Genetic Structure of *Mycosphaerella populorum* (Anamorph *Septoria musiva*) Populations in North-Central and Northeastern North America

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ABSTRACT

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In order to characterize the genetic variation of the poplar pathogen *Mycosphaerella populorum* (anamorph *Septoria musiva*), we have studied seven North American populations using the polymerase chain reaction random amplified polymorphic DNA (RAPD) technique. The fungal populations were sampled in 2001 and 2002 by obtaining 352 isolates from cankers and leaf spots in hybrid poplar plantations and adjacent eastern cottonwood (*Populus deltoides*). A total of 21 polymorphic RAPD markers were obtained with the six RAPD primers used. A fine-level scale analysis of the genetic structure within the populations revealed that subpopulations sampled on *P. deltoides* and on hybrid trees were not

significantly differentiated. In contrast, analyses performed on the entire data set showed high levels of haplotypic diversity and moderate to high genetic differentiation, with 20% of the expected genetic diversity found at the interpopulation level. Moreover, a high and significant correlation between genetic and geographic distances among populations was found, suggesting isolation by distance of the sampled populations. Although the occurrence of the sexual stage of this fungus remained unclear in field populations, five of the six populations were at gametic equilibrium for RAPD loci, suggesting the occurrence of recombination episodes in *Septoria musiva* populations. Overall, *S. musiva* appears to consist of differentiated subpopulations, with both asexual and sexual recombination contributing to the local level of genetic structure.

Additional keywords: multilocus analyses.

During the past 30 years, the area planted with interspecific hybrid poplar clones has been rapidly increasing in North America for the production of wood, fiber, and energy (2,10,44). However, this expansion of poplar culture in the north-central and northeastern regions of North America is limited because many poplar clones are susceptible to leaf spot and canker disease caused by the fungus *Septoria musiva* Peck (teleomorph *Mycosphaerella populorum* G. E. Thompson) (14,30,34). Necrotic lesions on leaves can cause early-season defoliation, and stems and branches often break at cankers in strong winds on susceptible clones. These have resulted in plantation failure during the mid-1970s in the north-central United States and in Ontario, Canada (30,31,45).

Selection and deployment of resistant clones is one of the best disease-control strategies (10,29) and has been considered for the control of *S. musiva* diseases. In southern parts of Québec, Canada, the breeding program and selection criteria are oriented largely toward selection for quantitative resistance to *Septoria* canker (27). Because the durability of host plant resistance is an evolutionary process that depends on pathogen fitness, recombination, mutation, and migration (26), breeding and deployment of durable resistance require an understanding of the diversity of the pathogen populations and ensuring that early-generation breeding lines are screened against a range of genotypes which reflect the diversity of pathogen populations. Unfortunately, the population

structure of *S. musiva* is poorly documented. Nothing is known about the global genetic diversity of this pathogen and how variability is distributed within and between populations, making it difficult to reliably determine whether putatively resistant poplar clones will be resistant over a wide geographic area.

The anamorph *S. musiva* and its teleomorph *M. populorum* belong to the class *Ascomycotina*, which includes fungi that are typically haploid during the asexual stage of their life cycle (48). The disease cycle of *S. musiva* is similar to the cycles of other foliage-infecting ascomycetes (16). This fungus produces asexual spores (conidia) in a pycnidium during its anamorphic state and sexual ascospores in a pseudothecium during its teleomorphic state. The conidia are dispersed by rain splash (36), hence having a supposed limited potential to move over long distances, whereas the ascospores are wind dispersed (36,37). The teleomorph has been observed on over-wintered leaves in Manitoba and Saskatchewan (53), Ontario (42), the north-central United States (16,30), and for the first time in 2004, Québec (D. Lamontagne, M. J. Mottet, and P. Périnet, *unpublished data*). Furthermore, the relative importance of sexual and asexual reproduction in the population genetics and dynamics of this organism remains unclear. Past epidemiological studies provided evidence that the primary inoculum leading to disease in hybrid poplar stands was airborne, suggesting that ascospores played a major role in initiating disease (16,30). However, other inoculum sources, such as over-wintering mycelia in branch cankers, are not excluded (16,30,36). In northeastern North America, the distribution of *S. musiva* is sympatric with native *Populus deltoides*, on which damage normally is limited to leaf spots (8). Endemic leaf spots on the local species *P. deltoides* are thought to be the initial source of inoculum for new hybrid poplar plantings (29).

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In contrast to virulence markers generally submitted to strong selection by the host, molecular markers are supposed to be selectively neutral and, therefore, may be useful to study the population evolutionary processes described previously (20,26). Analysis of random amplified polymorphic DNA (RAPD) polymorphisms (50,51) has become increasingly popular for estimating genetic diversity in pathogen populations (11,35,43). RAPD markers offer the advantage of technical simplicity and the ability to provide large numbers of polymorphic loci. Furthermore, analysis of RAPD variation is particularly well suited for genetic studies of haploid fungi because there is no loss of genetic information caused by the dominant inheritance of RAPDs. The general objective of the present research was to determine the genetic structure of North American populations of *S. musiva*. More specially, we investigated (i) the genetic structure of the pathogen at different spatial scales; (ii) the occurrence or not of sexual reproduction in *S. musiva* populations; and (iii) the relatedness between populations sampled in hybrid poplar stands and populations from endemic leaf spots on *P. deltoides*, and between one population from cankers and one from leaf spots.

MATERIALS AND METHODS

Populations surveyed. In all, 352 isolates of *S. musiva* were collected from seven host populations during the end of the epidemic period (August to October) in 2001 and 2002 (Table 1). The populations of Cornwall, St-Ours 2001 and 2002, Dane, and Calumet were subdivided in pairs, including one subpopulation sampled in a hybrid poplar stand and one other sampled on adjacent (distant from 3 km or less) native *P. deltoides* trees. The hybrid poplar stands of St-Ours, Thetford-Mines, Baby, Dane, and Calumet are experimental clone trials characterized by the presence of many clones carrying different levels of resistance, whereas Cornwall is a commercial hybrid poplar stand which contained only one clone. Regions near St-Ours, Thetford-Mines, and Baby are characterized by few hybrid poplar stands, whereas Cornwall is located near an area of intensive poplar cultivation with numerous plantations of *P. nigra* × *P. maximowiczii* clones. Moreover, native poplar and aspen populations located near St-Ours, Dane, Calumet, and Cornwall are composed in large part of *P. deltoides*, *P. grandidentata*, and *P. tremuloides*. The regions near Baby and Thetford-Mines are characterized by the presence of *P. tremuloides* and *P. balsamifera* as natives species.

Sampling of isolates. In 2001, the hybrid subpopulation in St-Ours was subjected to a three-level hierarchical sampling in order to investigate genetic structure at different spatial scales. Three leaves per tree were sampled on six hybrids trees and two or three

leaf spots were sampled per leaf, each spot corresponding to one isolate. In 2002, a two-level hierarchical sampling strategy was applied to the hybrid subpopulations of Dane, Calumet, St-Ours, and Thetford-Mines. In all, 6 to 11 leaves were collected on five hybrid trees and one isolate was taken from each of the infected leaves. In the population of Thetford-Mines, half of the isolates were sampled on leaf spots and the other half on cankers from the same trees.

In some instances, finding heavily diseased trees was difficult. This was the case for *P. deltoides* in all locations, and for hybrids in Baby and Cornwall. In these cases, subpopulations of *S. musiva* were sampled randomly on a large number of leaves from a variable number of trees per stand.

Isolation of *S. musiva*. Conidia were obtained by crushing pycnidia or from cirri produced after incubation of leaves in a moist chamber. Conidia were germinated on corn meal agar (supplemented with streptomycin at 50 mg/liter and chloramphenicol at 300 mg/liter) and individually transferred to potato dextrose agar or V8 juice agar. Identifications of isolates as *S. musiva* were based on symptom observation, microscopic examination of conidia (37), and molecular characteristics (N. Feau, unpublished data). Cultures were kept at 4°C until needed or stored at -80°C in potato dextrose broth containing 15% glycerol.

DNA extraction. Mycelium of *S. musiva* was grown in 125-ml Erlenmeyer flasks containing 50 ml of liquid V8 juice with 0.2% CaCO₃. The flasks were seeded with two mycelium plugs, placed on a rotary shaker at 110 rev/min, and incubated for 1 week at 22°C in darkness. The liquid cultures were centrifuged at 4,700 × g for 10 min; the supernatant was removed and the pellets were washed with sterile distilled water, lyophilized, and ground in liquid nitrogen. Total genomic DNA was extracted by the hexadecyl-

TABLE 2. Analysis of molecular variance for random amplified polymorphic DNA (RAPD) markers in the *Septoria musiva* population of St-Ours sampled in 2001 at three hierarchical levels^a

Hierarchical level	Variation (%)	Φ Statistic	P value
Φ _{st} (within leaves)	89.4	0.106	0.029 ^b
Φ _{sc} (among leaves within trees)	8.69	0.089	0.054 ^c
Φ _{ct} (among trees)	1.91	0.019	0.339 ^d

^a The variance representing total diversity was partitioned into components within and between leaves in the trees and between trees in the population.

^b Probability of obtaining equal or lower Φ statistic value determined by 1,000 randomizations of RAPD haplotypes among leaves among trees.

^c Probability of obtaining equal or larger Φ statistic value determined by 1,000 randomizations of RAPD haplotypes among leaves within trees.

^d Probability of obtaining equal or larger Φ statistic value determined by 1,000 randomizations of leaves among trees.

TABLE 1. Host, sample size, and geographic location of populations and subpopulations of *Septoria musiva* collected from poplars in northeastern and north-central North America in the given year

Populations (province or state), year	Subpopulations	Location (latitude, longitude)	Sampling strategy	Host (no. of isolates) ^a
Calumet (Wisconsin), 2002	Cal-hyb Cal-P.del	44°10'00"N, 88°30'00"W ...	Hierarchical Random	'NM6' (38) <i>P. deltoides</i> (35)
Dane (Wisconsin), 2002	Dane-hyb Dane-P.del	43°20'00"N, 89°22'00"W ...	Hierarchical Random	D × M (30) <i>P. deltoides</i> (28)
Cornwall (Ontario), 2002	Cor-hyb Cor-P.del	45°11'43"N, 74°57'02"W 45°13'13"N, 74°58'20"W	Random Random	'NM6' (15) <i>P. deltoides</i> (13)
Baby (Québec), 2002	...	47°28'29"N, 79°11'46"W	Random	<i>P. × berolinensis</i> (10); <i>P. × jackii</i> (6); other hybrids (2)
St-Ours (Québec), 2001	Sto01-hyb Sto01-P.del	45°52'39"N, 73°11'31"W 45°52'26"N, 73°11'04"W	Hierarchical Random	'NM6' (20); M(D × T) (24) <i>P. deltoides</i> (38)
St-Ours (Québec), 2002	Sto02-hyb Sto02-P.del	45°52'39"N, 73°11'31"W 45°52'26"N, 73°11'04"W	Hierarchical Random	M(M × T) (20+1); other hybrids (3) <i>P. deltoides</i> (29)
Thetford-Mines (Québec), 2002	Thet-leaf spots Thet-cankers	46°02'22"N, 71°24'28"W ...	Hierarchical Hierarchical	D × M (19) D × M (17); M(M × T) (4)

^a Includes the following hybrids. *Populus × berolinensis*: natural hybrid of *P. laurifolia* × *P. nigra*; *P. × jackii*: natural hybrid of *P. deltoides* × *P. balsamifera*; 'NM6': commercial cultivars, *P. nigra* × *P. maximowiczii*. When known, parentage is indicated as follows: *P. deltoides* (D); *P. maximowiczii* (M); *P. trichocarpa* (T). For numbers in parentheses, plain characters indicate isolates obtained from leaf spots and italicized characters indicate isolates obtained from cankers.

TABLE 3. Frequency of random amplified polymorphic DNA (RAPD) markers and expected genetic diversity (H_e) in North American *Septoria musiva* populations and subpopulations sampled in 2001 and 2002 using 21 RAPD loci

Populations	Frequency of the marker allele											
	OPK2				OPJ8				OPQ10			
	3000	2950	2000	1300	4200	4100	2500	1600	1440	1230	500	350
Calumet (WI)	0.644	0	0.671	0.877	0	0.877	1	0.11	0.123	0.096	0.904	0.069
Dane (WI)	0.466	0	0.621	0.983	0.052	0.81	1	0.086	0.052	0.086	0.897	0.276
Cornwall (ON)	1	0.071	0.143	1	0.25	0.643	1	0.429	0.25	0	0.893	0
Baby (QC)	0.722	0	0.667	1	0.333	0.667	1	0.278	0	0.111	1	0
St-Ours 2001(QC)	0.927	0.183	0.098	1	0.354	0.561	0.976	0.793	0.305	0.5	0.976	0.024
St-Ours 2002 (QC)	0.887	0.396	0.094	1	0.472	0.585	1	0.736	0.264	0.415	0.981	0.057
Thetford (QC)	1	0.3	0	1	0.55	0.35	1	0.875	0.575	0.575	1	0.1
G^{2b}	***	***	***	***	***	***	ns	***	***	***	*	***
Subpopulations												
Cal-hyb (WI)	0.605	0	0.658	0.895	0	0.868	1	0.184	0.053	0.026	0.921	0.053
Cal-P.del (WI)	0.686	0	0.686	0.857	0	0.886	1	0.029	0.2	0.171	0.886	0.086
Dane-hyb (WI)	0.533	0	0.667	0.967	0.033	0.833	1	0.133	0.1	0.133	0.867	0.2
Dane-P.del (WI)	0.393	0	0.571	1	0.071	0.786	1	0.036	0	0.036	0.929	0.357
Cor-hyb (ON)	1	0.067	0	1	0.2	0.667	1	0.333	0.2	0	0.933	0
Cor-P.del (ON)	1	0.077	0.308	1	0.308	0.615	1	0.539	0.308	0	0.846	0
Sto01-hyb (QC)	0.933	0.133	0.156	1	0.378	0.533	0.978	0.756	0.378	0.422	1	0.022
Sto01-P.del (QC)	0.919	0.243	0.027	1	0.324	0.595	0.973	0.838	0.216	0.595	0.946	0.027
Sto02-hyb (QC)	0.833	0.542	0.125	1	0.25	0.583	1	0.792	0.167	0.583	0.958	0.083
Sto02-P.del (QC)	0.931	0.276	0.069	1	0.655	0.586	1	0.69	0.345	0.276	1	0.035
Thet-leaf spots (QC)	1	0.368	0	1	0.579	0.316	1	0.842	0.632	0.579	1	0.053
Thet-canker (QC)	1	0.238	0	1	0.524	0.381	1	0.905	0.524	0.571	1	0.143
Total	0.79	0.142	0.324	0.972	0.261	0.659	0.994	0.48	0.23	0.284	0.946	0.085

(continued on next page)

^a Average unbiased Nei's genetic diversity over loci.

^b Log-likelihood statistic; *, **, and *** = significant at $P < 0.05$, 0.01, and 0.001, respectively; ns = not significant.

trimethylammonium bromide (CTAB) method according to Zolan and Pukkila (55). Total DNA was diluted 30-fold in Tris-EDTA (TE) buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8) and used as template for polymerase chain reaction (PCR)-RAPD amplifications.

RAPD amplification. Amplifications were carried out in volumes of 12.5 μ l containing 1 $\times$ *Taq* polymerase buffer (20 mM, Tris-HCl, pH 8, 5 mM KCl), 1.5 mM MgCl₂, 0.5 unit of *Taq* polymerase (Roche Diagnostics, Penzberg, Germany), 0.1 mM each dNTP, 2 μ M each primer, and 0.5 to 1 ng of genomic DNA. PCR amplifications were performed in a thermal cycler (model PTC100; MJ Research, Incline Village, NV) programmed for 20 cycles of 15 s at 94°C (denaturation), 15 s at 35°C (annealing), and 1 min at 72°C (extension); followed by 25 cycles of 15 s at 94°C, 30 s at 35°C, and 1.5 min at 72°C; followed by 5 s of auto-extension per cycle; and 10 min at 72°C (final extension). The PCR products were separated by electrophoresis on 1% agarose gels in 0.5 $\times$ Tris-acetate-EDTA buffer. Gels were stained with ethidium bromide and photographed under UV light. All amplifications were done at least twice to ensure reproducibility of results. Only well-resolved and reproducible bands were retained for analysis. Putative alleles at each polymorphic and reproducible RAPD locus were scored using a binary code, with a score of 1 or 0 corresponding to positive and null alleles for the RAPD loci, respectively. Each putative locus was named using a letter code corresponding to the primer used, followed by the size of the fragment. The possibility that, for a given primer, two fragments differing in size might represent allelic length mutation was addressed by verifying whether such fragments were mutually exclusive (i.e., never occurred together in the same haploid isolate).

Single locus analysis. Diversity statistics for the total population and for each population and subpopulation included allelic frequencies over loci and the average diversity over loci (H_e) using Nei's unbiased genetic diversity (expected heterozygosity) (28). When determining allelic frequencies, it was assumed that each fragment represented a unique, bi-allelic locus. Allelic frequencies and expected heterozygosity were calculated using Arle-

quin software (version 2.0; S. Schneider, D. Roessli, and L. Excoffier, Genetics and Biometry Laboratory, University of Geneva, Switzerland). For each locus, heterogeneity of the marker frequency between populations was calculated by the likelihood *G* test using SAS (version 8; SAS Institute, Cary, NC) (1,5).

Divergence statistics were estimated from Euclidean distances among all pairs of RAPD haplotypes using the hierarchical analysis of molecular variance (AMOVA) (7) using Arlequin software (version 2.0). A measure of genetic differentiation was obtained by Wright's *F* statistic analogs (Φ statistics). The variance components and Φ values were tested statistically by a nonparametric randomization test with 1,000 repetitions. Additionally, the genetic differentiation between subpopulation pairs was estimated using the statistic θ (49), an unbiased estimator of the Wright's *F* statistic. The estimator θ was used because it is unaffected by the number of individuals sampled per population or the number of populations sampled (49). The θ values and their significance were calculated for all pairwise subpopulations using the program FSTAT (9). Mantel tests (1,000 permutations) of correlation between geographic distances and estimated θ values or Nei's unbiased genetic distances (28) among pairs of populations or subpopulations were determined using TFPGA (version 1.3; M. Miller, Northern Arizona University, Flagstaff).

Multilocus analyses. Overall associations among loci were evaluated following the multilocus variance test (4,18) using allelic data for each isolate. We determined the variance in the number of pairwise allelic mismatches over all loci relative to that under the hypothesis of panmixia (i.e., random association of alleles). The index of association (I_A) was used as a measure of the degree of association between loci. I_A has an expected value of zero if there is no association between loci. The error variance of I_A , assuming no association was used to distinguish between values of I_A that significantly differed from zero and those that did not (4,18). A simulated sampling distribution of this error variance assuming no association, and the significance of its deviation from zero were obtained using Multilocus (version 1.2; P. M. Agapov and A. Burt, Imperial College, Berks, England). The exact test of pairwise linkage disequilibrium (LD) among loci for

TABLE 3. (continued from preceding page)

Frequency of the marker allele									Genetic diversity ($H_e \pm SD$) ^a
OPE12		OPC1				OPC15			
1400	900	1200	1000	800	600	1900	1500	600	
0.534	0.836	0.219	0.836	0.973	0.767	0.589	0.877	0.932	0.232 ± 0.157
0.379	0.931	0.069	0.914	0.948	0.448	0.586	0.776	0.983	0.228 ± 0.181
0.107	0.786	0.107	0.786	1	0.607	0.393	0.964	0.964	0.214 ± 0.188
0	0.889	0.167	0.889	1	0.556	0.444	0.556	0.944	0.225 ± 0.211
0.842	0.537	0	0.72	1	0.549	0.72	0.89	1	0.250 ± 0.197
0.208	0.623	0	0.849	1	0.698	0.698	0.66	0.981	0.270 ± 0.199
0.4	0.25	0	1	1	0.625	0.875	1	1	0.207 ± 0.220
***	***	***	***	ns	**	***	***	ns	...
0.474	0.79	0.29	0.79	0.974	0.763	0.632	0.816	0.921	0.238 ± 0.174
0.6	0.886	0.143	0.886	0.971	0.771	0.543	0.943	0.943	0.221 ± 0.160
0.433	0.933	0.067	0.933	1	0.4	0.5	0.867	0.967	0.227 ± 0.177
0.321	0.929	0.071	0.893	0.893	0.5	0.679	0.679	1	0.224 ± 0.197
0.2	0.667	0.067	0.8	1	0.533	0.333	1	0.933	0.200 ± 0.197
0	0.923	0.154	0.769	1	0.692	0.462	0.923	1	0.222 ± 0.210
0.867	0.556	0	0.756	1	0.578	0.667	0.822	1	0.256 ± 0.204
0.811	0.514	0	0.676	1	0.514	0.784	0.973	1	0.238 ± 0.199
0.208	0.667	0	0.75	1	0.792	0.542	0.625	0.958	0.278 ± 0.188
0.207	0.586	0	0.931	1	0.621	0.828	0.69	1	0.245 ± 0.204
0.316	0.316	0	1	1	0.737	0.842	1	1	0.207 ± 0.122
0.476	0.191	0	1	1	0.523	0.905	1	1	0.206 ± 0.121
0.455	0.682	0.074	0.841	0.986	0.614	0.645	0.835	0.974	0.289 ± 0.171

haploid data, which is an extension of Fisher's exact test for contingency tables (41), was applied using Arlequin software (version 2.0). Both digenic LDs and multilocus association could be influenced by the existence of a substructure between samples (i.e., an effect of population admixture equivalent to a Wahlund effect for multiple loci) (18,23); therefore, these were calculated and compared among each population independently. Moreover, because of its small sample size (under 20 isolates), the Baby population was discarded from the multilocus analysis (23). The extent to which clonal haplotypes are overrepresented in each population was quantitatively evaluated by calculating the probability of observing as many or more individuals with a particular haplotype, assuming a random distribution of alleles, as actually was observed in the sample (47,52). The probability (O_R) was determined using a QBASIC program (52) which shuffles alleles among strains at each polymorphic locus to mimic recombination and subsequently determines the frequency of the most common haplotypes observed in the shuffled data sets (47,52).

RESULTS

Genetic diversity. In order to test the effect of a finer scale sampling strategy on estimation of population structure, a preliminary genetic analysis was carried out in the population of St-Ours sampled in 2001 (Table 2). By using the AMOVA, we determined the intraleaves variability component to be $\approx 90\%$, whereas the diversity between leaves and between trees was 8.7 and 1.9%, respectively. The same analyses conducted at one higher hierarchical level in the subpopulations sampled on hybrid poplars in St-Ours, Thetford-Mines, Dane, and Calumet showed that the genetic variation distributed within the trees ranged from 94.34 to 98.44% (mean = $96.29 \pm 1.68\%$). The level of variability observed among trees within each of these four subpopulations ranged from 1.56 to 5.66% (mean = $3.71 \pm 1.68\%$) and was consistent with that observed in the population of St-Ours sampled in 2001 (data not shown).

The 21 reproducible bands retained were polymorphic on the seven populations studied and revealed a substantial level of polymorphism (Table 3). Based on the 99% criterion of polymorphism, 95% of the reproducible loci were considered polymorphic (i.e., by treating a locus as polymorphic if the most common al-

lele frequency was <0.99). Average expected gene diversity (28) across all loci was estimated with a mean value of $0.289 (\pm 0.171)$ for the entire data set, including populations sampled in 2001 and 2002. By excluding the population of St-Ours sampled in 2001, the estimate of this measure for the populations sampled in 2002 was similar, with a value of $0.284 (\pm 0.160)$.

Genetic differentiation between isolates sampled from cankers and leaf spots. RAPD data analysis of the Thetford-Mines population revealed similar marker frequencies at each locus for the 21 isolates sampled from cankers and for the 19 isolates sampled from leaf spots (Table 3). Similar levels of expected gene diversity calculated over all loci were observed in the two subpopulations (Table 3). Moreover, the values of differentiation between these subpopulations from each pair were low and nonsignificant ($\Phi_{st} = -0.019$, $P = 0.766$).

Temporal genetic differentiation between St-Ours populations. Although the values of expected genetic diversity within the populations of St-Ours sampled in 2001 and 2002 were similar (Table 3), the genetic structure of these populations differed significantly. Differentiation values between subpopulations of St-Ours from year to year varied from 0.082 to 0.103 (Table 4) and may be explained by differences in marker frequencies for loci OPE12₁₄₀₀ and OPK2₂₉₅₀ (Table 3).

Genetic differentiation between populations. Almost all RAPD markers scored in this study were present in the seven populations. Overall, allele frequencies differed significantly at the 0.001 level for 16 of 21 RAPD loci (Table 3). Three markers present in populations of southern Québec (Thetford-Mines and St-Ours) distinguished these populations particularly from those sampled in Wisconsin. Allele OPK2₂₉₅₀ was absent in populations of Calumet, Dane, and Baby, whereas this marker occurred at low frequencies in Cornwall (7.1%) and intermediate frequencies in the populations of St-Ours (18.3% in 2001 and 39.6% in 2002) and Thetford-Mines (30%). Marker OPJ8₄₂₀₀ was absent in Calumet and occurred at low frequency in Dane (5.2%) and intermediate frequencies in the other populations (25 to 55%). Differences also were observed for the OPJ8₁₆₀₀ marker, with frequencies ranging from 8.6 to 11% in the populations of Wisconsin, intermediate values of 27.8% in Baby and 42.9% in Cornwall, and higher frequencies in St-Ours (79.3% in 2001 and 73.6% in 2002) and Thetford-Mines (87.5%) (Table 3).

AMOVA conducted on the entire data set showed that 20% of the genetic diversity ($\Phi_{st} = 0.200$, $P = 0.000$) was found at the interpopulation level (data not shown). A more detailed analysis on nested AMOVA indicated low but statistically significant levels of genetic differentiation among subpopulations sampled in 2002 within regions ($\Phi_{st} = 0.09$, $P = 0.000$), and a higher and significant value of genetic differentiation ($\Phi_{st} = 0.17$, $P = 0.004$) among the Wisconsin region (subpopulations of Dane and Calumet), the Québec region (subpopulations of St-Ours 2002, Baby, and Thetford-Mines), and the Ontario region (subpopulations of Cornwall). The significant differentiation values obtained between regions were explained by significant θ between pairs of subpopulations from different regions (Table 4). The population of Baby, in northeastern Québec, was genetically more differentiated from the other subpopulations in southeastern Québec (0.164 to 0.165 with St-Ours and 0.354 with Thetford) than from the more geographically distant subpopulations of Wisconsin (0.068 to 0.073 with Dane and 0.075 to 0.124 with Calumet). The subpopulations of Cornwall showed a high range of θ values with other subpopulations, varying from 0.052 to 0.121 with Baby and 0.245 with Thetford-Mines. However, the relationships between the θ values and the geographical distances for all populations and subpopulations pairs were significant (Mantel $r = 0.66$, $P = 0.000$) at the geographical scale studied. Nei's unbiased genetic distances measured between all pairs of populations showed

identical patterns of differentiation among populations (Fig. 1) and were significantly correlated with geographical distances (Mantel $r = 0.70$, $P < 0.005$).

Genetic differentiation between subpopulations from hybrid poplars and from *P. deltoides*. RAPD analysis performed on the five pairs of subpopulations revealed similar marker frequencies at each locus for any given pair of subpopulations. For each locus and each pair of subpopulations, the alleles present in the subpopulations sampled on hybrid poplar clones were present in the adjacent subpopulation sampled on *P. deltoides*, except for loci OPK2₂₀₀₀ and OPE12₁₄₀₀ in Cornwall and locus OPQ10₁₄₄₀ in Dane. However, in these three cases, frequencies of the amplified allele were low (marker OPK2₂₀₀₀ was present in 31% of the samples from the subpopulation of Cornwall sampled on hybrid poplar clones and OPE12₁₄₄₀ in 20% of the samples of the subpopulation of Cornwall sampled on *P. deltoides* and in 10% in the subpopulation of Dane sampled on hybrids) (Table 3). Consequently, the intrasubpopulation gene diversity indices calculated over all loci showed that, within each population, the two subpopulations presented the same range (0.200 to 0.278) of expected gene diversity (Table 3). Moreover, differentiation between subpopulations from each pair was low and nonsignificant (0.002 to 0.056) (Table 4). The unweighted pair group method with arithmetic averages dendrogram based on pairwise measures of Nei's genetic distances resulted in a tree with five clusters, each corre-

TABLE 4. Pairwise subpopulations θ values calculated from the Weir and Cockerham differentiation estimator (above diagonal) and geographic distances in kilometers (below diagonal) between 12 North American *Septoria musiva* subpopulations^a

Subpopulations	Cal-hyb (WI)	Cal-P.del (WI)	Dane-hyb (WI)	Dane-P.del (WI)	Cor-hyb (ON)	Cor-P.del (ON)	Baby (QC)	Sto01-hyb (QC)	Sto01-P.del (QC)	Sto02-hyb (QC)	Sto02-P.del (QC)	Thetford (QC)
Cal-hyb (WI)	...	0.005 ns	0.030 ns	0.047 *	0.144 ***	0.124 ***	0.075 ***	0.215 ***	0.263 ***	0.219 ***	0.237 ***	0.367 ***
Cal-P.del (WI)	0.00	...	0.023 ns	0.073 **	0.167 ***	0.158 ***	0.124 ***	0.220 ***	0.275 ***	0.248 ***	0.269 ***	0.381 ***
Dane-hyb (WI)	139.93	139.93	...	0.006 ns	0.147 ***	0.140 **	0.068 *	0.226 ***	0.276 ***	0.233 ***	0.249 ***	0.379 ***
Dane-P.del (WI)	139.93	139.93	0.00	...	0.189 ***	0.175 ***	0.073 *	0.268 ***	0.317 ***	0.248 ***	0.255 ***	0.406 ***
Cor-hyb (ON)	995.03	995.03	1,103.83	1,103.83	...	0.002 ns	0.121 **	0.140 ***	0.185 ***	0.131 **	0.113 **	0.245 ***
Cor-P.del (ON)	993.44	993.44	1,102.43	1,102.43	3.00	...	0.052 ns	0.158 ***	0.211 ***	0.102 **	0.083 *	0.245 ***
Baby (QC)	765.96	765.96	892.05	892.05	389.41	386.30	...	0.234 ***	0.297 ***	0.164 ***	0.165 ***	0.354 ***
Sto01-hyb (QC)	1,134.76	1,134.76	1,246.96	1,246.96	156.58	156.74	463.22	...	0.010 ns	0.103 ***	0.082 **	0.094 ***
Sto01-P.del (QC)	1,135.30	1,135.30	1,247.48	1,247.48	156.90	157.07	463.92	0.70	...	0.100 **	0.114 ***	0.103 ***
Sto02-hyb (QC)	1,134.76	1,134.76	1,246.96	1,246.96	156.58	156.74	463.22	0	0.7	...	0.056 ns	0.140 ***
Sto02-P.del (QC)	1,135.30	1,135.30	1,247.48	1,247.48	156.90	157.07	463.92	0.7	0	0.7	...	0.072 ***
Thetford (QC)	1,271.69	1,271.69	1,383.77	1,383.77	290.97	291.64	584.77	139.02	138.50	139.02	138.50	...

^a P values were estimated from 10,000 resamplings of alleles over loci; *, **, and *** = significant at $P < 0.05$, 0.01, and 0.001, respectively; ns = not significant.

Fig. 1. Unweighted pair group method with arithmetic averages analysis of Nei's unbiased genetic distances among the seven studied populations divided into subpopulations according to host (hybrid poplars or *Populus deltoides*) or tissue sampled (stem cankers or leaf spots). Genetic distances were computed from 21 random amplified polymorphic DNA loci. Bootstrap values were calculated from 1,000 bootstrap replications over markers. Populations from Baby and Thetford-Mines were sampled from hybrid poplars only.

sponding to the subpopulation sampled on hybrid poplar clones and to their adjacent subpopulation sampled on *P. deltoides* trees (Fig. 1).

Multilocus analysis. Overall, considering all the *S. musiva* isolates sampled, 312 unique multilocus genotypes were identified in the sample of 352 individuals. Within each population, counting of multilocus haplotypes translated into high haplotype diversity (Table 5). The same pattern was observed among isolates within pairs of subpopulations (three haplotypes shared in the Dane pair of subpopulations, two in the Calumet pair, and two in the St-Ours 2001 pair) or populations from the same region (five haplotypes shared between the two populations in the Wisconsin region and one between the three populations sampled in 2002 in the Québec region). Only two haplotypes were shared between popu-

lations from different regions (one between Dane and Cornwall and one between Dane and Baby). In addition, isolates with identical RAPD haplotype were occasionally, but not always, found from the same tree or the same leaf.

Considering all the isolates in each population, the level of pairwise LD ranged from 3.8 to 16.9% (Table 6). Apart from the Calumet population, the clone-corrected samples showed lower estimates of LD. The highest values of LD were observed in the population of St-Ours sampled in 2002 (16.9% in the total population and 11.8% in the clone-corrected sample). The observed values of the index of multilocus association, I_A , and their significance levels for the total samples and the clone-corrected sample in each population are presented in Table 6. When clone-corrected samples were analyzed, the I_A levels were reduced and

TABLE 5. Occurrence of multilocus haplotypes detected at least twice among or within the 12 North American subpopulations of *Septoria musiva*^a

Multilocus haplotypes	Cal-hyb (WI)	Cal-P.del (WI)	Dane- hyb (WI)	Dane- P.del (WI)	Cor-hyb (ON)	Cor-P.del (ON)	Baby (QC)	Sto01- hyb (QC)	Sto01- P.del (QC)	Sto02- hyb (QC)	Sto02- P.del (QC)	Thetford (QC)	All ^b
10011011011001011101	...	...	...	...	...	...	...	...	...	2	...	...	2
100101100111010011011	...	...	...	...	...	...	...	...	...	2	...	...	2
110111110010000111111	...	...	...	...	...	...	...	...	...	...	2	...	2
100101100010100110011	...	...	...	1	1	...	...	...	...	...	...	...	2
101101100010010111011	...	...	...	...	...	...	2	...	...	...	...	...	2
100100110010000111111	...	...	...	...	...	...	1	...	...	...	1	...	2
100101100010010111011	...	...	1	...	...	...	1	...	...	...	...	...	2
001101100010010111011	1	...	1	2	...	...	...	...	...	...	...	...	4
001100100010110111111	...	...	1	1	...	...	...	...	...	...	...	...	2
100101100010010110111	1	...	1	1	...	...	...	...	...	...	...	...	3
001101100010110110111	...	1	1	...	...	...	...	...	...	...	...	...	2
001101100010110111011	...	1	1	...	...	...	...	...	...	...	...	...	2
101101100010110111011	...	2	1	...	...	...	...	...	...	...	...	...	3
001101100010010111101	...	...	...	2	...	...	...	...	...	...	...	...	2
000101100011110101101	...	...	...	2	...	...	...	...	...	...	...	...	2
101101100010010111111	2	1	...	...	...	...	...	...	...	...	...	...	3
001101100010010111111	2+1	1	...	...	...	...	...	...	...	...	...	...	4
001101100010110111111	...	2	...	...	...	...	...	...	...	...	...	...	2
100101101000111111111	...	2	...	...	...	...	...	...	...	...	...	...	2
100110111110000111111	...	...	...	...	...	...	...	...	...	...	...	2	2
100110110010110111111	...	...	...	...	...	...	...	2*	1	...	...	...	3
100101110010100111111	...	...	...	...	...	...	...	1	1	...	...	...	2
100110101110110011011	...	...	...	...	...	...	...	2*	...	...	...	...	2
101101110010010110111	...	...	...	...	...	...	...	2	...	...	...	...	2
100101111010110111001	...	...	...	...	...	...	...	2+1	...	...	...	...	3
100110111010100111111	...	...	...	...	...	...	...	1	...	...	...	1	1
100110111110110111111	...	...	...	...	...	...	...	...	1	...	...	1	1
100110110110100111111	...	...	...	...	...	...	...	...	1	...	...	1	1
100101110110110110111	...	...	...	...	...	...	...	...	2	...	...	...	2
110101111110010111011	...	...	...	...	...	...	...	...	2	...	...	...	2
100101100110000010111	...	...	...	...	...	...	...	...	2*+1	...	...	...	3
Total no. of haplotypes	35	32	30	25	15	13	17	39	34	22	28	39	—
No. of isolates	38	35	30	28	15	13	18	44	38	24	29	40	—

^a Multilocus haplotypes sampled on the same tree are bold and italicized and those from the same leaf are indicated with an asterisk; ... indicates haplotypes not observed in the populations.

^b All populations.

TABLE 6. Overrepresentation of haplotypes (O_R), multilocus associations (I_A), and number and percentage of significant ($P < 0.05$) pairwise linkage disequilibria (LDs) among 21 random amplified polymorphic DNA loci, considering all isolates of *Septoria musiva* and a clone-corrected sample

Populations	Number of		No. (and percentage) of pairwise LD ^a			<i>I</i> _A (<i>P</i> value) ^a		<i>O</i> _R ^b		
	Isolates	Haplotypes	Comparisons	All	Clone	All	Clone	4 ^c	3 ^c	2 ^c
Calumet (WI)	73	65	154	14 (9.1)	16 (10.4)	0.171 (0.03)	0.062 (0.24)	0.136	0.563	0.997
Dane (WI)	58	52	171	20 (11.7)	19 (11.1)	0.16 (0.02)	0.034 (0.34)	...	0.281	0.977
Cornwall (ON)	28	28	105	4 (3.8)	...	−0.15 (0.91)	...	...	...	...
St-Ours 2001 (QC)	82	71	136	14 (10.3)	8 (5.88)	0.134 (0.02)	0.102 (0.06)	...	0.211	0.977
St-Ours 2002 (QC)	53	51	136	23 (16.9)	16 (11.8)	0.144 (0.02)	0.125 (0.04)	...	0.021	0.547
Thetford (QC)	40	39	55	4 (7.3)	3 (5.5)	0.166 (0.02)	0.107 (0.09)	...	...	0.911

^a All = all isolates and Clone = clone-corrected isolates.

^b Probability that randomly mating populations (10,000 randomized data sets examined) contained a haplotype occurring at a number equal to or greater than that the number of times a common isolate haplotype occurs in the observed population; ... indicates the impossibility of carrying out the analysis.

^c Number of times a common isolate haplotype occurs in an observed population.

went from significant to nonsignificant values, except for the population of St-Ours sampled in 2002, where the value remained significantly higher than expected under random association of alleles at different loci ($P < 0.05$). This significant value of I_A observed in St-Ours 2002 could be explained by the moderate level of differentiation ($\theta = 0.056$) observed between the two subpopulations which composed this population. Based on probabilities obtained by repeated shuffling of the population sets, the occurrence of even two individuals with the same haplotype would be a frequent event (probabilities ranged from 0.900 to 0.997) under the null hypothesis of random mating, except in St-Ours sampled in 2002. In contrast, the predicted occurrence of haplotypes shared by three or more isolates within the shuffled population became a relatively less frequent event, with probabilities from 0.021 in St-Ours sampled in 2002 to 0.563 in Calumet (Table 6).

DISCUSSION

Molecular polymorphisms for neutral markers were obtained consistently with the six RAPD primers tested initially. All 21 reproducible RAPD markers generated were polymorphic and exhibited high levels of haplotype diversity coupled with moderate levels of gene diversity in the *S. musiva* populations studied. On average, the scale at which most of the diversity occurred was the same in the subpopulations sampled with a hierarchical strategy: >90% of the gene diversity was distributed within samples from the same trees. The amount and the distribution of haplotypes within poplar stands was consistent with the hypothesis that the majority of genetic variation was distributed at the tree scale: different haplotypes often were found within a single tree and, in the population of St-Ours sampled in 2001, most leaves on the same tree were colonized by different haplotypes. Although no single *S. musiva* haplotype predominated in this study, some isolates with identical haplotypes were detected within and among subpopulations, demonstrating the occurrence of asexual reproduction. However, the repeated isolation of certain haplotypes within subpopulations probably was a function of the sampling scale. For example, four clonal haplotypes were each sampled on the same leaf or tree when finer sampling scale was employed in the St-Ours hybrid subpopulation studied in 2001. Finding more clonal haplotypes when this finer sampling scale was employed was not unexpected and supported the idea that asexual spores of *S. musiva* move exclusively on short distances (37) and are dispersed by rain splash (36). The distribution of clonal haplotypes described at this fine-scale level here for *S. musiva* was similar to that found in the cereal pathogens belonging to the *Septoria* genus, such as *Stagonospora nodorum* (= *Septoria nodorum*), which causes a glume blotch on wheat (12,22), and *S. triticii*, the cause of leaf blotch on wheat (54), using restriction fragment length polymorphism markers. All three of these anamorph species produce asexual conidia that are dispersed by rain splash. Consequently, these pathogen populations appear to be arranged as clusters of genetically identical individuals grouped in the same location in the field. At the interpopulation level, the genetic differentiation observed among populations within regions and among regions was consistent with the correlation observed between genetic and geographic distances. We found a similar genetic structure between each of the closely located hybrid *P. deltoides* subpopulations, whereas the subpopulations within regions showed moderate differentiation levels, from nonsignificant values in the Wisconsin group to significant values <15% between St-Ours and Thetford-Mines. The correlation between genetic and geographical distances depends largely on the dispersal ability of an organism (19,40). Under this hypothesis, we confirmed a localized dispersal of the haplotypes supported by sampling of the same haplotype from trees located <3 km apart, as was the case for hybrid poplar subpopulations and their adjacent native *P. deltoides* stands. This suggests the importance of

asexual reproduction in the local dissemination of *S. musiva*, though we cannot be sure that individuals with the same haplotype were exact clones.

In the light of the different hybrid poplar clones sampled, the host selection as an alternative explanation for the genetic differentiation observed between subpopulations could be advocated. However, this hypothesis may be discarded for the two following reasons. First, we believe that the anonymous single-locus RAPD markers used in this experiment identify genetic variation that is, on average, selectively neutral (24). Moreover, the selection neutrality of our markers was supported by the low differentiation observed between subpopulations sampled on *P. deltoides* and hybrid poplar trees. Second, although it is possible that a marker would be tightly linked to a region of the genome involved in adaptation to the host, it is very unlikely that all loci showing strong differentiation are linked to such regions, particularly in fungi that are not strictly clonally reproducing (3,11).

The alternative explanation for the genetic differentiation and the genetic drift observed between populations may be explained by a limited gene flow between populations. The differentiation values estimated for *S. musiva* (overall $\Phi_{st} = 0.200$, $P = 0.000$) are considerably higher than those reported for other Coelomycetous pathogens, such as *S. triticii* in North America, with a differentiation value of 0.08 (54), and *S. nodorum*, with a value of 0.04 observed between two American and one Swiss populations (12). The low differentiation values observed between each of these fungal populations may be explained by the occurrence of wind-dispersed ascospores (3,12). In the case of *S. musiva*, the high differentiation values estimated were contradictory with the occurrence of a high dissemination potential. The ascospores of *S. musiva* were described as airborne ascospores (36,37), but their dissemination potential remains unknown. Similar moderate to high levels of differentiation also were observed for the chestnut blight fungus, *Cryphonectria parasitica* (25). The mean differentiation value for *C. parasitica* was 0.2 over 13 populations, indicating a limited gene flow for this fungus in eastern North America, even though it is supposed to produce wind-dispersed ascospores and conidia that are dispersed by water or animals vectors. Despite this potential for dispersal and a genetic diversity index of 0.430, genetic drift was retained as the most probable cause of differentiation among *C. parasitica* populations. It was hypothesized that genetic drift had occurred due to limited gene flow coupled with hypothetical founder effects or reduction in effective population size (25).

The significant correlation between the geographic distances and the population pairwise genetic differentiation indexes obtained is consistent with the idea of an equilibrium between gene flow and genetic drift (15,40). However, historical gene flow as well as recent colonization may affect equilibrium between genetic drift and current gene flow (38–40). In the light of this correlation, only 66% of the differentiation value and 70% of the genetic distances between populations may be explained by geographical distances and, in the case of the Baby population, we can hypothesize that the equilibrium has been affected by recent colonization events. The population of Baby is located in the Québec bioclimatic domain 3 (sugar maple–yellow birch) (33), where only the related species *S. populicola*, responsible for a leaf spot symptom similar to the *S. musiva* symptom, previously was reported. However, since 1986, the bioclimatic domain 3 includes plantations of poplar clones potentially susceptible to the *S. musiva* canker and, consequently, was expected to become infested by this pathogen (29). The first cankers eventually were detected in Baby in 2000 in 10- to 14-year-old hybrid poplar plantations. Although we cannot establish whether *S. musiva* already was present in this domain as a leaf spot pathogen, it was hypothesized that the population of Baby resulted from a recent colonization event (P. Périnet and M. J. Mottet, *personal communication*).

Although gene flow among *S. musiva* populations appears limited and the role of the *M. populorum* sexual stage of *S. musiva* in the field remains unclear, we cannot exclude the hypothesis that ascospores occur in *S. musiva* populations. Indeed, the amount and distribution of genetic variation in field populations of *S. musiva* were strikingly similar to those found for the cereal pathogens *S. tritici* (21) and *S. nodorum* (22). In these populations, the bulk of the gene diversity was distributed within samples from the same plants or trees. Moreover, the field populations of these fungi contained a high number of different haplotypes and the genetic variation was distributed on a small scale within a field. It was hypothesized that these results are consistent with a disease cycle in which the air-dispersed sexual ascospores were the main primary inoculum source (21,22).

As stated above, *S. musiva* can reproduce asexually and the presence of clones is certainly a direct consequence of clonal reproduction from asexual conidia. However, predominance of one or more haplotypes in *S. musiva* populations does not rule out the possibility of sexual recombination in relationships among the different haplotypes. For this reason, it was important to apply statistical tests for detecting recombination and indexing the extent of clonality within *S. musiva* populations (46). Results from the multilocus analysis of RAPD data also support the hypothesis that some *S. musiva* individuals originated from recombination. We relied on the I_A test and the measure of significant linkage disequilibrium over loci to assess the occurrence of recombination, and on a test of overrepresentation of multilocus haplotypes to measure the extent of clonality within populations. LD can arise in populations within which lineages are subject to temporal or geographical separations (Wahlund effect) that act as physical barriers to gene flow (18,23). To minimize such risks of artifacts, the statistical tests employed here were applied separately to individual populations. Moreover, by discarding the population of Baby ($n < 20$) from the analysis, and by coupling each nondifferentiated subpopulation pair, we minimized the chance that small sample sizes did not permit rejection of the null hypothesis (23). Once these potential biases had been discarded, we then could distinguish predominant clonal structure from putative epidemic clonality, defined as occasional spread of ephemeral clonal haplotypes in a basically sexual species (18). This was done by treating each haplotype as a single individual in these LD tests (17,18,32). Theoretically, if linkage persists in clone-corrected samples, this supports the hypothesis of predominant clonal evolution structure (17,18). When the distinct *S. musiva* haplotypes were treated as individual units within each population, less significant LD also was detected between several pairs of loci. In the same way, the multilocus association values were reduced and became nonsignificantly different from zero, indicating a history of recombination among the genetic loci analyzed and suggesting that sexual reproduction occurs or had occurred in *S. musiva* field populations. Thus, it is not surprising that we detected a genetic structure consistent with recombination, because ascocarps have been observed in the north-central United States (16,30), in central provinces of Canada (42,53), and, recently, in Québec (D. Lamontagne, M. J. Mottet, and P. Périnet, unpublished data). The last approach measured the extent of clonality by observing the occurrence of clones in simulated random mating populations. Within these randomized data sets, the occurrence of two isolates with identical haplotypes was not a relatively rare event, given the sample size and the 21 polymorphic loci examined. Consequently, the occurrence of clones of two isolates in the sampled populations of *S. musiva* (Calumet, Dane, St-Ours, and Thetford) was consistent with the structure of random mating populations. On the other hand, the probability of finding a haplotype shared by three or more isolates in randomized data sets became relatively low. This indicates an overrepresentation of haplotypes shared by more than two isolates and a primary role for clonal reproduction in generating clones with more than two isolates. Overall, these

results support the hypothesis that the populations studied undergo epidemic clonality rather than predominant clonal structure and that sexual reproduction, as hypothesized within epidemiological studies (16,30), could not be excluded within field populations of this fungus.

An important caveat to these analyses is that the I_A statistical test used in this study appears to be very sensitive, and it may only take a few individuals reproducing sexually per generation to create enough recombination to accept the null hypothesis (6,23). This makes it impossible to determine the extent of sexual reproduction and whether the underlying recombining genetic structure is due to past or present events. As a direct consequence, the importance of ascospore inoculum relative to conidial clonality remains difficult to determine in the disease cycle of this pathogen. Nevertheless, the teleomorph stage has been found in 2004 under field conditions in Québec (D. Lamontagne, M. J. Mottet, and P. Périnet, unpublished data), which indicates that sexual reproduction occurs in *S. musiva* to some extent in the life history of the pathogen.

S. musiva populations that cause poplar leaf spot and canker in northeastern North America appear to be composed of local subpopulations that are genetically diverse and recombinant. If pathogenicity and aggressiveness genes are distributed in a manner similar to the neutral markers we analyzed, this suggests a high potential for local adaptation of this pathogen. As a direct consequence, a broad range of genotypes reflecting local diversity of the pathogen is required when screening breeding material at any given location. Moreover, in an epidemiological perspective, it would seem important to control plant material exchanges in order to avoid homogenization of genetically differentiated subpopulations of the pathogen. However, the selectively neutral markers revealed here, while permitting a good assessment of pathogen population structure, do not necessarily provide information about pathotype variation. No evidence for selection by resistance loci was detected and, consequently, no pattern of differentiation due to host or symptom susceptibilities among the subpopulations and the populations sampled from different host clones or symptoms (leaf spots or cankers) was observed. As shown for other pathosystems, such as *Magnaporthe grisea* and rice (13), the possible effects of cultivar selection may be demonstrated by the predominance of a single clonal lineage in putatively recombining and genetically diverse populations. In our study, no single or few *S. musiva* haplotypes predominated and no subpopulation sampled on either *P. deltoides* or one particular hybrid poplar clone was dominated by a single clonal lineage. Finally, in order to determine whether variation in pathogenicity also indicates the potential for local host adaptation, a combination of approaches using both neutral genetic markers and pathogenicity assays might be ideal.

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