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Jeffrey Lehman
Otterbein University

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Jeffrey S. Lehman and Gregory Shaner

Department of Botany and Plant Pathology, Purdue University, 1155 Lilly Hall, West Lafayette, IN 47907-1155.
Current address of J. S. Lehman: Rutgers Blueberry and Cranberry Research Center, Lake Oswego Road, Chatsworth, NJ 08019.
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ABSTRACT

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The durability of partially resistant wheat cultivars to wheat leaf rust depends on the amount of genetic variation in parasitic fitness within populations of the pathogen *Puccinia recondita* f. sp. *tritici*. To assess the durability of partial resistance, phenotypic variation in latent period (a major component of parasitic fitness) was quantified and partitioned into genetic and nongenetic components for isolates of *P. recondita* f. sp. *tritici* on susceptible and partially resistant cultivars. Latent periods among isolates differed by 24 to 27% on individual partially resistant cultivars. In simulated epidemics, isolates with short latent periods caused 2 to 2.5 times more disease and overcame 13 to 35% of the resistance of four partially resistant cultivars. Heritability estimates for latent period of isolates of *P. recondita* f. sp. *tritici* on partially resistant cultivars ranged from 0.28 to 0.76. These results suggest that isolates with short latent periods should be favored by natural selection and could overcome a portion of the resistance of partially resistant wheat cultivars provided that short latent period is unlinked to other traits that reduce fitness. Despite a long latent period, wheat cultivar CI 13227 was anticipated to have the least durable resistance because pathogen isolates on 'CI 13227' were the most variable for latent period and because 'CI 13227' appeared to interact with pathogen isolates with the greatest specificity.

Additional keywords: general resistance, rate-reducing resistance, slow-rusting, *Triticum aestivum*.

The slow leaf-rusting characteristic of partially resistant wheat cultivars is a quantitative form of resistance that retards the establishment and reproduction of the fungus *Puccinia recondita* Roberge ex Desmaz. f. sp. *tritici*. On partially resistant cultivars, the fungus requires a longer time between infection and production of secondary inoculum (i.e., latent period) than on susceptible cultivars (27,29,38,40). In addition, uredinia of the fungus often are smaller (29,38,40), produce fewer spores (27,37,40), and are fewer in number (27,29,38,40). Because *P. recondita* f. sp. *tritici* is a polycyclic pathogen, these differences greatly reduce the rate of disease development in the field (5,10,38). Disease is, therefore, held to levels that are less detrimental to grain production.

Partial resistance to *P. recondita* f. sp. *tritici* may be more durable than high levels of hypersensitive resistance. Presently, however, there is little empirical evidence documenting the durability of partial resistance. Although the only true test of the durability of partial resistance is the evaluation of resistance during wide-scale cultivation for several years in environments favoring disease (4,18), various experimental approaches have been used to predict durability. The most common are methods that detect specificity of host-pathogen interaction with the assumption that specific resistance is less durable than nonspecific resistance (22,44,46). Specific resistance is resistance that is effective against only certain pathogen genotypes. Nonspecific resistance is resistance that is effective against all pathogen genotypes. Since the definition of the latter implies that the fungus is unable to overcome host resistance through genetic change, it is reasoned that nonspecific resistance should provide permanent disease control. Tests used to assess specificity, and indirectly durability, incorporate statistical procedures such as analysis of variance (ANOVA) (3,22,27,46), regression analysis (12,15), and comparisons of variances for adjusted disease severity (14). We proposed that, in addition to these approaches, quantifying the continuous variation in components of parasitic fitness and partitioning this variation into genetic and nongenetic sources may aid in understanding and predicting adaptation of pathogen populations toward partial resistance (i.e., the durability of host resistance).

Quantitative variation has been demonstrated for several traits (growth rate, spore size, and penicillin titer) of nonphytopathogenic fungi (*Schizophyllum commune*, *Neurospora crassa*, *Collybia velutipes*, and *Aspergillus nidulans*) (7,16,26,30,35,41,42,43). In contrast, few studies have attempted to address quantitative variation in parasitic fitness of foliar pathogens of plants or to partition this variation into genetic and nongenetic components. Those studies that have made this attempt report that components of parasitic fitness, such as disease efficiency, lesion length, and sporulation capacity, show continuous variation on host cultivars and are, for the most part, heritable (9,11,13,20,21). These studies conclude that natural selection should favor more fit pathogen isolates and that pathogen populations may overcome the quantitative resistance of partially resistant host cultivars. Because latent period is an important component of host resistance and parasitic fitness and it affects disease development of rust pathogens on cereal crops (1,2,17,31,32,33,38), the amount of genetic variation for this trait should determine, in part, the durability of partial resistance to *P. recondita* f. sp. *tritici*.

Corresponding author: J. S. Lehman; E-mail address: shaner@btny.purdue.edu

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Although the definition of latent period is clearest when applied to a single uredinium, most measurements of latent period refer to the time required for a population of uredinia on a leaf or plant to become infectious (36). Not all infections that occur at a particular time commence sporulation on the same day after inoculation (36,40). On wheat cultivars inoculated with urediniospores of *P. recondita* f. sp. *tritici*, uredinia erupt from infection sites over a period of many days after infection even if the fungal population is genetically uniform. Some infection sites may erupt in as few as 6 days after infection; others may not erupt until 15 or even 20 days after infection. The latent period for a plant inoculated with an isolate of the fungus may be expressed as the mean or median time for a population of infection sites to erupt.

Little is known about variation in latent period, a major component of fitness, for isolates of *P. recondita* f. sp. *tritici*. Our objective was to identify and partition into genetic and nongenetic components the phenotypic variation for latent period that exists among isolates of the fungus on a partially resistant cultivar. In addition, we investigated the existence of significant cultivar-pathogen interactions and estimated the proportion of resistance in the host that interacted specifically with fitness in the pathogen. Based on the level and range of latent period values, estimates of heritabilities, and the relative specificity in quantitative resistance, we concluded that genotypes of *P. recondita* f. sp. *tritici* do differ in fitness with respect to latent period and that use of slow-rusting wheat cultivars may exert selection pressure on the fungus for genotypes that would be more fit on these partially resistant cultivars.

MATERIALS AND METHODS

Fungal isolates. Single-uredinial isolates of *P. recondita* f. sp. *tritici* were made from seven cultures collected in Indiana and Kansas from 1975 to 1987 (Table 1). The isolates were increased on a susceptible wheat cultivar and evaluated for virulence phenotypes on 12 isogenic lines in a Thatcher background that comprise the *P. recondita* f. sp. *tritici* differential set (25).

Host cultivars. Isolates were evaluated for differences in latent period on five cultivars of winter wheat (*Triticum aestivum* L.), including the susceptible cultivar Monon (CI 13278) and cultivars Suwon 85 (PI 157600), Sw 72469-6 (Strampelli/69D-3607// Chokwang), L 574-1 (Wakeland/Blueboy), and CI 13227 (Wabash/American Banner//Klein Anniversario), previously reported as partially resistant (23,29,38). Seedlings of the cultivars were planted in flats containing a soil-peat mixture and were vernalized at 3°C for 6 to 8 weeks under 12 h of fluorescent light per day. After vernalization, seedlings were transplanted individually into 400-ml plastic pots containing a soil-peat mixture and grown in the greenhouse. Natural daylight was supplemented with incandescent and fluorescent light for 16 h/day (approximately 200 μE m^{-2} s^{-1}) from transplanting to maturity. Day and night temperatures were 20 to 24°C and 17 to 20°C, respectively.

TABLE 1. Seven single-uredinial isolates of *Puccinia recondita* f. sp. *tritici* were collected during 1975 to 1987 from *Triticum aestivum* grown in two states

Isolate[y]	Collection site and year	Race[z]
754	Kansas, 1975	TBT
7511	Kansas, 1975	TBT
757-1-2	Kansas, 1975	TBT
759-1	Kansas, 1975	PBT
771	Indiana, 1977	FBR
881	Indiana, 1987	FBR
882	Indiana, 1987	FBR

[y] Isolates collected from were obtained from L. E. Browder, Kansas State University.

[z] Among the seven isolates, three distinct races were detected. Races were determined from virulence patterns of isolates on 12 Thatcher isogenic lines in the *P. recondita* f. sp. *tritici* differential set (25). Phenotype TBT has virulence toward host genes *Lr*1, 2a, 2c, 3, 3ka, 11, 17, and 30; phenotype PBT has virulence toward host genes *Lr*1, 2c, 3, 3ka, 11, 17, and 30; and phenotype FBR has virulence toward host genes *Lr*2c, 3, 3ka, 11, and 30.

Evaluation of latent period. Flag leaves (the uppermost leaf) of each of the five cultivars were inoculated with an aqueous suspension of urediniospores of *P. recondita* f. sp. *tritici* at a concentration of 20 mg of spores/30 ml of water with a model 151 Devilbiss atomizer (Develbis Healthcare Inc., Somerset, PA). Inoculated plants were misted with a Tween 20 solution (1 drop of Tween 20/liter of H_2O) and placed in a moist chamber at 20 to 22°C for 12 to 14 h. On days 6 to 20 after inoculation, uredinia that had erupted from the adaxial surface of the middle 3 to 5 cm of flag leaves were counted. The average time required for a uredinium to erupt, the mean latent period (MLP), was calculated as:

$$\text{MLP} = \sum_{i=0}^{n} P_i \, t_i$$

in which P_i is the proportion of uredinia that appear on the ith day after inoculation relative to the final number of uredinia, t_i is the ith day after inoculation, and n is the number of days after inoculation when all uredinia have appeared.

Since the distribution of latent periods resembled a sigmoidal cumulative normal probability curve, probit analysis was also used to characterize latent period (36). With probit transformations, the proportion of erupted uredinia (Y) becomes a linear function of the number of days after inoculation (X) as described by the equation:

$$\text{Probit}\,(Y) = bX + a$$

in which b is the slope of the line and a is the y-intercept. From this equation, values for T_{50}, the day by which 50% of the uredinia erupt, were calculated. T_{50}, the median time for pustule eruption, is analogous to ED_{50} or LD_{50} of toxicological studies.

Experimental design and statistical analysis. Flag leaves of the five wheat cultivars were inoculated with the seven single-uredinial isolates. The experimental design was a randomized complete block design consisting of two experiments, four replications, five cultivars, and seven isolates. The linear additive model was:

$$Y_{ijkl} = \mu + L_i + R_{(i)j} + C_k + LC_{ik} + I_l + LI_{il} + CI_{kl} + LCI_{ikl} + E_{(i)jkl}$$

in which Y_{ijkl} = response of the $ijkl$th individual subunit; μ = overall mean; L_i = effect of ith experiment; i = experiment 1 or 2; $R_{(i)j}$ = effect of jth block nested within experiments; j = 1...4 replications; C_k = effect of kth wheat cultivar: k = 1...5 cultivars; LC_{ik} = interaction effect of the ith experiment and kth cultivar; I_l = effect of lth isolate: l = 1...7 isolates of *P. recondita* f. sp. *tritici*; LI_{il} = interaction effect of the ith experiment and lth isolate; CI_{kl} = interaction effect of the kth cultivar and lth isolate; LCI_{ikl} = interaction effect of the ith experiment, kth cultivar, and lth isolate; and $E_{(i)jkl}$ = random effect of the jklth subunit in the ith experiment (6).

A $\log_{10}$ transformation of MLP and T_{50} effectively eliminated the association between mean and variance that existed for the untransformed statistics. Duncan's new multiple-range test (α = 0.05) was used to separate means for cultivars, isolates, and interactions between cultivar and isolate (6,45).

Epidemiological simulations. The computer program SLORUS (39,47) was used to model disease development caused by isolates of *P. recondita* f. sp. *tritici* with either long, intermediate, or short latent periods on partially resistant cultivars. SLORUS was also used to model disease development of wild-type isolates on the susceptible cultivar Monon. The model begins with 10

primary infections per 10^4 mm^2 of leaf tissue. It assumes that infections occur daily after secondary inoculum is produced.

The model simulates disease progress in the field based on components of parasitic fitness and host resistance (e.g., latent period, sporulation, pustule size, and infectivity) measured in the greenhouse. With the exception of latent period, cultivar-dependent parameters were obtained from previous studies of wild-type rust populations and were constant for simulations run for the same cultivar (Table 2). The proportion of spores that land on a leaf, a parameter independent of cultivar resistance, was set at 0.018, and the epidemic was allowed to run for 45 days. Simulated disease development was plotted, and the area under the disease progress curve (AUDPC) was calculated by totaling percentage of severity values for days 0 to 45.

Estimates of heritability. Phenotypic variation was partitioned into genetic and nongenetic (i.e., environmental) components. Broad-sense heritabilities were estimated from mean squares for isolates, the interaction of experiment × isolate, and experimental error in ANOVA of $\log_{10}$ MLP and $\log_{10}$ T_{50} for *P. recondita* f. sp. *tritici* isolates on each wheat cultivar (28). Since MLP and T_{50} were calculated from a population of erupting uredinia, not an individual uredinium, heritabilities were calculated on a "family" or genotype basis, analogous to calculating heritabilities based on total plot yield instead of on individual plant yield (28). Heritability estimates of $\log_{10}$ MLP and $\log_{10}$ T_{50} for all isolates on a particular cultivar were calculated from formulas given in Table 3.

Estimates of relative specificity in quantitative resistance. We estimated the amount of specificity in quantitative resistance for latent period from $\log_{10}$ MLP values for cultivars. The $\log_{10}$ MLP for cultivar-isolate combinations was adjusted for the general fitness (general "virulence") of isolates. General fitness was estimated from $\log_{10}$ MLP values of each isolate of *P. recondita* f. sp. *tritici* on susceptible 'Monon' in a manner similar to that of Jenns and Leonard (14). Variances of adjusted values averaged for all pathogen isolates on partially resistant hosts (i.e., test cultivars) were calculated and expressed as a percentage of the highest value. The percentages were used to estimate the amount of specific resistance in test cultivars and to predict their relative durability.

RESULTS

Probit analysis effectively linearized the characteristic sigmoidal cumulative distribution of the eruption of infection sites on cultivars: 97% of the coefficients of determination for the regression line used in the calculation of T_{50} were greater than 0.85. T_{50} values were approximately 0.5 day shorter than values for MLP, and the conclusions drawn from the analyses of the two statistics were similar. Hence, except when results conflict, only the analysis of MLP will be presented.

Differences among wheat cultivars. All isolates produced susceptible infection types on the flag leaves of all cultivars. Thus, the partially resistant cultivars that we studied lacked effective genes for hypersensitive resistance in the adult plant. Averaged across isolates, cultivars differed significantly for $\log_{10}$ MLP (P = 0.005) (Table 4). $\log_{10}$ MLP values for all of the partially resistant cultivars were longer than those of 'Monon' (Fig. 1). Values for 'L 574-1', 'Suwon 85', and 'Sw 72469-6' were intermediate to those

TABLE 2. Parameters used in the computer model SLORUS to compare *Puccinia recondita* f. sp. *tritici* isolates with different latent period distributions on partially resistant cultivars of *Triticum aestivum*

	Cultivar-isolate phenotype combination												
	'CI 13227'			'L-574-1'			'Suwon 85'			'Sw 72469-6'			'Monon'
	Long[w]	Intermediate[w]	Short[w]	Long	Intermediate	Short	Long	Intermediate	Short	Long	Intermediate	Short	Short
Latent period parameters[x]													
P_6			0.01			0.01			0.01			0.01	0.01
P_7		0.01	0.52	0.08	0.10	0.52		0.07	0.52	0.01	0.08	0.52	0.52
P_8	0.01	0.01	0.33	0.19	0.39	0.33	0.05	0.40	0.33	0.12	0.23	0.33	0.33
P_9	0.01	0.24	0.08	0.12	0.21	0.08	0.26	0.24	0.08	0.17	0.42	0.08	0.08
P_{10}	0.04	0.14	0.03	0.13	0.12	0.03	0.26	0.19	0.03	0.14	0.15	0.03	0.03
P_{11}	0.10	0.13	0.01	0.16	0.10	0.01	0.18	0.05	0.01	0.18	0.09	0.01	0.01
P_{12}	0.13	0.14	0.02	0.13	0.05	0.02	0.13	0.03	0.02	0.16	0.02	0.02	0.02
P_{13}	0.17	0.13		0.06	0.03		0.04	0.01		0.09	0.02		
P_{14}	0.14	0.07		0.03	0.01		0.03	0.01		0.07			
P_{15}	0.11	0.05		0.03	0.01		0.03	0.01		0.05			
P_{16}	0.11	0.03		0.03			0.01	0.01		0.02			
P_{17}	0.09	0.01		0.03									
P_{18}	0.05	0.01		0.01									
P_{19}	0.02												
P_{20}	0.03												
Sporulation parameters[y]													
Intercept	3.85	3.85	3.85	4.49	4.49	4.49	3.87	3.87	3.87	4.10	4.10	4.10	4.70
Coefficient of log time	1.88	1.88	1.88	2.20	2.20	2.20	1.64	1.64	1.64	1.31	1.31	1.31	2.54
Coefficient of linear time	–0.18	–0.18	–0.18	–0.22	–0.22	–0.22	–0.09	–0.09	–0.09	–0.06	–0.06	–0.06	–0.25
Other parameters[z]													
Infection frequency	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.12
Uredinial size, mm^2	0.146	0.146	0.146	0.196	0.196	0.196	0.170	0.170	0.170	0.170	0.170	0.170	0.225

[w] Isolate phenotype "long" has a long latent period; isolate phenotype "intermediate" has an intermediate latent period; isolate phenotype "short" has a short latent period.

[x] Parameters P_6 to P_{20} are the proportion of uredinia that erupted on days 6 to 20 after inoculation.

[y] Parameters for sporulation are constants obtained from a previous study of wild-type *P. recondita* f. sp. *tritici* populations which described spore production as a gamma distribution. The parameters intercept, coefficient of log time, and coefficient of linear time were reported as ln K, $r - 1$, and γ, respectively, by Wilson and Shaner (47), in which K, r, and γ are constants derived from fitting the gamma distribution to daily spore production data.

[z] Parameters of infection frequency and uredinial size are the proportion of urediospores/mm^2 of leaf tissue that give rise to infections and the area (mm^2) of a fully expanded uredinium, respectively, obtained from a previous study of wild-type *P. recondita* f. sp. *tritici* populations.

of 'Monon' and 'CI 13227'. The MLPs (nontransformed) of the partially resistant cultivars CI 13227, Sw 72469-6, Suwon 85, and L 574-1 were 4.9, 2.5, 1.8, and 1.9 days longer than the MLP of susceptible cultivar Monon, respectively.

Differences among fungal isolates. The effect of isolate was nonsignificant for $\log_{10}$ MLP ($P = 0.75$) (Table 4). Duncan's new multiple-range test indicated no difference between means for isolates averaged across experiments, cultivars, and replications (Fig. 1). The interaction of experiment × isolate was significant for $\log_{10}$ MLP ($P = 0.008$) (Table 4) mainly because isolates 757-1-2 and 882 reversed rank between the two experiments. The interaction of experiment × isolate for $\log_{10} T_{50}$ was nonsignificant ($P = 0.12$).

Isolates differed for virulence phenotype. Three virulence phenotypes, TBT, PBT, and FBR, were detected among the isolates (Table 1). Isolates with the same virulence phenotype frequently varied for latent period on partially resistant cultivars.

Differences among cultivar × isolate combinations. The cultivar × isolate interaction was significant for $\log_{10}$ MLP ($P = 0.036$) (Table 4) because of the magnitude of differences in the latent period of isolates on different cultivars, as well as reversals in rank of isolate-cultivar combinations (Fig. 2). For example, on 'CI 13227', isolate 759-1 had a shorter latent period than did isolate 754; however, on 'L 574-1', the situation was reversed. Similarly, isolate 771 had one of the shortest latent periods on 'L 574-1'; yet on 'Suwon 85', its latent period was the longest. Isolate 882 also responded differentially on partially resistant cultivars. The susceptible cultivar Monon was the only cultivar upon which all isolates responded statistically the same for $\log_{10}$ MLP. On each of the four partially resistant cultivars, the shortest MLP was 24 to 27% shorter than the longest MLP (Fig. 2).

TABLE 3. Broad-sense heritability (H) was calculated from mean squares (MS) in the analysis of variance of latent period for seven single-uredinial isolates of *Puccinia recondita* f. sp. *tritici* on each of four partially resistant wheat cultivars

Source of variation	MS[y]	Expected MS[z]
Experiment (L)		
Replication (R)		
Isolate (I)	MS_I	$\sigma^2_\epsilon + r\sigma^2_{LI} + lr\sigma^2_I$
Experiment × isolate (LI)	MS_{LI}	$\sigma^2_\epsilon + r\sigma^2_{LI}$
Error (ϵ)	MS_ϵ	σ^2_ϵ
Formulas used to calculate H		
$\sigma^2_I = (MS_I - MS_{LI})/lr$		
$\sigma^2_{LI} = (MS_{LI} - MS_\epsilon)/r$		
$H = \sigma^2_I/[\sigma^2_I + (\sigma^2_{LI}/l) + (\sigma^2_\epsilon/lr)]$		

[y] Mean squares (MS) are for isolate (I), experiment × isolate (LI), and experimental error (ϵ).

[z] Variance components (σ^2) are for isolate (I), experiment × isolate (LI), and experimental error (ϵ). Letters r and l refer to levels of replications and experiments, respectively.

Simulated disease development. Differences in latent period distributions between fungal isolates on the same cultivar should result in differences in disease progress. The magnitude of this effect was explored with the simulation model SLORUS (39,47), which was used to predict disease progression in the field for 12 host-pathogen combinations.

Latent period distributions of isolates with the longest and shortest $\log_{10}$ MLP on each partially resistant cultivar were used in simulations. They represented isolates of different fitnesses for latent period on partially resistant cultivars. These isolate phenotypes are referred to as "long" and "intermediate", respectively (Table 2 and Fig. 3). Differences in the simulated epidemics produced by isolates with these latent period distributions are presumed to reflect disease differences between wild-type and partially adapted populations of *P. recondita* f. sp. *tritici* on partially resistant cultivars.

The average latent period distribution of all isolates on 'Monon' was also used in simulations with sporulation parameters that were characteristic for each partially resistant cultivar. These epidemics simulated disease development of isolates that have completely overcome the resistance for latent period in partially resistant cultivars, yet have overcome no other resistance components. These isolate phenotypes are referred to as "short" (Table 2 and Fig. 3).

To determine the relative effect of components of host resistance other than latent period, simulations were run with cultivar-dependent parameters typical of a susceptible "check". The parameters used in these simulations were from data of susceptible cultivar Monon inoculated with wild-type populations of *P. recondita* f. sp. *tritici*.

In simulated epidemics, disease progressed more rapidly for phenotypes with short latent periods than for phenotypes with long latent periods (Fig. 3). Similarly, disease increased more rapidly for phenotypes with intermediate latent periods compared with phenotypes with long latent periods. In all cases, the susceptible interaction (i.e., wild-type on 'Monon') resulted in more rapid disease progress than for any combination of a partially resistant cultivar with any isolate phenotype, regardless of the pathogen's latent period. AUDPCs calculated from simulated epidemics for isolates with long and intermediate latent periods were substantially smaller than those of isolates with short latent periods on partially resistant cultivars. For cultivars L 574-1, Suwon 85, and Sw 72469-6, average differences were approximately 23 and 50%, respectively (Fig. 3). On 'CI 13227', isolate phenotypes with long or intermediate latent periods produced 8 or 20% of the disease produced by isolates with short latent periods (Fig. 3).

TABLE 4. Analysis of variance of $\log_{10}$ MLP and $\log_{10} T_{50}$ for flag leaves of *Triticum aestivum* inoculated with single-uredinial isolates of *Puccinia recondita* f. sp. *tritici*[x]

Source of variation[y,z]	$\log_{10}$ MLP				$\log_{10} T_{50}$			
	df	Mean squares	*F* value	*P* value	*df*	Mean squares	*F* value	*P* value
Experiment (L)	1	0.0532			1	0.1130		
Replication (R)	3	0.0040			3	0.0061		
Cultivar (C)	4	0.3158	23.64	0.005	4	0.4143	12.47	0.016
$L \times C$	4	0.0134	6.36	0.0001	4	0.0332	9.98	0.0001
Isolate (I)	6	0.0035	0.56	0.750	6	0.0027	0.47	0.807
$L \times I$	6	0.0062	2.97	0.008	6	0.0058	1.73	0.116
$C \times I$	24	0.0075	2.12	0.036	24	0.0114	2.37	0.019
$L \times C \times I$	24	0.0035	1.69	0.028	24	0.0048	1.44	0.091
Error	204	0.0021			201	0.0033		

[x] MLP = the weighted mean number of days from infection to the production of secondary inoculum. T_{50} = the number of days required for 50% of the uredinia to erupt calculated from probit analysis (details in text).

[y] Text has the explanation of the sources of variation in the linear additive model used in the analysis.

[z] Mean squares for $L \times C$, $L \times I$, and $L \times C \times I$ were the denominators used to test C, I, and $C \times I$, respectively. The mean square for error was the denominator used to test $L \times C$, $L \times I$, and $L \times C \times I$.

Heritability of latent period. Natural selection of pathogen populations operates whenever fungal genotypes differ in fitness. Hence, knowledge of quantitative genetic variation in parasitic fitness is essential for understanding adaptation of populations of *P. recondita* f. sp. *tritici* toward partially resistant cultivars. To quantify genetic variation for latent period, we calculated broad-sense heritability, the ratio of genetic variation to phenotypic variation. Because *P. recondita* f. sp. *tritici* in North America is asexual and does not undergo sexual recombination, all the variations partitioned as genetic should be passed on to asexual offspring (i.e., dominance and epistasis which upwardly bias estimates of broad-sense heritability relative to narrow-sense heritability are not pertinent in the absence of recombination). Broad-sense heritabilities of latent period should represent the variation that influences adaptation of populations of *P. recondita* f. sp. *tritici* toward partially resistant cultivars for this trait.

Broad-sense heritabilities of latent period for isolates on each partially resistant cultivar ranged from 0.28 to 0.76 (Table 5). Estimates for isolates on 'L 574-1' and 'CI 13227' were intermediate and ranged from 0.41 to 0.49 for both measures of latent period. Values for isolates on 'Suwon 85' were approximately 40 and 60% higher for $\log_{10}$ MLP and $\log_{10}$ T_{50}, respectively, and ranged from 0.59 to 0.76. The heritability for $\log_{10}$ MLP of isolates on 'Sw 72469-6' was 0.28 or approximately 35% lower than heritabilities of isolates on 'L 574-1' and 'CI 13227'. In contrast, the heritability of isolates on 'Sw 72469-6' was similar to those of isolates on 'L 574-1' and 'CI 13227' for $\log_{10}$ T_{50}. Values for isolates on 'Monon' were negative because of error variance. This suggests that of the total phenotypic variation on 'Monon', none were attributable to genetic sources.

Estimates of relative specificity in quantitative resistance. We used the method of Jenns and Leonard (14) to estimate relative specificity in quantitative resistance for latent period based on $\log_{10}$ MLP data. Latent period length was assumed to be the product of genes for general and specific resistance that affect the latent period on partially resistant cultivars and of genes for general and specific fitness ("virulence") that affect the latent period of isolates of *P. recondita* f. sp. *tritici* (i.e., latent period length = (GS) + (GF) + (SS) + (SF), in which GS and SS are the contribution to latent period length due to general and specific susceptibility of the host, and GF and SF are the contribution to latent period length due to general and specific fitness of the pathogen). Values for MLP were adjusted for general fitness in which general fitness was estimated from $\log_{10}$ MLP values of individual fungal isolates on 'Monon', the cultivar in our study with functionally no resistance for latent period. Adjusted values were calculated from the equation: adjusted $\log_{10}$ MLP = ($\log_{10}$ MLP of cultivar-isolate combination) – ($\log_{10}$ MLP of 'Monon'-isolate combination) for the same isolate. Adjusted values represented the specific susceptibility of the host-pathogen combination. The variance of these adjusted values for each isolate on a resistant cultivar (i.e., the deviation from the average specific susceptibility for the cultivar) was assumed to be correlated with the number of specific host genes for resistance and, thus, was used to estimate the proportion of specific resistance in the test cultivar.

Values for adjusted $\log_{10}$ MLP and the variance of these values for each isolate on cultivars CI 13227, Suwon 85, L 574-1, and Sw 72469-6 are given in Table 6. Variances were expressed as a percentage of the highest value and were used to estimate the relative specificity in quantitative resistance. Based on relative ranking of the variance of adjusted $\log_{10}$ MLP, 'CI 13227' interacted with the seven isolates with the greatest degree of specificity; estimates for 'CI 13227' were considerably larger than those for all other partially resistant cultivars. Values for 'L 574-1', 'Suwon 85', and 'Sw 72469-6' were 37, 31, and 22% of that of isolates on 'CI 13227' (Table 6).

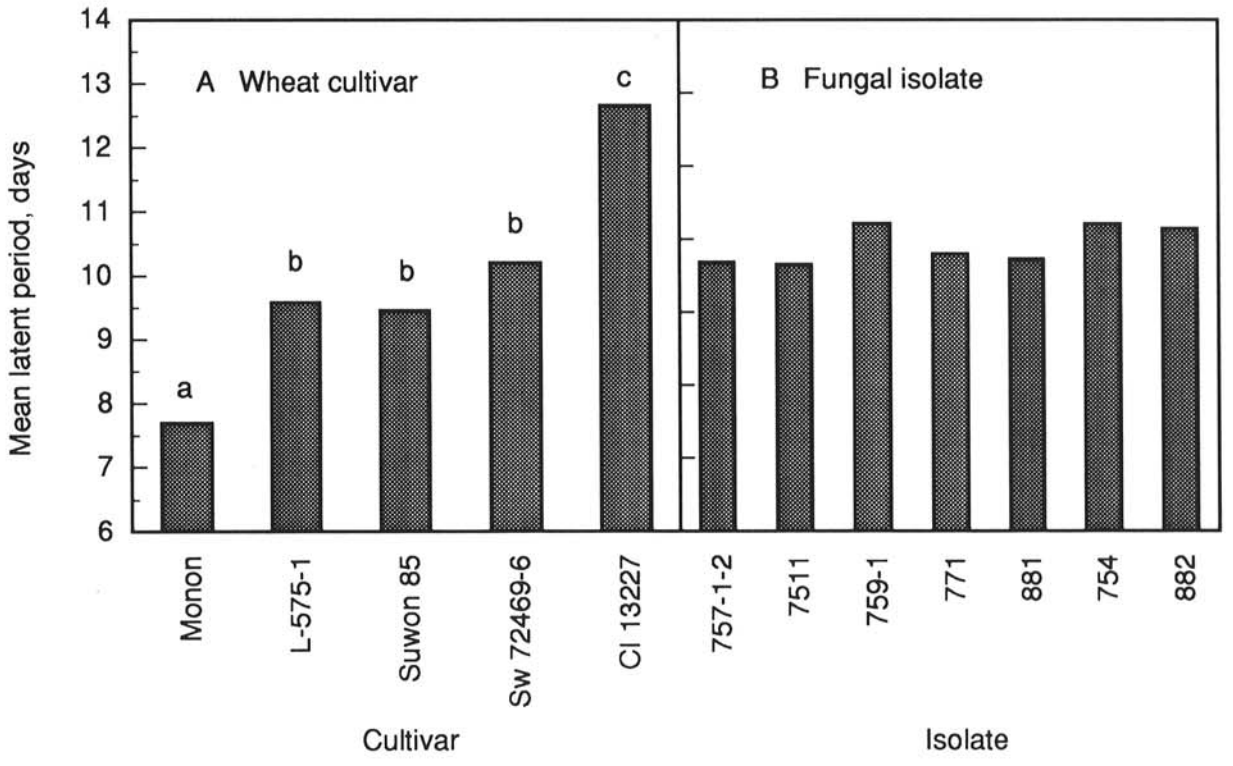

Fig. 1. Mean latent periods for flag leaves of five cultivars of *Triticum aestivum* inoculated with seven single-uredinial isolates of *Puccinia recondita* f. sp. *tritici*. Mean latent period is the average number of days from infection to the production of secondary inoculum. **A,** Cultivar means averaged across seven fungal isolates. **B,** Isolate means averaged across five wheat cultivars. Each bar is the mean of two experiments with four replications averaged across isolates of *P. recondita* f. sp. *tritici* or wheat cultivars. Cultivar means with a letter in common above the bar did not differ significantly for log-transformed data according to Duncan's new multiple-range test ($\alpha = 0.05$). Isolate means did not differ for log-transformed data.

DISCUSSION

Often, monocyclic infection experiments are used to measure the degree of partial resistance in a cultivar as a means to assess the value of its partial resistance in disease control. A more complete assessment should include an attempt to predict the durability of resistance (2,8).

Cultivar CI 13227 had the longest latent period we identified in any wheat cultivar. Because latent period partly determines disease development for a polycyclic (1,2,17,31,32,33,38), we anticipate the resistance of 'CI 13227' to be very effective at maintaining disease levels below an economically tolerable limit. Cultivars L 574-1, Suwon 85, and Sw 72469-6, by merit of having latent periods longer than that of 'Monon', are also expected to provide a degree of field resistance. Our findings and their implications agreed with field studies of these cultivars reported by Shaner and Finney (38) and Lehman and Shaner (23,24); cultivars with long latent period sustain less disease in the field.

Expectations regarding the durability of resistance are often based on the number of resistance genes in the host, irrespective of the genetics of fitness in the pathogen. Eenink (8) argues that there is no clear relationship between inheritance of host resistance and durability; little can be surmised about durability based on the genetics of host resistance alone. Rather, a more appropriate assessment of durability of resistance may be to explore the genetic potential of pathogen populations to adapt toward host resistance. Because of the close association between latent period and disease development of wheat leaf rust, we believe that studies of variation in latent period should provide insight to the adaptation of *P. recondita* f. sp. *tritici* populations toward wheat cultivars.

If adaptation of fungal populations results from genetic variation in parasitic fitness, then significant differences in latent period among fungal isolates on partially resistant cultivars suggest partial resistance may not be durable. Such differences exist among fungal isolates on the resistant cultivars that we examined. Values for $\log_{10}$ MLP of isolates phenotypically most fit for latent period were 24 to 27% shorter, respectively, than those of isolates that were phenotypically least fit. Because variation in latent period among isolates on 'CI 13227' was greater than that for other cultivars, *P. recondita* f. sp. *tritici* may be selected more rapidly for increased fitness on this cultivar than on the other partially resistant cultivars we studied. However, 'CI 13227' was more resistant than other cultivars, and greater change was required to overcome resistance.

In simulated epidemics, the differences in latent period among isolates of *P. recondita* f. sp. *tritici* resulted in 2 to 2.5 times more disease and were equivalent to the pathogen overcoming 13 to 35% of the resistance for latent period depending on the original level of resistance. The most fit isolate on cultivar CI 13327 overcame relatively less of the resistance of this cultivar in simulations than did the most fit isolate on 'L 574-1', 'Suwon 85', and 'Sw 72469-6'. However, instead of reflecting the potential durability of 'CI 13227' relative to the other partially resistant cultivars, we felt that this observation was because of the higher level of resistance in 'CI 13227'. This was supported by the observation that isolates on less resistant cultivars (i.e., cultivars with shorter latent periods and higher rates of disease progression) showed greater adaptation in computer simulations than isolates on more resistant cultivars, regardless of the amount of variation for latent period that isolates on these cultivars exhibited.

In general, the latent period of isolates on partially resistant cultivars was moderately heritable. Thus, selection should operate in favor of increased parasitic fitness of the pathogen. After exposure to partially resistant cultivars, we anticipate that individuals of *P. recondita* f. sp. *tritici* with shorter latent periods

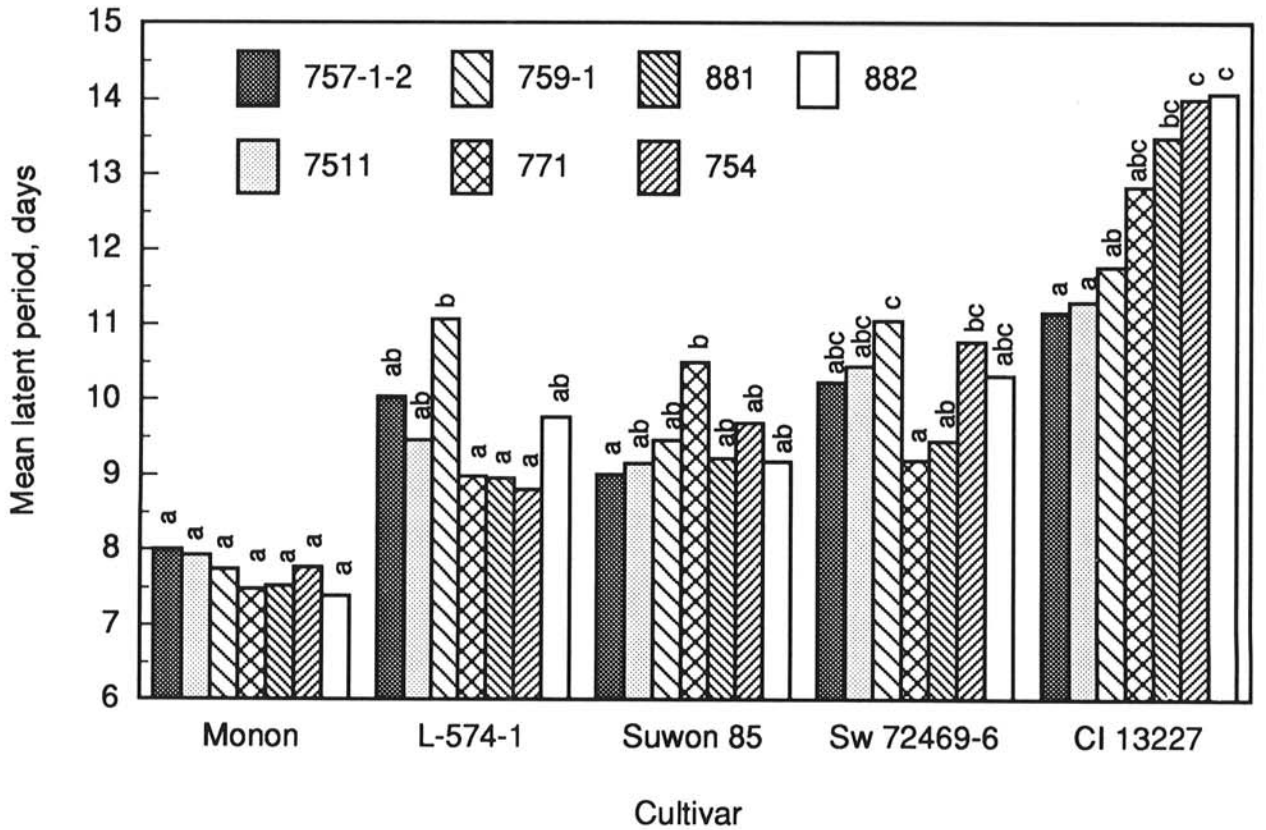

Fig. 2. Differential mean latent periods of isolates of *Puccinia recondita* f. sp. *tritici* on five cultivars of *Triticum aestivum*. The mean latent period of the "most fit" isolates were significantly shorter (24 to 27%) than those of the "least fit" on all except 'Monon'. Mean latent period is the average number of days from infection to the production of secondary inoculum. Each bar is the mean of two experiments with four replications. Means for isolates on the same cultivar with a letter in common above the bar did not differ significantly for log-transformed data according to Duncan's new multiple-range test ($\alpha = 0.05$).

should increase in frequency, provided these individuals do not also exhibit other traits which reduce fitness. Since no isolate appeared completely fit for latent period on partially resistant cultivars (i.e., no isolate on a partially resistant cultivar had a latent period as short as it did on 'Monon'), adaptation in the field may be incomplete and only a portion of the resistance may be rendered ineffective.

With the intent of indirectly assessing durability, ANOVA has been used to detect race-specific, quantitative interactions between host and pathogen. ANOVA, however, lacks sensitivity in detecting unique levels of disease for host-pathogen combinations and can not rank the relative specificity in quantitative resistance (14,34). Jenns and Leonard (14) developed a method to predict the relative durability of host resistance by estimating from disease severity data the amount of specificity in quantitative resistance of host cultivars inoculated in all possible combinations with a set of pathogen isolates. Their model assumes that genes for general virulence and resistance act additively and determine disease in each cultivar-isolate combination, whereas genes for specific resistance and virulence are assumed to interact additively in a gene-for-gene relationship. Based on the relative specificity in resistance, quantitatively resistant cultivars can be ranked for potential durability (stability) of resistance toward pathogen isolates.

Of all host-pathogen combinations tested, we hypothesized that gene(s) governing latent period in *P. recondita* f. sp. *tritici* interacted with the highest proportion of specificity with gene(s) governing latent period in 'CI 13227'. Values of relative specificity in quantitative resistance for isolates on 'CI 13227' were 3 to 5 times greater than for isolates on other cultivars. This suggests that resistance for latent period in 'CI 13227' would be less durable than that of other cultivars tested, provided that the variation of our seven isolates is similar to the variation present in the area of cultivar deployment. 'CI 13227' appeared to have four genes of unequal effects and with epistasis that control latent period (G. Shaner, G. Buechley, and W. Nyquist, *unpublished data*). Analysis of F_7 families of a recombinant inbred population indicated that genotypes that carried the plus allele for long latent period at the locus with the greatest effect had an average latent period that was 35% longer than the latent periods of genotypes with the opposite allele. If selection for greater fitness in the pathogen led to genotypes of *P. recondita* f. sp. *tritici* that overcame the effects of this one gene, they would reduce the MLP on 'CI 13227' from about 12.5 to 8.9 days according to this model. If a gene at one of the other three loci were overcome by the pathogen, this would reduce MLP from 12.5 to 10.6 days. This is about the magnitude of reduction in MLP we observed in the isolates most fit on 'CI 13227'. The adult-plant gene *Lr*34 might be involved in 'CI 13227's slow-rusting resistance and contribute to the greater degree of specificity observed for *P. recondita* f. sp. *tritici* isolates on 'CI 13227' (19). Isolates on cultivar Sw 72469-6 showed slightly less variation for latent period than isolates on 'CI 13227' and had a lower heritability for this trait. In addition, they showed the least specificity of host-pathogen interaction of all combinations tested. Thus, cultivar Sw 72469-6 should provide more durable disease control, despite having only moderate levels of partial resistance for latent period. The durability of 'L 574-1' and 'Suwon 85' are anticipated to be intermediate to those of 'CI 13227' and 'Sw 72469-6'.

In conclusion, the analysis of variation in traits affecting pathogen fitness and the partitioning of this variation into genetic and nongenetic components may improve our understanding of the adaptation of pathogen populations toward host resistance. In our studies, isolates of *P. recondita* f. sp. *tritici* on each resistant cultivar showed considerable variation for latent period. For the most part, this variation was moderately to highly heritable. Thus, isolates with short latent periods should be favored by natural selec-

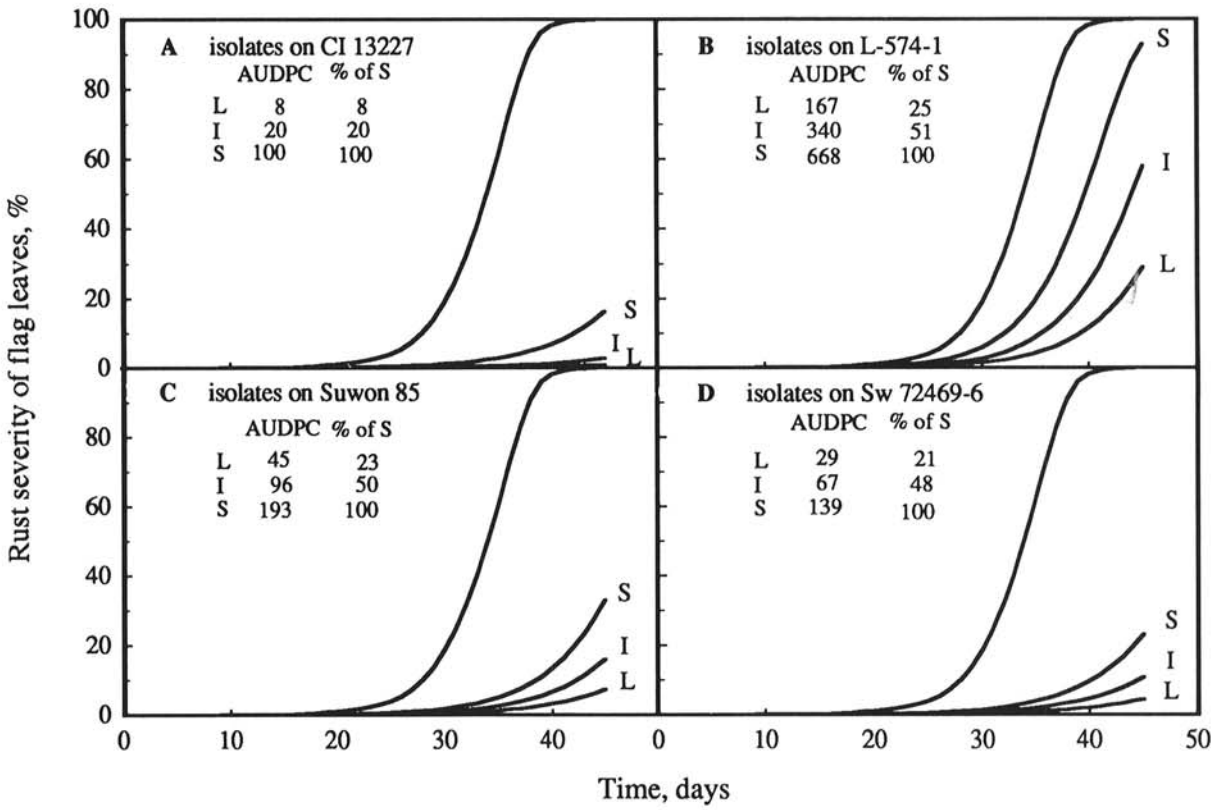

Fig. 3. Simulated development of disease for phenotypes of *Puccinia recondita* f. sp. *tritici* with long (L), intermediate (I), and short (S) latent periods on partially resistant wheat cultivars and for phenotypes with wild-type latent periods and sporulation parameters on susceptible cultivar Monon (uppermost simulated disease progress curve in **A through D**). On partially resistant cultivars **B,** L-574-1; **C,** Suwon 85; and **D,** Sw 72469-6, isolate phenotypes with intermediate latent periods caused on average 27% more disease than isolates with long latent periods. On **A,** 'CI 13227', the difference was only 12%. All isolates on partially resistant cultivars caused considerably less disease than did wild-type isolates on 'Monon'. AUDPC = area under the disease progress curve.

TABLE 5. Broad-sense heritabilities of $\log_{10}$ MLP and $\log_{10}$ T_{50} for isolates of *Puccinia recondita* f. sp. *tritici* on each of five cultivars of *Triticum aestivum*[z]

Trait	Cultivar				
	'Monon'	'L-574-1'	'Suwon 85'	'Sw 72469-6'	'CI 13227'
$\log_{10}$ MLP	–0.14	0.44	0.59	0.28	0.41
$\log_{10}$ T_{50}	–0.14	0.47	0.76	0.53	0.49

[z] MLP = the weighted mean number of days from infection to the production of secondary inoculum. T_{50} = the number of days required for 50% of the uredinia to erupt calculated from probit analysis (details in text). Estimates of heritability were calculated from mean squares of isolates, experiment × isolate interaction, and experimental error in the analysis of variance of latent period of isolates of *P. recondita* f. sp. *tritici* on wheat cultivars. Table 3 contains the formulas.

TABLE 6. Estimates of the relative specificity in quantitative resistance of wheat cultivars toward isolates of *Puccinia recondita* f. sp. *tritici*

Isolate	Adjusted $\log_{10}$ MLP[y]			
	'CI 13227'	'L-574-1'	'Suwon 85'	'Sw 72469-6'
754	0.25	0.06	0.10	0.14
757-1-2	0.15	0.10	0.10	0.11
759-1	0.19	0.15	0.09	0.16
7511	0.15	0.08	0.06	0.11
771	0.23	0.08	0.14	0.09
881	0.25	0.08	0.09	0.10
882	0.28	0.12	0.10	0.14
Variance for values of all isolates on cultivars	0.0028	0.0010	0.0008	0.0006
Relative specificity in resistance (%)[z]	100	37	31	22

[y] MLP = the weighted mean number of days from infection to the production of secondary inoculum (details in text). Values for MLP were adjusted for general fitness where general fitness is estimated from $\log_{10}$ MLP values of individual fungal isolates on 'Monon'. Adjusted values were calculated from the equation: adjusted $\log_{10}$ MLP = ($\log_{10}$ MLP of cultivar-isolate combination) – ($\log_{10}$ MLP of 'Monon'-isolate combination) for the same isolate.

[z] Values are the variance of adjusted $\log_{10}$ MLP for individual cultivars expressed as a percentage of the variance of isolates on 'CI 13227'.

tion, and partially resistant cultivars may be rendered less effective following their deployment, provided short latent period is unlinked to deleterious traits. Our results also suggest that the level of host resistance should not be the only criterion in determining the utility of germ plasm. In our study, the cultivar of greatest resistance may prove to be the least durable based on its relative specificity in quantitative resistance. Information pertaining to the durability of host resistance should be considered before incorporating partial resistance into agronomically adapted host genetic backgrounds.

LITERATURE CITED

1. Broers, L. H. M. 1989. Influence of development stage and host genotype on three components to resistance to leaf rust in spring wheat. Euphytica 44:187-189.
2. Broers, L. H. M. 1989. Partial resistance to wheat leaf rust in 18 spring wheat cultivars. Euphytica 44:247-258.
3. Broers, L. H. M. 1989. Race-specific aspects of partial resistance in wheat to wheat leaf rust, *Puccinia recondita* f. sp. *tritici*. Euphytica 44:273-282.
4. Caldwell, R. M. 1968. Breeding for general and/or specific resistance. Pages 263-272 in: Proc. Int. Wheat Genet. Symp., 3rd. Australian Academy of Science, Canberra.
5. Caldwell, R. M., Roberts, J. J., and Eyal, Z. 1970. General resistance ("slow rusting") to *Puccinia recondita* f. sp. *tritici* in winter and spring wheats. (Abstr.) Phytopathology 60:1287.
6. Carmer, S. G., Nyquist, W. E., and Walker, W. M. 1989. Least significant differences for combined analyses of experiments with two- or three-factor treatment designs. Agron. J. 81:665-672.
7. Croft, J. H., and Simchen, G. 1965. Natural variation among monokaryons of *Collybia velutipes*. Am. Nat. 99:451-462.
8. Eenink, A. H. 1976. Genetics of host-parasite relationships and uniform and differential resistance. Neth. J. Plant Pathol. 82:133-145.
9. Emara, Y. A., and Sidhu, G. 1974. Polygenic inheritance of aggressiveness in *Ustilago hordei*. Heredity 32:219-224.
10. Gavinlertvatana, S., and Wilcoxson, R. D. 1978. Inheritance of slow rusting of spring wheat by *Puccinia recondita* f. sp. *tritici* and host parasite relationships. Trans. Br. Mycol. Soc. 71:413-418.
11. Hamid, A. H., Ayers, J. E., Schein, R. D., and Hill, R. R., Jr. 1982. Components of fitness attributes in *Cochliobolus carbonum* race 3. Phytopathology 72:1166-1169.
12. Hamid, A. H., Ayers, J. E., and Hill, R. R., Jr. 1982. Host × isolate interactions in corn inbreds inoculated with *Cochliobolus carbonum* race 3. Phytopathology 72:1169-1173.
13. Hill, J. P., and Nelson, R. R. 1982. The heritability of three parasitic fitness attributes of *Helminthosporium maydis* race T. Phytopathology 72:525-528.
14. Jenns, A. E., and Leonard, K. J. 1985. Reliability of statistical analyses for estimating specificity in quantitative resistance in a model host-pathogen system. Theor. Appl. Genet. 69:503-513.
15. Jenns, A. E., Leonard, K. J., and Moll, R. H. 1982. Stability analyses for estimating relative durability of quantitative resistance. Theor. Appl. Genet. 63:183-192.
16. Jinks, J. L., Caten, C. E., Simchen, G., and Croft, J. H. 1966. Heterokaryon incompatibility and variation in wild populations of *Aspergillus nidulans*. Heredity 21:227-239.
17. Johnson, D. A., and Wilcoxson, R. D. 1979. Inheritance of slow rusting of barley infected with *Puccinia hordei* and selection of latent period and number of uredia. Phytopathology 69:145-151.
18. Johnson, R. 1984. A critical analysis of durable resistance. Annu. Rev. Phytopathol. 22:309-330.
19. Kolmer, J. A. Genetics of resistance to wheat leaf rust. Annu. Rev. Phytopathol. In press.
20. Kolmer, J. A., and Leonard, K. J. 1985. Genetic variation and selection for fertility in the fungus *Cochliobolus heterostrophus*. Heredity 55:335-339.
21. Kolmer, J. A., and Leonard, K. J. 1986. Genetic selection and adaptation of *Cochliobolus heterostrophus* to corn hosts with partial resistance. Phytopathology 76:774-777.
22. Kuhn, R. C., Ohm, H. W., and Shaner, G. E. 1978. Slow leaf-rusting resistance in wheat against twenty-two isolates of *Puccinia recondita*. Phytopathology 68:651-656.
23. Lehman, J. S., and Shaner, G. E. 1990. Adaptation of *Puccinia recondita* to slow-rusting wheat cultivars due to artificial selection. (Abstr.) Phytopathology 80:999.
24. Lehman, J. S., and Shaner, G. E. 1992. Correlation between pathogen fitness components and epidemics of wheat leaf rust. (Abstr.) Phytopathology 82:1161-1162.
25. Long, D. L., and Kolmer, J. A. 1989. A North American system of nomenclature for *Puccinia recondita* f. sp. *tritici*. Phytopathology 79:525-529.
26. Merrick, M. J. 1976. Hybridization and selection for penicillin production in *Aspergillus nidulans*–A biometrical approach to strain improvement. Pages 229-242 in: Genetics of Industrial Microorganisms. K. D. MacDonald, ed. Academic Press, Inc., New York.
27. Milus, E. A., and Line, R. F. 1980. Characterization of resistance to leaf rust in Pacific Northwest wheats. Phytopathology 70:167-172.
28. Nyquist, W. E. 1991. Estimation of heritability and prediction of selection response in plant populations. Crit. Rev. Plant Sci. 10:235-322.
29. Ohm, H. W., and Shaner, G. E. 1976. Three components of slow leaf-rusting at different growth stages in wheat. Phytopathology 66:1356-1360.
30. Papa, K. E., Srb, A. M., and Federer, W. T. 1966. Selection for increased growth rate in inter- and intra-strain crosses of *Neurospora*. Heredity 21:595-613.
31. Parlevliet, J. E. 1975. Partial resistance of barley to leaf rust, *Puccinia hordei*. I. Effect of cultivar and development stage on latent period. Euphytica 24:21-27.
32. Parlevliet, J. E., Leijn, M., and van Ommeren, A. 1985. Accumulating polygenes for partial resistance in barley to barley leaf rust, *Puccinia hordei*. II. Field evaluation. Euphytica 34:15-20.
33. Parlevliet, J. E., and van Ommeren, A. 1975. Partial resistance of barley to leaf rust, *Puccinia hordei*. II. Relationship between field traits, microplot tests, and latent period. Euphytica 24:293-303.
34. Parlevliet, J. E., and Zadoks, J. C. 1977. The integrated concept of disease resistance; A new view including horizontal and vertical resistance in plants. Euphytica 26:5-21.
35. Pateman, J. A. 1955. Polygenic inheritance in *Neurospora*. Nature 176:1274-1275.

36. Shaner, G. 1980. Probits for analyzing latent period data in studies of slow rusting resistance. Phytopathology 70:1179-1182.
37. Shaner, G. 1983. Growth of uredinia of *Puccinia recondita* in leaves of slow- and fast-rusting wheat cultivars. Phytopathology 73:931-935.
38. Shaner, G., and Finney, R. E. 1980. New sources of slow leaf rusting resistance in wheat. Phytopathology 70:1183-1186.
39. Shaner, G., and Hess, F. D. 1978. Equations for integrating components of slow leaf-rusting resistance in wheat. Phytopathology 68:1464-1469.
40. Shaner, G., Ohm, H. W., and Finney, R. E. 1978. Response of susceptible and slow leaf-rusting wheats to infection by *Puccinia recondita*. Phytopathology 68:471-475.
41. Simchen, G. 1965. Variation in dikaryotic populations of *Collybia velutipes*. Genetics 51:709-721.
42. Simchen, G. 1966. Monokaryotic variation and haploid selection in *Schizophyllum commune*. Heredity 21:241-263.
43. Simchen, G., and Jinks, J. L. 1964. The determination of dikaryotic growth rate in the basidiomycete *Schizophyllum commune*: A biometrical analysis. Heredity 19:629-649.
44. Simons, M. D. 1972. Polygenic resistance to plant disease and its use in breeding resistant cultivars. J. Environ. Qual. 1:232-240.
45. Steel, J. G. D., and Torrie, J. H. 1980. Principles and Procedures of Statistics: A Biometrical Approach, 2nd ed. McGraw-Hill Book Co., New York.
46. Vanderplank, J. E. 1968. Disease Resistance in Plants. Academic Press, Inc., New York.
47. Wilson, J., and Shaner, G. 1989. Individual and cumulative effects of long latent period and low infection type reactions to *Puccinia recondita* in triticale. Phytopathology 79:101-108.