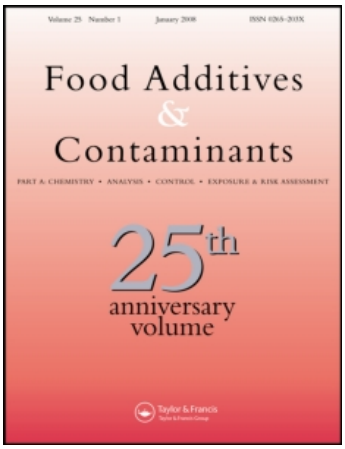

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Population genetic structure of *Gibberella zeae* isolated from wheat in Argentina

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Abstract
Gibberella zeae (anamorph *Fusarium graminearum*) causes *Fusarium* head blight of wheat. The authors used amplified fragment length polymorphisms (AFLPs) to characterize the genetic structure of two *G. zeae* populations from commercial wheat fields. The working hypothesis was that sufficient genetic exchange occurs between local populations to prevent significant partitioning of allelic variation. We analysed 216 AFLP loci for 113 isolates collected during the 2002 harvest season. All strains had AFLP profiles typical of *G. zeae* lineage 7. Both populations were genotypically diverse but genetically similar and potentially part of a larger, randomly mating population, with significant genetic exchange probably occurring between the two subpopulations. Linkage disequilibrium was low, but higher than reported for many other populations of *G. zeae*, and about 20% of the alleles detected were specific to one of the two subpopulations—results consistent with limited gene exchange between the two subpopulations. This study extends previous work with populations of *G. zeae* to include those found in Argentina, one of the world's largest wheat growing countries.

Keywords: *Amplified fragment length polymorphisms (AFLPs), wheat, gene flow, genetic diversity,* Gibberella zeae*, random mating.*

Introduction

Fusarium head blight (FBH) is economically one of the most important fungal diseases of wheat worldwide. FHB epidemics occurred in Argentina in 17 of the last 50 years, causing yield losses and price discounts due to reduced seed quality. The disease incidence has increased over the last decade, primarily in the central–north region, and can be attributed to increased soil conservation tillage practices, as well as the high frequency of maize as the preceding crop. *Gibberella zeae* (Schwein.) Petch (anamoph *Fusarium graminearum* Schwabe) is the principal causal agent of FHB in Argentina (Galich 1997, Ramirez et al. 2006). Contamination of wheat with mycotoxins produced by *G. zeae*, especially trichothecenes, that are toxic to both humans and animals (World Health Organization 2001) decrease further the value of the reduced amount of grain produced.

Plant cultivars that are highly resistant to the disease or tolerant to the toxin are not available and chemical control is limited by cost, application difficulties, and an incomplete understanding of factors that influence disease development (Goswami & Kistler 2004). Basic knowledge of the population structure of this pathogen is needed to answer fundamental questions about the adaptability of the pathogen to new cropping practices or cultivars, to determine the gene flow between populations, and to evaluate the dispersal of inoculum between fields and regions.

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A number of studies of the genetic diversity of *G. zeae* have been made with strains collected from diverse locations, including China (Gale et al. 2002, Gagkaeva & Yli-Mattila 2004), Nepal (Carter et al. 2000), parts of Europe (Miedaner et al. 2001, Gagkaeva & Yli-Mattila 2004, Tóth et al. 2005), USA (Walker et al. 2001, Zeller et al. 2003a, 2004, Schmale et al. 2006), Canada (Ouellet & Seifer 1993, Dusabenyagasani et al. 1999, Mishra et al. 2004, Fernando et al. 2006), Australia (Akinsanmi et al. 2006), and parts of South America—Brazil and Uruguay (Zeller et al. 2003b)—but there is no information on populations of this pathogen in Argentina.

We previously used vegetative compatibility groups (VCGs) to show that genotypic diversity in *G. zeae* populations isolated from wheat in Argentina is high (Ramirez et al. 2006). VCGs are good, but not infallible (Leslie 1993, Chulze et al. 2000), indicator of clonal identity and genotypic similarity among isolates. However, beyond 'same' or 'different', VCGs provide little information on the degree of genetic relatedness. A number of molecular markers including amplified fragment length polymorphisms (AFLPs) and restriction fragment length polymorphisms (RFLPs) have been used to determine the relationship(s) between populations and individual strains.

The objective of this study was to use AFLPs to characterize the genetic structure of *G. zeae* populations from wheat cultivated in Argentina. Our working hypothesis was that sufficient genetic exchange occurred between local populations for there to be no significant partitioning of allelic variation between them. This study extends the previous work with populations of *G. zeae* to include those found in Argentina, one of the world's largest wheat growing countries.

Materials and methods

Sampling

Wheat samples were collected from two commercial wheat fields located in Buenos Aires province (San Antonio de Areco and Alberti), Argentina. The two sites are 130 km from each other and located in the major wheat production area of Argentina, which has a temperate, humid climate. Both fields were planted with variety Pro Inta Granar, which is highly susceptible to FHB. The disease incidence was estimated as >70% at both locations. In each field, all of the samples were collected from a $5 \times 5\ m^2$ plot area during the 2002 harvest season. Rows were 20 cm apart, with a planting density of about 500 plants/m^2. Fifty spikes with symptoms of FHB were collected from each location, air dried, and stored at −20°C in paper envelopes. All of the isolates from a single field were regarded as members of the same population.

Strain isolation and morphological identification

Wheat seeds with symptoms of FHB were transferred to *Fusarium* selective medium containing pentachloronitrobenzene (Leslie & Summerell 2006). The plates were incubated at 25°C for 7 days with a 12/12 photo-period under cool white and black fluorescent lamps (Leslie & Summerell 2006). Single macroconidia were identified with a stereo microscope (70 × magnification) and transferred to carnation leaf agar (CLA) and potato dextrose agar (PDA), incubated as described above for 15 days for identification (Leslie & Summerell 2006). Strains identified as *F. graminearum* were kept as spore suspensions in 15% glycerol and frozen at −80°C.

Production of perithecia

Ability to produce homothallic perithecia was tested by culture on carrot agar and/or carnation leaf agar under the incubation conditions described above for 35 days.

DNA isolation

Isolates of *G. zeae* were grown in complete medium (Leslie & Summerell 2006) and incubated on an orbital shaker (150 rpm) for three days at 25 ± 1°C. The resulting mycelium was harvested by filtration through non-gauze milk filters (Ken AG, Ashland, OH, USA). Excess water was removed by blotting mycelia between clean paper towels. Dried mycelia were stored frozen at −20°C until ground. Fungal DNA was extracted with a cetyltrimethylammonium bromide (CTAB) method (Leslie & Summerell 2006).

AFLPs

AFLPs were performed as described by Vos et al. (1995), as modified by Leslie & Summerell (2006) in a PTC-2000 Thermal Cycler (MJ Research, Inc., Watertown, MA, USA). All buffers and DNA-modifying enzymes were used following either the manufacturer's instructions or standard protocols (Sambrook et al. 1989). Genomic DNA was digested to completion with *Eco*RI and *Mse*I and ligated to AFLP adapters in a single overnight reaction at room temperature (21–24°C). The digested and ligated templates were diluted in 9 vols of Tris-EDTA buffer before pre-amplification. Three primer-pair combinations with two selective nucleotides on each primer were used: *Eco*RI + AA/*MseI* + AT, *Eco*RI + CC/*MseI* + CG, and *Eco*RI + TG/*MseI* + TT. The *Eco*RI primer was

labelled with γ^{33}P for detection of bands by autoradiography.

Polymorphic AFLP bands ranging from about 200 to 500 bp in length were scored manually. Each band was treated as a distinct locus, with two alleles. *G. zeae* is haploid, so AFLP markers can be scored unambiguously as either 'band present' or 'band absent'. All bands in this size range were scored, including those that were monomorphic. Bands migrating at the same position were assumed to be homologous and to represent the same allele and locus. Bands of differing mobility were treated as independent loci with two alleles. AFLP data were recorded in a binary format (allele present = 1 or absent = 0). Unresolvable bands and missing data were scored as ambiguous in the context of the population genetic analyses. We included strains identified as belonging to *G. zeae* lineages 1–7 (O'Donnell et al. 2000) as standards on each gel.

Genotype diversity assessments and population genetic analyses

PAUP 4.0 (Swofford 1999) was used to identify distinct and clonal AFLP haplotypes within each *G. zeae* population. Allelic binary data were analysed with unweighted pair grouping by mathematical averaging (UPGMA). We identified indistinguishable *G. zeae* isolates, i.e. clones, as those isolates with AFLP haplotypes that shared ≥98% of the scored AFLP bands. Populations were clone censored, i.e. only one strain of each clone was retained for analyses of genetic diversity and linkage disequilibrium.

Genotypic diversity ($\hat{G}$) for each population was estimated as described by Milgroom (1996). Normalized genotypic diversity was calculated by dividing each estimated $\hat{G}$ by the number of unique AFLP haplotypes observed in each population.

We estimated (1) allele frequencies of polymorphic loci and gene diversity within and between populations as described by Nei (1973); (2) G_{ST} (fixation index) as described by Nei (1973); (3) N_m (effective migration rate) as described by McDermott and McDonald (1993), and (4) genetic identity among populations as described by Nei (1978) with the shareware program POPGENE version 1.32 (available at http://www.ualberta.ca/~fyeh; Yeh at al. 1997). All data analysed by this program were treated as haploid with dominant markers. G_{ST} and N_m were estimated with both the complete data set and with a subset of data that included only those loci for which both alleles were present at ≥5% frequency in at least one of the two populations. The results of these analyses were compared to determine if the inclusion of loci with rare alleles in these analyses altered the estimates of genetic differentiation of the populations. We also used POPGENE to estimate linkage disequilibrium for AFLP loci if both alleles at both loci were present in the population at a frequency of ≥5%. We calculated two-locus gametic disequilibria between all pairs of these loci, and conducted χ^2-tests for significance as described by Weir (1979).

Results

We isolated 113 *F. graminearum* strains; 69 from San Antonio de Areco and 44 from Alberti. All isolates produced fertile homothallic perithecia on carnation leaf agar and carrot agar; therefore all isolates were *G. zeae*.

A total of 216 AFLP bands were identified in the 200–500 bp range from the 113 analysed isolates when using the three primer pair combinations (Table I). Of these 216 AFLP loci, 91% were polymorphic in the San Antonio de Areco population and 88% were polymorphic in the Alberti population (Table I). All 113 isolates had AFLP profiles typical of *G. zeae* lineage 7.

Isolates with the same AFLP genotype (clones) were rare, and only nine of the 113 strains had an AFLP genotype that was the same as that of one of the other strains examined—two pairs of two strains each in the Alberti population and one pair of two and a set of three in the San Antonio de Areco population. No strains with the same AFLP haplotype were found in different locations. Normalized genotypic diversity ($\hat{G}$) was high (≥98% of the count) in both populations, with the highest number

Table I. Comparison of *Gibberella zeae* populations isolated from wheat fields in San Antonio de Areco, and Alberti, Argentina.

Population	San Antonio de Areco	Alberti
Sample size	69	44
Percent polymorphic loci	91	88
Number of haplotypes	66	42
Number of private alleles	23	17
Mean frequency of private alleles[a]	0.062	0.041
Range	0.015–0.121	0.024–0.095
$\hat{G}$[a]	0.99	0.99
Mean gene diversity[a,b]		
199 loci	0.267	0.238
148 loci	0.341	0.299

[a]Calculated as described by Milgroom (1996) from comparisons of AFLP allelic data at 161 AFLP loci. $\hat{G} = 1/\Sigma p_i^2$, where p_i is the observed frequency of the ith multilocus genotype in a population.
[b]Estimated for clone-censored populations. Clones were defined as isolates that shared ≥98% UPGMA similarity in AFLP banding pattern. Only one representative of each clone was retained for subsequent analyses.
[c]Calculated as in Nei (1973).

of clonal isolates found in the San Antonio de Areco population (Table I).

Allele frequencies were generally very similar between these two populations (data not shown), as were the mean gene diversities (Table I). There were 23 loci with private alleles (both allelic forms present in one population but not in the other) in the San Antonio de Areco population of which 13 had both alleles at a frequency of >5% (Table I). In the Alberti population there were 17 private alleles with both of the alleles at four of these loci present at a frequency of >5% (Table I). The mean frequency of the 40 private alleles across both populations was about 5.3%.

For the full set of 199 polymorphic loci, the average gene diversity was 0.25 for all of the isolates, 0.27 for the isolates from San Antonio de Areco, and 0.24 for isolates from Alberti (Table I). The higher gene diversity in the San Antonio de Areco population might be due to the larger number of polymorphic loci (182 loci) observed in that population relative to the population from Alberti (176 loci). When we removed the 51 loci with rare polymorphic alleles (frequency of the rarer allele <5% in both populations) from the analysis, the mean gene diversity estimates for the combined populations increased from about 0.25 to 0.32.

For the full set of 199 loci, values of G_{ST} (fixation index or differentiation among populations due to population subdivision) for individual loci ranged from a minimum of zero, i.e. either no divergence or equal allele frequencies, to a maximum of 0.185 (Table II). The mean G_{ST} across all 199 loci was 0.033, suggesting that the allele frequencies are similar in both populations (Table II). This range also suggests that significant genetic exchange has occurred between these two *G. zeae* populations ($N_m > 15$ across all 199 loci). We obtained similar results in the analysis of the subset of 148 loci for which the frequency of the rarer allele was >5% (mean $G_{ST} = 0.034$ and $N_m > 14$) (Table II).

For the calculation of two locus linkage disequilibria in *G. zeae* populations, there were 19 701 possible pair-wise comparisons for the 199 AFLP loci. We rejected the null hypothesis of two-locus linkage equilibrium ($p < 0.01$) in favour of the alternative hypothesis of two-locus linkage disequilibrium for 1479 pairs of loci (7.5%) for the Alberti population and for 1811 pairs of loci (9.2%) for the San Antonio de Areco population. At the $p < 0.05$ significance level, we rejected the null hypothesis of two-locus linkage equilibrium for 2422 pairs of loci (12%) from the Alberti population, for 3041 pairs of loci (15%) from the San Antonio de Areco population, and for 19 701 pairs of loci (19.7%) from the combined populations.

Table II. Statistics on population genetic differentiation between San Antonio de Areco and Alberti clone-censored populations of *Gibberella zeae* calculated from all polymorphic loci, and for the 148 loci for which the frequency of both alleles (band and no band) was >5%.

Statistic	199 loci	148 loci
Mean gene diversity[a]	0.252	0.320
Range	0.015–0.499	0.053–0.499
Fixation index (G_{ST})[a]	0.033	0.034
Range	0–0.185	0–0.185
Effective migration rate (N_m)[b]	15	14
Range	2.2–2000	2.2–2000
Genetic identity[c]	0.98	0.97

[a]Calculated as in Nei (1973).
[b]Calculated as in McDermott and McDonald (1993).
[c]Calculated as in Nei (1978).

Discussion

The present study is the first report on genetic diversity of *G. zeae* populations isolated from Argentina. We analysed AFLPs from 113 isolates of *G. zeae* lineage 7 (O'Donnell et al. 2000, 2004) from two different locations in the primary wheat-growing region of the country. Lineage 7 is the most widespread of the lineages of *G. zeae* described to date. The present results are the first report of this lineage from Argentina and are consistent with those of Zeller et al. (2003b) who found that lineage 7 was the dominant lineage in Uruguay and southern Brazil. The results are similar to those of Zeller et al. (2003a, 2004) and Schmale et al. (2006) from the USA in that we found only lineage 7 isolates in the samples. In both Brazil and Uruguay (Zeller et al. 2003b) and in Europe (Gagkaeva & Yli-Mattila 2004, Láday et al. 2004, Tóth et al. 2005) populations containing multiple lineages of *G. zeae* have been identified. In China, lineage 6 usually dominates and may be associated with the culture of rice (Gale et al. 2002).

Genetic diversity in populations of *G. zeae* has been assessed with various molecular markers including AFLPs (Zeller et al. 2003a, 2004, Schmale et al. 2006), RAPDs (Ouellet & Seifer 1993, Dusabenyagasani et al. 1999, Fernando et al. 2006), RFLPs (Gale et al. 2002, Tóth et al. 2005). Virtually all studies have identified high levels of genetic and genotypic diversity. Initially, this result seems surprising since homothallic sexual reproduction, of which this fungus is capable, is the genetic equivalent of self-replication. High levels of genotypic diversity with relatively few clones suggests that *G. zeae* outcrosses frequently enough to maintain a great deal of genetic heterogeneity in the population. Effective assessment of genetic and genotypic variation requires many markers that are randomly

distributed across the genome. AFLPs are relatively easy to generate in sufficiently large numbers and are well-enough distributed across the genome that they can be used to generate a genetic map of *G. zeae* (Jurgenson et al. 2002). The genetic map demonstrates that outcrossing and recombination can occur under laboratory conditions, and by inference under field conditions as well. We chose AFLPs for this study because of their ease of generation and interpretation in a haploid fungus, and because they have been used previously to characterize successfully field populations of *G. zeae* for population genetic purposes.

Previous studies of *G. zeae* populations from North America with AFLPs (Zeller et al. 2003a, 2004, Schmale et al. 2006) found that G_{ST} ranged from zero to 0.167. These values are similar to those observed in this study of zero to 0.185 (Table II). Thus the present results are consistent with the hypothesis that the two fields sampled are part of a larger population that includes, perhaps, the entire north central region of Argentina. This conclusion is tempered, however, by the relatively large number of loci (40/199; Table I) identified with private alleles in the studied populations. The relatively high frequency of some of these private alleles, up to 12% (Table I), suggests that these populations have been isolated from one another long enough for these private alleles to accumulate in the populations and that the observed migration levels could reflect historic, rather than contemporary, gene flow of population origins. Determining whether these private alleles are reflective of population subdivision or sampling artefacts will require analysis of additional populations.

Linkage disequilibrium is another character that can be used to assess genetic exchange within and between populations. Taking $p < 0.01$ as a cut-off, 12.4% of the locus pairs from the Alberti population, 17.4% of the locus pairs from the San Antonio de Areco population, and 12.9% of the loci in the combined populations were in linkage disequilibrium. These values are substantially larger than those found in the North America populations (Zeller et al. 2003a, 2004, Schmale et al. 2006) in which no more than 10% and usually less than 5% of the locus pairs examined were in linkage disequilibrium. Linkage disequilibrium has many possible causes, including inbreeding, and the relatively recent mixture of two (or more) different populations. Discerning the cause of the observed linkage disequilibrium will require further studies of more populations from more locations and collected at different times, but these results are consistent with the relatively recent introduction of a substantial amount of new genetic material into these populations or with the populations passing through a bottleneck from which they have mostly, but not completely recovered.

The data from our Argentinian populations, both in this study with AFLPs and in an earlier, more limited study with VCGs (Ramirez et al. 2006), as with the data from the North American populations (Zeller et al. 2003a, 2004, Schmale et al. 2006) is consistent with a high amount of outcrossing in these populations. We cannot estimate the relative amounts of heterothallic and homothallic sexual reproduction, but the laboratory estimate of 35% heterothallic crossing made by Bowden & Leslie (1999) would seem an upper bound. Heterothallic reproduction has been occurring for some time or the linkage disequilibrium values would be much higher than we observed. The lack of one, or a few, dominant genotypes suggests that the alleles for pathogenicity are either fixed, and thus are invariant, or that there are many ways to be an effective pathogen and that there is no intense selection for any of these individual multigenic phenotypes. Relatively little recombination is thought to be required to sustain relatively high levels of genotype diversity and to result in populations that appear to be randomly mating (Leslie & Klein, 1996). Clearly there is sufficient recombination in populations of *G. zeae* for the pathogen to be able to rapidly synthesize a multi-locus response to changes in selection pressures resulting from changes in host variety or the introduction of a novel biological or chemical control method.

In conclusion, the present study adds new information on populations of *G. zeae* from Argentina to the growing list of population studies of this fungus worldwide. The conclusions reflect the general conclusions on such populations, i.e. that these populations are genetically and genotypically diverse and that there is a significant amount of genetic exchange occurring between genetically proximate populations. In our case, however, these conclusions are tempered by the occurrence of a relatively large number of loci with private alleles and by higher than previously observed levels of linkage disequilibrium. Studies of additional populations collected over a broader area and multiple crossing seasons will be needed to determine whether Argentinian populations of *G. zeae* are typical of those found on wheat in other parts of the world.

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References

Akinsanmi OA, Backhouse D, Simpfendorfer S, Chakraborti S. 2006. Genetic diversity of Australian *Fusarium graminearum* and *F. pseudograminearum*. Plant Pathology 55:494–504.

Bowden RL, Leslie JF. 1999. Sexual recombination in *Gibberella zeae*. Phytopathology 89:182–188.

Carter JP, Rezanoor HN, Desjardins AE, Nicholson P. 2000. Variation in *Fusarium graminearum* isolates from Nepal associated with their host of origin. Plant Pathology 49:452–460.

Chulze SN, Torres A, Ramirez ML, Leslie JF. 2000. Genetic variation in *Fusarium* section Liseola from no-till maize. Applied and Environmental Microbiology 66:4378–4382.

Dusabenyagasani M, Dostaler D, Hamelin RC. 1999. Genetic diversity among *Fusarium graminearum* strains from Ontario and Quebec. Canadian. Journal of Plant Pathology 21:308–314.

Fernando WGD, Zhang JX, Dusabenyagasani M, Guo XW, Ahmed H, McCallum B. 2006. Genetic diversity of *Gibberella zeae* Isolates from Manitoba. Plant Disease 90:1337–1342.

Gagkaeva TY, Yli-Mattila T. 2004. Genetic diversity of *Fusarium graminearum* in Europe and Asia. European Journal of Plant Pathology 110:551–562.

Gale LR, Chen LF, Hernick CA, Takamura K, Kistler HC. 2002. Population analysis of *Fusarium graminearum* from wheat fields in eastern China. Phytopathology 92:1315–1322.

Galich MT. 1997. *Fusarium* head blight in Argentina. In: Duvin HJ, Gilchrist L, Reeves J, McNab A, editors *Fusarium* head scab: global status and future prospects. Mexico City: Centro Internacional de Mejoramiento de Maiz y Trigo. pp 19–28.

Goswami RS, Kistler HC. 2004. Heading for disaster: *Fusarium graminearum* on cereal crops. Molecular Plant Pathology 5:515–525.

Jurgenson JE, Bowden RL, Zeller KA, Leslie JF, Alexander NA, Plattner RD. 2002. A genetic map of *Gibberella zeae* (*Fusarium graminearum*). Genetics 160:1452–1460.

Láday M, Juhász A, Mulé G, Moretti A, Szécsi A, Logrieco A. 2004. Mitochondrial DNA diversity and lineage determination of European isolates of *Fusarium graminearum* (*Gibberella zeae*). European Journal of Plant Pathology 110:545–550.

Leslie JF. 1993. Fungal vegetative compatibility. Annual Review of Phytopathology 31:127–151.

Leslie JF, Klein KK. 1996. Female fertility and mating-type effects on effective population size and evolution in filamentous fungi. Genetics 144:557–567.

Leslie JF, Summerell BA. 2006. The *Fusarium* laboratory manual. Ames, IA: Blackwell Professional.

McDermott JM, McDonald BA. 1993. Gene flow in plant pathosystems. Annual Review of Phytopathology 31:353–373.

Miedaner T, Schilling AG, Geiger HH. 2001. Molecular genetic diversity and variation for aggressiveness in populations of *Fusarium graminearum* and *Fusarium culmorum* sampled from wheat fields in different countries. Journal of Phytopathology 149:641–648.

Milgroom MG. 1996. Recombination and multilocus structure of fungal population. Annual Review of Phytopathology 34:457–477.

Mishra PK, Tewuari JP, Clear RM, Turkington TK. 2004. Molecular genetic variation and geographical structuring in *Fusarium graminearum*. Annals of Applied Biology 145:299–307.

Nei M. 1973. Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Sciences, USA 70: 3321–3323.

Nei M. 1978. Estimation of average heterozygosities and genetic distance from a small number of individuals. Genetics 89:583–590.

O'Donnell K, Kistler HC, Tacke BK, Casper HH. 2000. Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of *Fusarium graminearum*, the fungus causing wheat scab. Proceedings of the National Academy of Sciences, USA 97: 7905–7910.

O'Donnell K, Ward TJ, Geiser DM, Kistler HC, 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 Genetics and Biology 41:600–623.

Ouellet T, Seifert KA. 1993. Genetic characterization of *Fusarium graminearum* strains using RAPD and PCR amplification. Phytopathology 83:1003–1007.

Ramirez ML, Reynoso MM, Farnochi MC, Chulze S. 2006. Vegetative compatibility among *Fusarium graminearum* (*Gibberella zeae*) isolates from wheat spikes in Argentina. European Journal of Plant Pathology 115:129–138.

Sambrook J, Fritsch EF, Maniatis T. 1989. Molecular cloning: A laboratory manual. 2nd. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Schmale DG III, Leslie JF, Zeller KA, Saleh AA, Shields EJ, Bergstrom GC. 2006. Genetic structure of atmospheric populations of *Gibberella zeae*. Phytopathology 96:1021–1026.

Swofford D. 1999. PAUP*. Phylogenetic analysis using parsimony (* and other methods), Version 4.0*. Sunderland, MA: Sinauer Associates.

Tóth B, Mesterházy A, Horváth Z, Bartók T, Varga M, Varga J. 2005. Genetic variability of central European isolates of the *Fusarium graminearum* species complex. European Journal of Plant Pathology 113:35–45.

Vos P, Hogers R, Bleeker M, Reijans M, Van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research 23:4407–4414.

Walker SL, Leath S, Hagler WMJ, Murphy JP. 2001. Variation among isolates of *Fusarium graminearum* associated with *Fusarium* head blight in North Carolina. Plant Disease 85:404–410.

Weir BS. 1979. Inferences about linkage disequilibrium. Biometrics 53:235–254.

World Health Organization. 2001. Deoxynivalenol. In: Safety evaluation of certain mycotoxins in food, WHO Food Additives Series No. 47. Geneva: WHO. pp 419–555.

Yeh FC, Yang RC, Boyle TBJ, Ze ZH, Mao JX. 1997. Popgene: Microsoft Windows®-based freeware for population genetic analysis, Version 1.32. University of Alberta and Centre for International Forestry Research, Canada; (free program available at: http://www.ualberta.ca/~fyeh).

Zeller KA, Bowden RL, Leslie JF. 2003a. Diversity of epidemic populations of *Gibberella zeae* from small quadrants in Kansas and North Dakota. Phytopathology 93:874–880.

Zeller KA, Bowden RL, Leslie JF. 2004. Population differentiation and recombination in wheat scab populations of *Gibberella zeae* from USA. Molecular Ecology 13:63–571.

Zeller KA, Vargas JI, Valdovinos-Ponce G, Leslie JF, Bowden RL. 2003b. Population genetic differentiation and lineage composition among *Gibberella zeae* (*Fusarium graminearum*) in north and South America. Fungal Genetics Newsletter 50(Suppl.):143.