Pathotypes of *Puccinia hordei* with Virulence for the Barley Leaf Rust Resistance Gene *Rph7* in the United States

BRIAN J. STEFFENSON and Y. JIN, Department of Plant Pathology, North Dakota State University, Fargo 58105; and C. A. GRIFFEY, Department of Crop and Soil Environmental Sciences, Virginia Polytechnic Institute and State University, Blacksburg 24061

ABSTRACT

Steffenson, B. J., Jin, Y., and Griffey, C. A. 1993. Pathotypes of *Puccinia hordei* with virulence for the barley leaf rust resistance gene *Rph7* in the United States. Plant Dis. 77:867-869.

The barley (*Hordeum vulgare*) accession Cebada Capa has been used extensively as a source of resistance to the leaf rust pathogen (*Puccinia hordei*) in the Virginia barley breeding program. Cebada Capa possesses the resistance gene *Rph7*; and in the mid-Atlantic region of the United States, cultivars derived from it have remained resistant to *P. hordei* since 1968. In 1990, outbreaks of leaf rust were observed in Virginia on cultivars carrying *Rph7*. Isolates of *P. hordei* collected from these cultivars were found to possess virulence on Cebada Capa in greenhouse and growth chamber experiments. In addition to Virginia, *P. hordei* isolates with *Rph7* virulence were identified in Pennsylvania and California. This is the first report of *Rph7* virulence in the North American population of *P. hordei*.

Barley leaf rust, caused by *Puccinia hordei* G. Otth, is considered a relatively minor disease of barley (*Hordeum vulgare* L.) in the United States, although sporadic outbreaks have occurred in the southeastern (14) and midwestern regions of the country (1,7). Most of the commercial barley cultivars grown in the United States are susceptible to *P. hordei,* with the exception of those bred in Virginia. In the mid-1950s, a program was initiated at Virginia Polytechnic Institute and State University to incorporate leaf rust resistance into barley cultivars (C. W. Roane, *personal communication*). The first resistant cultivar released from this program was James (CI 10659) in 1961 (20). James was derived from Bolivia (CI 1257), which possesses the resistance genes *Rph2* and *Rph6* (2). After the release of James, *Rph7* from Cebada Capa (CI 6193) was bred into nearly every barley cultivar released by the Virginia Experiment Station. These cultivars included Hanover (CI 13197), which was released in 1968 (21); Rapidan (CI 14006), released in 1970 (22); Surry (CI 15689), Henry (CI 15690), and Monroe (CI 15691) released in 1976 (16–18); Maury (CI 15692) released in 1977 (19); and Wysor (PI 501526) released in 1985 (23). In Virginia, *Rph7* has been very effective in controlling *P. hordei,* as little or no rust has been observed on these cultivars in the field (C. W. Roane, *personal communication*). For a time, *Rph7* was considered the most effective leaf rust resistance gene in barley after virulence for *Rph3* from Estate (CI 3410) became widespread in the *P. hordei* population of Europe (2). This situation changed in the late 1970s when pathotypes with virulence for the Cebada Capa resistance were identified in Israel (5) and later in Morocco (12). Virulence for *Rph7* has never been detected in populations of *P. hordei* in North America (1,9,10,13). However, recent outbreaks of leaf rust on previously resistant cultivars in Virginia indicated a possible change in the virulence spectrum of this pathogen. This report documents the occurrence of *P. hordei* pathotypes with virulence for *Rph7* in the United States.

North Dakota State University Agricultural Experiment Station Journal Series No. 2101.

Accepted for publication 4 May 1993.

MATERIALS AND METHODS

In 1990, 31 collections of *P. hordei* were made from barley by researchers at the USDA Cereal Rust Laboratory in St. Paul and by several state cooperators. Of these 31 collections, only 14 had viable urediniospores when inoculated onto the susceptible barley cultivar Larker (CI 10648). Urediniospores from a single uredinium of each isolate were collected and increased on Larker. The resulting urediniospores were collected, desiccated for about 16 hr (ca 20% RH at 21 C), and stored in an ultralow freezer at −80 C until needed for inoculation. The virulence phenotype of each *P. hordei* isolate was determined on a set of host differential genotypes possessing the leaf rust resistance genes *Rph1*–*Rph9* and *Rph12* (Table 1). Seed of the genotypes Clipper BC8 (*Rph10*) and Clipper BC67 (*Rph11*) (4) were not available for testing.

Seed of each differential genotype was sown in a plastic cone (3.8 cm in diameter and 21 cm deep) containing a 3:1 mixture of peat moss and perlite, and grown at 19–25 C in a greenhouse. Fertilizers, water soluble (435–535 ppm of 15N-0P-15K per cone) and controlled release (2.2 g of 14N-14P-14K per cone), were applied at planting. Urediniospores were removed from storage in the ultralow freezer, heat shocked for 3 min in a water bath at 45 C, and rehydrated for several hours before being suspended in a light mineral oil (Soltrol 170). Plants were inoculated with the *P. hordei* isolates (ca 2 mg of urediniospores per 0.2 ml of oil) 1 wk after planting, when the first leaves were fully expanded. The inoculated plants were placed in a chamber (20–21 C) maintained near saturation by intermittent mistings from an ultrasonic humidifier. After a 16-hr mist period, the chamber door was opened halfway to allow plants to dry slowly. Plants were then placed in a greenhouse at 21–24 C. Infection types on each host genotype were assessed 12–14 days after inoculation using a 0–4 scale (7). Infection types 0;, 1, and 2 indicated an incompatible reaction (low infection phenotype); and types 2^{-}, $3^{-}2$, 3, and 4, a compatible reaction (high infection phenotype).

In a preliminary experiment, we identified four pathogen isolates (90-12, 90-15, 90-23, and 90-34) that exhibited high infection phenotypes (types 3^{-} to 4) on Cebada Capa (Table 1). Because virulence for *Rph7* had never been reported in North America, two additional experiments were conducted to confirm our initial results. These additional evaluations were made with three different sources of Cebada Capa: one from the USDA National Small Grains Germplasm Research Facility, Aberdeen, Idaho (courtesy of D. M. Wesenberg); one from I.G.E.R. Welsh Plant Breeding Station, Aberystwyth, Wales (courtesy of B. C. Clifford); and one from Virginia Polytechnic Institute and State University, Blacksburg (courtesy of C. W. Roane). Also included in the experiments were La Estanzuela (PI number not assigned), another source of *Rph7*, and Larker, a susceptible check. Methods for experiment 1 were as described for the preliminary leaf rust evaluation. The same protocols also were used in experiment 2, except that plants were incubated in a growth chamber at 20–25 C (115 W VHO cool white bulbs emitting 120–225 $\mu E \cdot m^{-2} \cdot s^{-1}$ for 13 hr/day) instead of a greenhouse. Experiments were arranged in a completely randomized design with two replicates. Isolate ND8702 (UN race or pathotype 8 [7])

of *P. hordei* was used as a check in all inoculation experiments. Pathotype 8 has been a common virulence type in the midwest region of the United States during the last decade (1; B. J. Steffenson and Y. Jin, *unpublished*).

RESULTS AND DISCUSSION

Isolates 90-12, 90-15, 90-23, and 90-34 exhibited high infection phenotypes (types 3⁻ to 4) on Cebada Capa (*Rph7*) in all three tests (Table 1). Similar infection phenotypes were observed on the alternative *Rph7* source, La Estanzuela, in experiments 1 and 2. Virulence for *Rph7* was widely distributed in the United States, as *P. hordei* isolates with this virulence type were detected in Virginia (isolates 90-12 and 90-34), California (90-23), and Pennsylvania (90-15). The virulence spectra of isolates 90-12, 90-15, 90-23, and 90-34 were similar on the other differential host genotypes. These four isolates exhibited distinctly higher infection types than did isolate ND8702 on Peruvian (*Rph2*), Bolivia (*Rph6* + *Rph2*), and Cebada Capa (*Rph7*).

Virulence surveys of *P. hordei* have not been conducted on an annual basis in the United States. This, coupled with the fact that few isolates of the pathogen are collected each year, creates difficulties in making sound inferences on possible changes in the spatial and temporal frequencies of *P. hordei* pathotypes. The identification of *P. hordei* pathotypes with virulence for *Rph7* is significant, because this is the first time virulence for this important resistance gene has been reported in the United States. In previous surveys, the Cebada Capa resistance was effective against every isolate of the barley leaf rust pathogen collected from the United States (1,9,10,13; A. P. Roelfs, *personal communication*). Pathotypes of *P. hordei* with virulence for *Rph7* may be of recent origin in the United States, because their detection coincided with a sudden outbreak of leaf rust on previously resistant cultivars in Virginia.

The origin of *Rph7* virulent isolates in North America is not known. Virulence changes in cereal rust populations can result from sexual recombination, introduction, or mutation (8). In Israel, new virulence types of *P. hordei* were reported from the alternate hosts, *Ornithogalum narbonense* L., *O. montanum* Cyr., and *O. brachystachys* C. Koch (5), most likely as a result of sexual recombination. Although *O. umbellatum* L. is present in Virginia, natural infection by *P. hordei* has never been observed (C. W. Roane, *personal communication*). A second possibility is that these isolates were introduced into the Western Hemisphere from North Africa, where *Rph7* virulence is known. Pathotype 24 of the barley stripe rust pathogen (*Puccinia striiformis* Westend. f. sp. *hordei*) was probably introduced into South America from Europe (3). Since its introduction into Colombia in 1975, pathotype 24 has spread across South America (3) and is now present in Mexico and the United States (15). Mutation may be a more plausible explanation for the origin of *Rph7* virulence in North America. The selection pressure for mutants with *Rph7* virulence was likely great in Virginia because barley cultivars with the Cebada Capa resistance have been grown there since 1968.

The spread of *Rph7* virulence types in the eastern United States is easy to understand, because rust inoculum is known to be disseminated from the south (e.g., Virginia) to the north (e.g., Pennsylvania). Detection of a similar virulence type in distant California during the same year is difficult to explain. The virulence pattern of the California isolate (90-23) is distinctly different from two of the three isolates collected in the eastern United States. For example, isolate 90-23 confers a high infection phenotype on the barley land race genotype Tunisian 16, whereas isolates 90-12 and 90-15 confer low infection phenotypes (B. J. Steffenson and Y. Jin, *unpublished*). Isolate 90-34 from Virginia exhibits a virulence pattern that is similar to the California isolate on a number of additional host genotypes with leaf rust resistance. Further studies are needed to determine the relatedness of *Rph7* virulence types from the United States.

Barley leaf rust is present every year in Virginia. Under favorable conditions, *P. hordei* can cause yield losses as high as 20% in susceptible genotypes (C. A. Griffey, *unpublished*). Although the area of barley production in the mid-Atlantic region is not immense (ca 90,300–180,600 ha), the Cebada Capa resistance remained effective for 22 yr in different cultivars that were widely grown in Virginia and to a lesser extent in North Carolina, Maryland, Delaware, and Pennsylvania. The durable resistance of Virginia barley cultivars may have been due to more than just *Rph7*, because Parlevliet and Kuiper (11) reported three to four additional genes in Cebada Capa that confer a longer latent period.

Prior to 1990, virulence for *Rph7* was only known in Israel (5) and Morocco (12). Now that pathotypes with this virulence are present in North America, further spread is imminent. Barley

Table 1. Infection types of five *Puccinia hordei* isolates on barley seedlings

Host genotype[a]	Recognized *Rph* gene(s)	Isolate[b] 90-12	90-15	90-23	90-34	ND8702
Preliminary evaluation (greenhouse incubation)						
Larker	...	3⁻[c]	33⁺	4	34	33⁺
Sudan	*Rph1*	23⁻	33⁺	4	3	4
Peruvian	*Rph2*	23⁻	3⁻	23⁻	3	0;1
Estate	*Rph3*	0;	0;	0;	0;	0;
Gold	*Rph4*	34	3⁻2	3	3⁺	3
Magnif	*Rph5*	0;1	0;	0;1	0;	0;
Bolivia	*Rph6*⁺2	23⁻	3	4	4	0;1
Cebada Capa	*Rph7*	3⁻3	3	34	33⁻	0;
Egypt 4	*Rph8*	3⁻	3	3	3⁻	23⁻
Hor 2596	*Rph9*	0;1	2	0;1	0;1	0;
Triumph	*Rph12*[d]	0;	0;1	0;1	0;	0;
Experiment 1 (greenhouse incubation)						
Larker	...	3	3⁻	3⁻3	3⁻2	3⁻
Cebada Capa[e]	*Rph7*	3⁺	33⁺	33⁻	3	0;
La Estanzuela	*Rph7*	3	3	3	3	...[f]
Cebada Capa[g]	*Rph7*	33⁺	3⁻3	33⁺	3	0;
Cebada Capa[h]	*Rph7*	33⁺	3	3⁺3	33⁻	0;
Experiment 2 (growth chamber incubation)						
Larker	...	43	33⁺	3⁻3	3⁻	3⁻3
Cebada Capa[e]	*Rph7*	3⁺3	3	3⁺	33⁺	0;
La Estanzuela	*Rph7*	3	3⁻	33⁻	3⁻	0;
Cebada Capa[g]	*Rph7*	33⁺	3⁺3	33⁺	3⁺	0;
Cebada Capa[h]	*Rph7*	33⁺	33⁺	3⁺3	43	0;

[a] Seed of the differential barley genotypes provided courtesy of A. P. Roelfs, USDA Cereal Rust Laboratory, St. Paul, MN.

[b] Isolate 90-12 was collected in Painter, Virginia, on 15 May 1990; 90-15 in Lebanon, Pennsylvania, on 7 June 1990; 90-23 in Meridian, California, on 8 May 1990; 90-34 in Blacksburg, Virginia, on 2 November 1990; and ND8702 in Langdon, North Dakota, in 1987.

[c] Infection types are based on the 0–4 scale of Levine and Cherewick (7). The symbols ⁺ and ⁻ denote more or less sporulation, respectively.

[d] Gene designation for Triumph is based on Jin et al (6).

[e] Seed source courtesy of D. M. Wesenberg, USDA National Small Grains Germplasm Research Facility, Aberdeen, ID.

[f] Not tested.

[g] Seed source courtesy of B. C. Clifford, I.G.E.R. Welsh Plant Breeding Station, Aberystwyth, Wales.

[h] Seed source courtesy of C. W. Roane, Virginia Polytechnic Institute and State University, Blacksburg.

breeding strategies will have to be modified to combat the possible threat of these new virulence types in the United States. The continued use of single *Rph* genes in barley cultivars will likely result in ephemeral resistance, because virulence for all described leaf rust resistance genes is known in the global population of *P. hordei* (2; B. J. Steffenson and Y. Jin, *unpublished*). Greater durability of host resistance might be achieved through the transfer of several *Rph* genes into a single pure line cultivar; however, the detection of these genes in breeding lines will be difficult unless the appropriate "tester" cultures of *P. hordei* are available. An alternative strategy is to breed for the slow-rusting or type II resistance described by Clifford (2). This type of resistance has been used in Europe since the early 1970s and remains effective today.

ACKNOWLEDGMENTS

We thank C. W. Roane and B. C. Clifford for reviewing an early draft of this manuscript. This research was supported in part by the American Malting Barley Association of Milwaukee, Wisconsin.

LITERATURE CITED

1. Andres, M. W., Wilcoxson, R. D., Roelfs, A. P., and Pretorius, Z. A. 1983. Races of *Puccinia hordei* in the United States from 1979 through 1982. Plant Dis. 67:1336-1337.
2. Clifford, B. C. 1985. Barley leaf rust. Pages 173-205 in: The Cereal Rusts. Vol. II, Diseases, Distribution, Epidemiology, and Control. A. P. Roelfs and W. R. Bushnell, eds. Academic Press, New York.
3. Dubin, H. J., and Stubbs, R. W. 1986. Epidemic spread of barley stripe rust in South America. Plant Dis. 70:141-144.
4. Feuerstein, U., Brown, A. H. D., and Burdon, J. J. 1990. Linkage of rust resistance genes from wild barley (*Hordeum spontaneum*) with isozyme markers. Plant Breed. 104:318-324.
5. Golan, T., Anikster, Y., Moseman, J. G., and Wahl, I. 1978. A new virulent strain of *Puccinia hordei*. Euphytica 27:185-189.
6. Jin, Y., Statler, G. D., Franckowiak, J. D., and Steffenson, B. J. 1993. Linkage between leaf rust resistance genes and morphological markers in barley. Phytopathology 83:230-233.
7. Levine, M. N., and Cherewick, W. J. 1952. Studies on dwarf leaf rust of barley. U.S. Dep. Agric. Tech. Bull. 1056.
8. McIntosh, R. A. 1988. The role of specific genes in breeding for durable stem rust resistance in wheat and triticale. Pages 1-9 in: Breeding Strategies for Resistance to the Rusts of Wheat. N. W. Simmonds and S. Rajaram, eds. CIMMYT, Mexico, D.F. Mexico.
9. Moseman, J. G., and Greeley, L. W. 1965. New pathogenic strains of *Puccinia hordei* among physiologic races identified in United States from 1959 through 1964. Plant Dis. Rep. 49:575-578.
10. Moseman, J. G., and Roane, C. W. 1959. Physiologic races of barley leaf rust (*Puccinia hordei*) isolated in the United States from 1956 through 1958. Plant Dis. Rep. 43:1000-1003.
11. Parlevliet, J. E., and Kuiper, H. J. 1977. Resistance of some barley cultivars to leaf rust, *Puccinia hordei*; polygenic, partial resistance hidden by monogenic, hypersensitivity. Neth. J. Plant Pathol. 83:85-89.
12. Parlevliet, J. E., van der Beek, J. G., and Pieters, R. 1981. Presence in Morocco of brown rust, *Puccinia hordei*, with a wide range of virulence to barley. Cereal Rusts Bull. 9:3-8.
13. Reinhold, M., and Sharp, E. L. 1982. Virulence types of *Puccinia hordei* from North America, North Africa, and the Middle East. Plant Dis. 66:1009-1011.
14. Roane, C. W. 1962. Inheritance of reaction to *Puccinia hordei* in barley. I. Genes for resistance among North American race differentiating varieties. Phytopathology 52:1288-1295.
15. Roelfs, A. P., Huerta-Espino, J., and Marshall, D. 1992. Barley stripe rust in Texas. Plant Dis. 76:538.
16. Starling, T. M., Camper, H. M., Jr., and Roane, C. W. 1980. Registration of Surry barley. Crop Sci. 20:284.
17. Starling, T. M., Camper, H. M., Jr., and Roane, C. W. 1980. Registration of Henry barley. Crop Sci. 20:284.
18. Starling, T. M., Camper, H. M., Jr., and Roane, C. W. 1980. Registration of Monroe barley. Crop Sci. 20:284-285.
19. Starling, T. M., Camper, H. M., Jr., and Roane, C. W. 1980. Registration of Maury barley. Crop Sci. 20:285.
20. Starling, T. M., and Roane, C. W. 1966. Registration of James barley. Crop Sci. 6:303.
21. Starling, T. M., Roane, C. W., and Camper, H. M. 1970. Registration of Hanover barley. Crop Sci. 10:456.
22. Starling, T. M., Roane, C. W., and Camper, H. M., Jr. 1973. Registration of Rapidan barley. Crop Sci. 13:769-770.
23. Starling, T. M., Roane, C. W., and Camper, H. M., Jr. 1987. Registration of Wysor barley. Crop Sci. 27:1306-1307.