Selection of Virulence Phenotypes in a Heterogeneous, Asexual Population of *Puccinia recondita* f. sp. *tritici*

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ABSTRACT

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A heterogeneous population of *Puccinia recondita* f. sp. *tritici* was cultured for eight asexual uredinial generations on adult plants of the wheat lines Thatcher, Thatcher isogenic line *Lr13*, TC*Lr16*, and Chris (*Lr13* + *Lr34*). Frequencies of virulence phenotypes changed significantly from the initial generation in the populations cultured on TC*Lr13*, TC*Lr16*, and Chris. Little change was observed in frequencies of virulence phenotypes in the Thatcher population. The Chris, TC*Lr13*, and Thatcher populations maintained the phenotypic diversity found in the initial population. The TC*Lr16* population was dominated by one virulence phenotype after eight generations of selection. Rogers indexes of phenotypic overlap indicated that TC*Lr13* and Chris selected similar populations. Urediniospore production tests indicated that the TC*Lr16*, and Chris populations had become specifically adapted to their respective hosts relative to the initial population. The population cultured on TC*Lr13* showed little adaptation to TC*Lr13*, and the population cultured on Thatcher showed no adaptation. The changes in virulence phenotype frequencies and adaptation within the populations were most likely caused by nonrandom distribution of genetic variation for urediniospore production on the selection lines within the initial population.

Additional keywords: adult plant resistance, *Triticum*, virulence associations, wheat leaf rust.

Populations of *Puccinia recondita* f. sp. *tritici* (causal agent of wheat leaf rust) in Canada have been characterized as being relatively heterogeneous for virulence phenotypes (6,7,9) even though the sexual cycle of the fungus is not believed to be important in the epidemiology of the disease in North America (17). Leaf rust susceptible cultivars are generally grown in eastern Canada (southern Ontario and Quebec) and the Pacific region of southern Alberta and British Columbia. The leaf rust populations in these regions have higher levels of diversity for virulence phenotypes than the population in the prairie region of Manitoba and Saskatchewan, where resistant cultivars have been grown since 1937 (7,17). The annual surveys of physiologic specialization in the leaf rust fungus have concentrated on describing levels of specific virulence to seedling resistance genes (6,7,9). The leaf rust population in the prairie region has been characterized by directional increases in frequencies of virulence to resistance genes that have been released either in Canada or in the Great Plains region of the United States (7). The leaf rust populations in the eastern and Pacific regions of Canada have not been subjected to the same degree of directional selection for increased frequencies of virulence, and have consistently retained higher levels of phenotypic diversity than the prairie population (J. A. Kolmer, *unpublished data*).

Adult plant resistance gene *Lr13* has been in continuous use in the prairie region since 1966 when the cultivar Manitou was licensed and released for production (17). Gene *Lr13* was also used singly in Neepawa (1969) and Katepwa (1984), and in combination with *Lr16* in Columbus (1982) (18). This gene has also been used in combination with the adult plant gene *Lr34* in the American cultivars Chris and Era (16). The selective effects of adult plant resistance genes on populations of *P. recondita* have not been thoroughly characterized since it is difficult and time consuming to evaluate individual isolates of leaf rust for virulence on adult plants with *Lr13* and/or *Lr34*. If virulences to adult plant resistance genes are not randomly distributed throughout the leaf rust population, the continuous deployment of these genes may have an indirect selective effect on unrelated virulences to seedling resistance genes. Nonrandom associations among virulences to various resistance genes have been characterized in the eastern and prairie leaf rust populations (8).

This present study was undertaken to determine the selective effects of various adult plant and seedling resistance genes on a heterogeneous, asexual population of wheat leaf rust. A population of wheat leaf rust was cultured on a susceptible line, two lines with adult plant resistance genes, and a line with a seedling resistance gene. The selective effects of each line were determined by evaluating single pustule isolates from every other generation of selection on differential sets of isogenic lines with seedling resistance genes.

MATERIALS AND METHODS

Initial population and host selection lines. Forty-eight single-pustule isolates from the eastern 1987 population (6) were used as the initial population in the selection studies. The eastern population was chosen since it is more heterogeneous for virulence phenotypes than the population found in the prairie region. Five milligrams of urediniospores from each isolate were bulked together to form the initial population.

The leaf rust susceptible cultivar Thatcher, a Thatcher backcross line with adult plant resistance gene *Lr13* (TC*Lr13*), seedling resistant line TC*Lr16*, and the cultivar Chris (*Lr13* + *Lr34*) were chosen as the selective host lines. Gene *Lr13* has been used in many Canadian and American hard red spring wheats. *Lr16* is used in the Canadian cultivar Columbus (18) and American hard red winter wheats (12). The combination of *Lr13* + *Lr34* has been used in American hard red spring wheat breeding programs and has provided high levels of durable resistance (16). All isolates in the initial population produced avirulent infection types of 1+ to 2n (small to medium pustules surrounded by necrosis) on seedlings of TC*Lr16*; infection types of 2 to 2+ (medium to large pustules surrounded by chlorosis) on flag leaves of TC*Lr13*, and infection types of ;3+ (hypersensitive flecks with large pustules) on flag leaves of Chris.

General procedures. The experiments were based on a sequence of culturing separate populations of uredinia derived from the

initial population on each of the selective host lines for eight uredinial generations. The initial population was inoculated onto each of the selective host lines. Subsequently urediniospores were collected from each host line in each generation and inoculated onto plants of the same host line for the next generation. For each generation the selection lines were grown in a greenhouse at 15–20 C with 8 hr of supplemental fluorescent light/day in 10 6-in. fiberpots filled with a mixture of soil and peat moss, with 10 plants/pot. The plants were treated regularly with 20-20-20 N-P-K water soluble fertilizer. The selection lines were inoculated with 25 mg of urediniospores from the initial and subsequent selection generations when the lines were at the heading stage. The urediniospores were mixed with talc and deposited on the plants using a settling tower (15) to ensure an even distribution of uredinial infections and were incubated in a dew chamber for 16–24 hr. After incubation each selection line was maintained in a separate greenhouse section to prevent cross contamination among the selected rust populations. After 14 days rust was collected from each selection line every 2–3 days by tapping the infected plants over sheets of plastic cellophane. Collected urediniospores were vacuum dried, and stored at 4 C in sealed glass vials until used to inoculate the next generation.

Evaluation of virulence phenotypes. Thatcher lines isogenic for seedling resistance genes *Lr1*, *Lr2a*, *Lr2c*, *Lr3ka*, *Lr10*, *Lr11*, and *Lr24*, were chosen to identify virulence phenotypes in the selection generations based on degree of corresponding virulence polymorphism in the initial generation, and stability of their characteristic low infection types over different seasons in the greenhouse. Nine virulence phenotypes in the initial population could be grouped together using these seven differentials (Table 1). Ten 4-in. pots of the susceptible cultivar Little Club were inoculated using a settling tower with 5–10 mg of uredinia from generations 2, 4, 6, and 8 from each of the selection populations. Five or six single pustules from each pot of Little Club were isolated and increased on additional seedlings of Little Club. Fourteen to 20 days after isolation and increase, the isolates were inoculated onto differential sets of 7- to 8-day-old seedlings of the Thatcher near-isogenic differentials. Generally 50–60 single pustule isolates from each selection population were evaluated for virulence phenotype. The differential sets were incubated for 16–20 hr in a humidity chamber and then moved to a greenhouse bench at 15–20 C with 8 hr of supplemental fluorescent lighting per 24-hr period. The differential sets were read 12 days after inoculation; infection types 0; to 2 were recorded as avirulent; infection types 3 to 4 were recorded as virulent (11). Each single pustule isolate was assigned to a virulence phenotype group based on infection type to the seven differentials. Frequencies of the predominant virulence phenotypes were plotted over generations for each of the selection populations. Contingency tables (2,14) were used for each selection population to determine if there was a relationship between frequency of virulence phenotypes and generations. The Shannon index of phenotypic diversity (4) was plotted over generations for each selection population. The Rogers index of phenotypic proportional overlap (4) was calculated relative to the initial population over generations in each selection population. Rogers indexes within generations were calculated for paired combinations of selection populations. As a control, 66 single pustules were isolated from the initial population and evaluated for virulence phenotypes to determine if sampling variance in the absence of selection would be sufficiently large enough to cause significant differences in virulence phenotype frequencies.

TABLE 1. Grouping and frequencies of virulence phenotypes in the initial population of *Puccinia recondita* f. sp. *tritici*

Group no.	Resistance genes to which group members are virulent	Frequency
1	*Lr1, Lr10*	0.15
2	*Lr1, Lr10, Lr24*	0.129
3	*Lr1, Lr2a, Lr2c, Lr10*	0.236
4	*Lr1, Lr2c, Lr3ka, Lr10*	0.279
5	*Lr2a, Lr2c, Lr10*	0.042
6	*Lr2c*	0.021
7	*Lr11*	0.042
8	*Lr1, Lr11*	0.021
9	*Lr1, Lr3ka, Lr10*	0.063

Adaptation tests. The initial population, and the selection populations from generation eight were evaluated for spore production on adult plants of TC*Lr13*, TC*Lr16*, Chris, and Thatcher to determine if the selection populations had become specifically adapted to their respective host lines. For each selection population and the initial population (five tests in all), eight plants of each line were grown in two 6-in. fiberpots in growth cabinets at 18 C with 16 hr of fluorescent and incandescent light per 24-hr period. Plants for each test were inoculated simultaneously at the heading stage in a settling tower with 25 mg of urediniospores mixed with talc. The adult plants were then incubated in a dew chamber for 16 hr and then returned to the growth cabinet. After 12 days the infected flag leaves were numbered (eight leaves per line) and urediniospores were collected from each leaf with a cyclone spore collector into individual preweighed glass tubes. Rust was collected every 2 days for a total of five collections per leaf for each test. The weight of the glass tubes with urediniospores was determined and weight of the collected urediniospores for each leaf computed by subtraction. The flag leaves were then detached and area in square centimeters for each leaf was determined using a leaf area meter. Milligrams of urediniospores produced per square centimeter of leaf tissue was determined for each leaf. The significance of ranking of host lines within each test was determined with Tukey's studentized means test (19). The raw data was log transformed to equalize the variances within each test. Two-way analysis of variance to detect population by host line interaction was not performed since the five tests were carried out separately over a period of two months.

RESULTS

Frequency of virulence phenotypes. The population cultured on Thatcher changed relatively little in terms of virulence phenotype frequencies (Fig. 1), and had a nonsignificant value of x^2 in the contingency table analysis (Table 2), indicating no relationship between generations and frequency of virulence phenotypes. A nonsignificant x^2 statistic was also obtained in sampling the initial population ($x^2 = 5.63$, 6 df). In the population cultured on TC*Lr13* virulence phenotype 2 increased slightly and virulence phenotype 4 decreased after generation 2 (Fig. 1). The x^2 for this population (Table 2) indicated a significant relationship between generations and frequencies of virulence phenotypes. Virulence phenotype 4 predominated very rapidly in the population cultured on TC*Lr16* (Fig. 1) comprising more than 98% of the population in the eighth generation. The x^2 was extremely high for this population, as were the coefficient of contingency and Cramer's V (2) (Table 2). In the population cultured on Chris, virulence phenotypes 2 and 4 decreased, and virulence phenotype 7 increased over generations (Fig. 1). The x^2 (Table 2) indicated a significant relationship between virulence phenotype frequencies and generations.

Shannon indexes showed that populations cultured on Thatcher, TC*Lr13*, and Chris retained relatively high levels of diversity over eight generations, while phenotypic diversity in the TC*Lr16* population dropped rapidly (Fig. 2). Rogers indexes from the initial population paired with the selection populations showed that the populations cultured on Thatcher and TC*Lr13* changed the least relative to the initial population, and that the populations cultured on TC*Lr16* and Chris changed the most (Fig. 3A). Rogers indexes from paired combinations of selection populations within generations indicated that the population cultured on TC*Lr16* had relatively high indexes (0.50–0.93 in generations 4, 6, and 8) when paired with the TC*Lr13* and Chris populations (Fig. 3B). Rogers indexes for the populations cultured on Chris and TC*Lr13* were relatively low (0.13–0.39), as were the indexes for the Thatcher and TC*Lr13* populations (0.22–0.39). The Chris and

TC*Lr16* populations had intermediate indexes (0.27–0.57) when paired with the Thatcher population.

Adaptation tests. Thatcher and TC*Lr13* had the highest production of urediniospores per square centimeter of leaf tissue when inoculated with the initial population (Table 3), followed by TC*Lr16* and Chris at significantly lower levels. The population cultured on Thatcher had the highest levels of urediniospore production on Thatcher and TC*Lr13*, followed by TC*Lr16* at a level not significantly different from TC*Lr13*, and Chris at a significantly lower level. The population cultured on TC*Lr13* had the highest spore production level on TC*Lr13* and Thatcher at one level, followed by TC*Lr16* and Chris at lower levels. The population cultured on Chris had a similar ranking to TC*Lr13* except that Chris and TC*Lr16* were not significantly different at the lower level. The population cultured on TC*Lr16* produced the most spores on Thatcher and TC*Lr16* followed by TC*Lr13* at a level not significantly different from TC*Lr16*, and Chris at a significantly lower level (Table 3).

DISCUSSION

The populations cultured on TC*Lr13*, Chris, and TC*Lr16* changed significantly over generations in terms of virulence phenotype frequencies and wheat line rankings of urediniospore production relative to the initial population. The population cultured on Thatcher showed no significant change with regard to virulence phenotype frequencies, and little change in ranking of lines for urediniospore production. It is unlikely that the changes observed in the TC*Lr13*, Chris, and TC*Lr16* populations resulted from random drift since large numbers of uredinia were sampled in each generation, and changes in the frequencies of the predominant virulence phenotypes generally followed consistent trends. The nonsignificant x^2 statistic obtained in sampling the initial population indicates that sampling variance in the absence of selection would not likely contribute to significant changes in frequency of virulence phenotypes. The initial population adapted in various degrees to all of the selective hosts with resistance genes, yet did not adapt to the susceptible cultivar Thatcher. Clearly some genetic variation existed within the initial population for asexual reproduction on lines with resistance genes *Lr13, Lr16*, and *Lr34* even though all isolates in this population produced avirulent infection types on lines with these genes. Isolates with small or no differences in infection types may have large differences in spore production. This was previously observed in *Puccinia striiformis* by Johnson and Taylor (5). If genetic variation for spore production had not existed in the initial population, then no change in frequencies of virulence phenotypes would have been observed, as was the case in the Thatcher population.

It is unlikely that genetic variation for spore production is distributed evenly throughout isolates in the initial population.

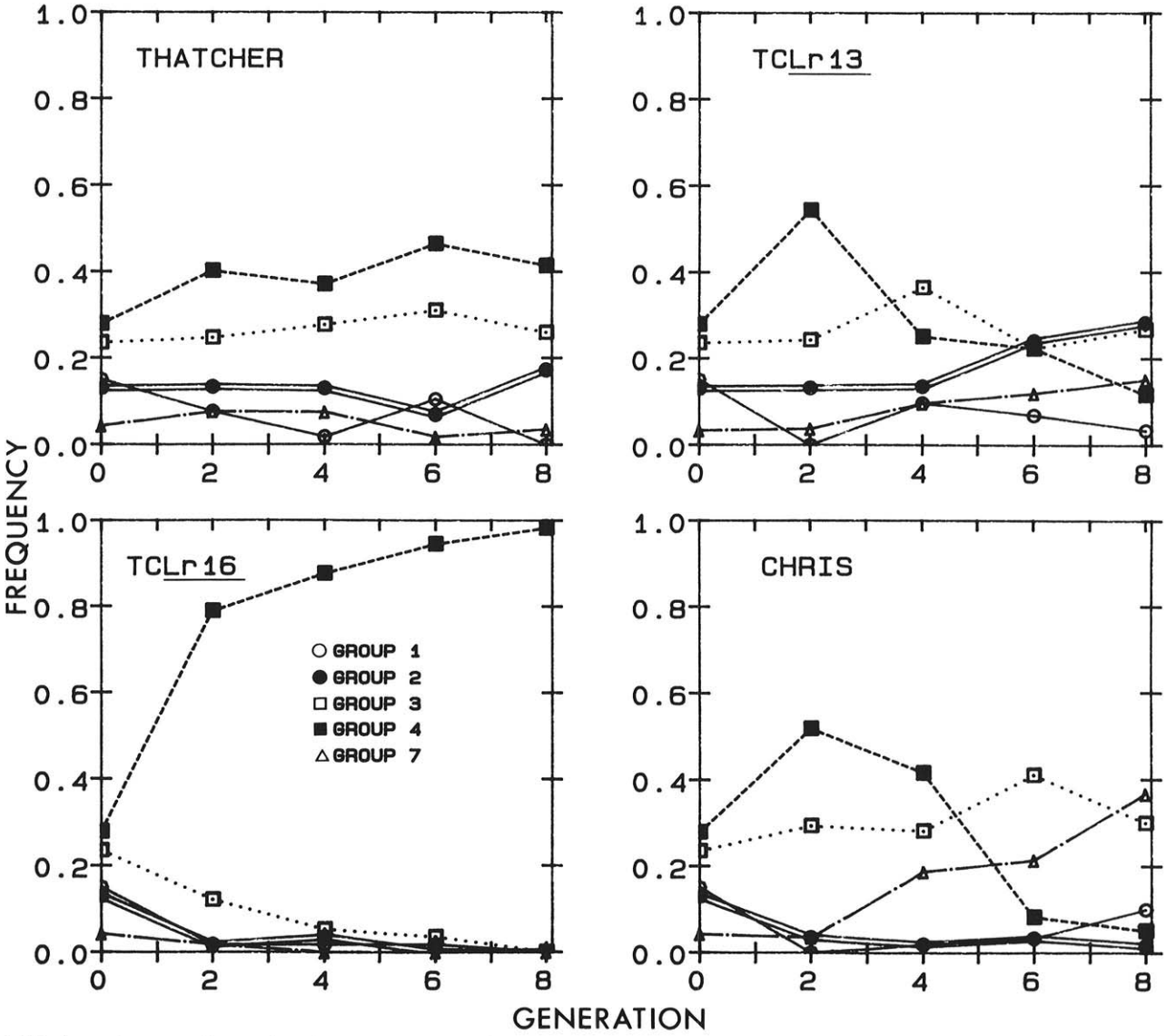

Fig. 1. Virulence phenotype frequencies of *Puccinia recondita* f. sp. *tritici* selected over eight uredinial generations on wheat lines Thatcher, TC*Lr13*, TC*Lr16*, and Chris. Virulence formulae of virulence phenotype groups are listed in Table 1.

By selecting the population on the resistant lines, indirect selection for certain virulence phenotypes that happen to have higher levels of spore production would also occur. Associations among virulences to seedling genes have been previously characterized in this population (8). Virulence associations have been indirectly measured in heterogeneous populations of *Uromyces appendiculatus* by selecting rust populations on one host line of bean and measuring the subsequent change in virulence frequencies on other host lines (3). Alexander et al (1) selected a heterogeneous field population of *U. appendiculatus* for five asexual generations on a bean line with partial resistance and measured the change in virulence frequency on four other bean lines. Frequency of virulence decreased on three of the lines, and increased on the fourth. Virulence to three of the lines would appear to be positively associated, while virulence to the fourth line would appear to be negatively associated from the other three in the original population.

The populations selected on Chris and TC*Lr13* would be expected to have some similarity since Chris has resistance gene *Lr13*. The increase of virulence phenotype 4 in generation 2 and subsequent decrease in generations 4 to 8 in the Chris and TC*Lr13* populations most likely resulted from changing temperatures in the greenhouse over time that would affect expression of the adult plant resistance in these lines, and hence their selection of virulence phenotypes. The population cultured on Thatcher showed the least differentiation from the other selection populations because there was not a significant relationship between virulence phenotype frequencies and generation. As such this population would

TABLE 2. Contingency table statistics for relationships between virulence phenotype frequencies and generations of *Puccinia recondita* f. sp. *tritici* within host selection lines

Statistic	Host selection lines			
	Thatcher	TC*Lr13*	TC*Lr16*	Chris
x^2	36.884	73.899[a]	109.323[a]	120.79[a]
Coefficient of contingency	0.345	0.4622	0.5327	0.551
Cramers V	0.1838	0.2606	0.3147	0.3302
df	32	32	32	32

[a] Significant at $P = 0.001$. The x^2 values for each population were tested for significance using the critical ratio described in Maxwell (14) since the expected number in many of the cells was less than 5.

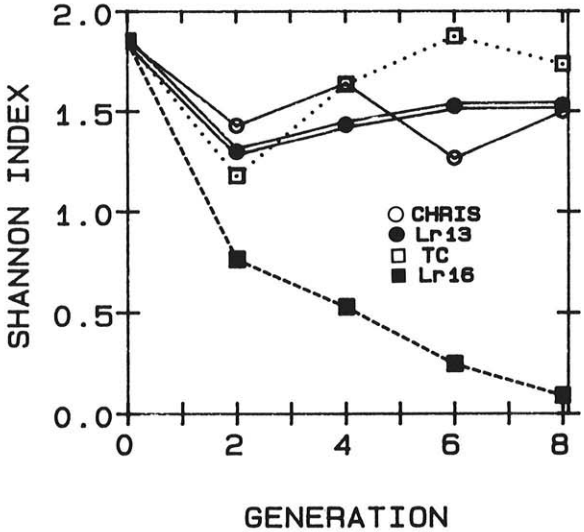

Fig. 2. Shannon indexes of phenotypic diversity for populations of *Puccinia recondita* f. sp. *tritici* selected for eight uredinial generations on four host lines of wheat. TC = population selected on Thatcher, Chris = population selected on Chris, Lr13 = population selected on TC*Lr13*, Lr16 = population cultured on TC*Lr16*.

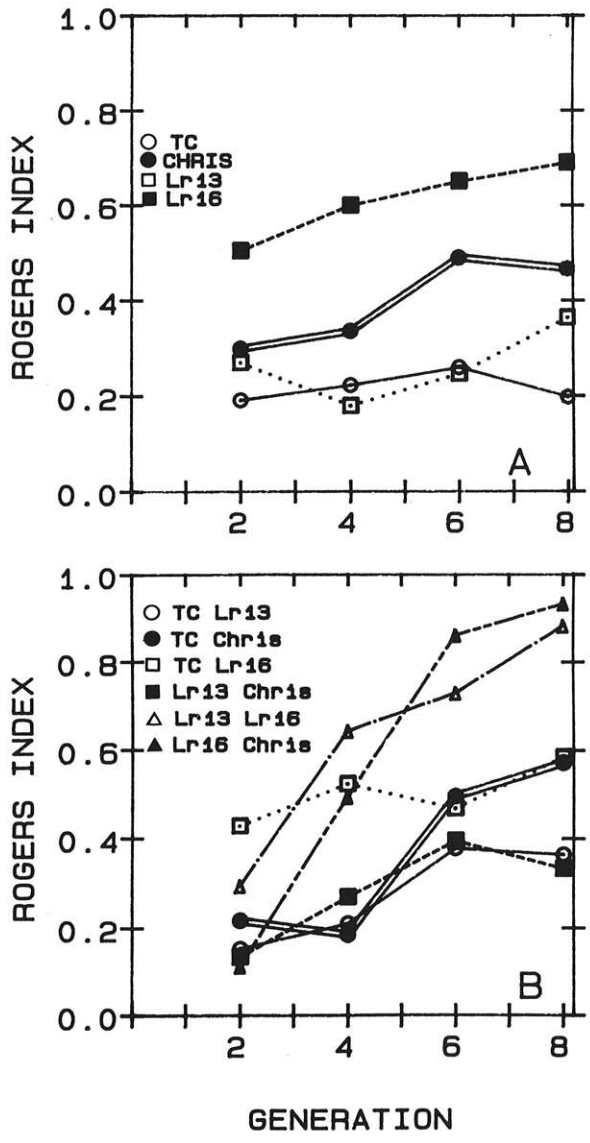

Fig. 3. Rogers indexes of proportional phenotypic overlap for populations of *P. recondita* f. sp. *tritici* selected for eight uredinial generations on four host lines of wheat. TC = population selected on Thatcher, Chris = population selected on Chris, Lr13 = population selected on TC*Lr13*, Lr16 = populations cultured on TC*Lr16*. **A,** Indexes of the selection populations relative to the initial population. **B,** Indexes of the selection populations in paired combinations within uredinial generations.

TABLE 3. Means and standard deviations of spore production of *Puccinia recondita* f. sp. *tritici* (mg/cm^2 of leaf tissue) within the initial and selection populations from generation 8 on four wheat lines

Wheat line	Initial population	Selection population			
		Thatcher	TC*Lr13*	TC*Lr16*	Chris
Thatcher	1.1368 A[a] (0.3754)	1.0880 A (0.1983)	1.5948 A (0.3486)	1.9401 A (0.5838)	0.9522 A (0.4303)
TC*Lr13*	1.1298 A (0.2128)	0.8462 AB (0.2140)	1.6799 A (0.5507)	0.9473 B (0.3726)	1.0134 A (0.4503)
TC*Lr16*	0.6548 B (0.1971)	0.6624 B (0.1920)	0.6639 B (0.1777)	1.3018 AB (0.4832)	0.3100 B (0.1114)
Chris	0.1433 C (0.0481)	0.2765 C (0.1439)	0.3969 C (0.1881)	0.2603 C (0.1611)	0.4015 B (0.2070)

[a] Significant differences within the initial and selection populations were determined at $P = 0.05$ using Tukey's studentized range means test (19). Means followed by the same letter are not significantly different. The raw data was log transformed to equalize the variances within each selection population.

not be significantly different from the initial population from which the selection populations developed.

The spore production tests further support the conclusion of directional and significant change in the TC*Lr16*, and Chris populations. The populations cultured on Chris and TC*Lr16* clearly adapted to their respective hosts relative to the initial population. The population cultured on TC*Lr13* showed no significant change relative to the initial population. The population cultured on Thatcher showed little change except that there was not a significant difference in spore production between TC*Lr13* and TC*Lr16*.

The populations cultured on Thatcher, TC*Lr13*, TC*Lr16*, and Chris retained the level of diversity found in the initial population. Results from virulence surveys have shown that the susceptible wheat cultivars that are generally grown in southern Ontario, British Columbia, and southern Alberta have maintained populations of *P. recondita* that have higher levels of phenotypic diversity than the population in the prairie region (6,7,9). Obviously isolates of leaf rust with unneeded genes for virulence in heterogeneous asexual populations are not at a general reproductive disadvantage relative to more simple isolates when cultured on susceptible hosts. This is not to say that Vanderplank's (20) concept of stabilizing selection is invalid as a rule for *P. recondita* since selection, virulence associations, and association between general fitness characters and virulences, rather than fitness attributes of the virulences per se, are most likely responsible for the observed levels of virulence phenotype diversity observed in this experiment and in the North American populations of leaf rust.

The results of a selection experiment with a heterogeneous pathogen population may depend on the distribution of virulence in the initial population. As an example, contradictory conclusions have been put forth regarding the importance of stabilizing selection in the oat stem rust disease. Using mixtures of oat stem rust races, Martens (13) determined over five uredinial generations that complex races had higher rates of reproduction than simple races on susceptible hosts in field plots. Leonard (10) cultured a heterogeneous population of oat stem rust that originated from pycnial infections on barberry, on a susceptible oat line for 10 generations and measured the changes in proportion of virulent infection types on resistant oat lines. He determined that stabilizing selection was effective in reducing the fitness of oat stem rust isolates with unneeded virulences relative to simple isolates. The differing results from the two experiments can most easily be explained by the differences in virulence distribution in the initial populations. Martens compared fitness of oat stem rust races that were currently predominant with races that were no longer commonly detected. Rather than examining the fitness effects of excessive virulence per se, Martens actually compared the fitness of different races (or virulence phenotypes) which would be an aggregate effect of background genotypes of the races with the pleiotropic effects of the virulence genes. In an initial population such as this, associations resulting from the asexual nature of the original population, rather than any selective advantage attributable to specific virulences would be important in determining race frequencies in the final generation. By using a sexual population of oat stem rust, Leonard randomized the fitness effects of genetic background relative to the virulence genes and was able to directly measure the effects of unneeded virulence in this population. However, Leonard did not measure the relative fitness of individual virulence phenotypes, and hence virulence associations resulting from reproductive advantage, since virulence frequencies were determined by counting the relative numbers of virulent and avirulent infection types on the resistant oat lines. A similar selection experiment to the one described in this article using an initial population derived from randomly mated pycnia is currently in progress to determine if different selective forces are reponsible for maintaining virulence polymorphisms in sexual and asexual populations of *P. recondita*.

Although genetic variation for reproductive capacity on Chris and TC*Lr13* was present and selected for in the initial population, it is unlikely that cultivars with only these resistance genes have strongly influenced levels of virulence to other resistance genes in the leaf rust population of the north central states and Manitoba and Saskatchewan. The changes in the prairie leaf rust population since the introduction of cultivars with *Lr13* and/or *Lr34* can be directly attributed to the use of seedling resistance genes *Lr1, Lr2a, Lr10, Lr24*, and *Lr26* in the hard red winter and spring wheats grown in the United States (6,7,9,12).

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