Latent Period and Infection Efficiency of *Puccinia recondita* f. sp. *tritici* Populations Isolated from Different Wheat Cultivars

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ABSTRACT

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Five bulk populations of *Puccinia recondita* f. sp. *tritici* were isolated from five susceptible wheat (*Triticum aestivum*) cultivars showing different levels of disease in the field. Each population was inoculated onto wheat plants of the cultivar from which they were originally isolated (their "own" cultivar) and onto the other four cultivars. Latent period and infection efficiency were measured in growth chambers for each of the population × cultivar combinations. No evidence of increased aggressiveness on the "own" cultivars was found for either infection efficiency or latent period. No significant cultivar × spore population interactions were detected in the infection efficiency analysis. However, significant interactions were detected with latent period. Spore populations differed in aggressiveness as measured by both latent period and infection efficiency. Rankings of the spore populations were different for infection efficiency vs. latent period.

Additional keywords: aggressiveness, leaf rust, rate-reducing resistance.

Understanding variability among pathogen populations for quantitatively measured traits such as latent period and infection efficiency is of great importance to the management of plant disease through host resistance. If such variability occurs within pathogen populations, increased aggressiveness (an increase in the extent of disease on a compatible host) could conceivably be selected for over time (1,4,8,13), perhaps reducing the durability of resistance (6,13,17). Significant pathogen variability also affects our ability to detect rate-reducing resistance, because some isolates of the pathogen will not be as aggressive on a cultivar as others. On the other hand, host-specific selection for pathogen aggressiveness may reduce the impact of complex pathogen races in cultivar mixtures (5,16).

Variability among pathogen populations for quantitatively measured traits has been documented in several plant/pathogen systems (4–7,9,11,15,20). For example, Whitney and Mackey (20) found an 18-fold difference in aggressiveness among different strains of *Erwinia carotovora* subsp. *betavasculorum* inoculated onto sugar beet (*Beta vulgaris*) cultivars.

Several authors have also investigated whether selection for increased aggressiveness occurred after several cycles of pathogen reproduction on the same host (1,6,8,10,13,14). Most authors who investigated this phenomenon in potato late blight found that

TABLE 1. Analysis of variance df, mean squares, and significance values ($P>F$) over all growth chambers for first latent period (FLPD), mid-latent period (MLPD), infection efficiency (INF)[a], and for the contrast between spore populations on the cultivar from which they were originally isolated vs. spore populations grown on cultivars from which they had not been isolated (own vs. other)

Source of variation[b]	df	FLPD		MLPD		INF	
		Mean square	$P>F$	Mean square	$P>F$	Mean square	$P>F$
Model	55	1,231.3	0.0001	1,524.4	0.0001	2,001.9	0.0001
Error	...[c]	265.5	...	324.4	...	244.8	...
SPOP	4	1,323.7	0.0006	152.8	0.7570	10,762.3	0.0001
CVAR	4	652.7	0.0451	2,282.1	0.0001	2,541.7	0.0001
GC	3	8,389.0	0.0001	9,696.7	0.0001	3,238.6	0.0001
LF	1	3,883.0	0.0002	6,867.9	0.0001	4,403.7	0.0001
SPOP × CVAR	16	582.2	0.0051	782.4	0.0018	339.3	0.1435
SPOP × LF	4	317.5	0.3121	60.1	0.9460	493.6	0.0911
SPOP × GC	11	815.4	0.0006	635.1	0.0314	1,774.5	0.0001
CVAR × GC	12	632.4	0.0057	923.4	0.0009	936.2	0.0001
Own vs. other	1	6.9	0.8720	0.6	0.9669	118.4	0.4870

[a]FLPD is the time from inoculation to first spore production, MLPD is the time from inoculation until the median uredinium begins to sporulate, and INF is the number of uredinia per unit leaf area.
[b]SPOP is spore populations, CVAR is cultivar, and GC is growth chambers.
[c]Degrees of freedom for error differed for the three measurements of aggressiveness due to differing numbers of missing values. The actual error df were 384, 382, and 461 for FLPD, MLPD, and INF, respectively.

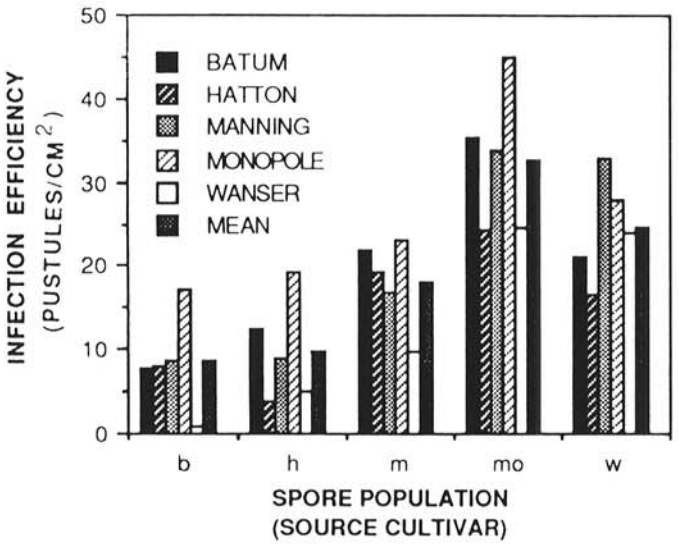

Fig. 1. Infection efficiency values for the combined (upper and lower) leaf data from *Puccinia recondita* populations collected from five different wheat cultivars in the field (source cultivars) and inoculated on the same five cultivars in growth chambers. Tukey's honestly significant difference value for the spore population mean at the 0.01 probability level = 6.92.

isolates of *Phytophthora infestans* were more aggressive on the potato (*Solanum tuberosum*) cultivars from which they were originally isolated, but found no evidence of increased aggressiveness after cycling (4,9,10,13). Clifford and Clothier (6) found similar results with barley (*Hordeum vulgare*) and leaf rust (*Puccinia hordei*). James and Fry (8), however, found no evidence for increased aggressiveness on the own cultivar, nor any evidence that indicated selection after cycling with the potato late blight system. On the other hand, Leonard (14) found that after subcycling a *Puccinia graminis* f. sp. *avenae* population on two different oat (*Avena sativa*) cultivars, each subpopulation produced more pustules on its own cultivar than on the other cultivar. The sporulation rate did not change significantly from the own to the other cultivar, however.

In this study, we examined the aggressiveness of populations of wheat leaf rust (*Puccinia recondita* f. sp. *tritici* Rob.) on the cultivar from which they were isolated and on four other cultivars. We quantified infection efficiency and two measures of latent period to determine the variability among bulk populations and to determine whether the populations were more aggressive on their own cultivars.

MATERIALS AND METHODS

Plants. Seeds for the cultivars used in this experiment, Monopole, Manning, Batum, Wanser, and Hatton, were obtained from the Oregon State University Wheat Breeding Project, Corvallis. All of these cultivars are hard red winter wheats and have been rated as susceptible to leaf rust of wheat.

Seeds of each were planted in 6.4-cm plastic pots, one plant per pot, in sterilized medium composed of pumice, peat, sand, and soil in a ratio of 2:1:1:1, and grown in a greenhouse at 24/18 C with a 16-h light/8-h dark regime. All plants were watered daily and fertilized every 2 wk with Miracle Gro (15-30-15). The fungicide fenarimol was vaporized in the greenhouse on a weekly basis to eliminate powdery mildew (*Erysiphe graminis*). Fenarimol has no effect on wheat leaf rust in the manner applied.

Pathogen populations. Urediniospores from 30–50 infected wheat leaves were collected from each of five different cultivars grown in a 1.5 × 4.3 m plot in each of two adjacent fields in Corvallis, OR. Populations from different cultivars were kept separate, but populations from a given cultivar collected from different fields were bulked. At the time of collection, the cultivars differed in disease severity. The cultivars Monopole and Manning had 90% of their leaves covered with pustules. The cultivar Hatton was 70% covered, and the cultivars Batum and Wanser were 50% and 40% covered, respectively. All plants in the field showed susceptible lesion types, except for the cultivar Batum, which had moderately susceptible type lesions. The collecting apparatus was cleaned after use on each cultivar with acetone, followed by a distilled water rinse.

After collection, the urediniospore populations were placed in a drying chamber with calcium phosphate for 36 h, weighed, and stored in liquid nitrogen. Upon removal from liquid nitrogen, spores were heat-shocked in a 48 C water bath for 8 min (3).

Inoculations. Five-week-old plants of each cultivar were used for each inoculation. The cultivars had been grown in randomized complete blocks in the greenhouse under the conditions described earlier. All cultivars were at the four-leaf stage at the time of inoculation.

Each spore population was inoculated onto each cultivar by placing 0.002 g of viable spores per plant in 6 ml of distilled water with two drops of Tween 80 per 50 ml added as a surfactant. Before inoculation, the percentage of viable spores per population was determined from a germination test on water agar plates. The third and fourth oldest leaves of each plant were inoculated. The spores were sprayed onto the leaves with an aspirator from a distance of about 0.3 m. The aspirator was rinsed with 3.0 ml of distilled water, which was also sprayed onto the plants. The leaves were turned during spraying so that both sides of

the leaves were coated. Once sprayed, the plants were moved to a dark, 18–20 C mist chamber for 12–16 h and then moved to a growth chamber at a constant 21 C and a 12-h day/night schedule.

There were three replications of each spore population × cultivar combination per block, and four blocks. There was only one replication of the spore population from Wanser in block three, and none in block four due to a shortage of inoculum. Each block of plants was inoculated separately and subsequently placed in separate growth chambers because all of the plants would not fit into the mist chamber or a single growth chamber. The use of a randomized complete block design in the statistical analysis allowed effects of inoculation time and growth chamber to be accounted for.

The leaves were examined for signs of sporulation beginning 6 days after inoculation. These examinations were conducted twice daily, approximately 7 h apart. Pustules were considered as sporulating if orange was visible and the leaf epidermis had been broken. All uredia appeared to be of a susceptible or moderately susceptible (sporulation with a little chlorosis) type, but no distinctions between reaction types were made during counting. Uredinia were counted in a 4-cm marked length on the leaf approximately 2 cm from the shoot. Both sides of the leaf were counted, because uredinia would often break through one side but not the other. Leaves were examined until the pustule count was the same for three successive time periods, usually around the 13th or 14th day after inoculation.

Infection efficiency was calculated by dividing the maximum number of uredinia counted by the area of the leaf. The lengths of the marked areas were measured on each individual leaf after final pustule number had been reached to account for any leaf growth that occurred after the initial marks were made. An average width was used for the marked areas of both the upper and lower leaves of each cultivar because some plants had been discarded before their widths were measured. The average was calculated from 10 to 20 plants of each cultivar. The variance for these measurements ranged from 0.69 mm to 0.24 mm.

Two latent periods were calculated; the first latent period was the time in hours from inoculation to first sporulation, and the second was the mid-latent period calculated as the time in hours when half of the maximum number of lesions were sporulating. A linear interpolation was used to estimate this time.

Statistical analysis. The experiment was subjected to analysis of variance and treated as a factorial randomized complete block

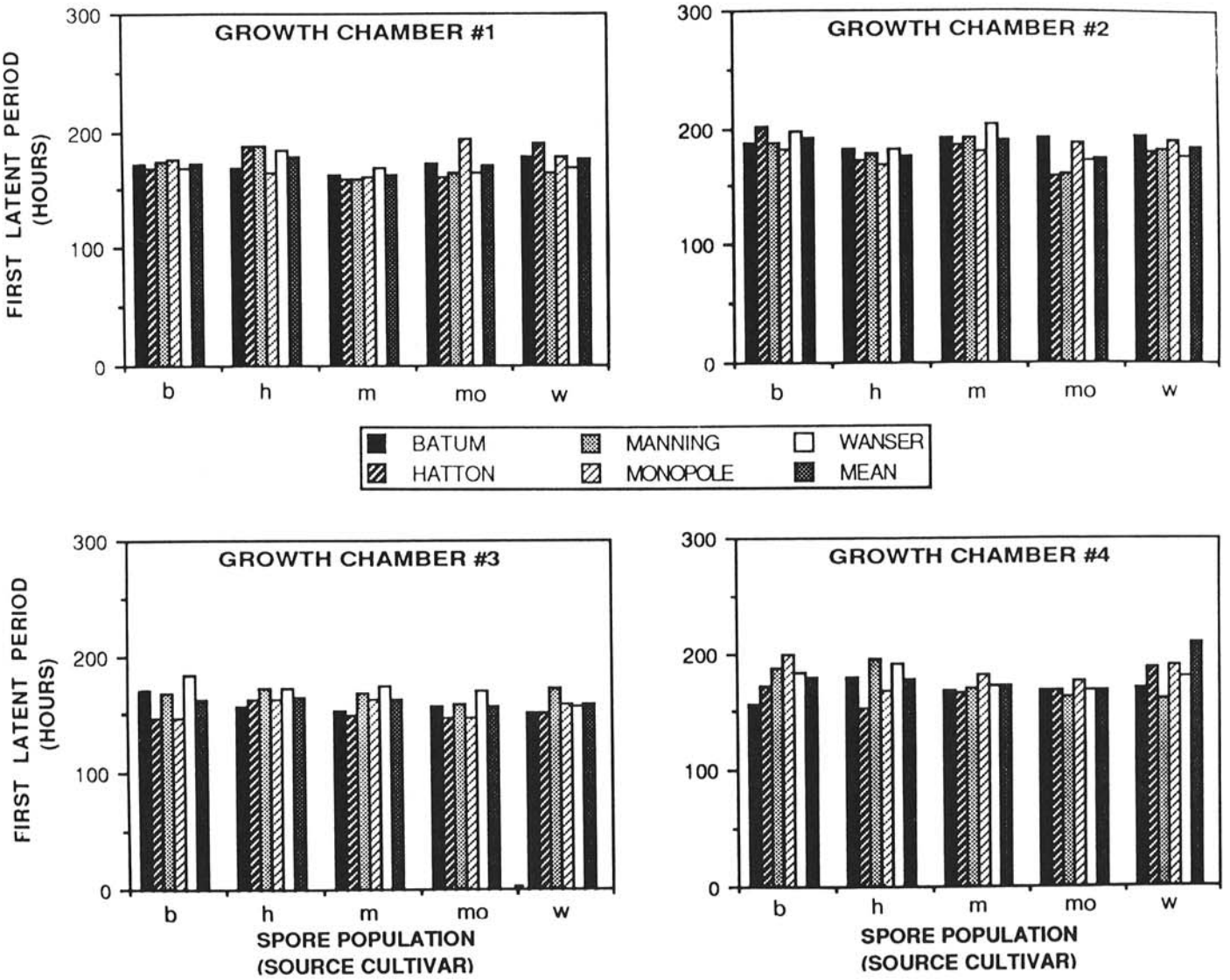

Fig. 2. First latent period values (time from inoculation to first spore production) resulting from *Puccinia recondita* populations collected from five different wheat cultivars in the field and inoculated onto the same five cultivars. Data are combined over two leaf positions. Tukey's honestly significant difference (HSD) for spore population × cultivar means at the 0.05 and 0.01 probability levels are 7.96 and 9.03, respectively, for GC 1, and 5.2 and 5.9, respectively, for GC 2. HSD values for the spore population means averaged over all cultivars at the 0.05 and 0.01 probability levels are 13.44 and 16.15, respectively, for GC 1, and 8.8 and 10.7, respectively, for GC 2. The spore population effects for GC 3 and GC 4 were not significant at the 0.05 level in the analysis of variance.

design with growth chambers (GC) as blocks, and cultivars (CVAR), spore populations (SPOP) and leaf position (LF) as main effects. Analyses were done with the general linear models procedure of the SAS statistics package (19) to account for missing data. Interaction terms were calculated for spore population × cultivar (SPOP × CVAR) spore population × leaf (SPOP × LF) spore population × growth chamber (SPOP × GC) and cultivar × growth chamber (CVAR × GC). Analyses for the latent period measurements were also run on single growth chambers because of statistically significant ($P = 0.01$) SPOP × GC interaction terms that nearly equaled in size the mean square for spore population (Table 1). Single growth chamber analyses were not run on the infection efficiency data because the SPOP × GC interaction term, though significant ($P = 0.0001$), was six times smaller than the SPOP main effect. A linear contrast (own vs. others) was run between spore populations on the cultivar from which they were isolated, their "own," and all other cultivars, "others."

Tukey's honestly significant difference (HSD) was calculated for those terms that were significantly different ($P = 0.05$) in the analysis of variance. HSD values for the infection efficiency spore population mean differences were reported only at the 0.01 probability level to ensure that no false differences were detected due to the significant GC × SPOP interaction. The values of the combined means (combined over upper and lower leaves) for all traits were tested for significant differences because the SPOP × LF interaction was not statistically significant ($P = 0.01$), except in one growth chamber for first latent period.

RESULTS

A significant difference between upper and lower leaves was found for each trait examined (Table 1). On average, the spore populations exhibited 25% higher infection efficiency, 3% shorter first latent periods, and 4% shorter mid-latent period on the upper leaves than the lower leaves. This could be because the upper leaves were more susceptible, or because the lower leaves were often more chlorotic or necrotic. The SPOP × LF interaction term, however, was not significant for any of the traits examined.

The spore population effect, which indicates the influence of the source cultivar on the aggressiveness of the pathogen, was significant at $P = 0.01$ for infection efficiency (Table 1). The spore population effect was also significant at $P = 0.05$ for first latent period in two growth chambers and in one growth chamber

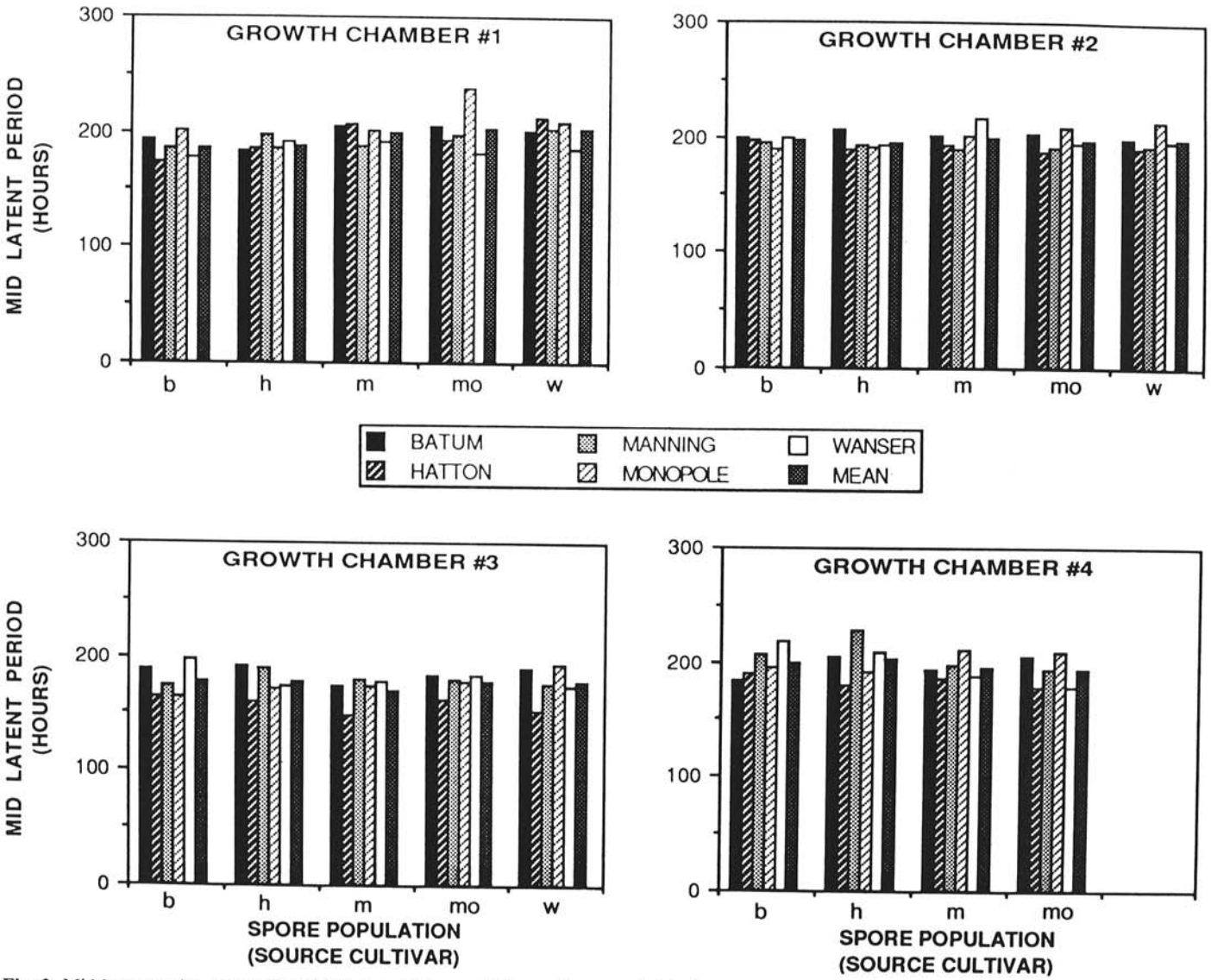

Fig. 3. Mid-latent period values (time from inoculation until the median pustule begins to sporulate) resulting from inoculation of five populations of *Puccinia recondita* collected from five different wheat cultivars in the field onto the same five cultivars. Data are combined over two leaf positions. Tukey's honestly significant difference value for spore population × cultivar means at the 0.05 and 0.01 probability levels are 9.5 and 10.8, respectively, for GC 1, and 4.9 and 5.7, respectively, for GC 2. HSD values for the spore population means averaged over all cultivars at the 0.05 and 0.01 probability levels are 16.0 and 19.2, respectively, for GC 1. The spore population effects for GC 2, GC 3, and GC 4 were not significant at the 0.05 level in the analysis of variance.

for mid-latent period. The influence of source cultivar on infection efficiency of the pathogen was considerable, with infection efficiency increasing fourfold from the least to the most favorable source (Fig. 1). There was no evidence of the pathogen being more aggressive on the cultivar from which it was isolated (Figs. 1–3), and the own vs. other contrast was not significant for any trait.

The importance of spore population × cultivar interactions depended on the trait measured. For infection efficiency, there were only minor changes in the ranking of the cultivars depending on the spore population used (Fig. 1). Consequently, the spore population × cultivar interaction was not statistically significant. With both measurements of latent period, the interactions were much larger. For example, with first latent period in growth chamber 1, the cultivar Monopole is ranked as least resistant with the spore population from Hatton, but most resistant with populations from Monopole (Fig. 2). The ranking of the cultivars for latent period varied among growth chambers as well as with different spore populations (Figs. 2–3).

DISCUSSION

The own vs. other contrast in this experiment was not significant for any of the traits measured. There were also no individual cases where pathogen populations were statistically more aggressive on their own cultivar than on any other cultivar (Figs. 1–3). Previous studies have revealed several instances where pathogen populations were more aggressive on the cultivar from which they were originally isolated (4–6,9,14), but a few studies had results similar to ours (8,17,20).

Significant pathogen × cultivar interactions for nonhypersensitive resistance reactions have been seen in several studies (2,6,11–13,17,18,20), including interactions between races of leaf rust and winter wheat cultivars (12,15). Increased aggressiveness of pathogens on their own cultivars clearly caused the interaction term in one case (6). For other studies (11,12,15,17,18,20), the source of the interaction could not be determined. In our case, the pathogen × cultivar interaction was not caused by increased pathogen aggressiveness on the own cultivars.

The effect of the source cultivars on population aggressiveness was evidenced by significant differences between the spore populations. There are two possible explanations for this result. One is that the source cultivars had different selective effects on the pathogen in the field. This would alter the composition of the populations collected from the field. Holub (7) found such selectivity in an experiment on *Aphanomyces euteiches*. He observed that if pea (*Pisum sativum*) was used as the baiting host, isolates of *A. euteiches* retrieved were more aggressive on pea. However, when other species were used as the baiting host, no differences in aggressiveness were observed. Holub (7) suggested that pea plants may have been more resistant than the other species and so selected only those isolates that were aggressive on pea.

Another explanation for the significant spore population effects in our study is that the populations we collected arose from immigration into the fields of genetically different isolates that maintained a degree of physical isolation within the fields. Thus, genetically different pathogen populations would be increasing in different plots. However, as several of the cultivars we sampled from were grown in adjacent plots, it is unlikely that physical separation of the plots could account for the deficiency in the fungal populations.

Our results indicate that pathogen populations originating from natural inoculum are much less uniform than is often assumed. Thus, host genotypes grown in the field may be tested with pathogen genotypes that differ considerably in their degree of aggressiveness. We also detected no significant increase in aggressiveness of any of the populations when inoculated onto their source cultivar. However, this study does not discount such interactions as we took only a few measurements of aggressiveness and were limited in our ability to detect more subtle differences.

LITERATURE CITED

1. Alexander, H. M., Groth, J. V., and Roelfs, A. P. 1985. Virulence changes in *Uromyces appendiculatus* after five asexual generations on a partially resistant cultivar of *Phaseolus vulgaris*. Phytopathology 75:449-453.
2. Bonman, J. M., and Bandong, J. M. 1989. Race-specific partial resistance to blast in temperate Japonica rice cultivars. Plant Dis. 73:496-499.
3. Bromfield, K. R. 1967. Some uredospore characteristics of importance in experimental epidemiology. Plant Dis. Rep. 51:248-252.
4. Caten, C. E. 1974. Intra-racial variation in *Phytophthora infestans* and adaptation to field resistance for potato blight. Ann. Appl. Biol. 77:259-270.
5. Chin, K. M., and Wolfe, M. S. 1984. Selection of *Erysiphe graminis* in pure and mixed stands of barley. Plant Pathol. 33:535-546.
6. Clifford, B. C., and Clothier, R. B. 1974. Physiologic specialization of *Puccinia hordei* on barley hosts with nonhypersensitive resistance. Trans. Br. Mycol. Soc. 63(3):421-430.
7. Holub, E. 1988. Pathogenic diversity of *Aphanomyces eutiches* on seedlings of pea, alfalfa, green bean, and red clover. Ph.D. dissertation. University of Wisconsin, Madison.
8. James, R. V., and Fry, W. E. 1983. Potential for *Phytophthora infestans* populations to adapt to potato cultivars with rate reducing resistance. Phytopathology 73:984-988.
9. Jefferey, S. I. B., Jinks, J. L., and Grindle, M. 1962. Intraracial variation in *Phytophthora infestans* and field resistance to potato blight. Genetica 32:323-338.
10. Jinks, J. L., and Grindle, M. 1963. Changes induced by training in *Phytophthora infestans*. Heredity 18(3):245-264.
11. Krupinsky, J. M. 1989. Variability in *Septoria musiva* in aggressiveness. Phytopathology 79:413-416.
12. Kuhn, R. C., Ohm, H. W., and Shaner, C. E. 1978. Slow leaf-rusting resistance in wheat against twenty-two isolates of *Puccinia recondita*. Phytopathology 68:652-656.
13. Latin, R. X., Mackenzie, D. R., and Cole, H., Jr. 1981. The influence of host and pathogen genotypes on the apparent infection rates of potato late blight epidemics. Phytopathology 71:82-85.
14. Leonard, K. J. 1969. Selection in heterogenous populations of *Puccinia graminis* f. sp. *avenae*. Phytopathology 59:1851-1857.
15. Milus, E. A., and Line, R. F. 1980. Characterization of resistance to leaf rust in Pacific Northwest wheat lines. Phytopathology 70:167-172.
16. Mundt, C. C., and Browning, J. A. 1985. Genetic diversity and cereal rust management. Pages 527-559 in: The Cereal Rusts. Vol. II. A. P. Roelfs and W. R. Bushnell, eds. Academic Press, Inc., New York.
17. Newton, A. C. 1989. Genetic adaptation of *Erysiphe graminis* f. sp. *hordei* to barley with partial resistance. J. Phytopathol. 126:133-148.
18. Parlevliet, J. E. 1977. Evidence of differential interaction in the polygenic *Hordeum vulgare–Puccinia hordei* relation during epidemic development. Phytopathology 67:776-778.
19. SAS Institute, Inc. 1988. SAS/STAT User's Guide, Release 6.03 edition. SAS Institute Inc., Cary, NC.
20. Whitney, E. D., and Mackey, B. E. 1989. Differences in aggressiveness of *Erwinia caratovora* subsp. *betavasculorum* strains and their reactions to sugar beet cultivars. Plant Dis. 73:220-222.
21. Vanderplank, J. E. 1978. Genetic and Molecular Basis of Plant Pathogenesis. Springer-Verlag, Berlin and New York.