Genetic Variation Among *Colletotrichum graminicola* Isolates from Four Hosts Using Isozyme Analysis

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ABSTRACT

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Anthraxnose basal rot (ABR) is a serious disease of turfgrasses that is caused by the pathogen *Colletotrichum graminicola*. The relationships of isolates causing ABR on turfgrasses to those causing disease on important crop hosts (maize, sorghum) remain unresolved. Genetic variation among isolates from annual bluegrass, creeping bentgrass, maize, and sorghum was evaluated based on host origin and geographic origin. Isozymes were used to estimate the genetic variation of the isolates. Five enzyme systems comprising 16 alleles from 5 loci were used. Allele frequencies, genetic distance, and linkage disequilibrium values were calculated for isolates based on both host and geographic origin. Isolates from creeping bentgrass and annual bluegrass were the most closely related based on Nei's genetic distance, while isolates from maize and sorghum were the most distantly related, consistent with their known species-level relationship. Isolates from annual bluegrass and creeping bentgrass had different genetic distances to isolates from both maize and sorghum. Annual bluegrass isolates from different geographic regions had the smallest genetic distance values observed in this study, indicating a very close relationship regardless of geographic origin. Based on these data, it appears that host origin, not geographic origin, plays a more important role in the genetic diversity of these fungi.

Additional keywords: *Agrostis palustris*, electrophoresis, *Poa annua*, *Zea mays*

Anthraxnose basal rot (ABR) is a serious disease of annual bluegrass (*Poa annua* L.) and creeping bentgrass (*Agrostis palustris* Huds.). Anthracnose basal rot and other diseases significantly diminish the ability of these grasses to survive periods of environmental stress. This disease presents a major obstacle in cultivating these grasses as desirable species (18). The disease begins as small, diffuse patches of chlorotic, dying turf that may increase in diameter to 15 cm or more (12). Initially, older leaves are discolored as a result of the diseased crown, but eventually the youngest leaf is discolored and plant death usually follows (12,13,18). The crown of an infected plant appears black and rotted and contains numerous acervuli.

One of the problems golf course superintendents face when turf is affected by ABR is the unpredictable ball roll caused by the small voids of bare soil left as a result of the disease. These voids also negatively affect the appearance of the playing surface. Because appearance is critically important in pleasing golfers,

ABR can cause significant problems for golf course superintendents.

The causal agent of ABR is presently identified as *Colletotrichum graminicola* (Ces.) G.W. Wils. (11,17). In most cases, *Colletotrichum* isolates that infect graminaceous hosts are identified as *C. graminicola* unless morphological or molecular characteristics show otherwise. Sutton (15) suggested that isolates of *C. graminicola* from sorghum (*Sorghum* spp.) and maize (*Zea mays* L.) be classified as separate species. The morphological separation was primarily based on appressorial characteristics, and Sutton (15) suggested that based on these differences isolates from sorghum be classified as a new species, *C. sublineolum* (14,15). Small morphological differences between isolates from maize, sorghum, and turfgrasses have been observed in several recent studies, but the overlapping range of measurements would preclude their use as a diagnostic criterion for identification as separate species (1,3). However, molecular genetic techniques support the concept that at least two species of *Colletotrichum* infect graminaceous hosts (1,3-5,10,16).

Vaillancourt and Hanau (16) used data generated from mtDNA restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), and crossing studies to demonstrate that *Colletotrichum* isolates from maize and sorghum are probably different species. Data from RAPD fingerprinting

showed only 45% similarity between isolates from different host origins, and only isolates originating from the same host could undergo sexual recombination (16). These data support the classification of *Colletotrichum* isolates originating on sorghum as a new species (16). Sherriff et al. (10) confirmed these results using the ribosomal DNA internal transcribed spacer region (ITS-1) sequence and suggested classifying sorghum isolates as a new species, *C. sublineolum*.

Several recent studies have attempted to clarify the genetic relationships of *Colletotrichum* spp. infecting amenity turfgrasses using molecular techniques (1,3,5). Backman et al. (1) used RAPDs to show that *Colletotrichum* isolates from annual bluegrass were more closely related to isolates from sorghum, and isolates from creeping bentgrass were more closely related to isolates from maize (1). Browning et al. (3) also used RAPDs to examine the relatedness of *Colletotrichum* isolates from the northeastern United States. Their results conflicted with those of Backman et al. (1), as isolates from maize and sorghum were more closely related to each other than to isolates from either annual bluegrass or creeping bentgrass. Both of these studies had experiments that tested the host specificity of the isolates, and both found that isolates were generally more virulent on the host from which the isolate originated. These studies also found that annual bluegrass could be infected by isolates from maize, sorghum, or creeping bentgrass. However, isolates from annual bluegrass were less virulent on other hosts. Hsiang and Goodwin (5) attempted to resolve the issue of classification of *Colletotrichum* isolates from turfgrasses using sequences of the ITS region of the ribosomal DNA of *C. graminicola*. Their results supported the findings of Browning et al. (3) suggesting that isolates from turfgrass were more similar to each other than to *C. graminicola* isolates from maize or to *C. sublineolum* isolates and, therefore, may belong to different biological species (5). The presence of geographic specialization and its impact on the relationships among isolates were not addressed by these studies.

Isozyme analysis provides a broader view of genetic variation than DNA analyses because it examines differences in the mobility of active metabolic proteins rather than differences in fragment sizes of DNA. Isozyme analysis generates genetic information using an efficient and cost-effective

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technique. Isozyme analysis has been used to study variation in other species of *Colletotrichum* (2,6,7). Bonde et al. (2) used 12 enzymes representing a total of 14 putative loci to examine *Colletotrichum* spp. infecting strawberries. They found that for the five species tested, all the isolates supported the current species designation with the exception of *C. acutatum* and *C. gloeo-*

sporioides. Isolates from these two groups seemed to be related at a subspecies level rather than comprising distinct species (2). Their findings demonstrate that isozymes provide an effective method for distinguishing among *Colletotrichum* species (2).

Our objectives were: (i) to examine the genetic diversity present in isolates of *C. graminicola* originating on *P. annua*,

A. palustris, *Z. mays*, and *Sorghum* spp. based on host origin, and (ii) to examine whether geographic specialization was also a factor in the genetic diversity of isolates of *C. graminicola* from turfgrasses.

MATERIALS AND METHODS

Isolate collection. Isolates of *C. graminicola* were obtained from several

Table 1. Isolates used in this study and their host origin, geographic region, electrophoretic phenotype (EP), geographic origin, and source

Isolate	Host	Region ^a	EP	Geographic origin	Source ^b
QR 95-1	<i>Poa annua</i>	A	1	Scarsdale, NY	Browning et al. (3)
SUN 1	<i>Poa annua</i>	A	1	Johnston, PA	Backman et al. (1)
DB 95-1	<i>Poa annua</i>	M	1	Detroit, MI	—
IW 94-1	<i>Poa annua</i>	M	1	Detroit, MI	—
WH 95-1	<i>Poa annua</i>	M	1	Lansing, MI	—
LS 95-1	<i>Poa annua</i>	M	1	Chicago, IL	—
KWIL 95-1	<i>Poa annua</i>	M	1	Chicago, IL	—
EV 95-1	<i>Poa annua</i>	M	1	Chicago, IL	—
WF 95-1	<i>Poa annua</i>	A	2	Wakefield, RI	Browning et al. (3)
OAK 3	<i>Poa annua</i>	A	2	Pittsburgh, PA	Backman et al. (1)
VAL 1	<i>Poa annua</i>	A	2	State College, PA	Backman et al. (1)
MCC 95-1	<i>Poa annua</i>	A	2	Madison, CT	Browning et al. (3)
AP 95-1	<i>Poa annua</i>	A	2	Cranston, RI	Browning et al. (3)
SPC 95-1	<i>Poa annua</i>	A	2	Sands Point, NY	Browning et al. (3)
CAM	<i>Poa annua</i>	A	2	State College, PA	Backman et al. (1)
OAK 2	<i>Poa annua</i>	A	2	Pittsburgh, PA	Backman et al. (1)
IWIL 95-1	<i>Poa annua</i>	M	2	Chicago, IL	—
RK 96-1	<i>Poa annua</i>	M	2	Detroit, MI	—
GR 95-1	<i>Poa annua</i>	M	2	Grand Rapids, MI	—
IV 95-1a	<i>Poa annua</i>	M	2	Toledo, OH	—
CW 95-1	<i>Poa annua</i>	M	2	Vienna, OH	—
CCL 95-1	<i>Poa annua</i>	M	3	Lansing, MI	—
BA 95-1	<i>Poa annua</i>	—	3	Bel Aire, CA	—
CL 95-1	<i>Poa annua</i>	M	4	Missouri	—
WGC 95-1	<i>Poa annua</i>	M	4	Weirton, WV	—
ICC 95-2	<i>Agrostis palustris</i>	A	3	Ipswich, MA	Browning et al. (3)
KCC 95-2	<i>Agrostis palustris</i>	A	3	Yarmouthport, MA	Browning et al. (3)
ACC 95-2	<i>Agrostis palustris</i>	A	3	Plymouth, MA	Browning et al. (3)
PCC 95-2	<i>Agrostis palustris</i>	A	3	Litchfield, NH	Browning et al. (3)
PQ 95-4	<i>Agrostis tenuis</i>	—	3	Bristol, ME	Browning et al. (3)
AT 96-2	<i>Agrostis palustris</i>	—	3	Atlanta, GA	—
CCN 95-2	<i>Agrostis palustris</i>	—	3	Pinehurst, NC	—
FF 96-2	<i>Agrostis palustris</i>	—	3	Frankfort, KY	—
GM 95-2b	<i>Agrostis palustris</i>	—	3	Missouri	—
KEN-1	<i>Agrostis palustris</i>	—	3	Lexington, KY	Backman et al. (1)
CH 95-2	<i>Agrostis palustris</i>	A	5	Southbridge, MA	Browning et al. (3)
SS 95-2	<i>Agrostis palustris</i>	A	5	Ridgefield, CT	Browning et al. (3)
BOB 95-2	<i>Agrostis palustris</i>	A	6	Brewster, NY	Browning et al. (3)
AV 95-2	<i>Agrostis palustris</i>	A	6	Weston, CT	Browning et al. (3)
PA 95-2	<i>Agrostis palustris</i>	A	6	Bellingham, MA	Browning et al. (3)
SCC 95-2	<i>Agrostis palustris</i>	A	6	Stonington, MA	Browning et al. (3)
VAL 2	<i>Agrostis palustris</i>	A	6	State College, PA	Backman et al. (1)
WFCC 95-2	<i>Agrostis palustris</i>	A	6	Wethersfield, CT	Browning et al. (3)
GW 95-2	<i>Agrostis palustris</i>	A	6	Greenwich, CT	Browning et al. (3)
FCC 95-2	<i>Agrostis palustris</i>	A	7	Farmington, CT	Browning et al. (3)
MA 95-2	<i>Agrostis palustris</i>	—	8	Kennesaw, GA	—
UNK 95-2	<i>Agrostis palustris</i>	—	9	Chicago, IL	—
COR 2	<i>Zea mays</i>	—	10	Rock Springs, PA	Backman et al. (1)
Cg 151	<i>Zea mays</i>	—	11	New York	G. C. Bergstrom
Cg 46	<i>Zea mays</i>	—	11	Unknown	D. G. White
Cg ASK 88	<i>Zea mays</i>	—	12	Unknown	D. G. White
Cg M6	<i>Zea mays</i>	—	12	Missouri	R. L. Nicholson
Cg 17	<i>Zea mays</i>	—	12	Unknown	D. G. White
Cg 122	<i>Zea mays</i>	—	12	Indiana	R. L. Nicholson
Mo 940 D	<i>Zea mays</i>	—	12	Indiana	R. L. Nicholson
Cg M9 Mo	<i>Zea mays</i>	—	13	Missouri	R. L. Nicholson
COR 3	<i>Zea mays</i>	—	14	New York	Backman et al. (1)
Cg 1042 (2-3-9)	<i>Sorghum halepense</i>	—	15	Stuttgart, AR	D. TeBeest
Cg 1034	<i>Sorghum halepense</i>	—	16	Devil's Den, AR	D. TeBeest
FRM 1993 236I	<i>Sorghum bicolor</i>	—	17	Griffin, GA	U. L. Rosewich
Cg 1031	<i>Sorghum halepense</i>	—	18	College Station, TX	D. TeBeest

^a Region for geographic data analysis: M = Midwest, A = Atlantic, and — = not included in analysis.

^b Source of isolates, — = isolated by authors.

sources and represent several different geographic locations (Table 1). Twenty-five isolates from *P. annua*, 21 from *A. palustris*, 1 from *Agrostis tenuis*, 10 from *Z. mays*, and 4 from *Sorghum* spp. were obtained from single spores and stored as a spore suspension in 20% (wt/vol) glycerol solution at -80°C . Several isolates were also stored as colonized potato dextrose agar (PDA) (Difco Laboratories, Detroit, MI) plugs in 1 ml of sterile heavy mineral oil at room temperature. Isolates were maintained in storage for up to 2 years until they were needed for experiments.

Culture preparation. Isolates were cultivated from storage by inoculating 15 ml of V8 juice broth (200 ml of low Na V8, 800 ml of distilled H_2O , 6 g of CaCO_3) with 200 μl of spore suspension and incubating them in a 25-cm² tissue culture flask under a 24-h photoperiod for 4 days.

Extraction and electrophoresis. The techniques for isozyme analysis were modified as described below from Quiros (9). Proteins were extracted from hyphae immediately prior to electrophoresis. The contents of the tissue culture flask were harvested, and a small portion of mycelium (2 to 3 cm²) was excised and blotted dry with paper towels. The mycelium was placed in a sample well of a Plexiglas grinding tray that was placed on ice to prevent protein degradation. Cold 2% glutathione extraction buffer (120 μl) was added to each sample to further prevent protein degradation. The mycelium was macerated with a blunt Plexiglas rod to release the proteins into the extraction buffer. Extracted proteins from one sample were absorbed into four filter paper wicks (Whatman, Maidstone, England) measuring approximately 3 $\times$ 8 mm. Both a pH 8.3 system and a pH 5.7 system were used to separate enzymes present in the crude extract. Horizontal starch gels (12% wt/vol) were prepared using the appropriate gel buffers for each system (Table 2). The gel origin was sliced perpendicular to the direction of the electrical current, and two wicks from each sample were placed vertically at the origin of the gel. The gels and appropriate running buffers (Table 2) were maintained at 4°C , and electrical current was applied across the gel at 55

mA for the pH 8.3 system and at 35 mA for the pH 5.7 system, and the current was monitored closely throughout each 3-h run. The protein front was allowed to progress for about 40 min before the wicks were removed from the origin. Following wick removal, the gel was covered with transparency film followed by placement of a glass plate on top of the film. Ice bags were placed on the glass plate to cool the gel and prevent protein denaturation.

Visualization of enzyme activity. Following electrophoresis, gels were sliced into four 1-mm-thick slabs, and each slab was placed in a separate enzyme stain. Enzyme activity was assayed for each enzyme system (Table 2) using histochemical stains that produce an insoluble dye where enzyme activity is present. The staining protocols were modified from Vallejos (17) by optimizing the intensity of the staining through adjustments to the substrate amount in the stain. To ensure the repeatability of the zymograms, each isolate was run on two replicate gels. A total of 16 enzyme systems were assayed for activity and resolution in two buffer systems. Seven enzyme systems were resolved in isolates originating from *A. palustris* and *P. annua*, while only five enzyme systems were resolved in isolates from *Z. mays* and *Sorghum* spp. (Table 2). The remaining nine enzyme systems were not repeatedly resolved, and therefore, they were not investigated further.

Analysis of data. Data generated from each gel run were recorded in a matrix identifying the presence (1) or absence (0) of a particular band. Putative allele frequencies were calculated from the presence/absence matrices and subdivided based on host origin or geographic location. Nei's unbiased genetic distance, *D* (corrected for small sample sizes), (8) was calculated for each group of isolates. As the value of *D* approaches 0, the two populations being compared are more closely related and approach identity. As the value of *D* gets larger, the two populations are more distant and share less genetic similarity. Exact tests for linkage disequilibrium were also calculated to test all possible pairs of loci for significant disequilibrium.

RESULTS

Of the 16 enzymes originally tested, seven enzymes produced distinct and repeatable zymograms in isolates from *A. palustris* and *P. annua*. Only five enzymes produced repeatable zymograms in isolates from *Z. mays* or *Sorghum* spp. The enzymes 6-PGDH (E.C. number 1.1.1.44) and IDH (E.C. number 1.1.1.42) were dropped from all analyses because they were monomorphic in the *A. palustris* and *P. annua* groups of isolates, and were not resolvable in the *Z. mays* or *Sorghum* spp. isolate groups. Therefore, only the five enzymes that were resolved in all isolates were used for analysis.

Sixteen putative alleles were resolved with these five enzymes. Eighteen unique electrophoretic phenotypes (EPs) were observed in the 61 isolates sampled. Putative allele frequencies were calculated for isolates grouped by host origin and geographic origin (Table 3). All isolates produced single-banded phenotypes consistent with a haploid genetic condition in all enzyme systems tested. Significant linkage disequilibrium was evident among alleles at all loci except Pgm/Tpi and Aat/Tpi. However, no allele at any locus was unique to a single host or geographic origin (Table 4). The observed pattern demonstrates that genetic distance analyses are appropriate for examining the pattern of genetic variation in these fungi.

Electrophoretic phenotypes 1, 2, and 4 were comprised solely of isolates from *P. annua*, while EPs 5 to 9 contained isolates from *A. palustris*. *Z. mays* isolates were grouped into EPs 10 to 14, and *Sorghum* isolates were grouped into EPs 15 to 18 (Table 1). There was only one EP that contained members from multiple host origins. Electrophoretic phenotype 3 contained nine members from *A. palustris*, one member from *A. tenuis*, and two members from *P. annua*.

Geographically, EPs 1 and 2 were comprised of *P. annua* isolates from both the Midwest and Atlantic regions. Electrophoretic phenotype 4 was unique to Midwestern *P. annua* isolates, and EPs 5 to 7 were unique to Atlantic *A. palustris* isolates. Electrophoretic phenotype 3 contained both Midwestern *P. annua* isolates and Atlantic *A. palustris* isolates.

Table 2. Isozymes used in electrophoretic analysis of *Colletotrichum graminicola* and *C. sublineolum* isolates

Enzyme ^a	Abbreviation	EC no.	Activity ^b	Electromorphs ^c	Buffer system ^d
Aspartate aminotransferase	AAT	2.6.1.1	A,B	4	1
Isocitric dehydrogenase	IDH	1.1.1.42	A	1	2
Malate dehydrogenase	MDH	1.1.1.37	A,B	3	2
Phosphoglucose isomerase	PGI	5.3.1.9	A,B	4	2
Phosphoglucomutase	PGM	5.4.2.2	A,B	2	1
6-Phosphogluconate dehydrogenase	6PGDH	1.1.1.44	A	1	2
Triosephosphate isomerase	TPI	5.3.1.1	A,B	3	1

^a All stain recipes from (17).

^b Visualized in isolates from: A = amenity turfgrasses, B = crop plants.

^c Number of different activity bands visualized in each enzyme studied.

^d Buffer systems (17) 1 = Lithium borate/Tris-citrate, pH 8.3, 55 mA; 2 = Histidine-citrate, pH 5.7, 35 mA.

Using the frequencies for each putative allele (Table 3), Nei's unbiased genetic distance (8) indicated that isolates from *P. annua* and *A. palustris* were the most closely related. Isolates from turfgrasses were intermediate in their relationships to crop isolates. Isolates from *Z. mays* and *Sorghum* were the most distantly related (Table 5).

Atlantic and Midwestern isolates from *P. annua* had the smallest genetic distance ($D = 0.0746$) of all comparisons in the study, indicating a close relationship independent of geographic origin (Table 5).

Atlantic isolates from *P. annua* and *A. palustris* (Table 1) had a larger genetic distance ($D = 0.3704$) than the distance calculated for the same comparison over all isolates ($D = 0.1602$) irrespective of geographic origin (Table 5).

DISCUSSION

This study differs from other recent work because genetic variation among isolates of *Colletotrichum* from amenity turfgrasses was evaluated based on both geographic and host origin. We found that the primary source of variation among

Colletotrichum isolates tested was host specialization, not geography.

Our work agrees with other research, which concluded that isolates from maize and sorghum are genetically distinct and represent different species, *C. graminicola* and *C. sublineolum*, respectively (10,16). While the sample sizes of isolates from *Z. mays* and *Sorghum* spp. were small, the samples were larger than those of previous studies (1,3) examining the relationship of these fungi to those *Colletotrichum* spp. infecting turfgrass. Our results are also consistent with Browning et al.'s (3) and

Table 3. Allele frequencies for isozyme loci tested, summarized by host origin and geographic location

	N ^a	Pgi11 ^b	Pgi12	Pgi13	Pgi14	Mdh11	Mdh12	Mdh13	Pgm11	Pgm12	Aat11	Aat12	Aat13	Got14	Tpi11	Tpi12	Tpi13
Host origin																	
<i>Poa annua</i>	25	0.680	0.000	0.320	0.000	0.680	0.000	0.320	0.680	0.320	0.400	0.080	0.000	0.520	1.000	0.000	0.000
<i>Agrostis palustris</i>	20	0.546	0.000	0.000	0.455	0.500	0.000	0.500	0.909	0.091	0.409	0.591	0.000	0.000	1.000	0.000	0.000
<i>Zea mays</i>	10	0.000	0.500	0.500	0.000	0.000	0.900	0.100	1.000	0.000	1.000	0.000	0.000	0.000	0.100	0.600	0.300
<i>Sorghum</i> spp.	4	0.250	0.500	0.250	0.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.250	0.750	0.750	0.250	0.000
Geographic location																	
Midwest <i>P. annua</i>	14	0.600	0.000	0.400	0.000	0.600	0.000	0.400	0.600	0.400	0.533	0.133	0.000	0.333	1.000	0.000	0.000
Atlantic <i>P. annua</i>	10	0.800	0.000	0.200	0.000	0.800	0.000	0.200	0.800	0.200	0.200	0.000	0.000	0.800	1.000	0.000	0.000
Atlantic <i>A. palustris</i>	14	0.330	0.000	0.000	0.670	0.330	0.000	0.670	0.933	0.067	0.533	0.467	0.000	0.000	1.000	0.000	0.000

^a Number of isolates used from each group.

^b Enzyme alleles were numbered sequentially from the anodal end of the gel using one number for the putative locus and a second number for the putative allele.

Table 4. Unique electrophoretic phenotypes (EPs) of the 61 isolates used in this study showing presence/absence of alleles^a

	Pgi 11	Pgi 12	Pgi 13	Pgi 14	Mdh 11	Mdh 12	Mdh 13	Pgm 11	Pgm 12	Aat 11	Aat 12	Aat 13	Aat 14	Tpi 11	Tpi 12	Tpi 13
EP-1	0	0	1	0	0	0	1	0	1	1	0	0	0	1	0	0
EP-2	1	0	0	0	1	0	0	1	0	0	0	0	1	1	0	0
EP-3	1	0	0	0	1	0	0	1	0	0	1	0	0	1	0	0
EP-4	1	0	0	0	1	0	0	1	0	1	0	0	0	1	0	0
EP-5	0	0	0	1	0	0	1	1	0	0	1	0	0	1	0	0
EP-6	0	0	0	1	0	0	1	1	0	1	0	0	0	1	0	0
EP-7	0	0	0	1	0	0	1	0	1	1	0	0	0	1	0	0
EP-8	1	0	0	0	1	0	0	0	1	0	1	0	0	1	0	0
EP-9	1	0	0	0	0	0	1	1	0	1	0	0	0	1	0	0
EP-10	0	1	0	0	0	1	0	1	0	1	0	0	0	1	0	0
EP-11	0	1	0	0	0	1	0	1	0	1	0	0	0	0	0	1
EP-12	0	0	1	0	0	1	0	1	0	1	0	0	0	0	1	0
EP-13	0	1	0	0	0	1	0	1	0	1	0	0	0	0	1	0
EP-14	0	1	0	0	0	0	1	1	0	1	0	0	0	0	0	1
EP-15	0	1	0	0	0	0	1	0	1	0	0	1	0	1	0	0
EP-16	0	1	0	0	0	0	1	0	1	0	0	0	1	1	0	0
EP-17	0	0	1	0	0	0	1	0	1	0	0	0	1	1	0	0
EP-18	1	0	0	0	0	0	1	0	1	0	0	0	1	0	1	0

^a 0 = absent, 1 = present.

Table 5. Genetic distances^a of *Colletotrichum* isolates based on host origin and geographic location

	<i>Poa annua</i>	<i>Agrostis palustris</i>	<i>Zea mays</i>	Midwest <i>P. annua</i>	Atlantic <i>P. annua</i>
Host origin					
<i>Agrostis palustris</i>	0.1602				
<i>Zea mays</i>	0.9046	0.8718			
<i>Sorghum</i> spp.	0.4726	0.8254	1.6290		
Geographic location					
Midwest <i>P. annua</i>					0.0746
Atlantic <i>A. palustris</i>				0.2260	0.3704

^a Nei's unbiased genetic distance (8).

Hsiang and Goodwin's (5) conclusions that isolates from *P. annua* and *A. palustris* are genetically similar and are distinct from *C. graminicola* isolates from maize and *C. sublineolum* isolates from sorghum. Browning et al. (3) suggested in their discussion that geography may play a role in the specialization of *Colletotrichum* spp.; however, our results disagree with this conclusion. Our results also differ from those of Backman et al. (1), who found that *A. palustris* isolates were more similar to maize isolates and *P. annua* isolates were more similar to sorghum isolates. As suggested by Hsiang and Goodwin (5), it appears that further division of *Colletotrichum* may be necessary to accommodate the genetic differentiation between turfgrass isolates and crop isolates.

Interestingly, Hsiang and Goodwin (5) found that, with one exception, the ITS sequences of the turfgrass isolates clustered with isolates of *C. dematium* and *C. coccodes*. There was one isolate from annual bluegrass that clustered with sorghum isolates. However, the authors concluded that since other studies (1,3) had established that sorghum isolates could infect annual bluegrass, and the isolate was from Texas, a sorghum growing region, that it was possible that a sorghum isolate infected the annual bluegrass plant (5).

Based on Nei's unbiased genetic distance (8), the expected distance between isolates from different locations would be large if geographic specialization existed between the Atlantic and Midwestern groups of *P. annua* isolates (Table 1). However, the genetic distance between isolates from these two geographic regions ($D = 0.0746$) revealed that Midwestern and Atlantic isolates from *P. annua* share a very close relationship. This distance was the smallest of all the comparisons made by either host type or geography. Several

different factors may be responsible for the lack of geographic specialization, including isolate migration, a lack of genetic diversity in the isolates that comprised the original founding population, or the presence of a large population that would limit genetic differentiation via genetic drift.

Future work on this disease should include a comprehensive study of host specificity. Also, population studies that compare host and geographic population structures should be performed to better understand how the pathogen is structured. A complete taxonomic study is needed to resolve species relationships, particularly with regard to the interesting finding by Hsiang and Goodwin (5) that turfgrass isolates may be more closely related to *C. dematium* and *C. coccodes*.

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