Diversity of Virulence Within and Among Populations of *Puccinia recondita* f. sp. *tritici* in Different Areas of the United States

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ABSTRACT

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Data from surveys of pathogenic races of *Puccinia recondita* f. sp. *tritici* from 1988 to 1990 were analyzed to compare phenotypic diversity within and among pathogen populations in eight areas of the United States. Collections from nurseries were significantly more diverse than those from commercial fields in five of seven areas. Populations from the southern, central, and northern Great Plains were phenotypically similar. The population from California and other southwestern states was distinctly different from populations from all other areas. Leaf rust pathogen populations in the Pacific Northwest were no more similar to those of the Great Plains than to populations in areas east of the Mississippi River. The midwestern, northeastern, and southeastern populations were sufficiently distinct to indicate local sources of primary inoculum with limited exchange among those areas.

Additional keywords: epidemiology, *Triticum aestivum*, wheat leaf rust

The principal objectives of annual surveys of pathogenic races of cereal rust fungi are to estimate the relative prevalence and distribution of known races of the pathogens and to discover new and potentially dangerous races soon after they appear (13). The second objective can be met most effectively by sampling rust pathogen isolates from nurseries, which contain diverse host lines, including those with resistance genes that have not yet been used in commercial production. The first objective, however, may be better met by collecting isolates from fields of commercial cultivars. This should provide a less biased assessment of the actual composition of the pathogen population in a given region. Unbiased assessments of pathogen populations are important for analyzing the diversity of pathogen populations and for characterizing evolutionary changes in them (1-4,12).

For the past 3 yr, we have collected and tested the virulence of isolates of the wheat (*Triticum aestivum* L.) leaf rust fungus (*Puccinia recondita* Roberge ex. Desmaz. f. sp. *tritici* (Eriks. & E. Henn.) D. M. Henderson) from eight areas of the United States. Isolates were collected both from breeders' nurseries and from commercial fields. The results of these surveys have been reported in the manner of previous surveys of cereal rust pathogen races (7). In this article, we have further analyzed the data with two main objectives. The first was to compare genetic diversity among isolates collected from nurseries with that among isolates from fields in each of eight areas of the United States. The second objective was to assess the degree of similarity of wheat leaf rust pathogen populations across those areas as an indication of whether they constitute distinct epidemiological units. Finally, we also compared analyses to determine how much the assessment of genetic similarity among populations from different areas depends on whether the isolates were collected from fields or from nurseries.

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MATERIALS AND METHODS

Methods used in collecting and testing the isolates of *P. r. tritici* that provided the data for our analyses were described by Long et al (7). Isolates were grouped according to collection source in eight agroecological areas (Fig. 1). These are characterized as follows: area 1 = mainly soft red winter wheats adapted to the southern climate; areas 2 and 3 = mostly soft red and white winter wheats adapted to the northern climate, with the two areas separated by geographical features; area 4 = primarily hard red winter wheat with a mixture of other types; area 5 = hard red winter wheat; area 6 = primarily hard red spring and durum wheats with some hard red winter wheat; area 7 = spring wheats planted in late fall; and area 8 = primarily soft white winter wheats with a mixture of other types. Wheat cultivars grown in area 6 have the most resistance to leaf rust and those in areas 1 and 8 have the least. On average, wheat cultivars grown in areas 3 and 4 have better resistance than those in areas 2, 5, and 7, although cultivars commonly grown in each of these areas range from susceptible to resistant.

Isolates collected in 1988, 1989, and 1990 in each area were combined for analyses of diversity, but isolates from nurseries and fields were kept separate for comparison. Nursery collections came mostly from small plot cultivar trials and advanced germ plasm lines. Isolates from trap plots susceptible to leaf rust in area 6 were included among the nursery collections. Isolates from trap plots made up 17% of the nursery isolates from area 6. For areas 1-6, there were 71-206 field isolates and 112-771 nursery isolates per area over the 3 yr (7). Areas 7 and 8 had eight and one field isolate, respectively, and 78 and 16 nursery isolates.

Although cultivars and lines in nurseries of each area generally resemble cultivars grown commercially in the area, nurseries contain a wider range of variation in both agronomic traits and resistance genotypes. Some overlap occurs among nurseries in different areas of the United States. For example, soft red winter wheat cultivars adapted to area 3 are often included in the parentage of breeding lines in area 1. Some cultivars from area 5 are included in cultivar trials in area 4, and vice versa. Cultivars in areas 7 and 8 are more distinct. Those in area 7 are mostly derived from CIMMYT breeding lines. Cultivars in area 8 are not widely adapted to other areas and are rarely found in nurseries outside area 8.

Data analysis. Race designations for isolates were based on reactions on the standard set of 12 isolines with different single genes for leaf rust resistance (6) plus two additional isolines with leaf rust resistance genes *Lr*10 and *Lr*18. The Gleason and Shannon indexes of genetic diversity (2) were calculated from occurrence and frequency of races in each area.

The Gleason index (H_g), which reflects the number of races per sample, is calculated as $H_g = (r - 1)/\log_e(N)$, in which r is the number of races in the sample and N is the total number of isolates in the sample. Thus, the Gleason index takes into account the principle that the probability of detecting a new

race with each new isolate declines as the total number of isolates in the sample increases. For example, $H_g = 5.4$ is obtained for a sample of 50 isolates with 22 races or a sample of 100 isolates with 26 races. However, the Gleason index is sensitive to sample size; a sample of 10 isolates with the maximum of 10 races has a Gleason index of only 3.9.

The Shannon index (H_w) reflects not only the number of races per sample but also the relative evenness of their frequencies (i.e., lack of dominance) in the sample. The Shannon index is calculated by the equation $H_w = -\Sigma p_i \log_e(p_i)$, in which p_i is the frequency of the ith race. Standard errors were calculated and paired Shannon indexes were compared statistically by the t test as described by Poole (9).

For comparison with the Gleason index, a sample of 100 isolates with 25 races each at a frequency of 0.04 had a Shannon index of 3.2. However, if 24 of the 25 races appeared only once (at a frequency of 0.01) and the 25th race occurred at a frequency of 0.76, $H_w = 1.4$. The maximum value of H_w for a sample of 100 isolates with 100 races is 4.6, and the minimum value for a sample with only one race is 0.0. For a more detailed discussion of the uses and relative merits of these and other indexes of diversity, see Groth and Roelfs (2).

Rogers' index and an adaptation of Nei's standard genetic distance were calculated for all possible paired comparisons of populations in areas 1–8. Rogers' index is a measure of the similarity of racial composition of populations and is calculated as $H_r = 0.5\ \Sigma|p_{Ai} - p_{Bi}|$, in which $|p_{Ai} - p_{Bi}|$ is the absolute value of the difference between the frequency of the ith race in population A and the ith race in population B (2). Rogers' index varies from 1.0 for populations with no shared races to 0.0 for populations that have identical races at identical frequencies.

Nei's (8) standard genetic distance (D) is based on frequencies of alleles at a number of genetic loci in the respective populations. It is calculated as $D = -\log_e I$, in which $I = J_{AB}/(J_A J_B)^{0.5}$. For dimorphic loci, $J_{AB} = (1/L)\Sigma[p_{Ai}p_{Bi} + (1 - p_{Ai})(1 - p_{Bi})]$; $J_A = (1/L)\Sigma[p_{Ai}^2 + (1 - p_{Ai})^2]$; and $J_B = (1/L)\Sigma[p_{Bi}^2 + (1 - p_{Bi})^2]$, in which L is the number of loci, p_{Ai} and $(1 - p_{Ai})$ are the frequencies of the two alleles at the ith locus in population A, and p_{Bi} and $(1 - p_{Bi})$ are the frequencies of the two alleles at the ith locus in population B. $I = 1$ when two populations have identical allele frequencies over all loci that are tested, and $I = 0$ when the two populations share no alleles. Thus, D can vary between 0 and infinity.

Because we did not know whether isolates of *P. r. tritici* were heterozygous or homozygous, we based our calculations of genetic distance on frequencies of phenotypes rather than alleles. That is, the frequency of virulence on the *Lr*1 isoline was treated as the frequency of the allele for virulence corresponding to the *Lr*1 resistance gene, and so on.

Rogers' index and the adapted Nei's distance were calculated both for field and nursery collections from the eight areas, with the exception of area 8, for which there was only one field isolate from 1988 to 1990. Phenograms based on Rogers' index and Nei's distance were computed by the unweighted pair-group method with arithmetic mean (5,8) to display relationships among populations.

RESULTS

Collections of *P. r. tritici* isolates from nurseries had significantly ($P < 0.05$) greater Shannon indexes of diversity than collections from fields in areas 1, 2, 6, and 7 (Fig. 2). In areas 3–5, the diversity of races within collections from nurseries did not differ significantly from that of collections from fields. There was only one field isolate from area 8, so no comparison could be made between field and nursery isolates of area 8. The Gleason index values, though not tested statistically, also were greater for nursery collections than field collections in areas

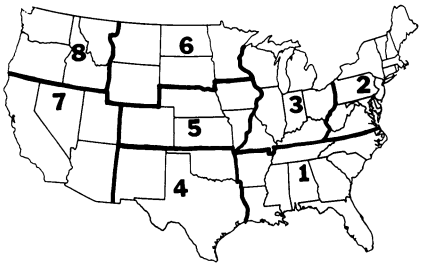

Fig. 1. Agroecological areas of wheat production in the United States. Diversity within and among populations of *Puccinia recondita* f. sp. *tritici* in these areas was analyzed. Area 1 = mainly southern-adapted soft red winter wheats; areas 2 and 3 = mostly northern-adapted soft red and white winter wheats; area 4 = a mixture of wheat types but primarily hard red winter; area 5 = hard red winter wheats; area 6 = mixed wheat types, but primarily hard red spring wheat and durum; area 7 = spring wheats planted in late fall; and area 8 = mixed wheat types, but primarily soft white winter types.

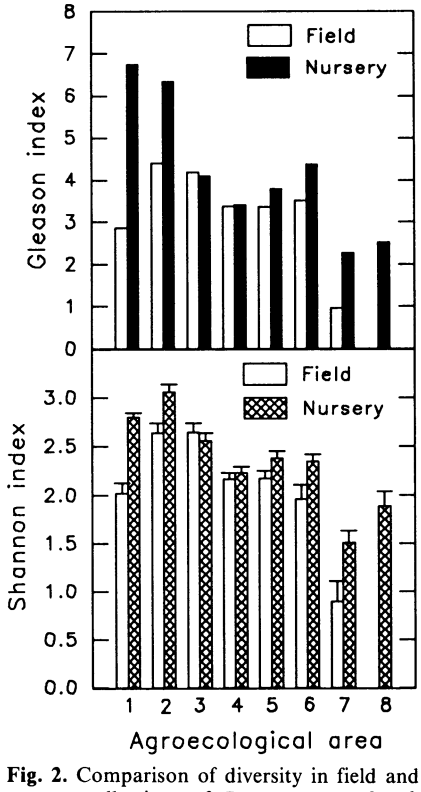

Fig. 2. Comparison of diversity in field and nursery collections of *Puccinia recondita* f. sp. *tritici* from areas 1–8 in the United States from 1988 to 1990. Two diversity indexes, Shannon and Gleason, were calculated; 95% confidence intervals are indicated for Shannon indexes. Diversity could not be determined for the field population in area 8 because only one field isolate was collected there.

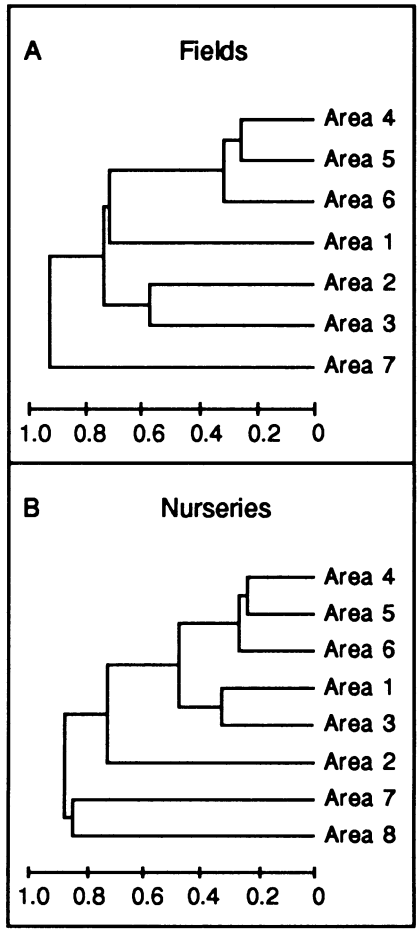

Fig. 3. Phenograms of similarities of racial composition based on Rogers' indexes among collections of *Puccinia recondita* f. sp. *tritici* from **(A)** fields in areas 1–7 and **(B)** nurseries in areas 1–8 of the United States during 1988–1990. There were too few isolates from fields in area 8 to allow comparison with other areas.

1, 2, 6, and 7, whereas they were more similar in areas 3–5.

The northeastern (area 2) and the midwestern (area 3) field populations were significantly ($P < 0.05$) more diverse than the field populations from all other areas as measured by the Shannon index. The southwestern population (area 7) was significantly less diverse than the other populations. Shannon indexes from areas 4 and 5 in the Great Plains were nearly identical. The field population in area 6 (northern plains) was slightly, though not significantly, less diverse than those in areas 4 and 5.

The phenogram for Rogers' indexes based on field collections (Fig. 3A) indicates that the collections from the three Great Plains areas (areas 4–6) were very similar. Collections from fields in the Midwest (area 3) were more similar to those in the Northeast (area 2) than to the field population in area 4, which has been suggested as an occasional source of inoculum for the Midwest (14). The collections from the Southwest (area 7) were distinctly different from those of all other areas.

The phenogram for Rogers' indexes based on nursery isolates (Fig. 3B) also shows the close similarity among areas 4–6 in the Great Plains. Rogers' indexes based on nursery collections indicated a closer relationship between the populations in areas 1 and 3 than was reflected in the Rogers' indexes calculated for field isolates. Rogers' indexes also indicated that nursery collections of *P. r. tritici* from the Northeast (area 2) were no more closely related to those of other areas east of the Mississippi River (areas 1 and 3) than they were to those of the Great Plains areas (Fig. 3B). Based on field collections, the population in area 2 was slightly more closely related to that in area 3 than to the populations in the Southeast (area 1) and the Great Plains (Fig. 3A).

Phenograms for the adapted Nei's standard genetic distance also showed the close similarities among collections from areas 4–6 in the Great Plains (Fig. 4). For both the field and nursery collections, Nei's genetic distance indicated that collections from areas 1 and 3 were closely related. The population in area 2 was less closely related to the other populations east of the Mississippi River. In fact, virulence frequencies from nurseries in area 2 were more similar to those from the Pacific Northwest (area 8) than to those from the Great Plains, the Midwest, or the Southeast.

The population of *P. r. tritici* in the Southwest (area 7) was distinctly different from all other populations of leaf rust. This was shown by both Rogers' index and Nei's distance and for both field and nursery collections (Figs. 3 and 4). Even the population in the Pacific Northwest (area 8) showed little similarity to that in area 7.

Nei's genetic distances among nursery collections from areas 1–7 were less than those among field collections (Fig. 4). Rogers' indexes, however, were relatively similar in comparisons among areas with either field or nursery collections (Fig. 3).

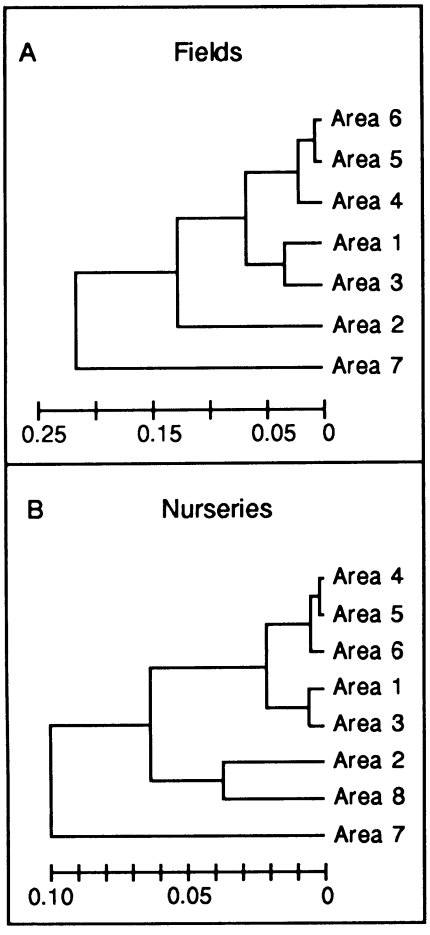

Fig. 4. Phenograms of similarities of virulence frequencies based on an adaptation of Nei's standard genetic distance among collections of *Puccinia recondita* f. sp. *tritici* from **(A)** fields in areas 1–7 and **(B)** nurseries in areas 1–8 of the United States during 1988–1990. There were too few isolates from fields in area 8 to allow comparison with other areas. Note the difference in scale between A and B.

DISCUSSION

Nursery collections of *P. r. tritici* contained significantly greater diversity than was found within field collections in four of seven areas. This illustrates that collections from nurseries are useful for detecting new races that might not appear among a similar number of isolates collected from fields. On the other hand, our results show that analysis of nursery collections will usually overestimate the genetic diversity in field populations of the pathogen.

Area 1 in the Southeast ranked relatively low among the agroecological areas with regard to diversity among field isolates as indicated by both the Shannon and Gleason indexes. In contrast, among nursery collections, area 1 showed greater diversity than all areas except area 2. This indicates that the Southeast provides a greater reservoir of rare races of *P. r. tritici* that can be detected in nurseries but do not appear often in field collections. The alternative hypothesis that nurseries in the Southeast somehow impose greater selection for pathogen diversity is unlikely. Wheat cultivars and breeding lines of the Southeast have less resistance to leaf rust than those of areas 2–7.

The Shannon index values found in this study are in the same range as the values 2.37 and 1.52 reported by Groth and Roelfs (1) from earlier studies of asexual populations of *P. r. tritici*. Kolmer (3) reported similar Shannon indexes of 2.36 and 1.81 for *P. r. tritici* in eastern Canada and the Prairie Provinces in 1988. This level of diversity is less than that for *P. coronata* Corda f. sp. *avenae* W. P. Fraser & Ledingham, the oat crown rust pathogen, but greater than that for *P. graminis* Pers.:Pers f. sp. *tritici* Eriks. & E. Henn., the wheat stem rust pathogen (1,2). The *P. graminis* populations, however, were more diverse before the eradication of barberry, their alternate host (1,2).

The high diversity of *P. r. tritici* populations in areas 2 and 3 suggests that there may be multiple overwintering sites in those areas with limited movement of inoculum among local subpopulations. Kolmer (3) compared the genetic diversity in populations of *P. r. tritici* in the three main wheat-growing areas of Canada in 1988. He found that the population in the Prairie Provinces was less diverse than those in the Eastern and Pacific areas. This is consistent with our conclusion that the populations of areas 2 and 3, which are nearest eastern Canada, are more diverse than those of the Great Plains, which provide inoculum for the Prairie Provinces. Kolmer (3) suggested that the greater diversity among isolates from eastern Canada may be attributable to the greater opportunities for *P. r. tritici* to overwinter there, as well as to the presence of susceptible cultivars that have not imposed strong selection pressure on the pathogen population in eastern Canada. He also found evidence of some migration of races from the Prairie Provinces to eastern Canada.

The greatest difference between Kolmer's (3) results and ours is that he found the population in the Pacific area to be most diverse, whereas we found that area 7 was the least diverse of the eight areas that we compared. Area 8, which is adjacent to the Canadian Pacific area, showed relatively low diversity among nursery isolates in our survey. Our survey included few isolates from area 8, so it may be that our estimate of diversity in the Pacific Northwest is too low. On the other hand, Kolmer's estimate of diversity in the Canadian

Pacific area may be inflated by his inclusion of southern Alberta in that area (4). Populations of *P. r. tritici* in southern Alberta share some virulence characteristics of both the population in British Columbia and that in the Prairie Provinces (J. A. Kolmer, *personal communication*).

Kolmer (3) showed that the racial compositions of the leaf rust populations in eastern Canada and in the Prairie Provinces originally were similar, but they diverged from 1945 to 1984. Kolmer attributed that divergence to selection pressures resulting from the introduction of genes for race-specific resistance into cultivars in the Prairie Provinces after 1937. After 1984, the two populations appeared to converge somewhat, but this is based on frequencies of virulence to only the four differential lines in the modified Unified Numeration set. When compared over the 12 differential lines in the standard North American set, the *P. r. tritici* populations of eastern Canada and the Prairie Provinces are still distinct (4). We have not compared racial compositions of populations in the various areas of the United States before 1988.

The similarity of racial compositions and frequencies of specific virulence genes among field collections from areas 4–6 in our study can be explained by the well-documented movement of urediniospores from the southern to the northern plains along the "*Puccinia* path" as the wheat crop develops during the growing season (10,14). The wheat types, and especially their genes for leaf rust resistance, differ considerably among areas 4–6. Therefore, the similarity of leaf rust populations in the Great Plains shows that selection imposed by host cultivars is not always the primary force determining the frequency of pathogenic races within geographical areas. When long-distance dispersal of urediniospores is a major factor in rust epidemiology, as it is in the Great Plains, the effects of migration of rust genotypes among areas may overwhelm differences in selection within the areas. The effect of long-distance dispersal in the Great Plains in recent years is particularly evident in the close similarity of frequencies of virulence to lines with leaf rust resistance genes *Lr*11, *Lr*24, and *Lr*26 in areas 4–6 and the Prairie Provinces of Canada (4,7). These genes have been used in hard red winter wheats in areas 4 and 5 but not in the hard red spring wheats of area 6 or the Prairie Provinces.

There was less similarity between field collections from areas 3 and 4 than between areas 4 and 6. This calls into question the postulated movement of urediniospores from the southern plains into the Midwest east of the Mississippi River. Racial compositions of populations from areas 1–3 east of the Mississippi River were more distinct from each other than were those in areas 4–6 in the Great Plains. This is in spite of the fact that many wheat cultivars grown in area 3 have also been important in area 1. Therefore, it appears that leaf rust epidemiology east of the Mississippi River depends more on local overwintering and local increase than in the Great Plains. Long-distance dispersal of urediniospores, which is a major factor in epidemiology in the Great Plains, appears to have much less importance east of the Mississippi River.

In comparing the field collections, it is interesting that the western populations (areas 7 and 8) were no more similar to the Great Plains populations than they were to those east of the Mississippi River. Kolmer (3) also found that the Canadian Pacific population of *P. r. tritici* differed as much from the prairie population as from the population in eastern Canada. Although there is evidence for occasional exchange of wheat stem rust races across the Rocky Mountains (11), such events must be rare. If they also occur with leaf rust, they have not made a noticeable impact on the population structure except in Alberta, where the leaf rust pathogen population shares characteristics of both the Canadian Pacific and Prairie regions.

Although the general patterns of phenograms based on Rogers' index and the adapted Nei's standard genetic distance were similar, there are two apparent differences. Based on Nei's genetic distance, field collections from the Southeast (area 1) were closely related to those from the Midwest (area 3), but no close relationship was evident with Rogers' index. Also, nursery collections from the Northeast (area 2) were related to those of the Northwest (area 8) by Nei's distance but not by Rogers' index.

Neither Rogers' index nor Nei's standard genetic distance is entirely satisfactory for assessing degrees of genetic differences among populations of asexually reproducing rust fungi. Rogers' index does not take into account the degree of similarity among pathogenic races. Each race is treated as an equally distinct phenotype even though one race may differ from another at only a single virulence locus. On the other hand, Nei's standard genetic distance does not take into account the high degree of linkage disequilibrium that typically exists in asexual populations of rust fungi. Populations with similar frequencies of virulence genes might consist of distinctly different races depending on the way in which the virulence genes are associated.

Until a better measure of genetic distance among asexual populations is developed, it is worthwhile to use both Rogers' index and Nei's genetic distance. When the two measures give similar results, one can be confident in the conclusions. When they give different results, some further interpretation is needed. The relatively small Nei's distance between field collections from areas 1 and 3 implies similar frequencies of specific virulence genes. The relatively large Rogers' index for areas 1 and 3 shows that the virulence genes are linked in different combinations in these two areas. Similarly, nursery collections from areas 2 and 8 share similar frequencies of virulence genes, but their races are different.

Within four of seven areas, the collections of *P. r. tritici* from nurseries had more races and less dominance of any single race (i.e., greater diversity) than did collections from fields. This greater diversity of races within nurseries was not reflected in comparisons among areas. Rogers' indexes for comparisons among areas based on nursery collections were generally similar to those based on field collections. On the other hand, Nei's distances among areas were smaller for nursery collections than for field collections.

Within areas, the greater diversity of host lines in nurseries than in growers' fields should lead to greater diversity among the pathogen isolates that are collected. On the other hand, this diversity within nurseries may be similar across areas. Thus, rare races that appear only in nurseries of one area also may appear in nurseries of other areas.

These differences between field and nursery collections illustrate the problems of using nursery collections when comparing diversity within pathogen populations or assessing genetic similarities among pathogen populations. Although nursery collections are valuable for detecting rare races, they may not accurately reflect the levels of diversity within and among the larger populations of the pathogen as they occur in growers' fields.

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