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An adaptive evolutionary shift in Fusarium head blight pathogen populations is driving the rapid spread of more toxigenic *Fusarium graminearum* in North America

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Abstract

Analysis of Fusarium head blight (FHB) pathogen diversity revealed that 3ADON producing *Fusarium graminearum* are prevalent in North America and identified significant population structure associated with trichothecene chemotype differences ($F_{ST} > 0.285$; $P < 0.001$). In addition, we identified a trichothecene chemotype cline in Canada and documented a recent and significant shift in FHB pathogen composition by demonstrating that the 3ADON chemotype frequency in western Canada increased more than 14-fold between 1998 and 2004. On average, isolates from 3ADON populations produced significantly ($P < 0.05$) more trichothecene and had significantly ($P < 0.005$) higher fecundity and growth rates than isolates from the 15ADON population. These results indicate that selection is driving the rapid spread of an introduced pathogen population that is more toxigenic and potentially more vigorous. The discovery of this previously unrecognized pathogen diversity has significant implications for food safety and cereal production in North America.

Keywords: Adaptation; Cline; Fusarium head blight; Trichothecene; Mycotoxin; 3ADON; 15ADON; Deoxynivalenol; Population substructure

1. Introduction

Fusarium head blight (FHB) is among the most destructive and economically important diseases of wheat, barley and other small grain cereals worldwide. In the last 25 years, epidemic outbreaks of FHB were frequent in North America, and FHB of wheat and barley caused losses of more than $3 billion to agriculture in the United States and Canada during the 1990s (McMullen et al., 1997; Windels, 2000). In addition, recent outbreaks in Asia, Europe, and South America demonstrate that FHB is a growing threat to the world's food supply (Goswami and Kistler, 2004). FHB infection of cereals significantly lowers grain yield and quality, and can result in contamination of grain with trichothecene mycotoxins that act as virulence factors on sensitive hosts (Jansen et al., 2005; Proctor et al., 1995). Trichothecene contamination also poses a significant risk to food safety and animal health because trichothecenes inhibit eukaryotic protein synthesis and modify immune function (Pestka and Smolinski, 2005; Ueno et al., 1973).

The primary etiological agents of FHB belong to the *Fusarium graminearum* species complex (*Fg* complex),

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which consists of at least 11 phylogenetically distinct species (O'Donnell et al., 2000; O'Donnell et al., 2004; Starkey et al., 2007). Members of the *Fg* complex and five closely related species comprise the B-trichothecene lineage of FHB pathogens (B-FHB), and they produce one of three strain-specific profiles (chemotypes) of B-trichothecene metabolites (Miller and Greenhalgh, 1991; O'Donnell et al., 2004): nivalenol and acetylated derivatives (NIV chemotype), deoxynivalenol and primarily 3-acetyldeoxynivalenol (3ADON chemotype), and deoxynivalenol and primarily 15-acetyldeoxynivalenol (15ADON chemotype). Interestingly, B-trichothecene chemotype polymorphism is transspecific and has been maintained through multiple speciation events by natural selection, indicating that chemotype differences can have a significant impact on pathogen fitness (Ward et al., 2002).

Despite the global species and trichothecene chemotype diversity represented by FHB pathogens, previous studies indicated that *F. graminearum* strains with the 15ADON chemotype were largely responsible for FHB in North America (Abbas et al., 1986; Abramson et al., 1993, 2001; Miller and Greenhalgh, 1991). Initial analyses of population subdivision (Dusabenyagasani et al., 1999; Zeller et al., 2003, 2004) led to the suggestion that local populations of *F. graminearum* likely represent subsets of a larger panmictic population covering much of the wheat-growing areas of North America (Zeller et al., 2003, 2004). However, two recent studies indicated localized heterogeneity among North American *F. graminearum* populations. Starkey et al. (2007) identified six *F. graminearum* isolates with a NIV or 3ADON chemotype in Louisiana, and Gale et al. (2007) identified a genetically divergent population of *F. graminearum* characterized by a 3ADON chemotype in six Minnesota and North Dakota counties. In this study, we demonstrate that population subdivision among North American *F. graminearum* is widespread, directly contradicting the hypothesis of a panmictic FHB pathogen population in North America. More importantly, we document a recent and significant shift among *F. graminearum* populations in which a highly toxigenic population of *F. graminearum* with a 3ADON chemotype has been rapidly replacing the dominant FHB pathogen in North American wheat fields.

2. Materials and methods

2.1. Isolates and DNA sequence analysis

Isolates are identified by NRRL numbers from the U.S. Department of Agriculture, Agricultural Research Service (ARS) Culture Collection, Peoria, IL. Strain histories are provided in Supplemental Table 1 and through the ARS Culture Collection website (http://nrrl.ncaur.usda.gov/cgi-bin/usda). Isolates used for molecular surveillance in Canada were collected at harvest from single producers. Sampling was based on crop districts, and efforts were made to collect only one sample from any individual town within a crop district and to sample each of the provincial crop districts, where possible, to ensure that isolates were representative of the entire agricultural area of sampled provinces. DNA sequences from 19 genes were examined for species and chemotype-specific nucleotide variation among a panel of 53 B-FHB isolates (O'Donnell et al., 2000; O'Donnell et al., 2004; Starkey et al., 2007; Ward et al., 2002).

2.2. Multilocus genotyping assay

We developed a multiplex PCR (MLGT multiplex; Table 1) to enable simultaneous determination of species identity and trichothecene chemotype via a multilocus genotyping (MLGT) assay. MLGT multiplex amplifica-

Table 1
Multiplex composition and amplification primers

Multiplex	Primer	Primer sequence	Chromosome/Scaffold[a]
MLGT	RED-f	AGACTCATTCCAGCCAAG	2/2
	RED-r	TCGTGTTGAAGAGTTTGG	
	TRI101-f	CAAGATACAGCTCGACACC	4/4
	TRI101-r	CTGGGTAGTTGTTCGAGA	
	EF1-f	CGACCACTGTGAGTACCA	2/5
	EF1-r	GTCAAGAACCCAGGCGTA	
	MAT-f	TTCTCAGGAACGACTCAAC	2/5
	MAT-r	TGTCGGTTCAGACGATCA	
	TRI3-f	AACCTGAGCCCTCCAGT	2/2
	TRI3-r	TGGCAAAGACTGGTTCAC	
	TRI12-f	CATGAGCATGGTGATGTC	2/2
	TRI12-r	AAGCATCAGCCTCTGCTC	
VNTR1	HK1043-f	ACAGGCATCCAAGGACATTT	1/1
	HK1043-r	GTTTGATGGCGCATTCAAAG	
	HK1073-f	TATGATGCAGCGAATGCAAC	4/6
	HK1073-r	TAGAGACCTGGCCCATACCA	
	HK913-f	GCAGGACCTGGATGATGAA	1/1
	HK913-r	ATGTGTGCAGCCATGAGATT	
VNTR2	HK977-f	AAACGTAAACGGATCAACGG	3/3
	HK977-r	AGATTCGCAACTTTGTGCTG	
	HK1059-f	AAGACTGGTCAGCAGTAGGGA	3/3
	HK1059-r	TGAGAGCGAGACTGAGCATGA	
	HK967-f	AAGAGGGCGTGTCTCTGTTTT	2/2
	HK967-r	CGCTTCCTTCCTTTCAATTC	
VNTR3	HK957-f	TCCGAAGGTAGAAGCGTTGT	1/1
	HK957-r	TCAAGCCCATCTATGCTGTT	
	HK917-f	ATCTCCCAAGCTGGCTAATT	1/1
	HK917-r	AGAACCGGCAAAGTTCGATT	
	HK630-f	TGGATATGGTTCCCCAGCT	4/6
	HK630-r	TACTGACCTTGAGGAGCAC CATAC	
TRI3	3CON	TGGCAAAGACTGGTTCAC	2/2
	3NA	GTGCACAGAATATACGAGC	
	3D15A	ACTGACCCAAGCTGCCATC	
	3D3A	CGCATTGGCTAACACATG	
TRI12	12CON	CATGAGCATGGTGATGTC	2/2
	12NF	TCTCCTCGTTGTATCTGG	
	12-15F	TACAGCGGTCGCAACTTC	
	12-3F	CTTTGGCAAGCCCGTGCA	

[a] Based on genetic and physical map of *F. graminearum* (Gale et al., 2005).