Temporal Variation in *Setosphaeria turcica* Between 1974 and 1994 and Origin of Races 1, 23, and 23N in the United States

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ABSTRACT

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Setosphaeria turcica causes northern leaf blight, an economically important disease of maize throughout the world. Survey collections of *S. turcica* isolates from 1974 to 1994 provided a unique opportunity to examine temporal diversity in the eastern United States. Two hundred forty-two isolates of *S. turcica* from maize were studied with random amplified polymorphic DNA (RAPD) markers, mating type, and virulence on maize differential inbred lines with known *Ht* resistance genes to examine changes over time. One hundred forty-nine RAPD haplotypes were identified. Nearly 20% of haplotypes recurred in more than one year. Race 0 isolates declined in frequency from 83% in 1974 to near 50% in the 1990s, most likely in response to the widespread deployment

of *Ht1* in commercial maize hybrids. Races 23 and 23N were present in the collection at low levels throughout the study period and were also found among isolates from Virginia in 1957. The frequency of *MAT1-2* isolates increased sharply after 1979 and was associated with the emergence of race 1 during the same period. RAPD markers were used to investigate the genetic diversity among a subset of isolates collected in the United States from 1976 to 1982, the period in which this dramatic shift in race frequency occurred. Multilocus haplotypes were not exclusively associated with known races of *S. turcica*. Based on shared haplotypes and cluster analysis, race 1 isolates share greater similarity with race 0 than with 23 or 23N isolates, indicating race 1 probably evolved from multiple lineages of race 0. *Sorghum* spp.-infecting isolates share greater similarity with one another than with maize-infecting isolates and represent a distinct subgroup.

Northern leaf blight (NLB) is a foliar disease of maize (*Zea mays* L.) caused by *Setosphaeria turcica* (Luttrell) Leonard and Suggs (anamorph *Exserohilum turcicum*). This ascomycete causes economically important disease losses in many maize-growing areas around the world, both in temperate climates, and in mid-altitude and highland areas of the subtropics and tropics (2,17, 34,44,52). The disease has a long history in the United States. The disease was present in nearly all states to the east and some states immediately west of the Mississippi River as early as 1923 (10). Notable epidemics of NLB in the United States occurred in the early 1940s, in 1951 (19) in northwestern North Carolina (24), and in the Gulf Coast of Texas in 1985 (44), throughout Texas in 1992 (22), and frequently on sweet maize in Florida (33).

Resistant cultivars are the most effective and widely used method of NLB control (44,49,50,52). Sources of resistance may be either qualitative or quantitative; however the usefulness of qualitative sources (*Ht* genes) is limited by race specificity. Several pathogenic races of *S. turcica* have been reported, based on their avirulence/virulence to the resistance genes *Ht1*, *Ht2*, *Ht3*, and *Hm1* (*HtN*) (4,14,20,21,27,43,46,48,51,54). Due to virulence to *Ht* genes in *S. turcica* populations, quantitative NLB resistance has greater appeal to most maize breeders (44,49,50,52).

Population genetic structure of *S. turcica* on a spatial scale has been studied in tropical (Kenya, Mexico, and Southern China) and

temperate climates (Europe, Northern China, and Eastern United States) to gain insight into how migration and reproductive biology shape populations (5,6,7,13). Populations of *S. turcica* from tropical climates showed high genotypic diversity, no or weak gametic phase disequilibrium, and similar frequencies of the two mating types, which suggested sexual recombination (5). In European populations of *S. turcica*, common haplotypes were shared among German, Swiss, and French samples, indicating substantial migration within Europe, limited only when populations were separated by the Alps (6). There were other indications that migration over extremely long distances was possible. A French outgroup shared common alleles with African isolates, and Austrian haplotypes were similar to isolates from Mexico (6). Populations in Europe, Northern China, and highland Kenya showed lower genotypic diversity, gametic disequilibrium, and uneven distributions of mating type consistent with infrequent sexual recombination (5).

Results of geographic studies of *S. turcica* in the eastern United States showed that migration over long distances was likely, and genotypic diversity was higher than in temperate populations previously analyzed (13). Asexual reproduction has an important role, particularly in causing epidemics on the local level.

Changes in RAPD marker frequencies were used to examine genetic diversity over time in Kenyan populations of *S. turcica*, both within a single growing season (early versus late infections) and in two subsequent years of maize cultivation (7). Even in populations with high genotypic diversity, clones were conserved over years and within a season. Clonality increased within a single growing season, but diversity increased slightly in subsequent years.

Yearly surveys of *S. turcica* conducted in the eastern United States from 1974 to 1994 provide an opportunity to examine tem-

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poral diversity in the pathogen population (41,42). We examined samples from these surveys to determine temporal changes in frequencies of RAPD markers, virulence, and mating type. Studying genetic change over time can provide insight into the selective forces that are most important in shaping pathogen populations in the eastern United States and contribute to the process of breeding for disease resistance.

Insight may be gained into the evolution of races of *S. turcica* in the United States by examining associations of haplotypes of *S. turcica* isolates sampled between 1976 and 1982. This survey period spans the time in which race 1, 23, and 23N were first reported (43,46,48), and provides a unique opportunity to examine the pathogen population in an historical perspective. By focusing on this period, we have an opportunity to determine if race 1 originated in North America by mutation within a single race 0 lineage or arose in multiple lineages. Several isolates from 1957 were also included for comparison to more current isolates.

MATERIALS AND METHODS

Isolate collection. Isolates of *S. turcica* were obtained from several sources. Most of the isolates were sampled by DeKalb Genetics, Inc. (DeKalb, IL, subsidiary of Monsanto, Inc.) in yearly surveys of maize foliar pathogens (41,42). DeKalb Genetics, Inc. generously supplied samples of *S. turcica* on dried leaf tissue for our study (J. Kinsey, J. M. Perkins, and D. R. Smith). Samples were collected from 25 maize inbred lines planted at 23 locations in 17 states representing different environments in which maize is commonly grown. Plots were sampled five times during a given growing season and leaf tissue was sent to a central laboratory for identification and isolation of pathogens. Identifications were conducted using microscopic techniques and inoculation of suspected pathogenic isolates on selected maize genotypes under controlled conditions. Infected greenhouse-grown leaf tissue from their inoculations was dried and stored in labeled envelopes at 4°C. Isolates of *S. turcica* stored on dried leaf tissue were readily recovered in our study. Additional isolates collected in the Midwest and North Carolina (M. L. Carson) and in Florida (J. K. Pataky) were included in our studies. The collection consisted of 242 *S. turcica* isolates, and included isolates collected on maize from 1974 to 1994 in the eastern United States (includes IA, OH, IL, GA, NC, SC, VA, MN, MI, WI, NY, PA, and FL). Four isolates collected in 1957 from Virginia (C. W. Roane) were also included to test for similarity to later samples.

To address the origin of virulence changes in the North American population of *S. turcica*, we focused on a subset of 106 isolates, the majority (89) of which were collected from 1976 to 1982, a critical period of selection and change in relation to the deployment of host resistance genes. During the 1960s and 1970s, *Ht1* was used widely in commercial maize hybrids in North America. Virulence to *Ht1* was first reported in the continental United States in 1979 (48). In the years that followed, race 1 of *S. turcica* increased in frequency and distribution in North America (20,21,24,42). Races 23 and 23N were first reported in the United States in 1976 (43) and 1986 (46), respectively. By focusing on samples from this transitional period, inferences may be made about the origins of new races in the pathogen population in the United States. Four additional isolates of *S. turcica* from 1957, seven isolates characterized as race 23 from 1983 to 1993 and six isolates from *Sorghum bicolor* and *S. halepense* were also included.

Isolation of *S. turcica* from leaf tissue. Characteristic NLB lesions were excised from dried leaf tissue and surface-disinfested by rinsing for 1 min in 0.5% sodium hypochlorite solution, followed by rinsing in sterile distilled H₂O for 30 to 60 s. Field-collected tissue was surface disinfested in the same manner after rinsing for one min in 70% (vol/vol) ethanol. Surface-disinfested

tissue was incubated on moist sterile filter paper for 2 to 3 days at 20 to 25°C under 12 h fluorescent lighting.

Single conidia of *S. turcica* from leaf tissue were germinated on water agar and transferred to lactose casein hydrolysate agar (LCA) (47). Cultures were grown under 12 h lighting for 10 to 14 days at 20°C to produce abundant conidia. Conidia of each isolate were harvested in sterile glycerol at 15% (vol/vol), placed in duplicate labeled vials and stored at -80°C.

Race determinations. To confirm race identity, we assessed virulence of isolates on differential inbred lines. Lines used to identify virulence were: Pa91 (no *Ht* genes), Pa91*Ht1*, Pa91*Ht2*, Pa91*Ht3*, and B68*Hm1* (formerly *HtN*). Pots (30.5-cm diameter, clay) were filled with a 1:1 mixture of Pro-Mix 'BX' (Premier Horticulture Ltd., Dorval, QUE, Canada): Steam-sterilized soil. All differential lines were planted together in one pot, three seeds per line, lines appropriately labeled, and inoculated with a single isolate per pot. Two pots containing all five lines were inoculated with each isolate to be race-typed. Plants were grown in the greenhouse at moderate temperatures, 20 to 22°C day/15 to 18°C nights, with supplemental lighting at 25 and 50 klux (325 to 650 µE/m²/s) average photosynthetic photon flux density, consistent with conditions in Leonard et al. (25). Reactions associated with genes *Ht1*, *Ht2*, and *Ht3* have been shown to be sensitive to varying temperature and light intensity (23). Plants were grown for 19 to 21 days and inoculated after the fourth leaf fully emerged.

Isolates of *S. turcica* to be race-typed, and isolates of each known race, were grown from first generation subcultures frozen at -80°C (transferred from leaf tissue to medium for storage) on LCA. Cultures were grown for 7 to 10 days at 20 to 25°C under 12 h light/dark regime to induce sporulation. Conidia were dislodged with a glass rod and rinsed from the petri plates with sterile H₂O containing Tween 20 (10 µl/liters). Filtered conidial suspensions were quantified with a hemacytometer and diluted to approximately 1,000 conidia/ml. Aliquots of 0.1 ml of conidial suspensions were pipetted into the leaf whorl of maize seedlings in the 4- to 6-leaf stage. Inoculated plants were incubated overnight (16 h) at 20 to 22°C in a mist chamber and removed to greenhouse benches.

Plants were inspected for symptoms of infection on the differential lines at 14 and 20 days after inoculation. Lesions, in which tissue appeared typically wilted, gray-green, necrotic, and lacked chlorosis, were scored as susceptible reactions. Symptoms expressed as necrotic lesions surrounded by chlorosis were scored as resistant reactions. Isolates were identified according to the race scheme outlined in Leonard et al. (25).

Genomic DNA extraction. DNA extraction methods were adapted from DuTeau and Leslie (11). Our modifications included use of a different lysis buffer, phenol/chloroform solution for extraction, and final treatment of extracted samples with RNase. First generation isolates transferred from stock cultures (-80°C storage) to LCA were grown 4 to 6 days on oatmeal agar (75 g of ground rolled oats, 15 g of agar per liter deionized H₂O) at 20°C with 12 h fluorescent lighting to encourage mycelial growth. Mycelium was harvested and frozen with liquid nitrogen prior to adding heated (65°C) lysis buffer. Lysis buffer consisted of 10 mM Tris (pH 7.4), 100 mM lithium chloride, 0.5% sodium dodecyl sulfate, 10 mM EDTA and proteinase K at 0.1 mg/ml. Following phenol-chloroform extraction, DNA was precipitated overnight in ammonium acetate and 95% ethanol, dried under vacuum, resuspended in Tris-EDTA buffer (pH 7.4) (36), and treated with RNase (1 µg/µl). DNA was quantified by visual comparison of at least two dilutions per isolate to λ-DNA standards in 1.0% agarose gels run in 1× Tris-borate-EDTA (TBE) (36) and stained with ethidium bromide.

Determination of mating type. We used a polymerase chain reaction (PCR) assay developed by Arie et al. (3) to identify mating type of *S. turcica* isolates in our collection. The two primers

they designed based on flanking sequences (ChHMG1 and ChHMG2) in *Cochliobolus heterostrophus* generates a 0.3-kb product from *MAT1-2*, but not from *MAT1-1* strains of *S. turcica* in a simple PCR procedure. The primers ChHMG1 and ChHMG2 were synthesized by Sigma-Genosys (Woodlands, TX), dissolved in sterile distilled water at 100 μ M concentration and stored at -20°C . Reaction mixtures containing 10 ng of genomic DNA in a total reaction volume of 25 μ l were prepared and PCR conducted as outlined by Arie et al. (3). The procedure was tested using three isolates each of known mating type. Ten microliters of each PCR product was subjected to electrophoresis in 1.5% agarose gel in 1 $\times$ TBE (36) containing ethidium bromide for 4 h at 75 volts and photographed. All reactions were run with control isolates of each mating type and repeated for each sample to confirm results. Presence of the 0.3-kb fragment corresponded to *MAT1-2*, absence of the fragment to *MAT1-1*. In repeated tests, 37 isolates of the 242 were inconclusive for *MAT1-1* or *MAT1-2* (failed to consistently produce a PCR product) in the PCR assay. These same DNA preparations consistently yielded PCR products when RAPD primers were used, so it was presumed that the lack of consistent amplification in the mating type assay was not due to DNA quality.

Inconclusive isolates were paired in conventional tests in culture with mating type tester isolates. Six tester isolates of *MAT1-1* and five of *MAT1-2* were used. Sterile Johnson grass (*Sorghum halepense*) culms (2 cm) were embedded in the center of 9-cm petri dishes (29) containing modified Sach's agar. First generation subcultures were grown 4 to 6 days on potato dextrose agar (PDA). Agar plugs (No. 1 cork borer, 3 mm) from testers of *MAT1-1* or *MAT1-2* and unknown cultures were placed 1 cm from the *S. halepense* culm opposite each other. Tester isolates were selfed, paired in all combinations with testers of opposite mating type, as well as testers of the same mating type to prevent ambiguous results. Successful mating only occurred between testers of opposite mating type. Duplicate pairings of sample isolates of unknown mating type were conducted with three tester isolates of each mating type. Petri dishes containing paired isolates were sealed and placed in the dark at 20°C and were examined under a stereoscope at 3 and 4 weeks after plating for the presence of pseudothecia. Pseudothecia are usually visible in fertile combinations after 10 days and ascospores generally mature after 21 days (29). When mating occurred, it was exclusively with a tester of one mating type. Ascus and ascospore production was not assessed.

RAPD-PCR. Extracted DNA of *S. turcica* was amplified by PCR with random decamer primers (53). A total volume of 25 μ l was used for RAPD reactions. Components of the reaction mixture are as follows: 1 $\times$ buffer (2.5 μ l stock Promega 10 $\times$ buffer consisting of 500 mM KCl, 100 mM Tris-HCl, pH 9.0, at 25°C , and 1% Triton X-100), 0.2 mM dNTP, 2.5 μ l of primer (10 μ g/ μ l stock), 1.5 mM MgCl₂, 5 ng of template DNA, 0.04 units of Taq polymerase (Promega). RAPD-PCR reactions were run in a PCT-100 thermal cycler (MJ Research, Inc., Waltham, MA), with the following protocol: (i) denature at 92°C for 30 s, (ii) anneal at 35°C (primer-dependent) for 1 min, (iii) ramp to 7°C at a rate of $1^{\circ}\text{C}/8$ s over 5 min, (iv) extend at 72°C for 2 min, (v) repeat 45 cycles (steps 1 to 4), (vi) hold at 72°C for 7 min, (vii) hold at 4°C . Alternative thermocycling protocols were used occasionally, depending upon annealing temperatures appropriate for given primers. Amplification products were analyzed by gel electrophoresis. A loading dye containing ethidium bromide was added to each completed reaction. Fifteen microliters of each sample was loaded on an agarose gel (1.5%) and run in 1 $\times$ TBE (36) for 5 to 6 h at 75 V. Amplified fragments were visualized under UV light and photographed.

Decamer primers from Operon Technologies (Alameda, CA) and the University of British Columbia (Vancouver, Canada) previously screened in studies of spatial diversity in the eastern

United States (13) also were used to analyze temporal change in *S. turcica*. Data included information from seven primers that amplified 20 markers that were reproducible and polymorphic. Positive and negative control isolates, one that reliably produced the marker fragment and one that did not, were used in each reaction to confirm results.

Data analysis. Isolates were analyzed to examine temporal changes in RAPD marker, virulence, and mating type frequencies. RAPD marker frequencies were compared year by year (17 years represented), in four multiple-year groups, and within the four groups to determine if frequencies were homogeneous through time, confirm appropriateness of multiple-year groupings, and to examine associations among molecular and phenotypic markers over time.

Fragments amplified by the RAPD-PCR procedure showing polymorphism between isolates correspond to one allele at one marker locus in our analysis. Other alleles, not amplified, are unknown. Absence of a particular marker fragment does not necessarily indicate an alternate allele. Multilocus haplotypes were based on presence or absence of 20 RAPD marker loci.

Comparisons between two isolates were made based on the Dice similarity coefficient (45), $S_d = 2n_{xy}/(2n_{xy} + n_x + n_y)$, where x and y are two isolates to be compared and n_{xy} = number of markers found in both isolates, n_x = markers found only in x , and n_y = markers found only in y . NTSYS-pc (35) was used to calculate similarities and perform principal coordinates analyses. Dice similarities were transformed into scalar products with DCENTER followed by EIGEN, which calculates eigenvalues and eigenvectors used to plot relationships among isolates in multiple dimensions. Principal coordinate plots in two and three dimensions were developed based on RAPD haplotypes.

Frequencies of RAPD markers, race, and mating type were compared among subsequent year's samples and over two decades by chi-square tests of homogeneity. Year samples deemed homogeneous for marker frequencies and were pooled for comparison to other pooled multiple year-groups.

Multilocus haplotypes determined by RAPD markers alone, and by RAPDs in conjunction with race and mating type were examined for recurrence over the years of the survey to determine survival of isolates between cropping seasons. Recurrence of clones in consecutive years as well as those in nonconsecutive years and their relative frequencies overall were determined. Associations among RAPD markers, virulence, and mating type in *S. turcica* over time were assessed by tests of pairwise linkage disequilibrium (26,39,40).

Genotypic diversity based on Shannon index. The Shannon genotypic diversity index was calculated based on RAPD haplotypes for years and year-groups (18). The measure corrects for different sample sizes as described by Sheldon (37). The equation used was: $H_S = \{-\sum_i [g_i \ln(g_i)]\}/\ln N$, where g_i is the frequency of the i th haplotype in one sample and N is the sample size. This type of index accounts for both the abundance of genotypes and evenness of their distribution in the population (15). Genotypic diversity estimates, H_S values, were compared with a simple t test (16,18).

Gene diversity. Nei's average gene diversity over all loci was measured using the index: $H_N = \sum_l h_l/L = \sum_l (1 - \sum_a p_a^2)/L$, where h_l is the gene diversity at the l th marker locus, L the number of marker loci sampled, and p_a the frequency of the a th allele at the locus in question (32). The gene diversity estimate for haploid populations h_l is the same as that described for a homozygous autogamous (self-fertilizing) population. The sampling variance of the gene diversity was calculated by the following equation: $\text{Variance}(H_N) = \sum_l (h_l - H_N)2/[(L - 1) \times L]$. Gene diversity estimates for multiple year-groups were compared pairwise by t tests (32).

Index of association. To assess the importance of sexual recombination in multiple year groups of *S. turcica* I_A , the index of multilocus association (8) was calculated as: $I_A = (V_O/V_E) - 1$,

where V_o is the observed and V_e , the expected variance of k . The variable k is the number of loci at which two isolates differ, computed for all possible pairs of isolates, $n(n-1)/2$. We established a null hypothesis of no association of alleles at different loci ($H_o: I_A = 0$), which is consistent with random mating in a population. This hypothesis was tested with an upper confidence limit of V_o , the observed variance of k , which is calculated using the error variance of V_e . The data set was clone-corrected, considering only one isolate per clone based on RAPD markers, virulence (race), and mating type haplotype within multiple-year groups (31).

Similarities among the 106 isolates from the 1976 to 1982 period were subjected to unweighted pair group method using arithmetic averages (UPGMA) cluster analysis using NTSYS-pc in the SAHN module (35) to examine groupings of races based on RAPD haplotypes. The pairwise similarity matrix generated using the Dice coefficient was subjected to the matrix-correspondence or Mantel test (30). Comparing the similarity matrix to the co-phenetic value matrix (COPH and MXCOMP modules) is a means of determining the goodness of fit of the UPGMA dendrogram to the haplotype data. Reliability of the clustering was evaluated by bootstrapping haplotype data 1,000 times by repeated sampling with replacement using WinBoot software (55). The frequency of occurrence of the groupings reflects the robustness of the clusters in the dendrogram.

RESULTS

Comparison of yearly samples from 1974 to 1994 (consecutive except for 1979 and 1987 to 1989; insufficient number of

samples) were heterogeneous in frequencies of race, mating type, and all 20 RAPD markers as determined by chi-square tests of homogeneity. When consecutive years' samples were placed into four multiple year-groups (1974 to 1977, 1979 to 1982, 1983 to 1986, and 1990 to 1994), multiple year-groups were heterogeneous in frequencies for race, and five RAPD markers, Op I12-0.51, 0.77, UBC417-1.75, UBC77-1.45, and 1.6 (Table 1).

Within year-groups, chi-square tests were conducted to determine appropriateness of pooling samples from successive years. Samples from 1974 to 1977 were homogeneous for race, mating type and 19 of 20 RAPD markers except Op B14-2.1. Isolates from 1979 to 1982 were only heterogeneous for frequencies of race and a single RAPD marker, Op B11-1.7. Samples from 1983 to 1986 were smaller and may not be representative of the population as a whole, but were heterogeneous only in frequencies of race and three RAPD markers (Op B11-1.7, Op I12-0.51, UBC417-1.75). Isolates collected from 1990 to 1994 were heterogeneous for frequencies of race, mating type, and six of the 20 RAPD markers (Op B11-1.7, UBC100-2.52, UBC23-1.75, UBC417-0.75 and 1.75).

A single race 1 isolate was found in 1976, but race 1 did not become prominent in the collection until 1979 (48) (Table 1). In the early 1970s, race 0 isolates made up the largest proportion of isolates. The frequency of race 0 fluctuated from 82% of isolates in 1974 and declined to near 50% in the 1990s. Race 23N was present at low numbers throughout the collection period, even though it was not described until 1986 (46). The race having lowest frequency among our isolates was race 23. It was not found in our collection until 1981 and remained at low levels in

TABLE 1. Frequencies of virulence, mating type, and random amplified polymorphism DNA (RAPD) markers through years collected, and summary of year-groups of *Setosphaeria turcica* isolates collected in the eastern United States from 1974 to 1994

Year-group ^a	1974–1977 ^b	1979–1982 ^c	1983–1986 ^d	1990–1994 ^e	Total
N ^f	49	60	29	104	242
RAPD genotypes ^g	38	46	27	62	149
R0 ^h *	0.82	0.33	0.48	0.49	0.52
R1*	0.02	0.43	0.34	0.36	0.31
R23*	0.00	0.07	0.07	0.03	0.04
R23N*	0.14	0.17	0.10	0.12	0.13
MAT1-1 ⁱ	0.51	0.28	0.28	0.39	0.38
MAT1-2	0.49	0.72	0.72	0.61	0.62
Op B11 ^j 1.7	0.22	0.38	0.17	0.34	0.31
Op I12 0.51	0.33	0.25	0.38	0.09	0.21
0.55	0.92	0.95	0.90	0.96	0.94
0.77	1.00	0.97	0.86	0.99	0.97
1.6	1.00	0.98	0.97	0.99	0.99
2.18	0.98	0.95	1.00	0.98	0.98
Op B14 2.1	0.98	0.98	0.93	0.95	0.95
2.52	0.49	0.52	0.38	0.33	0.41
Ubc23 0.82	0.82	0.77	0.62	0.79	0.77
1.35	0.86	0.98	0.90	0.95	0.93
1.75*	0.16	0.08	0.10	0.08	0.10
Ubc417 0.75	0.41	0.27	0.45	0.26	0.31
1.52	0.98	0.98	0.90	0.93	0.95
1.75	0.86	0.57	0.48	0.42	0.55
Ubc77 0.65	1.00	0.97	0.97	0.98	0.98
1.25	0.98	0.98	0.97	1.00	0.99
1.45*	0.71	0.92	0.9	0.93	0.88
1.6*	0.47	0.68	0.31	0.64	0.58
1.7	0.90	0.90	0.97	0.98	0.94
Ubc100 2.05	0.96	0.95	0.93	0.88	0.92
2.52	0.49	0.52	0.38	0.33	0.41

^a Asterisk indicates markers whose frequencies differed among years sampled by chi-square tests of homogeneity ($P \leq 0.05$).

^b Isolates were combined for analysis from 1974, 1975, 1976, and 1977; includes a single isolate of race 3 collected in 1975.

^c Isolates were combined for analysis from 1979, 1980, 1981, and 1982.

^d Isolates were combined for analysis from 1983, 1984, 1985, and 1986.

^e Isolates were combined for analysis from 1990, 1991, 1992, 1993, and 1994.

^f N = number of *S. turcica* isolates in year groups analyzed.

^g Number of haplotypes which share common RAPD banding patterns.

^h R0, R1, R23 and R23N refer to races of *S. turcica* distinguishable by reaction to *Ht* resistance genes in maize (25).

ⁱ MAT1-1 and MAT1-2 refer to mating types identified in *S. turcica* by PCR, confirmed by in vitro pairings.

^j Primers used are followed by fragments sizes compared with DNA size standards given in kilobases.

the population, never reaching more than 0.5% of isolates. However, a few isolates of *S. turcica* collected in 1957 in Virginia were found to be race 23 and 23N.

Frequencies of mating types differed among year-groups in the collection (Table 1) with an increasing proportion of *MAT1-2* isolates through time. Mating type frequencies were approximately equal from 1974 to 1977 and shifted to *MAT1-2* isolates after 1979. This increase in *MAT1-2* frequency coincided with an increase in virulence to *Ht1*.

Haplotypes based on combined RAPD markers, race, and mating type were used to examine shared similarity of *S. turcica* isolates over time. Of 242 isolates studied, there were 189 distinct haplotypes. Twenty-one haplotypes recurred through time (Table 2). Seven haplotypes occurred only in consecutive years, while 13 recurred in nonconsecutive years. Haplotypes present at the highest frequencies were A51 (2.1%), A32, A38, A52, A84, and A89, (each at 1.6%). The most frequent haplotype, A51, was present in both 1980 and 1994.

There were 149 haplotypes based on presence/absence of RAPD markers alone among the 242 isolates analyzed. Twenty-eight RAPD haplotypes recurred in more than one year (Table 2). Three occurred only in consecutive years, while 25 haplotypes recurred in more than one nonconsecutive year. The most common RAPD haplotype, 40, occurred at a frequency of 5.4%, recurring in 1974, 1976, 1980, 1981, 1993, and 1994. Three other haplotypes each occurred at 2.9% while two others occurred at frequencies of 2.5% among the 242 isolates.

Principal-coordinates analysis of haplotype similarities (combined RAPD, race, and mating type data) produced two major groups in 2D- and 3D-eigen plots (Fig. 1). These groups did not reflect differences strictly related to the time period in which the isolates were collected, but rather reflect race groupings. The first two dimensions accounted for 14.4 and 11.2% of the variation among haplotypes, the third dimension accounting for an additional 9.4%, cumulatively amounting to 35% of variation among haplotypes.

Gene and genotypic diversity comparisons. Genotypic diversity estimated by the Shannon index did not differ significantly

among any single year's sample between 1974 and 1994. All of these yearly (1974 to 1994) samples were at or very near the maximum amount of diversity possible. Grouping yearly samples together also did not result in significant differences among time periods, although genotypic diversity appeared to decrease after 1982 (Table 3). Nei's average gene diversity estimates did not differ significantly among multiple year groups compared, although lowest gene diversity was found in the sample from 1990 to 1994 (Table 3). Gene and genotypic diversity were lowest in the most recent time period sampled.

Linkage disequilibrium tests. Association among virulence to *Ht*-resistance genes in maize, mating type, and RAPD markers,

Fig. 1. Principal coordinates analysis of haplotypes of *Setosphaeria turcica* determined by random amplified polymorphism DNA markers, mating type, and virulence to *Ht* genes in maize. Isolates were collected from 1974 through 1994 in the Eastern United States. Isolates are grouped by years of collection: A, 1974 to 1977, B, 1979 to 1982, C, 1983 to 1986, and D, 1990 to 1994. Coordinates 1 and 2 accounted for 25% of the variation among isolates.

TABLE 2. Haplotypes (based on race, mating [MAT] type and random amplified polymorphism DNA [RAPD] markers or RAPD markers alone) shared among yearly samples of *Setosphaeria turcica*, collected from 1974 to 1994 in the eastern United States

Haplotype (RAPD markers + MAT + race)	Years found	Haplotype (RAPD markers alone)	Years found
A28	1977,1993	27	1977,1992,1993
A32	1993,1994	29	1993,1994
A38	1983,1990,1993	35	1983,1990,1991,1993
A41	1992,1993	36	1993,1994
A44	1974,1975	37	1974,1975,1994
A50	1974,1976,1993	39	1983,1985,1990,1994
A51	1980,1994	40	1974,1976,1980,1981,1993,1994
A52	1993,1994	42	1984,1991
A58	1980,1990	43	1980,1990,1992
A71	1986,1993	44	1979,1980,1983,1986
A84	1981,1991,1993	51	1986,1993
A89	1974,1976,1977	62	1981,1982,1990,1991,1993
A90	1976,1981,1994	64	1974,1976,1977
A91	1976,1994	65	1976,1981,1994
A92	1977,1980,1990	66	1977,1980,1981,1990,1994
A98	1983,1990	68	1983,1990,1991
A131	1990,1991	69	1977,1981
A133	1975,1977	97	1977,1981
A134	1976,1982	98	1990,1991
A184	1979,1981	100	1975,1976,1977,1982
A200	1981,1982	113	1981,1990,1993
		122	1982,1990
		128	1976,1982
		132	1974,1976
		133	1990,1994
		141	1982,1990
		143	1979,1981
		157	1981,1982,1993

was examined in pairwise tests of linkage disequilibrium of isolates sampled from 1974 to 1994 (Table 4). Reaction with known maize resistance genes was associated in the following ways, (i) virulence to *Ht1* occurred alone and not in conjunction with virulence to other *Ht* genes, (ii) virulence to *Ht2* and *Ht3* occurred together (with one exception), and (iii) virulence to *Hm1* always occurred in conjunction with virulence to *Ht2* and *Ht3*. Mating type was significantly associated with virulence to *Ht1* and *Hm1*. Race 1 isolates were predominantly *MAT1-2* mating type (73%), whereas slightly more race 23N isolates were *MAT1-1* (53%). The

TABLE 3. Genotypic diversity calculated by the Shannon index (H_S), Nei's average gene diversity (H_N), and significance of recombination (Brown's index of multilocus association, I_A) in *Setosphaeria turcica* isolates sampled over time

Years collected	Gene diversity ^a		Genotypic diversity ^b			Multilocus allele association ^c		
	<i>B</i>	H_N	<i>N</i>	<i>M</i>	H_S^d	I_A	V_O	<i>L</i>
1974–1977	22	0.234	49	38	0.91	1.02	7.75 >	6.09
1979–1982	25	0.243	60	46	0.91	0.92	7.52 >	6.14
1983–1986	24	0.264	29	27	0.86	0.44	6.69 <	6.72 ^e
1990–1994	24	0.216	104	62	0.81	0.42	5.44 <	5.89 ^e
Total	25	0.239	242	148	0.86			

^a *B* indicates the number of polymorphic loci in each year group.

^b *N* = sample size, *M* = number of multilocus haplotypes determined by random amplified polymorphism DNA (RAPD) markers.

^c I_A = Brown's index of multilocus association based on RAPD markers, virulence to *Ht* genes, and mating type in clone-corrected data set; V_O = observed variance (test statistic); and *L* = lower confidence limit at $\alpha = 0.05$.

^d Values are not significantly different at $\alpha = 0.05$, according to pairwise *t* tests.

^e H_O : $I_A = 0$ at $\alpha = 0.05$, conclude that there is little or no association between loci examined.

number of race 23 isolates was small, but dominated by *MAT1-2* isolates (78%). Race 0 isolates were represented by 40% *MAT1-1* and 60% *MAT1-2*.

Specific virulence to *Ht* genes and mating type were also found to be associated with RAPD markers (Table 4). Virulence to *Ht1* was associated with the RAPD markers, Op B11-1.7kb, UBC23-0.82, and UBC417-0.75, but no markers were exclusively present or absent in isolates of race 1. Virulence to *Ht2* and *Ht3*, expressed in both races 23 and 23N, was associated with four RAPD markers; Op B11-1.7kb, Op I12-1.6kb, UBC417-0.75kb, and UBC77-0.65kb. A single isolate was found which could successfully infect maize with *Ht3* alone. Virulence to *Htn1*, expressed in our collection only by race 23N isolates, was associated with RAPD markers UBC417-0.75kb, UBC77-0.65kb, and UBC77-1.6kb.

Several RAPD markers were significantly associated with mating type, although there were not large numerical differences in the frequencies of markers by mating type. Those RAPD markers significantly associated with mating type were Op B11-1.7kb, UBC100-2.05kb, UBC417-0.75kb and 1.1kb.

Associations among RAPD markers were also found. The percentage of associated loci per RAPD marker locus ranged from 12.5 (Op I12-0.51kb) to 41.7% (UBC77-1.25kb and Op I12-0.55kb). The RAPD markers Op I12-0.55kb, 0.77kb, and 2.18kb, UBC77-1.25kb, 1.45kb, 1.6kb and 1.7kb were each associated with 7 to 10 other markers. The remaining RAPD markers were associated with 3 to 6 other markers.

Index of association. To address the role of clonal vs. recombining reproduction, Brown's index of association (I_A) was calculated for multiple year groups and associations of phenotypic markers, race, and mating type were examined (Table 3). Index values calculated for 1974 to 1977 and 1979 to 1982 in clone-corrected data sets differed significantly from those expected with

TABLE 4. Tests of pairwise linkage disequilibrium indicating association among specific virulence, mating type, and random amplified polymorphism DNA (RAPD) markers expressed in *Setosphaeria turcica* isolates collected in the United States from 1974 to 1994

Marker	Ht1 ^a	Ht2/3	Htn1	Mat ^b	B11 1.7 ^c	I12 0.51	I12 0.55	I12 0.77	I12 1.6	I12 2.2	B14 2.1	UBC100 2.05	UBC100 2.52	UBC23 0.82	UBC23 1.35	UBC23 1.75	UBC417 0.75	UBC417 1.1	UBC417 1.52	UBC417 1.75	UBC77 0.65	UBC77 1.25	UBC77 1.45	UBC77 1.6
Ht1	...	+	+	+	+									+			+							
Ht2/3	+	...	+	+	+				+								+				+			
Htn1	+	+	...	+	+												+				+			+
Mat	+	+	+	...	+							+					+	+						+
B11 1.7	+	+		+	...							+												
I12 0.51						...										+		+			+			
I12 0.55							...	+		+			+	+	+					+	+	+	+	+
I12 0.77								+	+	+						+		+			+			+
I12 1.6		+							+	...				+				+			+			+
I12 2.2								+	+	+	...				+					+	+	+	+	+
B14 2.1											...				+					+	+	+	+	
UBC100 2.05				+	+							...	+					+	+		+			+
UBC100 2.52							+					+	...	+		+								+
UBC23 0.82	+						+		+				+	...										
UBC23 1.35							+			+	+				...						+	+	+	
UBC23 1.75								+								...			+				+	
UBC417 0.75	+	+	+	+								+					...			+			+	
UBC417 1.1				+				+				+						...	+	+				
UBC417 1.52																		+	...			+		
UBC417 1.75							+			+	+									...				+
UBC77 0.65		+	+			+	+	+		+	+	+			+						...			+
UBC77 1.25							+			+	+				+		+					...	+	+
UBC77 1.45							+			+	+				+	+						+	...	+
UBC77 1.6		+					+	+	+	+	+		+							+	+		+	...

^a Virulence of isolates on *Ht* resistance genes in maize.

^b Mating type of *Setosphaeria turcica* isolates, *MAT1-1* versus *MAT1-2*.

^c Decamer RAPD primers beginning with a single letter are Operon primers. Primers beginning with UBC were supplied by University of British Columbia. Below the primer used are the fragment sizes generated listed in kilobases as compared to DNA standards.

^d Plus symbols indicate significant associations of virulence, mating type, or RAPD loci at $P = 0.05$.

strictly random mating. The more recent collections from 1983 to 1986 and 1990 to 1994 had multilocus association values consistent with randomly mating populations, although genotypic diversities calculated for these year groups were lower than values for the first two groups mentioned.

Phenetic analysis. An UPGMA phenogram constructed among RAPD haplotypes from the 1976 to 1982 period contained four major clusters (Fig. 2A to D), with the largest cluster further subdivided into five closely related groups (B1, B2, B3, B4, and B5).

Cluster A included nine maize haplotypes and the *Sorghum bicolor*-infecting haplotype 19. These RAPD haplotypes were mostly of Midwestern origin from IL, MI, MN, WI, VA, IA, and also NC. Three races were represented: 0, 1, and 23. The single *S. bicolor*-infecting isolate was from a recent collection from North Carolina. Mating types *MAT1-1* and *MAT1-2* occurred in near-equal proportions in this cluster.

Cluster B contained the most predominant haplotypes, encompassed the majority of isolates analyzed, and was further subdivided into five subgroups. Subgroup B1 includes 13 RAPD haplotypes from IA, IN, IL, MN OH, VA, OH, NC, and SC. Three races were represented (0, 1, and 23N) with race 0 predominating.

Subgroup B2 contained only five haplotypes, most of which were *MAT1-2*. They originated from PA, VA, IL, and FL and represented races 0, 1, and 23N. Clustering closest to B2 was subgroup B3, which contained 10 haplotypes of nearly equal proportions of mating types that were collected from OH, IN, IL, MI, MN, and PA, and included races 0, 1, 23, and 23N. Subgroup B4 included five haplotypes from IL, MI, OH, VA, NC, and MN, which were all race 0 except a single isolate of race 23. Placed alone between subgroups B4 and B5 was isolate 203 of RAPD haplotype 63 collected in 1957 in VA. The final cluster in cluster B, subgroup B5, included 13 haplotypes from PA, VA, WI, NC, MN, and IL, although most were from PA and VA. Most of the isolates were race 1, with a lesser number of races 0 and 23N.

Cluster C did not form a single coherent cluster, but consisted of smaller subgroups of 2 to 6 isolates each. At the bottom of the dendrogram (Fig. 2) is cluster D consisting of four RAPD haplotypes, all isolated from either *S. halepense* or *S. bicolor*. Maize-infecting isolates from the 1950s grouped independently of one another. Isolate 198 (RAPD haplotype 67) from 1957 clustered with isolates in cluster B5, indicative that this may represent an original clonal lineage for the cluster of VA and PA isolates. Other *Sorghum*-infecting isolates that clustered with maize isolates were 204, 203, and 205. Isolate 204 grouped with isolate 24 from SC (race 23N) collected in 1974. Isolate 203 clustered between B4 and B5 among maize haplotypes. Finally, 205 grouped with group B2 as detailed above.

DISCUSSION

Temporal stability of *S. turcica*. Examination of genetic change among isolates of *S. turcica* over time indicated the ability of specific haplotypes to persist in North America. When RAPD marker, mating type, and race frequencies were compared among all 17 year's samples in our study, significant differences occurred among the years tested (chi-square test of homogeneity, $P \leq 0.05$). Several haplotypes, or clones, survived between growing seasons and recurred over several years while new genotypes appeared. Persistence of haplotypes over two decades in the eastern United States indicated high fitness of those isolates. New genotypes may have arisen by migration, via local sexual recombination, by mutation, or in view of the high degree of genetic diversity in the population, may have appeared infrequently in the survey due to small sample size. Borchardt et al. (7) analyzed temporal variation in *S. turcica* isolates in a single field population in Kenya. Isolates collected in two consecutive growing cycles, as well as early and late within a growing season, did not vary in frequen-

cies of RAPD markers and were composed of the same haplotypes. Even in an environment where recombination is likely, clonal reproduction was of primary importance, and genotypes were conserved within and between growing seasons.

Principal coordinates analyses of haplotypes did not group isolates based on time of collection, neither in individual years nor by multiple year groups. In two-dimensional eigen plots of combined RAPD marker, race, and mating type haplotypes, two clusters of isolates formed, but were not related to time of collection. Race was a key factor in grouping isolates, with race 23 and 23N isolates clustering together.

Gene and genotypic diversity did not differ significantly over time. However, isolates collected in the most recent sampling period, 1990 to 1994, exhibited the lowest gene and genotypic diversities of time periods analyzed. With the continuous deployment of new maize hybrids in the United States, selection may have occurred for isolates that are both highly adapted to the climate and physical environment, and able to successfully infect a range of maize hybrids. Increasing clonality within a growing season was demonstrated in a Kenyan field population of *S. turcica* when early and late-season infections were compared (7). A similar situation may be plausible in the United States, in the absence of new major selective forces, such as the introduction of new sources of resistance.

Linkage disequilibrium analysis. Nonrandom association between mating type, virulence, and RAPD markers would be expected in *S. turcica* collections since clonal reproduction is suggested based on a priori information about reproductive biology. Liu and Kolmer (28) compared asexual and sexual populations of *Puccinia triticina* Erikss. to examine associations between virulence and RAPD markers. Associations were strongest and most frequent in the asexual population compared with the sexual population. The collection of *S. turcica* examined here shows evidence for clonal reproduction in the associations noted among molecular markers, virulence, and mating type. However, none of these associations was absolute.

RAPD bands which were highly associated with other RAPD markers, as well as virulence and mating type would be most useful in population studies. In this study, no RAPD markers were associated exclusively with virulence, so they would not be suitable for race identification. Our study does, however, show evidence for clonal reproduction in the levels of association noted among molecular markers, virulence and mating type.

S. turcica has been subject to varying degrees of selection pressure over time. Changes in the patterns of use of major resistance genes in maize over the past three decades placed selection pressure on the population. The widespread use of *Ht1* in the 1970s and 80s may have resulted in a slight decline in genotypic diversity, particularly notable in the most recent collections from 1990 to 1994. However, when clone-corrected data sets of isolates from multiple year-groups are tested using Brown's index of association (I_A), significant association was found among loci examined in collections from 1974 to 1982. Clone-corrected collections from 1983 to 1994 did not differ significantly from random mating. Although markers showed little association in those later year-groups, genotypic diversity was lower and several haplotypes were represented more often than in earlier collections.

Nonrandom associations between mating type, virulence and molecular markers were expected in *S. turcica* collections, since asexual reproduction is its primary mode of reproduction. The sexual stage has not been observed in the field, although genetic evidence strongly suggests that some level of recombination is occurring, particularly in tropical environments (5,7). The near equal frequency of both mating types, the relatively low level of gametic disequilibrium, and the high levels of genetic diversity present in American collections indicate a role for recombination at some stage in the life cycle (13). It is unclear where and when

Fig. 2. Unweighted pair group method using arithmetic averages phenogram of random amplified polymorphic DNA (RAPD) haplotypes of 106 isolates of *Setosphaeria turcica* from the Eastern United States, the majority of which were collected from 1976 through 1982. Isolates are designated by isolate number, state and year collected, race, and mating type. **A to D** denote significant clusters of RAPD haplotypes.

recombination occurs and with what frequency it would be necessary to maintain diversity in populations on par with randomly mating populations.

Origin of race 1 in the United States. Within the group of 27 race 1 isolates of *S. turcica* collected in the Eastern United States from 1976 to 1982, there was a high level of diversity as determined by RAPD markers. Frequencies of markers expressed in race 1 isolates differed from those of race 0 isolates for three markers (Table 5) compared to races 23, 3, and 23N isolates, whose marker frequencies differed from race 1 isolates for six markers each. In several instances, RAPD haplotypes were shared by multiple races and/or mating types (Fig. 2). A greater number of haplotypes were shared between race 0 and race 1 isolates than were common to other races. The majority (81%) of race 1 isolates are of type *MAT1-2*, which may indicate evolution from an *MAT1-2* ancestor, likely of race 0. Although not as common, *MAT1-1* isolates of race 1 were found, underscoring the possibility of multiple origins of race 1. Based on studies of population structure (5,13), sexual recombination may have been integral to the evolution of race 1 in *S. turcica*. Propagules resulting from sexual recombination may reach the United States from diverse populations in Central America, creating potential for development of aggressive isolates that vary in ability to infect maize with different *Ht* genes. Loss of avirulence to *Ht1* may have existed in North American populations of *S. turcica* at undetectable levels before *Ht1* resistance was used commercially and selection simply allowed these isolates to increase in frequency to reach detectable levels. The data suggest that virulence to *Ht1* may reduce fitness of race 1 isolates in the absence of *Ht1*, although fitness was not directly evaluated in our studies. Continued presence of race 1 in the population may be attributed to continued presence of *Ht1* in many commercial maize hybrids in North America. Race 1 isolates most likely arose from race 0 isolates in North America. The combination of virulence to the four *Ht* genes, *Ht1*, *Ht2*, *Ht3*, and *Hm1* was not found among our isolates but has been reported previously from laboratory crosses (12) and rarely in the field (14,51). Combined virulence may so reduce fitness that any race 123N would not survive. Other virulence combinations as yet unreported in the field include 12, 13, 3N, and 13N, which may corroborate this argument.

Variation based on isozymes versus RAPD markers. Simcox et al. (38) used isozymes to characterize diversity among 40 *S. turcica* isolates collected in the United States Maize Belt from 1979 to 1987. Eight distinct isozyme patterns were identified and allowed them to clearly distinguish *S. turcica* races. Race 0 isolates shared similar isozyme phenotypes across years and locations. They identified four different isozyme phenotypes among race 1 isolates, with a single phenotype predominating. Race 23 isolates studied produced a single diagnostic isozyme profile. Race 1 isolates were more similar to race 0 than to race 23 isolates, suggesting an ancestral link between race 0 and race 1. Associations of geographical location and unique isozyme pro-

files were found, and variation within race 1 isolates acknowledged. Abadi et al. (1) used RAPD markers to analyze variation among 18 isolates of *S. turcica* from Israel, Africa, and the United States. Race 0 isolates were most similar to race 1 isolates, although no race-specific RAPD patterns were found. Race 23 isolates grouped separately from races 0 and 1, while isolates from Johnsongrass (*Sorghum halpense* L.) were more distant from maize isolates. In our studies, RAPDs identified greater levels of diversity among isolates than that shown by isozyme analysis. This is likely due in part to a more expansive collection analyzed in our study, to greater variation detectable in *S. turcica* using RAPD's versus isozymes, and also may be influenced by sampling effects.

Origins of races 23 and 23N. Races 23 and 3 differed from race 0 isolates in frequency of four RAPD markers (Table 5). Race 23N isolates differed in frequency of only two markers from race 0 isolates. Three haplotypes of the 13 characterized as races 23 and 3 were common to race 0. A similar percentage was shared between race 1 and race 0 isolates. Race 23N isolates shared the highest percentage of haplotypes with race 0 isolates. Comparatively few isolates of races 23, 3, and 23N shared a haplotype with race 1 isolates; a single haplotype was shared among all races. UPGMA cluster analysis grouped race 1, 23, 3, and 23N isolates closely based on Dice similarity measures, indicating possible common origins for these races. Races 23 and 23N were found as early as 1957 in our collection and are present in regions where *Ht2*, *Ht3*, and *Hm1* have reportedly not been deployed in maize (5,7,14,51), indicating that races 23 and 23N have co-existed with race 0 isolates prior to the discovery of *Ht2*, *Ht3*, and *Hm1* genes for resistance.

***Sorghum* spp.-infecting isolates.** Among the small sample of non-maize-infecting isolates collected from *Sorghum bicolor* and *S. halepense*, eight of 12 RAPD markers differed in frequency compared with race 0 isolates from maize. Four markers were exclusively absent and two others exclusively present among the *Sorghum*-infecting isolates. No haplotypes were shared among maize- and *Sorghum*-infecting isolates and five of the six *Sorghum*-infecting isolates grouped into a distant cluster in our UPGMA dendrogram. A single isolate collected in 1998 in NC from *S. bicolor* grouped with maize-infecting isolates. *Sorghum* spp.-infecting isolates of *S. turcica* are likely to be reproductively isolated from maize-infecting isolates in nature although compatible pairings can be made in the laboratory.

In summary, the presence of race 1 in multiple, diverse RAPD lineages indicates that virulence to *Ht1* either arose by mutation multiple times in several race 0 lineages in North America, or arose in a single race 0 lineage and subsequently spread to other genetic backgrounds via sexual recombination. The apparent absence of race 1 before the wide usage of *Ht1* hybrids in the United States may indicate that virulence to *Ht1* reduces fitness. The presence and persistence of races 23 and 23N in the absence of *Ht2*, *Ht3*, and *HtN*, however, suggests no loss of fitness due to

TABLE 5. Frequency of random amplified polymorphism DNA (RAPD) markers among a subset of 106 *Setosphaeria turcica* isolates (the majority collected from 1976 to 1982) possessing different virulence to *Ht* resistance genes in maize, as well as *Sorghum*-infecting isolates

Virulence	N ^a	M ^b	B11 1.66 ^c	I12- 0.51	UBC100 2.52 ^d	UBC23 0.82	UBC23 1.35	UBC23 1.75	UBC417 0.75	UBC417 1.52	UBC417 1.75	UBC77 1.45	UBC77 1.6	UBC77 1.71
Race 0 ^e	40	31	0.2	0.3	0.55	0.8	0.93	0.13	0.3	0.98	0.68	0.88	0.63	0.93
Race 1	27	24	0.67	0.3	0.37	0.7	1.0	0.11	0.19	0.96	0.56	0.85	0.59	0.89
Race 23 & 3	13	11	0.23	0.15	0.54	0.46	0.92	0.23	0.38	0.85	0.62	0.85	0.69	0.92
Race 23N	20	19	0.15	0.4	0.5	0.7	0.9	0	0.65	0.95	0.8	0.9	0.65	0.8
Nonmaize	6	6	0	0.17	0.5	0	0.17	0	0.33	0	0.33	1.0	0.67	1.0

^a N = number of isolates of each race type included in the analysis.

^b M = number of multilocus RAPD haplotypes represented in each race group.

^c B11 and I12 are both primers from Operon Technologies, followed by the size of marker produced (kb).

^d Ubc primers included here are University of British Columbia, primer numbers are followed by the size of marker produced (kb).

^e Race designations refer to the reaction of isolates to *Ht* resistance genes in maize. The naming scheme followed in that from Leonard et al. 1989 (25). Nonmaize infecting isolates were originally isolated from *Sorghum* spp. and were not infective to maize.

unnecessary virulence to these genes. Races 23 and 23N have coexisted with race 0 over an extended time. Virulence to *Ht2*, *Ht3*, and *HtN* is often not expressed under field conditions (9), so it is unclear whether virulence to these genes would offer much advantage, even in the presence of these genes in the host population. The absence of isolates with virulence to *Ht1* in combination with virulence to *Ht2*, *Ht3*, or *HtN* in the population suggests that either these virulence combinations dramatically reduce fitness or that sexual recombination between race 1, and races 23 and 23N is rare or nonexistent in the United States. Further research on the effects of unnecessary virulence on fitness and pathogen aggressiveness in *S. turcica* is clearly needed.

Studies provided evidence of both asexual reproduction and sexual recombination. This could be expected with a predominantly asexual fungus that either reproduces sexually sporadically and/or has a constant influx of individuals from neighboring tropical populations where sexual recombination occurs more frequently.

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