# Virulence and Polymorphic DNA Relationships of *Puccinia striiformis* f. sp. *hordei* to Other Rusts

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## ABSTRACT

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The relationships of *Puccinia striiformis* f. sp. *hordei* (stripe rust of barley) to *P. striiformis* f. sp. *tritici* (stripe rust of wheat) and *P. striiformis* f. sp. *poae* (stripe rust of bluegrass) in North America were determined by virulence and random amplified polymorphic DNA (RAPD) analyses. Their relationships to *P. hordei* (leaf rust of barley), *P. recondita* f. sp. *tritici* (leaf rust of wheat), and *P. graminis* f. sp. *tritici* (stem rust of wheat) were determined by RAPD assay. All isolates of *P. striiformis* f. sp. *hordei* were virulent on some cultivars of wheat, and some isolates of *P. striiformis* f. sp. *tritici* were virulent on some cultivars of barley. Isolates of *P. striiformis* f. sp. *hordei* and *P. striiformis* f. sp. *tritici* did not infect bluegrass, and isolates of *P. striiformis* f. sp. *poae* did not infect barley and wheat cultivars. Of 31 barley genotypes tested, 9 were susceptible and 8 were resistant to all isolates of *P. striiformis* f. sp. *hordei*. The remaining 14 genotypes showed differential reactions. Fourteen races (pathotypes) of *P. striiformis* f. sp. *hordei* were identified using eleven selected barley genotypes based on avirulence/virulence patterns. A system for naming and designating races of *P. striiformis* f. sp. *hordei* was presented to distinguish races of *P. striiformis* f. sp. *hordei* from races of *P. striiformis* f. sp. *tritici*. RAPD analyses separated the isolates of *P. striiformis* f. sp. *hordei*, *P. striiformis* f. sp. *tritici*, and *P. striiformis* f. sp. *poae*. *P. striiformis* f. sp. *hordei* and *P. striiformis* f. sp. *tritici* were more closely related to each other than they were to *P. striiformis* f. sp. *poae*. Based on molecular variation, none of the three formae speciales of *P. striiformis* were more closely related to *P. hordei*, *P. recondita* f. sp. *tritici*, or *P. graminis* f. sp. *tritici*.

*Additional keywords*: *Hordeum vulgare*, host-pathogen interaction, *Poa pratensis*, specialization, *Triticum aestivum*, yellow rust.

Stripe rust (yellow rust) of barley (*Hordeum vulgare* L.), caused by *Puccinia striiformis* West. f. sp. *hordei*, was first differentiated from other stripe rusts by Eriksson in 1894 (9). Barley stripe rust has occurred in Europe and Asia for many years (41), and severe epidemics of the disease have been reported in northwestern and central European countries (11,14,41), India (17,18,33,37), Bangladesh (41), Nepal (16), China (32,45,46,47), and Japan (15,26). Since 1975, barley stripe rust has been a problem in several South American countries (8). Most recently, the disease has appeared in the United States (24,35). In South America, *P. striiformis* f. sp. *hordei* was first observed near Bogota, Colombia, in 1975 and was postulated by Dubin and Stubbs (8) to have been introduced from Europe. The disease spread southward as far as Argentina by 1982 (8) and northward to Mexico by 1987 (4). Yield losses of 30 to 70% occurred in these regions (8).

There were no reports of *P. striiformis* f. sp. *hordei* in the United States before 1991. However, *P. striiformis* f. sp. *tritici* (stripe rust of wheat) had occurred in North America since 1915 (3,12). A herbarium specimen collected in 1892 was determined to be *P. striiformis* (12), but the forma specialis was not determined. *P. striiformis* f. sp. *tritici* has been observed on barley in the Pacific Northwest of the United States but has never developed to intensities that would cause significant losses. Prior to 1991, stripe rust was observed on and collected from wild grasses, including *Hordeum* spp. and cultivated barley (13,22), but all isolates tested were *P. striiformis* f. sp. *tritici*.

Once barley stripe rust appeared in Mexico, there was concern that it would spread to the United States. The disease was first observed in a barley breeding nursery near Uvalde, TX, during April 1991 (24,35). By 1992, the disease had spread to Oklahoma, New Mexico, and Colorado (2,24). Brown et al. (2) reported severe losses of commercial barley in the San Luis Valley, CO, in 1992. Severe epidemics of barley stripe rust occurred in Arizona during the spring of 1993, and by August the disease was detected in southern Idaho and Montana. Also in 1993, stripe rust was observed on barley in California and was confirmed as *P. striiformis* f. sp. *hordei* (24). In 1994, severe stripe rust was observed in barley fields in Utah and California. In 1995, barley stripe rust was severe in plots at Logan, UT, and was observed in western Oregon and both western and eastern Washington. Samples from all of these states were confirmed to be *P. striiformis* f. sp. *hordei* when tested in the greenhouse. During July 1995, a severe barley stripe rust epidemic occurred in the major barley-growing region of northern California and southwestern Oregon. Thus, as of the summer of 1995, barley stripe rust has occurred in Texas, Oklahoma, New Mexico, Arizona, Colorado, Utah, California, Idaho, Montana, Oregon, and Washington.

The environment in the Pacific Northwest of the United States is highly favorable for survival of the pathogen and development

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of stripe rust epidemics; therefore, if not controlled, the disease has a high potential for causing major losses in barley production since cultivars currently grown in the region are susceptible.

The races (pathotypes) of the barley stripe rust pathogen in South American countries were identified by Dubin and Stubbs (8) as race 24 and variants of race 24. These races also were prevalent in Europe; therefore, they postulated that race 24 was introduced into Colombia from Europe. Marshall and Sutton (24) tested 273 barley stripe rust isolates collected in Texas, Oklahoma, and New Mexico from 1991 to 1994 and reported that 255 isolates were like race 24, 14 were like race 23, which is also in Europe (8,41), and 4 had a different virulence pattern.

In general, information about the characteristics of the population of *P. striiformis* f. sp. *hordei* and the relationships of *P. striiformis* f. sp. *hordei* to other rusts, especially other formae speciales of *P. striiformis*, is limited. The primary objectives of this study were to determine the variability of *P. striiformis* f. sp. *hordei* in the United States based on virulence and polymorphic DNA analyses and the relationships of *P. striiformis* f. sp. *hordei* to other rusts, especially *P. striiformis* f. sp. *tritici* and *P. striiformis* f. sp. *poae*.

TABLE 1. Origin of isolates of *Puccinia* spp.

| Isolate[a] | | Origin of collection | |
|---|---|---|---|
| Number | Designation | State | Host |
| *P. hordei* | | | |
| 1 | PH-1 | NC | Barley |
| 2 | PH-2 | ND | Barley |
| 3 | PH-3 | VA | Barley |
| *P. striiformis* f. sp. *hordei* | | | |
| 4 | PSH93TX-1 | TX | Sussex barley |
| 5 | PSH93TX-2 | TX | Sussex barley |
| 6 | PSH93TX-3 | TX | Sussex barley |
| 7 | PSH93TX-4 | TX | Sussex barley |
| 8 | PSH93AZ-1 | AZ | Barley |
| 9 | PSH93AZ-2 | AZ | Wheat |
| 10 | PSH93AZ-3 | AZ | Wheat |
| 11 | PSH93AZ-4 | AZ | Wheat |
| 12 | PSH93AZ-5 | AZ | Wheat |
| 13 | PSH93AZ-6 | AZ | Wheat |
| 14 | PSH93CO-1 | CO | Barley |
| 15 | PSH94CO-1 | CO | Barley |
| 16 | PSH94CO-2 | CO | Barley |
| 17 | PSH93ID-1 | ID | Shurler barley |
| 18 | PSH93ID-2 | ID | Nebar barley |
| 19 | PSH94ID-1 | ID | WB50 barley |
| 20 | PSH94ID-2 | ID | WB50 barley |
| 21 | PSH93MT-1 | MT | Barley |
| 22 | PSH94MT-1 | MT | Barley |
| A1 | PSH94ID-3 | ID | Nebar barley |
| A2 | PSH94CO-3 | CO | Fong Tien barley |
| A3 | PSH94CO-4 | CO | Crystal barley |
| *P. striiformis* f. sp. *poae* | | | |
| 23 | PSP92WA-1 | WA | Kentucky bluegrass |
| 24 | PSP91OR-1 | OR | Kentucky bluegrass |
| *P. striiformis* f. sp. *tritici* | | | |
| 25 | PST93-210-1 | MT | Composition barley cross |
| 26 | PST93-210-2 | MT | Composition barley cross |
| 27 | PST93-205 | WA | Russell barley |
| 28 | PST93-206 | WA | Russell barley |
| 29 | PSTCDL-1 | WA | Chinese 166 wheat |
| 30 | PSTCDL-21 | CA | Triticale |
| 31 | PSTCDL-45 | WA | Hyak wheat |
| *P. recondita* f. sp. *tritici* | | | |
| 32 | PRT93WA-1 | WA | Stephens wheat |
| *P. graminis* f. sp. *tritici* | | | |
| 33 | PGT91WA-1 | WA | Stephens wheat |

[a] Isolates A1, A2, and A3 were used in the virulence analyses but not in the random amplified polymorphic DNA analyses.

## MATERIALS AND METHODS

**Virulence analysis of rust isolates.** The geographic and host sources of urediospore isolates used in the study are shown in Table 1. The source of the rust collections was either harvested spores or sporulating uredia on leaves. The isolates were propagated on barley, wheat (*Triticum aestivum* L.), or bluegrass (*Poa pratensis* L.) in a greenhouse as previously described (5).

Virulence patterns of the stripe rust isolates (isolates 4 to 23, A1 to A3, and 25 to 31) were determined on seedlings of barley, wheat, and bluegrass as described by Line et al. (21,22). The virulence tests were repeated at least twice to confirm the consistency of the virulence patterns. Inoculated plants were kept in double plastic booths placed in a growth chamber in a greenhouse to minimize cross-contamination. Conditions for growing plants before and after inoculation and the method of recording infection types were as described by Chen and Line (5). Since stripe rust sometimes produces different infection types on the first and secondary leaves, seedlings were inoculated after the second leaves had developed, and infection types were recorded on both the first and second leaves 18 to 21 days after inoculation.

**DNA extraction and amplification.** DNA was extracted from urediospores as described by Chen et al. (6). Approximately 0.2 μg of DNA was obtained from 20 mg of urediospores. The DNA was dissolved in 200 μl of Tris-EDTA and kept at –20°C for later use. A working DNA solution was made by diluting the stock DNA solution to about 0.1 ng/μl. Twelve random primers (Operon Technologies, Alameda, CA) that previously were shown to amplify rust DNA (6) were chosen for analyzing the 33 isolates. The primers were OPA-19 (5′-CAAACGTCGG-3′), OPB-08 (5′-GTCCACACGG-3′), OPB-15 (5′-GGAGGGTGGT-3′), OPB-17 (5′-AGGGAACGAG-3′), OPC-08 (5′-TGGACCGGTG-3′), OPD-03 (5′-GTCGCCGTCA-3′), OPD-07 (5′-TTGGCACGGG-3′), OPD-13 (5′-GGGGTGACGA-3′), OPD-18 (5′-GAGAGCCAAC-3′), OPE-07 (5′-AGATGCAGCC-3′), OPF-02 (5′-GAGGATCCCT-3′), and OPG-05 (5′-CTGAGACGGA-3′). Conditions for DNA amplification, gel electrophoresis, photography, and recording DNA bands were as previously described (6). Amplification with each primer was repeated at least twice to ensure the consistency of the banding patterns.

**Analyses of data.** Dendrograms were constructed based on virulence and random amplified polymorphic DNA (RAPD) data using the Numerical Taxonomy System for personal computers (NTSYS-pc) version 1.80 (36). Only bands repeatable in at least two independent experiments with the same primer were used in the phenetic analysis. The presence or absence of a RAPD band was considered an alternative character and was coded as "1" or "0," respectively. Similarly, virulence was coded as "1" and avirulence as "0." Virulence data of *P. striiformis* f. sp. *hordei* isolates produced on 11 selected barley differential cultivars were used to construct the virulence dendrogram. Of the 11 by 22 isolate-cultivar interactions, only cultivar Astrix inoculated with isolates 22 and A1 and cultivar Hiproly inoculated with isolate 21 had intermediate infection types. The intermediate infection types were treated as avirulent.

For analyses of the RAPD data, a similarity matrix was generated using the SIMQUAL program based on the Dice coefficient $2a/(2a + b + c)$, where $a$ is the number of positive DNA bands shared by the two isolates in a pair and $b$ and $c$ are the number of positive DNA bands present in only one of the isolates in a pair (7). For analyses of virulence data of isolates of *P. striiformis* f. sp. *hordei*, a similarity matrix was generated using the simple matching coefficient $m/n$, where $m$ is the total number of cultivars on which both isolates in a pair were virulent or avirulent, and $n$ is the total number of tested cultivars. Cluster analysis was conducted using the unweighted pair group arithmetic mean (UPGMA), complete-link, and single-link methods in the SAHN program (36). The dendrogram with the best fit to the

similarity matrix based on cophenetic values and matrix comparison (MXCOMP) was chosen. The statistical stability of the branches in the cluster was estimated by bootstrap analysis with 2,000 replicates using the Winboot computer program (27).

Correlation of the RAPD and virulence data was determined by comparison of the two similarity matrices using MXCOMP (36). A 3-D illustration for the relationships among the formae speciales of *P. striiformis* and their relationships to *P. hordei*, *P. recondita* f. sp. *tritici*, and *P. graminis* f. sp. *tritici* was constructed based on mean dissimilarity values of each isolate to isolates of *P. striiformis* f. sp. *hordei*, *P. striiformis* f. sp. *tritici*, and *P. striiformis* f. sp. *poae*, using the MOD3D program in NTSYS-pc.

## RESULTS

**Virulence analysis.** Thirty-one barley, twenty-five wheat, and two bluegrass genotypes were used in the virulence analysis. The barley genotypes consisted of commercial cultivars and genotypes that may have potential for differentiating races of *P. striiformis* f. sp. *hordei* and germ plasm resistant to barley stripe rust. The wheat genotypes consisted of cultivars that were either susceptible to *P. striiformis* f. sp. *tritici* or that differentiated races of the pathogen. Isolates 4 to 22 and A1 to A3, which were collected from Texas, Arizona, Colorado, Idaho, and Montana in 1993 and 1994 (Table 1), were identified as *P. striiformis* f. sp. *hordei*. Of the 21 isolates, 5 were originally from wheat and 16 from barley. Four isolates (isolates 25 to 28) collected from barley in Montana and Washington in 1993 were identified as *P. striiformis* f. sp. *tritici*.

To interpret the results, isolates that produced infection type IT 0 to IT 3 were considered avirulent, isolates that produced IT 4 to IT 6 were considered intermediate in virulence, and isolates that produced IT 7 to IT 9 were considered virulent. Isolates 4 to 22 and A1 to A3 were virulent on seedlings of barley cvs. Russell (PI483127), Steptoe (CI015229), Larker (CI010648), Morex (CI015773), Robust (PI476976), Luttichaus Landger (PI328824), Fong Tien, Hokkiado Chevalier, and Topper and avirulent on 20 of the 25 wheat cultivars. All of the 22 isolates produced IT 0 on Yamhill (CI014503) and Madsen (PI511673); IT 1 on Lemhi (CI011415), Heines VII (PI201195), Moro (CI013740), Paha (CI014485), PS279 (SU92/3*Omar), Druchamp (CI013723), Riebesel 47/51 (PI295999), Stephens (CI017596), Fielder (CI017268), Heines Kolben (PI180619), Tyee (CI017773), Hyak (PI511674), and Chinese Spring (CI014108); and IT 2 on Produra (CI017406), Lee (CI012488), Tres (CI017917), Michigan Amber (PI315203), and Nugaines (CI013968). Based on virulence analysis, isolates 4 to 22 and A1 to A2 were *P. striiformis* f. sp. *hordei*.

Of the 31 barley genotypes tested, Stauffers Obersulzer, Abyssinian 14, BBA 2890, Grannelose Zweizeilige (PI548740), Hor 1428 (PI548708), Hor 2926 (PI548734), Hor 3209 (PI548747), and I 5 (PI288187) were resistant to all isolates of *P. striiformis* f. sp. *hordei*. IT 3 was produced on Stauffers Obersulzer, and IT 1 to 2 were produced on the other cultivars. The results indicate that the eight genotypes may be potential sources in breeding for resistance to barley stripe rust.

Differential reactions were observed on barley genotypes Cambrinus (PI321779), Heils Franken (PI290183), Emir (CI013541), Astrix (CI013862), Hiproly (CI003947), Varunda (PI410865), Abed Binder 12 (PI327961), Trumpf (PI548762), Mazurka (PI399501), Bigo (CI011795), Nakai Zumizairai (CI011561), Zephyr (PI339815), BBA 809, and Hor 4020. The results show that virulence of the North American population of *P. striiformis* f. sp. *hordei* is highly variable and that some of the barley genotypes can be used to differentiate races of the pathogen in the United States. Of the 14 barley genotypes, 9 (Heils Franken, Emir, Astrix, Hiproly, Varunda, Abed Binder 12, Trumpf, Mazurka, and Bigo) were selected as differential cultivars. Cambrinus, Nakai Zumizairai, and Zephyr were not selected because they consisted of plants with both low and high reactions to the isolates. BBA 809 and Hor 4020 were not selected because they had intermediate reactions to

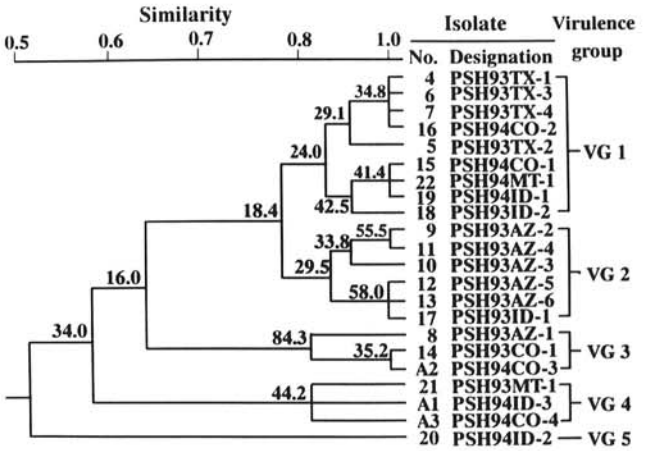


**Fig. 1.** Dendrogram showing the similarities of 22 isolates of *Puccinia striiformis* f. sp. *hordei* (PSH) from Texas, Arizona, Colorado, Idaho, and Montana based on virulence patterns produced on the barley differential cultivars listed in Table 2 by the unweighted pair group arithmetic mean program of NTSYS-pc (version 1.80) (36). The number at each branch shows the percentage of times the group of isolates in that branch occurred, based on 2,000 cycles in bootstrap analysis using the Winboot program (27).

TABLE 2. Virulence and avirulence of races of *Puccinia striiformis* f. sp. *hordei* (PSH) on barley differential cultivars

| Type race | PSH isolate[a] | Avirulence/virulence designation | Infection types produced on barley differential cultivars[b] | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| PSH-1 | 18 | 3,4,5,6,7,8,9,10,11/1,2 | 9 | 9 | 1 | 2 | 2 | 1 | 1 | 2 | 1 | 1 | 1 |
| PSH-2 | 15,19 | 4,5,6,7,8,9,10,11/1,2,3 | 9 | 9 | 9 | 2–3 | 2 | 2 | 1 | 1 | 2 | 1–2 | 2 |
| PSH-3 | 10 | 3,5,6,7,8,9,10,11/1,2,4 | 9 | 9 | 2 | 8 | 2 | 2 | 2 | 2 | 1 | 1 | 2 |
| PSH-4 | 12,13,17 | 3,4,6,7,8,9,10,11/1,2,5 | 9 | 9 | 2 | 2–3 | 8–9 | 1–2 | 1–2 | 1 | 1–2 | 1 | 1 |
| PSH-5 | A1 | 2,3,4,5,8,9,10,11/1,6,7 | 9 | 2 | 3 | 4 | 3 | 9 | 9 | 2 | 2 | 2 | 2 |
| PSH-6 | 4,6,7,16 | 5,6,7,8,9,10,11/1,2,3,4 | 9 | 9 | 7–8 | 7–8 | 1–2 | 1–2 | 0–1 | 1–2 | 1–2 | 2 | 2 |
| PSH-7 | 9, 11 | 3,6,7,8,9,10,11/1,2,4,5 | 9 | 9 | 2 | 8 | 9 | 2 | 1–2 | 1–2 | 1–2 | 1 | 2 |
| PSH-8 | 22 | 4,5,6,8,9,10,11/1,2,3,7 | 9 | 9 | 7 | 5 | 2 | 2 | 9 | 3 | 2 | 2 | 2 |
| PSH-9 | 5 | 5,7,8,9,10,11/1,2,3,4,6 | 9 | 9 | 8 | 7 | 2 | 7 | 0 | 1 | 2 | 2 | 2 |
| PSH-10 | A3 | 2,4,8,9,10,11/1,3,5,6,7 | 9 | 2 | 8 | 2 | 7 | 9 | 9 | 2 | 2 | 1 | 2 |
| PSH-11 | 21 | 2,4,5,9,10,11/1,3,6,7,8 | 9 | 1 | 8 | 1 | 4 | 8 | 9 | 7 | 2 | 2 | 2 |
| PSH-12 | 8 | 6,7,9,10,11/1,2,3,4,5,8 | 9 | 9 | 8 | 7 | 7 | 2 | 0 | 8 | 2 | 2 | 2 |
| PSH-13 | 20 | 4,5,7,11/1,2,3,6,8,9,10 | 9 | 9 | 8 | 2 | 2 | 7 | 1 | 9 | 8 | 8 | 2 |
| PSH-14 | 14,A2 | 9,10,11/1,2,3,4,5,6,7,8 | 9 | 7–9 | 8–9 | 7–8 | 7–8 | 8–9 | 9 | 8 | 2 | 2 | 2 |

[a] The isolate numbers are the same as those in Table 1.

[b] Infection types are based on a 0 to 9 scale (21). Barley differential cultivars: 1 = Topper; 2 = Heils Franken; 3 = Emir; 4 = Astrix; 5 = Hiproly; 6 = Varunda; 7 = Abed Binder 12; 8 = Trumpf; 9 = Mazurka; 10 = Bigo; and 11 = I 5.

specific isolates. Topper, which was susceptible (IT 9), and I 5, which was resistant (IT 1 to 2) to all isolates of *P. striiformis* f. sp. *hordei*, were added to the set of differential cultivars. Based on virulence/avirulence patterns on the barley differential set, 14 races of *P. striiformis* f. sp. *hordei* were identified. The virulence/avirulence patterns and infection types of the 14 *P. striiformis* f. sp. *hordei* (PSH) races are shown in Table 2.

All isolates of *P. striiformis* f. sp. *hordei* were highly virulent (IT 9) on PI574357, PI574377, and PI478214 wheat entries from the National Small Grain Collections grown in a plot in Arizona from which isolates 8 to 13 were collected. All isolates of *P. striiformis* f. sp. *hordei*, except isolates 10, 11, and A1, were moderately virulent (IT 4 to 7) on the wheat differential cultivar Chinese 166 (CI011765). The wheat cultivar Morocco (PI377890) was either moderately resistant (IT 3 to 4) or highly susceptible (IT 9) to the *P. striiformis* f. sp. *hordei* isolates. The results show that some wheat cultivars are susceptible to *P. striiformis* f. sp. *hordei*.

TABLE 3. Number of specific random amplified polymorphic DNA (RAPD) bands for *Puccinia* spp. and their combinations out of 172 reproducible RAPD bands produced using 12 primers

| *Puccinia* species, forma specialis, or combination | No. of specific RAPD bands[a] |
|---|---|
| *P. striiformis* f. sp. *hordei* (PSH) | 7 |
| *P. striiformis* f. sp. *tritici* (PST) | 5 |
| *P. striiformis* f. sp. *poae* (PSP) | 14 |
| *P. hordei* (PH) | 19 |
| *P. recondita* f. sp. *tritici* (PRT) | 10 |
| *P. graminis* f. sp. *tritici* (PGT) | 6 |
| PSH + PST | 16 |
| PSH + PSP | 1 |
| PST + PSP | 2 |
| PSH + PST + PSP | 6 |
| PSP + PH | 3 |
| PST + PGT | 2 |
| PRT + PGT | 1 |
| PSH + PST + PH | 1 |
| PSH + PSP + PGT | 1 |
| PSH + PST + PSP + PH | 1 |
| PSH + PST + PSP + PRT | 2 |
| PSH + PST + PSP + PGT | 2 |
| PH + PRT | 2 |
| PH + PGT | 1 |

[a] Species or formae specialis combinations without specific bands are not listed.

Isolates 25 and 26 collected from barley in Montana and isolates 27 and 28 collected from barley in Washington had avirulence/virulence patterns of *P. striiformis* f. sp. *tritici* type races CDL-3, CDL-22, CDL-41, and CDL-45, respectively. These isolates, plus isolate 31 (type race CDL-45 of *P. striiformis* f. sp. *tritici*), were virulent on Russell, and isolate 28 was virulent on Steptoe. However, some plants of Russell and Steptoe were resistant to the isolates. All of the isolates of *P. striiformis* f. sp. *tritici* were avirulent on the remaining 29 barley genotypes. These results confirm previous observations that barley can be a host for *P. striiformis* f. sp. *tritici*, but in general, barley genotypes are resistant to the forma specialis.

Isolate 23 from bluegrass in Washington did not attack any of the barley and wheat cultivars (IT 0). None of the barley and wheat isolates attacked the bluegrass germ plasm (IT 0). The results indicate that barley and wheat are not hosts for *P. striiformis* f. sp. *poae*, and bluegrass is not a host for *P. striiformis* f. sp. *hordei* or *P. striiformis* f. sp. *tritici*.

Using the UPGMA method of cluster analysis, the *P. striiformis* f. sp. *hordei* isolates (isolates 4 to 22 and A1 to A3) were separated into five virulence groups (VG) based on 80% similarity as a cut-off point (Fig. 1). VG 1 consisted of races PSH-1, PSH-2, PSH-6, PSH-8, and PSH-9 (Table 3); VG 2 consisted of races PSH-3, PSH-4, and PSH-7; VG 3 consisted of races PSH-12 and PSH-14; VG 4 consisted of races PSH-5, PSH-10, and PSH-11; and VG 5 consisted of race PSH-13. VG 1 and 2 were more closely related and would have been combined if the cut-off point was 75% similarity. VG 1 and 2 were virulent on two to five differential cultivars. Except for PSH-1 (the least virulent race), races in VG 1 were virulent on Emir, whereas races in VG 2 were avirulent on Emir.

The unique feature of VG 3 was that the races were virulent on differential cultivars Topper, Heils Franken, Emir, Astrix, Hiproly, and Trumpf. Unlike other virulence groups, races in VG 4 were avirulent on Heils Franken. The virulence on Mazurka and Bigo made race PSH-13 (VG 5) a distinct virulence group. The com-

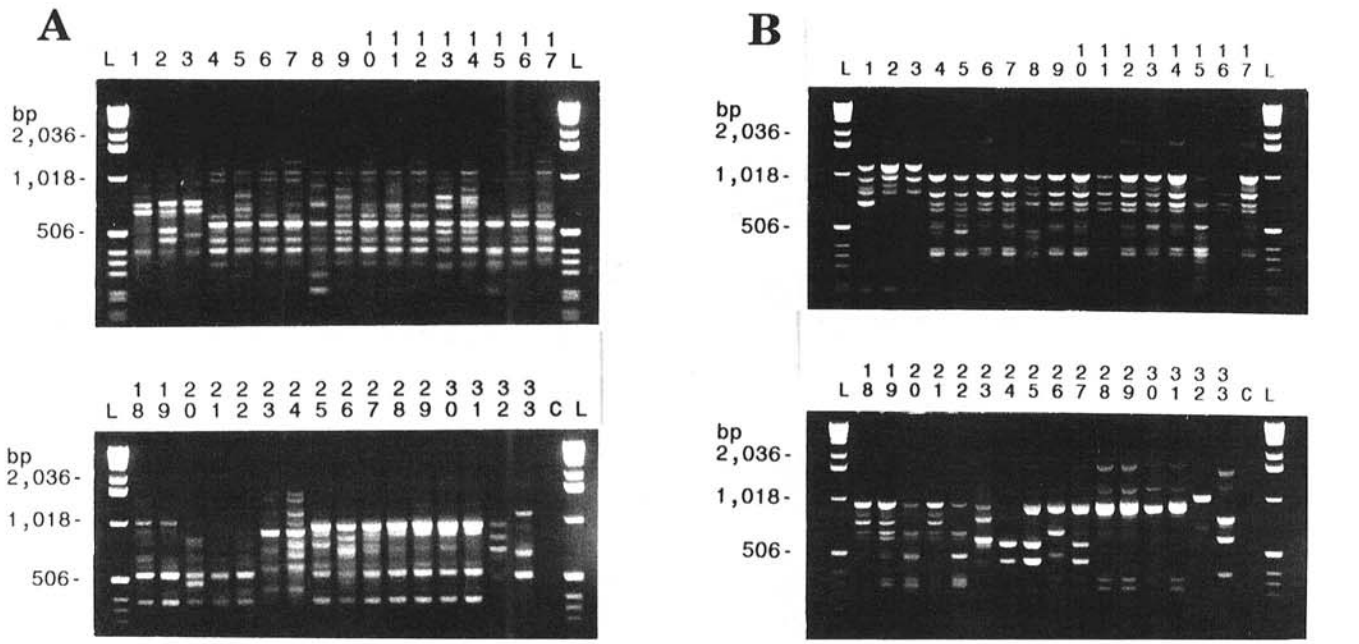


**Fig. 2.** Random amplified DNA polymorphisms of 33 isolates of *Puccinia* spp. with primers **A,** OPD-13 and **B,** OPG-05 showing different banding patterns. Lanes 1 through 3, isolates of *P. hordei*; lanes 4 through 22, isolates of *P. striiformis* f. sp. *hordei*; lanes 23 and 24, isolates of *P. striiformis* f. sp. *poae*; lanes 25 through 31, isolates of *P. striiformis* f. sp. *tritici*; lane 32, *P. recondita* f. sp. *tritici*; and lane 33, *P. graminis* f. sp. *tritici*. Lane L, 1-kb ladder DNA, and lane C, control with sterile water.

plete-link and single-link dendrograms were similar to the UPGMA dendrogram, but their correlation coefficients ($r$ = 0.82 for both complete-link and single-link methods) were not as high as the correlation coefficient for the UPGMA method ($r$ = 0.88). Bootstrap analysis showed that these groups varied in their statistical stability. Of the five virulence groups, VG 3 had the highest bootstrap value (84%).

**DNA polymorphism analyses.** DNA polymorphism was assessed by RAPD analyses. A total of 172 reproducible bands were obtained with 12 random primers. None of the bands were common to all of the isolates that were tested. Figures 2A and B and 3 show examples of banding patterns. Figure 2A shows the banding patterns of the 33 isolates using primer OPD-13. Isolates 4 to 22 (*P. striiformis* f. sp. *hordei*) and isolates 25 to 31 (*P. striiformis* f. sp. *tritici*) shared two common bands (about 550 and 380 bp). A 900-bp band was present in all isolates of *P. striiformis* f. sp. *tritici* but not in all isolates of *P. striiformis* f. sp. *hordei*. *P. striiformis* f. sp. *poae* (isolates 23 and 24), *P. hordei* (isolates 1 to 3), *P. recondita* f. sp. *tritici* (isolate 32), and *P. graminis* f. sp. *tritici* (isolate 33) had distinct banding patterns. Figure 2B shows that the banding patterns of the 33 isolates using primer OPG-05 were similar among the isolates within a forma specialis of *P. striiformis* and within *P. hordei* but were distinct among formae speciales of *P. striiformis* and among species. There were two bands (about 950 and 650 bp) present only in the isolates of *P. striiformis* f. sp. *hordei* (isolates 4 to 22). There was one band (about 900 bp) present only in isolates of *P. striiformis* f. sp. *tritici*. Figure 3 shows that there was considerable variation in RAPD banding patterns among the isolates of *P. striiformis* f. sp. *hordei*.

Table 3 shows the comparison of the number of unique bands detected in the species, formae speciales, and their combinations. Of the 172 reproducible RAPD bands, 7 were present only in the isolates of *P. striiformis* f. sp. *hordei*, 5 were present only in the isolates of *P. striiformis* f. sp. *tritici*, and 14 were present only in the isolates of *P. striiformis* f. sp. *poae*. These unique bands have the potential to be developed into forma specialis-specific markers. The results show that RAPD assay readily differentiated among the three formae speciales of *P. striiformis* and differentiated them more readily from other rusts. Isolates of *P. striiformis* f. sp. *hordei* and *P. striiformis* f. sp. *tritici* shared 16 bands that were not present in any other rusts, whereas *P. striiformis* f. sp. *hordei* shared one band and *P. striiformis* f. sp. *tritici* shared two bands with *P. striiformis* f. sp. *poae*. The results indicate that *P. striiformis* f. sp. *hordei* and *P. striiformis* f. sp. *tritici* are more closely related to each other than they are to *P. striiformis* f. sp. *poae*.

The same relationships also were obtained when the mean similarities within and among the rust groups were compared (Table 4). Between isolates within a forma specialis of *P. striiformis* similarities ranged from 84 to 88%; between formae speciales within *P. striiformis* similarities ranged from 33 to 63%; and between different species similarities ranged from 13 to 32%. These data also show that *P. striiformis* f. sp. *hordei* and *P. striiformis* f. sp. *tritici* are more closely related to each other than they are to *P. striiformis* f. sp. *poae* and that the three formae speciales of *P. striiformis* are more closely related to each other than to *P. hordei*, *P. recondita* f. sp. *tritici*, or *P. graminis* f. sp. *tritici*.

Figure 4 shows the dendrogram for isolates of *P. striiformis* f. sp. *hordei*, *P. striiformis* f. sp. *poae*, *P. striiformis* f. sp. *tritici*, *P. hordei*, *P. recondita* f. sp. *tritici*, and *P. graminis* f. sp. *tritici* generated using UPGMA based on the 172 RAPD bands generated using 12 primers. The correlation coefficient of the dendrogram

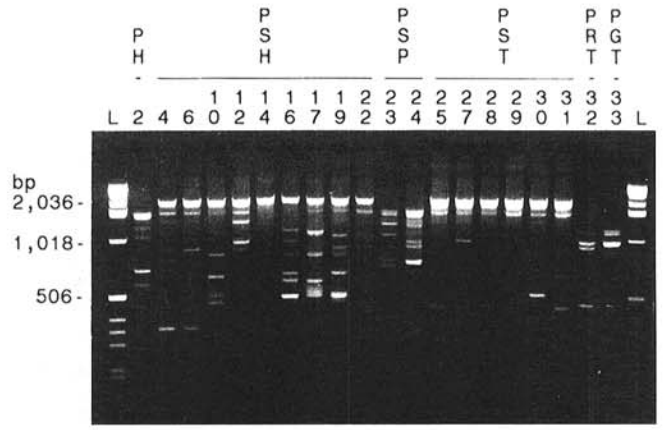


**Fig. 3.** Random amplified DNA polymorphisms of 20 isolates of *Puccinia* spp. with primer OPB-17 showing the differences and commonalities among isolates of *P. striiformis* f. sp. *hordei* (PSH) and the commonalities between *P. striiformis* f. sp. *hordei* and *P. striiformis* f. sp. *tritici* (PST) compared to *P. hordei* (PH), *P. striiformis* f. sp. *poae* (PSP), *P. recondita* f. sp. *tritici* (PRT), and *P. graminis* f. sp. *tritici* (PGT). Lane L, 1-kb ladder DNA, and lane C, control with sterile water.

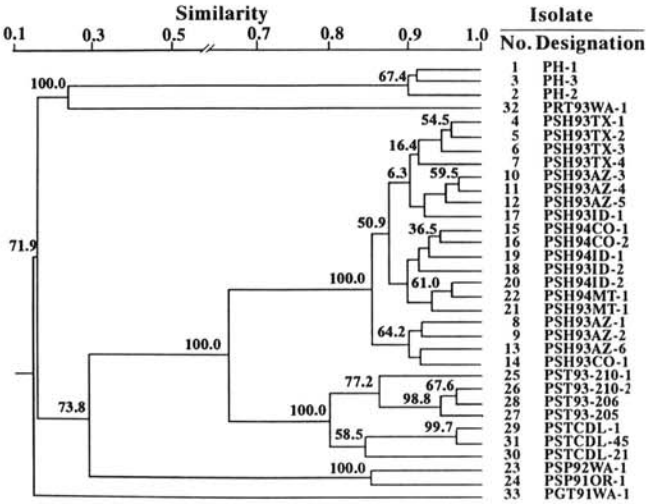


**Fig. 4.** Dendrogram of 33 isolates of *Puccinia* spp. based on 172 random amplified polymorphic DNA bands generated by the unweighted pair group arithmetic mean program of NTSYS-pc (version 1.80) (36) showing similarities among isolates of species and formae speciales of *Puccinia*. The number at each branch shows the percentage of times the group of isolates in a branch occurred, based on 2,000 cycles in bootstrap analysis using the Winboot program (27).

TABLE 4. Mean and standard deviation of group comparisons for *Puccinia* spp.

| | Mean and standard deviation of similarities within and between groups | | | | | |
|---|---|---|---|---|---|---|
| *Puccinia* species or forma specialis | PSH | PST | PSP | PH | PRT | PGT |
| *P. striiformis* f. sp. *hordei* (PSH) | 0.877 ± 0.032 | 0.628 ± 0.029 | 0.331 ± 0.027 | 0.159 ± 0.021 | 0.206 ± 0.033 | 0.134 ± 0.041 |
| *P. striiformis* f. sp. *tritici* (PST) | | 0.840 ± 0.069 | 0.394 ± 0.044 | 0.132 ± 0.030 | 0.146 ± 0.020 | 0.200 ± 0.043 |
| *P. striiformis* f. sp. *poae* (PSP) | | | 0.852 ± 0.000 | 0.221 ± 0.019 | 0.175 ± 0.010 | 0.316 ± 0.030 |
| *P. hordei* (PH) | | | | 0.905 ± 0.004 | 0.232 ± 0.028 | 0.158 ± 0.013 |
| *P. recondita* f. sp. *tritici* (PRT) | | | | | ...[a] | 0.176 ± 0.000 |
| *P. graminis* f. sp. *tritici* (PGT) | | | | | | ... |

[a] ... = no data.

compared to the similarity matrix was high ($r = 0.99$). The four *Puccinia* species (*P. hordei*, *P. striiformis*, *P. recondita*, and *P. graminis*) were separated at 25% similarity. The three formae speciales of *P. striiformis* (*P. striiformis* f. sp. *hordei*, *P. striiformis* f. sp. *tritici*, and *P. striiformis* f. sp. *poae*) were separated at 65% similarity. *P. striiformis* f. sp. *hordei* and *P. striiformis* f. sp. *tritici* were more closely related; they only had 35% similarity with *P. striiformis* f. sp. *poae*. Among isolates of *P. striiformis* f. sp. *tritici*, isolates 25 to 27, which were recently collected from barley, were clustered together and appeared to be different from isolates 29 to 30, which were type races that were collected earlier from wheat. The dendrograms generated using the complete-link and single-link methods were similar to the UPGMA dendrogram and had about the same correlation coefficients ($r = 0.98$ for both complete-link and single-link dendrograms). Separation of the species and formae speciales was strongly supported by bootstrap analysis with bootstrap values ranging from 67.4 to 100%.

By plotting the mean dissimilarity values based on RAPD data of each isolate using *P. striiformis* f. sp. *hordei*, *P. striiformis* f. sp. *tritici*, and *P. striiformis* f. sp. *poae* as the three axes, the intragroup and intergroup relationships could be illustrated in a 3-D diagram. As shown in Figure 5, isolates within a forma specialis were clustered together, and the isolates of *P. striiformis* f. sp. *hordei* and *P. striiformis* f. sp. *tritici* were more closely related to each other than they were to the isolates of *P. striiformis* f. sp. *poae*. As expected, the three formae speciales of *P. striiformis* were not closely related to the other rusts (*P. hordei*, *P. recondita* f. sp. *tritici*, and *P. graminis* f. sp. *tritici*). *P. hordei*, *P. recondita* f. sp. *tritici*, and *P. graminis* f. sp. *tritici* were used as an outgroup in Figure 5 to show their distance from the three formae speciales of *P. striiformis*. No attempt was made to show their relationships to one another.

When the similarity matrices based on RAPD and virulence analyses were compared for isolates of *P. striiformis* f. sp. *hordei*, the correlation coefficient was low ($r = 0.32$). These results indicate that DNA polymorphism and virulence variation are not highly associated.

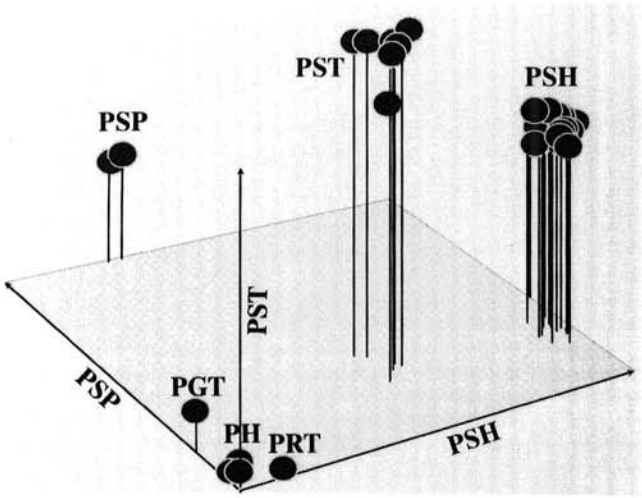


**Fig. 5.** Three-dimensional illustration showing the intra- and intergroup relationships among isolates of *Puccinia striiformis* f. sp. *hordei* (PSH), *P. striiformis* f. sp. *tritici* (PST), and *P. striiformis* f. sp. *poae* (PSP) and their relationships to *P. hordei* (PH), *P. recondita* f. sp. *tritici* (PRT), and *P. graminis* f. sp. *tritici* (PGT) based on random amplified polymorphic DNA data. The illustration was constructed based on the mean dissimilarity values of each isolate to *P. striiformis* f. sp. *hordei*, *P. striiformis* f. sp. *tritici*, and *P. striiformis* f. sp. *poae* using the MOD3D program of NTSYS-pc (version 1.80) (36). The PSH-axis refers to the similarities to *Puccinia striiformis* f. sp. *hordei*, the PST-axis refers to the similarities to *P. striiformis* f. sp. *tritici*, and the PSP-axis refers to the similarities to *P. striiformis* f. sp. *poae*. *P. hordei*, *P. recondita* f. sp. *tritici*, and *P. graminis* f. sp. *tritici* are used as outgroups to show their relationships to the formae speciales of *P. striiformis*; their distant relationships to each other are not shown.

## DISCUSSION

Virulence analyses show that stripe rust isolates from barley in Texas, Arizona, Colorado, Idaho, and Montana and isolates from wheat in Arizona collected during 1993 and 1994 are *P. striiformis* f. sp. *hordei*. The results confirm that stripe rust of barley caused by *P. striiformis* f. sp. *hordei* is spreading within barley-growing regions of the western United States. Both virulence and RAPD analyses show that the primary pathogen of barley stripe rust is a forma specialis of *P. striiformis*.

In 1894 Eriksson (9) reported that there were five formae speciales of *P. striiformis* based on host genus: *P. striiformis* f. sp. *tritici* on wheat, *P. striiformis* f. sp. *hordei* on barley, *P. striiformis* f. sp. *secalis* on rye, *P. striiformis* f. sp. *elymi* on *Elymus* spp., and *P. striiformis* f. sp. *agropyri* on *Agropyron* spp. Later, three more formae speciales of *P. striiformis* were reported: *P. striiformis* f. sp. *dactylidis* on orchard grass (*Dactylis glomerata*) (23,43,48), *P. striiformis* f. sp. *poae* on Kentucky bluegrass (*Poa pratensis*) (1, 25,43,44), and *P. striiformis* f. sp. *leymi* on *Leymus secalinus* (31).

The subdivision of *P. striiformis* into formae speciales, especially *P. striiformis* f. sp. *tritici* and *P. striiformis* f. sp. *hordei*, has been questioned because of the overlapping host ranges (10,30, 39,42). However, Zadoks (48) and Stubbs (41) considered them to be different formae speciales based on greenhouse and field data. Newton et al. (28) showed that *P. striiformis* f. sp. *hordei* and *P. striiformis* f. sp. *tritici* were different based on isozyme and double-stranded RNA analyses. Our virulence and RAPD data support separation of *P. striiformis* f. sp. *hordei*, *P. striiformis* f. sp. *tritici*, and *P. striiformis* f. sp. *poae* into formae speciales. Although *P. striiformis* f. sp. *hordei* primarily attacks barley and *P. striiformis* f. sp. *tritici* primarily attacks wheat, we found that *P. striiformis* f. sp. *hordei* is virulent on some wheats, and *P. striiformis* f. sp. *tritici* is virulent on some barleys. Also, infection of barley and wheat by both *P. striiformis* f. sp. *hordei* and *P. striiformis* f. sp. *tritici* has been reported by others (10,19,29,39, 40,41). Both formae speciales have been observed on other grass species, and the two formae speciales have some common hosts.

RAPD analyses also support the separation of *P. striiformis* f. sp. *hordei*, *P. striiformis* f. sp. *tritici*, and *P. striiformis* f. sp. *poae* into formae speciales. Assuming that isolates of *P. striiformis* f. sp. *hordei* in the United States originated from Europe via South America and Mexico (4,8,35), the separation of *P. striiformis* f. sp. *hordei* from *P. striiformis* f. sp. *tritici* by RAPD analyses could be due to geographic isolation. However, even though the two forms have coexisted for many years in Europe (9), isolates of the two forms from Europe were readily separated. Using isozyme and double-stranded RNA analyses, Newton et al. (28) clearly separated isolates of *P. striiformis* f. sp. *hordei* from those of *P. striiformis* f. sp. *tritici*, but within each forma specialis, Newton et al. (28) could not separate the United Kingdom isolates from isolates from other European countries. Therefore, *P. striiformis* f. sp. *hordei* and *P. striiformis* f. sp. *tritici* must have evolved into two clearly separated groups.

By comparison, isolates of *P. striiformis*, *P. hordei*, *P. recondita*, and *P. graminis* also are separated clearly by RAPD analysis. The results agree with our previous RAPD studies comparing *P. striiformis* f. sp. *tritici*, *P. graminis* f. sp. *tritici*, and *P. recondita* f. sp. *tritici* (X. M. Chen, R. F. Line, and H. Leung, *unpublished data*) and with studies of phylogenetic relatedness among *P. striiformis*, *P. graminis*, *P. recondita*, and *P. hordei* inferred by sequence analysis of the rDNA internal transcribed spacer region (49). The separation of *P. striiformis* f. sp. *hordei* from *P. hordei* by RAPD and restriction fragment length polymorphism analyses also was reported by Smith et al. (38).

Considerable variation in virulence was detected within *P. striiformis* f. sp. *hordei* (Table 2). We detected five distinct virulence patterns based on avirulence/virulence reactions on barley differential cultivars (Topper, Cambrinus, Mazurka, Varunda, Bigo,

and Emir) used by Dubin and Stubbs (8) and Stubbs (41) to differentiate races of *P. striiformis* f. sp. *hordei* in Europe and South America. In 1986, 11 years after *P. striiformis* f. sp. *hordei* was found in Colombia, Dubin and Stubbs (8) identified four virulence patterns (race 24 and three variants of race 24 based on additional virulence on Mazurka and Varunda) and reported that the same virulence patterns also were prevalent in Europe. Based on collections from Texas, New Mexico, and Oklahoma, Marshall and Sutton (24) reported three races (races 23 and 24 and race TXG, which was similar to race 24 but also virulent on Mazurka, Varunda, Bigo, and I 5). Using the differential cultivars of Dubin and Stubbs (8), we detected three of the four virulence patterns reported in South America.

Our results show that the previous differential set used by Stubbs (41) and Dubin and Stubbs (8) does not adequately differentiate races of *P. striiformis* f. sp. *hordei* in the United States. Based on differential reactions, we selected 11 barley cultivars (Table 3) as differentials. The differential set includes all cultivars used by Dubin and Stubbs (8) to differentiate races of *P. striiformis* f. sp. *hordei* in Europe and South America, except that Cambrinus was replaced by Heils Franken because both cultivars differentiated the same races and the Cambrinus seed was contaminated. Cultivar I 5, which was resistant to all isolates of *P. striiformis* f. sp. *hordei* tested, was included as a differential because it differentiated race TXG reported by Marshall and Sutton (24). By adding Astrix, Hiproly, Abed Binder 12, and Trumpf, we more clearly differentiated the virulence patterns (Table 2).

The use of the archaic designation for races of *P. striiformis* f. sp. *hordei* has led to much confusion, especially with race 24. Our system of naming and describing the races of *P. striiformis* f. sp. *hordei* is based on the concept used for describing races of *P. striiformis* f. sp. *tritici* in North America (21,22). We used the prefix PSH in naming the races to separate the *P. striiformis* f. sp. *hordei* races from the *P. striiformis* f. sp. *tritici* races.

Our results support the hypothesis that *P. striiformis* f. sp. *hordei* in the United States originated from Europe and entered the country from Mexico by way of South America. Based on virulence patterns, Dubin and Stubbs (8) postulated that *P. striiformis* f. sp. *hordei* was introduced into Colombia from Europe. There is little doubt that the pathogen has spread from South America to Mexico and from Mexico to the United States (35). In this study, we were able to differentiate many of the races using European cultivars. This would be expected if the pathogen had evolved under the influence of barley cultivars grown in Europe. The considerable diversity revealed by virulence and DNA polymorphism suggests that *P. striiformis* f. sp. *hordei* entered into the United States as a mixture of different races and genetic lineages (RAPD clusters). Susceptibility of the commercial barley cultivars and wild *Hordeum* species (14,24,34) may have contributed to the maintenance of diversity. In contrast, many of the *P. striiformis* f. sp. *tritici* races have evolved under the selection pressure of previously resistant cultivars. Only a few races of *P. striiformis* f. sp. *tritici* were originally detected in North America. New virulent races have appeared after release of cultivars with specific resistance, and new combinations of virulence followed (20,22).

The information on the diversity of the *P. striiformis* f. sp. *hordei* population and on the resistance of barley lines is necessary for disease-resistance breeding. The differentiation between *P. striiformis* f. sp. *hordei* and *P. striiformis* f. sp. *tritici* shown by DNA polymorphism suggests that *P. striiformis* f. sp. *hordei* may not cause epidemics on wheat and *P. striiformis* f. sp. *tritici* may not cause epidemics on barley. However, some wheats may serve as hosts for barley stripe rust and provide a source of inoculum. Therefore, the distribution of barley and wheat stripe rust races should be taken into account in breeding and growing wheat and barley cultivars. Since the population of *P. striiformis* f. sp. *hordei* in the United States is genetically diverse, screening for resistance should involve several races or races selected for the greatest range of virulence. Race-specific seedling resistance, as detected in this study, can be used in breeding programs for developing resistant barley cultivars. The variability of *P. striiformis* f. sp. *hordei* detected in this study also increases the possibility of identifying other types of resistance, such as adult-plant resistance and race-specific seedling resistance.

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