Trichothecene Profiling and Population Genetic Analysis of *Gibberella zeae* from Barley in North Dakota and Minnesota

Rishi R. Burlakoti, Stephen M. Neate, Tika B. Adhikari, Sanjaya Gyawali, Bacilio Salas, Brian J. Steffenson, and Paul B. Schwarz

First, second, third, and fourth authors: Department of Plant Pathology, North Dakota State University (NDSU) Department 7660, P.O. Box 6050, Fargo 58108-6050: fifth author: Pest Detection, Diagnostics, and Management Laboratory, United States Department of Agriculture, Edinburg, TX 78541-5033; sixth author: Department of Plant Pathology, 495 Borlaug Hall, 1991 Upper Buford Circle, University of Minnesota, St. Paul 55108-6030; and seventh author: Department of Plant Sciences, NDSU Department 7670, P.O. Box 6050, Fargo.
Current address of first author: Weather Innovations Incorporated, 7159 Queens Line, Chatham, ON, Canada.
Current address of second author: Agri-Science Queensland DEEDI, P.O. Box 2282, Toowoomba, Queensland 4350 Australia.
Accepted for publication 4 December 2010.

ABSTRACT

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

Gibberella zeae, the principal cause of Fusarium head blight (FHB) of barley, contaminates grains with several mycotoxins, which creates a serious problem for the malting barley industry in the United States, China, and Europe. However, limited studies have been conducted on the trichothecene profiles and population genetic structure of *G. zeae* isolates collected from barley in the United States. Trichothecene biosynthesis gene (*TRI*)-based polymerase chain reaction (PCR) assays and 10 variable number tandem repeat (VNTR) markers were used to determine the genetic diversity and compare the trichothecene profiles of an older population (n = 115 isolates) of *G. zeae* collected in 1997 to 2000 with a newer population (n = 147 isolates) collected in 2008. Samples were from across the major barley-growing regions in North Dakota and Minnesota. The results of *TRI*-based PCR assays were further validated using a subset of 32 and 28 isolates of *G. zeae* by sequence analysis and gas chromatography, respectively. *TRI*-based PCR assays revealed that all the *G. zeae* isolates in both populations had markers for deoxynivalenol (DON), and the frequencies of isolates with a 3-acetyldeoxynivalenol (3-ADON) marker in the newer population were ≈11-fold higher than those among isolates in the older population. *G. zeae* populations from barley in the Midwest of the United States showed no spatial structure, and all the isolates were solidly in clade 7 of *G. zeae*, which is quite different from other barley-growing areas of world, where multiple species of *G. zeae are* commonly found in close proximity and display spatial structure. VNTR analysis showed high gene diversity (H = 0.82 to 0.83) and genotypic diversity but low linkage disequilibrium (LD = 0.02 to 0.07) in both populations. Low genetic differentiation (F_{ST} = 0.013) and high gene flow (Nm = 36.84) was observed between the two populations and among subpopulations within the same population (Nm = 12.77 to 29.97), suggesting that temporal and spatial variations had little influence on population differentiation in the Upper Midwest. Similarly, low F_{ST} (0.02) was observed between 3-ADON and 15-acetyldeoxynivalenol populations, indicating minor influence of the chemotype of *G. zeae* isolates on population subdivision, although there was a rapid increase in the frequencies of isolates with the 3-ADON marker in the Upper Midwest between the older collection made in 1997 to 2000 and the newer collection made in 2008. This study provides information to barley-breeding programs for their selection of isolates of *G. zeae* for evaluating barley genotypes for resistance to FHB and DON accumulation.

Additional keywords: *Fusarium graminearum*, *Hordeum vulgare*, population genetics.

Fusarium head blight (FHB), principally caused by *Gibberella zeae* (Schwein.) Petch (anamorph: *Fusarium graminearum* Schwabe), is one of the most destructive and widespread diseases of barley (*Hordeum vulgare* L.) in the United States (20,41) and other barley-producing regions of the world (31). The fungus produces different mycotoxins such as deoxynivalenol (DON), nivalenol (NIV), 3- and 15-acetyldeoxynivalenol (3-ADON and 15-ADON), and zearalenone (ZEA). DON is the most predominant mycotoxin detected on FHB-infected barley grains in the Upper Midwest of the United States (36). These mycotoxins create problems for the malt, food, and feed industries (20). In particular, a major concern of brewing industries is FHB-infected grains because Fusarium hydrophobins have been shown to cause beer gushing, and a portion of the DON present on barley malt can be transferred to the resultant beer (13).

Corresponding author: S. Neate;
E-mail address: Stephen.Neate@DEEDI.qld.gov.au

doi:10.1094/PHYTO-04-10-0101
© 2011 The American Phytopathological Society

G. zeae is a haploid and homothallic fungus, and has both sexual and asexual stages (5). The ascospores are produced initially on soil or residues and cause primary infection on the plant host and, with conducive conditions, conidia are produced on the host from the flowering stage and cause secondary infection (19). Knowledge of population genetic structure of plant pathogens is essential for predicting disease epidemics, and for screening and developing resistant cultivars (18). In particular, the nature of the genetic diversity and gene flow patterns of plant pathogens and their association with phenotypic characters such as aggressiveness and mycotoxin production are useful for the deployment of durable resistance and management of fungicide resistance (21).

North Dakota and Minnesota constitute the largest barley production region in the United States. This region accounted for ≈40% of both barley acreage and barley production in the United States during 2007 and 2008 (45). The climatic conditions, soils, and agronomic practices are suitable for barley cultivation in the region. However, warm temperatures and high rainfall and humidity frequently occur at the flowering and post-anthesis stages of barley in the region (17), which is highly favorable for FHB

epidemics and mycotoxin contamination. FHB reemerged as an important disease in the early 1990s or late 1980s in the Upper Midwest, causing serious negative impacts on grain production, grain quality and processing, and food and feed security and safety (20,41).

The genetic structure of *G. zeae* isolated from wheat has been analyzed from different countries using different molecular markers (2,12,14,22,24,34,52). Using sequence information of trichothecene biosynthesis gene (*TRI*) clusters, *TRI*-based polymerase chain reaction (PCR) assays were developed to identify trichothecene profiles of *G. zeae* (9,40,47). Chemical methods such as gas chromatography (GC) and mass spectrometry (MS) have been used to identify specific trichothecenes, and to determine the quantities produced in grains by the fungus (23).

In the United States, studies on the genetic structure of *G. zeae* populations and mycotoxin profiling have been conducted almost exclusively on isolates from wheat. In previous studies, Burlakoti et al. (6,7) analyzed limited isolates (n = 28) of *G. zeae* from barley and compared their genetic relationships and mycotoxin profiles with the isolates from wheat, potato, and sugar beet. Based on these analyses, the isolates from barley were genetically similar to those from the other three hosts (7); however, the isolates with the 3-ADON marker were not detected from barley (6). The isolates of *G. zeae* analyzed in the previous study (6) were mainly collected from a single field in Fargo, ND; therefore, the small sample size might have influenced the estimation of genetic diversity. In the current study, we included an expanded collection of isolates collected in 1997 to 2000 (older population) and 2008 (newer population). Both populations were collected from the same major barley-growing areas in North Dakota and Minnesota. Using these two *G. zeae* populations, we examined their relationships between molecular genetic variation and mycotoxin profiles. Although the same pathogen causes FHB infection in barley and wheat, host response to pathogen attack in barley is quite different than in wheat (41). For example, spread of pathogen within the spike is common in wheat but rare in barley. Barley also remains susceptible from flowering until harvest and mycotoxin accumulation in barley is higher than in wheat.

We hypothesize that temporal and spatial variations can influence the genetic exchange among *G. zeae* populations from barley in the Upper Midwest region of the United States, leading to changes in the frequencies of *G. zeae* isolates associated with different trichothecene-specific genetic markers. Our main goal was to investigate temporal and spatial influence on population genetic structure and trichothecene profiles of *G. zeae* from barley to assess whether barley behaved similarly to the other major small grain host, wheat. The specific objectives of this study were to (i) assess the incidence of *G. zeae* on barley collected in 2008 and (ii) analyze and compare the population genetic structure and trichothecene profiles of the newer (n = 147 isolates) and older (n = 115 isolates) populations of *G. zeae* from barley. Information from this study would assist in establishing a baseline to monitor the distribution and spread of different genotypes of *G. zeae* in barley-producing regions in the United States.

MATERIALS AND METHODS

Collection, isolation, and identification of *G. zeae*. Barley grain samples were collected 1997, 2000, and again in 2008 as a part of the annual regional barley crop-quality survey (4). Representative grain samples weighing 0.5 to 1 kg were collected during harvest from random farms and deliveries to county elevators in all North Dakota counties and from barley-growing counties in Minnesota. In 2008, we attempted to match the number and location of isolates to those made in 1997 to 2000.

The crop-reporting districts represented included 2 counties in Minnesota and 7 counties in North Dakota in the northern region of the states, 3 counties in Minnesota and 9 counties in North Dakota in the central region of the states, and 2 counties in Minnesota and 16 counties in North Dakota in the southern region of the barley-growing area (Tables 1 and 2).

To isolate *G. zeae*, in 1997 to 2000, 50 randomly selected kernels from each grain sample were plated mostly on acidified half-strength potato dextrose agar (AHSPDA). In 2008, 20 FHB-damaged barley kernels were randomly selected from each grain sample and plated on Komada's media (16) or AHSPDA. Only one isolate was recovered from each seed. A single-spore isolate was recovered for each of the *G. zeae* isolates as described previously (7,8). Each isolate was identified as *G. zeae* based on morphological characteristics (27,38) and molecular markers specific to *G. zeae* (28), as described previously (7,8). In all, 115 *G. zeae* isolates collected in 1997 to 2000 (herein after referred to as the older population) and 147 isolates collected in 2008 (herein after referred to as the newer population) were analyzed for trichothecene profile and population genetic structure (Table 2). For long-term storage, mycelial plugs grown in half-PDA were either dried in a laminar flow unit, stored in a cryopreservation vial at –20°C, or grown on PDA slants and stored at 5°C.

Incidence of *G. zeae* infection on barley seed in 2008. From the 2008 seed collection only, the incidence of *G. zeae* on barley seed was recorded and percent recovery of *G. zeae* in barley seed was calculated for each sample (Table 1). For the newer population, 33 barley samples from the northern region, 49 samples from the central region, and 58 samples from the southern region of North Dakota and Minnesota were analyzed for recovery of *G. zeae* isolates. From these 140 barley samples, 246 isolates of *G. zeae* were recovered. To balance isolates between the older population (n =115) and the newer population, 147 isolates were randomly selected from the 246 isolates collected in 2008.

DNA extraction. Preparation of mycelium culture and extraction of genomic DNA from each isolate of *G. zeae* were performed as described previously (7,8). For sequence analysis, the Qiagen DNeasy Plant Mini Kit (Qiagen Inc.) was used to extract genomic DNA from each isolate. Each DNA sample was quantified using a NanoDrop spectrophotometer (Model ND-1000; Thermo Scientific Inc., Waltham, MA), and working DNA concentration was adjusted to 10 ng/µl by adding sterile distilled water.

Sequence analysis. The portions of phosphate permease (PHO) and translation elongation factor-1α (TEF) genes of 32 randomly selected isolates (≈13% isolates from each population) of *G. zeae* (13 isolates from the older population and 19 isolates from the newer population) were amplified by PCR primer sets PHO1 × PHO6 and EF1T × EF2T, respectively (29), and sequenced. All PCR and thermal cycle conditions were used as described previously (7). The amplified PCR products were purified using QIAquick PCR purification kit (Qiagen Inc.); 3 µl of purified PCR was run in 1.5% agarose gel to confirm the amplification and the rest of the reaction was used for sequencing. Sequencing of these genes was performed in both sense and antisense directions at the Molecular Cloning Laboratories DNA sequencing service (San Francisco).

Using the Staden Package (39), the raw sequence data were edited manually. Both forward and reverse sequences of each isolate were aligned in MultAlin (10), and overlap was deleted to prepare a single sequence. The Basic Local Alignment Search Tool (3) confirmed the sequence identity of the two genes of all 32 isolates of *G. zeae*. To identify the phylogenetic group of these *G. zeae* isolates, sequence data of nine phylogenetically distinct clades of *F. graminearum* complex, and three other *Fusarium* spp. (*F. pseudograminearum*, *F. culmorum*, and *F. cerealis*) downloaded from previous studies (29,30), were used as reference sequences. Maximum parsimony analyses were performed using heuristic search in phylogenetic analysis using parsimony (PAUP* version 4.0b10) (43) and phylogenetic trees were constructed. Bootstrap analysis was performed with 1,000 pseudoreplicates

and 60% consensus levels. The nucleotide sequence data for the PHO and TEF genes of the 32 representative 2008 and 1997 to 2000 isolates were submitted to GenBank with the accession numbers HQ008647 to HQ008710. Phylograms have been submitted to TreeBase with the accession number 510830.

Trichothecene profiling of *G. zeae* populations by *TRI*-based PCR assays. *TRI*-based PCR assays (9,40,47) were used to determine the trichothecene profiles of *G. zeae* isolates. A *TRI13*-based PCR assay (9) was used to identify the isolate specific to the DON marker or NIV marker. Two *TRI*-based multiplex PCR assays (*TRI3* and *TRI12*) (40,47) were performed to further differentiate isolates with the DON marker into isolates with the 3-ADON marker and the 15-ADON marker. All primers, PCR reactions, and thermal cycle conditions were used as described previously (6). The primers were synthesized by Integrated DNA Technologies, Inc. (IDT, Coralville, IA) and all reagents used for PCR assays were from the Promega Corporation (Madison, WI), except *Taq* polymerase (purchased from New England Biolabs Inc., Ipswich, MA). For a negative control, water was used instead of template DNA. To estimate DNA band size in each sample, either a 100- or 1-kb DNA ladder (Invitrogen Corporation, Carlsbad, CA) was used. The PCR products were separated on 1.5% (wt/vol) prestained agarose (Amresco, Solon, OH) gels and stained with GelRed nucleic acid gel stain (Biotium Inc. Hayward, CA) as instructed by the manufacturer. Photographs were taken with a Fluorochem 2200 Image system (Alpha Innotech Corp., San Leandro, CA).

Population genetics analysis with variable number tandem repeat markers. Variable number tandem repeat (VNTR) marker repeatability and determination of allele robustness were confirmed from the DNA samples of *G. zeae* isolates extracted from two independent methods (phenol:chlorform:isoamyal alcohol [25:24:1] methods and Qiagen DNeasy Plant Mini Kit [Qiagen Inc.]). *G. zeae* reference isolate no. NRRL 31084 was kindly provided by Dr. Kerry O'Donnell, the United States Department of Agriculture (USDA) Agricultural Research Service (National Center for Agricultural Utilization Research, Peoria, IL) and was used for a positive control. Primers and water were used as negative controls. All PCR regents were purchased from Promega Corporation, except *Taq* polymerase (purchased from New England Biolabs Inc.). All primers were synthesized by IDT. Using the 10 VNTR primers (42), both *G. zeae* populations from barley were analyzed. PCR reactions were amplified in a PTC-100 Thermal Cycler (MJ Research, Watertown, MA). All PCR reac-

TABLE 1. Details of sampling sites, number of barley seed samples, and percent recovery of *Gibberella zeae* from barley seed in 2008 from major crop-reporting districts (CRDs) of barley production in North Dakota (ND) and Minnesota (MN)

CRD	Counties	State	Barley seed samples collected for 2008	Barley seed samples used for *G. zeae* isolation	Recovery of *G. zeae* in barley seed (%)	Total isolates of *G. zeae* recovered
Northern region						
MN 1	Kittson	MN	2	2	15	6
MN 1	Roseau	MN	2	2	15	6
ND 1	Burke	ND	5	5	0	0
ND 1	Divide	ND	2	2	0	0
ND 1	Renville	ND	10	5	13	13
ND 2	Bottineau	ND	14	5	3	3
ND 2	Rolette	ND	6	5	10	10
ND 3	Cavalier	ND	10	5	37	37
ND 3	Pembina	ND	2	2	28	11
Subtotal			53	33		86
Central region						
MN 1	Marshall	MN	11	5	17	17
MN 1	Pennington	MN	2	2	5	2
MN 1	Red Lake	MN	2	2	8	3
ND 1	Mountrail	ND	6	5	1	1
ND 1	Ward	ND	8	5	1	1
ND 2	Benson	ND	11	5	11	11
ND 2	Pierce	ND	6	5	6	6
ND 2	McHenry	ND	9	5	6	6
ND 3	Ramsey	ND	15	5	5	5
ND 3	Walsh	ND	4	2	28	11
ND 3	Grand Forks	ND	3	3	18	11
ND 3	Nelson	ND	6	5	16	16
Subtotal			83	49		90
Southern region						
MN 1	Clay	MN	2	2	25	10
MN 1	Polk	MN	5	5	11	11
ND 5	Eddy	ND	5	5	7	7
ND 5	Foster	ND	7	5	8	8
ND 5	Kidder	ND	2	2	3	1
ND 5	Sheridan	ND	6	5	0	0
ND 5	Stutsman	ND	7	5	0	0
ND 5	Wells	ND	8	5	0	0
ND 6	Barnes	ND	3	3	10	6
ND 6	Cass	ND	2	2	0	0
ND 6	Griggs	ND	4	4	16	13
ND 6	Steele	ND	2	2	18	7
ND 6	Traill	ND	2	2	10	4
ND 9	Ransom	ND	2	2	8	3
ND 9	LaMoure	ND	2	2	0	0
ND 9	McIntosh	ND	2	2	0	0
ND 9	Richland	ND	2	2	0	0
ND 9	Logan	ND	3	3	0	0
Subtotal	…	…	66	58	…	70
Total	…	…	202	140	–	246

tions and conditions were the same as described previously (7). The amplified PCR fragments (10 µl of each sample) were separated in 6% urea nondenaturing polyacrylamide gel system as described previously (37). The gel system was prestained by adding ethidium bromide (20 µl of 10 mg/ml) in buffer of lower reservoirs and was run at 350 V for 1 h. After prestaining, amplified PCR products were loaded in the gel, run at 350 V for 1.5 h, and photographed with a Fluorochem 2200 Image system (Alpha Innotech Corp.). Allele sizes were estimated by comparing DNA bands relative to a 50- or 100-bp DNA ladder (Invitrogen Corporation). *G. zeae* isolates with the same DNA band were considered as the same allele for each VNTR marker.

To analyze VNTR marker data, the *G. zeae* isolates from barley were classified into older or newer populations. Each population was further divided into three subpopulations (northern, central, and southern) according to the location of collections (Tables 1 and 2). The *G. zeae* isolates also were classified as belonging to the 3-ADON population or the 15-ADON population according to their chemotype. POPGENE version 1.32 (51) was used to analyze allele frequencies, gene diversity (*H*), (25,26), Nei's unbiased genetic identity (*I*) (26), and the overall estimate of gene flow (*Nm*). Allele frequency was determined for each allele for a single locus. *H* is a function of the number and frequencies of alleles at each locus and was estimated in the total population (H_T) and within population (H_S) (25,26). Multilocus 1.3 software (1) was used to calculate multilocus genotype (*G*), genotype diversity (*GD*), and multilocus linkage disequilibrium (*LD*). *GD* is defined as the probability that two individuals taken at random have different genotypes and was calculated by $(n/n - 1)\ (1 - \Sigma pi^2)$, where *pi* is the frequency of the *i*th genotype and *n* is the number of individuals sampled (1). The *LD* measures the nonrandom association of alleles at different gene loci in a population. The test of significance was determined by using 1,000 randomizations in all populations and subpopulations. GENALEX 6 (32) was used to analyze analysis of molecular variance (AMOVA) and pairwise gene flow (*Nm*). *Nm* was calculated based on F_{ST} as $Nm = 0.5\ [(1/F_{ST}) - 1]$, where F_{ST} was calculated as the proportion of the variance among populations relative to the total. To analyze the genetic variation in populations and subpopulations, AMOVA (11) was performed, where the variance was partitioned into covariance components to calculate three Φ fixation indices (Φ_{PR}, Φ_{RT}, and Φ_{PT}), and their levels of significance ($P < 0.05$ or $P < 0.01$) were determined using 1,000 permutations (11).

Chemical analysis of trichothecenes by GC. Using GC (44), the results of *TRI*-based genetic markers were verified with chemical analysis of trichothecene (DON, 3-ADON, 15-ADON, and NIV) produced by the 28 randomly selected representative isolates (17 from the newer population and 11 from the older population) in rice culture. In all, 13 isolates with the 3-ADON marker and 15 isolates with the 15-ADON marker were included in this experiment. Methods for growing the fungal isolates in rice culture and preparing samples for chemical analysis were as described previously (6). The experiments were repeated and each experiment was laid out with randomized complete block design with three replicates. Mirex (Absolute Standards, Inc., Hamden, CT) was used as an internal standard and the standard at 1 µg/g was injected that has all four trichothecenes for identification. *Fusarium* spp. trichothecene standards for DON, 3-ADON, 15-ADON, and NIV were purchased from Sigma-Aldrich (St. Louis). The detection limit was 0.5 µg/g for each trichothecene, and selected ion monitoring mode was used for each trichothecene analysis as described previously (8).

Data analysis for trichothecenes profiling. Polymorphic DNA fragments were scored manually based on the presence or absence of DNA bands generated by *TRI*-based PCR assays. To test the temporal and spatial distributions of isolates with 3-ADON and 15-ADON markers, the χ^2 test was performed using the Statistical Analysis System (SAS, version 9.1; SAS Institute, Cary, NC). Mycotoxin assayed at multiple times was considered as replicates. Standard errors for mycotoxins were calculated to observe the difference between different cultures for the same isolates. All values for mycotoxins produced by *G. zeae* isolates were presented with mean values ± standard errors. In addition, Pearson's correlation analysis was performed to determine the relationships between *TRI*-based PCR genetic markers and chemical analyses.

RESULTS

Incidence of *G. zeae* infection on barley seed in 2008. Percent recovery of *G. zeae* from barley seed was higher in the barley grain samples collected from the eastern northern region than from the western northern and southern region (Table 1). In

TABLE 2. Frequency distribution of *Gibberella zeae* isolates with 3-acetyldeoxynivalenol (3-ADON) and 15-acetyldeoxynivalenol (15-ADON) markers in newer (2008) and older (1997 to 2000) populations from major crop-reporting districts (CRDs) of barley production in North Dakota (ND) and Minnesota (MN) determined by trichothecene biosynthesis gene-based multiplex polymerase chain reaction assays

			Newer population			Older population		
CRD	State	Counties	*n*	3-ADON	15-ADON	*n*	3-ADON	15-ADON
Northern Region								
MN 1	MN	Kittson, Roseau	9	5	4	6	1	5
ND 1	ND	Renville	12	3	9	6	0	6
ND 2	ND	Bottineau, Rolette	8	3	5	2	0	2
ND 3	ND	Cavalier, Pembina, Towner	17	10	7	12	0	12
Subtotal	…	…	46	21	25	26	1	25
Central region								
MN 1	MN	Marshall, Pennington, Red Lake	18	5	13	10	0	10
ND 1	ND	Montrail, Ward	2	1	1	4	0	4
ND 2	ND	Benson, Pierce, McHenry	8	3	5	12	1	11
ND 3	ND	Grand Forks, Nelson, Ramsey, Walsh	18	12	6	12	0	12
Subtotal	…	…	46	21	25	38	1	37
Southern region								
MN 1	MN	Polk, Clay, Mahnomen,[a] Norman [a]	19	7	12	12	1	11
ND 5	ND	Eddy, Foster, Kidder, Sheridan, Wells [a]	14	3	11	12	0	12
ND 6	ND	Barnes, Cass,[a] Griggs, Steele,[a] Traill	18	4	14	14	1	13
ND 9	ND	Ransom, Dickey,[a] LaMoure,[a] McIntosh,[a] Richland,[a] Sargent [a]	4	0	4	13	0	13
Subtotal	…	…	55	14	41	51	2	49
Total	…	…	147	56	91	115	4	111

[a] *G. zeae* isolates from these counties were only from the older population (1997 to 2000).

particular, North Dakota ND 3 Crop Reporting District had the highest level of recovery of *G. zeae* (Cavalier 37% and Pembina 28%). In contrast, *G. zeae* was not recovered from barley samples collected from northwestern crop counties: Burke and Divide (ND 1); Sheridan, Wells, and Stutsman (ND 5); and Cass (ND 6) and LaMoure, Logan, McIntosh, and Richland of ND 9. In the central region, recovery of *G. zeae* isolates was higher in the samples collected at Walsh, Grand Forks, and Nelson (ND 3) than the samples collected from other sites (Table 1). In Minnesota, samples from Kittson, Roseau, Marshall, and Clay (MN 1) had higher recovery of *G. zeae* than other crop-reporting districts. In all, 246 isolates of *G. zeae* were recovered, and 147 isolates were randomly selected and used for trichothecene profiling and population structure analysis.

Sequence analysis. The maximum parsimony analysis from sequence data of PHO and TEF genes of the 32 representative isolates of *G. zeae* identified that all the isolates belonged to clade 7 of *G. zeae* (Fig. 1).

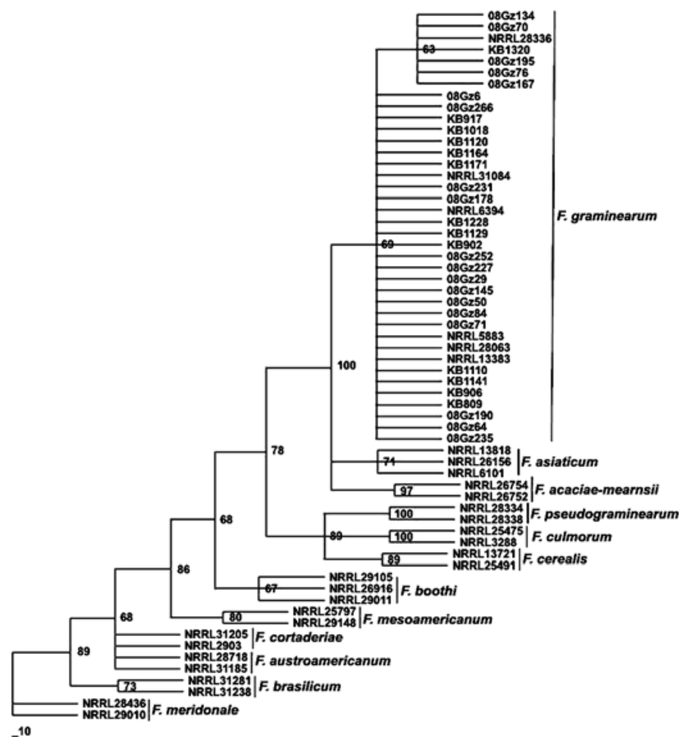

Fig. 1. Maximum parsimony phylogram of *Fusarium graminearum* isolates from this study and isolates of *Fusarium* spp. from O'Donnell et al. (29,30) generated using sequence from phosphate permease (PHO1 × PHO6) genes. Isolates from the new population begin with 08Gz and isolates with the old population begin with KB. Sequences of isolates beginning with NRRL were downloaded from the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/BLAST) deposited by O'Donnell et al. (29,30).

Trichothecene profiling of *G. zeae* isolates using *TRI*-based PCR assays. All of the isolates of *G. zeae* analyzed had characteristic DON markers, as indicated by amplification of an ≈799-bp fragment size in the *TRI13*-based PCR assay. Further differentiation of the isolates with DON markers into isolates with 3-ADON markers or 15-ADON markers was performed using two sets of multiplex PCR assays (*TRI3*- and *TRI12*-based assays). Both *TRI3*- and *TRI12*-based multiplex PCR assays showed consistent results.

The frequency of *G. zeae* isolates with the 3-ADON marker in the newer population (38%) was ≈11-fold higher than in the older population (3.5%), indicating that there was a rapid increase in the frequency of isolates with 3-ADON markers in the Upper Midwest region of United States over 8-10 years (Table 2). The χ^2 analysis showed a highly significant difference ($\chi^2 = 43.38$, $P = 0.001$) between the newer and older populations for the frequency distribution of genetic markers for 3-ADON and 15-ADON. In the newer population, the *G. zeae* isolates from the southern region were significantly different from isolates from northern and central regions ($\chi^2 = 4.53$, $P = 0.03$) for the frequency distribution of genetic markers for 3-ADON and 15-ADON. In particular, isolates from ND 3 (spanning northern and central counties) in North Dakota had higher frequencies of 3-ADON markers compared with isolates from other crop-reporting districts (Table 2).

Population genetic analysis with VNTR markers. The 10 VNTR markers used in this study were highly polymorphic and the number of alleles in each marker (locus) was 7 to 13 (Table 3). The allele sizes were 110 to 300 bp (Table 3). Allele frequencies of each locus were 0.005 to 0.27. All 262 isolates of *G. zeae* analyzed from the combined older and newer populations were distinct genotypes, indicating that there was 100% genotypic diversity (Table 4). High H was observed in both populations ($H = 0.85$ to 0.86) and in three subpopulations (northern, central, and southern) of each population ($H = 0.81$ to 0.83). Very low linkage disequilibrium ($LD = 0.02$ to 0.07, $P < 0.01$) was observed in both populations and all subpopulations (Table 4).

High overall Nm (36.84) and low F_{ST} (0.013) was observed between the older population and the newer population, indicating that a low level of genetic differentiation occurred in *G. zeae* populations from barley in the Upper Midwest. The overall I was also high (0.86) between the two populations. Within each population, high values of I (0.75 to 0.92) and Nm (12.77 to 29.97) were observed among subpopulations from northern, central, and southern regions (Table 5), suggesting that spatial variation has a very low effect on population subdivision in *G. zeae*. However, pairwise I and Nm were higher between northern and central subpopulations than other pairs of subpopulations showing some regional influence. Hierarchical AMOVA revealed low genetic variation between the two populations (1%) and between subpopulations within the population (2%) (Table 6). In contrast, genetic variation among the isolates within subpopulation was high (97%) (Table 6).

TABLE 3. Variable number tandem repeat (VNTR) markers their amplicon size range, number of alleles, and allele frequencies produced by *Gibberella zeae* isolates of barley[a]

Locus	Allele size range (bp)	Number of alleles	Allele frequencies
HK630/Sc6/Ct1.371/112 325	207–283	8	0.04–0.23
HK913/Sc1/Ct1.73/664	205–262	7	0.06–0.23
HK917/Sc1/Ct1.82/2471	190–262	9	0.02–0.21
HK957/ Sc1/ Ct.191/ 16 055	150–298	13	0.03–0.16
HK965/Sc2/Ct1.154/51 671	195–300	9	0.03–0.18
HK967/Sc2/Ct1.154/53 868	188–255	9	0.03–0.27
HK977/Sc3/Ct1.208/47 696	190–248	9	0.05–0.19
HK1043/Sc1/Ct1.52/41 839	190–286	10	0.038–0.21
HK1059/Sc3/Ct1.196/164 228	185–248	7	0.09–0.21
HK1073/Sc6/Ct1.398/70 812	110–260	13	0.005–0.13

[a] VNTR markers from Suga et al. (42).

When the isolates were grouped into the 3-ADON and 15-ADON populations, the overall estimate of Nm was high (23.64) and F_{ST} was low (0.02), confirming low genetic differentiation between the 3-ADON and 15-ADON populations. AMOVA showed 3% genetic variation between them (data not shown). High estimates of the pairwise comparisons of gene flow (Nm) (11.24 to 28.71) were observed between subpopulations of 3-ADON or 15-ADON (Table 7).

Chemical analysis of trichothecenes by GC. All 28 representative isolates analyzed produced DON and either 3-ADON or 15-ADON (Table 8). None of the isolates produced NIV. Analysis of variance results showed that the isolates differed significantly ($P < 0.01$) in production of DON, 3-ADON, and 15-ADON. Pearson's correlation analysis showed 100% correlation among results from *TRI*-based PCR assays and chemical analysis by GC. The isolates from the newer population produced significantly higher amounts of DON ($P = 0.007$, t test) and 3-ADON ($P = 0.012$, t test) compared with isolates from the older population (Table 8). The average DON and 3-ADON produced by isolates of the newer population was 495 and 307 µg/g, respectively, while the average DON and 3-ADON production by isolates of the older population was 128 and 67 µg/g, respectively. In addition, 3-ADON-producing isolates produced significantly ($P = 0.007$, t test) higher amounts of DON (605 µg/g) than 15-ADON producing isolates (141 µg/g).

DISCUSSION

Our main goal was to determine the population dynamics of *G. zeae* from barley over time and space using computational, trichothecene profiling and population genetic analysis approaches. In this study, we analyzed two populations (the newer population was collected in 2008 and older population was collected in 1997 to 2000) of *G. zeae* from the Upper Midwest of the United States. Sequence analyses with PHO and TEF genes from representative isolates confirm the identity of isolates to clade 7 of *G. zeae* (7). For this proof of concept, we further utilized VNTR and *TRI*-based molecular markers and chemical analysis (GC) to detect

TABLE 4. Genetic diversity and multilocus linkage disequilibrium analysis of *Gibberella zeae* isolates from the newer population (2008) and the older population (1997 to 2000) in the Upper Midwest of the United States

Population	*N*[a]	*G*[b]	*GD*[c]	*H*[d]	*LD*[e]
Newer population					
Northern	46	46	1	0.83	0.03**
Central	46	46	1	0.82	0.06**
Southern	55	55	1	0.83	0.06**
Subtotal	147	147			
Older population					
Northern	25	25	1	0.81	0.07**
Central	38	38	1	0.83	0.06**
Southern	52	52	1	0.83	0.02**
Subtotal	115	115	…	…	…
Total	262	262	…	…	…

[a] Sample size.
[b] Number of distinct genotypes.
[c] Genetic diversity (GD) within population was calculated as $GD = (n/n - 1)(1 - \Sigma pi^2)$, where pi is the frequency of the ith genotype and n is the number of individuals sampled (1).
[d] Gene diversity (H) (Nei) within populations (25,26).
[e] Measure of multilocus linkage disequilibrium (LD) (1); ** = significant at $P < 0.01$.

TABLE 5. Pairwise comparisons of gene flow (above diagonal) and genetic identity (below diagonal) of *Gibberella zeae* subpopulations from barley in the upper Midwest of the United States[a]

	Newer[b]			Older[b]		
Population	Northern	Central	Southern	Northern	Central	Southern
Newer, Northern	…	23.82	16.06	13.80	21.97	13.51
Newer, Central	0.84	…	12.77	10.58	10.37	10.75
Newer, Southern	0.78	0.75	…	16.34	15.08	20.40
Older, Northern	0.73	0.69	0.77	…	29.97	16.10
Older, Central	0.81	0.68	0.76	0.92	…	27.75
Older, Southern	0.75	0.71	0.82	0.76	0.84	…

[a] Gene flow (Nm) was calculated as $Nm = 0.5[(1/F_{ST}) - 1]$ using GENALEX 6 (32), where F_{ST} was calculated as the proportion of the variance among populations relative to the total variance. Probability of obtaining equal or lower F_{ST} value was determined by 1,000 randomizations by permuting individuals among populations. Genetic identity = Nei's unbiased genetic identity (26).
[b] Newer denotes isolates made in 2008 and older denotes isolates made in 1997 to 2000.

TABLE 6. Analysis of molecular variance for *Gibberella zeae* populations of barley in the upper Midwest of the United States

Hierarchical analysis[a]	df	Estimated variance	Variation (%)	Φ[b]	*P* value[c]
Between population (ΦRT)	1	0.05	1	0.01	0.001
Between subpopulations within population (ΦPR)	4	0.11	2	0.02	0.001
Individuals within subpopulations (ΦPT)	256	4.24	97	0.03	0.001

[a] Variance was partitioned into two populations as newer population (2008) and older population (1997 to 2000), among three subpopulations (northern, central, and southern) within population, and individuals within three subpopulations of newer or older population.
[b] ΦRT was calculated as the proportion of the variance among groups, relative to the total variance. ΦPR was calculated as the proportion of variance among subpopulations within population, relative to the variance among and within subpopulations. ΦPT was calculated as proportion of variance among populations and subpopulations of individuals, relative to the total variance (11).
[c] Probability of obtaining equal or lower Φ value was determined by 1,000 random permutations.

genetic and trichothecene variations in the two populations of *G. zeae*. Although high genetic diversity and high genetic exchange were detected between the older and newer populations, no genetic differentiation was demonstrated, confirming that a single large population of *G. zeae* exists in the Upper Midwest (7), which is quite different from other continents where multiple species are commonly found together. However, trichothecene profiling revealed large changes in the frequency distribution of *G. zeae* isolates with 3-ADON and 15-ADON markers over 8 to 10 years, which is a fundamental difference between the newer and older populations of *G. zeae*.

A higher percentage of *G. zeae* incidence was observed from northern and central-western parts of barley-growing counties (most of the counties of ND 3 of North Dakota, some counties of MN 1 and ND 1 of Minnesota and North Dakota) compared with northeastern and southern counties in 2008. Salas et al. (33) found

TABLE 7. Pairwise comparisons of gene flow (above diagonal) and genetic identity (below diagonal) among 3-acetyldeoxynivalenol (3-ADON) and 15-acetyldeoxynivalenol (15-ADON) populations of *Gibberella zeae* from barley in the upper Midwest of the United States[a]

Population	Newer, 3ADON	Older, 15ADON	Newer, 15ADON
Newer, 3ADON	…	11.24	16.47
Older, 15ADON	0.72	…	28.71
Newer, 15ADON	0.80	0.87	…

[a] Gene flow (*Nm*) was calculated as $Nm = 0.5[(1/F_{ST}) - 1]$ using GENALEX 6 (32), where F_{ST} was calculated as the proportion of the variance among populations relative to the total variance. Probability of obtaining equal or lower F_{ST} value was determined by 1,000 randomizations by permuting individuals among populations. Because only four isolates of 3ADON producers were found in the older populations, we excluded them in the pairwise comparison. Genetic identity = Nei's unbiased genetic identity (26). Newer and older = populations from 2008 and 1997 to 2000, respectively.

G. zeae to be the dominant species (≈64%) among other species of *Gibberella* from the Upper Midwest. These barley-growing areas have had the highest FHB severity and rejection for malting barley during the past 10 years (35). East-central North Dakota and western Minnesota is the largest region of barley production in Minnesota and North Dakota and high FHB severity has been observed during the 1990s FHB epidemics. Climatic conditions such as rainfall and humidity are higher in these regions compared with western and southern regions.

Trichothecene profiling of *G. zeae* isolates showed that all the isolates had the DON marker and none of isolates had NIV markers in both newer and older populations. Yang et al. (50) found 98% isolates with DON markers among the isolates in clade 7 of *G. zeae* from barley collected from seven provinces in China. The frequency of *G. zeae* isolates with the 3-ADON marker increased 11-fold in barley in the Upper Midwest region over 8 to 10 years. This result is in agreement with the previous studies in wheat in this region (15,48), which showed a recent increase in the frequency of *G. zeae* isolates with the 3-ADON marker. A significant shift in the frequency of *G. zeae* isolates with the DON marker to isolates with the NIV markers has also been reported from Europe (46). Zhang et al. (53) reported recent increase in *F. asiaticum* isolates with the 3-ADON marker replacing isolates with the 15-ADON marker in southern China. Yang et al. (50) also reported that *F. asiaticum* (clade 6) isolates with DON markers have been replacing the traditional isolates with NIV markers in barley-growing areas in China. They further suggested that the isolates with DON markers were more virulent and aggressive than the isolates with NIV markers and that this may be a factor in the change.

To date, no clear evidence has been provided for the cause of rapid change in trichothecene-producing isolates of *G. zeae* in wheat. Ward et al. (48) hypothesized that the wheat isolates with

TABLE 8. Trichothecene production by 28 representative *Gibberella zeae* isolates of barley from newer (2008) and older (1997–2000) populations by gas chromatography and presence of trichothecene-specific markers detected by trichothecene biosynthesis gene-based polymerase chain reaction assays[a]

Isolate[b]	Assay result for markers[c]			Mean concentration (µg/g) ± SE[d]		
	DON	3ADON	15-ADON	DON	3-ADON	15-ADON
08Gz6	+	–	+	10 ± 7	ND	2 ± 1
08Gz29	+	+	–	790 ± 361	572 ± 286	13 ± 11
08Gz50	+	+	–	1217 ± 494	422 ± 200	1 ± 1
08Gz64	+	–	+	10 ± 2	ND	3 ± 1
08Gz70	+	–	+	323 ± 242	2 ± 1	19 ± 6
08Gz71	+	+	–	1657 ± 445	697 ± 251	6 ± 3
08Gz84	+	+	–	1235 ± 388	336 ± 99	1 ± 1
08Gz134	+	+	–	445 ± 95	147 ± 36	ND
08Gz145	+	–	+	304 ± 136	2 ± 1	9 ± 2
08Gz167	+	+	–	584 ± 429	112 ± 59	ND
08Gz178	+	–	+	266 ± 150	ND	13 ± 5
08Gz190	+	–	+	256 ± 185	ND	10 ± 4
08Gz195	+	+	–	499 ± 188	241 ± 85	ND
08Gz227	+	+	–	616 ± 338	192 ± 121	1 ± 1
08Gz235	+	–	+	45 ± 20	ND	17 ± 7
08Gz252	+	+	–	39 ± 12	42 ± 19	ND
08Gz266	+	–	+	113 ± 86	ND	5 ± 1
KB1018	+	–	+	26 ± 15	ND	2 ± 1
KB1110	+	–	+	76 ± 23	ND	1 ± 0.4
KB1120	+	–	+	125 ± 19	ND	7 ± 0.4
KB1129	+	+	–	43 ± 8	22 ± 9	ND
KB1141	+	–	+	118 ± 55	ND	12 ± 3
KB1164	+	–	+	156 ± 92	1 ± 1	15 ± 12
KB1228	+	+	–	419 ± 206	106 ± 33	ND
KB1320	+	+	–	23 ± 6	15 ± 9	ND
KB809	+	–	+	5 ± 2	ND	2 ± 1
KB902	+	+	–	293 ± 71	126 ± 18	ND
KB906	+	–	+	69 ± 50	1 ± 1	10 ± 5
Control	…	…	…	ND	ND	ND

[a] DON = deoxynivalenol, 3-ADON = 3-acetyldeoxynivalenol, and 15-ADON = 15-acetyldeoxynivalenol.
[b] Isolates from the newer population begin with 08Gz and isolates with the older population begin with KB.
[c] Presence and absence indicated by + and –, respectively.
[d] SE = standard error and ND = not detected.

3-ADON markers existed in eastern Canada originally and moved into western Canada and the Upper Midwest of the United States via global seed trade. Several researchers suggested that selection forces such as use of resistant wheat cultivars and fungicides could be responsible for a shift in frequency of *G. zeae* isolates with 3-ADON and 15-ADON markers (15,48). Our previous study showed no significant difference in the frequency distribution of *G. zeae* isolates with 3-ADON and 15-ADON markers between isolates collected from resistant and susceptible wheat cultivars (6). Another possible explanation could be that cropping patterns and climatic factors might be responsible for the shift in species or chemotypes (46,48). However, Yang et al. (50) did not find significant influence of climatic conditions and crop rotations in the distribution of *G. zeae* isolates with DON and NIV markers in China. Ward et al. (47) reported that polymorphism in trichothecene-specific strains of *G. zeae* has occurred in the process of multiple speciations and is maintained by balancing selection. However, there is no evidence for balancing selection playing a role in increasing the frequency of the 3-ADON-producing isolates. It is difficult to speculate on the cause of the rapid change in the frequency of trichothecene-specific isolates of *G. zeae* without extensive further studies.

Less spatial variation was observed in overall distribution of isolates with 3-ADON and 15-ADON markers. Intriguingly, the frequencies of isolates with 3-ADON markers were higher in the northern and central region compared with the southern region. In particular, the frequencies of isolates with 3-ADON markers were higher from the barley-cropping counties of ND 3 (Cavalier, Pembina, Grand Forks, and Nelson) of North Dakota than other counties. High FHB severity and rejection of malting barley due to DON contamination during the past 10 years was observed in these counties (35). Aggressiveness of the pathogen is one factor in increased disease severity. High aggressiveness associated with 3-ADON isolates may be the cause for the presence of a high frequency of isolates with the 3-ADON marker from counties with high FHB epidemics. In chemical analysis of trichothecene by growing the fungal isolates in rice culture, 3-ADON-producing isolates also produced significantly ($P = 0.007$, t test) higher amount of DON (605 μg/g) than 15-ADON-producing isolates (141 μg/g). These results are in agreement with the previous finding of Ward et al. (48), who also stated that 3-ADON-producing isolates were more aggressive and may have higher fitness advantages than 15-ADON-producing isolates.

Sequence analysis of the 32 representative isolates revealed that all the isolates were in clade 7 of *G. zeae*, indicating that, in barley in the Midwest, the *F. graminearum* complex is composed of a single species (clade). Several studies showed that most *G. zeae* isolates from different hosts from North America were in clade 7 (7,15,30). Our study showed that the *G. zeae* population from barley in the Midwest is quite different from other barley-growing areas of world, where multiple species of *F. graminearum* were commonly reported and also displayed spatial structure (50). Yang et al. (50) reported that, in barley samples from seven provinces of China, only 2.4% of species were in clade 7 of *G. zeae* and 90% were in clade 6 (*F. asiaticum*). Population genetic analysis with VNTR markers showed very high values of H in all *G. zeae* populations, with 100% distinct genotypes from both populations. VNTR markers were highly polymorphic, as indicted by higher numbers of alleles and wide range of allele frequencies. The genetic diversity of the fungal populations from barley was higher than our previous population analysis of *G. zeae* isolates from wheat and barley (7) and most of the studies from North America and other cereal-growing parts of the world (2,50,52). Our findings suggest that primary diverse ascospores (sexual spores) could have a major role in FHB epidemics of barley. The LD values were very low in all populations (0.02 to 0.07), indicating that those populations were close to a randomly mating population.

No evidence for population subdivision was observed in the Upper Midwest over 8 to 10 years, as indicated by low F_{ST} values and high Nm and I values between the newer population and the older population of *G. zeae*. Similarly, minimal influence of space on population differentiation was observed, as indicated by high pairwise Nm values among three subpopulations (northern, central, and southern) within each population. However, higher pairwise Nm was observed between northern and central subpopulations compared with other subpopulation pairs, suggesting little existence of population at regional level. Wright (49) stated that those populations having ≥4.0 Nm values are considered to be part of a large single population. Our result indicated that all subpopulations within a population are part of a single large population of *G. zeae* on barley in the Upper Midwest region. These results agree with our previous study (7) and other findings of population studies of *G. zeae* from wheat (34,52).

When overall population was grouped into 3-ADON and 15-ADON populations, low F_{ST} and high Nm values were observed between overall 3-ADON and 15-ADON populations and between 3-ADON and 15-ADON populations within the newer or older population. Although the frequency of isolates with 3-ADON markers has increased rapidly, our results indicated that frequency distribution of trichothecene-specific isolates of *G. zeae* had no effect on population differentiation in the Upper Midwest barley-growing region over 8 to 10 years. These findings are in contrast to the previous findings (15,48), that reported that population subdivision was due to chemotype differences in *G. zeae*.

In 2008, barley production declined (≈32%) in North Dakota, Idaho, and Montana (35). FHB epidemics and associated DON contamination in barley was one of the major causes for the decrease in barley acreage and production in the past two decades (35). In this perspective, the development of effective management strategies is imperative to cope with FHB epidemics and DON contamination in barley. Population genetic analysis and trichothecene profiling of *G. zeae* revealed the overall dynamics of the fungal populations in the region over time. More importantly, the recent population of 3-ADON-producing *G. zeae* has developed. Therefore, this information should be taken into consideration for breeding barley with resistance to FHB. Additionally, regular monitoring of the distribution and spread of different genotypes of *G. zeae* in the Upper Midwest and other barley-producing states in the United States is necessary.

ACKNOWLEDGMENTS

We thank K. O'Donnell (USDA) for providing representative strains of the *G. Zeae*, H. Chawla for technical assistance in laboratory work, and S. Mamidi for his valuable suggestions in data analysis. This work was funded by a grant from the American Malting Barley Association and the collection of barley samples was funded by the North Dakota Barley Council.

LITERATURE CITED

1. Agapow, P. M., and Burt, A. 2001. Indices of multilocus linkage disequilibrium. Mol. Ecol. Notes 1:101-102.
2. Akinsanmi, O. A., Backhouse, D., Simpfendorfer, S., and Chakraborty, S. 2006. Genetic diversity of Australian *Fusarium graminearum* and *F. pseudograminearum*. Plant Pathol. 55:494-504.
3. Alstchul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller W., and Lipman, D. J. 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucleic Acids Res. 25:3389-3402.
4. Barr, J. M., and Schwarz, P. B. 2008. 2008 Barley Crop Quality Report. North Dakota Barley Council, Fargo.
5. Bowden, R. L., and Leslie, J. F. 1999. Sexual recombination in *Gibberella zeae.* Phytopathology 89:182-188.
6. Burlakoti, R. R., Ali, S., Secor, G. A., Neate, S. M., McMullen, M. P., and Adhikari, T. B. 2008. Comparative mycotoxin profiles of *Gibberella zeae* populations from barley, wheat, potatoes, and sugar beets. Appl. Environ. Microbiol. 74:6513-6520.

7. Burlakoti, R. R., Ali, S., Secor, G. A., Neate, S. M., McMullen, M. P., and Adhikari, T. B. 2008. Genetic relationships among populations of *Gibberella zeae* from barley, wheat, potato, and sugar beet in the Upper Midwest of the United States. Phytopathology 98:969-976.
8. Burlakoti, R. R., Estrada, R., Jr., Rivera, V. V., Boddeda, A., Secor, G. A., and Adhikari, T. B. 2007. Real-time PCR quantification and mycotoxin production of *Fusarium graminearum* in wheat inoculated with isolates collected from potato, sugar beet and wheat. Phytopathology 97:835-841.
9. Chandler, E. A., Simpson, D. R., Thomsett, M. A., and Nicholson, P. 2003. Development of PCR assays to *Tri7* and *Tri13* trichothecene biosynthetic genes and characterization of chemotypes of *Fusarium graminearum*, *Fusarium culmorum* and *Fusarium cerealis*. Physiol. Mol. Plant Pathol. 62:357-367.
10. Corpet, C. 1988. Multiple sequence alignment with hierarchical clustering. Nucleic Acids Res. 16:10881-10890.
11. Excoffier, L., Smouse, P. E., and Quattro, J. M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotype: Application to human mitochondrial DNA restriction sites. Genetics 131:479-491.
12. Fernando, W. G. D., Zhang, J. X., Dusabenyagasani, M., Guo, X. W., Ahmed, H., and McCallum, B. 2006. Genetic diversity of *Gibberella zeae* isolates from Manitoba. Plant Dis. 90:1337-1342.
13. Gabe, L.-A., Schwarz, P., and Ehmer, A. 2009 Beer gushing. Pages 185-212 in: Beer. A Quality Perspective. C. W. Bamforth, ed. Academic Press, New York.
14. Gale, L. R., Chen, L.-F, Hernick, C. A., Takamura, K., and Kistler, H. C. 2002. Population analysis of *Fusarium graminearum* from wheat fields in eastern China. Phytopathology 92:1315-1322.
15. Gale, L. R., Ward, T. J., Balmas, V., and Kistler, H. C. 2007. Population subdivision of *Fusarium graminearum* sensu stricto in the Upper Midwestern United States. Phytopathology 97:1434-1439.
16. Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soils. Rev. Plant Prot. Res. 8:114-125.
17. Markell, S. G., and Francl, L. J. 2003. Fusarium head blight inoculum: Species prevalence and *Gibberella zeae* spore type. Plant Dis. 87:814-820.
18. McDonald, B. A., and McDermott, J. M. 1993. Population genetics of plant pathogenic fungi. Bioscience 43:311-319.
19. McMullen, M. P., Enz, J., Lukach, J., and Stover, R. 1997. Environmental conditions associated with Fusarium head blight epidemics of wheat and barley in the Northern Great Plains, North America. Cereal Res. Commun. 25:777-778.
20. McMullen, M. P., Jones, R., and Gallenberg, D. 1997. Scab of wheat and barley: A re-emerging disease of devastating impact. Plant Dis. 81:1340-1348.
21. Miedaner, T., Cumagun, C. J. R., and Chakraborty, S. 2008. Population genetics of three head blight pathogens *Fusarium graminearum*, *F. pseudograminearum*, and *F. culmorum*. J. Phytopathol. 156:129-139.
22. Miedaner, T., Schilling, A. G., and Geiger, H. H. 2001. Molecular genetic diversity and variation for aggressiveness in populations of *Fusarium graminearum* and *Fusarium culmorum* sampled from wheat fields in different countries. J. Phytopathol. 149:641-648.
23. Mirocha, C. J., Abbas, H. K., Windels, C. E., and Xie, W. 1989. Variation in deoxynivalenol, 15-acetyl-deoxynivalenol, 3-acetyl-deoxynivalenol, and zeralenone production by *Fusarium graminearum* isolates. Appl. Environ. Microbiol. 55:1315-1316.
24. Mishra, P. K., Tewari, J. P., Clear, R. M., and Turkington, T. K. 2004. Molecular genetic variation and geographical structuring in *Fusarium graminearum*. Ann. Appl. Biol. 145:299-307.
25. Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA 70:3321-3323.
26. Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:83-590.
27. Nelson, P. E., Toussoun T. A., and Marasas, W. F. O. 1983. *Fusarium Species*: An Illustrated Manual for Identification. Pennsylvania State University Press, University Park.
28. Nicholson, P., Simpson, D. R., Weston G., Rezanoor, H. N., Lees, A. K., Parry, D. W., and Joyce, D. 1998. Detection and quantification of *Fusarium culmorum* and *Fusarium graminearum* in cereals using PCR assays. Physiol. Mol. Plant Pathol. 53:13-17.
29. 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. USA 97:7905-7910.
30. 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.
31. Parry, D. W., Jenkinson, P., and McLeod, L., 1995. Fusarium ear blight (scab) in small grain cereals—a review. Plant Pathol. 44:207-238.
32. Peakall, R., and Smouse, P. E. 2006. GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes 6:288-295.
33. Salas, B., Steffenson, B. J., Casper, H. H., Tacke, B., Prom, L. K., Fetch, T. G., Jr., and Schwarz, P. B. 1999. *Fusarium* species pathogenic to barley and their associated mycotoxins. Plant Dis. 83:667-674.
34. Schmale, D. G., III, Leslie, J. F., Zeller, K. A., Saleh, A. A., Shields, E. J., and Bergstrom, G. C. 2006. Genetic structure of atmospheric populations of *Gibberella zeae*. Phytopathology 96:1021-1026.
35. Schwarz, P., Horsley, R., and Hertsgaard, K. 2008. Survey of barley producers in Idaho, Montana, and North Dakota. Master Brew. Assoc. Am. 45:99-107.
36. Schwarz, P. B. 2003. Impact of *Fusarium* head blight on malting and brewing quality of barley. Pages 395-418 in: Fusarium Head Blight of Wheat and Barley. K. J. Leonard and W. R. Bushnell, eds. The American Phytopathological Society Press, St. Paul, MN.
37. Shi, J., Ward, R., Wang, D., and Lewis, J. 2001. Application of a high throughput, low cost, non-denaturing polyacrylamide gel system for wheat microsatellite mapping. Pages 25-30 in: National Fusarium Head Blight Forum, Erlanger, KY. M. Canty, J. Lewis, L. Silver, and R. W. Ward, eds. Kinko's Publisher, Okemos, MI.
38. Stack, R. W., and McMullen, M. 1985. Head blighting potential of *Fusarium* species associated with spring wheat heads. Can J. Plant Pathol. 7:79-82.
39. Staden, R. 1994. The Staden package. Pages 9-170 in: Methods in Molecular Biology, Vol. 25. A. M. Griffin, and H. G. Griffin, eds. Humana Press, Totowa. NJ.
40. Starkey, D. E., Ward, T. J., Aoki, T., Gale, L. R., Kistler, H. C., Geiser, D. M., Suga, H., Toth, B., Varga, J., and O'Donnell, K. 2007. Global molecular surveillance reveals novel *Fusarium* head blight species and trichothecene toxin diversity. Fungal Genet. Biol. 44:1191-1204.
41. Steffenson, B. J. 2003. *Fusarium* head blight of barley: Impact, epidemics, management, and strategies for identifying and utilizing genetic resistance. Pages 241-295 in: Fusarium Head Blight of Wheat and Barley. K. J. Leonard and W. R. Bushnell, eds. The American Phytopathological Society Press, St. Paul, MN.
42. Suga. H., Gale L. R., and Kistler, H. C. 2004. Development of VNTR markers for two *Fusarium graminearum* clade species. Mol. Ecol. Notes 4:468-470.
43. Swofford, D. L. 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4.0b10. Sinauer Associates, Sunderland, MA.
44. Tacke, B. H., and Casper, H. H. 1996. Determination of deoxynivalenol in wheat, barley and malt by column cleanup and gas chromatography with electron capture detection. J. AOAC Int. 79:472-475.
45. U.S. Dep. Agric. NASS. 2008. National Agricultural Statistics, USDA. www.nass.usda.gov.
46. Waalwijk, C., Kastelein, P., De Vries, I., Kerenyi, Z., van der Lee, T., Hesselink, T., Kohl, J., and Kema, G. 2003. Major changes in *Fusarium* spp. in winter wheat in the Netherlands. Eur. J. Plant Pathol. 109:743-754.
47. 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. USA 99:9278-9283.
48. Ward, T. J., Clear R. M., Rooney, A. P., O'Donnell, K., Gaba, D., Patrick, S., Starkey, D. E., Gilbert, J., Geiser, D. M., and 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.
49. Wright, S. 1951. The genetical structure of populations. Ann. Eugen. 15:323-354.
50. Yang, L., van der Lee, T., Yang, X., Yu, D., and Waalwijk, C. 2008. *Fusarium* populations on Chinese barley show a dramatic gradient in mycotoxin profiles. Phytopathology 98:719-727.
51. Yeh, F. C., Yang, R-C., Boyle, T. B. J., Ye, Z-H., and Mao, J. X. 1997. POPGENE, the User-Friendly Shareware for Population Genetic Analysis. Molecular Biology and Biotechnology Center, University of Alberta, Canada.
52. Zeller, K. A., Bowden, R. L., and Leslie, J. F. 2004. Population differentiation and recombination in wheat scab populations of *Gibberella zeae* from the United States. Mol. Ecol. 13:563-571.
53. Zhang, H., Zhang, Z., van der Lee, T., Chen, W. Q., Xu, J. S., Yang, L., Yu, D., Waalwijk, C., and Feng, J. 2010. Population genetic analyses of *Fusarium asiaticum* populations from barley suggest a recent shift favoring 3ADON producers in southern China. Phytopathology 100:328-336.