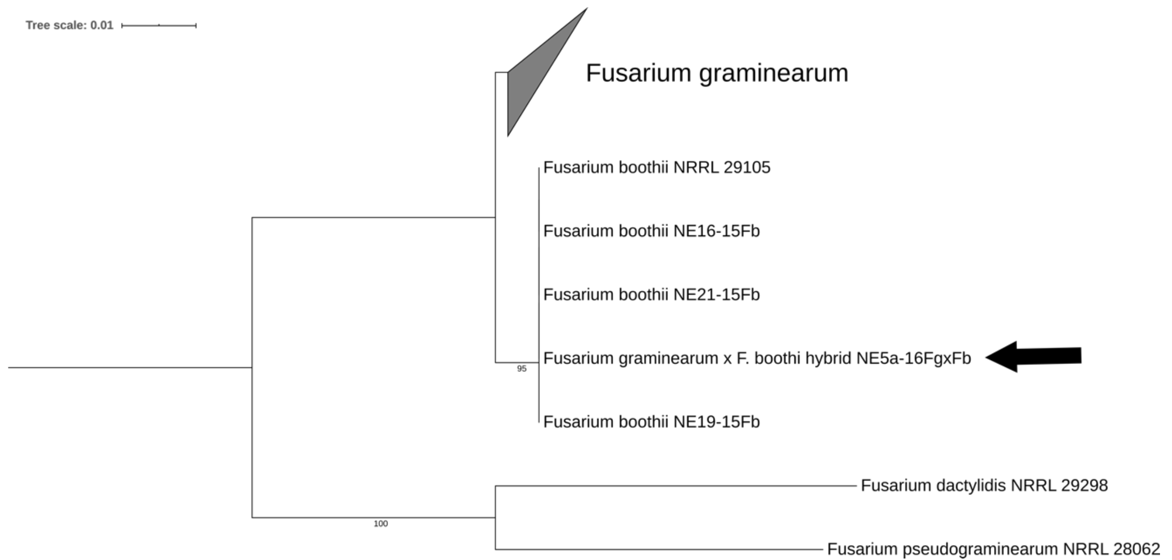


Figure 8. Same as Figure 7. Isolates clustering together with *F. graminearum* NRRL 29169 were compressed for better visualization and are represented by a dark triangle. The position of the *F. graminearum* × *F. boothii* hybrid isolate is indicated with an arrow.

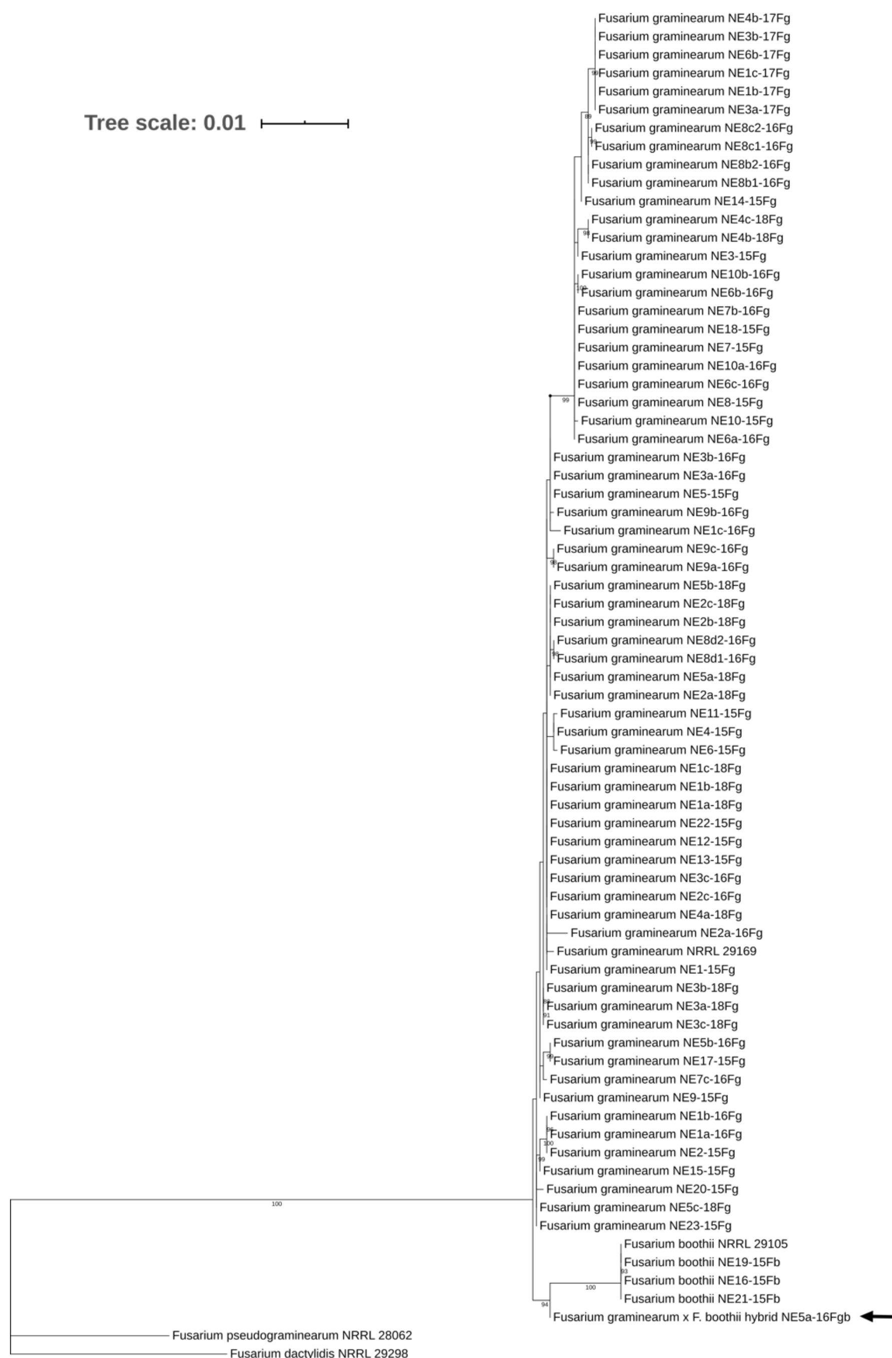


Figure 9. Maximum likelihood phylogram for combined *TEF1*, *TRI101* and *RED* sequences from 70 *Fusarium* isolates collected from Nebraska wheat in relation to reference isolates (denoted with NRRL accession numbers). Phylogram was built using the TNe + I model of evolution and numbers on branches indicate bootstrap support from 1000 UFboot replications. There were a total of 2740 positions in the final dataset, including 2505 invariant, 187 variable and 138 informative sites. The position of the *F. graminearum* × *F. boothii* hybrid isolate is indicated with an arrow.

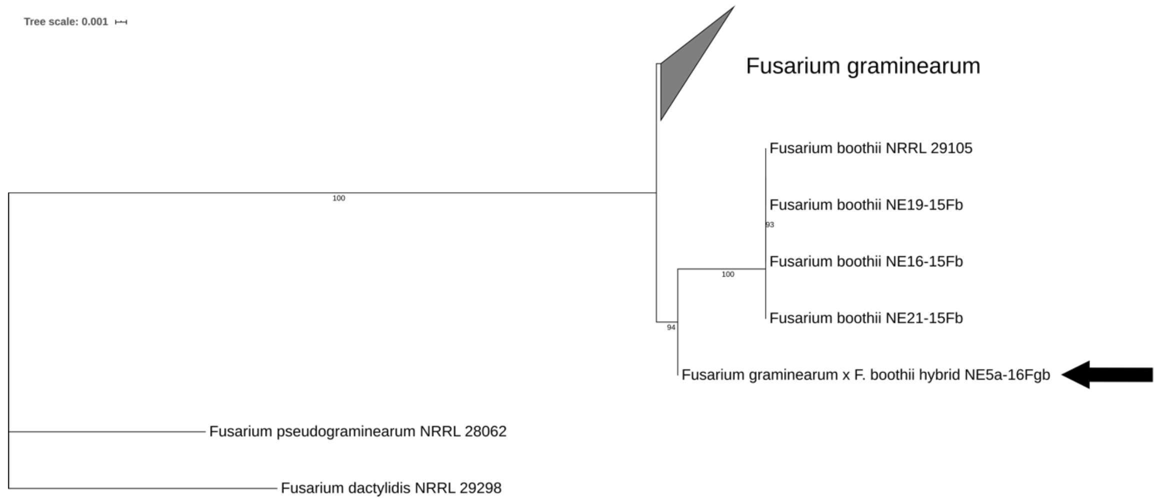


Figure 10. Same as Figure 9. Isolates clustering together with *F. graminearum* NRRL 29169 were compressed for better visualization and are represented by a dark triangle. The position of the *F. graminearum* × *F. boothii* hybrid isolate is indicated with an arrow.

CHAPTER 3

Fusarium boothii from Nebraska: Fulfillment of Koch's postulates

Portions of this material have previously appeared in the following publication:

Wegulo SN, Valverde-Bogantes E, Bolanos-Carriel C, Hallen-Adams H, Bianchini A, McMaster N, Schmale DG. 2018. First report of Fusarium boothii *causing head blight of wheat in the United States. Plant Dis. 102(12):2646.*

Abstract

Fusarium head blight (FHB) is a significant disease of wheat. In the U.S., the main causal agent is *Fusarium graminearum*, but multiple *Fusarium* species can cause FHB. In 2015, severe and widespread FHB epidemics occurred in wheat in Nebraska. As part of a survey of FHB pathogens conducted in the state, three isolates from Nebraska wheat – two from Chase County in the southwest and one from Box Butte County in the northwest – were identified as *F. boothii*. Since *F. boothii* had not been previously reported from wheat in the country, this study was designed to fulfill Koch's postulates with *F. boothii* isolates from Nebraska, in order to formally report *F. boothii* as a causal agent of FHB of wheat in the U.S. Spikes of FHB-susceptible spring wheat cultivar Wheaton were spray-inoculated at anthesis with spores of two *F. boothii* isolates or a single isolate of *F. graminearum* collected in the same survey (approximately 1×10^5

spores/ml), or not inoculated. A randomized complete block design with five or six replications was used in two separate experiments. At 21 days after inoculation, FHB severity (percent of bleached spikelets on a spike) ranged from 17.5 to 86.6%. Mature grain was ground to flour and tested for deoxynivalenol (DON) and its acetylated derivatives 3-ADON and 15-ADON using gas chromatography/mass spectrometry. All three isolates produced DON and had the 15-ADON chemotype. Recovery of single-spored cultures of the three isolates from symptomatic kernels obtained from both experiments fulfilled Koch's postulates. The three isolates produced fertile perithecia on carrot agar after incubation at room temperature for 7 weeks, and their colonies on PDA resembled typical *F. graminearum* clade colonies. Measurements of ascospores (17.50 to 32.50 × 3.75 to 7.50 µm) and macroconidia (36.25 to 67.50 × 3.75 to 6.25 µm) of the three isolates were similar to those reported for species in the *F. graminearum* clade. *Fusarium boothii* has been reported in the United States on maize and as the cause of FHB of wheat or Gibberella ear rot of maize in several countries including Mexico and South Africa. To our knowledge, this is the first report of *F. boothii* causing FHB of wheat in the United States. Additional research will be needed to determine the distribution, aggressiveness, and impact on yield of *F. boothii* compared to *F. graminearum*.

Introduction

Fusarium head blight (FHB) is one of the most devastating and economically important diseases affecting wheat, barley, oats, and other small grain cereals (Goswami and Kistler 2004). Most of the FHB pathogens are grouped within the *Fusarium graminearum* species complex (FGSC), which is part of the broader *Fusarium sambucinum* species complex lineage 1 (FSAMSC-1). The FGSC includes the following species: *F. acaciae-mearnsii*, *F. aethiopicum*, *F. asiaticum*, *F. austroamericanum*, *F. boothii*, *F. brasilicum*, *F. cortaderiae*, *F. gerlachii*, *F. graminearum*, *F. louisianense*, *F. meridionale*, *F. mesoamericanum*, *F. nepalense*, *F. ussurianum*, and *F. vorosii* (Aoki et al. 2012). In the U.S., *F. graminearum* is the main causal agent of FHB. Other species that have been reported causing FHB in the country include *F. gerlachii* in the upper Midwest (Starkey et al. 2007), as well as *F. asiaticum* (Gale et al. 2011) and *F. louisianense* (Sarver et al. 2011) in Louisiana. The species within the FGSC are also known maize pathogens, causing diseases such as Fusarium root rot, Gibberella ear rot (GER) and Gibberella stalk rot (GSR) of maize.

In wheat, FHB causes premature bleaching of spikes that are either sterile or produce shriveled and soft grains, also known as *Fusarium*-damaged kernels, scabby kernels, or “tombstones”, that have reduced weight and functional qualities (Trail 2009). The *Fusarium*-damaged kernels may be contaminated with mycotoxins, primarily type B trichothecenes, which are unsafe for human and animal consumption. Mycotoxin contamination further reduces marketability and prices, in many instances forcing farmers to sell at animal feed prices or to be penalized with huge discounts (McMullen et al. 1997).

In 2015, a period of excessive rainfall before and during wheat flowering allowed for the development of severe and widespread epidemics of FHB in Nebraska, causing significant yield losses and price discounts in most of the wheat fields in the state (Lilleboe 2015). Symptomatic wheat spike samples were collected from affected fields as part of a survey of FHB pathogens described in chapter 2 of this dissertation. The overwhelming majority of isolates obtained were identified as *F. graminearum*. However, three isolates from Chase County in the southwest (isolates NE16-15Fb and NE19-15Fb) and Box Butte County (isolate NE21-15Fb) in the northwest were identified as *F. boothii*, representing the first isolation of this species from wheat in the U.S. Previously, *F. boothii* had been reported on maize from Texas (Aoki et al. 2012) and as the cause of FHB of wheat or GER of maize in several countries including Mexico (Cerón-Bustamante et al. 2018) and South Africa (Boutigny et al. 2011).

The objective of this study was to fulfill Koch's postulates with *F. boothii* isolates from Nebraska, in order to formally report *F. boothii* as a causal agent of FHB of wheat in the U.S.

Materials and Methods

Fusarium isolates

Two *F. boothii* isolates (NE16-15Fb, NE19-15Fb) from Nebraska were used in this study. An *F. graminearum* isolate (NE20-15Fg) was also included for comparison. These isolates were collected, single-spored and identified as part of a survey of FHB

pathogens from Nebraska described in chapter 2 of this dissertation. The *F. boothii* isolates were collected from wheat heads sampled from Chase County in the West Central region of the state, and the *F. graminearum* isolate was from Kimball County in the Panhandle. All three isolates had the 15-ADON genotype, determined using a multiplex PCR with genotype-specific primers based on trichothecene 15-*O*-acetyltransferase (*TRI3*) and trichothecene efflux pump (*TRI12*) genes (Starkey et al. 2007).

In addition to the sequences obtained for these isolates in chapter 2 of this dissertation, a portion of the translation elongation factor 1α (*TEF1*) gene was amplified using primers EF-1f (CGACCACTGTGAGTACCA) and EF-1r (GTCAAGAACCCAGGCGTA) (Ward et al. 2008) and bidirectionally sequenced. The sequences were deposited in GenBank under accession numbers MH379136 to MH379138.

Pathogenicity tests in the greenhouse

Experiment design

Evaluation of pathogenicity of the *Fusarium* isolates on wheat plants was carried out under greenhouse conditions in two different experiments. Four treatments, consisting of each *F. boothii* and *F. graminearum* isolate, and untreated check plants, were organized in a randomized complete block design in both experiments. The first experiment had six replications, and the second experiment had five replications. Each pot with wheat spikes inoculated with one isolate was considered an experimental unit.

Inoculum preparation

In the first experiment, the isolates were grown on potato dextrose agar (PDA; Becton Dickinson and Company, MD) for 18 days at 25 ºC in a Precision™ Low Temperature Incubator (Thermo Fisher Scientific Inc., Waltham, MA) under a 12:12 light:dark cycle. A spore suspension was prepared by adding sterile distilled water to each plate and resuspending spores with an L-shaped plastic rod followed by filtration through four layers of cheesecloth.

In the second experiment, the inoculum was prepared as previously described by Hallen-Adams et al. (2011) with slight modifications. Briefly, the isolates were grown in 250-ml flasks with 100 ml of carboxymethyl cellulose medium (CMC; 15 g carboxymethyl cellulose sodium salt, 1 g NH_4NO_3, 1 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, and 1 g yeast extract per liter of medium). The flasks were incubated for 6 days at room temperature on an orbital shaking incubator (MaxQ 4000; Thermo Fisher Scientific Inc., Waltham, MA) set to 200 rpm. The cultures grown on CMC were filtered through sterile Miracloth (Millipore, Bedford, MA) into sterile centrifuge bottles. The bottles were centrifuged at 4000 × *g* for 5 minutes and the spores were washed twice with sterile distilled water. The spores were resuspended in sterile distilled water.

All spore suspensions were standardized to a final concentration of approximately 1×10^5 spores/ml with a hemocytometer, stored at 4°C, and used on the day of preparation.

Plant materials, inoculation and disease rating

Plants of the FHB-susceptible spring wheat cv. Wheaton (Busch et al. 1984) were grown in 15-cm pots in a greenhouse room, at a rate of 12 plants per pot (experimental unit). The potting mix consisted of 33% clay loam soil, 33% peat soil, 16.5% sand, and 16.5% vermiculite. Plants were watered daily and fertilized together with irrigation using Peter's Peat Lite Special Fertilizer, 20-10-20 (Everris, Marysville, Ohio) at 250 μg/ml. Lighting was provided by Lumigrow Pro 325 LED Grow Lights (Lumigrow Inc., Emeryville, CA) for 14 hours a day. Mean temperature in each room was 23.4ºC and ranged from 19.3ºC to 32.4ºC. Mean relative humidity was 57% and ranged from 28% to 95%.

In total, 5 to 18 spikes from each pot were inoculated at full anthesis. Inoculated spikes were labeled numerically with tape for identification during disease evaluation. Each spike was inoculated with approximately 1 ml of spore suspension (or water for the check plants) using a manual spray bottle and covered with a 16.5 cm × 8.2 cm transparent Ziploc bag for 72 h to maintain high humidity, which favors infection. Severity (percentage of symptomatic spikelets) was evaluated visually on each spike 21 and 28 days after inoculation (DAI) for the first experiment, and 14 and 21 DAI for the second experiment.

Mycotoxin quantification and re-isolation of Fusarium *from symptomatic heads*

Wheat spikes were harvested by hand at maturity. The grain from each experimental unit was threshed and winnowed individually with a single-spike thresher

(Precision Machine, Lincoln, NE). A portion of the grain was ground to flour prior to mycotoxin quantification using a cyclone sample laboratory mill (UDY Corporation, Fort Collins, CO). Quantification of DON, 15-ADON, 3-ADON and NIV in the grain was conducted on an Agilent 6890/5975 GC/MS system. *Fusarium* was re-isolated from a portion of the grain, single-spored and identified as described in chapter 2 of this dissertation.

Data analysis

Data from each experiment were analyzed separately. The statistical analyses were conducted in R version 3.6.0 (R Core Team 2019). An α level of 0.05 and P values of ≤ 0.05 were considered statistically significant. Block effects were considered random effects. The aov function in the *stats* package was used to fit linear mixed models to analyze severity, DON, 15-ADON and 3-ADON data. Fisher's least significant difference (LSD, $P = 0.05$) was calculated using the LSD.test function in the *agricolae* package (Mendiburu 2019). All resulting figures were generated using the *ggplot2* package (Wickham 2016).

Cultural and morphological characteristics of *Fusarium* isolates

Colony morphology was determined from growth on PDA in 9-cm plastic petri plates. Cultures were incubated at room temperature for 7 days. Photos of the plates were taken with an IUL Flash & Go camera (Neutec Group Inc., Farmingdale, NY).

Macroconidia measurements were made on cultures grown at room temperature for 7 days on Bilay's media (Hallen-Adams et al. 2011). A spore suspension was prepared in sterile distilled water from culture plates. The length and width of 100 randomly chosen macroconidia with 5 septa were measured for each isolate.

Homothallic sexual development was evaluated as described by Hallen-Adams et al. (2011). Briefly, each isolate was inoculated into carrot agar (carrots, 350 g; agar, 20 g per liter of medium) in 6-cm petri plates and incubated at room temperature for 4-5 days until the mycelia covered the surface of the agar. Aerial mycelia were scrapped off the plates without disturbing the surface of the agar, and 800 µl of a 2.5% Tween 60 solution was spread on the surface using a glass rod. The carrot agar plates were incubated in a single layer at room temperature until mature perithecia were obtained. The length and width of 100 mature ascospores from crushed perithecia chosen randomly were measured for each isolate.

Results

Pathogenicity tests in the greenhouse

Greenhouse-grown plants of spring wheat cv. Wheaton inoculated at flowering with the *F. boothii* and *F. graminearum* isolates exhibited premature bleaching of spikelets, which is characteristic of FHB (Figures 1-3). Minimal levels of disease and mycotoxins were observed in the check plants due to unintentional contamination. Symptoms were observed in all three isolate treatments when the Ziploc bags were

removed, 3 DAI. In the first experiment, disease severity in the inoculated plants ranged from 17.5% to 52.3% by 21 DAI, and from 32.7% to 63.5% by 28 DAI. In the second experiment, disease severity ranged from 59.0% to 71.7% by 14 DAI, and from 74.4% to 86.6% by 21 DAI (Table 1). In general, the *F. graminearum* isolate caused more disease than the *F. boothii* isolates on most disease assessment dates, although the difference was not significant. Single-spored cultures of the three isolates were recovered from symptomatic kernels obtained from both experiments.

All isolates produced quantifiable amounts of DON in the grain under greenhouse conditions in both experiments. All isolates produced predominantly 15-ADON, consistent with their trichothecene genotype. None of the samples had detectable NIV. Mean values of DON concentration in the grain ranged from 2.72 to 14.80 μg/g in the first experiment, and from 13.30 to 38.14 μg/g in the second experiment. Mean values of 15-ADON were between 0.20 and 1.02 μg/g in the first experiment, and between 0.26 and 1.10 μg/g in the second experiment. The *F. graminearum* isolate produced consistently more DON and 15-ADON than each of the *F. boothii* isolates ($P < 0.05$) (Table 1).

Cultural and morphological characteristics of *Fusarium* isolates

On PDA, *F. boothii* and *F. graminearum* produced similar colony morphologies, with abundant mycelia varying from white to yellow in color. Production of red pigments was observed on the reverse of the plates (Figure 4). Macroconidia morphology was similar in both species. Macroconidia produced by *F. boothii* isolates measured 35 to

66.25 µm × 3.75 to 6.25 µm, whereas *F. graminearum* produced macroconidia that measured 37.5 to 67.5 µm × 3.75 to 6.25 µm (Figure 5).

All isolates produced mature perithecia homothallically on carrot agar after approximately 7 weeks of incubation. Ascospore morphology was similar in both species. Ascospores produced by *F. boothii* isolates measured 17.5 to 32.5 µm × 3.75 to 7.50 µm, whereas *F. graminearum* produced ascospores that measured 18.75 to 27.5 µm × 3.75 to 5.75 µm (Figure 6). Figure 7 shows examples of perithecia and ascospores produced by the *Fusarium* isolates.

Discussion

The results from this study confirm that *F. boothii* is a causal agent of FHB in Nebraska. The *F. boothii* isolates used here were initially collected from wheat heads showing FHB symptoms in Chase County, Nebraska. In two different experiments, inoculation of susceptible plants with single-spore isolates of *F. boothii* produced symptoms typical of FHB under greenhouse conditions (Figures 1-3). Re-isolation of *F. boothii* from inoculated plants completed Koch's postulates. This represents the first report of *F. boothii* causing FHB of wheat in the United States.

Isolates of *F. boothii* and *F. graminearum* from Nebraska were indistinguishable morphologically and culturally. Culture morphology on PDA, as well as macroconidia and ascospore features were similar to those reported for species in the *F. graminearum* clade (O'Donnell et al. 2004; Leslie et al. 2006). All *F. boothii* isolates found to date in

Nebraska had the 15-ADON genotype and chemotype, which agrees with previous reports for this species (Cerón-Bustamante et al. 2018; Aoki et al. 2012).

Fusarium boothii has been isolated from maize in Texas (Aoki et al. 2012), and South Dakota (Okello et al. 2019), suggesting a wider distribution of *F. boothii* in the U.S. Phylogenetic studies indicate that *F. boothii* evolved in Mesoamerica along with *F. mesoamericanum* (Aoki et al. 2012). In southern Mexico, *F. boothii* is the main pathogen causing FHB of wheat, which agrees with the hypothesis of its origins (Cerón-Bustamante et al. 2018). However, *F. boothii* has been reported infecting maize and wheat in multiple locations in Africa, Asia, Europe and South America (Boutigny et al. 2011; Desjardins and Proctor 2011; Duan et al. 2016; Láday et al. 2004; Malihipour et al. 2012; Sampietro et al. 2011; Tóth et al. 2005; Zhang et al. 2016). Additional studies have reported *F. boothii* causing disease in other unrelated hosts, such as soybean (*Glycine max*) (Chiotta et al. 2015), tomato (*Solanum lycopersicum*) (Gomes et al. 2015), pecan (*Carya illinoinensis*) and camel thorn (*Vachellia erioloba*) (Gryzenhout et al. 2016).

Most studies have isolated *F. boothii* from maize, and host-adaptation of *F. boothii* to maize has been suggested. A survey conducted in South Africa revealed that while *F. boothii* was the almost exclusive pathogen causing GER of maize, *F. graminearum* was the main pathogen causing FHB of wheat in the same geographic locations (Boutigny et al. 2011). Comparative aggressiveness studies using South African isolates of *F. acaciae-mearnsii*, *F. boothii*, *F. cortaderiae*, *F. graminearum* and *F. meridionale* collected by Boutigny et al. (2011) showed that *F. boothii* was the most aggressive species on maize under greenhouse conditions (Beukes et al. 2018). The authors also found that wheat grains from plants inoculated with *F. boothii* contained

significantly less DON than when *F. graminearum* was inoculated. In China, Zhang et al. (2016) observed that *F. boothii* isolates were less aggressive on wheat than *F. graminearum*. The authors also concluded that wheat-maize crop rotations tend to favor *F. graminearum*, whereas continuous maize favors *F. boothii* (Zhang et al. 2016).

The results described here showed that, under greenhouse conditions, the *F. boothii* isolates from Nebraska accumulated less DON and seemed to be less aggressive on wheat than the *F. graminearum* isolate included, in agreement with the studies from South Africa and China (Beukes et al. 2018; Zhang et al. 2016). Although maize was not sampled in the survey described in chapter 2 of this dissertation, it is likely that *F. boothii* occurs in Nebraska maize, in addition to wheat. Maize is one of the main crops in Nebraska, and maize and wheat are often included in crop rotation systems in the state; therefore, a host adaptation of *F. boothii* to maize could pose a threat to maize production in Nebraska. Additional sampling of wheat and maize from Nebraska, as well as comparative aggressiveness and population structure studies including both wheat and maize isolates will be required to evaluate whether the *F. boothii* population present in the state has a host-preference for maize.

Conclusions

The results presented here confirm *F. boothii* as a causal agent of FHB of wheat in the U.S. Reports of *F. boothii* from maize in South Dakota and Texas suggest that it is likely that *F. boothii* has a wider distribution in North America. Additional research will be needed to determine the distribution, aggressiveness, and impact on yield of *F. boothii*

in the U.S. compared to *F. graminearum*. Increased vigilance and constant monitoring of pathogen populations are required in order to detect possible changes in the populations infecting crops and the toxin risks associated.

The next chapter of this dissertation describes a greenhouse experiment designed to evaluate the aggressiveness and mycotoxin production of a larger number of *F. boothii* and *F. graminearum* isolates from Nebraska.

References

Aoki, T., Ward, T. J., Kistler, H. C., and O'Donnell, K. 2012. Systematics, Phylogeny and Trichothecene Mycotoxin Potential of Fusarium Head Blight Cereal Pathogens. JSM Mycotoxins. 62:91–102.

Beukes, I., Rose, L., van Coller, G., and Viljoen, A. 2018. Disease development and mycotoxin production by the *Fusarium graminearum* species complex associated with South African maize and wheat. Eur. J. Plant Pathol. 150:893–910.

Boutigny, A.-L., Ward, T. J., Coller, G. J. Van, 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.

Busch, R., McVey, D., Rauch, T., Baumer, J., and Elsayed, F. 1984. Registration of Wheaton Wheat. Crop Sci. 24:622.

Cerón-Bustamante, M., Ward, T. J., Kelly, A., Vaughan, M. M., McCormick, S. P.,

Cowger, C., et al. 2018. Regional differences in the composition of Fusarium Head Blight pathogens and mycotoxins associated with wheat in Mexico. Int. J. Food Microbiol. 273:11–19.

Chiotta, M. L., Zanon, M. S. A., Giaj-Merlera, G., Tessmann, D., Barros, G. G., and Chulze, S. 2015. Phylogenetic analyses of the *Fusarium graminearum* species complex isolated from soybean in Argentina and Brazil. Australas. Plant Dis. Notes. 10:32.

Desjardins, A. E., and Proctor, R. H. 2011. Genetic diversity and trichothecene chemotypes of the *Fusarium graminearum* clade isolated from maize in Nepal and identification of a putative new lineage. Fungal Biol. 115:38–48.

Duan, C., Qin, Z., Yang, Z., Li, W., Sun, S., Zhu, Z., et al. 2016. Identification of Pathogenic *Fusarium* spp. Causing Maize Ear Rot and Potential Mycotoxin Production in China. Toxins. 8:186.

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.

Gomes, A. A. M., Machado, A. R., Ferreira, A. P. S., Dutra, D. C., and Pereira, O. L. 2015. Postharvest Fruit Rot of Tomato in Brazil Caused by *Fusarium boothii*. Plant Dis. 99:555.

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

Gryzenhout, M., Khooa, B., and Landman, L. 2016. First report of *Fusarium boothii* from pecan (*Carya illinoinensis*) and camel thorn (*Vachellia erioloba*) trees in South Africa. South African J. Bot. 105:158–162.

Hallen-Adams, H. E., Cavinder, B. L., and Trail, F. 2011. *Fusarium graminearum* from Expression Analysis to Functional Assays. In Fungal Genomics: Methods and Protocols, eds. Jin-Rong Xu and Burton H Bluhm. New York: Humana Press, p. 79–101.

Láday, M., Juhász, Á., Mulè, G., Moretti, A., Szécsi, Á., and Logrieco, A. 2004. Mitochondrial DNA Diversity and Lineage Determination of European Isolates of *Fusarium graminearum* (*Gibberella zeae*). Eur. J. Plant Pathol. 110:545–550.

Leslie, J. F., Summerell, B. A., and Bullock, S. 2006. *The Fusarium Laboratory Manual*. Blackwell Publishing, Ames, IA.

Lilleboe, D. 2015. FHB in 2015: a vexing year, overall. Retrieved 13 January 2019 from https://scabusa.org/pdfs/USWBSI-Article_2015FHB-Summary_10-26-15.pdf.

Malihipour, A., Gilbert, J., Piercey-Normore, M., and Cloutier, S. 2012. Molecular Phylogenetic Analysis, Trichothecene Chemotype Patterns, and Variation in Aggressiveness of *Fusarium* Isolates Causing Head Blight in Wheat. Plant Dis. 96:1016–1025.

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.

Mendiburu, F. 2019. Agricolae: Statistical Procedures for Agricultural Research.

Available at: https://cran.r-project.org/web/packages/agricolae/agricolae.pdf.

O'Donnell, K., Ward, T. J., Geiser, D. M., Kistler, H. C., and Aoki, T. 2004. Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the *Fusarium graminearum* clade. Fungal Genet. Biol. 41:600–623.

Okello, P. N., Petrović, K., Kontz, B., and Mathew, F. M. 2019. Eight Species of *Fusarium* Cause Root Rot of Corn (*Zea mays*) in South Dakota. Plant Heal. Prog. 20:38–43.

R Core Team. 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

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.

Sarver, B. A. J., Ward, T. J., Gale, L. R., Broz, K., Kistler, H. C., Aoki, T., et al. 2011. Novel Fusarium head blight pathogens from Nepal and Louisiana revealed by multilocus genealogical concordance. Fungal Genet. Biol. 48:1096–1107.

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.

Tóth, B., Mesterházy, Á., Horváth, Z., Bartók, T., Varga, M., and Varga, J. 2005. Genetic

Variability of Central European Isolates of the *Fusarium graminearum* Species Complex. Eur. J. Plant Pathol. 113:35–45.

Trail, F. 2009. For Blighted Waves of Grain: *Fusarium graminearum* in the Postgenomics Era. Plant Physiol. 149:103–110.

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.

Wickham, H. 2016. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. Available at: https://ggplot2.tidyverse.org.

Zhang, H., Brankovics, B., Xu, J., Xu, J. S., Guo, C., Guo, J. G., et al. 2016. Crops are a main driver for species diversity and the toxigenic potential of *Fusarium* isolates in maize ears in China. World Mycotoxin J. 9:701–715.

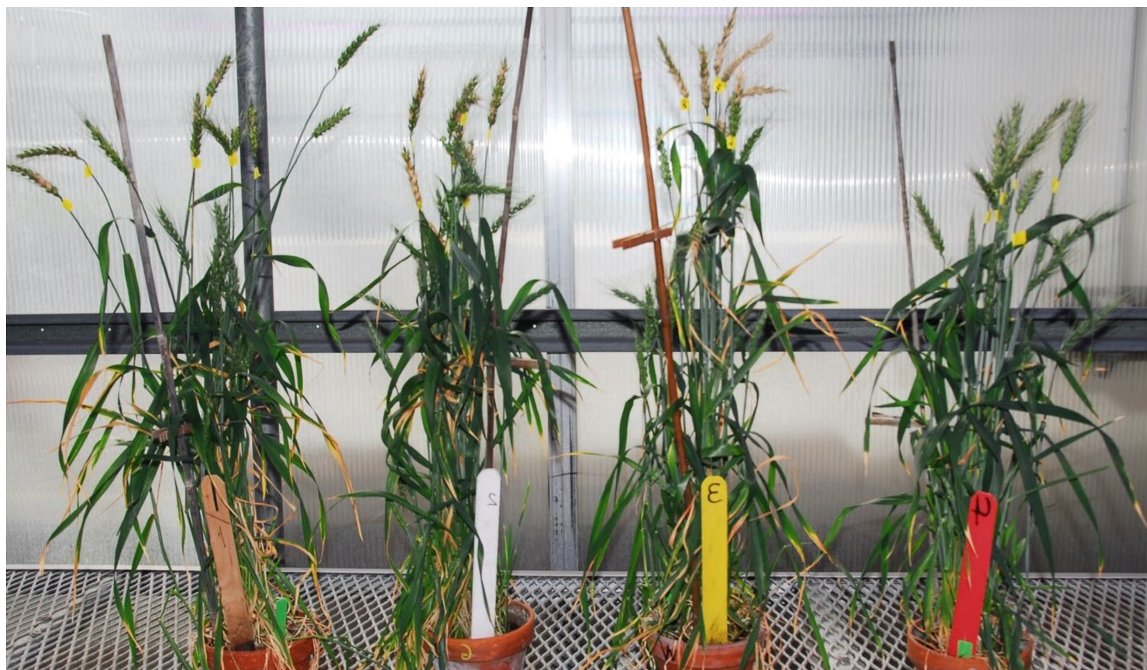


Figure 1. Wheaton plants inoculated with *Fusarium* isolates showing premature bleaching characteristic of FHB. From left to right: *F. boothii* isolate NE16-15Fb, *F. boothii* isolate NE19-15Fb, *F. graminearum* isolate NE20-15Fg, uninoculated control. Photo credits: Dr. Carlos Bolanos Carriel.

A

B

Figure 2. (A) Premature bleaching of spikelets characteristic of FHB on Wheaton wheat 21 days after inoculation with *F. boothii* isolate NE16-15Fb. (B) Red arrow shows caryopsis abortion in a floret. Photo credits: Dr. Carlos Bolanos Carriel.

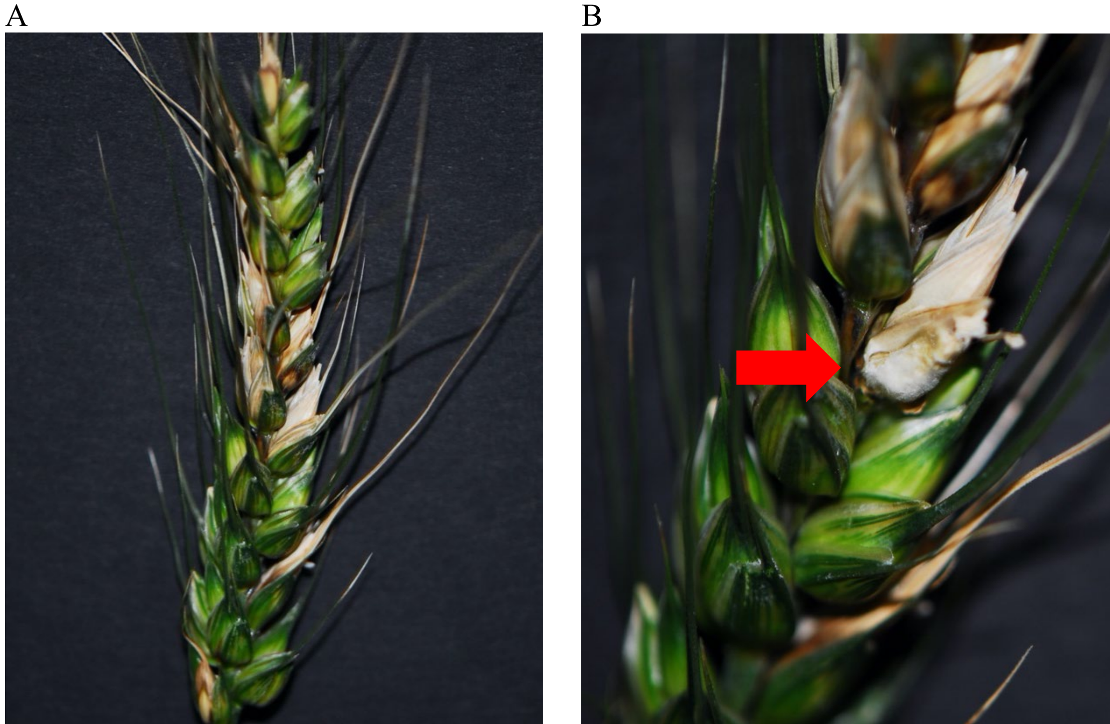


Figure 3. (A) Premature bleaching of spikelets characteristic of FHB on Wheaton wheat 21 days after inoculation with *F. boothii* isolate NE19-15Fb. (B) Red arrow shows *Fusarium* damaged kernel in a floret. Photo credits: Dr. Carlos Bolanos Carriel.

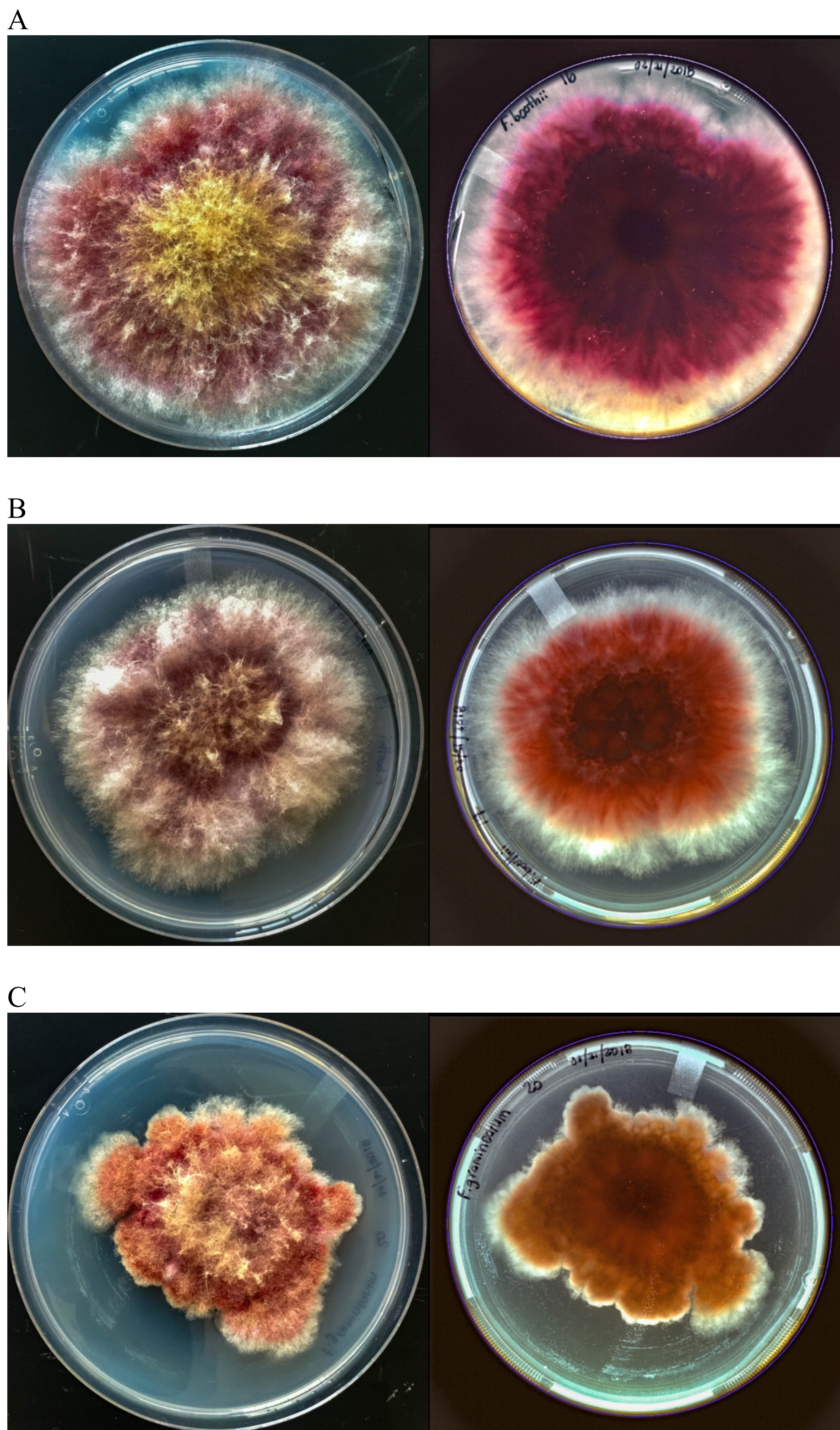


Figure 4. Front (left) and reverse (right) colony morphology of *F. boothii* isolates NE16-15Fb (A), NE19-15Fb (B), and *F. graminearum* isolate NE20-15Fg (C) on PDA incubated at room temperature for 7 days.

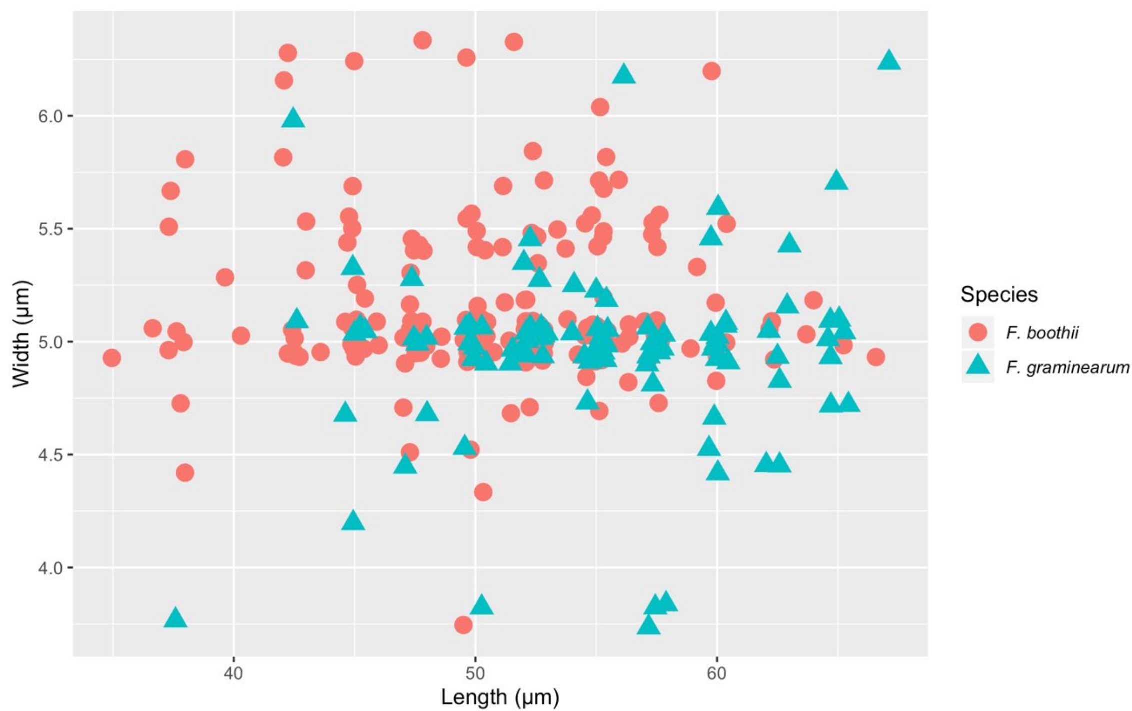


Figure 5. Length and width in µm of 5-septate conidia of *F. boothii* and *F. graminearum* isolates from Nebraska grown on Bilay's media.

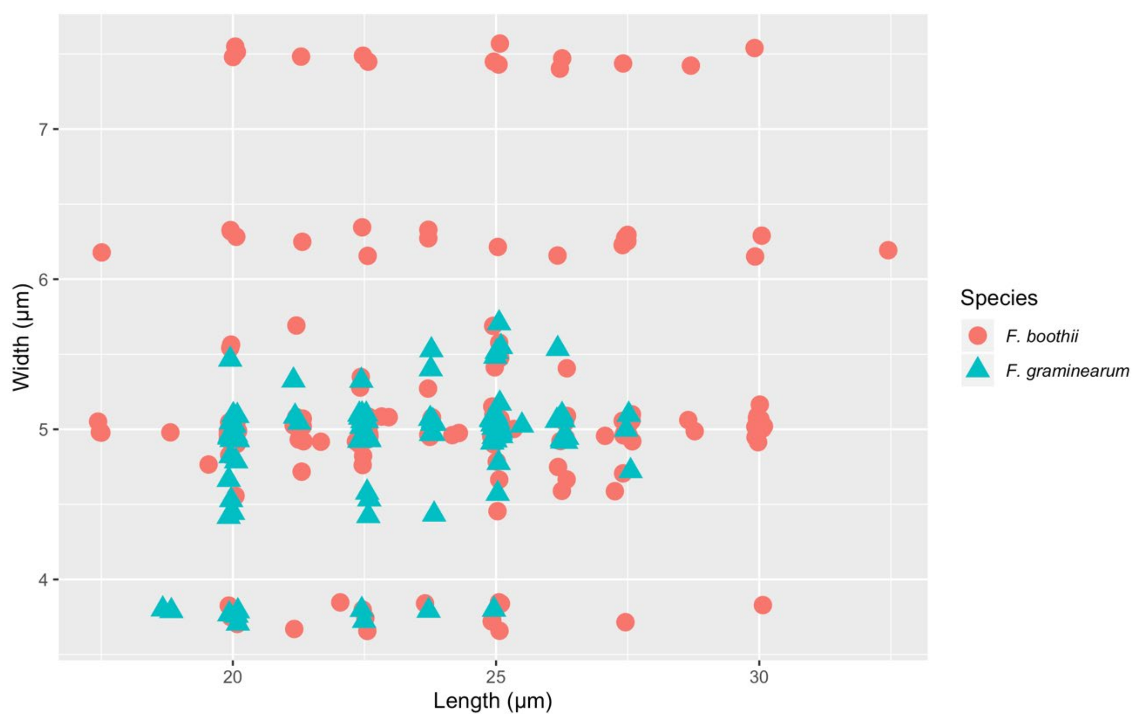


Figure 6. Length and width in µm of mature ascospores of *F. boothii* and *F. graminearum* isolates from Nebraska grown on carrot agar.

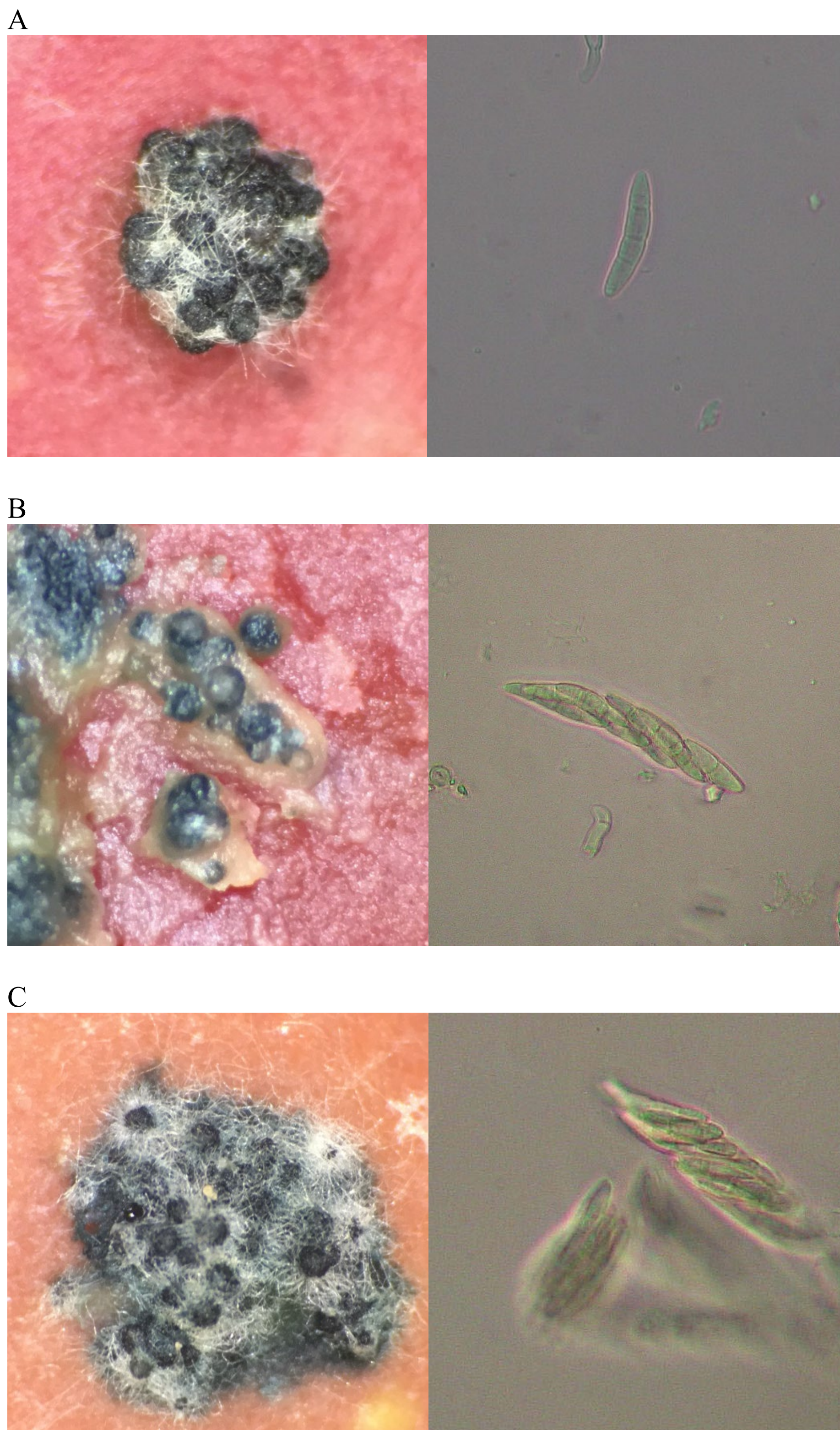


Figure 7. Examples of perithecia and ascospores produced by *F. boothii* isolates NE16-15Fb (A), NE19-15Fb (B), and *F. graminearum* isolate NE20-15Fg (C) on carrot agar. Photographs are not to scale.

Table 1. Overall means and Fisher's least significant difference (LSD) for FHB severity (percentage of symptomatic spikelets), deoxynivalenol (DON; µg/g), 15-acetyldeoxynivalenol (15-ADON; µg/g) and 3-acetyldeoxynivalenol (3-ADON; µg/g) in spring wheat cultivar Wheaton grown under greenhouse conditions inoculated with *F. boothii* and *F. graminearum* isolates from Nebraska. Mycotoxin limit of quantification (LOQ) was 0.05 µg/g. DAI: days after infection.

Treatment	Experiment 1					Experiment 2				
	Severity 21 DAI (%)	Severity 28 DAI (%)	DON (µg/g)	15-ADON (µg/g)	3-ADON (µg/g)	Severity 14 DAI (%)	Severity 21 DAI (%)	DON (µg/g)	15-ADON (µg/g)	3-ADON (µg/g)
F. boothii **NE16-15Fb**	17.5	32.7	2.72	0.20	<LOQ	59.0	74.4	13.30	0.26	0.05
F. boothii **NE19-15Fb**	49.2	63.7	6.80	0.42	<LOQ	66.4	78.2	13.65	0.40	0.11
F. graminearum **NE20-15Fg**	52.3	63.5	14.80	1.02	0.12	71.7	86.6	38.14	1.10	0.36
Check	6.8	9.5	0.34	<LOQ	<LOQ	0.2	0.5	0.94	<LOQ	<LOQ
LSD (0.05)	9.8	16.2	4.21	0.34	0.06	13.1	13.9	10.34	0.24	0.11

CHAPTER 4

Aggressiveness and deoxynivalenol production of Nebraska isolates of *Fusarium boothii* and *F. graminearum*

This article has been submitted for publication in Plant Health Progress. *(*Valverde-Bogantes E, Bolanos-Carriel C, Hallen-Adams HE, McMaster N, Schmale III DG, and Wegulo SN, "Aggressiveness and deoxynivalenol production of Nebraska isolates of* Fusarium boothii *and* F. graminearum*" (submitted for publication January, 2020).)*

Abstract

Significant losses in wheat result from Fusarium head blight (FHB) and its associated mycotoxin deoxynivalenol (DON). The predominant FHB pathogen in North America is *Fusarium graminearum*. *F. boothii* was recently confirmed for the first time in the United States as a causal agent of FHB in Nebraska wheat fields. This greenhouse study compared the aggressiveness and DON production in wheat among 13 *F. graminearum* and three *F. boothii* isolates from Nebraska. Spikes of the susceptible spring wheat cultivar Wheaton at anthesis were spray-inoculated with spores of the isolates. Severity data were used to calculate the area under the disease progress curve (AUDPC). DON concentration in the grain was quantified by gas chromatography/mass spectrometry. *F. graminearum* isolates were more aggressive and produced more DON than *F. boothii* isolates. Mean AUDPC values were 1,171 and 885 percent days for *F. graminearum* and

F. boothii, respectively. Mean DON values were 41.0 and 13.6 µg/g for *F. graminearum* and *F. boothii*, respectively. Although only three available *F. boothii* isolates were used, the results are in agreement with previous studies that found *F. graminearum* be more aggressive and toxigenic in wheat than *F. boothii*.

Keywords: Fusarium head blight, trichothecene mycotoxins, wheat, area under the disease progress curve, *Fusarium graminearum* species complex

Fusarium head blight (FHB) causes major losses in wheat and other small grain cereals worldwide, posing a threat to both food security and food safety due to yield reduction and contamination of grain with mycotoxins (McMullen et al. 2012). The disease is caused mainly by *Fusarium graminearum*, but several other species of *Fusarium* are causal agents. The majority of infections occur on wheat spikes in wet and humid weather during the anthesis growth stage and are incited mainly by ascospores released from mature perithecia on cereal crop residue. Symptoms are manifested as premature whitening of spikes. Infected spikelets are sterile or produce shriveled, chalky white or pink kernels known as *Fusarium*-damaged kernels, scabby kernels, or "tombstones" (McMullen et al. 1997). These kernels not only have reduced weight and quality but are also contaminated with mycotoxins (McMullen et al. 1997, 2012; Trail 2009). The main group of toxins produced by these fungi is type B trichothecenes, which includes deoxynivalenol (DON), 15-acetyldeoxynivalenol (15-ADON), 3-acetyldeoxynivalenol (3-ADON) and nivalenol (NIV) (O'Donnell et al. 2000). Type B trichothecenes are protein synthesis inhibitors that are harmful to humans and animals. Depending on the trichothecenes produced, FHB pathogens are classified into three different chemotypes: 3-ADON (producing DON and 3-ADON), 15-ADON (producing DON and 15-ADON) and NIV (producing nivalenol and its acetylated derivatives). Molecular tests have been developed to determine trichothecene genotypes and thus predict trichothecene chemotypes (Starkey et al. 2007; Ward et al. 2002). In addition to trichothecenes, most of these pathogens can also produce the estrogenic mycotoxin zearalenone.

The predominant causal agents of FHB are grouped within the *Fusarium graminearum* species complex (FGSC), which is part of the broader *Fusarium sambucinum* species complex lineage 1. The FGSC includes the following species: *F. acaciae-mearnsii*, *F. aethiopicum*, *F. asiaticum*, *F. austroamericanum*, *F. boothii*, *F. brasilicum*, *F. cortaderiae*, *F. gerlachii*, *F. graminearum*, *F. louisianense*, *F. meridionale*, *F. mesoamericanum*, *F. nepalense*, *F. ussurianum*, and *F. vorosii*. These species can also infect maize, causing diseases such as Gibberella ear and stalk rots. Significant biogeographic structure within the FGSC suggests that these species originated independently from populations that evolved allopatrically (Aoki et al. 2012). The FHB pathogen that predominates in the USA is *F. graminearum* with the 15-ADON genotype (Ward et al. 2008), although other species and genotypes have also been reported (Gale et al. 2011; Sarver et al. 2011; Starkey et al. 2007).

In Nebraska, FHB epidemics are sporadic due to variability in year-to-year environmental conditions. Recent major epidemics occurred in the state in 2007, 2008, 2015 and 2019 (Lilleboe 2015, 2019; Mengistu et al. 2007; Wegulo et al. 2008). Previous studies from Nebraska have reported variability in the aggressiveness and DON production of *F. graminearum* isolates (Hernandez Nopsa et al. 2014; Panthi et al. 2014). Knowledge of the levels of aggressiveness and toxigenicity in FHB pathogen populations infecting wheat in a particular region is important not only in assessing the risk to the crop but also in the selective deployment of management strategies and tactics such as cultivar resistance, fungicide application, and crop rotation. Recently, three isolates of *F. boothii* with the 15-ADON genotype were identified for the first time in the USA causing FHB of wheat in Nebraska wheat fields (Wegulo et al. 2018). The objectives of this study

were to (i) compare the aggressiveness and mycotoxin production on wheat among Nebraska isolates of *F. graminearum* and the three recently identified *F. boothii* isolates and (ii) determine the relationship between DON concentration and FHB intensity using regression analysis.

Greenhouse experiments

Pathogen isolates.

The 16 single-spore isolates used in this study were obtained from symptomatic wheat heads collected from Nebraska wheat fields in 2015 and 2016 (Fig. 1). These isolates were previously identified in our lab as *F. boothii* (n=3) and *F. graminearum* (n=13) using partial sequences from the translation elongation factor 1-α (*TEF1*), reductase (*RED*) and trichothecene 3-*O*-acetyltransferase (*TRI101*) genes. Trichothecene genotypes were determined using a multiplex polymerase chain reaction with genotype-specific primers based on trichothecene 15-*O*-acetyltransferase (*TRI3*) and trichothecene efflux pump (*TRI12*) genes (Starkey et al. 2007). All isolates had the 15-ADON genotype, which is the only trichothecene genotype that has been reported in Nebraska.

Inoculum.

Spore suspensions were prepared according to the following protocol adapted from Hallen-Adams et al. (2011) with slight modifications. Each isolate was inoculated into 350 ml of carboxymethyl cellulose medium (CMC; 15 g carboxymethyl cellulose sodium salt, 1 g NH_4NO_3, 1 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$ and 1 g of yeast extract per liter of medium) in a 500 ml conical flask. The flasks were incubated for 5 days at room

temperature on a shaker set to 200 rpm. The cultures grown on CMC were filtered through sterile Miracloth (Millipore, Bedford, MA) into sterile centrifuge bottles. The bottles were centrifuged at 4000 × *g* for 5 minutes and the spores were washed twice with sterile distilled water. The spores were resuspended in sterile distilled water, and each spore suspension was standardized to a final concentration of 5×10^5 spores/ml with a hemocytometer, stored at 4°C, and used on the day of preparation.

Plant material and inoculation.

The FHB-susceptible spring wheat cultivar Wheaton (Busch et al. 1984) was seeded in 15-cm-diameter clay pots (12 seeds/pot) and grown in the greenhouse. The potting mix consisted of 33% clay loam soil, 33% peat soil, 16.5% sand, and 16.5% vermiculite. Plants were watered daily and fertilized together with irrigation using Peter's Peat Lite Special Fertilizer, 20-10-20 (Everris, Marysville, Ohio) at 250 µg/g. Spikes used for disease assessment were inoculated at full anthesis on 21 October 2018. In total, 10 to 23 flowering spikes in each pot, depending on the number of tillers obtained from the seeds planted, were labeled numerically with tape for identification during disease evaluation. Each spike was inoculated with approximately 1 ml of spore suspension using a manual spray bottle and covered with a 16.5 cm × 8.2 cm transparent Ziploc bag for 72 h to maintain high humidity, which favors infection. The remaining wheat spikes were inoculated six days after the first inoculation regardless of growth stage in order to maximize the amount of infected grain for mycotoxin quantification, but they were not used for disease evaluation.

Experimental design.

Treatments (*F. boothii* and *F. graminearum* isolates) were arranged in a randomized complete block design with four replications. Each pot with wheat spikes inoculated with one isolate was considered an experimental unit. Two replicate experiments were conducted simultaneously in separate greenhouse rooms with the same environmental conditions. Lighting was provided by Lumigrow Pro 325 LED Grow Lights (Lumigrow, Emeryville, CA) for 14 h a day. Mean temperature in each room was 23.3ºC and ranged from 19.4ºC to 30.5ºC. Mean relative humidity was 55% and ranged from 14% to 84%.

Disease assessment, harvesting, and mycotoxin analysis.

Disease severity (percentage of symptomatic spikelets) was evaluated visually on each spike 3, 6, 9, 12, 15, 18 and 21 days after inoculation (DAI). Severity data were used to calculate the area under the disease progress curve (AUDPC) according to the equation by Madden et al. (2007). Wheat spikes were harvested by hand 58 days after the first inoculation. The grain from each experimental unit was threshed and winnowed individually with a single-spike thresher (Precision Machine, Lincoln, NE). Fan speed was adjusted to prevent removal of *Fusarium*-damaged kernels. The grain was ground to flour at 1,600 rpm for 4 min with 2-min intervals in 15-ml vials with two 11-mm steel balls (OPS Diagnostics, Lebanon, NJ) using a Geno/Grinder 2025 (SPEX SamplePrep, Metuchen, NJ). Quantification of DON, 15-ADON, 3-ADON and NIV in the grain was conducted on an Agilent 6890/5975 gas chromatography/mass spectrometry system.

Data analysis.

Data were analyzed in R version 3.6.0 (R Core Team 2019). An α level of 0.05 and *P* values of ≤ 0.05 were considered as statistically significant. Block effects were considered random effects. Homogeneity of error variances between experiments was tested using Bartlett's test and the Fligner-Killeen test provided in the *stats* package. The aov function in the *stats* package was used to fit linear mixed models to analyze severity, AUDPC, DON, and 15-ADON data. Fisher's least significant difference (LSD, $P = 0.05$) was calculated using the LSD.test function in the *agricolae* package (Mendiburu 2019). To compare aggressiveness and mycotoxin concentration between species, each isolate was considered a replicate within each species. Linear regressions were calculated using the lm function in the *stats* package to evaluate the relationship between (i) AUDPC and DON, (ii) AUDPC and 15-ADON, and (iii) DON and 15-ADON. All resulting figures were generated using the *ggplot2* package (Wickham 2016). Error variances between experiments were homogeneous ($P > 0.05$); therefore, data from both experiments were combined for analysis.

Isolate aggressiveness.

All isolates caused typical FHB symptoms on cultivar Wheaton under greenhouse conditions. Disease severity increased over time, ranging from 2.2% to 16.7% at 3 DAI, and from 58.7% to 93.8% by 21 DAI (Table 1). Mean AUDPC varied significantly among the 16 isolates ($P < 0.001$), with values ranging from 715 to 1,466 percent days (Table 1, Supplementary Figure S1). The three most aggressive isolates, in descending order, were *F. graminearum* isolates NE2c-16Fg, NE1c-16Fg and NE20-15Fg, whereas the three least aggressive isolates were *F. graminearum* isolates NE10b-16Fg and

NE8b1-16Fg and *F. boothii* isolate NE19-15Fb. Notably, the least aggressive isolate (NE19-15Fb, *F. boothii*) had significantly lower AUDPC values ($P < 0.05$) than all other isolates and consistently had the lowest FHB severity on all seven disease assessment dates. The more aggressive species was *F. graminearum*, with a mean AUDPC value of 1,171 percent days. A significantly lower mean AUDPC value of 885 percent days was obtained from spikes inoculated with *F. boothii* ($P < 0.05$; Table 2).

Mycotoxin production.

All isolates produced detectable DON and 15-ADON in the grain under greenhouse conditions, consistent with their trichothecene genotype. None of the samples had detectable 3-ADON or NIV. Mean values of DON in the grain ranged from 9.5 to 68.2 μg/g (Table 1, Supplementary Figure S2), and mean values of 15-ADON were between 0.20 and 1.82 μg/g (Table 1). There were significant differences in DON among the 16 isolates ($P < 0.001$) (Table 1, Supplementary Figure S2). The three isolates that produced the highest concentrations of DON were, in descending order, *F. graminearum* isolates NE2c-16Fg, NE20-15Fg and NE22-15Fg. In contrast, the three isolates that produced the lowest concentrations of DON were the three *F. boothii* isolates (NE21-15Fb, NE16-15Fb and NE19-15Fb). *F. graminearum* isolates produced approximately three times more DON and 15-ADON than *F. boothii* isolates ($P < 0.05$) (Table 2).

Relationships between AUDPC and mycotoxin concentrations.

A strong positive relationship ($R^2 = 0.51$, $P < 0.0001$) was observed between AUDPC and DON concentration in the grain (Fig. 2). Correspondingly, the most aggressive isolate (*F. graminearum* NE2c-16Fg) was the most toxigenic, and the least

aggressive isolate (*F. boothii* NE19-15Fb) was the least toxigenic. Similar strong positive relationships were obtained between AUDPC and DON concentration when data from each species were analyzed separately (*F. graminearum*: $R^2 = 0.41$, $P < 0.0001$; *F. boothii*: $R^2 = 0.40$, $P = 0.0009$) (Fig. 2). Within each species, the most aggressive *F. graminearum* isolate (NE2c-16Fg) was the most toxigenic, whereas the least aggressive *F. graminearum* isolate (NE8b1-16Fg) produced the least DON. The same pattern was observed for *F. boothii*, with the most aggressive isolate (NE21-15Fb) being the most toxigenic and the least aggressive isolate (NE19-15Fb) producing the least DON. There were strong positive relationships between AUDPC and 15-ADON when data from both species were analyzed together ($R^2 = 0.32$, $P < 0.0001$) and separately (*F. graminearum*: $R^2 = 0.19$, $P < 0.0001$; *F. boothii*: $R^2 = 0.41$, $P = 0.0007$). A very strong positive relationship ($R^2 = 0.72$, $P < 0.0001$) was observed between DON and 15-ADON.

Conclusions and Implications for Disease Management

In this study, the aggressiveness and mycotoxin production of 13 *F. graminearum* and three *F. boothii* isolates from Nebraska were evaluated on the spikes of the FHB-susceptible spring wheat cultivar Wheaton under greenhouse conditions. The results indicated that *F. graminearum* isolates were more aggressive and produced more DON than *F. boothii* isolates. In China, Zhang et al. (2016) similarly found *F. graminearum* isolates to be more aggressive on wheat spikes than *F. boothii* isolates. In South Africa, Beukes et al. (2018) did not find significant differences in FHB incidence on wheat spikes among five *Fusarium* species including *F. graminearum* and *F. boothii*, but showed that *F. graminearum* isolates colonized wheat grain better and produced more

DON than isolates of the other four species. Cerón-Bustamante et al. (2018) reported that an isolate of *F. boothii* from Mexico was more aggressive than the North American *F. graminearum* reference isolate Gz3639. In contrast, Malihipour et al. (2012) reported that *F. graminearum* isolates from Canada and Iran were twice as aggressive on wheat as *F. boothii* isolates from Mexico.

Knowledge of the aggressiveness and toxigenicity of *F. graminearum* and *F. boothii* where they co-occur can be useful in the selective deployment of FHB management strategies and tactics such as the integration of cultivar resistance, fungicide application, and crop rotation. Previous research has shown a host preference or adaptation for both species, with greater adaptation of *F. boothii* to maize and *F. graminearum* to wheat (Beukes et al. 2018; Boutigny et al. 2011; Zhang et al. 2016). In addition, research by Zhang et al. (2016) indicated that wheat-maize rotation selects for *F. graminearum* whereas continuous maize culture selects for *F. boothii*. In states, countries, or regions where both crops are grown and routinely rotated as in Nebraska, FHB management tactics targeting the more aggressive and toxigenic *F. graminearum* can be deployed to more effectively control the disease. Host adaptations have also been demonstrated for other members of the FGSC. For example, *F. asiaticum* appears to be adapted to rice (Gomes et al. 2015; Lee et al. 2009; Zhang et al. 2016), whereas *F. meridionale* prefers maize (Sampietro et al. 2011).

There were significant differences in AUDPC and DON among the *F. graminearum* isolates in this study, with very aggressive and toxigenic isolates as well as mildly aggressive isolates that produced low concentrations of DON. The variation observed among these isolates is in agreement with previous studies that assessed the

aggressiveness and mycotoxin production of isolates of *F. graminearum* from Nebraska (Hernandez Nopsa et al. 2014; Panthi et al. 2014) and other parts of the world (Beukes et al. 2018; Malihipour et al. 2012; Zhang et al. 2016). The variability observed among the isolates suggests the existence of different populations of *F. graminearum* in Nebraska, which has been documented in other parts of North America (Kelly and Ward 2018).

The strong positive relationships between AUDPC and DON are similar to those reported in previous studies (Beukes et al. 2018; Hernandez Nopsa et al. 2014; Panthi et al. 2014). These results are consistent with the findings in this and the previous studies that the more aggressive isolates produced higher concentrations of DON, whereas the less aggressive isolates produced lower concentrations of the mycotoxin. The strong positive relationships between AUDPC and 15-ADON and between DON and 15-ADON were expected since 15-ADON is a derivative of DON.

In this study, only three *F. boothii* isolates were available, and their aggressiveness and toxigenicity were compared with 13 isolates of *F. graminearum*. Although the results are in agreement with previous studies (Beukes et al. 2018; Zhang et al. 2016), it is desirable to test more Nebraska isolates of *F. boothii* to further confirm the findings from the current study. The distribution and population structure of *F. boothii* in Nebraska and the pathogen's aggressiveness and toxigenicity on maize are currently unknown. Due to the large acreage of maize and the routine wheat-maize rotation in the state (USDA 2019), research is needed to fill these knowledge gaps as well as to elucidate the impact of these crops on the distribution of members of the FGSC in the state.

Acknowledgements

This research was funded in part by the Nebraska Wheat Board and the Nebraska Agricultural Experiment Station Hatch Multistate Research capacity funding program (Accession Number 1006715) from the USDA National Institute of Food and Agriculture.

Literature cited

Aoki, T., Ward, T. J., Kistler, H. C., O'Donnell, K. 2012. Systematics, Phylogeny and Trichothecene Mycotoxin Potential of Fusarium Head Blight Cereal Pathogens. JSM Mycotoxins 62:91-102.

Beukes, I., Rose, L., van Coller, G., Viljoen, A. 2018. Disease development and mycotoxin production by the *Fusarium graminearum* species complex associated with South African maize and wheat. Eur. J. Plant Pathol. 150:893-910.

Boutigny, A., Ward, T. J., Van Coller, G. J., Flett, B., Lamprecht, S. C., O'Donnell, K., Viljoen, A. 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.

Busch, R., McVey, D., Rauch, T., Baumer, J., Elsayed, F. 1984. Registration of Wheaton Wheat. Crop Sci. 24:622-622.

Cerón-Bustamante, M., Ward, T. J., Kelly, A., Vaughan, M. M., McCormick, S. P., Cowger, C., Leyva-Mir, S. G., Villaseñor-Mir, H. E., Ayala-Escobar, V., Nava-Díaz,

C. 2018. Regional differences in the composition of Fusarium Head Blight pathogens and mycotoxins associated with wheat in Mexico. Int. J. Food Microbiol. 273:11-19.

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

Gomes, L. B., Ward, T. J., Badiale-Furlong, E., Del Ponte, E. M. 2015. Species composition, toxigenic potential and pathogenicity of *Fusarium graminearum* species complex isolates from southern Brazilian rice. Plant Pathol. 64:980-987.

Hallen-Adams, H. E., Cavinder, B. L., Trail, F. 2011. *Fusarium graminearum* from Expression Analysis to Functional Assays. Pages 79-101 in: Fungal Genomics: Methods and Protocols. J. Xu and B. H. Bluhm, eds. Humana Press, New York.

Hernandez Nopsa, J. F., Wegulo, S. N., Panthi, A., Hallen-Adams, H. E., Harris, S. D., Baenziger, P. S. 2014. Characterization of Nebraska Isolates of Causing Head Blight of Wheat. Crop Sci. 54:310-317.

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

Lee, J., Chang, I., Kim, H., Yun, S., Leslie, J. F., Lee, Y. 2009. Genetic Diversity and Fitness of *Fusarium graminearum* Populations from Rice in Korea. Appl. Environ. Microbiol. 75:3289-3295.

Lilleboe, D. 2015. FHB in 2015: a vexing year, overall. Retrieved 26 December 2019 from https://scabusa.org/pdfs/USWBSI-Article_2015FHB-Summary_10-26-15.pdf.

Lilleboe, D. 2019. Fusarium head blight in 2019: serious impact in several states; minimal to moderate in others. Retrieved 26 December 2019 from https://scabusa.org/pdfs/USWBSI-Article_2019_FHB-Summary_11-18-19.pdf.

Madden, L. V., Hughes, G., van den Bosh, F. 2007. Temporal Analysis I: Quantifying and Comparing Epidemics. Pages 63-116 in: The Study of Plant Disease Epidemics. G. Hughes and F. van den Bosh, eds. American Phytopathological Society, St. Paul.

Malihipour, A., Gilbert, J., Piercey-Normore, M., Cloutier, S. 2012. Molecular Phylogenetic Analysis, Trichothecene Chemotype Patterns, and Variation in Aggressiveness of *Fusarium* Isolates Causing Head Blight in Wheat. Plant Dis. 96:1016-1025.

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.

Mengistu, N., Baenziger, P. S., Wegulo, S., Breathnach, J., and Cousell, J. 2007. Scab epidemic in Nebraska. Page 205 in: Proc. 2007 Fusarium Head Blight Forum. S. Canty, A. Clark, D. Ellis, and D. Van Sanford, eds. Kansas City, MO.

Mendiburu, F. 2019. Agricolae: Statistical Procedures for Agricultural Research. Retrieved 16 May 2019 from https://cran.r-project.org/web/packages/agricolae/agricolae.pdf.

O'Donnell, K., Kistler, H. C., Tacke, B. K., Casper, H. H. 2000. Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of *Fusarium graminearum*, the fungus causing wheat scab. Proc. Natl. Acad. Sci. U.S.A. 97:7905-7910.

Panthi, A., Hallen-Adams, H., Wegulo, S. N., Nopsa, J. H., Baenziger, P. S. 2014. Chemotype and aggressiveness of isolates of *Fusarium graminearum* causing head blight of wheat in Nebraska. Can. J. Plant Pathol. 36:447-455.

R Core Team. 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

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

Sarver, B. A. J., Ward, T. J., Gale, L. R., Broz, K., Corby Kistler, H., Aoki, T., Nicholson, P., Carter, J., O'Donnell, K. 2011. Novel Fusarium head blight pathogens from Nepal and Louisiana revealed by multilocus genealogical concordance. Fungal Genet. Biol. 48:1096-1107.

Starkey, D. E., Ward, T. J., Aoki, T., Gale, L. R., Kistler, H. C., Geiser, D. M., Suga, H., Tóth, B., Varga, J., O'Donnell, K. 2007. Global molecular surveillance reveals novel

Fusarium head blight species and trichothecene toxin diversity. Fungal Genet. Biol. 44:1191-1204.

Trail, F. 2009. For Blighted Waves of Grain: *Fusarium graminearum* in the Postgenomics Era. Plant Physiol. 149:103-110.

USDA. 2019. Crop Production 2018 Summary. Retrieved 29 May 2019 from https://www.nass.usda.gov/.

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

Ward, T. J., Clear, R. M., Rooney, A. P., O'Donnell, K., Gaba, D., Patrick, S., Starkey, D. E., Gilbert, J., Geiser, D. M., Nowicki, T. W. 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.

Wegulo, S. N., Baenziger, P. S., Nelson, L. A., Nopsa, J. H., Millhouse, J. C., Mengistu, N., and Breathnach-Stevens, J. 2008. The 2008 Fusarium head blight epidemic in Nebraska. Page 72 in: Proc. 2008 National Fusarium Head Blight Forum. S. Canty, A. Clark, E. Walton, D. Ellis, J. Mundell, and D. Van Sanford, eds. Indianapolis, IN.

Wegulo, S. N., Valverde-Bogantes, E., Bolanos-Carriel, C., Hallen-Adams, H., Bianchini Huebner, A., McMaster, N., Schmale D. G. 2018. First Report of *Fusarium boothii* Causing Head Blight of Wheat in the United States. Plant Dis. 102:2646.

Wickham, H. 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York. https://ggplot2.tidyverse.org.

Zhang, H., Brankovics, B., Luo, W., Xu, J., Xu, J. S., Guo, C., Guo, J. G., Jin, S. J., Chen, W. Q., Feng, J., van Diepeningen, A. D., van der Lee, T., Waalwijk, C. 2016. Crops are a main driver for species diversity and the toxigenic potential of *Fusarium* isolates in maize ears in China. World Mycotoxin J. 9:701-715.

Table 1. Means and Fisher's least significant difference (LSD) for deoxynivalenol (DON), 15-acetyldeoxynivalenol (15-ADON), Fusarium head blight (FHB) severity, and area under the disease progress curve (AUDPC) in the susceptible spring wheat cultivar Wheaton grown under greenhouse conditions and inoculated with three *F. boothii* and 13 *F. graminearum* isolates from Nebraska

				FHB severity (%)							
Isolate	Species	DON (μg/g)	15-ADON (μg/g)	3 DAI	6 DAI	9 DAI	12 DAI	15 DAI	18 DAI	21 DAI	AUDPC
NE16-15Fb	*F. boothii*	11.5	0.25	5.2	35.5	45.2	52.8	60.6	68.2	71.6	910
NE19-15Fb	*F. boothii*	9.5	0.20	2.2	28.4	35.2	40.8	48.0	54.5	58.7	715
NE21-15Fb	*F. boothii*	19.9	0.45	7.0	43.9	53.5	60.0	66.5	73.7	77.0	1,030
NE3-15Fg	*F. graminearum*	35.7	0.59	8.9	53.2	61.5	68.2	72.8	78.4	80.4	1,150
NE18-15Fg	*F. graminearum*	46.6	0.90	8.6	57.7	68.1	78.4	84.9	89.8	91.8	1,300
NE20-15Fg	*F. graminearum*	54.1	1.04	13.0	59.9	70.5	79.4	84.3	88.7	90.3	1,322
NE22-15Fg	*F. graminearum*	49.4	1.22	4.6	57.8	69.1	77.3	83.7	87.7	90.0	1,275
NE1a-16Fg	*F. graminearum*	42.9	0.95	8.4	56.7	67.5	74.6	80.9	85.2	87.5	1,251
NE1c-16Fg	*F. graminearum*	40.8	1.02	6.0	61.7	72.9	81.1	87.8	90.9	92.2	1,340
NE2c-16Fg	*F. graminearum*	68.2	1.82	16.7	73.1	80.7	87.4	91.0	92.8	93.8	1,466
NE6b-16Fg	*F. graminearum*	29.8	0.58	5.5	40.0	51.4	61.0	69.4	75.6	80.8	1,030
NE7b-16Fg	*F. graminearum*	36.0	0.97	4.8	43.4	55.1	64.9	74.3	80.4	84.9	1,096
NE7c-16Fg	*F. graminearum*	45.0	1.11	6.0	47.4	57.2	66.7	74.9	79.5	82.5	1,119
NE8b1-16Fg	*F. graminearum*	23.0	0.65	3.4	31.4	39.6	51.1	60.6	72.4	76.2	890
NE8c1-16Fg	*F. graminearum*	35.7	0.82	4.1	45.3	55.1	63.7	71.8	79.5	82.1	1,081
NE10b-16Fg	*F. graminearum*	25.8	0.70	4.0	31.5	40.2	51.7	61.0	72.8	76.2	898
LSD (0.05)		15.4	0.37	3.3	9.4	9.3	8.8	8.5	9.3	8.7	142

Table 2. Fusarium head blight area under the disease progress curve (AUDPC), deoxynivalenol (DON) and 15-acetyldeoxynivalenol (15-ADON) in spring wheat cultivar Wheaton grown under greenhouse conditions and inoculated with three *Fusarium boothii* and 13 *F. graminearum* isolates from Nebraska[z]

Species	AUDPC	DON (μg/g)	15-ADON (μg/g)
F. boothii	885 a	13.6 a	0.30 a
F. graminearum	1,171 b	41.0 b	0.95 b

[z] Means that have different letters are statistically different when compared using a *t* test at $P < 0.05$.

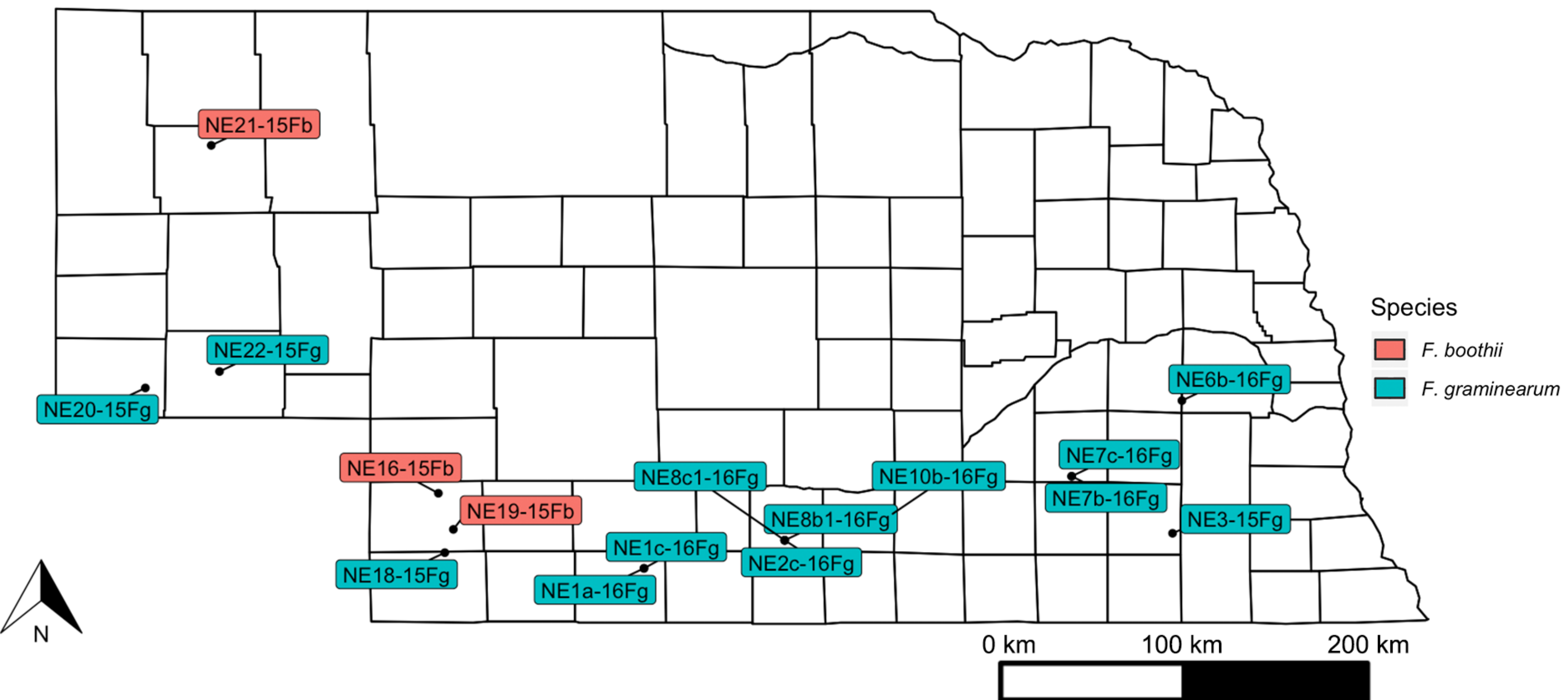


FIGURE 1
Map of Nebraska showing the geographic origin of the *Fusarium boothii* and *F. graminearum* isolates used in this study.

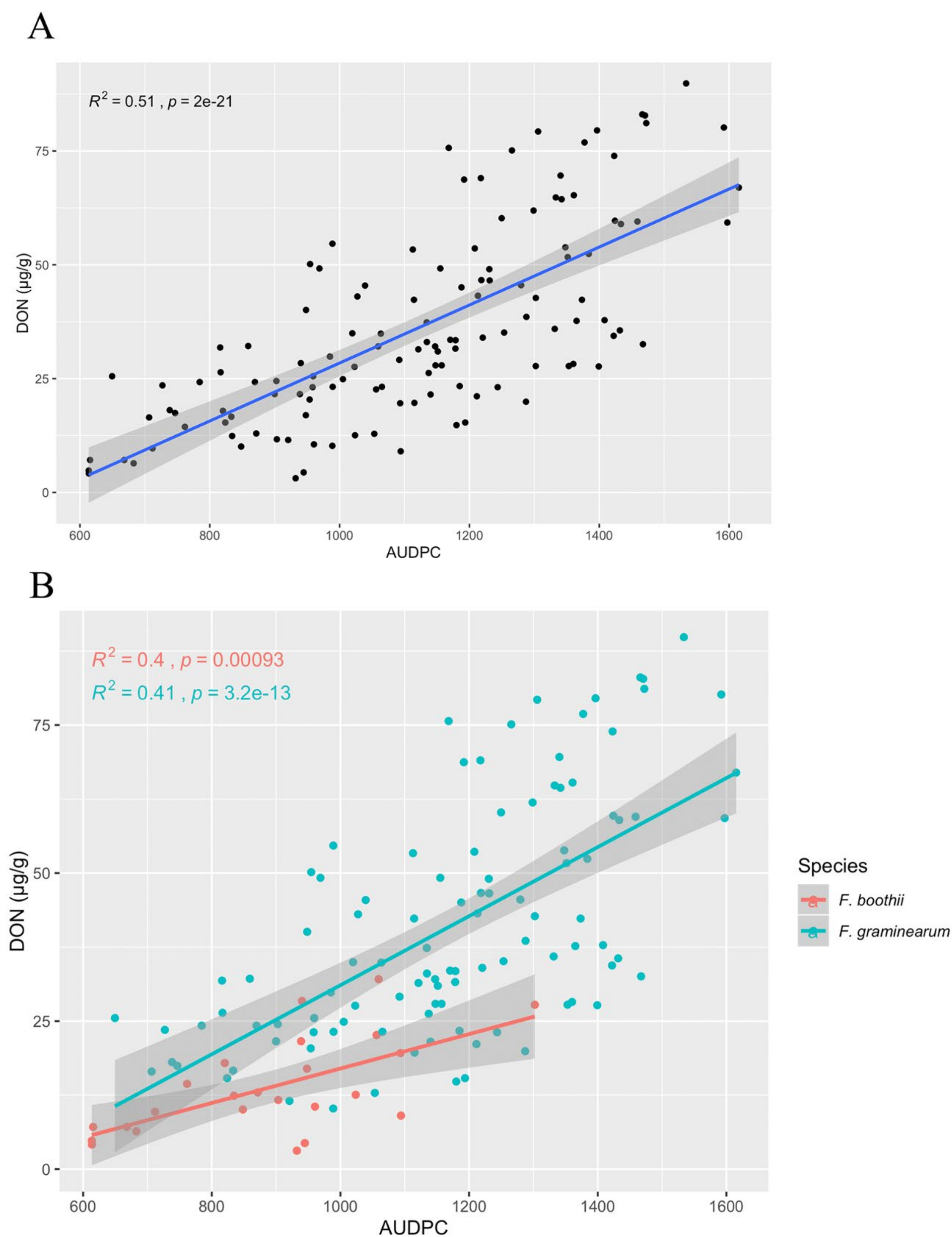
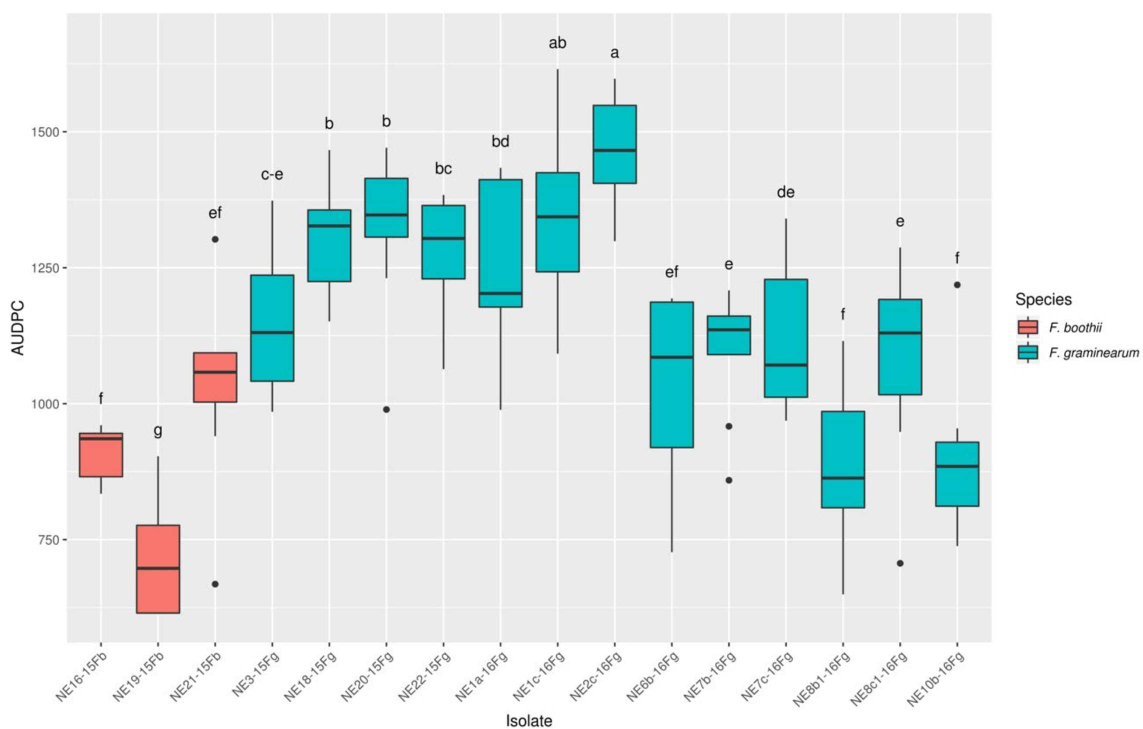
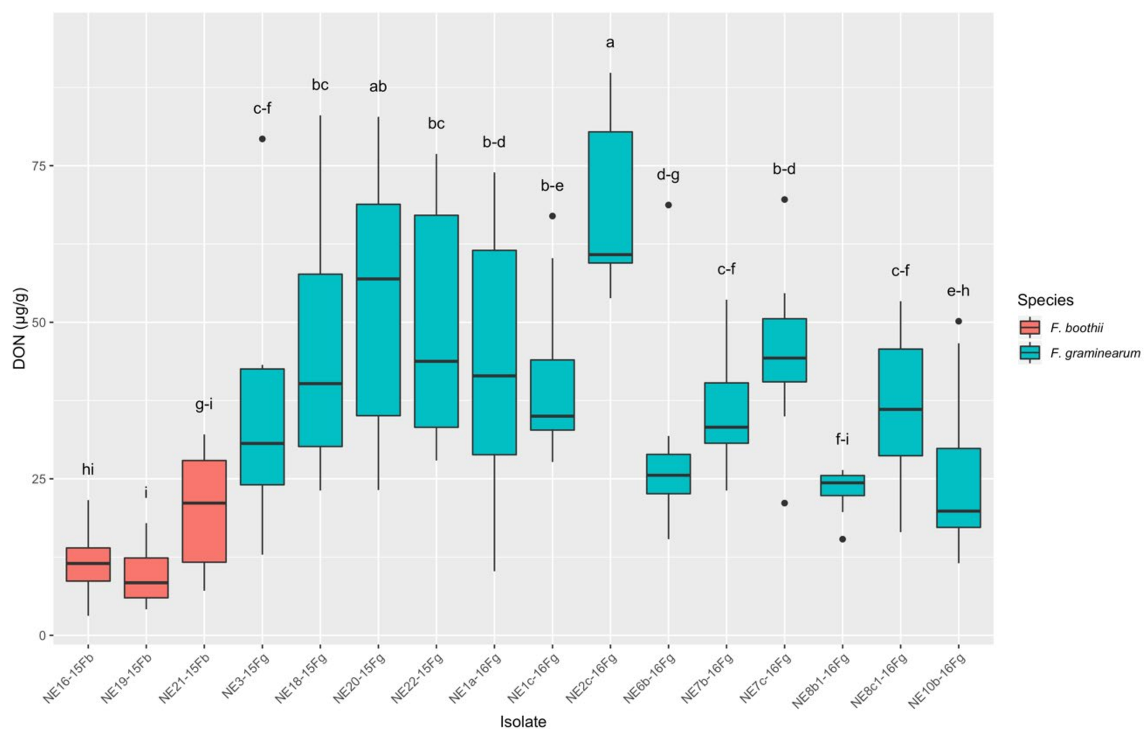


FIGURE 2
Regressions of deoxynivalenol (DON) concentration in wheat grain on area under the disease progress curve (AUDPC), combined (**A**) and by species (**B**), for three *Fusarium boothii* and 13 *F. graminearum* isolates from Nebraska.



SUPPLEMENTARY FIGURE S1

Area under the disease progression curve (AUDPC) values of three *F. boothii* and 13 *F. graminearum* isolates from Nebraska. Isolates with the same letters are not significantly different according to the least significant difference test ($P < 0.05$).



SUPPLEMENTARY FIGURE S2

Deoxynivalenol (DON) concentration in grain for three *F. boothii* and 13 *F. graminearum* isolates from Nebraska. Isolates with the same letters are not significantly different according to the least significant difference test ($P < 0.05$).

CHAPTER 5

A novel PCR-based method for the identification of Fusarium head blight pathogens

Abstract

Members of the *Fusarium sambucinum* species complex (FSAMSC) are the main etiological agents of Fusarium head blight (FHB) in most of the wheat growing regions of the world. In the U.S. and Canada, *F. graminearum* is the main species infecting wheat. *F. boothii* was recently reported causing FHB of wheat for the first time in the U.S. Other related species, such as *F. asiaticum*, *F. gerlachii*, *F. culmorum,* and *F. cerealis*, have also been reported. Accurate species identification of isolates is important for pathogen surveillance and disease management; however, most of the species causing FHB of wheat are morphologically indistinguishable. DNA sequencing and a multilocus genotyping assay provide reliable identification, but they might not be readily available to most labs or they might be cost prohibitive for population studies. The aim of this research is to develop a simpler and more affordable PCR-based assay to identify FHB pathogens. In order to achieve this aim, primers were designed based on lineage-specific single nucleotide polymorphisms (SNPs) in the trichothecene 3-*O*-acetyltransferase (*TRI101*) gene. On top of the fixed SNP, an additional mismatch 1-3 bases from the 3' end of the primer was introduced using the SNAPER program (http://ausubellab.mgh.harvard.edu/) to provide better allele differentiation. Amplification was carried out using a touchdown method with a combined annealing/extension step.

Individual reactions to differentiate *F. graminearum*, *F. boothii*, *F. asiaticum*, *F. gerlachii*, and *F. culmorum* from related species, as well as a multiplex reaction to target these five species in the same reaction.

Introduction

Fusarium head blight (FHB) of wheat is one of the most important diseases affecting wheat and other small grain cereals in all growing regions of the world (Stępień and Chełkowski 2010; McMullen et al. 1997). FHB causes significant yield reductions that are exacerbated by the production of mycotoxins that make grain consumption unsafe (Stępień and Chełkowski 2010).

Members of the *Fusarium graminearum* species complex (FGSC), which is part of the broader *F. sambucinum* species complex (FSAMSC) are the main etiological agents of FHB. The FGSC includes the following species: *F. acaciae-mearnsii*, *F. aethiopicum*, *F. asiaticum*, *F. austroamericanum*, *F. boothii*, *F. brasilicum*, *F. cortaderiae*, *F. gerlachii*, *F. graminearum*, *F. louisianense*, *F. meridionale*, *F. mesoamericanum*, *F. nepalense*, *F. ussurianum* and *F. vorosii* (Aoki et al. 2012). Other members of the FSAMSC that are important FHB pathogens are *F. culmorum*, *F. poae*, *F. sporotrichioides*, and *F. langsethiae*. Some species within the *F. tricinctum* species complex (FTSC), such as *F. avenaceum*, and *F. tricinctum*, cause significant losses in certain regions (Yli-Mattila 2010; Cerón-Bustamante et al. 2018; Stępień and Chełkowski 2010). In the U.S. and Canada, *F. graminearum* accounts for most of the FHB of wheat (Ward et al. 2008). However, *F. boothii* was reported recently causing FHB of wheat for the first time in the U.S. (Wegulo et al. 2018). Other species, such as *F. asiaticum*, *F. gerlachii*, *F. louisianense*, *F. culmorum,* and *F. cerealis*, have also been reported (Gale et al. 2011; Starkey et al. 2007; Bissonnette et al. 2018; Schmale et al. 2011; Sarver et al. 2011).

As stated by Wingfield et al. (2012), it is well known that accurate species identification is critical for all aspects of plant pathology, including studies of pathogen biology, population dynamics, and genomics, as well as disease surveillance, reporting and management. Recent reports have shown that, in many parts of the world, populations of FHB pathogens are dynamic and change constantly in response to selection imposed by different factors, most of them largely unknown, which include host distribution, climate change and disease management practices (Valverde-Bogantes et al. 2019). The members of the FGSC are morphologically indistinguishable due to their morphological simplicity and overlapping conidial features combined with within-species variability (O'Donnell et al. 2004). Therefore, in the context of constantly changing populations, molecular methods are required for an accurate identification.

Several primers have been designed for the differentiation of *F. graminearum* from other related FHB pathogens, including Fg16F/Fg16R primers (Nicholson et al. 1998), UBC85 primers (Schilling et al. 1996) and GO primers (de Biazio et al. 2008). However, these primers are no longer valid, since they are not specific for *F. graminearum* and also unable to distinguish between members of the FGSC because they were designed before *F. graminearum* was split into multiple species (Hafez et al. 2019; Valverde-Bogantes 2017; Garmendia et al. 2018).

Currently, two main methods are used to identify FHB pathogens to species level: (i) sequencing of informative genes and (ii) the multilocus genotyping (MLGT) assay developed by Ward et al. (2008). Informative genes include the translation elongation factor 1α (*TEF1*), reductase (*RED*), trichothecene 3-*O*-acetyltransferase (*TRI101*) genes, as well as the DNA-directed RNA polymerase II largest (*RPB1*) and second largest

subunit (*RPB2*) genes (O'Donnell et al. 2000, 2015). Another alternative for species identification is the MLGT, which is a Luminex-based multiplex assay targeting fixed single nucleotide polymorphisms (SNPs) in informative genes that allows simultaneous identification of most FHB pathogens and their trichothecene genotype in a high-throughput platform. Both sequencing and the MLGT assay provide reliable identification; however, they might not be readily available to most labs or they might be cost prohibitive for population studies. As a consequence, there is a need for simpler and more inexpensive assays, such as species-specific PCR-based methods.

The aim of this research was to develop a simpler and more affordable PCR-based assay to identify FHB pathogens. In this study, species-specific SNPs in the *TRI101* gene were used to design primers that can easily discriminate between five different species of FHB pathogens that have been reported in the U.S.: *F. graminearum*, *F. boothii*, *F. culmorum*, *F. asiaticum*, and *F. gerlachii*. This new procedure allows rapid and inexpensive identification of isolates from these species.

Materials and Methods

Fungal cultures and DNA extraction

Reference isolates of FHB pathogens representing 21 different species were obtained from the U.S. Department of Agriculture, Agricultural Research Service (ARS) Culture Collection in Peoria, IL. Northern Regional Research Laboratory (NRRL) accession numbers for the reference strains are provided in Table 1. Existing *TRI101* gene nucleotide sequences from these isolates were obtained from the GenBank database

(https://www.ncbi.nlm.nih.gov/genbank/). For DNA extraction, isolates were grown for 7 days at room temperature on half-strength V8 juice agar (V8 juice, 100 ml; $CaCO_3$, 1.5 g; agar, 15 g per liter of medium). Approximately 500 mg of mycelium from each isolate was transferred into a 1.5-ml tube using a sterile toothpick or a flame sterilized scalpel without disturbing the agar surface. The mycelia were disrupted inside the tube using a pestle and DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) per manufacturer's instructions.

The primers were further tested with suspensions of DNA from isolates of *F. boothii*, *F. culmorum*, *F. graminearum*, *F. gerlachii*, *F. cerealis* and *F. louisianense* (Table 2) generously provided by Dr. Todd Ward (Mycotoxin Prevention and Applied Microbiology research unit, Agricultural Research Service, US Department of Agriculture).

Primer design

Forward primers were designed using species-specific SNPs in the *TRI101* gene for five different species: *F. graminearum*, *F. boothii*, *F. gerlachii*, *F. asiaticum* and *F. culmorum* (Table 3). These species-specific SNPs were identified and validated previously by O'Donnell et al. (2004) and Ward et al. (2008). The forward primers designed here contained the fixed nucleotide on the 3' end. In order to increase allele specificity, an additional mismatch was introduced within 1-3 bases from the 3'-terminal nucleotide using the SNAPER program (http://ausubellab.mgh.harvard.edu/; Drenkard et al. 2000). This program generates a list of primers with different additional mismatch

alternatives, ranking them by specificity based on empirical data. Drenkard et al. (2000) named these primers containing an additional mismatch as SNAP primers, for single nucleotide amplified polymorphisms.

Reverse primers generated by the SNAPER program were not used. Instead, a reverse primer universal for all five target species (TRI101rev; Table 3) was designed based on a conserved sequence in the *TRI101* gene determined by aligning sequence data in MEGA7 (Kumar et al. 2016) using the MUSCLE algorithm. This provided a method for identifying markers of different fragment lengths for each isolate.

PCR amplification

Specificity of the SNAP primers for their target species was evaluated with DNA from isolates listed in Tables 1 and 2. Optimization of PCR components and conditions was done empirically in order to amplify the specific alleles preferentially over nonspecific alleles. Amplification reactions were performed in a total volume of 20 µl using the Platinum® Taq DNA Polymerase (Invitrogen, Carlsbad, CA, USA) and prepared as described in Table 4. Reactions were tested as singleplexes (containing only one forward primer) or as multiplexes (containing more than one forward primer in the same reaction). Size differences between products for each species were large enough to allow a multiplex reaction targeting all 5 species in the same reaction. PCR was carried out in a T100 Thermal Cycler (Bio-rad, Hercules, CA) using a touchdown method with a combined annealing/extension step. The following cycle parameters were used: 94°C for 5 min, 15 cycles of 94°C for 30 s and 71°C (−0.5°C/cycle) for 1 min; followed by 20

cycles of 94°C for 30 s, and 64ºC for 1 min. A final extension step at 72°C for 10 min was included. A negative control sample containing all reagents and primers but no template DNA was included in each run.

The amplification products were separated by electrophoresis in 0.7% agarose gels with 0.5 µg/mL ethidium bromide. Molecular weight markers (GeneRuler 1 kb DNA ladder; Thermo Scientific, Vilnius, Lithuania) were used to estimate the size of the amplicons. Expected product sizes for each primer are shown in Table 3.

Results

Under the conditions described here, the SNAP primers designed for *F. boothii*, *F. culmorum*, *F. graminearum*, *F. asiaticum*, and *F. gerlachii* generated specific products of expected sizes with DNA from their respective targets (Figures 1-5). Specificity was evaluated against DNA from 21 different species of FHB pathogens. Expected product sizes of the SNAP primers with the TRI101rev reverse primer were: 1036 bp for *F. boothii* (FbooSNAP1), 843 bp for *F. culmorum* (FculSNAP1), 495 bp for *F. graminearum* (FgramSNAP1), 390 bp for *F. asiaticum* (FasiSNAP1), and 291 bp for *F. gerlachii* (FgerSNAP1). No other amplicons of different sizes were detected, and no products were observed in the template-free reactions.

The reactions were optimized in terms of cycling conditions, as well as concentration of DNA polymerase, $MgCl_2$, deoxynucleotides (dNTPs), and primers. In order to obtain adequate specificity, a hot start polymerase was required. It was observed that using DNA templates at concentrations higher than 50 ng/µl yielded no products or

caused smearing in the agarose gels. Bands produced by the *F. culmorum* and *F. gerlachii* primers tended to be weaker than the other primers (Figures 2 and 5), thus a higher primer concentration was required for these reactions.

Size differences in the amplicons produced for each species allowed for the development of a multiplex reaction with all five SNAP primers. Amplicons generated in multiplex reactions were easily differentiated after separation by agarose electrophoresis, allowing for adequate discrimination of *F. boothii*, *F. culmorum*, *F. graminearum*, *F. asiaticum*, and *F. gerlachii* in a single reaction (Figure 6). No evident interaction or interference was observed between the SNAP primers in the multiplex reactions.

Further testing of the multiplex reaction with DNA from isolates of representative species (Table 2) generated expected products for *F. boothii*, *F. culmorum*, *F. graminearum*, *F. asiaticum*, and *F. gerlachii* (Figure 7). However, the expected product was not obtained with *F. gerlachii* isolate NRRL 38405. Sequence analysis of the *TRI101* gene from this isolate showed that it has a mutation in the SNP targeted by the *F. gerlachii* primer (FgerSNAP1). Faint amplicons of the size expected for *F. graminearum* were obtained with isolates *F. gerlachii* NRRL 38405, and *F. louisianense* NRRL 54196 and 54197.

Discussion

This study introduces a novel PCR-based method for the identification of five species of FHB pathogens: *F. boothii*, *F. culmorum*, *F. graminearum*, *F. asiaticum*, and *F. gerlachii*. These species were chosen to target most of the FHB-causing species

present in the U.S. and Canada. In the context of dynamic and changing populations of FHB pathogens, accurate species identification is fundamental not only for disease surveillance, but also for disease management, pathogen biology, population dynamics, and genomics (Wingfield et al. 2012).

Previously described PCR tests for identification of *F. graminearum* have been proven to be nonspecific. Schilling et al. (1996) described the UBC85F/UBC85R primer set based on a fragment obtained from random amplification of polymorphic DNA unique to *F. graminearum*. The Fg16F/Fg16R primer pair described by Nicholson et al. (1998) targets the *Fg16* gene and was designed to identify *F. graminearum*. However, both UBC85 and Fg16 primer pairs were described when all of the members of the FGSC were thought to be a single panmictic species. In 2004, O'Donnell et al. (2004) divided the panmictic *F. graminearum* into different species which these primers are not able to distinguish (Garmendia et al. 2018; Hafez et al. 2019; Valverde-Bogantes 2017). Many authors have used the UBC85 primers as FGSC-specific primers (Suga et al. 2008; Karugia et al. 2009; Puri and Zhong 2010; Shen et al. 2012); however, false negatives have been reported for these primers when tested against different species within the FGSC (Hafez et al. 2019).

The galactose oxidase (*GO*) gene was used by de Biazio et al. (2008) to design primers to identify *F. graminearum*. Despite having been designed following the description of the FGSC, the GO primers were designed by aligning *GO* genes from *F. graminearum*, *Fusarium venenatum*, *Aspergillus oryzae* and *Fusarium sporotrichioides*, without including other species within the FGSC. The GO primers have been shown to be

unable to differentiate *F. graminearum* from related species, including species outside of the FGSC, such as *F. cerealis* (Valverde-Bogantes 2017).

A PCR-RFLP method was recently developed to differentiate *Fusarium graminearum* from other species within the *Fusarium graminearum* species complex based on differential digestion of the *TEF1* gene by the restriction enzyme *BsaH*I (Garmendia et al. 2018). However, in silico analysis has revealed that some *F. asiaticum*, *F. culmorum* and *F. pseudograminearum* isolates could yield the same digestion pattern as *F. graminearum*, leading to misidentification (Hafez et al. 2019).

Sequencing of informative genes and the Luminex MLGT mentioned above are currently used to identify FHB pathogens to species level. However, these methods can be laborious, time consuming or expensive depending on the number of samples. The assay described here provides an easy and rapid PCR-based assay for species identification. In order to use proper species circumscriptions, the primers from the present study were designed to target polymorphisms in the *TRI101* gene which had been previously described by O'Donnell et al. (2004) and Ward et al. (2008). The *TRI101* gene was targeted because it is a single-copy informative gene that has been used extensively in phylogenetic studies of this group of pathogens (O'Donnell et al. 2000; Sarver et al. 2011; O'Donnell et al. 2008; Yli-Mattila et al. 2009; Ward et al. 2008; Starkey et al. 2007; O'Donnell et al. 2004). Unfortunately, most of the interspecies polymorphisms in this gene are SNPs. It is known that primers designed with a single mismatch on the 3' end do not provide adequate allele specificity (Kwok et al. 1994); therefore, an additional mismatch was introduced 1-3 bases from the 3' end using the SNAPER program in order to destabilize the 3' terminus and increase allele discrimination (Drenkard et al. 2000).

The components and conditions of the reactions were optimized in this study in order to improve specificity of the primers and avoid the amplification of misprimed products. The use of a hot-start polymerase was necessary in order to avoid mispriming at low temperatures. In agreement with Kwok et al. (1994), a lower concentration of PCR components (DNA polymerase, $MgCl_2$, dNTPs, and primers) and higher annealing temperatures tended to increase the stringency of the amplification. The addition of 15 touchdown cycles at the beginning of the amplifications provided enhanced specificity to the reactions. Furthermore, the higher temperatures in the combined annealing/extension step provided sufficient advantage to the specific allele over the nonspecific allele. Despite efforts to optimize the reactions, it is possible that faint bands of nonspecific products may be generated with the primer for *F. graminearum*. The *F. graminearum* SNP targeted here was a G/T mismatch, which has been shown to have a weaker destabilization strength compared to other nucleotide mismatches (Kwok et al. 1994). Thus, faint *F. graminearum* bands should be confirmed by other methods, such as the *F. graminearum*-specific FgssF/FgssR primers designed by Hafez et al. (2019).

Compared to methods currently used for species identification of FHB pathogens, such as DNA sequencing or the Luminex MLGT assay, the PCR protocol describe here has the advantage of potential cost savings and that it has a shorter processing time (~5 h, including DNA extraction). This method does not require sophisticated equipment that might not be accessible to most labs. Multiplexing the reactions allows the targeting of *F. graminearum*, *F. boothii*, *F. asiaticum*, *F. gerlachii*, and *F. culmorum* in the same reaction, which can be adjusted according to the needs of each particular region. For instance, laboratories in Nebraska can target the only two species that have been reported

in the state so far: *F. graminearum* and *F. boothii*, which should identify most of the isolates. Since species-specific SNPs have been described for most FHB pathogens, the design of primers for other species outside of the U.S. and Canada using the approach described here should be relatively straightforward.

Potential future limitations of this assay include the possibility of obtaining faint nonspecific allele bands with the *F. graminearum* primer due to the weaker destabilization of the mismatch, which should be confirmed using alternative identification methods. Targeting a SNP in a single locus will not allow for detection of interspecies hybrids, such as the *F. graminearum* × *F. boothii* hybrid from Nebraska reported in chapter 2 of this dissertation. This could also lead to false negatives or misidentification of isolates that have mutations in the target SNPs such as the *F. gerlachii* isolate NRRL 38405 shown here, which, although rare, have been also observed for the SNPs targeted by the Luminex MLGT assay (Boutigny et al. 2014). Due to the recent divergence of the species in the FGSC and the low number of isolates available from some species, it is also possible that the species-specific SNPs used here have not been fixed in all the diverse isolates of a species.

Conclusions

The PCR protocol described here provides an alternative and feasible option for species identification of FHB pathogens. This assay was designed to target species-specific SNPs in the *TRI101* gene in order to identify isolates of *F. graminearum*, *F. boothii*, *F. culmorum*, *F. asiaticum*, and *F. gerlachii*. The primers tested for these species

can be used individually in singleplexes or simultaneously in a multiplex, and adjusted according to regional needs. This method is comparatively rapid and does not need special equipment, which is expected to facilitate rapid pathogen surveillance in a relatively inexpensive and high-throughput way.

This assay is currently being validated in a blinded test by Dr. Todd Ward's lab (Mycotoxin Prevention and Applied Microbiology research unit, Agricultural Research Service, US Department of Agriculture) using a larger number of isolates for each species.

References

Aoki, T., Ward, T. J., Kistler, H. C., and O'Donnell, K. 2012. Systematics, Phylogeny and Trichothecene Mycotoxin Potential of Fusarium Head Blight Cereal Pathogens. JSM Mycotoxins. 62:91–102.

Bissonnette, K. M., Wharton, P., Chen, J., and Marshall, J. M. 2018. Survey of *Fusarium* Species Associated with Fusarium Head Blight of Spring Wheat (*Triticum aestivum*) in Southeastern Idaho. Plant Heal. Prog. 19:125–127.

Boutigny, A.-L., Ward, T., Ballois, N., Iancu, G., and Ioos, R. 2014. Diversity of the *Fusarium graminearum* species complex on French cereals. Eur. J. Plant Pathol. 138:133–148.

Cerón-Bustamante, M., Ward, T. J., Kelly, A., Vaughan, M. M., McCormick, S. P., Cowger, C., et al. 2018. Regional differences in the composition of Fusarium Head

Blight pathogens and mycotoxins associated with wheat in Mexico. Int. J. Food Microbiol. 273:11–19.

de Biazio, G. R., Leite, G. G. S., Tessmann, D. J., and Barbosa-Tessmann, I. P. 2008. A new PCR approach for the identification of *Fusarium graminearum*. Brazilian J. Microbiol. 39:554–560.

Drenkard, E., Richter, B. G., Rozen, S., Stutius, L. M., Angell, N. A., Mindrinos, M., et al. 2000. A simple procedure for the analysis of single nucleotide polymorphism facilitates map-based cloning in *Arabidopsis*. Plant Physiol. 124:1483–1492.

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.

Garmendia, G., Umpierrez-Failache, M., Ward, T. J., and Vero, S. 2018. Development of a PCR-RFLP method based on the transcription elongation factor 1-α gene to differentiate *Fusarium graminearum* from other species within the *Fusarium graminearum* species complex. Food Microbiol. 70:28–32.

Hafez, M., Abdelmagid, A., Adam, L. R., and Daayf, F. 2020. Specific Detection and Identification of *Fusarium graminearum* Sensu Stricto Using a PCR-RFLP Tool and Specific Primers Targeting the Translational Elongation Factor 1α Gene. Plant Dis. 104:1076–1086.

Karugia, G. W., Suga, H., Gale, L. R., Nakajima, T., Tomimura, K., and Hyakumachi, M. 2009. Population Structure of the *Fusarium graminearum* Species Complex from a Single Japanese Wheat Field Sampled in Two Consecutive Years. Plant Dis. 93:170–

174.

Kumar, S., Stecher, G., and Tamura, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol. Biol. Evol. 33:1870–1874.

Kwok, S., Chang, S. Y., Sninsky, J. J., and Wang, A. 1994. A guide to the design and use of mismatched and degenerate primers. PCR Methods Appl. 3:S39-47.

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.

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.

O'Donnell, K., Kistler, H. C., Tacke, B. K., and Casper, H. H. 2000. Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of *Fusarium graminearum*, the fungus causing wheat scab. Proc. Natl. Acad. Sci. 97:7905–7910.

O'Donnell, K., Ward, T. J., Aberra, D., Kistler, H. C., Aoki, T., Orwig, N., et al. 2008. Multilocus genotyping and molecular phylogenetics resolve a novel head blight pathogen within the *Fusarium graminearum* species complex from Ethiopia. Fungal Genet. Biol. 45:1514–1522.

O'Donnell, K., Ward, T. J., Geiser, D. M., Kistler, H. C., and Aoki, T. 2004. Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within

the *Fusarium graminearum* clade. Fungal Genet. Biol. 41:600–623.

O'Donnell, K., Ward, T. J., Robert, V. A. R. G., Crous, P. W., Geiser, D. M., and Kang, S. 2015. DNA sequence-based identification of *Fusarium*: Current status and future directions. Phytoparasitica. 43:583–595.

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.

Sarver, B. A. J., Ward, T. J., Gale, L. R., Broz, K., Kistler, H. C., Aoki, T., et al. 2011. Novel Fusarium head blight pathogens from Nepal and Louisiana revealed by multilocus genealogical concordance. Fungal Genet. Biol. 48:1096–1107.

Schilling, A. G., Moller, E. M., and Geiger, H. H. 1996. Polymerase chain reaction-based assays for species-specific detection of *Fusarium culmorum*, *F. graminearum*, and *F. avenaceum*. Phytopathology. 86:515–522.

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.

Shen, C., Hu, Y., Sun, H., Li, W., Guo, J., and Chen, H. 2012. Geographic Distribution of Trichothecene Chemotypes of the *Fusarium graminearum* Species Complex in Major Winter Wheat Production Areas of China. Plant Dis. 96:1172–1178.

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.

Stępień, Ł., and Chełkowski, J. 2010. Fusarium head blight of wheat: pathogenic species and their mycotoxins. World Mycotoxin J. 3:107–119.

Suga, H., Karugia, G. W., Ward, T., Gale, L. R., Tomimura, K., Nakajima, T., et al. 2008. Molecular Characterization of the *Fusarium graminearum* Species Complex in Japan. Phytopathology. 98:159–166.

Valverde-Bogantes, E. 2017. Species and Trichothecene Genotypes of Fusarium Head Blight Pathogens in Nebraska, USA in 2015-2016. MS thesis. University of Nebraska-Lincoln.

Valverde-Bogantes, E., Bianchini, A., Herr, J. R., Rose, D. J., Wegulo, S. N., and Hallen-Adams, H. E. 2019. Recent population changes of Fusarium head blight pathogens: drivers and implications. Can. J. Plant Pathol. DOI: 10.1080/07060661.2019.1680442.

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.

Wegulo, S. N., Valverde-Bogantes, E., Bolanos-Carriel, C., Hallen-Adams, H., Bianchini, A., McMaster, N., et al. 2018. First Report of *Fusarium boothii* Causing Head Blight of Wheat in the United States. Plant Dis. 102:2646.

Wingfield, M. J., De Beer, Z. W., Slippers, B., Wingfield, B. D., Groenewald, J. Z.,

Lombard, L., et al. 2012. One fungus, one name promotes progressive plant pathology. Mol. Plant Pathol. 13:604–613.

Yli-Mattila, T. 2010. Ecology and evolution of toxigenic *Fusarium* species in cereals in northern Europe and Asia. J. Plant Pathol. 92:7–18.

Yli-Mattila, T., Gagkaeva, T., Ward, T. J., Aoki, T., Kistler, H. C., and O'Donnell, K. 2009. A novel Asian clade within the *Fusarium graminearum* species complex includes a newly discovered cereal head blight pathogen from the Russian Far East. Mycologia. 101:841–852.

Table 1. *Fusarium* isolates used in this study.

Species	NRRL accession number
F. acaciae-mearnsii	26752
F. aethiopicum	46710
F. asiaticum	6101
F. austroamericanum	28585
F. boothii	26916
F. brasilicum	31238
F. cerealis	25805
F. cortaderiae	29306
F. culmorum	25475
F. gerlachii	36905
F. graminearum	5883
F. louisianense	54196
F. lunulosporum	13393
F. meridionale	28436
F. mesoamericanum	25797
F. nepalense	54220
F. praegraminearum	39664
F. pseudograminearum	28062
F. sporotrichioides	3299
F. ussurianum	45665
F. vorosii	37605

Table 2. DNA suspensions provided by Dr. Todd Ward (Agricultural Research Service, US Department of Agriculture).

Species	NRRL accession number
F. boothii	26915
	29011
	29020
	29105
F. culmorum	25475
F. graminearum	5883
F. asiaticum	26156
F. gerlachii	36905
	38380
	38405
F. cerealis	25805
F. louisianense	54196
	54197

Table 3. Primers used in this study. Species-specific SNPs on the 3’ end and additional mismatches introduced with the SNAPER program are highlighted in bold.

Target	Primer name	Sequence (5'-to-3')	Product size (bp)
F. boothii	FbooSNAP1	CCAGCTACCAGGCCTCCTTT**TGG**	1036 bp
F. culmorum	FculSNAP1	TCCCTTTTGAGGACGTTCCTCGTGT**AA**	843 bp
F. graminearum	FgramSNAP1	GCAAGACGATAGTTCCTTACCTTGAAAAC**CAT**	495 bp
F. asiaticum	FasiSNAP1	TCAGTGCAAGCTGGGCGTTCT**A**CA**A**	390 bp
F. gerlachii	FgerSNAP1	CGACTGACGATGCTCTTTCG**C**C**C**	291 bp
Reverse	TRI101rev	TGTTGTGCAGGTACGTCGCGAG	-

Table 4. PCR setup for the identification of *F. boothii*, *F. culmorum*, *F. graminearum*, *F. asiaticum*, and *F. gerlachii* using the Platinum® Taq DNA Polymerase kit (Invitrogen, Carlsbad, CA, USA).

Reagent	Stock concentration	Volume (µl)
PCR buffer	10X	2
Forward SNAP primer(s)[a]	60 ng/µl each	1 or 2[b]
Reverse primer (TRI101rev)	60 ng/µl	1
$MgCl_2$	50 mM	0.6
Deoxynucleotides (dNTPs; Invitrogen)	2.5 mM each	1
DNA Polymerase	2 U/µl	0.2
DNA template	<50 ng/µl	1
Nuclease-free water	-	To final volume of 20 µl

[a] The reaction may be performed as a singleplex or as a multiplex targeting all five species in the same reaction.

[b] Primers FbooSNAP1, FgramSNAP1 and FasiSNAP1 require 1 µl each, whereas primers FculSNAP1 and FgerSNAP1 require 2 µl.

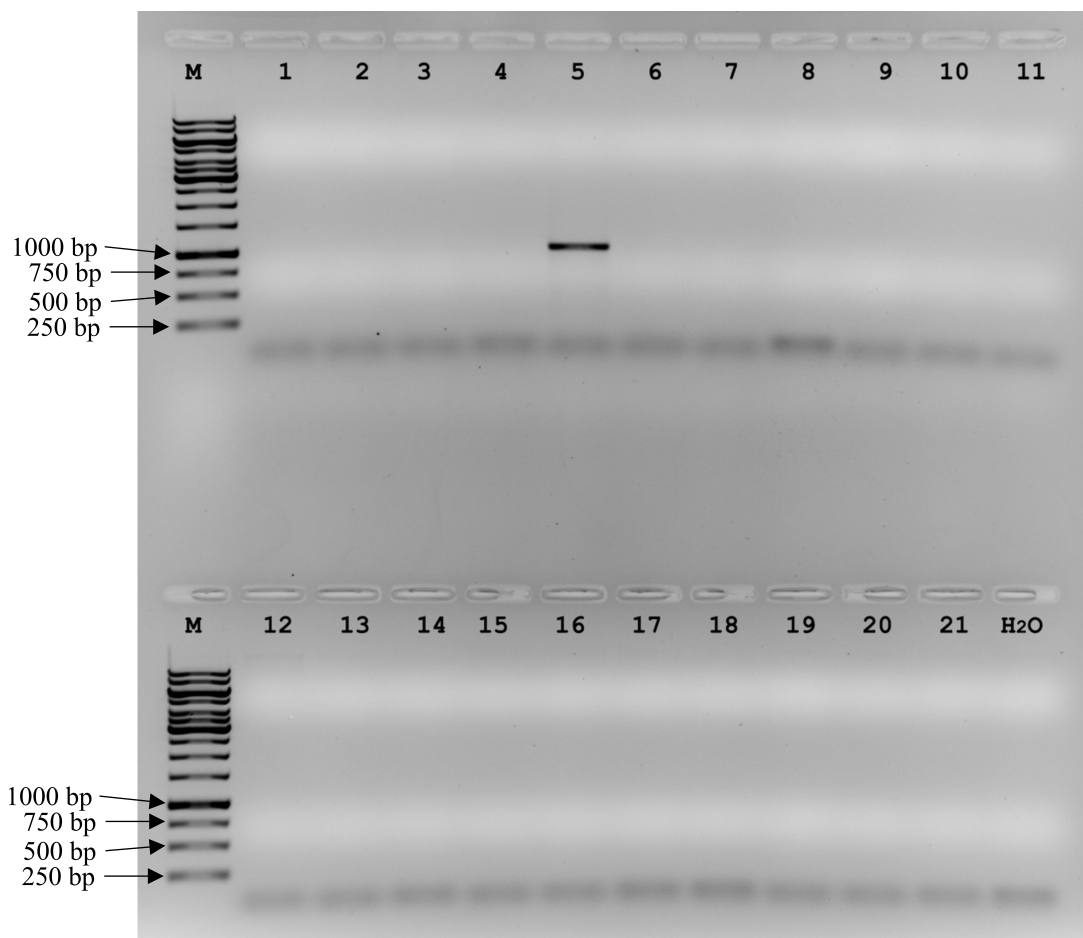


Figure 1. Agarose gel electrophoresis showing results obtained after singleplex amplification with the SNAP primer targeting *F. boothii* (FbooSNAP1) and reverse primer (TRI101rev). The expected 1036 bp product was generated only with *F. boothii* DNA. (1) *F. acaciae-mearnsii* NRRL 26752, (2) *F. aethiopicum* NRRL 46710, (3) *F. asiaticum* NRRL 6101, (4) *F. austroamericanum* NRRL 28585, (5) *F. boothii* NRRL 26916, (6) *F. brasilicum* NRRL 31238, (7) *F. cerealis* NRRL 25805, (8) *F. cortaderiae* NRRL 29306, (9) *F. culmorum* NRRL 25475, (10) *F. gerlachii* NRRL 36905, (11) *F. graminearum* NRRL 5883, (12) *F. louisianense* NRRL 54196, (13) *F. lunulosporum* NRRL 13393, (14) *F. meridionale* NRRL 28436, (15) *F. mesoamericanum* NRRL 25797, (16) *F. nepalense* NRRL 54220, (17) *F. praegraminearum* NRRL 39664, (18) *F. pseudograminearum* NRRL 28062, (19) *F. sporotrichioides* NRRL 3299, (20) *F. ussurianum* NRRL 45665, (21) *F. vorosii* NRRL 37605. Lane M, molecular weight marker (1 kb). H_2O: template-free negative control.

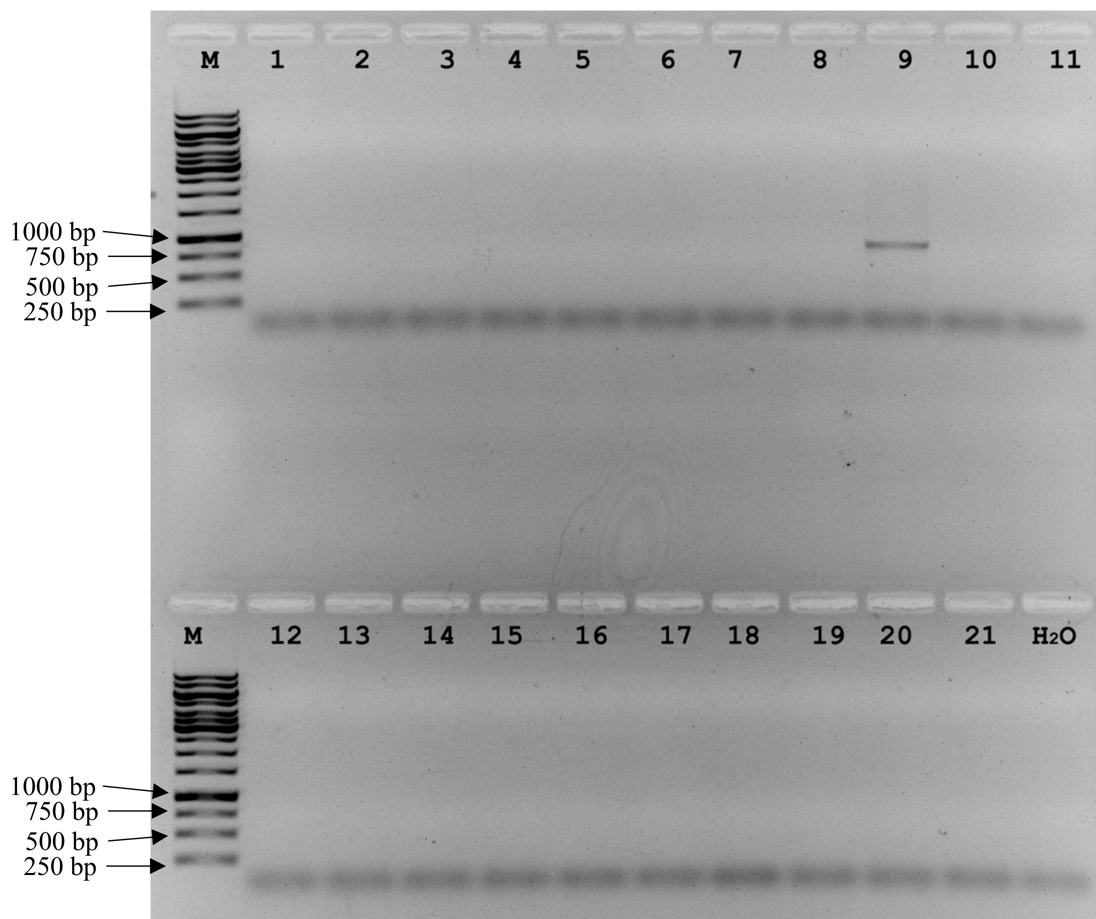


Figure 2. Agarose gel electrophoresis showing results obtained after singleplex amplification with the SNAP primer targeting *F. culmorum* (FculSNAP1) and reverse primer (TRI101rev). The expected 843 bp product was generated only with *F. culmorum* DNA. (1) *F. acaciae-mearnsii* NRRL 26752, (2) *F. aethiopicum* NRRL 46710, (3) *F. asiaticum* NRRL 6101, (4) *F. austroamericanum* NRRL 28585, (5) *F. boothii* NRRL 26916, (6) *F. brasilicum* NRRL 31238, (7) *F. cerealis* NRRL 25805, (8) *F. cortaderiae* NRRL 29306, (9) *F. culmorum* NRRL 25475, (10) *F. gerlachii* NRRL 36905, (11) *F. graminearum* NRRL 5883, (12) *F. louisianense* NRRL 54196, (13) *F. lunulosporum* NRRL 13393, (14) *F. meridionale* NRRL 28436, (15) *F. mesoamericanum* NRRL 25797, (16) *F. nepalense* NRRL 54220, (17) *F. praegraminearum* NRRL 39664, (18) *F. pseudograminearum* NRRL 28062, (19) *F. sporotrichioides* NRRL 3299, (20) *F. ussurianum* NRRL 45665, (21) *F. vorosii* NRRL 37605. Lane M, molecular weight marker (1 kb). H_2O: template-free negative control.

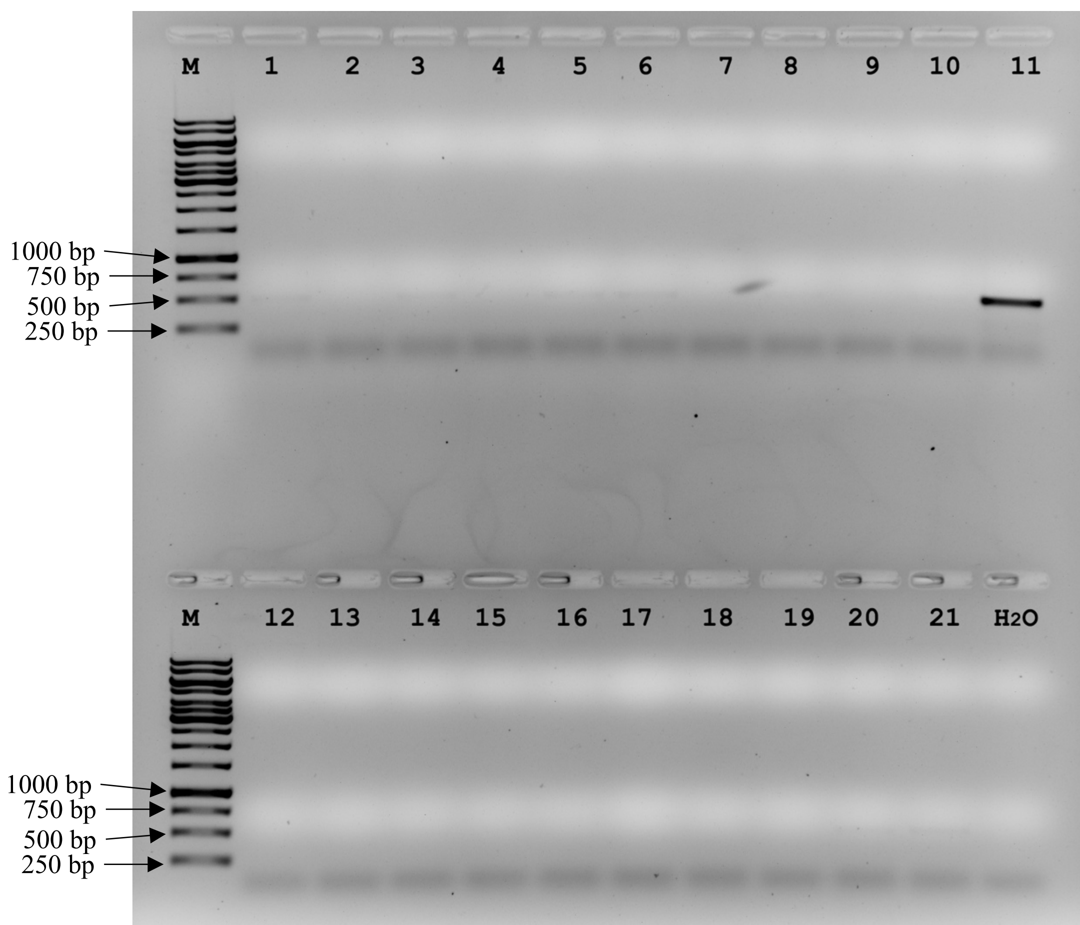


Figure 3. Agarose gel electrophoresis showing results obtained after singleplex amplification with the SNAP primer targeting *F. graminearum* (FgramSNAP1) and reverse primer (TRI101rev). The expected 495 bp product was generated only with *F. graminearum* DNA. (1) *F. acaciae-mearnsii* NRRL 26752, (2) *F. aethiopicum* NRRL 46710, (3) *F. asiaticum* NRRL 6101, (4) *F. austroamericanum* NRRL 28585, (5) *F. boothii* NRRL 26916, (6) *F. brasilicum* NRRL 31238, (7) *F. cerealis* NRRL 25805, (8) *F. cortaderiae* NRRL 29306, (9) *F. culmorum* NRRL 25475, (10) *F. gerlachii* NRRL 36905, (11) *F. graminearum* NRRL 5883, (12) *F. louisianense* NRRL 54196, (13) *F. lunulosporum* NRRL 13393, (14) *F. meridionale* NRRL 28436, (15) *F. mesoamericanum* NRRL 25797, (16) *F. nepalense* NRRL 54220, (17) *F. praegraminearum* NRRL 39664, (18) *F. pseudograminearum* NRRL 28062, (19) *F. sporotrichioides* NRRL 3299, (20) *F. ussurianum* NRRL 45665, (21) *F. vorosii* NRRL 37605. Lane M, molecular weight marker (1 kb). H_2O: template-free negative control.

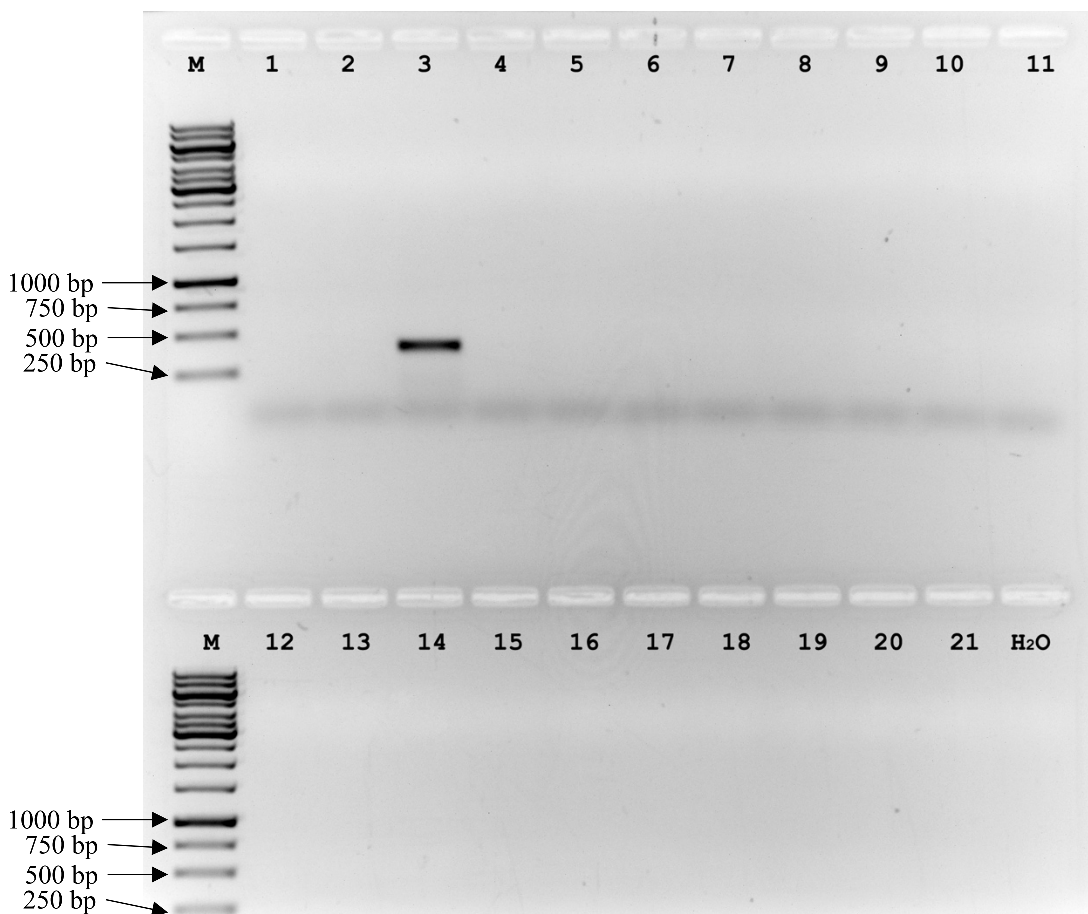


Figure 4. Agarose gel electrophoresis showing results obtained after singleplex amplification with the SNAP primer targeting *F. asiaticum* (FasiSNAP1) and reverse primer (TRI101rev). The expected 390 bp product was generated only with *F. asiaticum* DNA. (1) *F. acaciae-mearnsii* NRRL 26752, (2) *F. aethiopicum* NRRL 46710, (3) *F. asiaticum* NRRL 6101, (4) *F. austroamericanum* NRRL 28585, (5) *F. boothii* NRRL 26916, (6) *F. brasilicum* NRRL 31238, (7) *F. cerealis* NRRL 25805, (8) *F. cortaderiae* NRRL 29306, (9) *F. culmorum* NRRL 25475, (10) *F. gerlachii* NRRL 36905, (11) *F. graminearum* NRRL 5883, (12) *F. louisianense* NRRL 54196, (13) *F. lunulosporum* NRRL 13393, (14) *F. meridionale* NRRL 28436, (15) *F. mesoamericanum* NRRL 25797, (16) *F. nepalense* NRRL 54220, (17) *F. praegraminearum* NRRL 39664, (18) *F. pseudograminearum* NRRL 28062, (19) *F. sporotrichioides* NRRL 3299, (20) *F. ussurianum* NRRL 45665, (21) *F. vorosii* NRRL 37605. Lane M, molecular weight marker (1 kb). H_2O: template-free negative control.

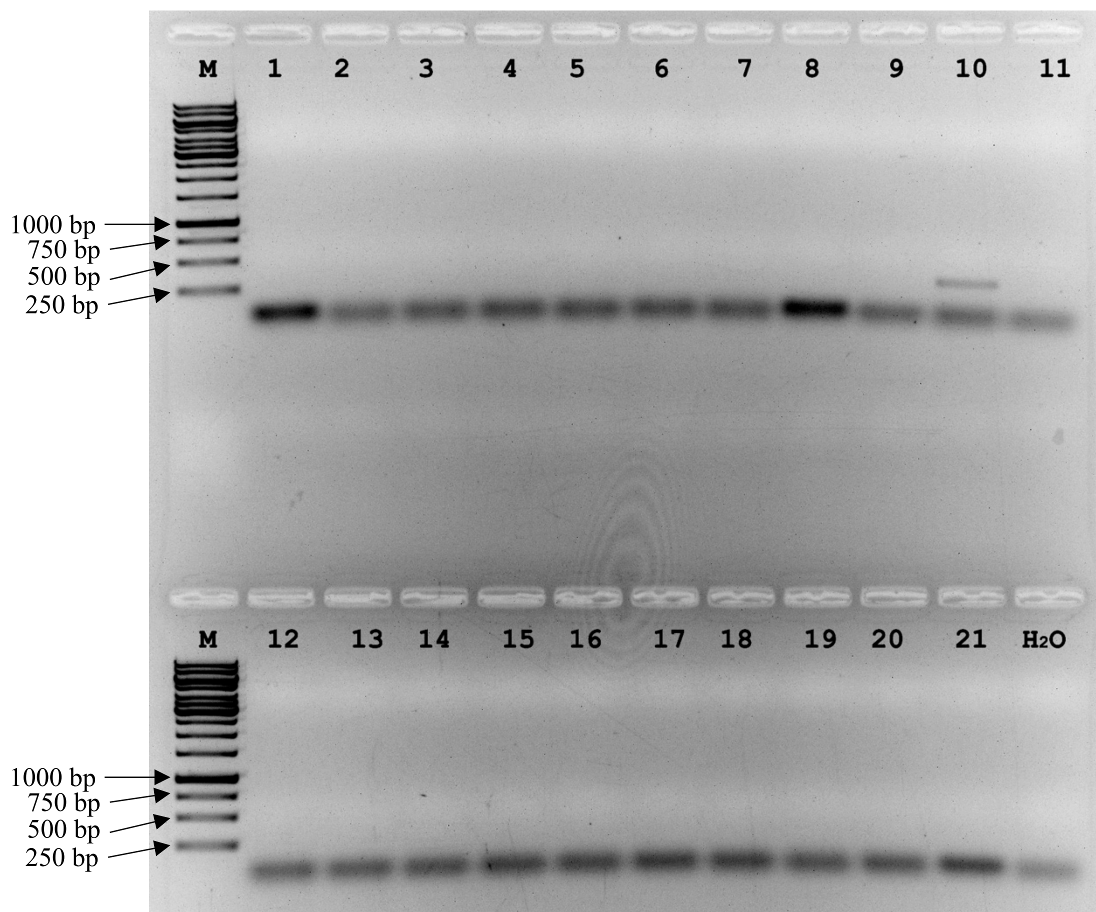


Figure 5. Agarose gel electrophoresis showing results obtained after singleplex amplification with the SNAP primer targeting *F. gerlachii* (FgerSNAP1) and reverse primer (TRI101rev). The expected 291 bp product was generated only with *F. gerlachii* DNA. (1) *F. acaciae-mearnsii* NRRL 26752, (2) *F. aethiopicum* NRRL 46710, (3) *F. asiaticum* NRRL 6101, (4) *F. austroamericanum* NRRL 28585, (5) *F. boothii* NRRL 26916, (6) *F. brasilicum* NRRL 31238, (7) *F. cerealis* NRRL 25805, (8) *F. cortaderiae* NRRL 29306, (9) *F. culmorum* NRRL 25475, (10) *F. gerlachii* NRRL 36905, (11) *F. graminearum* NRRL 5883, (12) *F. louisianense* NRRL 54196, (13) *F. lunulosporum* NRRL 13393, (14) *F. meridionale* NRRL 28436, (15) *F. mesoamericanum* NRRL 25797, (16) *F. nepalense* NRRL 54220, (17) *F. praegraminearum* NRRL 39664, (18) *F. pseudograminearum* NRRL 28062, (19) *F. sporotrichioides* NRRL 3299, (20) *F. ussurianum* NRRL 45665, (21) *F. vorosii* NRRL 37605. Lane M, molecular weight marker (1 kb). H_2O: template-free negative control.

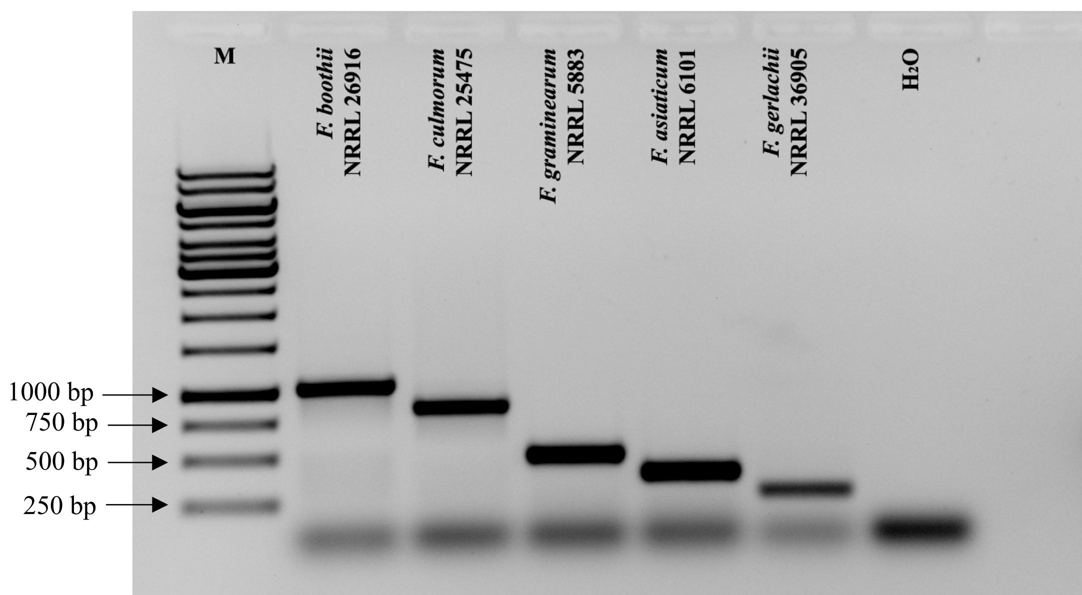


Figure 6. Agarose gel electrophoresis showing results obtained after multiplex amplification with SNAP primers targeting *F. boothii* (FbooSNAP1), *F. culmorum* (FculSNAP1), *F. graminearum* (FgramSNAP1), *F. asiaticum* (FasiSNAP1), *F. gerlachii* (FgerSNAP1), and reverse primer (TRI101rev). Expected product sizes were: 1036 bp for *F. boothii*, 843 bp for *F. culmorum*, 495 bp for *F. graminearum*, 390 bp for *F. asiaticum* and 291 bp for *F. gerlachii*. Lane M, molecular weight marker (1 kb). H_2O: template-free negative control.

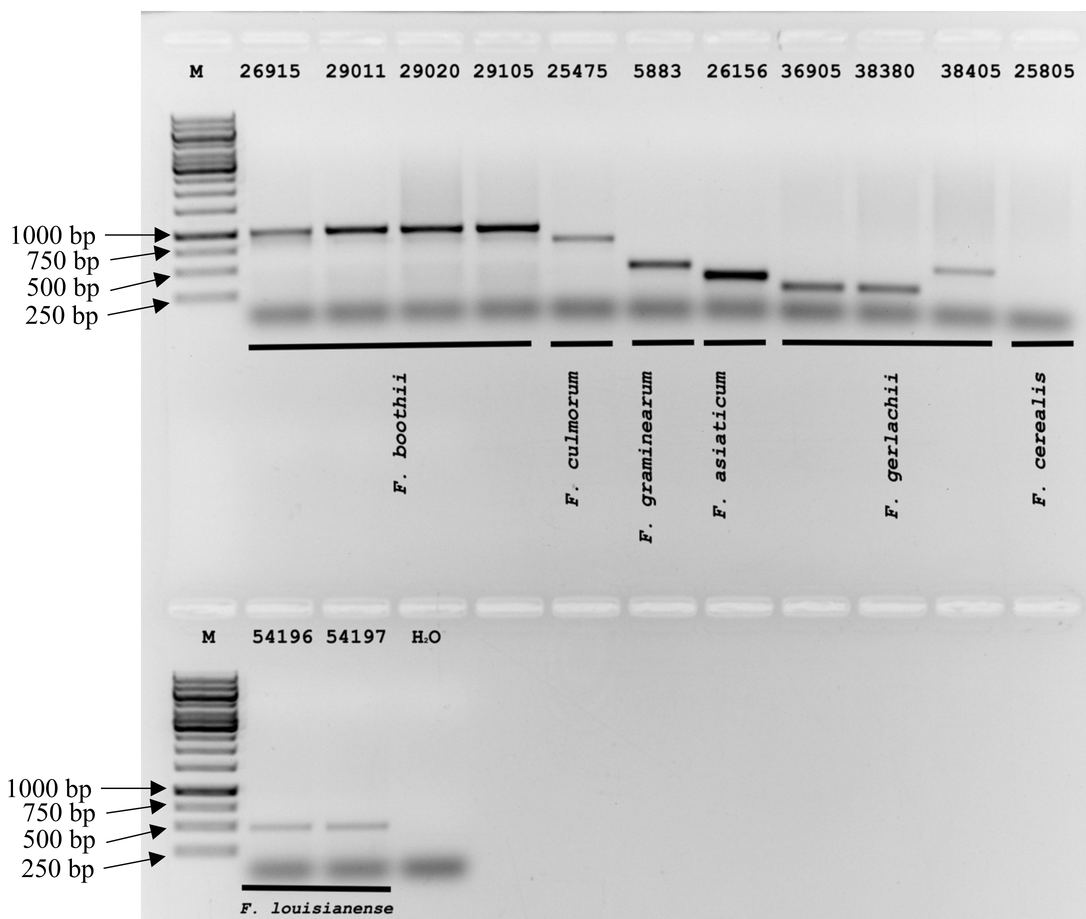


Figure 7. Agarose gel electrophoresis showing results obtained after multiplex amplification with SNAP primers targeting *F. boothii* (FbooSNAP1), *F. culmorum* (FculSNAP1), *F. graminearum* (FgramSNAP1), *F. asiaticum* (FasiSNAP1), *F. gerlachii* (FgerSNAP1), and reverse primer (TRI101rev). Expected product sizes were: 1036 bp for *F. boothii*, 843 bp for *F. culmorum*, 495 bp for *F. graminearum*, 390 bp for *F. asiaticum* and 291 bp for *F. gerlachii*. Lane M, molecular weight marker (1 kb). H_2O: template-free negative control.

CHAPTER 6

Summary of conclusions and future work

In this dissertation, the *Fusarium* species causing Fusarium head blight (FHB) in Nebraska, their aggressiveness and mycotoxin production were studied. This chapter summarizes the main findings and conclusions from each of the previous chapters of this dissertation, which are discussed in greater detail in each of the respective chapters.

This dissertation started with a detailed review of recent population changes of FHB pathogens around the world, in order to identify the main forces driving these changes as well as their implications. The literature review showed that most of the causes of population shifts have not been completely elucidated and remain largely unknown. However, there were some instances for which the effects of climate and anthropogenic activities on FHB pathogens at the population level is evident. For example, climate change and increased maize production in Europe has been associated with the replacement of *F. culmorum* by *F. graminearum* in the region. The replacement of *F. graminearum* by *F. poae* in Italy has been hypothesized to be due to yearly variations in environmental conditions.

The inadvertent introduction of exotic populations into new areas was also shown to cause dramatic changes in the population composition and toxigenic profiles when local conditions are favorable to the introduced population. This was demonstrated by the expansion of the more aggressive and more toxigenic *F. graminearum* population NA2 –

composed mainly of 3-acetyldeoxynivalenol (3-ADON) producers – in Canada and the Upper Midwest of the U.S., replacing the existing NA1 population – composed mainly of 15-acetyldeoxynivalenol (15-ADON) producers. The NA2 population is believed to have been introduced to North America from Europe.

The review also summarized recent introductions of novel populations into new areas, highlighting the importance of phytosanitary regulations to prevent unintentional introductions. Continuing surveillance of FHB pathogen populations was recommended not only for species and trichothecene genotype, but also for the genetic population structure of the circulating strains in a region, due to reports of population level replacements, such as the NA2 population of *F. graminearum* replacing the NA1 population in North America. Furthermore, genetic population structure and gene flow information will be useful for the development of effective disease management strategies, due to the fact that different populations could be affected selectively.

The second chapter of this dissertation described a survey conducted in Nebraska between 2015 and 2018 with the purpose of determining the species and trichothecene genotype of FHB pathogens infecting wheat in the state. In all, 42 wheat head samples showing FHB symptoms were collected during the 2015, 2016, 2017, and 2018 growing seasons. Species identities were determined by sequencing portions of the translation elongation factor 1α (*TEF1*), trichothecene 3-*O*-acetyltransferase (*TRI101*), and reductase (*RED*) genes. A multiplex PCR using two alternative sets of primers based on the trichothecene 15-*O*-acetyltransferase (*TRI3*) and trichothecene efflux pump (*TRI12*) genes was used to determine trichothecene genotype.

The results from the survey revealed that, out of 74 single-spore *Fusarium* isolates obtained from symptomatic wheat heads, most of the isolates were identified as *F. graminearum* (n=67). However, three isolates were identified as *F. boothii* (n=3), which was the first time that *F. boothii* was isolated from wheat in the U.S. Additional species identified included *F. poae* (n=2) and *F. acuminatum* (n=1). One isolate was identified as an interspecific hybrid between *F. graminearum* and *F. boothii* (n=1) because it had *F. graminearum* alleles at the *TEF1* and *TRI101* genes, and a *F. boothii* allele at the *RED* gene. Finally, all *F. graminearum* and *F. boothii* isolates had the 15-ADON genotype, which is the only trichothecene genotype that has been reported in Nebraska.

Since *F. boothii* had not been previously reported from wheat in the country, it became necessary to fulfill Koch's postulates with *F. boothii* isolates from Nebraska, in order to formally report *F. boothii* as a causal agent of FHB of wheat in the U.S. Thus, a greenhouse study was conducted to fulfill Koch's postulates with these isolates. This greenhouse experiment was described in chapter 3 of this dissertation. Briefly, spikes of FHB-susceptible spring wheat cultivar Wheaton were spray-inoculated at anthesis with spores of either of two of the *F. boothii* isolates, or a single isolate of *F. graminearum* collected in the same survey. Plants that were not inoculated were included as controls.

In two separate experiments, all *F. boothii* and *F. graminearum* isolates included were able to cause FHB symptoms. At 21 days after inoculation, FHB severity (percent of bleached spikelets on a spike) ranged from 17.5 to 86.6%. Mature grain was ground to flour and tested for deoxynivalenol (DON) and its acetylated derivatives 3-ADON and 15-ADON using gas chromatography/mass spectrometry. All three isolates produced

DON and had the 15-ADON chemotype. Recovery of single-spored cultures of the three isolates from symptomatic kernels obtained from both experiments fulfilled Koch's postulates for *F. boothii* isolates from Nebraska.

The three isolates included in the Koch's postulates experiments also produced fertile perithecia on carrot agar after incubation at room temperature for 7 weeks, and their colonies on potato dextrose agar resembled typical *F. graminearum* clade colonies. Measurements of ascospores and macroconidia of the three isolates were similar to those reported for species in the *F. graminearum* clade.

After confirming *F. boothii* as an FHB pathogen in the U.S., a more elaborate greenhouse study (described in chapter 4 of this dissertation) was designed to compare the aggressiveness and DON production in wheat among *F. graminearum* and *F. boothii* isolates from Nebraska. In total, 13 *F. graminearum* and three *F. boothii* isolates were included in this study. Spikes of the susceptible spring wheat cultivar Wheaton at anthesis were spray-inoculated with spores of the isolates and severity data, collected every 3 days over a 21-day period, were used to calculate the area under the disease progress curve (AUDPC). The concentrations of DON and its acetylated derivatives in the grain were quantified by gas chromatography/mass spectrometry.

The *F. graminearum* isolates were more aggressive and produced more DON than the *F. boothii* isolates. Mean AUDPC values were 1171 and 885 percent days for *F. graminearum* and *F. boothii*, respectively. Mean DON values were 41.0 and 13.6 μg/g for *F. graminearum* and *F. boothii*, respectively. A positive relationship was observed between AUDPC and DON concentration in the grain.

Although only the 3 available *F. boothii* isolates were used, the results obtained are in agreement with previous studies from China and South Africa that found *F. graminearum* be more aggressive and toxigenic in wheat than *F. boothii*. A host-adaptation of *F. boothii* to maize has been suggested by several studies based on the fact that most studies have isolated *F. boothii* from maize, and that the distribution of *F. boothii* and *F. graminearum* in places where they co-occur, such as South Africa, indicates that *F. boothii* predominates in maize, whereas *F. graminearum* predominates in wheat.

Additional isolates of *F. boothii* from Nebraska will be needed to further confirm that the circulating *F. boothii* strains in the state are less aggressive and less toxigenic than the circulating *F. graminearum* strains. More research is needed to determine the distribution and population structure of *F. boothii* in Nebraska, both in wheat and maize, as well as the pathogen's aggressiveness and toxigenicity on maize. Due to the potential host preference of *F. boothii* for maize, the large acreage of maize, and the routine wheat-maize rotation in Nebraska, more research is needed to elucidate the impact of these crops on the distribution of FHB pathogens in the state. It is recommended that in states, countries, or regions where both crops are grown and routinely rotated as in Nebraska, FHB management tactics targeting the more aggressive and toxigenic *F. graminearum* can be deployed to more effectively control the disease.

As with any plant pathogen, accurate species identification of isolates present in a region is important for pathogen surveillance and disease management; however, most of the species causing FHB of wheat are morphologically indistinguishable, and thus, require molecular methods for final identification. DNA sequencing and a multilocus

genotyping assay provide reliable results, but they might not be readily available to most labs or they might be cost prohibitive for population studies. This motivated the research presented in chapter 5 of this dissertation, in which a PCR-based assay was designed as an alternative to current methods used to identify FHB pathogens.

The PCR assay used primers designed based on lineage-specific single nucleotide polymorphisms (SNPs) in the *TRI101* gene. On top of the fixed SNP, an additional mismatch 1-3 bases from the 3' end of the primer was introduced using the SNAPER program (http://ausubellab.mgh.harvard.edu/) to provide better allele differentiation. Amplification was carried out using a touchdown method with a combined annealing/extension step.

This assay was designed to differentiate isolates of *F. graminearum*, *F. boothii*, *F. culmorum*, *F. asiaticum*, and *F. gerlachii* from related species. Some of the advantages of this method are that the primers for these species can be used individually in singleplexes or simultaneously in a multiplex, and adjusted according to regional needs. This method is comparatively rapid and does not need special equipment, which is expected to facilitate rapid pathogen surveillance in a relatively inexpensive and high-throughput way.

Future work and recommendations

Overall, this project has provided a baseline for future survey studies of *Fusarium* species in Nebraska. Because FHB pathogen populations can change over time, it is important to continue surveillance efforts. Due to the fact that these fungi are able to

infect maize, and the potential host adaptation of *F. boothii* to maize, pathogen monitoring should focus not only on wheat, but also on maize. Collecting additional *F. boothii* isolates from Nebraska will provide a better insight into the impacts that this species has on wheat and maize production in the state. The PCR method described here will be useful to monitor the main FHB pathogen species that have been reported in the U.S.

This project identified the strains infecting wheat in Nebraska at the species and trichothecene genotype level. Moving forward, research should also investigate the genetic population structure of the FHB pathogens, which could be achieved using variable number of tandem repeat (VNTR) or whole genome sequence data. Including this information will provide information on the structure, gene diversity, dynamics and gene flow between the circulating populations, which will be important for disease management in the future due to the fact that different populations of the same species could differ in host preference, virulence, mycotoxin production and effectiveness of disease control strategies. It is desirable to collect more *F. boothii* isolates and conduct co-infection studies between species, populations of the same species, and between isolates from the same genetic population to evaluate potential interactions. The aggressiveness and mycotoxin production of *F. boothii* and *F. graminearum* isolates on corn should be determined as well, to evaluate the risk to this important crop in Nebraska.

Finally, the approach described in chapter 5 for the development of a PCR assay could be used to also target other species of FHB pathogens that are important in other regions of the world. This will facilitate global species monitoring and further knowledge on the global distribution of this important group of pathogens.