Comparison of *Puccinia graminis* f.sp. *tritici* from South America and Europe

B. D. McCallum\*†, A. P. Roelfs\b, L. J. Szabo\b and J. V. Groth\a

\aDepartment of Plant Pathology, University of Minnesota; and \bUSDA-ARS Cereal Disease Laboratory, St Paul, Minnesota, USA

Twenty isolates of *Puccinia graminis* f.sp. *tritici* from South America were compared with 19 from Europe using virulence, isozymes and random amplified polymorphic DNA (RAPD) markers. The isozyme and virulence patterns for these isolates were also compared with those of 11 isolates representative of the common race clusters in North America. All three types of marker showed a level of similarity between the South American and European isolates comparable with that between isolates from the same continent. The average similarity coefficients between the South American and European isolates were 0·65 for virulence, 0·67 for isozymes, and 0·70 for RAPD markers. Among South American isolates the values were 0·63 for virulence, 0·64 for isozymes and 0·72 for RAPDs. For the South American and European isolates, correlation between the similarity matrices based on RAPDs and on isozyme markers, respectively ($r = 0·52$), was higher than that between the RAPD and virulence matrices ($r = 0·32$) or between isozyme and virulence matrices ($r = 0·16$). The North American isolates had a comparable level of similarity for virulence and isozymes to both the South American and European populations. There was no clear distinction between the South American, North American and European isolates, which is consistent with the hypothesis that these populations may have had a common origin.

**Keywords:** introduction, population studies, stem rust, wheat

**Introduction**

Many organisms which cause plant disease have been moved between continents by human activity. Accidental introductions from Europe into America include the causal agents of Dutch elm disease, chestnut blight, and potato wart disease (Yarwood, 1983). Determining the migration and source of these pathogens can help, through quarantine, to limit further spread and assist in selecting sources of host resistance. Race QCCJ of *Puccinia graminis* f.sp. *tritici*, which has caused economic damage to barley in the North American Great Plains, was recently introduced to the area, most probably from the western side of the Rocky Mountains (Roelfs et al., 1997).

*Puccinia graminis* f.sp. *tritici* is believed to have originated in central Asia and eastern Africa, where *Berberis* and *Mahonia* have existed along with many secondary hosts in the Gramineae (Wahl et al., 1984), spread along with *Berberis* spp., and radiated onto grass hosts (Anikster & Wahl, 1979). The most important alternative host, *Berberis vulgaris* (barberry), is native to mountainous regions of southeastern Europe and Asia (Stakman, 1923). During the Middle Ages, monks brought the barberry into northern and central Europe (Stakman, 1923). Arthur (1929) believed that *P. graminis* f.sp. *tritici* was introduced into North America as very few susceptible indigenous hosts for it occur there. In North America wheat was grown as early as 1621 and wheat stem rust was probably present as early as 1660 (Bidwell & Falconer, 1925). *Puccinia graminis* f.sp. *tritici* was also probably introduced into South America, where Arthur (1923) noted the absence of infection on native species of barberry. Wheat was introduced into Brazil in 1534 but *P. graminis* f.sp. *tritici* was not reported until 1811, at which time it became a limiting factor in wheat production (Bastos Lagos 1983). Early immigrants could have introduced *P. graminis* f.sp. *tritici* into South America directly from Europe, or via North America, by the importation of urediospores, infected grasses or barberry plants.

Genetic distance analysis of populations can help to determine the migration or introduction of plant pathogenic fungi. Isozyme markers were used to complement virulence analysis of *P. graminis* f.sp. *tritici*...
to demonstrate that changes in the Australian population resulted from an exogenous introduction rather than mutations in the pre-existing population (Burdon et al., 1982). Isozyme markers and DNA fingerprinting were used to study the origin of A2 mating type isolates of *Phytophthora infestans* which recently appeared in Europe (Fry et al., 1992) and probably originated in Mexico, the only known region in which genotypes similar to the recently introduced strains occur.

To determine whether *P. graminis* f.sp. *tritici* could have been introduced into South America from Europe, either directly or via North America, isolates from South America were compared with isolates from Europe using virulence, isozyme and random amplified polymorphic DNA (RAPD) markers. The virulence and isozyme banding patterns of these isolates were also compared with those of isolates representing the common North American races published previously (Roelfs et al., 1997). If the South American population arose from a relatively recent introduction from Europe (1800s), a high degree of similarity to the current European population for nonselected markers would be expected. There should also be a degree of similarity between the North and South American populations and the European population since it is believed that the North American population was introduced from Europe.

**Materials and methods**

**Isolates**

To compare the South American and European *P. graminis* f.sp. *tritici* populations, 20 single pustule isolates from South America and 19 from Europe were analysed. The isolates were originally chosen because they represented a broad spectrum of the diversity for virulence on wheat that exists in South America and Europe. *Puccinia graminis* f.sp. *tritici* is an obligate parasite, so urediniospores produced from sporulating infections were used in both isozyme and RAPD analyses. To generate large quantities of urediniospores, each isolate was inoculated onto seedlings of the susceptible wheat cultivar McNair 701. Urediniospores were suspended in Soltrol oil (Phillips Petroleum, Bartlesville, OK, USA) and sprayed onto the plants which were then placed in a lighted humidity chamber overnight. The seedlings were grown in clear plastic chambers in the greenhouse to prevent contamination resulting from the movement of urediniospores. Urediniospores were harvested from sporulating pustules at 14 and 21 days after inoculation using a cyclone spore collector.

**Virulence markers**

Virulence determinations for all isolates were made by inoculating each onto the 16 single gene differential wheat lines used for the annual North American stem rust surveys (Roelfs et al., 1993). The differential host series consisted of wheat lines with the resistance genes Sr5, 6, 7b, 8a, 9a, 9b, 9d, 9e, 9g, 10, 11, 17, 21, 30, 36, and Tmp. Inoculated plants were grown in the greenhouse until sporulating pustules were evident. Infection type for each differential line/isolate combination was scored as either resistant (fleck; 0, 1 or 2 type pustules) or susceptible (3 or 4 type pustules) 10–12 days after inoculation. The pathotype for each isolate was determined using the nomenclature system of Roelfs & Martens (1988).

**Isozyme markers**

Horizontal starch gel electrophoresis was carried out on extracts of homogenized urediniospores as described previously (Burdon & Roelfs, 1985a). Approximately 50 mg of urediniospores were ground in a few drops of universal buffer (10 mM MES-potassium salt, 15% v/v ethylene glycol and 2% v/v 2-mercaptoethanol adjusted to pH 6.8 with HCl) using a mortar and pestle. Crude spore extracts were then absorbed onto paper wicks, which were loaded into 11% hydrolyzed starch gels. One continuous buffer system (histidine, pH 8.0) and two discontinuous buffer systems (borate, pH 7.8, and citrate, pH 8.0) were used for electrophoresis. The enzyme systems leucine aminopeptidase (LP: EC 3.4.11.1) and dihydrolipoamide reductase (synonym NADD diaphorase, NADHD: EC 1.6.4.3) were assayed on citrate gels, glutamate oxalate transaminase (synonym aspartate aminotransferase, GOT: EC 2.6.1.1), and glucosephosphate isomerase (synonym phosphoglucoisomerase, GPE: EC 5.3.1.9) were assayed on borate gels, and phosphoglucomutase (PGM: EC 2.7.5.1) was assayed on histidine gels. Isozyme banding patterns of the isolates were compared with the known banding pattern of the North American *P. graminis* f.sp. *tritici* isolates 90MN357-2 (race QCCJ) and 90MT28-2 (race TPMK) which were run in every gel (bands were assigned letters according to Burdon & Roelfs, 1986). All tests were repeated until unequivocal results were obtained.

**RAPD markers**

For RAPD analysis approximately 50 mg of urediniospores were germinated overnight by dusting the spores onto 500 mL of germination buffer (500 mL distilled and filtered H2O, 5 μL nonyl alcohol, 25 μL Tween 20, 0.5 mL ethanol) using a fine screen mesh. The germinated spores were collected the following morning and lyophilized before storing at −80°C. Then DNA was extracted from at least 1-g lyophilized germinating pads using phenol–chloroform extraction as described by Kubelik & Szabo (1995). The RAPD conditions were: 0.2 mM dNTPs, 10 mM Tris pH 8.3, 50 mM KCl, 3.5 mM MgCl2, 0.001% gelatin, 10 ng DNA, 0.4 μM primer, 0.5 Units Amplitaq (Perkin-Elmer, Norwalk, CT, USA), and water to a total of 25-μL reaction mixture which was overlaid with a drop of mineral oil. The thermocycler

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(MJ Research, Watertown, MA, USA) program was 94°C, 1 min; 36°C, 1 min; 72°C, 2 min repeated 40 times, then 72°C, 10 min final extension; and finally held at 4°C. The reaction products were run out the full length of 1X TBE gels containing 1.5% agarose. Various primers were screened to identify 10 primers that gave reliable amplification for all isolates; those used were Operon (Operon Technologies, Alameda, CA, USA) OPB-10, OPC-2, OPA-13, OPO-16, and the high G + C content primers CR1L7, 9, 11, 16, 14, and 22 (Kubelik & Szabo, 1995). Each primer isolate combination was repeated at least twice to confirm banding patterns.

<table>
<thead>
<tr>
<th>Country</th>
<th>Isolate</th>
<th>Pathotype</th>
<th>Ineffective resistance genes</th>
<th>LAP</th>
<th>NADHD</th>
<th>GOT</th>
<th>PGM-2</th>
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<td>RRTS</td>
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<td>AB</td>
<td>CC</td>
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<td>AB</td>
<td>CC</td>
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<td>21,9e,7b,9g,17,9a,9d,10,Tmp</td>
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<td>CC</td>
<td>BC</td>
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<tr>
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<td>BB</td>
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<tr>
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<td>TTRT</td>
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<td>CD</td>
<td>BB</td>
<td>CD</td>
<td>BB</td>
</tr>
</tbody>
</table>

Table 1: Pathotype and isozyme banding pattern for 20 South American, 19 European and 11 North American Puccinia graminis f.sp. tritici isolates

**a** Differentials and coding system as described by Roelfs & Martens (1988).

**b** Those differential genes for which the isolate possessed virulence.

**c** Data for the North American isolates are from Roelfs et al. (1997).
Data analysis

Data on the isozyme and RAPD banding patterns were recorded as the presence or absence of each scoreable band. Virulence was treated as a marker by scoring a susceptible reaction as the presence of a marker and a resistant reaction as the absence of a marker. The total numbers of polymorphic markers were: 16 for virulence, 19 for isozymes and 118 for RAPDs. The isozyme banding patterns and virulence reaction of 11 isolates representative of the North American race clusters published previously (Roelfs et al., 1997) were included in the analysis. The program NTSYS version 1.60 was used to analyse the data (Rohlf, 1990); each type of marker was analysed separately. Pairwise similarity comparisons between each of the isolates were made using the simple matching coefficient, $SMC = (a + d)/n$ (Nygaard et al., 1989), for the virulence and isozyme data, and the Dice coefficient, $2a/(2a + b + c)$, for the RAPD data. $a = [1,1]$, $b = [0,1]$, $c = [1,0]$, and $d = [0,0]$. Phenograms were constructed from the similarity matrices using the cluster program UPGMA (Rohlf, 1990). Bootstrap analysis was carried out on the similarity matrices using WinBoot v 2.02 (Yap & Nelson, 1996). The similarity matrices based on virulence, isozymes and RAPDs, respectively, were exported from NTSYS to a spreadsheet program to compute average similarity between the isolates.

Results

Virulence markers

Virulence and isozyme banding patterns for the isolates are shown in Table 1. Overall, there was a high degree of similarity between the South American, North American and European populations. The average SMC for virulence was 0·63 among South American isolates, 0·71 among European isolates, 0·72 among the North American isolates, 0·65 between the European and South American isolates, 0·65 between the North American isolates and European isolates (Table 2). Cluster analysis did not show that the South American, North American and European populations were distinctly different from each other or that there were any clearly distinct clusters of isolates (Fig. 1).

Isozyme markers

Isozyme markers also showed that the South American isolates were on average as closely related to the European isolates as they were to each other. The average SMC for isozymes was 0·64 among South American isolates, 0·67 among European isolates, 0·79 among North American isolates, 0·67 between European and South American isolates, 0·65 between

| Continent | Continent | Virulence* | Isozymes* | RAPDs*
<table>
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<tr>
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<td>0·64</td>
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<td>North America</td>
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<td>0·66</td>
<td>ND</td>
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<tr>
<td>Europe</td>
<td>North America</td>
<td>0·65</td>
<td>0·65</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Simple matching coefficient for virulence and isozymes and Dice coefficient for RAPDs.
†Data for the North American isolates are from Roelfs et al. (1997).
ND, no data.

Table 2 Average similarity within and between South American, Europe and North American isolates of Puccinia graminis f.sp. tritici based on virulence, isozymes and RAPD markers
European and North American isolates, and 0.66 between North and South American isolates (Table 2). None of the enzyme alleles found in this study was unique to South America. When considering all the isozyme systems together, two isozyme banding patterns were common to both South American and European isolates, two patterns were common to both South American and North American isolates, two were common to both European and North American isolates, and two patterns were common to isolates from all three continents (Fig. 2).

All isozyme alleles detected among the South American isolates were also found among the European isolates. The LAP 'A' allele was found in a group of South American isolates (SA-ARG1, SA-BOL1, SA-BOL2, SA-CHI2, SA-ECU1, SA-PAR1 and SA-PAR2) but was rare in the European isolates (EU-FRA2) and absent in those from the North American Great Plains. The GOT 'D' allele was found only in the South American isolates SA-BRA4 and SA-COL2, the European isolates EU-HUN1, EU-ITA2 and EU-TUR1 and the North American Great Plains race NA-RCRS.

RAPD markers
A total of 113 RAPD markers were used to assess relationships between the European and South American isolates. As with virulence and isozyme markers, the RAPD markers showed a level of similarity between the European and South American isolates comparable with that between isolates from the same continent (Fig. 3). The RAPD markers showed the clearest distinction of the isolates, based on continent of origin, of the three types of markers. The average Dice similarity coefficient was 0.578.

![Figure 2 Phenogram of simple matching similarity between South American, North American and European isolates of Puccinia graminis f.sp. tritici using isozyme markers. Branches with bootstrap values greater than 80% are indicated by bold lines.](image2)

![Figure 3 Phenogram of Dice similarity between South American and European isolates of Puccinia graminis f.sp. tritici using RAPD markers. Branches with bootstrap values greater than 80% are indicated by bold lines.](image3)
amongst the South American isolates was 0.72; the average among the European isolates was 0.73; and the average between South American and European isolates was 0.70 (Table 2). The high $G+C$ content primers produced more bands than the conventional primers. An example of the RAPD banding patterns produced for these isolates using CRL9 (a $G+C$ content primer) is shown in Fig. 4.

The RAPD and isozyme markers were more closely correlated with each other than with virulence markers. When only the South American and European isolates were included the correlations between the virulence similarity matrix and the RAPD matrix ($r = 0.32$) and between the virulence and isozyme matrices ($r = 0.16$) were lower than the correlation between the RAPD and isozyme matrices ($r = 0.52$).

Small clusters of isolates were very similar for each type of marker. In a few cases these clusters contained both South American and European isolates. These clusters included SA-ARG1, SA-BOL1, SA-BOL2, SA-CHI2, SA-ECU1, SA-PAR1, SA-PAR2 and EU-FRA2; EU-HUN1, EU-ITA2 and SA-COL2; and SA-BRA2, SA-BRA3, SA-CHI1, SA-CHI3, SA-ECU3 and EU-MOR3.

**Discussion**

The high degree of similarity between the South American, North American and European populations of *P. graminis f.sp. tritici* supports the hypothesis that they may have all arisen from a common ancestral population in Europe. Isolates from other parts of the world may also show a degree of similarity if compared in this way. There were many RAPD markers common to both South American and European isolates. Isolates from all three continents shared isozyme banding patterns and had virulence profiles that were similar. In general there appears to be a level of similarity between the European and South American populations consistent with the hypothesis that *P. graminis f.sp. tritici* was introduced from Europe into South America. Considering the isozyme and virulence similarities between the isolates from North and South America, it is also possible that at least a portion of the South American population could have originated from Europe, via North America. The late appearance of *P. graminis f.sp. tritici* in Brazil relative to the introduction of wheat (Bastos Lagos, 1983) also supports the hypothesis that this fungus was introduced into South America.
The investigation showed that RAPD and isozyme markers, which are presumed to be selectively neutral, grouped the isolates fairly similarly, although the correlation between the similarity matrices was low ($r = 0.52$). Groupings generated using virulence markers, however, differed somewhat from those generated by RAPD and isozyme markers. Many of the resistance genes in the host differentials used in this study have been used in commercial cultivars and therefore it is likely that the corresponding virulence genes have been selected for in the *P. graminis* f.sp. *tritici* populations. Chen et al. (1993) also found a low degree of association between RAPD and virulence patterns in North American populations of *Puccinia striiformis* f.sp. *tritici*. Similarly, there was no clear correlation between restriction fragment length polymorphism (RFLP) or RAPD markers and race phenotype in Australian isolates of *Uromyces appendiculatus* (Braithwaite et al., 1994).

The population structures of the South American and European populations of *P. graminis* f.sp. *tritici* appear to be much more diverse than the Great Plains population in North America. The North American Great Plains population is thought to be a clonal asexual population because of the absence of the alternative host, and is made up of 11 distinct clonal groups of races (Roelfs et al., 1997). Races within each group have exactly the same isozyme banding pattern and only differ from one another by one to three virulence genes. Each group has a distinct isozyme banding pattern and the groups differ from each other by an average of 11 virulence genes. In contrast, the North American Pacific Northwest population is thought to be a sexual population with no large clonal groups (Burdon & Roelfs, 1985b). The South American and European populations of *P. graminis* f.sp. *tritici* do not appear to be composed of small numbers of clonally reproducing groups, based on the data from this study; if they are composed of clonal groups, therefore the number of groups in both populations must be much higher than in the North American Great Plains population.

In Europe, stem rust inoculum has been reported to be blown in from northern Africa as well as arising locally from barberry in some locations (Hogg et al., 1969) and the sexual cycle may play a part in increasing diversity. *Berberis vulgaris* occurs in South America but was not considered an important inoculum source for cereal crops and overwintering was thought to be primarily in the uredinal stage (Leppik, 1961). The importance of the sexual stage in generating diversity in South America is not clear.

Since the eradication of barberry from the North American Great Plains region, *P. graminis* f.sp. *tritici* overwinters only in the uredinal stage. Overwintering is usually limited to the extreme southern portion of the United States because of freezing temperatures further north. This relatively small effective population creates a genetic bottleneck that may reduce diversity in the population. In South America and Europe, stem rust may have a much greater overwintering population because of larger and more geographically diverse areas that can support uredinal populations during the winter. The lack of any population bottleneck may also account for the greater degree of diversity in the South American and European *P. graminis* f.sp. *tritici* populations than in the North American Great Plains population.

The mode of entry of *P. graminis* f.sp. *tritici* into South America is still unknown. In North America it is thought that the rust was introduced when early colonists imported barberry (Yarmow, 1983). It may have travelled from Europe to South America via North America or from Europe directly, or both routes may have been used. It is possible that the rust came into South America on infected *Berberis vulgaris* plants but other sources such as teliospores on straw or uredinospores mixed with seed cannot be excluded.

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