Relatedness of *Macrophomina phaseolina* isolates from tallgrass prairie, maize, soybean, and sorghum

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ABSTRACT

Agricultural and wild ecosystems may interact through shared pathogens such as *Macrophomina phaseolina*, a generalist clonal fungus with more than 284 plant hosts that is likely to become more important under climate change scenarios of increased heat and drought stress. To evaluate the degree of subdivision in populations of *M. phaseolina* in Kansas agriculture and wildlands, we compared 143 isolates from maize fields adjacent to tallgrass prairie, nearby sorghum fields, widely dispersed soybean fields, and isolates from eight plant species in tallgrass prairie. Isolate growth phenotypes were evaluated on a medium containing chlorate. Genetic characteristics were analyzed based on amplified fragment length polymorphisms (AFLPs) and the sequence of the rDNA-ITS region. The average genetic similarity was 58% among isolates in the tallgrass prairie, 71% in the maize fields, 75% in the sorghum fields, and 80% in the dispersed soybean fields. The isolates were divided into four clusters: one containing most of the isolates from maize and soybean, two others containing isolates from wild plants and sorghum, and a fourth containing a single isolate recovered from *Solidago canadensis* in the tallgrass prairie. Most of the sorghum isolates had the dense phenotype on media containing chlorate, while those from other hosts had either feathery or restricted phenotypes. These results suggest that the tallgrass prairie supports a more diverse population of *M. phaseolina* per area than do any of the crop species. Subpopulations show incomplete specialization by host. These results also suggest that inoculum produced in agriculture may influence tallgrass prairie communities, and conversely that different pathogen subpopulations in tallgrass prairie can interact there to generate "hybrids" with novel genetic profiles and pathogenic capabilities.

INTRODUCTION

Several generalist pathogen species occur in both naturally-established and agricultural plant populations. A critical trait of these pathosystems is the degree to which the same pathogen populations are present in both agricultural and natural ecosystems. Analysis of the population genetic structure of generalist pathogens is one approach to understanding this dynamic, and may be simpler if the pathogen reproduces clonally (Anderson & Kohn 1995). For example, the distribution of mitochondrial DNA haplotypes of the generalist *Sclerotinia sclerotiorum* in agricultural sites and native plant populations indicated that the more common types could be recovered from agricultural systems while there were more haplotypes recovered from native species even at smaller spatial scales (Kohli & Kohn 1996). Gordon et al. (1992) concluded that *Fusarium oxysporum* isolates, collected in an uncultivated area and an agricultural field, were part of the same population, although there was more genetic variation in the strains from the uncultivated area. *Barley yellow dwarf virus* (BYDV) maintained in an invasive weed, wild oats, had very damaging effects on wild grass populations in California (Malmstrom et al. 2006), an example of pathogen spillover where pathogens adapted to agriculture move to native systems (Power & Mitchell 2004). In contrast, BYDV strains recovered from Kansas tallgrass prairie did not include the dominant strain in Kansas wheat production (Garrett et al. 2004). While plant diversity in wildland systems can provide ecosystem services through regulation of plant disease, more careful evaluation is needed in cases where generalist pathogens can infect both wild and agricultural species (Cheatham et al. 2009).

Macrophomina phaseolina (Tassi) Goid. is a soil- and seed-borne generalist fungal pathogen that has a global distribution and can infect more than 284 plant species including monocot and dicot plant hosts (Farr et al. 1995). In the United States, *M. phaseolina* causes charcoal rot

disease of many agronomically important crops such as sorghum (*Sorghum bicolor* Moench), maize (*Zea mays* L.), soybean (*Glycine max* L. Merrill), and cotton (*Gossypium arborum* L. and *G. hirsutum* L.) (Baird & Brock 1999; Bradley & del Río 2003; Smith & Wyllie 1999; Stack 2002; Yang & Navi 2005). *Macrophomina phaseolina* also can infect many wild plants and is an opportunistic pathogen of humans (Tan et al. 2008). Drought and high temperature stress increase crop losses due to *M. phaseolina* (Mihail 1989). In contrast to the many pathogens favored by change to moister conditions (Garrett et al. 2006), *M. phaseolina* may become more problematic in agricultural areas where climate change results in longer drought periods and higher temperatures (Mihail 1992; 1989), such as predicted for the US Great Plains.

As has been the case for many pathogens, especially generalists and pathogens that reproduce clonally, the taxonomic identity of *M. phaseolina* has challenged researchers. *Macrophomina phaseolina* has many synonyms, including *M. phaseoli* (Maubl.) Ashby, *Macrophoma corchori* Sawada, *Macrophoma cajani* Syd. & Butl., *Sclerotium bataticola* Taub., *Rhizoctonia bataticola* (Taub.) Briton-Jones, and *Tiarosporella phaseolina* (Tassi) van der Aa (Crous et al. 2006; Mihail 1992; Holliday & Punithaligam1970). Crous et al. (2006) used morphological characters and 28S rDNA sequences to confirm the identity of *M. phaseolina* (anamorph stage) and included it in the ascomycete family *Botryosphaeriaceae*, indicating that *Rhizoctonia bataticola* and *Tiarosporella phaseolina* are inappropriate names for this fungus.

Although only one species has been identified in the genus *Macrophomina*, isolates from different hosts and soils differ in their morphology, e.g. formation of sclerotia, production of pycnidia, colony morphology, pathogenicity, and growth on media containing $KClO_3$. Pearson et al. (1986; 1987a) used chlorate resistance to divide isolates into three phenotypic groups that differentially infect various host plants. Isolates with a dense phenotype were more commonly

recovered from maize, rice, sorghum, and onion, whereas isolates with feathery or restricted phenotypes were more typically recovered from soybean, sunflower, mungbean, and okra (Aboshosha et al. 2007; Pearson et al. 1986; 1987a; 1987b; Mihail & Taylor 1995; Purkayastha et al. 2006; Cloud & Rupe 1991). The distribution of strains of these phenotypes also may be affected by crop rotation and heat and drought stress (Pearson et al. 1986; 1987a; 1987b; Purkayastha et al. 2006; Singh et al. 1990).

Molecular markers, e.g. random amplified polymorphic DNA (RAPD), amplified fragment length polymorphic DNA (AFLP), and restriction fragment length polymorphisms in the rDNA region (RFLP-rDNA), have been used to characterize populations of *M. phaseolina* from various plant hosts and geographic locations. For example, in the United States, *M. phaseolina* isolates from four different hosts (maize, soybean, sorghum and cotton) were distinguished by RAPD genotyping, while chlorate phenotype was less predictive of host (Su et al. 2001). Similarly, in India, *M. phaseolina* isolates from cluster bean were genetically distinct from those isolates recovered from other crops such as cotton and soybean regardless of their geographic origin or chlorate phenotype (Purkayastha et al. 2006). There was a clear association between isolate genotype and host of origin, and the chlorate resistant phenotypes were genetically distinct from the chlorate sensitive ones within each host (Purkayastha et al. 2006; Su et al. 2001). AFLP fingerprints of *M. phaseolina* isolates from Mexico also were associated with geographic origin and source host species (Mayek-Pérez et al. 2001). In contrast, a number of studies have found no association between DNA genotypes and geographic origin or host origin in populations from the United States or elsewhere (Reyes-Franco et al. 2006; Vandemark et al. 2000). However, when Reyes-Franco et al. (2006) reanalyzed the AFLP subgroups of *M. phaseolina*, they could discriminate genetically between the isolates from Mexico and isolates from other countries.

Although molecular markers have not been used to identify significant differences in pathogenicity of isolates, some investigators report that isolates with dense chlorate phenotype were more aggressive than those with restricted or feathery phenotypes (Purkayastha et al. 2006; Reyes-Franco et al. 2006).

We evaluated and compared populations of *M. phaseolina* from agricultural systems in Kansas and from the indigenous tallgrass prairie. The objective of this study was to determine the genetic diversity and differentiation of these populations, which were recovered from eleven host species and five geographic locations. We tested the hypothesis that populations of *M. phaseolina* are host specialized and that the level of genetic diversity in and differentiation of populations varies by host and location. We also determined whether chlorate phenotypes were consistent with previous studies, in which maize and sorghum tended to yield chlorate-resistant (dense) phenotypes while soybean tended to yield chlorate-sensitive (feathery or restricted) phenotypes (Pearson et al. 1986; Pearson et al. 1987a; Su et al. 2001), and whether isolates with the same chlorate phenotype were more closely related than those with different phenotypes. This study provides new perspectives on a generalist pathogen common to native and agro-ecosystems and the extent to which these populations are genetically differentiated.

MATERIALS AND METHODS

Fungal isolate collection

Macrophomina phaseolina isolates were collected from agricultural fields (maize, sorghum and soybean) in Kansas and from tallgrass prairie in the Konza Prairie Biological Station (KPBS) near Manhattan, Kansas. Maize plants were collected from two fields that were adjacent to the

main gate of KPBS, where maize has rarely been grown. Sorghum plants were collected from two fields at the Kansas State University Agronomy Department's Ashland Bottoms Research Farm (ABRF) a few km from the main gate of KPBS. For maize and sorghum fields, symptomatic plant tissues were collected in a systematic sampling grid and *M. phaseolina* isolates were recovered from plants with microsclerotia visible in stems or leaves. Soybean fields were not common in this area; soybean plants were collected in systematic sampling sampling grids from three sites that were 56 (Rossville), 180 (Leavenworth), and 402 (Columbus) km from KPBS. Native tallgrass prairie plants were sampled from three undisturbed tallgrass prairie sites within KPBS dominated by the grass *Andropogon gerardii*. Isolates were recovered from eight different native prairie plant species (Tables 1 and S1). For soybean and native prairie plant samples, *M. phaseolina* isolates were collected from roots.

Fungal isolation and identification

Fungal isolates from roots were collected by removing infected sections (0.5 cm in length) after rinsing with tap water. Symptoms on the generally smaller roots of the prairie species were difficult to interpret. The root sections were surface sterilized with a 0.5% NaOCl solution for 5 to 10 minutes, and then dried with paper towels. Surface-sterilized root pieces were placed in Petri dishes containing a semiselective medium (39 g potato dextrose broth powder (Difco, Detroit, MI), 10 g agar, 250 mg streptomycin sulfate (Sigma-Aldrich, St. Louis, MO), and 160 mg Chloroneb (Sigma-Aldrich), in 1L distilled water; Singleton et al. 1992), and incubated for a week at 22°C. The protocol for strain isolation from maize and sorghum stems was similar, except that 0.5 cm sections of symptomatic stem tissue were collected and surface sterilized prior to incubation on the semiselective medium. To isolate *M. phaseolina* from sorghum, Lima bean

agar (Singleton et al. 1992) was used to reduce contamination by *Fusarium* spp. We identified 143 isolates of *M. phaseolina* based on the production of microsclerotia in culture, with 126 isolates from agricultural fields and 17 from tallgrass prairie (Table 1). One *M. phaseolina* isolate from Arizona was included as a standard (Mihail & Taylor 1995). Three to five small agar pieces containing fungal mycelia were transferred to 125-ml flasks containing 40 ml of a complete medium broth (Leslie & Summerell 2006). The flasks were incubated at 22°C for 24 hrs without agitation, and then transferred to an orbital shaker (150 rpm) and incubated for 3 to 4 days at room temperature (22-25°C). Mycelia were harvested by filtering the medium through milk filter discs (Ken AG, Ashland, OH), dried by blotting with paper towels and kept in aluminum foil at -20°C until ground under liquid nitrogen.

The chlorate sensitivity/resistance of the *M. phaseolina* isolates was determined on minimal media (MM) containing 1.5% $KClO_3$ (Pearson et al. 1986). Colony growth phenotypes were scored after incubation in the dark for one week at room temperature (22-25°C). Three phenotypic classes were identified: dense, feathery, and restricted (Pearson et al. 1986).

DNA extraction and AFLP analysis

Frozen mycelia were ground to a powder under liquid nitrogen in a mortar and pestle. The ground mycelia were transferred to 1.5 ml microcentrifuge tubes. Fungal DNA was extracted by the CTAB method (Murray & Thompson 1980) as modified by Leslie and Summerell (2006).

AFLPs (Vos et al. 1995) were generated as previously described (Leslie & Summerell 2006) with minor modifications. Four AFLP primer-pairs were used, *Eco*RI-AA/*Mse*I-AA, *Eco*RI-AA/*Mse*I-AC, *Eco*RI-AA/*Mse*I-AG, and *Eco*RI-AA/*Mse*I-AT (Reyes-Franco et al. 2006). The *Eco*RI-AA primer was fluorescently labeled with 6-carboxyfluorescein (6-FAM) (IDT,

Coralville, IA). An ABI DNA 3730 sequencer was used to identify the AFLP PCR-amplicons (KSU Genotyping Laboratory, Manhattan, Kansas). Each AFLP reaction contained a Genescan™ 500 LIZ™ size standard (Applied Biosystems, Foster City, CA). GeneMarker version 1.6 software (SoftGenetics, State College, PA) was used to score and analyze the AFLP data. We recorded the presence/absence of each AFLP band ≥ 200 bp in length.

Sequencing of rDNA-ITS region

The ITS region of the rDNA repeat was amplified from 28 isolates representing the four AFLP groups identified using the following forward and reverse primers: ITS5 (SR6R) 5′-GGAAGTAAAAGTCGTAACAAGG-3′ and LR5 5′-TCCTGAGGGAAACTTCG-3′ (Vilgalys & Hester 1990; White et al. 1990). The PCR reaction contained 1 ng/µl genomic DNA, 1× PCR buffer (Bioline, Randolph, MA), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.25 µM of each primer, and 1.0 unit BIOLASE™ DNA polymerase (Bioline, Randolph, MA) in a 30 µl reaction volume. The PCR program was: one cycle at 94°C for 3 min, 31 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min, and one cycle of 72°C for 5 min, and then held at 4°C. PCR amplicons were purified with a QIAquick PCR Purification Kit (Qiagen, Valencia, CA) and then sequenced directly at the KSU sequencing facility. The DNA sequences were edited and aligned with BioEdit software (http://www.mbio.ncsu.edu/Bioedit/bioedit.html). PAUP version 4.0b10 (Swofford 2003) was used to identify haplotypes and determine phylogenetic relationships among the 28 sequences. Other rDNA sequences of *M. phaseolina* previously deposited in GenBank were analyzed along with those identified in this study.

Genetic data analyses

Unique and clonal AFLP haplotypes within and among the various populations of *M. phaseolina* were identified by using the unweighted pair-group method with arithmetic mean algorithm (UPGMA) implemented in PAUP. The Dice coefficient (Nei & Li 1979) was used to calculate pairwise genetic distances within and among the populations using SAS software release 9.1 (SAS Institute, Cary, NC).

To determine the level of genetic variability and genetic differentiation within and among populations of *M. phaseolina*, the allele frequencies of polymorphic AFLP loci, and the genetic diversity within and between different populations of *M. phaseolina* (Nei 1973), were calculated with POPGENE version 1.32 (available at http://www.ualberta.ca/~fyeh/index.htm, Yeh & Boyle 1997). POPGENE also was used to estimate: Nei's unbiased genetic identity and the genetic distance (Nei 1978), the genetic fixation index (G_{st}) (McDermott & McDonald 1993) and the effective migration rate (Nm) (Nei 1987). A locus was considered polymorphic when the band was present at a frequency between 5 and 95%.

Structure software version 2.0 (Pritchard et al. 2000) was used to detect population genetic structure of *M. phaseolina* and identify individuals that were admixed or had migrated. Each isolate was assigned to one or more of K clusters. Cluster numbers from $K = 1$ to $K = 10$ were tested (each simulation of K was replicated five times) based on the statistics described by Evanno et al. (2005) to select the number of clusters that best matched the population structure of *M. phaseolina*.

The variation in AFLP marker patterns among isolates was examined further using principal component analysis (SAS). Major principal components were subjected to analysis of variance to test for effects of host, chlorate phenotype within host, and location within host.

RESULTS

Chlorate growth phenotypes

The growth of *M. phaseolina* on MM amended with chlorate divided the isolates into three phenotype classes: dense, feathery, and restricted. All of the isolates recovered from the soybeans and the KPBS-prairie were either feathery or restricted. Most of the isolates from KPBS-maize also were either feathery or restricted, but two isolates had the dense phenotype. Eighty percent of the isolates from ABRF-sorghum had the dense phenotype.

AFLP genotyping

The four AFLP primer-pairs were equally informative. One hundred-fifty AFLP markers were scored across the 144 isolates, including the reference isolate from Arizona. No monomorphic markers were observed. The greatest genetic diversity (Table 2) occurred amongst isolates collected from KPBS-prairie, with an average genetic similarity of 58%, followed by the KPBS-maize population with an average genetic similarity of 71%. The least genetic diversity was seen amongst isolates from soybean fields with an average genetic similarity of 80%. The highest pairwise genetic similarity was between the KPBS-maize and soybean populations. ABRF-sorghum and soybean populations were the most dissimilar (Table 2).

Unique and repeated AFLP haplotypes within and amongst different populations of *M. phaseolina* were identified with PAUP software. Based on the 150 AFLP markers, there were 134 unique AFLP haplotypes (Tables 1 and S1). Most of the AFLP haplotypes were represented by a single isolate; however, eight haplotypes were represented by more than one isolate (Figure

1). Of these eight haplotypes, three were recovered from different hosts. One haplotype was recovered once from KPBS-maize and twice from ABRF-sorghum. Another haplotype was recovered once from KPBS-maize and once from KPBS-*Helianthus* sp. The third haplotype was recovered once from KPBS-maize and twice from soybeans collected from Rossville, KS (Figures 1, 2).

Based on the UPGMA clustering analysis, the isolates were grouped in four clusters with bootstrap support of 58-100% (Figure 1). The first cluster contained most of the isolates from KPBS-maize and soybean, and a few isolates from ABRF-sorghum and KPBS-prairie. The *M. phaseolina* isolate from Arizona also was in this cluster (Figure 1). All isolates in cluster 1 had either feathery or restricted growth on chlorate medium. Eleven of the 17 isolates from KPBS-prairie grouped in the second cluster, and most of the ABRF-sorghum isolates grouped in cluster 3. All isolates in cluster 3 had the dense phenotype on chlorate medium, except for two isolates that grew poorly on minimal medium with or without chlorate. Cluster 4 contained a single feathery isolate collected from KPBS-*Solidago*. This isolate was genetically distant from the other isolates (Figure 1).

rDNA-ITS sequencing

To confirm the identity of *M. phaseolina* isolates, the rDNA-ITS region and a portion of the rDNA 28S gene were amplified and sequenced from 28 isolates representing the four AFLP clusters (Figure 1 and Table S1). The 28 rDNA sequences were deposited in the GenBank database under the accession numbers FJ395220-FJ395247. The size of the PCR products ranged from 1420 to 1423 bp. Ten single nucleotide polymorphisms (SNPs) were identified and used to group the 28 isolates into five rDNA haplotypes (Figure 1 and Table S3). Most of the SNPs

(7/10) were located in the ITS-1 region. Only one SNP was detected in the ITS-2 region and no SNPs were detected in the 5.8S nrRNA gene. Two SNPs were detected in the portion of the 28S nrRNA gene. Two haplotypes were represented by only one isolate each (MPKS026 and MPKS323), both of which were collected from KPBS-*Solidago*. The third and fourth haplotypes were represented by two (collected from KPBS-*Lespedeza* and KPBS-*Solidago*) and five isolates (collected from ABRF-sorghum and KPBS-maize), respectively. The fifth haplotype was represented by 18 isolates that were collected from KPBS-maize, ABRF-sorghum, soybean, KPBS-*Helianthus*, and KPBS-*Panicum*, in addition to the standard Arizona isolate (Figure 1).

The four AFLP groups were correlated with the five rDNA haplotypes. AFLP cluster 2, which contained most of the *M. phaseolina* isolates collected from KPBS-prairie, was associated with two rDNA haplotypes (Figure 1). When the five rDNA haplotypes were compared with previously deposited rDNA sequences of *M. phaseolina*, most of the deposited rDNA sequences belonged to a single haplotype, which was recovered at all six of our sample sites and was the most common in GenBank (Table S4). This common haplotype also was recovered from other countries such as Australia, Canada, China, India, and Spain in addition to the United States. The sequence of the rDNA haplotype isolated from KPBS-*Lespedeza* and KPBS-*Solidago*, was identical to that of a *M. phaseolina* isolate reported to infect humans (GenBank accession number EF570500). The other three rDNA haplotypes were unique to Kansas populations (GenBank accession numbers FJ395220, FJ395226, and FJ395246). There were a few rDNA sequences deposited in Genbank and attributed to *M. phaseolina* with ITS regions that could not be aligned with the *M. phaseolina* ITS region obtained in this study, and with more than six SNPs in the 5.8S nuclear rRNA gene when aligned with the *M. phaseolina* 5.8S nuclear rRNA

gene (e.g. GenBank accession numbers DQ314733, DQ233663, DQ233665, DQ359740, DQ682587).

When we compared restriction maps of rDNA regions, generated by the NEBcutter program (Vincze et al. 2003), of the ITS-rDNA sequences from the present study with the five RFLP patterns reported by Purkayastha et al. (2006) for *M. phaseolina* from India, we found significant differences (Table S2). The average total length of the amplified ITS-rDNA sequence was ~ 1422 bp from the Kansas isolates and ~ 1600 bp from the Indian isolates. The restriction enzymes used by Purkayastha et al. (2006) would result in a single RFLP-rDNA banding pattern for all of the Kansas sequences, although five banding patterns were reported from the Indian population (Table S2). Some of these discrepancies can be explained by errant size estimates from the gels, *e.g.*, *Eco*RI, *Taq*I, *Hae*III, *Mbo*I and *Rsa*I, but the *Hind* III site in the Indian isolates is completely missing from the ITS-rDNA sequences of the Kansas isolates. Su et al. (2001) also amplified the same region from 45 isolates collected from four plant hosts from Louisiana, USA, and used restriction enzymes *Eco*RI, *Taq*I, *Hae*III, *Mbo*I and *Rsa*I. They identified only one RFLP-rDNA haplotype, which is identical to the one reported in this study. If the Indian rDNA sequences are used to search GenBank, then three of the unique RFLP-rDNA haplotypes match with sequences of fungi other than *M. phaseolina*. In particular, the rDNA sequence of isolate OS-5467-D (GenBank accession number DQ233665) was the same as that of *Dothideomycete* sp. (GenBank accession number EU680526), rDNA from isolate CB-100-D (GenBank accession number DQ233663) was the same as that of *Cochliobolus lunatus* (GenBank accession number DQ836799), and rDNA from isolate GH-50-D (GenBank accession number DQ314733) was the same as that of *Alternaria*. sp. (GenBank accession number

FJ176475). There also are other cases where fungal isolates were misidentified as *Macrophomina* based on rDNA sequences, e.g. Posada et al. (2007).

Population genetic differentiation

POPGENE was used to evaluate the genetic variation within and among *M. phaseolina* populations and to determine if these populations were genetically differentiated. When the isolates from KPBS-prairie, KPBS-maize, ABRF-sorghum, and soybean fields were compared, the overall estimated genetic fixation index was $G_{st} = 0.367$. This high fixation index suggests great genetic differentiation amongst the four location-host groups, *i.e.*, 37% of the total genetic variation could be attributed to genetic differences due to group. The overall effective migration rate (*Nm*) across the four groups was < 1, indicating that gene flow between these populations is not extensive.

When the groups were compared pairwise, the KPBS-maize and soybean groups had the lowest fixation index ($G_{st} = 0.04$) and the highest effective migration number ($Nm = 13$). These values suggest that significant genetic exchange occurs between these two groups and that there are few barriers to genetic exchange (Table 3). The highest G_{st} values separated the ABRF-sorghum group from the other three groups and ranged from 0.34 to 0.45 (Table 3). Thus, the *M. phaseolina* population from ABRF-sorghum is genetically differentiated from the other groups, and is the most genetically divergent of these four groups. The pairwise estimate for G_{st} between the groups from the KPBS-prairie grasses and KPBS-maize was $G_{st} = 0.15$, which indicates that some genetic exchange occurs between these two groups ($Nm < 3$). The pairwise genetic identity between KPBS-prairie grasses and the KPBS-maize groups also was > 90%, which is consistent with a hypothesis of genetic exchange between these groups (Table 2).

Structure 2.0 also was used to evaluate the population genetic structure of the 144 analyzed isolates and to identify migrant and admixed individuals. ΔK (Evanno et al. 2005), which is based on the rate of change in the log-likelihood between successive K values (K = 1 to 10), was used to determine the likely number of clusters. For $K > 3$, the number of populated clusters did not increase, although the number of admixed individuals did. Thus, three clusters suffice to circumscribe the genetic structure of the *M. phaseolina* populations. The results of the Structure analysis were consistent with the results of the AFLP and rDNA analyses in which all isolates from soybeans and most of those from KPBS-maize were assigned to the same cluster that also contained a few individuals from ABRF-sorghum and KPBS-prairie (Figure 3). Most of the ABRF-sorghum isolates were assigned to a second cluster (Figure 3). The isolates from KPBS-prairie were assigned to two clusters (Figure 3). Admixed individuals were detected in soybean/KPBS-maize (8 out of 64 isolates), ABRF-sorghum (2 out of 62), and KPBS-prairie (5 out of 17) clusters. Interestingly, the standard Arizona isolate was one of the admixed individuals (Figure 3).

Principal component analysis

The first four principal components of AFLP data explained 58% of the genetic variation among isolates. The first, third and fourth components displayed significant ($p \leq 0.001$) host effects, while the first and fourth components varied ($p \leq 0.001$) among chlorate phenotypes within a given host (Table 4). Significant location within host (for soybean) effects were not observed for any of the major principal components.

Host means are plotted for the first and third principal components in Figure 4A. The first principal component primarily separated ABRF-sorghum isolates from the other isolates, while

the third component separated isolates into two groups, with one group comprised entirely of KPBS-prairie isolates and the other group comprised of isolates from agronomic plants plus those from *Helianthus* sp., *Panicum virgatum*, and Arizona soil. The fourth principal component separated the Arizona isolate from all other isolates (data not shown).

Chlorate phenotypes for isolates from KPBS-maize, ABRF-sorghum, and soybean were separated by the first and fourth principal components (Figure 4B). Isolates generally clustered by chlorate phenotype, with dense isolates separated from feathery and restricted isolates by the first component and feathery isolates separated from restricted isolates by the fourth component.

DISCUSSION

Populations of pathogens that infect multiple host species may be partitioned geographically and with respect to host, and the degree of partitioning may differ for native or agricultural populations. For example, Kohn (1995) and Kohli and Kohn (1996) reported more widespread distribution of haplotypes of *Sclerotinia sclerotiorum* in agricultural populations than in non-agricultural populations. For *M. phaseolina* in Kansas, we found a common rDNA haplotype of *M. phaseolina* across all the locations and the three crop species sampled. However, there also were rDNA haplotypes that were recovered from only a subset of locations and hosts. Higher dissimilarity was found amongst isolates from tallgrass prairie plants, although this may be explained at least in part by the greater number of host species sampled there (Gordon et al., 1992). Unlike the apparent separation in serotypes observed for BYDV between tallgrass prairie and wheat in Kansas (Garrett et al., 2004), the results from the present study suggest that a portion of the *M. phaseolina* haplotypes in Kansas are shared between tallgrass prairie plants and crop species cultivated in the region. The most common rDNA haplotype, which was recovered

385 from all six sampling sites, also was the most common in GenBank, and has been recovered from Australia, Canada, China, India, and Spain in addition to the United States.

Macrophomina phaseolina is a complex and widely dispersed fungal species in terms of both host and geographic distribution. For a putatively asexual species, it also is quite diverse when evaluated for a number of genetic and physiological characters. In general, isolates from
390 the same location-host clustered together in all analyses. Although the soybean isolates were collected from three widely-dispersed locations, they were the most homogeneous group, with an average genetic similarity of 80%, and all of these isolates belonged to the same AFLP cluster and had the same ITS-rDNA sequence. Most of the isolates from ABRF-sorghum also belonged to a single AFLP cluster with an average similarity of 75%. These results are similar to those of
395 others, *e.g.*, Purkayastha et al. (2006) and Su et al. (2001), who found that *M. phaseolina* isolates from the same host were genetically more similar to one another and genetically more distinct from isolates from other hosts.

The KPBS-prairie population was genetically the most diverse. These isolates were recovered from different hosts, as there were too few isolates from each host species to treat each
400 group as a separate population. Yet most of the KPBS-prairie isolates, regardless of the host, grouped in the same AFLP cluster. Native tallgrass prairie plants could serve as a reservoir for diversity within *M. phaseolina*. Crop acreage may be a greater overall source of inoculum for *M. phaseolina* in the region, but tallgrass prairie provides an environment in which isolates from different hosts may be in immediate contact as host roots intertwine. Heat and drought stress
405 broaden the effective host range of *M. phaseolina* (Pearson et al. 1986, 1987a). The impact of *M. phaseolina* on tallgrass prairie plant communities is not known, but to the extent that climate

change increases heat and drought stress, native prairie plant communities should be more heavily impacted by this pathogen.

The uniqueness of the sorghum population was unexpected, but could result if the *M. phaseolina* isolates that colonize this crop originated in Africa and accompanied sorghum to the United States. However, isolates capable of acting as sorghum pathogens also might be recovered from weedy sorghum relatives, *e.g.* Johnson grass and shattercane, that were not sampled in this study. The recovery of clonal isolates from multiple locations and the presence of presumably genetically admixed isolates suggest that both geographic and genetic mixing is occurring in the populations of this fungal pathogen. Thus, selectively advantageous genetic changes could be retained and shared across all of the *M. phaseolina* populations.

The KPBS-maize population was the most complex, with isolates belonging to three AFLP clusters, two ITS-rDNA sequences, and three chlorate phenotype groups. This variation could result from several factors, including the location of the sampled field next to tallgrass prairie, with much higher plant diversity than typical agricultural systems, and crop rotation within the field. Crop rotation may affect the proportion of various strain types recovered (Pearson et al. 1986; 1987a), probably by altering the selection pressure as different hosts are present. For example, Su et al. (2001) reported more colonization of maize plants by *M. phaseolina* occurred when the field in which the maize was growing was infested by *M. phaseolina* isolates from maize compared to when the infesting fungal isolates were from sorghum, soybean or cotton.

The three population subdivisions of the *M. phaseolina* isolates identified in Structure were consistent with the grouping of the isolates based on the molecular, AFLP and ITS-rDNA, and chlorate phenotype data. Isolates carrying the most common ITS-rDNA sequence clustered in the same AFLP cluster and Structure subpopulation regardless of their host or geographic origin,

suggesting that members of this group were the oldest and most cosmopolitan. Admixed isolates, i.e. isolates that may have mixed ancestry, also were detected (Figure 3). The presence of admixed individuals provides evidence for genetic exchange amongst different isolates of *M. phaseolina*, perhaps through hyphal fusion followed by a parasexual recombination process (Jones et al. 1998; Mihail & Taylor 1995).

While subdivision of *M. phaseolina* into more than one species has been considered, there generally has not been sufficient evidence to support splitting (Crous et al. 2006; Rossman & Palm-Hernández 2008). Numerous studies have used diverse molecular markers to evaluate strains from even more diverse hosts and geographic locations and have identified tremendous levels of genetic variation, but there still are no data that suggest that this species should be subdivided into multiple species (Crous et al. 2006; Reyes-Franco et al. 2006; Jana et al. 2005; Jones et al. 1998; Su et al. 2001). Our results are consistent with these reports in that the lowest genetic similarity between two isolates based on AFLPs in our study was 58%, a value consistent with that for a single species in other fungi in which inter- and intra-specific comparisons of AFLP diversity have been evaluated (*e.g.* Leslie et al. 2001; Marasas et al. 2001; Zeller et al. 2003). Moreover, the rDNA showed that it is very conserved amongst the strains used in this study. Consequently, we suggest that the isolates evaluated in this study probably belong to a single fungal species with a global distribution.

Interpretation of the population genetic structure of *M. phaseolina* is challenging, in part because of a general lack of knowledge about dispersal processes for soilborne pathogens. The hypothesis that expanding populations will be less genetically diverse at their frontiers than in the center of their range (e.g., Milgroom et al. 2008) would be interesting to test in this system, but information about the historical range of *M. phaseolina* in the central United States is not

available. All the haplotypes recovered might have been present throughout the region sampled prior to the introduction of agriculture, followed by selection imposed by cropping systems for particular haplotypes. There also may be current movement between the systems studied; characterizing the connectivity of agricultural and natural components of the landscape for a generalist pathogen such as *M. phaseolina* will be an important step in understanding landscape connectivity, while host specialists are much simpler to address (e.g., Margosian et al., 2009). Another change resulting from agricultural cultivation is the much greater potential for dispersal of soilborne pathogens through wind erosion of soils, or for wind dispersal of infected plant debris. In contrast, tallgrass prairie provides ecosystem services such as protection from wind erosion of soil, and a reduced probability of long-distance dispersal of soilborne pathogens, through extensive perennial root systems.

The importance of *M. phaseolina* is likely to increase in both agricultural and natural ecosystems where climate change scenarios include greater heat and drought stress. An increase in infection by *M. phaseolina* in native ecosystems may alter the relative frequency of plant species in the community and could even result in the extinction of species that are particularly sensitive to this fungus. A larger fungus biomass should also increase the diversity of *M. phaseolina* in both the natural setting and agriculture, and so increase the likelihood of developing more pathogenic lines of agricultural importance. Thus, the abundance and diversity of *M. phaseolina* may become an important indicator of natural and agro-ecosystem health in response to global climate change.

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REFERENCES

1. Aboshosha SS, Atta Allah SI, El-Korany AE, El-Argawy E (2007) Characterization of *Macrophomina phaseolina* isolates affecting sunflower growth in El-Behera governorate, Egypt. International Journal of Agriculture and Biology, 9, 807-815.
2. Almeida AMR, Abdrlnoor RV, Arias CAA, Carvalho VP, Jacoud Filho D S, Marin SRR, Benato LC, Pinto MC, Carvalho CGP (2003) Genotypic diversity among Brazilian isolates of *Macrophomina phaseolina* revealed by RAPD. Fitopatologia Brasileira, 28, 279-285.
3. Anderson JB, Kohn LM (1995) Clonality in soilborne, plant-pathogenic fungi. Annual Review of Phytopathology, 33, 369-391.
4. Baird RE, Brock JH (1999) First report of *Macrophomina phaseolina* on cotton (*Gossypium hirsutum*) in Georgia. Plant Disease, 83, 487.
5. Bradley CA, del Río LE (2003) First report of charcoal rot on soybean caused by *Macrophomina phaseolina* in North Dakota. Plant Disease, 87, 601.

6. Cheatham MR, Rouse MN, Esker PD, Ignacio S, Pradel W, Raymundo R, Sparks AH, Forbes GA, Gordon TR, Garrett KA (2009) Beyond yield: Plant disease in the context of ecosystem services. Phytopathology, 99, 1228-1236.
7. Cloud GL, Rupe JC (1991) Morphological instability on a chlorate medium of isolates of *Macrophomina phaseolina* from soybean and sorghum. Phytopathology, 81, 892-895.
8. Crous PW, Slippers B, Wingfield M, Rheeder J, Marasas WFO, Philips AJL, Alves A, Burgess T, Barber P, Groenewald JZ (2006) Phylogenetic lineages in the *Botryosphaeriaceae*. Studies in Mycology, 55, 235-253.
9. Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology, 14, 2611-2620.
10. Farr DF, Bills GF, Chamuris GP, Rossman AY (1995) Fungi on plants and plant products in the United States, 2nd edn. APS Press, St. Paul, Minnesota.
11. Gaetán SA, Fernandez L, Madia M (2006) Occurrence of charcoal rot caused by *Macrophomina phaseolina* on canola in Argentina. Plant Disease, 90, 524.
12. Garrett KA, Dendy SP, Frank EE, Rouse MN, Travers SE (2006) Climate change effects on plant disease: Genomes to ecosystems. Annual Review of Phytopathology, 44, 489-509.
13. Garrett KA, Dendy SP, Power AG, Blaisdell GK, Alexander HA, McCarron JK (2004) Barley yellow dwarf disease in natural populations of dominant tallgrass prairie species in Kansas. Plant Disease, 88, 574.
14. Gordon TR, Okamoto D, and Milgroom MG (1992) The structure and interrelationship of fungal populations in native and cultivated soils. Molecular Ecology, 1, 241-249.
15. Holliday P, Punithalingam E (1970) *Macrophomina phaseolina*. CMI Description of Pathogenic Fungi and Bacteria No. 28.

16. Jana TK, Singh NK, Koundal KR, Sharma TR (2005) Genetic differentiation of charcoal rot pathogen, *Macrophomina phaseolina*, into specific groups using UP-PCR. Canadian Journal of Microbiology, 51, 159-164.

17. Jones RW, Canada S, Wang H (1998) Highly variable minichromosomes and highly conserved endonuclease genes in the phytopathogenic fungus *Macrophomina phaseolina*. Canadian Journal of Botany, 76, 694-698.

18. Kohli Y, Kohn LM (1996) Mitochondrial haplotypes in populations of the plant-infecting fungus *Sclerotinia sclerotiorum*: Wide distribution in agriculture, local distribution in the wild. Molecular Ecology, 5, 773-783.

19. Kohn LM (1995) The clonal dynamic in wild and agricultural plant-pathogen populations. Canadian Journal of Botany, 73, S1231-S1240.

20. Leslie JF, Summerell BA (2006) The *Fusarium* Laboratory Manual, Blackwell Professional Publishing, Ames, Iowa.

21. Leslie JF, Zeller KA, Summerell BA (2001) Icebergs and species in populations of *Fusarium*. Physiological and Molecular Plant Pathology, 59, 107-117.

22. Malmstrom CM, Stoner CJ, Brandenburg S, Newton LA (2006) Virus infection and grazing exert counteracting influences on survivorship of native bunchgrass seedlings competing with invasive exotics. Journal of Ecology, 94, 264-275.

23. Marasas WFO, Rheeder JP, Lamprecht SC, Zeller KA, Leslie JF (2001) *Fusarium andiyazi* sp. nov., a new species from sorghum. Mycologia, 93, 1203-1210.

24. Margosian ML, Garrett KA, Hutchinson JMS, With KA (2009) Connectivity of the American agricultural landscape: Assessing the national risk of crop pest and disease spread. BioScience, 59, 141-151.

25. Mayek-Pérez N, Lopez-Castaneda C, Gonzalez-Chavira M, Garcia-Espinosa R, Acosta-Gallegos J, Martinez de la Vega O, Simpson J (2001) Variability of Mexican isolates of *Macrophomina phaseolina* based pathogenesis and AFLP genotype. Physiological and Molecular Plant Pathology, 59, 257-264.

26. McDermott JM, McDonald BA (1993) Gene flow in plant pathosystems. Annual Review of Phytopathology, 16, 353-374.

27. Mihail JD (1989) *Macrophomina phaseolina*: Spatio-temporal dynamics on inoculum and of disease in a highly susceptible crop. Phytopathology, 79, 848-855.

28. Mihail JD (1992) *Macrophomina*. Pages 134-136 in: Methods for Research on Soilborne Phytopathogenic Fungi. Singleton LL, Mihail JD, Rush CM, APS Press, St. Paul, Minnesota.

29. Mihail JD, Taylor SJ (1995) Interpreting variability among isolates of *Macrophomina phaseolina* in pathogenicity, pycnidium production and chlorate utilization. Canadian Journal of Botany, 73, 1596-1603

30. Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Research, 8, 4321-4325.

31. Nei M (1973) Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Sciences of the USA, 70, 3321-3323.

32. Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, 89, 583-590.

33. Nei M (1987) Molecular Evolutionary Genetics, Columbia University Press, New York.

34. Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences of the USA, 76, 5269-5273.

35. Pearson CAS, Leslie JF, Schwenk FW (1986) Variable chlorate resistance in *Macrophomina phaseolina* from corn, soybean and soil. Phytopathology, 76, 646-649.

36. Pearson CAS, Leslie JF, Schwenk FW (1987a) Host preferences correlated with chlorate resistance in *Macrophomina phaseolina*. Plant Disease, 71, 828-831.

37. Pearson CAS, Leslie JF, Schwenk FW (1987b) Nitrogen source utilization by chlorate-resistance and chlorate-sensitive isolates of *Macrophomina phaseolina*. Transactions of the British Mycological Society, 88, 497-502.

38. Posada F, Aime MC, Peterson SW, Rehner SA, Vega FE (2007) Inoculation of coffee plants with the fungal entomopathogen *Beauveria bassiana* (*Ascomycota: Hypocreales*). Mycological Research, 748–757

39. Pritchard JK, Stephens M, and Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics, 155, 945-959.

40. Power AG, Mitchell CE (2004) Pathogen spillover in disease epidemics. The American Naturalist, 164, S79-S89.

41. Purkayastha S, Kaur B, Arora P, Bisyer I, Dilbaghi N, and Chaudhury A (2008) Molecular genotyping of *Macrophomina phaseolina* isolates: comparison of microsatellite primed PCR and repetitive element sequence-based PCR. Journal of Phytopathology, 156,372-381.

42. Purkayastha S, Kaur B, Dilbaghi N, and Chaudhury A (2006) Characterization of *Macrophomina phaseolina*, the charcoal rot pathogen of cluster bean, using conventional techniques and PCR-based molecular markers. Plant Pathology, 55, 106-116.

43. Reyes-Franco MC, Hernández-Delgado S, Beas-Fernández R, Medina-Fernández M, Simpson J, and Mayek-Pérez N (2006) Pathogenic and genetic variability within

Macrophomina phaseolina from Mexico and other countries. Journal of Phytopathology, 154,447-453.

44. Rossman AY, Palm-Hernández ME (2008) Systematics of plant pathogenic fungi: Why it matters. Plant Disease, 92, 1376-1386.

45. Singh SK, Nene YL, and Reddy MV (1990) Influence of cropping systems on *Macrophomina phaseolina* populations in soil. Plant Disease, 74, 812-814.

46. Singleton LL, Mihail JD, and Rush CM (1992) Methods for Research on Soilborne Phytopathogenic Fungi, APS Press, St. Paul, Minnesota.

47. Smith GS, Wyllie TD (1999) Charcoal rot. Pages 29–31 in: Compendium of Soybean Diseases, 4th ed. Hartman GL, Binclair JB, and Rupe JC, APS Press, St. Paul, Minnesota.

48. Stack JP (2002) Recurring and emerging sorghum diseases in North America. Pages 449-456 in: Sorghum and Millets Diseases, Leslie JF, Iowa State University Press, Ames, Iowa.

49. Su G, Suh S-O., Schneider RW, and Russin JS (2001) Host specialization in the charcoal rot fungus *Macrophomina phaseolina*. Phytopathology, 91, 120-126.

50. Summerbell RC, Moore MK, Strink-Willemse M, and Van Iperen A (2007) ITS barcodes for *Trichophyton tonsurans* and *T. equinum*. Medical Mycology, 45, 193-200.

51. Swofford DL (2003) PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.

52. Tan DHS, Sigler L, Gibas CFC, and Fong IW (2008) Disseminated fungal infection in a renal transplant recipient involving *Macrophomina phaseolina* and *Scytalidium dimidiatum*: case report and review of taxonomic changes among medically important members of the Botryosphaeriaceae. Medical Mycology, 46, 285-292.

53. Vandemark G, Martinez O, Pecina V (2000). Assessment of genetic relationships among the isolates of *Macrophomina phaseolina* using simplified AFLP technique and two different methods of analysis. Mycologia, 92, 656-664.

54. Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology, 172, 4238-4246.

55. Vincze T, Posfai J, Roberts RJ (2003) NEBcutter: a program to cleave DNA with restriction enzymes. Nucleic Acids Research, 31, 3688-3691

56. 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.

57. White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Shinsky JJ, White TJ, eds. PCR Protocols: A Guide to Methods and Applications, Academic Press, San Diego.

58. Yang XB, Navi SS (2005) First report of charcoal rot epidemics caused by *Macrophomina phaseolina* in soybean in Iowa. Plant Disease, 89, 526.

59. Yeh FC, Boyle TJB (1997) Population genetic analysis of co-dominant and dominant markers and quantitative traits. Belgian Journal of Botany, 129, 157.

60. Zeller KA, Summerell BA, Leslie JF (2003) *Gibberella konza* (*Fusarium konzum*) sp. nov. from prairie grasses, a new species in the *Gibberella fujikuroi* species complex. Mycologia, 95, 943-954.

Table 1. Isolates of *M. phaseolina* included in the analyses and their sources.

Population Family	**Sampling location**	**Host(s)**	**Isolates/ AFLP haplotypes**
Natural	**KPBS**		17/17
Asteraceae		*Ambrosia* sp. (AM)[a]	1/1
Asclepiadaceae		*Asclepias* sp. (AS)	2/2
Cornaceae		*Cornus drummondii* (D)	1/1
Asteraceae		*Helianthus* sp. (H)	3/3
Fabaceae		*Lespedeza capitata* (L)	1/1
Poaceae		*Panicum virgatum* (PA)	2/2
Solanaceae		*Physalis* sp. (PH)	1/1
Asteraceae		*Solidago canadensis*(SO)	6/6
Maize	**KPBS**		
Poaceae		*Zea mays* (C)	20/20
Soybean			44/42
Fabaceae	Rossville,	*Glycine max* (SR)	15/14
	Leavenworth	*Glycine max* (SL)	20/20
	Columbus	*Glycine max* (SC)	9/8
Sorghum	**ABRF**		
Poaceae		*Sorghum bicolor* (G)	62/57

[a]The abbreviation of species name at the end of isolate designation

[b]The number of clones is not additive because some haplotypes contain isolates from more than one population.

Table 2. The pairwise average genetic similarities using the Dice coefficient (between populations above the diagonal and within populations on the diagonal) and pairwise calculations of Nei's unbiased genetic identity (below the diagonal) for four different populations of *M. phaseolina*.

640

	KPBS-Maize	Soybean	ABRF-Sorghum	KPBS-prairie
KPBS-Maize	0.712	0.767	0.643	0.611
Soybean	0.989	0.797	0.601	0.691
ABRF-Sorghum	0.732	0.656	0.746	0.623
KPBS-prairie	0.908	0.882	0.718	0.584

Table 3. Pairwise estimates of fixation index (G_{st}) (above diagonal) and effective migration number (Nm) (below diagonal) based on the 88 AFLP markers from four populations of *M. phaseolina*.

645

Population	Maize	Soybean	Sorghum	Native prairie
Maize	-	0.04	0.34	0.15
Soybean	13.0	-	0.45	0.21
Sorghum	1.0	0.61	-	0.34
Native prairie	2.8	1.9	0.98	-

Table 4. Analysis of variance of major principal components for AFLP patterns of

650 *Macrophomina phaseolina* isolates from Kansas.

	Mean square			
	PC1	PC2	PC3	PC4
Effect	(24.3)[1]	(39.4)	(50.5)	(57.8)
Host	96.92*	24.79	89.52*	129.67*
Phenotype (Host)	87.90*	22.65	10.67	1.49*
Location (Host)	0.27	0.07	0.14	0.31
Isolate	4.63	16.26	9.53	0.39

[1]Cumulative % variance

*Statistically significant for $\alpha = 0.001$.

Figure captions:

Figure 1. Phylogenetic tree constructed by UPGMA method based on 150 AFLP markers for the 144 isolates of *M. phaseolina*. The numbers above the branches represent the bootstrap values for 1000 replicates. The last letters of isolate names indicate the source host from which the isolates were collected (see Table 1). The ITS-rDNA region was amplified from the isolates indicated in bold font. The three chlorate phenotypes, F = feathery, R = restricted, and D = dense (CH PHE); and five rDNA haplotypes are indicated.

Figure 2. Venn diagram illustrating the overlap of the four groups of *M. phaseolina* from different plant hosts and locations. Shared rDNA (in Roman numerals) and AFLP (letters in circles) haplotypes among groups are in intersections.

Figure 3. Structure summary blot showing the assignment of *M. phaseolina* isolates from different locations/hosts to K= 3 clusters, where each cluster is indicated by a different shading. Each fungal isolate is represented by a thin vertical region partitioned into membership fractions of the three assigned clusters. Vertical black lines separate groups of fungal isolates from different locations/hosts. Arabic numbers, 1-8, indicate the following hosts: 1-*Solidago*, 2-*Helianthus*, 3-*Panicum*, 4-*Asclepias*, 5-*Cornus*, 6-*Lespedeza*, 7-*Ambrosia*, and 8-*Physalis*. Number 9 indicates the standard isolate from Arizona.

Figure 4. Scatterplot for (A) host species means of the first and third principal components and (B) chlorate phenotype means of the first and fourth principal components for *M. phaseolina* AFLP patterns.