Demonstration of Pathotype Specificity in Stem Rust of Perennial Ryegrass

W. Pfender

USDA-ARS National Forage Seed Production Research Center/Oregon State University Department of Botany and Plant Pathology, 3450 SW Campus Way, Corvallis 97331.

Accepted for publication 25 April 2009.

ABSTRACT

Pfender, W. 2009. Demonstration of pathotype specificity in stem rust of perennial ryegrass. *Phytopathology* 99:1185-1189.

Rust diseases cause significant damage in forage and seed crops of perennial ryegrass (*Lolium perenne*), which is highly heterozygous and heterogeneous and thus presents difficulty in genetic analysis. There has been no definitive demonstration of the existence of pathotypes for stem rust or other rusts of perennial ryegrass, although experiments with crown rust (*Puccinia coronata*) of this host are strongly suggestive of pathotype

specificity. We made single-pustule isolates of *P. graminis* subsp. *graminicola*, and applied them individually to a set of genetically diverse, clonally propagated individuals of *L. perenne*. There were clear examples of different patterns of virulence among isolates across the different plant clones, including qualitative and quantitative differences in resistance. These data demonstrate the existence of pathotype specificity in stem rust of *L. perenne*, information which will be useful in breeding for disease resistance.

Perennial ryegrass (*Lolium perenne* L.), a cool-season species used for forage and turf, is an obligately-outcrossing grass. Cultivars are therefore highly heterozygous and heterogeneous, presenting a significant challenge for elucidating genetics of disease resistance. In the major seed-producing region for ryegrasses in the United States, and in some other parts of the world, stem rust (*Puccinia graminis* Pers. subsp. *graminicola* Urban) is the most damaging disease for seed crops (14,20). Although there is a range in susceptibility to stem rust among cultivars grown in the United States (13), none is sufficiently resistant to reliably produce a seed crop without the use of fungicides where stem rust is common.

Genetic specificity in rust pathosystems is well known, exemplified by Flor's foundational gene-for-gene research with flax rust (5). Numerous host genes for resistance to rusts are known in cereal grass crops such as wheat (2). Many of these genes condition a qualitative resistance which prevents infection or greatly restricts growth for some pathotypes of the rust pathogen, a reaction manifested as a low infection type (IT) (16). Some of these qualitative resistance genes, such as *Sr6* (19), have been effective against a broad range of pathotypes whereas others have a more restricted range of activity. Other resistance genes such as *Sr2* (for wheat stem rust), *Yr36* (for stripe rust), and *Lr34* (for leaf rust) condition a partial resistance which appears to be effective against all pathotypes of the respective pathogens. Some of these genes have been identified and their effects have been demonstrated to be a reduction in pathogen growth rather than a hypersensitive response (6,9). In the case of *Lr34*, the resistance is manifested as a reduced number of pustules rather than a reduction in infection type (15).

In contrast to the information available for cereal rusts, relatively little research has been published concerning host resis-

tance to stem rust in perennial ryegrass (7,17), and all the reported research on this pathosystem has used bulked, genetically undefined populations of the pathogen. On the other hand, there is a larger, rapidly growing literature concerning resistance in perennial ryegrass to crown rust (*Puccinia coronata* Corda f. sp. *lolii*), which is the more important rust disease on this host in Europe and parts of Australasia. With few exceptions, this research also has used genetically undefined, and thus presumably mixed, populations of the fungus. Nonetheless, some of these reports suggest strongly that pathotypes exist in the *Lolium*-*P. coronata* pathosystem. In northern Europe, different quantitative trait loci (QTLs) for crown rust resistance were identified among different field test sites, suggesting genetic differences among local pathogen populations (18). Field collections of *P. coronata* from geographically diverse areas in Australia differed in their virulence patterns on a set of *Lolium* perennial genotypes (1). In a QTL analysis demonstrating a crown rust resistance locus on linkage group 2 of perennial ryegrass, the mixed pathogen population from several geographic locations used in selecting parents for the mapping population produced a different response from the geographically restricted (though still genetically mixed) pathogen population used for detailed QTL analysis (4). There is an example of research with a single-pustule isolate of *P. coronata* in *Lolium* (21). In that research, two half-sib families of the host displayed resistance consistent with a single major gene whereas responses of four other half-sib families were consistent with multi-gene, quantitative resistance. Although Wilkins' study (21) used only one genetically defined pathogen isolate, its results are strongly suggestive of pathotypes with respect to host genotypes. Recent research in Australia has demonstrated a high degree of genetic diversity in field collections of *P. coronata* from *L. perenne*, by expressed sequence tag-based simple sequence repeat marker analysis of genetically homogenized isolates (3). Comparative virulence of these isolates was not reported, but the genetic diversity among them is suggestive of the probable existence of pathotypes.

Characterization of pathotypes for rust fungi on *Lolium* would facilitate discovery, utilization, and deployment of host resistance (8). Data in this paper demonstrate the existence of pathotypes in

Corresponding author: W. Pfender; E-mail address: pfenderw@onid.orst.edu

doi:10.1094/PHYTO-99-10-1185

This article is in the public domain and not copyrightable. It may be freely reprinted with customary crediting of the source. The American Phytopathological Society, 2009.

the stem rust pathogen *P. graminis* subsp. *graminicola*, shown by isolate-specific qualitative resistance in *L. perenne*. Evidence for quantitative resistance is presented also.

MATERIALS AND METHODS

Plant material. Individual plants resistant or susceptible to stem rust were selected from the New Zealand cultivar Kingston (PGG Wrightson Seeds), which previously was found to display some resistance to the pathogen in field plantings in Oregon (13). To make the selections, seeds of 'Kingston' perennial ryegrass were planted in flats of potting soil in a greenhouse. After 3 weeks, plantings were thinned to 25 plants per flat. Four weeks later, the plants were inoculated with a suspension of urediniospores of *P. graminis* subsp. *graminicola* that had been collected from field plantings of several perennial ryegrass cultivars (including 'Kingston') and bulked together. The spores were applied as a suspension in Soltrol oil (11) (2 mg of spores per ml of oil), and plants were incubated under conditions favorable for infection as described in previous reports (12). Inoculated plants were maintained for 16 days in a greenhouse, then evaluated for disease. Plants with fewer than five or more than 50 pustules were retained, and the others were discarded. Foliage of the retained plants was cut, the plants were allowed to regrow for 4 weeks, and then reinoculated. Sixteen days later, plants that again appeared resistant (fewer than 2 pustules) or susceptible (more than 50 pustules) were retained for further use. These plants were numbered with the prefix R for the resistant plants and S for the susceptible plants.

Several crosses were made between R and S plants in order to develop mapping populations for other related research that is in progress. For the research reported here, we used parents and some of the progeny from one of these crosses (R2 × S20), and several other R and S plants. Vegetative clones (ramets) of each plant were made by periodically dividing plants and repotting three to five tillers per pot. Ramets were grown in cylindrical pots (3.8 cm diameter × 24 cm tall) of soil in a greenhouse, fertilized at 2-week intervals with pH-adjusted NPK solution, and periodically trimmed to a height of 8 cm.

Inoculum. To isolate single-pustule cultures of *P. graminis* subsp. *graminicola*, plants were inoculated and closely observed towards the end of the latent period (10) for a well-isolated, nonerumpent pustule on a section of the leaf that could support good pustule development. All other leaves were cut off, leaving one leaf bearing a single, nonerumpent pustule. The plant was then isolated from others by inserting its cylindrical pot through a hole in the bottom of a 1-gallon jar of clear plastic, so that the plant was enclosed in the jar and the bottom of the pot could be kept in water. The lid of the jar was fitted with Whatman No. 1 filter paper to allow air and moisture exchange while maintaining isolation from contaminating spores. Several days later, when the pustule had produced abundant urediniospores, the spores were collected with a small cyclone collector (G-R Manufacturing, Manhattan, KS) attached to vacuum pump. The spores were suspended in Soltrol oil (11) and used to reinoculate the same

plant, which was returned to its isolation jar. Spores collected from the resultant pustules then were used to inoculate a 50-pot set of the highly susceptible cultivar Jet, which was kept in a small greenhouse room with no other rust-infected plants. Urediniospores were collected, desiccated overnight in an atmosphere of 30% relative humidity, and then stored at -60°C until used (11).

Six single-pustule isolates were obtained. They differed in the source of the bulk inoculum from which they were derived, and the host plants on which the initial single pustule occurred. Isolates 101 and 102 were derived from a bulk inoculum collected in 2001 from a mixture of perennial ryegrass cultivars grown near Corvallis, OR (44°38'N, 123°12'W), and applied to the cultivar Morningstar. Isolates 103 and 104 were derived from a bulk inoculum collected in 2000 from several cultivars of tall fescue (*L. arundinaceum* Schreb. S.J. Darbyshire) grown near Corvallis, OR, and applied to 'Morningstar' perennial ryegrass. Isolates 106 and 108 were derived from a bulk inoculum collected in 2007 from several perennial ryegrass cultivars, including 'Morningstar', 'Jet', and 'Linn', and applied to several of the resistant (R) 'Kingston' plants identified during selection of the parents for the R × S cross.

Rust reaction of single-pustule isolates on plant clones.

Ramets of 22 perennial ryegrass clones were inoculated (Table 1). The day before inoculating, the plant in each pot was trimmed to a height of 10 cm. Inoculum of each single-pustule isolate was removed from cold storage, heat-shocked and suspended in Soltrol oil at 6 mg/ml (11). The spore suspension was constantly agitated on a stir plate during the inoculation procedure to maintain a consistent concentration as aliquots were removed for application. Spore suspension was sprayed onto plants in randomized, small groups placed in an inoculation chamber. Each plant received approximately 30 µl of spore suspension. Between the use of different isolates, the inoculation chamber was vacated with an exhaust fan and misted with a continuous water spray for 1 min to remove any residual airborne spores. To check for efficacy of this cleaning procedure, three noninoculated plants of the susceptible cultivar Jet were placed in the inoculation chamber for 2 min after the misting procedure, and then removed for later inclusion with other plants in the infection-favorable overnight environment. Pustules on these plants would indicate cross-contamination. After inoculation, oil was permitted to dry from the plants before they were placed into an environmental enclosure under conditions to favor infection (12). Humidifiers misted distilled water in the enclosure to maintain a thin film of water on plant leaves. The plants were held in darkness at a temperature of 18 to 22°C overnight, then exposed to natural light as the temperature warmed to 25°C. After 4 h of these conditions the plants were allowed to dry, then they were moved to a greenhouse bench. Greenhouse temperature was approximately 12°C at night and 18°C in the day, and plants were watered from below to avoid wetting leaves. Air temperature in the greenhouse was recorded at 30-min intervals with an automated data logger (CR10X, Campbell Scientific, Logan, UT) for the calculation of heat units to monitor progress of the latent period (10).

TABLE 1. Disease severity^y for six single-pustule isolates of *Puccinia graminis* subsp. *graminicola* on 22 clones^z of *Lolium perenne*

Isolate	S16	27	34	23	25	S20	R3R	30	13	2	R7	35	38	R8	22	32	R13	R2	36	R4	28	29
101	1.2 a	1.4 ab	0.7 b	0.6 b	1.0 a	0.4 b	0.9 a	1.0 a	0.4 a	0.1 c	0.1 c	0.0 c	0.0 b	0.0 b	0.0 b	0.0 c	0.0 b	0.1 b	0.0 b	0.0 c	0.0 b	0.2 a
102	1.3 a	1.2 bc	1.2 ab	0.5 b	0.7 a	0.8 b	0.8 a	1.0 a	0.2 a	0.1 c	0.0 c	0.0 c	0.0 b	0.0 b	0.0 b	0.0 c	0.1 b	0.1 b	0.0 b	0.0 c	0.0 b	0.2 a
103	1.5 a	0.9 c	1.2 ab	0.5 b	0.2 a	0.6 b	0.4 a	0.9 a	0.1 a	0.4 bc	0.0 c	0.1 c	0.0 b	0.0 b	0.0 b	0.0 c	0.0 b	0.0 b	0.0 b	0.0 c	0.0 b	0.1 a
104	1.4 a	0.9 c	0.8 b	1.5 a	0.5 a	1.1 ab	0.7 a	1.6 a	0.9 a	0.5 ab	0.1 c	0.0 c	0.1 b	0.0 b	0.0 b	0.0 c	0.2 b	0.0 b	0.1 b	0.0 c	0.1 b	0.1 a
106	1.5 a	1.6 ab	1.6 a	0.8 b	0.3 a	1.0 b	0.8 a	0.9 a	0.7 a	1.0 a	2.1 a	1.5 b	1.6 a	1.4 a	1.1 a	1.4 a	1.9 a	1.4 a	1.4 a	0.8 b	0.7 a	0.6 a
108	1.6 a	1.7 a	1.7 a	1.0 b	1.1 a	1.5 a	0.9 a	1.4 a	0.8 a	0.8 ab	1.8 b	1.7 a	1.5 a	1.3 a	1.2 a	0.7 b	1.6 a	1.3 a	1.2 a	1.3 a	1.0 a	0.4 a

^y Severity = log₁₀(1 + number of pustules per plant). Each value is the average of two experiments with three replicates per experiment. Numbers within a column followed by the same letter do not differ (*P* = 0.05) according to the Student-Newman-Keuls test.

^z All clones that are indicated by a number without a letter prefix are progeny of a cross between S20 and R2.

There were 132 host–pathogen combinations (22 plant clones, 6 pathogen isolates). Three replicate ramets (i.e., three pots) per clone were inoculated for each host–pathogen combination. The pots were arranged in the incubation enclosure and on the greenhouse bench in three randomized complete blocks. The experiment was repeated once.

Data and analysis. For each inoculated plant, the number of erumpent stem rust pustules was determined twice. The first assessment was done when the cumulative number of heat units after inoculation had reached a value previously determined (10) to correspond to the latent period duration for 50% of the pustules on a standard susceptible variety (designated LP₅₀). The second assessment was done at approximately two times the LP₅₀ value, when all pustules would be expected to have opened in any compatible interaction. The second value for pustule number was taken as the severity. The ratio of the first value to the second value, designated herein as the LP ratio, was taken as an indicator of latent period duration. In an interaction with a short latent period, the LP ratio is larger than it is in an interaction with a relatively long latent period. On the second assessment date, we also recorded the infection type (IT) of the interaction on a 0 to 4 scale. The scale is a modified form of the standard stem rust infection type scale (16). Scores of 0 and “fleck” indicate that the pathogen is unable to establish a viable infection. Scores of 1, 2, and 3 indicate successful infections with sporulating areas of <1 mm, 1 to 2 mm, and > 2 mm diameter, respectively. In almost all cases, pustules without chlorotic halos of some extent were inconsistently present, therefore our IT values do not include a distinction for pustules without halos, and we did not use the standard IT of 4 which indicates a large pustule with no halo (16). A score of 3+ was used indicate a relatively large pustule, but with a halo.

Initial assessment of the severity data (counts of pustules) showed significant heterogeneity of variance. These data were

therefore transformed to log₁₀(1 + number of pustules), after which the variances were homogeneous. To assess differences among isolates, a two-way analysis of variance (ANOVA) was done in SigmaStat (SPSS, Inc., Chicago, IL) for the severity data among isolates within each plant genotype, with experiment and plant genotype as the treatments. In addition to the ANOVA results, the severity also was categorized into four classes: low (L), <0.5 pustule per plant; moderately low (mL), 0.5 to 2.5; moderately high (mH), 2.5 to 15; and high (H), >15 pustules per plant. Results suggested the isolates could be grouped into two pathotypes, so additional analyses were conducted in which data from all isolates in each pathotype were combined. These combined data were used for ANOVAs of severity values as well as LP ratios across plant clones within pathotypes. The latent period ratio was transformed to arcsine square root before analysis. A *t* test was used to compare LP ratios for each clone between the two pathotypes. A *P* value of 0.05 was used in all statistical tests, and the Student-Newman-Keuls procedure was used for means separation.

RESULTS

Differences were observed among the single-pustule *P. graminis* subsp. *graminicola* isolates in the severity of disease they caused across the *L. perenne* clones. There was no significant experiment-by-isolate interaction within any clone for severity (number of pustules), so severity data for the two experiments were averaged for presentation. No disease developed on the plants of ‘Jet’ used to check for cross contamination in the inoculation chamber.

Among the 22 plant clones tested, 16 had significant differences in severity among the six isolates (Table 1). As a general pattern, isolates 106 and 108 were usually (17 of 22 clones) similar to each other in their severities, and the other isolates (101, 102, 103, and 104) also were usually (19 of 22 clones)

TABLE 2. Qualitative and categorical observations of disease reaction^x in perennial ryegrass clones^y inoculated with isolates of two pathotypes^z of *Puccinia graminis* subsp. *graminicola*

Plants		Pathotype 101		Pathotype 106	
Grouping	Clone	IT	Severity	IT	Severity
IA	S16	3+	H	3+	H
	27	3+ (3)	mH	3+	H
	34	3+	mH	3+	H
	23	3+	mH	3+	mH
	25	3+ (3)	mH	3	mH
	S20	3+	mH	3+ (3)	H
	R3R	3+ (2)	mH	3 (2)	mH
IB	30	3 (2)	H	3 (fl)	H
	13	3, fl (1)	mL	3 (2, fl)	mH
	2	3+	mL	3+ (3)	mH
IIA	R7	0	L	3	H
	35	0	L	3+	H
	38	0	L	3+	H
	R8	0	L	3	H
	22	fl	L	3+	mH
	32	fl	L	3 (1,2)	mH
IIB	R13	0 (2)	L	3	H
	R2	0 (1,2)	L	3+ (3)	H
	36	fl (1)	L	3	H
III	R4	0 (fl)	L	1 (fl)	mH
	28	fl	L	1 (fl)	mH
	29	1 (3)	L	2, fl	mL

^x IT = infection type, 0 to 4, which is a modification of a standard cereal rust scale. Predominant type is followed by common but less prevalent type(s) in parentheses. Disease severity categories: H = high, >15 pustules per plant; mH = moderately high, 2.5 to 15 pustules; mL = moderately low, 0.5 to 2.5 pustules; and L = low, <0.5 pustules per plant.

^y Plant clones are grouped by rust reaction.

^z Pathotype 101 includes data from inoculations with isolates 101, 102, 103, and 104; pathotype 106 includes data from inoculations with isolates 106 and 108.

similar among themselves in their severities. For 15 of the 22 clones, there was a significant difference between the severity caused by 101, 102, 103, or 104 versus severity caused by 106 or 108. For convenience, these two isolate groups will be referred to hereafter as pathotype 101 (isolates 101, 102, 103, and 104) and pathotype 106 (isolates 106 and 108), respectively. The plant clones are grouped in Table 2 according to their reactions to these pathotypes.

Plants classed as group I have a high infection type (3 or 3+) as their predominant reaction, and most display a high susceptibility (severity = H, i.e., >15 pustules per plant, or mH), to both pathotypes. This group includes plant clones S16, 27, 34, 23, 25, S20, R3R, 30, 13, and 2. Two subgroups (IA and IB) differ somewhat in that IB plants had some additional pustules with a lower infection type (1, 2, or fleck) and/or a lower severity (mL = 0.5 to 1.5 pustules per plant) than the others. A second distinct group (IIA) comprises plants with a resistant IT (0 or fleck) and low severity (<0.5 pustule per plant) when exposed to pathotype 101 isolates, but high IT and H or mH severity when exposed to pathotype 106 isolates. Plants in this group are numbers R7, 35, 38, R8, 22, and 32. Plants 22 and 32 show a slightly different level of resistance (IT of fleck instead of 0 to pathotype 101, and severity of mH instead of H to pathotype 106) than the other plants in this group. The plants categorized as group IIB (plants R13, R2, and 36) are similar in reaction to group IIA plants, except that they show some pustules with IT of 1 or 2 to pathotype 101 in addition to the predominant 0 or fleck reaction. Plants categorized as group III (plants R4, 28, and 29) have IT of 2 or less for all isolates, low severity to pathotype 101 and mH or mL severity for pathotype 106. Plant 29 is unique among those tested in having L or mL severity to all isolates.

Analysis of disease severity (number of pustules per plant) across plant clones within each of the two pathotypes indicated quantitative differences in disease reaction (Tables 3 and 4). Among plants for which pathotype 101 was capable of establishing an infection (Table 3), plant clones S16, 30, and 27 had significantly higher severities than clones 25, 13, 2, or 29. For disease caused by pathotype 106, clones R7, R13, 34, 27, 35, and 38 had significantly higher severities than clones 23, R3R, 28, 13, 25, or 29. There was no significant effect of experiment or experiment-by-clone interaction in these severity analyses. Most plant-isolate combinations are not distinguishable in latent period duration as assessed by the LP ratio (Tables 3 and 4), but within

TABLE 3. Severity and latent period indicator for perennial ryegrass clones infected by pathotype 101^x of *Puccinia graminis* subsp. *graminicola*

Plant clone	Severity ^y	LP ratio ^z
S16	20.0 a	0.33
30	11.9 ab	0.45
27	11.5 ab	0.31
34	8.1 abc	0.29
23	4.8 bcd	0.29
S20	4.4 bcd	0.20
R3R	4.3 bcd	0.33
25	3.0 cd	0.26
13	1.6 de	0.49
2	0.8 e	0.35
29	0.4 e	0.37

^x Tabulated values are combined data from plants inoculated with isolates 101, 102, 103, and 104.

^y Severity is back-transformed from the mean of $\log_{10}(1 + \text{number of pustules per plant})$. Values are averages of two experiments, three replicates per isolate per plant clone in each experiment. Values followed by the same letter do not differ ($P = 0.05$) according to the Student-Newman-Keuls test.

^z LP ratio = latent period ratio, proportion of the pustules that had erupted at the time when a standard cultivar is expected to have approximately 50% of its pustules erupt. A small value reflects a long latent period, a large value indicates a short latent period. There were no significant differences among these LP ratio values.

pathotype 106, 1 clone (R4) had a significantly longer latent period than three of the clones (25, 13, and 32). There were no significant differences in LP ratio among clones for pathotype 101 isolates. In t test comparisons between pathotypes for each clone, only clone 25 showed a significant difference in LP ratio between pathotype 101 and pathotype 106 reactions (analysis not shown).

DISCUSSION

These experiments demonstrate clearly the existence of pathotype specificity in stem rust of perennial ryegrass. Qualitative resistance (IT = 0 or fleck) was observed in the reaction of the group IIA plant clones (R7, 35, 38, R8, 22, and 32) to pathotype 101 (isolates 101, 102, 103, and 104) (Table 2). We also observed a range of severities among those plants that did support infection, indicating quantitative resistance to isolates by some clones (Tables 3 and 4). Among clones susceptible to infection, some showed significant quantitative resistance (clones 2, 13, and 29) or significant susceptibility (clones S16, 27, and 34) against both pathotypes. In contrast clone R3R had significantly less disease than many clones when inoculated with pathotype 106, but showed severities similar to most clones when inoculated with pathotype 101. It is apparent that there is both qualitative and quantitative resistance to stem rust in this plant population. Several of the individual clone-pathotype combinations on clones R13, R2, 36, R4, 28, and 29 displayed a range of infection types and sometimes lower severities, reflecting the underlying complexity of what may be intergrading resistance mechanisms spanning a continuum between qualitative and quantitative resistance (15).

For this pathosystem, in which there has been no previous work on pathotype specificity, documentation of a range of qualitative

TABLE 4. Severity and latent period indicator for perennial ryegrass clones infected by pathotype 106^x of *Puccinia graminis* subsp. *graminicola*

Plant clone	Severity ^y	LP ratio ^z
R7	86.1 a	0.40 ab
R13	49.8 ab	0.39 ab
34	46.5 ab	0.43 ab
27	41.5 ab	0.32 ab
35	38.7 ab	0.34 ab
38	35.0 abc	0.38 ab
S16	32.3 abcd	0.34 ab
R2	21.6 abcde	0.23 ab
R8	21.3 abcde	0.23 ab
36	17.5 bcde	0.53 ab
S20	15.9 bcdef	0.30 ab
30	12.4 bcdef	0.27 ab
22	12.0 bcdef	0.48 ab
R4	10.2 bcdef	0.02 b
32	10.1 bcdef	0.61 a
2	7.1 cdef	0.59 ab
23	6.6 def	0.52 ab
R3R	6.0 ef	0.35 ab
28	5.9 ef	0.44 ab
13	4.6 ef	0.72 a
25	4.2 ef	0.61 a
29	2.2 f	0.49 ab

^x Tabulated values are combined data from plants inoculated with isolates 106 and 108.

^y Severity is back-transformed from the mean of $\log_{10}(1 + \text{number of pustules per plant})$. Values are averages of two experiments, three replicates per isolate per plant clone in each experiment. Values within a column followed by the same letter do not differ ($P = 0.05$) according to the Student-Newman-Keuls test.

^z LP ratio = latent period ratio, proportion of the pustules that had erupted at the time when a standard cultivar is expected to have approximately 50% of its pustules erupt. A small value reflects a long latent period, a large value indicates a short latent period. Values within a column followed by the same letter do not differ ($P = 0.05$) according to the Student-Newman-Keuls test.

and quantitative resistance is important in interpreting and utilizing genetic resistance. Given the occurrence of more than one pathotype in a bulk collection, it becomes clear that an individual plant expressing moderately low severity when exposed to field-collected inoculum may be the result of either quantitative resistance across the applied pathotypes (similar to *Lr34* resistance in wheat leaf rust) or qualitative resistance to some of the pathotypes and susceptibility to others. Occurrence of multiple specific resistances is almost certainly the case also for crown rust of perennial ryegrass, where extensive work has been done on the genetics of resistance to bulk populations of the pathogen (1,4, 8,18) but pathotype specificity has not yet been demonstrated explicitly. Currently there is no published research on the molecular genetics of pathogen diversity in *P. graminis* subsp. *graminicola*, as there is for *P. coronata* f. sp. *lolii* (3).

The pathogen collections from which the single-pustule isolates were derived for our research represent a very limited scope in geography and cultivar provenance. More diverse collections will be required to describe the full extent of pathotype variability. After a diverse collection is established, it can be used to test a range of perennial ryegrass cultivars and produce a description of the pathosystem genetics in this crop. Isolates 103 and 104, which were selected from a bulk inoculum originally collected on tall fescue, were very similar in virulence pattern to isolates 101 and 102 from a perennial ryegrass bulk collection. We previously noted (11) that inoculum from tall fescue is capable of infecting perennial ryegrass, although its host range across other plant species differs somewhat from that of isolates commonly collected from perennial ryegrass. A larger sample of single-pustule isolates would be required to assess pathotype differences between populations from these two hosts.

The range in rust reactions among the numbered plant clones in this study is representative of that seen in a larger sample of F1 plants from the same cross (R2 × S20). Knowledge of pathotype specificity and quantitative resistance presented in this study will be instrumental in further research on mapping and QTL analysis for stem rust in this pathosystem.

ACKNOWLEDGMENTS

I thank S. Seguin and L. Evans-Marks for excellent technical assistance in this research.

LITERATURE CITED

1. Aldaoud, R., Anderson, M. W., Reed, K. F. M., and Smith, K. F. 2004. Evidence of pathotypes among Australian isolates of crown rust infecting perennial ryegrass. *Plant Breed.* 123:395-397.
2. Bonman, M. J., Bockelman, H. E., Jin, Y., Hijmans, R. J., and Gironella, A. I. N. 2007. Geographic distribution of stem rust resistance in wheat landraces. *Crop Sci.* 47:1955-1963.
3. Dracatos, P. M., Dobrowolski, M. P., Lamb, J., Olle, R. S., Gendall, A. R., Cogan, N. O. I., Smith, K. F., and Forster, J. W. 2009. Development of genetically homogenised populations of the crown rust pathogen (*Puccinia coronata* f. sp. *lolii*) for disease trait dissection in perennial ryegrass (*Lolium perenne* L.). *Austral. Plant Pathol.* 38:55-62.
4. Dumsday, J. L., Smith, K. F., Forster, J. W., and Jones, E. S. 2003. SSR-based genetic linkage analysis of resistance to crown rust (*Puccinia coronata* f. sp. *lolii*) in perennial ryegrass (*Lolium perenne*). *Plant Pathol.* 52:628-637.
5. Flor, H. H. 1956. The complementary genetic system in flax and flax rust. *Adv. Gen.* 8:29-54.
6. Fu, D., Uauy, C., Distelfeld, A., Blecht, A., Epstein, L., Chen, X., Sela, H., Fahima, T., and Dubcovsky, J. 2009. A kinase-START gene confers temperature-dependent resistance to wheat stripe rust. *Science* 323:1357-1360.
7. Jo, Y.-K., Barker, R., Pfender, W., Warnke, S., Sim, S., and Jung, G. 2008. Comparative analysis of multiple disease resistance in ryegrass and cereal crops. *Theor. Appl. Gen.* 117:531-543.
8. Kimbeng, C. A. 1999. Genetic basis of crown rust resistance in perennial ryegrass, breeding strategies, and genetic variation among pathogen populations: A review. *Austral. J. Exp. Agric.* 39:361-378.
9. Krattinger, S. G., Lagudah, E. S., Spielmeier, W., Singh, R. P., Huerta-Espino, J., McFadden, H., Bossolini, E., Selter, L. L., and Keller, B. 2009. A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. *Science* 323:1360-1363.
10. Pfender, W. F. 2001. A temperature-based model for latent period duration in stem rust of perennial ryegrass and tall fescue. *Phytopathology* 91:111-116.
11. Pfender, W. F. 2001. Host range differences between populations of *Puccinia graminis* subsp. *graminicola* obtained from perennial ryegrass and tall fescue. *Plant Dis.* 85:993-998.
12. Pfender, W. F. 2003. Prediction of stem rust infection favorability, by means of degree-hour wetness duration, for perennial ryegrass seed crops. *Phytopathology* 93:467-477.
13. Pfender, W. F. 2004. Effect of autumn planting date and stand age on severity of stem rust in seed crops of perennial ryegrass. *Plant Dis.* 88:1017-1020.
14. Pfender, W. 2009. A damage function for stem rust of perennial ryegrass seed crops. *Phytopathology* 99:498-505.
15. Poland, J. A., Balint-Kurti, P. J., Wissner, R. J., Pratt, R. C., and Nelson, R. J. 2009. Shades of gray: The world of quantitative disease resistance. *Trends Plant Sci.* 14:21-29.
16. Roelfs, A. P., Singh, R. P., and Saari, E. E. 1992. Rust diseases of wheat: Concepts and methods of disease management. CIMMYT, Mexico, D.F.
17. Rose-Fricker, C. A., Meyer, W. A., and Kronstad, W. E. 1986. Inheritance of resistance to stem rust (*Puccinia graminis* subsp. *graminicola*) in six perennial ryegrass (*Lolium perenne*) crosses. *Plant Dis.* 70:678-681.
18. Studer, B., Boller, B., Bauer, E., Posselt, U. K., Widmer, F., and Kölliker, R. 2007. Consistent detection of QTLs for crown rust resistance in Italian ryegrass (*Lolium multiflorum* Lam.) across environments and phenotyping methods. *Theor. Appl. Genet.* 115:9-17.
19. Tsilo, T. J., Chao, S., Jin, Y., and Anderson, J. A. 2009. Identification and validation of SSR markers linked to the stem rust resistance gene Sr6 on the short arm of chromosome 2D in wheat. *Theor. Appl. Genet.* 118: 515-524.
20. Welty, R. E., and Azevedo, M. D. 1994. Application of propiconazole in management of stem rust in perennial ryegrass grown for seed. *Plant Dis.* 78:236-240.
21. Wilkins, P. W. 1975. Inheritance of resistance to *Puccinia coronata* Corda and *Rhynchosporium orthosporum* Caldwell in Italian ryegrass. *Euphytica* 24:191-196.