Population Subdivision of *Fusarium graminearum* Ssensu Stricto in the Upper Midwestern United States

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ABSTRACT

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A collection of 712 *Fusarium graminearum* sensu stricto (s.s.) strains, predominantly gathered between 1999 and 2000 from nine states within the United States, was examined for population structure and polymerase chain reaction-based trichothecene type. Most strains belonged to a cohesive genetic population characterized by a 15-acetyldeoxynivalenol (15ADON) trichothecene type. However, using a Bayesian model-based clustering method, we also identified genetically divergent groups of strains in some sampled locations of Minnesota and North Dakota. Strains of the major group of divergent populations were of a 3ADON

trichothecene type and formed a distinct cluster with a collection of previously gathered strains from Italy, which displayed all three trichothecene types (15ADON, 3ADON, and nivalenol). The co-existence of genetically divergent populations of *F. graminearum* s.s. in the Upper Midwest allows for the rejection of the hypothesis that *F. graminearum* s.s. in the United States consists of a single population. These results also suggest that recombination has been insufficiently frequent in this homothallic (selfing) fungal species to homogenize the divergent populations observed in the Upper Midwest.

Additional keywords: deoxynivalenol, *Fusarium* head blight, *Gibberella zeae*.

Fusarium head blight (FHB) of wheat and barley is among the worst plant diseases for U.S. agriculture, with many wheat- and barley-producing states being plagued by severe epidemics in years when environmental conditions are conducive to disease development (11,12,27). Since 1993, considerable annual losses have occurred in the United States. For example, a recent report (17) estimated the direct and secondary economic impact of FHB for 3 years (1998 to 2000) at \$870 million and \$1.8 billion, respectively, in nine states of the Northern Great Plains and Central United States. The neighboring states of North Dakota and Minnesota suffered almost half of all losses. In regions that have experienced epidemics in consecutive years, including the Red River Valley in Minnesota and North Dakota, this disease has had a devastating impact on farmers and their communities (11,27). The significance of FHB as a plant disease stems from the fact that the pathogens not only damage kernels and cause yield loss but also contaminate grains with trichothecene mycotoxins, which in the United States are predominantly deoxynivalenol (DON) and its derivative, 15-acetyldeoxynivalenol (15ADON) (13,23). Depending on toxin levels, contaminated grain may be unsuitable for use as feed or food.

Much research effort for FHB has been directed toward cultivar development, chemical control, and disease forecasting systems. Substantial progress also has been made toward elucidating the biology of the causal fungal organisms. FHB in wheat and barley is caused by a variety of *Fusarium* spp. (8), and several of these

are widespread and economically important in many European countries (1) and also are present in low frequency in samples from the United States (23,28). Nevertheless, the most troublesome causal agents of FHB globally are members of the *Fusarium graminearum* species complex (18). Once considered a single species (*F. graminearum* Schwabe; teleomorph: *Gibberella zeae* (Schwein.) Petch), genealogical concordance phylogenetic species recognition has allowed for formal description of 11 distinct species within the *F. graminearum* species complex (18,19,24) with *F. graminearum* sensu stricto (s.s.) being the major causal agent of FHB in wheat and barley in the United States, Europe, and portions of Asia (2,6,19,28).

The population structure of *F. graminearum* s.s. in the United States recently was investigated using amplified fragment length polymorphisms (28). This study reinforced the belief that the U.S. population of *F. graminearum* s.s. is relatively homogeneous. Species within the *F. graminearum* species complex other than *F. graminearum* s.s. were not detected. High genotypic diversity, little gametic disequilibrium, and significant amounts of gene flow also were attributed to the *F. graminearum* s.s. population in the United States. Though some population subdivision was detected that was correlated with distance, regional differentiation in the U.S. population of *F. graminearum* s.s. was deemed minimal (28).

Due to the global heterogeneity of the *F. graminearum* species complex (19) and the regionally incomplete information currently existing on population structure of *F. graminearum* s.s. in the United States, additional assessments of population diversity are required to further characterize the FHB populations. Therefore, the specific objectives of this study were to (i) characterize Midwestern U.S. strains of *F. graminearum* s.s. collected over large epidemic regions under the hypothesis put forward by Zeller et al. (28) that *F. graminearum* s.s. in the United States essentially con-

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sists of a single population, and (ii) establish trichothecene type diversity in U.S. populations of *F. graminearum* s.s.

MATERIAL AND METHODS

Fungal strains. Many of the strains examined in this study were gathered during the course of wheat rust surveys that are conducted annually by the United States Department of Agriculture, Agricultural Research Service (USDA-ARS), Cereal Disease Laboratory, St. Paul, MN. These surveys cover routes of $\approx 20,000$ km covering the Midwest and the Gulf States of the southern United States (7,9). FHB-infected plant material was gathered in 1999 and 2000 from surveyed fields, collecting ≈ 3 to 15 wheat heads per field. In addition, more intense sampling of 14 commercial wheat fields and 1 barley field in three counties of northwestern Minnesota (Norman, Polk, and Clay) was carried out in July 2000.

Collaborators also provided a number of strains from wheat and barley or infected wheat heads: 23 strains of *F. graminearum* s.s. from wheat originating from three counties in South Dakota (Yue Jin, USDA-ARS, Cereal Disease Laboratory, St. Paul, MN), three wheat heads from Dodge County, WI (Alemu Mengistu, University of Wisconsin, Madison, WI), and 13 barley strains from three counties in North Dakota (Linnea Skoglund, Busch Agricultural Resources, Inc., Ft. Collins, CO). Nineteen strains from the Istituto Sperimentale per la Patologia Vegetale (ISPAVE) located in Rome, Italy also were included in the analyses to allow preliminary comparison between U.S. and European populations of *F. graminearum* s.s.

Individual seed from wheat or barley heads showing typical head blight symptoms were dislodged and surface sterilized before plating on agar as previously described (5). In cases where no fungal cultures could be established or when symptoms were questionable, the entire wheat or barley head was plated. Although one to six cultures were established from severely diseased heads in 1999, only up to two strains per head were established for the 2000 collection. Isolation, culturing and single-spore, long-term storage, and DNA extraction procedures have been described previously (5,22).

Determination of trichothecene types and generation of restriction fragment length polymorphism data. Assignment of strains to trichothecene type were by a previously described multiplex polymerase chain reaction (PCR) method (24,26) that makes use of discrete polymorphisms in *TRI3* and *TRI12* sequences in the trichothecene gene cluster that are conserved for the three trichothecene profiles (chemotypes) found in the *F. graminearum* species complex (i.e., 15ADON: DON producers that mainly produce 15ADON in addition to DON, and 3ADON: DON producers that mainly produce 3-acetyldeoxynivalenol in addition to DON) and nivalenol (26). The multiplex PCR tests for both *TRI3* and *TRI12* accurately predict chemotype (24,26).

DNA from all strains was subjected to restriction fragment length polymorphism (RFLP) analysis. The generation of a genomic library from *F. graminearum* s.s. strain NRRL 29169 (GZ 3639) and the screening for polymorphic RFLP probes have been described previously (5,22). Among 116 RFLP probes screened, only 10 were polymorphic among a small subset of *F. graminearum* s.s. strains from the United States. These 10 probes were used to generate allelic data and multilocus RFLP genotypes for strains examined in this study. DNA fingerprinting patterns, generated with the telomeric probe pNla17, allowed for the identification of clonally related strains as previously described (5). Strains also were assessed for their RFLP patterns using six diagnostic probes that distinguished between seven species within the *F. graminearum* species complex (5). Additionally, the assignment of strains to *F. graminearum* s.s. was confirmed for a subset of 37 strains that represented the diversity in our collection, using DNA sequences from the EF-1 α and reductase genes.

Assigning strains to genetic clusters. The program STRUCTURE (version 2.1; available online) (4,20) was used for initial inference of population structure. STRUCTURE, a Bayesian model-based clustering method, assigns multilocus genotypes probabilistically to user-defined *K* clusters, each characterized by specific allele frequencies at examined loci. The model is based on maximizing linkage equilibrium within clusters and disequilibrium between them. We did not make a priori assumptions that clusters necessarily represent populations as cautioned (20,25), but rather used the results as a guide to define genetic populations as recommended (25). STRUCTURE requires exploratory runs and, in these, we allowed for values of *K* from 1 to 5, with 10,000 or 100,000 iterations of Markov Chain Monte Carlo (MCMC), after a burn-in period of 10,000 or 100,000 without any prior information on the origin of each sampled individual. The time-series plots for individual parameters were stable even after short runs; therefore, we performed final analysis for a burn-in period of 10,000 with 10,000 MCMC iterations. Based on results from exploratory runs, we decided to use the admixture model in which the fraction of ancestry from each cluster is estimated for each individual. The parameter of individual admixture, α , was allowed to be different for individual clusters and was given a uniform prior. During exploratory runs, a variety of values for α was used, varying between 0.001 and 1; α was set to 0.25 for the final runs. Allele frequencies were assumed to be uncorrelated between populations. Otherwise, default settings were used.

Population genetic analyses. Strains that were placed into specific populations after reviewing STRUCTURE results were included in further analyses at the population level that included number of alleles and their frequencies and gene diversity in specific populations, in addition to genetic distance between populations. Several software programs were used: Arlequin, version 2.000 (available online; S. Schneider, D. Roesli, and L. Excoffier, Laboratoire de Genetique et Biometrie, Université de Genève, Switzerland), Popgene 1.32 (available online; F. C. Yeh, R.-C., Yang, T. J. B. Boyle, Z.-H. Yeh, and J. X. Mao, Molecular Biology and Biotechnology Centre, University of Alberta, Canada), and Tools for Population Genetic Analysis (TFPGA) (available online; M. P. Miller, Department of Biology, Utah State University, Logan).

RESULTS

Fungal cultures and data collection. Initial positive identification of *F. graminearum* s.s. was based on visual assessment of conidia under a microscope during single-spore, followed by the use of diagnostic RFLP probes (5) that could distinguish *F. graminearum* s.s. from *F. culmorum*, *F. asiaticum*, *F. pseudograminearum*, and related species. We collected RFLP and trichothecene type data for a total of 713 U.S. strains that were identified as *F. graminearum* s.s. by use of the diagnostic RFLP probes. Strains with identical multilocus RFLP genotypes from the same field were examined for their hybridization pattern using the telomeric pNla17 to identify clones. After clone correction and eventual data removal for two strains (see below), 587 U.S. strains were further analyzed, together with 19 strains from Italy (Fig. 1; Table 1).

Multiplex PCR-tests based on *Tri3* and *Tri12* sequences (24,26) classified most U.S. strains of *F. graminearum* s.s. (94.7%) as having a 15ADON trichothecene type. A small sampling from Minnesota and North Dakota (30 strains or 5.1% of total number of strains) were of 3ADON trichothecene type. All 3ADON trichothecene-type strains from Minnesota (25 total) were detected in an area covering ≈ 750 km²; specifically, in eight fields located in three neighboring counties located just east of the Red River Valley that forms part of the border between North Dakota and Minnesota (Fig. 2). The five 3ADON trichothecene-type strains detected in samples from North Dakota were from five fields in three counties (Fig. 2).

Two nivalenol tricothecene-type strains also were identified in the collection. Strain 00-551 originated from a wheat field in the Red River Valley (Polk County, MN); the other strain, 00-100, was from southwestern Missouri (Vernon County, near Nevada, MO). Strain 00-551 (=NRRL 36905) since has been determined to belong to a newly described species, *F. gerlachii* T. Aoki, Starkey, Gale, Kistler, O'Donnell, sp. nov. (24). Strain 00-100 was removed from further analyses due to its uncommon tricothecene type in the current collection.

In order to put in perspective the extent of population subdivision and tricothecene diversity within the United States, we compared the U.S. strains to a collection of strains from Italy. As a whole, the small collection of Italian strains exhibited more diversity in tricothecene type, because all three tricothecene types were represented at appreciable frequencies (68% 58ADON, 21% 3ADON, and 21% nivalenol).

Preliminary examination of the RFLP data revealed that most U.S. 3ADON tricothecene-type and Italian strains displayed multilocus RFLP genotypes that were dissimilar from U.S. 15ADON tricothecene-type strains. Thus, we tested the hypothesis of population subdivision in *F. graminearum* s.s. using STRUCTURE 2.1 without a priori assumptions or constraints to avoid subjective classification of perceived populations (10). Initial simulations were performed assuming cluster numbers from

$K = 2$ to 5. Whereas $K = 2$ resulted in two discrete clusters, with most strains being firmly placed in either cluster, an increase in K simply resulted in “admixed” individuals without clear structure. Therefore, we concluded that the existence of two clusters best explained the data. Most (93.4%) 15ADON tricothecene-type strains from the United States fit into cluster 1, with a very high average membership value (q) (avg. $q = 0.988$, standard deviation [s.d.] = 0.012). However, 19 15ADON strains from seven states with a predominant cluster 1 genotype ($q > 0.5$) had q values < 0.9 (0.53 to 0.88). In addition, two 3ADON strains (00-552 and 00-556) from the United States and one Italian strain (01-36) had genotypes consistent with cluster 1 ($q > 0.9$).

Most (90.0%) 3ADON strains from the United States as well as 89.5% of Italian strains fit well into cluster 2 (avg. $q = 0.936$, s.d. = 0.015 and avg. $q = 0.943$, s.d. = 0.017, respectively). In addition, 12 15ADON tricothecene-type strains were placed in cluster 2 displaying fairly consistent, but lower q values than the remainder of cluster 2 members (avg. $q = 0.861$, s.d. = 0.025).

Fig. 1. Approximate county locations in nine states of the United States from which samples of *Fusarium graminearum* sensu stricto were obtained.

Fig. 2. Counties in northwestern Minnesota (MN) and in North Dakota (ND) in which strains belonging to the Upper Midwestern 3-acetyldeoxynivalenol (UMW 3ADON) and the tentative UMW 15ADON populations were identified: CL = Clay (UMW 3ADON and UMW 15ADON), N = Norman (UMW 3ADON and UMW 15ADON), P = Polk (UMW 3ADON and UMW 15ADON), CA = Cavalier (UMW 3ADON and UMW 15ADON), T = Towner (UMW 3ADON and UMW 15ADON), F = Foster (UMW 3ADON), B = Bottineau (UMW 15ADON), and W = Walsh (UMW 15ADON).

TABLE 1. Strains of *Fusarium graminearum* sensu stricto (clone corrected) from the United States and Italy analyzed in this study and their origins

Location	Number of			County or region ^a
	Counties	Fields	Strains	
United States				
Illinois	2	2	14	Macon, Stephenson
Indiana	6	7	65	La Porte (N) (2), Parke, Randolph (N), Sullivan, Tippecanoe (N), Tipton
Kansas	1	1	1	McPherson
Minnesota	13	28	318	Clay (2), Dakota (N × 2), Itasca (N), Kittson, Lincoln (2), Marshall, Norman (7), Polk (7), Redwood (2), Stearns, Stevens (N), Waseca (N), Wilkin
Missouri	2	2	3 ^b	Henry, Vernon
North Dakota	17	30	113	Barnes, Bottineau, Cavalier (5), Dickey, Divide, Foster (N × 2), Grant, LaMoure (2), McHenry, Pierce, Richland (2), Rolette, Towner (2), Walsh (2), Ward (3), Wells (3), Williams (2)
Ohio	3	3	20	Henry (N), Mercer, Williams
South Dakota	6	8	39	Aurora, Brookings (N × 2), Brown, Day, Hamlin, Roberts, Union, Walworth
Wisconsin	2	2	12	Columbia, Dodge
U.S. total	52	83	587	...
Italy	8 ^c	NA ^d	19 ^e	Emilia-Romagna, Lazio, Lombardia, Marche, Piemonte, Sicilia, Umbria, Veneto

^a N indicates nursery; N × 2 indicates that the same nursery was sampled in both years; these specific locations were counted as only one field. Numbers in parentheses indicate the number of fields sampled from a specific county, if more than one.

^b Excludes 00-100, an isolate of *F. graminearum* s.s. with a nivalenol chemotype.

^c Regions in Italy.

^d NA = not available.

^e Some of these strains have been analyzed previously by Corazza et al. (3).

Eight additional strains (six 15ADON strains from five states, one 3ADON strain from MN, and one Italian nivalenol strain) were placed into cluster 2, but with lower q values (0.642 to 0.785).

Based on the results from STRUCTURE and taking into account other observed strain attributes (i.e., trichothecene type and geographic information), we defined the genetic populations that were further used for analyses at the population level. Cluster 1 strains that were of a 15ADON trichothecene type with high average q membership values ($q > 0.9$) were considered members of a genetic population that was named Midwestern (MW) 15ADON. Due to their geographic separation, the Italian strains and the 3ADON trichothecene-type strains from Minnesota and North Dakota that were solidly placed ($q > 0.9$) into cluster 2 were considered independent genetic populations and were designated as the Italian population and the Upper Midwestern (UMW) 3ADON population, respectively. The lower average q values displayed by the 12 15ADON trichothecene-type strains with cluster 2 membership, in addition to relatively low q value variability, were seen as an indicator for the possible existence of yet another population. To further examine this hypothesis, we tentatively classified this group of strains as a separate population, UMW 15ADON, and treated it as such in ensuing population level analyses.

In all, $\approx 5\%$ of the U.S. collection (30 strains) could not be confidently placed in any population based on these methods. Setting aside data from these strains, information from the remaining 576 strains was rerun in STRUCTURE using the same settings. Although the q values for the individual strains remained virtually unchanged, the estimated ln probability of data improved from -3035.2 to -2794.6 , the mean value of ln likelihood went from -2984.6 to -2767.2 , and the variance of ln likelihood decreased from 101.2 to 54.8.

Population level analyses. Based on population assignments detailed above, strains were arranged into four populations that were subjected to population level analyses: the common MW 15ADON population ($n = 520$), the UMW 3ADON population ($n = 27$), the tentative UMW 15ADON population ($n = 12$), and the Italian population ($n = 17$).

The MW 15ADON population was, on average, the most diverse for all parameters examined (i.e., mean number of alleles/locus, gene diversity across all loci, and pairwise differences between multilocus RFLP genotypes) (Table 2). Although allelic and gene diversity overall was elevated in the MW 15ADON population, this was not the case for all loci. Observed alleles for seven loci and their frequencies are detailed in Table 3. Each of these seven loci highlights differences and similarities between

the four populations. Allele frequency differences between the four populations are further summarized as genetic distances and levels of population differentiation between them (Table 4). Results from these analyses underscore the following: (i) the dis-

TABLE 3. Allele frequencies at seven restriction fragment length polymorphism (RFLP) loci in four populations of *Fusarium graminearum* sensu stricto^a

Locus, allele ^b	MW 15ADON	UMW 3ADON	UMW 15ADON	Italy
pGz121				
1	0.423	0	0	0
2	0.381	0	0	0
3	0.085	1	1	1
4	0.108	0	0	0
5	0.002	0	0	0
6	0.002	0	0	0
pGz129				
1	1	0.111	0.917	0.059
2	0	0.889	0.083	0.941
pGz86				
1	0.902	0.556	0.083	0.235
2	0.089	0	0	0
3	0	0.444	0.917	0.765
4	0.006	0	0	0
5	0.002	0	0	0
6	0.002	0	0	0
pGz117				
1	0.914	0.667	1	0.412
2	0.087	0.333	0	0.588
pGz29				
1	1	0	0	0
0	0	1	1	1
pGz60				
1	0.402	0.852	0.167	0.882
2	0.594	0.148	0.833	0.118
0	0.004	0	0	0
pGz106				
1	0.800	0.222	0.833	0.882
2	0.198	0.778	0.167	0.118
3	0.002	0	0	0

^a Allele frequencies were calculated in POPGENE. Alleles for the remaining three RFLP loci (pGz54, pGz110, and pGz62) were polymorphic in the midwestern (MW) 15-acetyldeoxynivalenol (15ADON) population, whereas they were fixed for the most common allele in the remaining three populations. The four populations—MW 15ADON, Upper Midwestern (UMW) 15ADON, UMW 3ADON, and Italy—are genetically defined and subdivided according to results by use of STRUCTURE and by taking into account other attributes, i.e., trichothecene type and geographic origin).

^b Allele 0 = null allele (no hybridization observed).

TABLE 2. Observed number of alleles (na) and gene diversity (h) at 10 restriction fragment length polymorphism (RFLP) loci for four populations of *Fusarium graminearum* sensu stricto^a

RFLP locus	MW 15ADON ($n = 520$)		UMW 3ADON ($n = 27$)		UMW 15ADON ($n = 12$)		Italy ($n = 17$)	
	na ^b	h ^b	na	h	na	h	na	h
121	6	0.657	1	0	1	0	1	0
129	1	0	2	0.198	2	0.153	2	0.111
86	5	0.179	2	0.494	2	0.153	2	0.360
54	2	0.085	1	0	1	0	1	0
29	1	0	1	0	1	0	1	0
60	3	0.485	2	0.252	2	0.278	2	0.208
110	6	0.631	1	0	1	0	1	0
117	2	0.158	2	0.444	1	0	2	0.484
62	2	0.230	1	0	1	0	1	0
106	2	0.320	2	0.346	2	0.278	2	0.208
Mean	3	0.275	1.5	0.173	1.4	0.086	1.5	0.137
P.D. ^c	...	2.750	...	1.801	...	1.045	...	1.456

^a The four populations—Midwestern (MW) 15-acetyldeoxynivalenol (15ADON), Upper Midwestern (UMW) 15ADON, UMW 3ADON, and Italy—are genetically defined and subdivided according to results by use of STRUCTURE and by taking into account other attributes (i.e., trichothecene type and geographic origin).

^b According to Nei (15,16); means of na and h were calculated in POPGENE.

^c Mean number of pairwise differences between multilocus RFLP genotypes within populations were calculated in ARLEQUIN.

parity between the MW 15ADON and the remaining populations; (ii) the close genetic relationship between the Italian and the UMW 3ADON populations, the only population pair that was not significantly different from each other using an exact test of population differentiation; and (iii) the uncertain genetic relationship of the UMW 15ADON population with the other populations.

DISCUSSION

Analysis of DNA polymorphism data from a geographically extensively sampled collection of the pathogen allowed for rejection of the hypothesis that *F. graminearum* s.s. in the United States consists of a single population. Instead, we identified genetically diverse populations within the species that co-existed in several sample locations. Population subdivision in the United States, initially discovered using the program STRUCTURE, was strongly correlated with trichothecene type, not only demonstrating its usefulness as a genetic marker but also leading to our current population classification for U.S. strains of *F. graminearum* s.s. that is partially based on trichothecene type. The MW 15ADON population was predominant and ubiquitous, and 89% of U.S. strains examined clearly could be assigned to this population; undoubtedly, this represents the population previously identified and described in detail by Zeller et al. (28). On the other hand, STRUCTURE grouped all but three 3ADON trichothecene-type strains from the United States into a second cluster, and we deduced the existence of a second population, UMW 3ADON, from these results. UMW 3ADON strains were identified without exception from samples originating from North Dakota and Minnesota. Although we identified members of the UMW 3ADON population from relatively few locations (Fig. 2), they were generally at considerable frequency, averaging 9.4% (27/288) in counties from which they were identified.

A smaller collection of 12 strains tentatively classified as the UMW 15ADON population had essentially the same geographic distribution as the UMW 3ADON population (Fig. 2). The overall frequency of the UMW 15ADON population in those counties in which they were identified amounted to 4.1% of total strains that could be assigned to populations.

Population level analyses were conducted for a total of 576 strains subdivided into four populations: the common MW 15ADON, the tentative UMW 15ADON, the UMW 3ADON, and the Italian population. The MW 15ADON was more diverse than the remaining populations, supporting the idea that this is the native *F. graminearum* s.s. population in the United States. The UMW 3ADON population was more diverse than the Italian population, and the UMW 15ADON population was the least diverse of all.

Calculations of genetic distances between populations essentially supported results from STRUCTURE. The MW 15ADON

population was found to be genetically distinct from the other three populations. Our current working hypothesis that considers 15ADON strains that were grouped into cluster 2 a separate population (UMW 15ADON) also was supported by population level analyses, because genetic distances to the other populations were moderately high. Nevertheless, additional analyses with more markers and larger sample sizes are deemed necessary for final conclusions.

Surprisingly, the UMW 3ADON population could not be differentiated from a small collection of strains from Italy based on STRUCTURE analysis. The close genetic relationship between the Italian and UMW 3ADON populations was confirmed by population analyses, supporting the hypothesis that the UMW 3ADON and the Italian population are derived from the same or closely related populations. Although the UMW 3ADON population was somewhat more diverse than the Italian population based on RFLP markers, the Italian population was more diverse for trichothecene type, because all three types were found in the small collection from Italy at appreciable frequencies. Taking into account the geographically restricted occurrence of the UMW 3ADON population in the United States, the lack of diversity for trichothecene type, and its close genetic relationship with the Italian population, we currently speculate that the UMW 3ADON population may have had its origin in Europe. However, limited surveys indicate that the 3ADON trichothecene type of *F. graminearum* s.s. may be rare in Europe and has been identified previously for only three Swedish isolates (2) and four isolates in England and Wales (6). Further comparisons with populations from around the world and evaluation of global population structure for *F. graminearum* s.s. are needed to further evaluate the hypothesis of European descent of the UMW 3ADON population.

In counties of Minnesota and North Dakota where the UMW 3ADON population was identified, its average frequency was sizeable (9.4%). This observation indicates the need for monitoring and additional phenotypic characterization of this population, especially in regard to virulence toward recently deployed resistant cultivars and to their sensitivity to the fungicide Follicur which, in recent years, has been applied routinely in regions with high disease potential. Fungal collections from 2003 and 2004 currently are being analyzed to further determine the potential for change in frequency and the exact distribution of these emergent populations (UMW 3ADON and the tentative UMW 15ADON) in North Dakota, Minnesota, and South Dakota.

Results presented here, in conjunction with another recent study (24) that reports on the presence of a highly divergent population of *F. graminearum* s.s. from the U.S. Gulf Coast that displays all three trichothecene types, prove that *F. graminearum* s.s. in the United States does not consist of a single homogeneous population. The fact that we were able to identify discrete populations co-existing in the same locations also allows for the conclusion that recombination has been insufficiently frequent in this homothallic (selfing) fungal species to homogenize the divergent populations observed in the Upper Midwest. This may be due to an overall limited amount of recombination in the species or a recent introduction of these divergent populations into the region.

We also hypothesize that currently available data have not unearthed all diversity and complexities present in U.S. populations of *F. graminearum* s.s., requiring further sampling and additional analyses of those strains that we could not place into specific populations in the current study. A previous statement by Zeller et al. (28) also needs to be reevaluated: "If pathogenicity and aggressiveness genes are distributed in a manner similar to the molecularly detected genetic variation, then resistance breeding material planted at any single location should be exposed to a representative range of the pathogenic variation present in the continental *G. zeae* population, and differences between locations should probably be attributed to factors other than differences in genetic composition of the pathogen population." In light of changing

TABLE 4. Nei's genetic distance D (below diagonal) and *P* values from exact tests for population differentiation (above diagonal) between four populations of *Fusarium graminearum* sensu stricto^a

Populations	MW 15ADON	UMW 3ADON	UMW 15ADON	Italy
MW 15ADON	...	0.000	0.000	0.000
UMW 3ADON	0.547	...	0.000	0.123
UMW 15ADON	0.368	0.234	...	...
Italy	0.605	0.074	0.201	...

^a D (14) was calculated in Tools for Population Genetic Analysis (TFPGA); population differentiation was according to Raymond and Rousset (21) as implemented in TFPGA (1,000 dememorization steps, 10 batches, 2,000 permutations per batch). The four populations—Midwestern (MW) 15-acetyldeoxynivalenol (15ADON), Upper Midwestern (UMW) 15ADON, UMW 3ADON, and Italy—are genetically defined and subdivided according to results by use of STRUCTURE and by taking into account other attributes (i.e., trichothecene type and geographic origin).

agricultural practices (ongoing resistance breeding, deployment of resistant cultivars, and widespread fungicide applications in epidemic regions), the presence of genetically distinct strains or populations should be of concern per se, even if they are currently at low frequency, because it is conceivable that selection may increase the frequency of genetically divergent populations or strains to problematic proportions under suitable conditions.

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