Population Structure of Seedborne *Phaeosphaeria nodorum* on New York Wheat

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ABSTRACT

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Population genetic and epidemiological studies have resulted in different hypotheses about the predominant source of primary inoculum in the *Phaeosphaeria nodorum*–wheat pathosystem (i.e., sexually derived, windborne ascospores versus asexual or seedborne inoculum). We examined the genetic structure of seedborne populations of *P. nodorum* as a further step toward evaluating the hypothesis that seedborne inoculum is an important contributor to foliar epidemics in New York's rotational wheat fields. In all, 330 seedborne isolates from seven field populations were genotyped at 155 amplified fragment length polymorphism loci. Seedborne populations possessed high levels of genotypic diversity, with virtually every isolate (326/330) having a unique haplotype. As in previ-

ous population genetic studies of *P. nodorum*, we found low levels of gametic disequilibrium, although we could reject the null hypothesis of random mating with the index of association test for two populations. Thus, genotypically diverse and seemingly panmictic populations of *P. nodorum* that have been observed in wheat foliage could be derived from seedborne primary inoculum. Although sexual reproduction and recombination may contribute to the diversity of foliar populations of *P. nodorum*, population genetic data do not rule out seed as a source of primary inoculum. Further experimentation will be needed to determine definitively the relative importance of windborne ascospores and seedborne asexual inoculum in epidemics of *Stagonospora nodorum* blotch in New York.

Additional keyword: inoculum sources.

In recent years, the application of population genetic analyses to the study of pathogen populations has provided numerous and previously unattainable insights into pathogen biology (2,9,32,37,48). Despite the ease with which population genetic data may be obtained and analyzed, the interpretation of results in an ecological or epidemiological context—as opposed to a purely genetic context—is not always simple and straightforward. In particular, high genotypic diversity and random association of alleles at different loci (i.e., lack of gametic or linkage disequilibrium) do not necessarily mean that sexual reproduction occurs frequently or that sexually produced propagules (e.g., ascospores) are the prevalent source of inoculum (9,35). In theory, only a small amount of recombination is necessary to maintain genotypic diversity and the appearance of random mating (8,10,20,30). Moreover, nonrecombining populations, if sufficiently large, can maintain high levels of genetic diversity from past recombinant events because diversity will not be lost quickly through drift (17).

Population genetic data for *Phaeosphaeria nodorum* (E. Müller) Hedjadroude (anamorph: *Stagonospora nodorum* (Berk.) Castellani & E. G. Germano) on wheat have been interpreted in an epidemiological context. A genetic structure consistent with a diverse and randomly mating population was found in mostly foliar populations of *P. nodorum* and was used to conclude that ascospores are the main source of primary inoculum for foliar epidemics (22,23,33). Although pseudothecia of *P. nodorum* have been found in Australia (5), western Canada (14), and Europe (3,39), they have not been seen in eastern North American wheat-production areas (12,38; G. C. Bergstrom, unpublished data). Al-

though ascospores may be an important source of primary inoculum in some geographic regions, we question their predominance in the rotational cropping systems for wheat practiced in much of eastern North America.

Several lines of epidemiological evidence support the hypothesis that infected seed is a significant source of inoculum. First, the ability of *P. nodorum* to infect seed has been well documented (11,18,27) and significant levels of seed infection in commercial seed lots is common (11,18,44). Second, transmission from naturally infected seed to coleoptiles and primary leaves is efficient over the broad range of temperatures that would be encountered in the field (45). Third, significant transmission occurs even in seedlings derived from seed treated with fungicide (21). Fourth, levels of seed infection are positively correlated with disease severity in foliage (4,26,46). Finally, genetically marked seedborne isolates were recovered from foliar and spike infections, demonstrating that seedborne *P. nodorum* can serve as a source of primary inoculum for foliar epidemics and then be transmitted to the next generation of infected seed (46).

The apparent discrepancy between population genetic and epidemiological evidence for the different sources of primary inoculum of *P. nodorum* could be reconciled by considering several hypotheses. First, for the most part, population genetic and epidemiological studies have been conducted in disjunct geographic regions. Geographic differences in the biology and epidemiology of *P. nodorum* due to various unknown biological or environmental influences may account for conflicting inferences from genetic and epidemiological studies. Second, high levels of genetic diversity may be present in populations infecting seed. These seedborne populations, serving as sources of primary inoculum, could give rise to foliar epidemics caused by equally diverse populations. The diverse foliar populations then could be transmitted to the next generation of seed, with the cycle repeating itself to maintain continuous populations of *P. nodorum* on sub-

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sequent generations of seed. Third, both ascospores and infected seed could contribute primary inoculum, but the former have not been detected in some regions.

Our overall goal was to test the hypothesis that seedborne populations of *P. nodorum*, which are transmitted asexually to wheat seedlings, are genetically diverse with a panmictic population structure. To avoid differences in biology or epidemiology that may be associated with different geographic regions, we tested this hypothesis on seedborne populations in New York, where extensive epidemiological studies have implicated infected seed as an important source of primary inoculum (21,44–47). Specifically, our objectives for seedborne populations were to (i) estimate gene and haplotype diversity, (ii) analyze the multilocus structure of populations to test the hypothesis of random mating, and (iii) test for genetic differentiation among geographic locations and between cultivars.

MATERIALS AND METHODS

Sampling. We sampled seven soft white winter wheat fields for *P. nodorum* to examine population structure and to look for differentiation among cultivars and locations. In July 2002, a field each of cvs. Caledonia and Harus was sampled near harvest from three New York certified seed-producing farms (farms 1 to 3) in three locations (Fig. 1A to C). The two cultivars were grown within 8 km of each other at each of the locations, and the locations were separated from each other by 80 to 150 km. Fields were sampled by collecting spikes from 36 to 50 1-m² sites within each field. Sites were separated from each other by 5 m to reduce the number of clones collected from splash-dispersed pycnidiospores. Because the three farms produced certified seed, the two cultivars each shared common seed sources (i.e., two foundation producers in different states had grown the two different cultivars for distribution to certified seed producers). Spikes from an additional field of cv. Harus also were sampled from location A, farm 4 (Fig. 1), but with a minimum of 2.5 m between the collection sites.

For a preliminary comparison of seed-derived populations to a foliar population, we examined a small sample of foliar isolates collected previously (6). Thirty-three foliar isolates of *P. nodorum* had been collected in spring 2000 from the Robert Musgrave Research Farm in Aurora, NY (Fig. 1D, farm 5). Samples were taken on 2 days from 24 sites in a single field, with a minimum of 10 m between each site.

Isolation, culturing, and DNA extraction. *P. nodorum* was isolated from wheat spikes by selecting seed from symptomatic glumes and plating them on *S. nodorum* agar for wheat (SNAW) (29) after surface sterilization. Plugs of *P. nodorum*-like isolates were transferred to V8 juice agar (200 ml of V8 juice, 3 g of CaCO₃, and 15 g of agar per 800 ml of distilled water) and placed under near-UV light to induce sporulation. After approximately 9 days, single-spore cultures were obtained from mature *P. nodorum* pycnidia by plating a dilution of spores onto SNAW and, after 24 h, picking out a single germinating conidium onto V8 agar with an insect pin.

For DNA extraction, mycelia were grown on Czapek-Dox agar (Difco Laboratories, Detroit) overlain with sterile cellophane discs. Harvested mycelia were lyophilized and ground to a powder with a sterile plastic pestle before DNA extraction with Qiagen's DNeasy Plant Mini-kit (Qiagen, Chatsworth, CA).

Amplified fragment length polymorphism procedure. The basic amplified fragment length polymorphism (AFLP) protocol (50), adapted for use with fluorescently labeled primers (55), was followed. Two AFLP primer pairs were used: *Eco*RI-C/*Mse*I-CG and *Eco*RI-C/*Mse*I-CT (*Eco*RI-C: 5'GACTGCGTACCAATTCC; *Mse*I-Cx: 5'GATGAGTCCCTGAGTAACx). The *Eco*RI primer was 5' end labeled with 6-FAM fluorescent dye. Polymerase chain reaction (PCR) products were run on an ABI 3730 capillary

machine (Applied Biosystems, Foster City, CA); unambiguous loci were identified with the program Genemapper (Applied Biosystems) and binned using a macro program (C. Harjes, unpublished data). Chromatograms also were checked visually.

The reproducibility of the AFLP data was measured previously by repeating the entire procedure using two replicate growth conditions and DNA extractions on two *P. nodorum* isolates (6). Three PCR reactions were performed per replicate and an estimated AFLP error rate of <1% was found within replications.

Markers with simple Mendelian inheritance are highly desirable for most population genetic analyses. Therefore, we obtained AFLP data from a single ordered tetrad (Sn26-1 × Sn27-1 [13], the only one available to us at this time) to identify loci inherited in a Mendelian manner (hereafter referred to as Mendelian loci).

Data analyses. Isolates obtained from one field were considered as a single population. Gene and genotypic diversity analyses were conducted with PowerMarker version 2.73 (K. Liu and S. Muse) and MultiLocus v1.32 (P.-M. Agapow and A. Burt, Imperial College, London), respectively. Gene diversity was calculated according to Weir (52) and genotypic diversity according to Nei (41). Overall population differentiation was measured by Weir's θ (52) with MultiLocus using 1,000 randomizations. We also estimated θ for differentiation between the two cultivars and among the three locations.

Measures of multilocus gametic disequilibrium (GD) were calculated and tested for significance with 1,000 randomizations in MultiLocus, using (i) proportion of phylogenetically compatible pairs of loci (15) (alternatively, two loci are incompatible if all four two-locus haplotypes are found within a population); (ii) index of association (I_A) (8,31); and (iii) an alternative measure of index of association ($\bar{r}_d$) that is less sensitive to the number of loci (1). Because loci with skewed frequencies have a disproportionate effect on GD estimates (7,8), GD analyses were conducted using loci with allele frequencies between 10 and 90%. In addition to the multilocus estimates of GD, we used χ^2 tests to assess the number of significant comparisons among all pairs of loci within each population. We generated a null distribution for significance testing by randomly permuting alleles at each locus, performing χ^2 tests, and determining the number of significant associations for all possible locus pairs. The probability of obtaining the observed number of significant associations or more under the null hypothesis of no association was obtained from 10,000 permutations.

Fig. 1. Sample locations of *Phaeosphaeria nodorum* from wheat fields in New York state. Locations A, B, and C are separated from each other by 80 to 150 km. The population collected from D is from foliar lesions. Details of locations (towns) and cultivars are in Table 1.

RESULTS

A total of 330 seedborne isolates was collected from 284 sites in the seven fields. We recovered two or more isolates of *P. nodorum* from 60 sites, and no isolates from 23 sites. We scored a total of 155 AFLP loci from the two primer pairs, of which 124 were polymorphic. From the tetrad, 17 Mendelian loci were identified; other AFLP loci were not detected or were monomorphic in the parents. We found 326 unique multilocus haplotypes among the 330 isolates; isolates with identical haplotypes were always found within the same 1-m² site. Because asexual reproduction of *P. nodorum* by rain-splashed pycnidiospores is the likely reason for sampling the same haplotype more than once, we analyzed only one isolate per clone and conducted GD and differentiation analyses on the clone-corrected sample ($n = 326$). We found 29 haplotypes among the 33 foliar isolates. Combined with seedborne isolates, 129 loci in all were polymorphic.

Genotypic and gene diversity. Genotypic diversity of seedborne and foliar populations was nearly at its maximum because few clonal haplotypes were found among isolates assayed (Table 1). Gene diversity averaged over all 155 loci, on the other hand, was 0.11 for all seedborne populations and 0.12 for the foliar population (Table 1).

Population differentiation. There was little evidence for population differentiation among the seedborne populations from seven fields or between cultivars, despite the fact that the location 1, farm 3 field had a different seed source than the other Harus fields (Table 2). In contrast, we detected significant differentiation among the three locations except when using the Mendelian subset of loci (Table 2). Between the seed and foliar isolates, we also found significant differentiation, but this comparison is confounded by the foliar isolates coming from a different location and year. Estimates of θ , however, were close to zero for all comparisons, indicating only weak differentiation, even when significantly greater than zero.

Gametic disequilibrium. We tested the hypothesis of random mating by estimating gametic disequilibrium. A preliminary analysis of GD between pairs of loci showed that six pairs were associated consistently across most populations. One locus from each of these pairs was arbitrarily removed from further analyses because of the possibility of physical linkage affecting GD estimates. After removing one locus from each putatively linked locus pair, we found between 30 and 39 loci with allele frequencies between 10 and 90% (Table 3). Because we found no

genetic differentiation among fields within a given location (data not shown), we combined samples within locations to obtain larger sample sizes for greater power in testing the significance of GD estimates (Table 3).

In testing for the proportion of phylogenetically compatible pairs of loci, we rejected the null hypothesis of random mating if there were fewer locus pairs with all four combinations of two-locus haplotypes than expected; this is equivalent to observing more compatible locus pairs than expected under random mating. Random mating was not rejected by this test in any of the seven seedborne populations or from the single foliar population. However, we rejected the random mating hypothesis for the combined data from location B ($P = 0.005$).

With the index of association test, we rejected the null hypothesis of random mating for two of the seven seed-derived populations: farm 1, Caledonia, with the intermediate-frequency

TABLE 2. Population differentiation in populations of *Phaeosphaeria nodorum* from wheat in New York estimated from amplified fragment length polymorphism data

Comparison	θ^a	P^a
Seedborne populations		
All loci	0.002	0.157
Intermediate ^b	0.002	0.190
Mendelian	<0.001	0.652
Cultivars ^c		
All loci	<0.001	0.571
Intermediate	<0.001	0.578
Mendelian	<0.001	0.434
Locations ^d		
All loci	0.005	0.004*
Intermediate	0.007	<0.001*
Mendelian	0.001	0.256
Seed versus foliar ^e		
All loci	0.055	<0.001*
Intermediate	<0.001	0.594
Mendelian	<0.001	0.538

^a Weir's estimate of F_{ST} for haploids (52); P values estimated with 1,000 randomizations (Multilocus version 1.32); * indicates significant at $P \leq 0.05$.

^b Intermediate loci with frequencies ≥ 10 or $\leq 90\%$.

^c Test of cultivar specialization: seed isolates from Harus versus seed isolates from Caledonia.

^d Test of geographic differentiation among the three locations from which the seed isolates were collected.

^e Seed isolates collected in 2002 compared with a single foliar population (Table 1) collected in 2000.

TABLE 1. Genotypic and gene diversity for populations of *Phaeosphaeria nodorum* sampled from wheat in New York estimated from amplified fragment length polymorphism (AFLP) data

Population				<i>n</i> ^b	No. of haplotypes	No. of polymorphic loci	Genotypic diversity ^c	Gene diversity ^d
Source	Location ^a	Farm	Cultivar					
Seed	A	1	Harus	47	46	82	0.999	0.113
	A	1	Caledonia	49	49	77	1.0	0.112
	A	4	Harus	38	38	71	1.0	0.108
	B	2	Harus	44	43	76	0.998	0.111
	B	2	Caledonia	49	47	85	0.999	0.110
	C	3	Harus	53	53	82	1.0	0.110
	C	3	Caledonia	50	50	79	1.0	0.113
	Total seedborne	...	...	330	...	...	...	0.113
Foliar ^e	D	5	Caledonia	33	29	74	0.992	0.123

^a Locations in New York: A = Albion, B = Groveland, C = Seneca Falls, and D = Aurora.

^b Number of isolates.

^c Genotypic diversity is estimated as $\frac{n}{n-1} \left(1 - \sum_i p_i^2 \right)$, where p_i is the frequency of the i th haplotype and n is the number of individuals sampled (41).

^d Weir's (52) biased estimator of gene diversity at the l th locus, $\hat{D}_l = \sum_{u=1}^k p_{lu}^2$, calculated for all 155 AFLP loci and averaged, where p_{lu} is the frequency of the alleles u at the l th locus, and n is the number of individuals sampled.

^e Subset of foliar isolates collected as part of a previous study (6).

loci, and farm 2, Caledonia with the Mendelian loci (Table 2). The values of I_A and $\bar{r}_d$ for all populations were low. Combined data from each location produced no significant values of the index of association. The foliar population exhibited levels of I_A and $\bar{r}_d$ similar to that of the seed-derived populations, but neither were significantly different from 0.

We also estimated GD in each population by using pairwise tests among loci (Table 2) after removing locus pairs that were consistently significantly associated across populations in preliminary analyses (see above). The number of significant pairwise comparisons ranged from 0 to 7%, but none of the populations had more significantly associated locus pairs than expected at random.

DISCUSSION

Our results show that populations of *P. nodorum* from wheat seed have high genotypic diversity, significant but weak genetic differentiation between locations, and low levels of gametic disequilibrium (with few significant associations). In these respects, seedborne populations of *P. nodorum* from New York are quite similar to foliar populations studied in other regions. Previous studies (22,23,33) found essentially the same population structure and the authors concluded that *P. nodorum* frequently reproduces sexually and that ascospores are the dominant source of primary inoculum initiating foliar epidemics on wheat. However, our findings of essentially the same population structure in seedborne populations mean that—from a genetic perspective—seed are an equally plausible source of primary inoculum as ascospores. The seedborne inoculum hypothesis cannot be rejected because of strong epidemiological evidence (4,26,46) and, now, genetic evidence for high levels of diversity and panmictic population structure.

We propose the following scenario to explain the genetic and epidemiological observations in this system. We hypothesize that the high levels of genotypic diversity found in foliar populations

are retained in seed populations, which then contribute primary inoculum asexually to subsequent foliar epidemics. This hypothesis previously was rejected (22) because of expectations of finding more evidence of clonality if foliar epidemics were initiated from infected seed—for example, finding the same haplotypes distributed more widely over a field. Furthermore, it was thought that genetic drift and selection would decrease genetic diversity over time in asexual seedborne populations. However, seedborne populations comprise highly diverse haplotypes. Even if multiple seed are infected by the same haplotypes, routine handling of seed prior to planting is likely to physically mix *P. nodorum* haplotypes. With high genotypic diversity and mixing of seed, very large numbers of primary lesions would need to be sampled at a field scale to find the same haplotypes multiple times.

Our proposed scenario of asexual seedborne inoculum and the hypothesis that sexual reproduction occurs regularly in *P. nodorum* populations are not mutually exclusive. Ascospores produced on wheat debris (3,5,14) and dispersing to wheat also could be a source of primary inoculum. However, further epidemiological studies are needed to address the quantitative contribution of ascospores as primary inoculum. The problem with inferring epidemiology from population genetic data is that only a small amount of recombination may be sufficient to give a population the appearance of random mating (8,10,20,30). Lack of GD in *P. nodorum* only means that recombination has occurred sometime in the past, but does not tell anything about when or how often it occurs. In the *Ascochyta rabei*–chickpea pathosystem, ≈ 40 generations of enforced experimental asexual reproduction in a field population failed to erode evidence of past recombination (42). Therefore, the failure to find significant GD cannot be interpreted as evidence that sex is the dominant mode of reproduction (9,35,36) and that sexual propagules are the dominant source of inoculum.

Even if recombination occurs only rarely, large effective populations can maintain genetic diversity generated by previously recombinant ancestors (17). Given favorable weather conditions,

TABLE 3. Gametic disequilibrium for populations of *Phaeosphaeria nodorum* from wheat in New York estimated from amplified fragment length polymorphism data

Population						Compatible locus pairs ^c			Index of association ^d			Pairwise comparisons ^e	
Source	Location	Farm	Cultivar	n^a	Loci ^b	Observed	Expected	P	I_A	$\bar{r}_d$	P	No. (%)	P
Seed	A	1	Harus	46	32	0.083	0.077	0.313	0.014	<0.001	0.398	25/496 (5)	0.304
	...	...	...	...	10 (M)	0.022	0.039	0.84	0.024	0.003	0.331	3/45 (7)	0.196
Seed	A	1	Caledonia	49	36	0.100	0.111	0.862	0.208	0.006	0.012*	31/630 (5)	0.298
	...	...	...	...	13 (M)	0.128	0.105	0.27	0.132	0.011	0.056	5/78 (6)	0.115
Seed	A	4	Harus	38	32	0.079	0.089	0.837	0.092	0.003	0.169	16/496 (3)	0.927
	...	...	...	...	11 (M)	0.109	0.099	0.466	<0.001	<0.001	0.591	2/55 (4)	0.505
Seed	A	All A ^f	...	124	32	0.008	0.004	0.163	0.014	<0.001	0.348	...	...
Seed	B	2	Harus	43	30	0.078	0.071	0.299	<0.001	<0.001	0.98	17/435 (4)	0.730
	...	...	...	...	9 (M)	0	0.016	1	<0.001	<0.001	0.66	0/36 (0)	0.846
Seed	B	2	Caledonia	47	31	0.095	0.083	0.179	<0.001	<0.001	0.893	20/465 (4)	0.585
	...	...	...	...	9 (M)	0.028	0.017	0.465	0.140	0.018	0.017*	3/36 (8)	0.114
Seed	B	All B	...	90	30	0.037	0.017	0.005*	<0.001	<0.001	0.986	...	...
Seed	C	3	Harus	53	33	0.066	0.069	0.622	<0.001	<0.001	0.662	21/528 (4)	0.729
	...	...	...	...	11 (M)	0.036	0.041	0.699	0.052	0.005	0.209	4/55 (7)	0.131
Seed	C	3	Caledonia	50	33	0.063	0.061	0.484	0.110	0.003	0.084	25/528 (5)	0.459
	...	...	...	...	11 (M)	0	0.012	1	0.040	0.004	0.223	4/55 (7)	0.279
Seed	C	All C	...	103	33	0.017	0.014	0.302	0.002	<0.001	0.463	...	...
Foliar	D	5	Caledonia	29	39	0.135	0.163	0.991	0.058	0.002	0.303	38/741 (5)	0.338
	...	...	...	...	15 (M)	0.171	0.198	0.821	0.106	0.008	0.139	5/105 (5)	0.354
Total	...	...	...	355	...	...	...	...	...	...	...	...	...

^a Clone-corrected sample size (i.e., number of unique haplotypes).

^b Number and type of loci. Only loci with frequencies between 10 and 90% are included and one locus of putatively linked locus pairs was removed from gametic disequilibrium analyses. Loci confirmed to have Mendelian segregation in an ordered tetrad are denoted by (M).

^c Proportion of compatible locus pairs (1); P values were estimated from 1,000 randomizations; * indicates significant at $P \leq 0.05$.

^d Index of association (I_A) (8,31) and $\bar{r}_d$, a modified index of association (1); P values were estimated from 1,000 randomizations and are identical for I_A and $\bar{r}_d$.

^e Number of significant pairwise gametic disequilibrium comparisons for loci. Numbers in parentheses are the percentage of locus pairs that have significant gametic disequilibrium. P values were estimated from 10,000 permutations to generate a null distribution.

^f All A, B, or C farms = all fields within a given location were combined to increase sample size because no significant genetic differentiation was detected (data not shown).

agricultural ecosystems often are ideal environments for explosions in pathogen populations. The high planting densities for wheat (typically in the range of 200 to 500 seed m⁻² in eastern North America) may mitigate any bottlenecks created from low incidences of seed infection in seed lots grown in unfavorable disease environments. With seed lots containing 0.5% infected seed, for example, one potentially still could find an infected plant in each square meter of the field. The low gene diversity (as opposed to genotypic diversity) we found in *P. nodorum* may support this hypothesis; low levels of gene diversity have been used to infer past population expansions (40) because newly generated alleles have a greater chance of persisting in large populations. Although previous studies using restriction fragment length polymorphisms (RFLPs) in *P. nodorum* found high levels of gene diversity (22,23), this marked difference to our results may be due to the fact that the RFLP probes had been selected for detecting polymorphisms and, thus, were not a random sample of loci in the genome (28). Furthermore, *P. nodorum* populations, either in this study or previous ones (23), are not strongly genetically differentiated. Therefore, the effective population size may be large, with drift acting only weakly to reduce genetic variation.

Our results begin to reconcile the discrepancy between genetic and epidemiological evidence for sources of primary inoculum in this pathosystem. The hypothesis of a seedborne source of primary inoculum cannot be rejected by population genetic data because seedborne populations are characterized by comparable high genotypic diversity and low levels of GD as observed in foliar populations. The importance of infected seed relative to other potential sources in this pathosystem, however, is unclear. Other potential sources of primary inoculum include asexual or sexual spores from within-field crop debris (16,19) or alternate gramineous hosts (24,51,53), and windborne ascospores from crop debris in surrounding or more distant fields (3,5,34,43). *P. nodorum* appears to exhibit sufficient host specialization to rule out alternate gramineous hosts as significant sources of inoculum (24,25). *P. nodorum* does not survive long in soil environments (49); therefore, tillage, as typically practiced in the eastern United States, is considered an effective means of reducing the production of sexual and asexual inoculum from infected wheat debris (16). For these reasons, we believe that alternate hosts and within-field wheat debris are not likely to be as important as infected seed or windborne ascospores as inoculum sources in rotational cropping systems. *P. nodorum* has the potential to reproduce sexually because both mating types, required for sexual reproduction in this heterothallic fungus, are present in New York populations (6). However, windborne ascospore inoculum coming from other fields would result in spatial disease patterns similar to those of seedborne inoculum. Infected seed may prove to be the dominant source of primary inoculum early in the growing season, with windborne ascospores contributing inoculum later. This hypothesis would explain the observed genetic structure while accounting for the epidemiological evidence supporting seed as an important source. The small but significant genetic differentiation we detected among locations supports the hypothesis of relatively unrestricted migration between locations, which could be accounted for by either airborne ascospore inoculum or movement of inoculum on seed. Because it is difficult to distinguish the contribution of these two sources using either traditional epidemiological approaches or population genetic analyses alone, we currently are tracking marked isolates (54) in the field to determine experimentally the relative importance of each as an inoculum source.

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