Virulence Changes in *Uromyces appendiculatus* After Five Asexual Generations on a Partially Resistant Cultivar of *Phaseolus vulgaris*

Helen Miller Alexander, J. V. Groth, and A. P. Roelfs

First and second authors are former research associate and associate professor, Department of Plant Pathology, University of Minnesota, St. Paul 55108; third author is research plant pathologist, Cereal Rust Laboratory, ARS, USDA, St. Paul 55108. Present address of the first author is Department of Biology, University of Louisville, Louisville, KY 40292.

Cooperative investigations, U.S. Department of Agriculture and the University of Minnesota. Scientific Journal Series Paper 13,919 of the Minnesota Agricultural Experiment Station.

We are grateful to Eden Debebe for technical assistance and to J. J. Burdon and F. Gould for valuable discussions. This research was supported in part by USDA Competitive Grant 59-2271-1-1-687-0.

Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

Accepted for publication 23 October 1984.

ABSTRACT

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.

A field population of *Uromyces appendiculatus*, the bean rust pathogen, was obtained from the susceptible cultivar Pinto 111 in southwestern Minnesota, and maintained for five asexual generations on a partially resistant bean cultivar, Slimgreen. Changes in latent period, pustule size, and urediniospore production on Slimgreen and Pinto 111 over the generations were small or not detectable. In contrast, there was a large increase in the proportion of the pathogen population virulent on bean cultivar US#3 and large decreases in the proportion virulent on the cultivars Early Gallatin, Roma, and B1349 over the course of the experiment. Although Minnesota populations of *U. appendiculatus* are polymorphic for virulence on all four cultivars (virulence frequencies between 15 and 55%), none of the four is grown in the state in more than small amounts. These experimental results illustrate that changes in virulence gene frequencies may be independent of pathogen exposure to host resistance.

Additional key words: correlated response to selection, pathogenicity association, selection, "unnecessary" virulence.

Virulence associations occur when isolates with particular combinations of host-specific virulence and avirulence for different lines or cultivars of a host species occur at frequencies in the pathogen population different than would be expected by chance. The primary source of data on virulence associations has been descriptive race surveys, where isolates collected over a geographic area are inoculated on differential host lines to determine their virulence phenotype. Virulence associations can develop because of linkage in the pathogen genome, allelism of pathogen genes controlling the traits, pleiotropic effects of a single pathogen gene affecting the disease response on both cultivars, or selection, if genotypes with particular combinations of genes controlling virulence have higher survival and/or reproduction than other genotypes. In the case of many fungal pathogens, the predominance of asexual reproduction (effectively complete linkage of the entire genome) is probably responsible for most virulence associations (1,22). Unfortunately, methods of collecting and analysis in race surveys can lead to documentation of spurious associations if isolates collected from different cultivars or different geographic regions are combined in analyses (14,22).

The selection experiment, used widely by population geneticists (8 [pages 188-206]), is an experimental tool for determining if there is a genetic basis for virulence associations. Given genetic variation, when a pathogen population is maintained on one cultivar for several generations, genotypes best able to survive and reproduce on the cultivar will increase in frequency. By inoculating other cultivars with samples of the population during the course of the experiment, it is possible to determine if the selection process has indirectly led to changes in virulence on other host lines. By using this approach, Leonard (15) observed negative correlations and Gould (9) found both positive and negative correlations in the ability of pathogens or herbivorous mites to infect or infest different cultivars or species.

Ideally, experimental genetic studies of pathogens should be integrated with descriptive data on the genetic composition of pathogen populations. For example, the discovery of negative

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

virulence associations in race survey data has led to the suggestion that cultivars that contain the respective resistance genes may offer long-lasting disease control (3,19–21). Given the problems inherent in interpreting descriptive data, we feel it is unwise to base a breeding program solely on such evidence. However, if these virulence genes were also negatively associated in selection experiments, there would be more compelling reasons for breeders to develop cultivars that combine their corresponding resistance genes. Experimental genetic studies may also suggest explanations for the occurrence of high frequencies of apparently "unnecessary" genes for virulence in pathogen survey data; such virulence genes may be positively associated with other genes that are essential to the pathogen population. Selection experiments with insects have revealed many positive and a few negative correlations between resistance to different insecticides, thus demonstrating the genetic basis for "cross-resistance" and suggesting more effective control strategies (4).

With the causal agent of bean rust, *Uromyces appendiculatus* (Pers.) Unger, field surveys of the magnitude needed to provide information on virulence associations have not been done. It is clear, however, that populations of *U. appendiculatus* are genetically variable (10) and the short life of rust resistance in the two newly recommended and grown bean cultivars Fleetwood and Olathe in Minnesota in 1982 (J. V. Groth, *unpublished*) suggests that information on the population genetics of virulence of this pathogen is needed.

The goal of this study was to determine whether some components of fitness and virulence frequencies on several cultivars changed when an asexually reproducing population of *U. appendiculatus* was maintained for five generations on a partially resistant cultivar.

MATERIALS AND METHODS

Origin of the experimental population. The experimental population of *U. appendiculatus* was obtained from urediniospores collected by vacuum aspiration from approximately 60 heavily rusted leaves of Pinto 111 from a 400 m^2 area in a commercial field in Renville County, MN, on 26 August 1982. Pinto 111, a dry bean, is very susceptible to bean rust and possesses no known resistance genes to isolates of the pathogen from dry bean areas of Minnesota (11). Urediniospores were suspended in Soltrol 170 light oil (Phillips Petroleum Co.) and a small atomizer inoculator was used to spray the suspension on partially expanded unifoliolate leaves of Pinto 111. After leaves were allowed to dry for 1 hr, plants were put in a high-humidity atmosphere for 20 hr at 25 C. Urediniospores were collected by using cyclone spore collectors from 1,000–1,500 pustules 1 wk later. These were desiccated over silica gel 1–2 days and then stored in an ultrafreezer at −80 C. This population of urediniospores, one generation removed from the field population, is referred to as the "original" collection.

The selection experiment. Urediniospores of the original collection were used to initiate a selection experiment on plants of cultivar Slimgreen, a green snap bean that produces small non-necrotic pustules (No. 5 on the Bean Rust Grading Scale) when infected by several Minnesota isolates of *U. appendiculatus* (11). A bulked mixture of Slimgreen seed grown in 1976 and 1977 was used for the entire experiment.

The population of *U. appendiculatus* was maintained on Slimgreen for five successive asexual generations. To start each generation, partially expanded unifoliolate leaves of 7-day-old Slimgreen plants were inoculated with urediniospores from the previous generation. A quantitative inoculator (17) was used to deposit a suspension of urediniospores in 0.3% Bacto agar (1 mg of urediniospores: 1 ml of water agar) on a circle of leaf tissue approximately 1.5 cm in diameter; a water-driven magnetic stirrer kept the suspension homogeneous. After inoculation, plants were kept at 18 C in a mist chamber for 20–22 hr. For each generation, approximately 50 plants were inoculated; 8–12 plants with 25–35 pustules per leaf were then selected for the experiment (pustule density affects spore production per pustule [23], thus we selected plants to maintain the same pustule density per leaf throughout the experiment). The total number of uredinia per generation, summed over the 8–12 plants that were used, was: first, 444; second, 408; third, 461; fourth, 482; and fifth, 474. The chosen plants for each generation were maintained in plastic-covered cages in a greenhouse with natural lighting; this part of the study was done between June and September in 1983. Temperature, daylength, and light intensity changed during this period, but not in a unidirectional manner.

When sporulation began (approximately 10 days following inoculation), urediniospores were collected from the chosen plants every 1–3 days for a 10-day period, dried over silica gel, and then stored in the ultrafreezer. Prior to inoculation of the next generation, collections made on different days were mixed thoroughly.

Components of pathogen fitness on Slimgreen and Pinto 111. To compare attributes of the pathogen's fitness on the cultivar on which the pathogen population was maintained (Slimgreen) and on the original cultivar from which the population was isolated (Pinto 111), subsamples of stored urediniospores of the original collection, the second generation, and the fifth generation were each inoculated in October 1983 on 40 plants of Slimgreen and 40 plants of Pinto 111. Inoculation techniques were identical to those used in the selection experiment and all plants (all combinations of cultivar and pathogen generations) were inoculated on the same day so treatments were exposed to the same environmental conditions. Plants of the different cultivar/generation combinations were randomly placed on the greenhouse bench, so that slight variations in environmental conditions were equally experienced by all treatments. The total number of pustules was counted on each leaf of the experimental plants 10 days after inoculation. Fourteen leaves were chosen per treatment so that the distribution of pustule densities for each treatment was similar (all six treatments had average pustule densities of 21–23 per leaf with standard deviations between 4.4 and 6.7). Only one leaf per plant was chosen; the other primary leaf and the trifoliolate leaf shoot apex were removed.

Latent period was measured on the 9th day after inoculation by counting the number of sporulating pustules (pustules with ruptured peridia) and recording this as a percentage of the total number of sporulating and nonsporulating pustules. One day earlier, all pustules were nonsporulating, and 1 day later, over 90% of the pustules for all treatments were sporulating; thus, measurements made on the 9th day offered the best chance for detecting differences among treatments.

Pustule diameter was measured with a ×15 ocular micrometer on the four most distal pustules on each leaf on the 11th, 14th, and 31st day after inoculation (pustules on the leaf edge or very close to major veins or other pustules were avoided). A diagram of each leaf was made so that the same pustules could be measured each time. Measurements on the first two dates were of the original pustule; measurement on the last date was of the maximum diameter represented by the secondary satellite pustules.

Urediniospore production was determined by collecting urediniospores from each leaf in a separate pre-weighed plastic centrifuge tube using a modified cyclone collector. Collections were made every 1–2 days for 3 wk, starting 11 days after inoculation. Urediniospore collections were terminated on the 31st day after inoculation when over 95% of the pustules were producing teliospores. Open centrifuge tubes were stored over silica gel and then weighed after the final collection to obtain the weight, by subtraction, of the urediniospores produced. (Measurements were made to the nearest 0.1 mg; the smallest value of spore weight per leaf was 1.3 mg.)

Virulence frequencies on four bean lines. Urediniospores from the original collection, the second generation, and the fifth generation were also inoculated on 12 plants of each of four other bean lines, to examine if virulence frequencies changed on cultivars to which the pathogen population had not been exposed. Three lines were green snap beans (US#3 [a bean rust differential line (12)], Roma [a flat-podded Italian bean developed by Rogers Brother Seed Co., Idaho Falls and Twin Falls, ID], and Early Gallatin [a bean rust differential line developed by Gallatin Valley

Seed Co., Twin Falls, ID]). The fourth line was a dry bean of Mexican origin (B1349 [=Negro de Chincha]). For Minnesota isolates of *U. appendiculatus*, all four lines produce either a pustule or a necrotic fleck when infected (11, and J. V. Groth, *unpublished*). One reason for choosing these bean lines was that they produce discrete, easily recognizable flecks; such flecks are readily distinguishable by shape, color, and distribution from flecks caused by other biotic and abiotic agents. Earlier work (5) established that the number of flecks produced by each of two isolates of *U. appendiculatus* on Early Gallatin was statistically similar to the numbers of pustules obtained with the same number of urediniospores applied. The original field collection of *U. appendiculatus* used in this experiment, prior to ultrafreezer storage, was polymorphic for virulence on all cultivars (percent virulence: US#3, 57.0%; Early Gallatin, 30.3%; Roma, 39.5%; and B1349, 18.6%).

The inoculation procedure consisted of spraying a suspension of spores from storage in light oil on the plant, drying the leaves 1 hr, and placing the plants in the mist chamber (18 C) overnight; all combinations of cultivar and pathogen generation were inoculated on the same day. Virulence frequencies were determined 10 days after inoculation from counts of the number of pustules taken as a percentage of the total number of pustules and necrotic flecks counted on each leaf or part of a leaf (parts of leaves with very high pustule densities were not counted). Virulence frequencies of the original collection had also been estimated 10 mo earlier by measuring smaller samples; these values were used to determine if virulence frequencies had changed during the time urediniospores were stored at −80 C.

RESULTS

Components of pathogen fitness on Slimgreen and Pinto 111. Latent period progressively increased on Pinto 111 over the generations studied, but no trend was detected for latent period changes on Slimgreen (percent sporulation on the 9th day for Pinto 111/Slimgreen: original = 80.8/59.2, second generation = 77.1/68.3, and fifth generation = 68.4/51.5 (all percentages were based on $n > 300$ pustules). Changes in the latent period of *U. appendiculatus* over the course of the selection experiment could be dependent on the host studied; a three-way contingency test bulking all leaves in one treatment (18) revealed that the interaction between cultivar, pustule status (sporulating versus nonsporulating on day 19), and pathogen generation was of borderline significance with $G = 5.94$, df = 2 ($G_{0.05,\,df=2} = 5.99$).

Although pustule diameter was always greater for infections on Pinto 111 than on Slimgreen, there was no change in pustule size as a result of the selection process on either cultivar (Table 1). The average dry weight of spores produced per pustule on Slimgreen was lowest for the original collection, while the lowest average spore production per pustule on Pinto 111 occurred in the fifth generation (Table 2). Consequently, the ratio of average spore weight per pustule on Slimgreen to average spore weight per pustule on Pinto 111 increased over the course of the experiment (Table 2). The differences between the cultivars in the relationship between spore production and the generation of the selection process caused a significant cultivar × generation interaction in a two-way analysis of variance with the 14 leaves as replicates ($F_{2,57} = 3.57$, $P < 0.05$; due to loss of some spore samples, all treatments were reduced to 10 replicates by random exclusion of samples to maintain a balanced design).

Virulence frequencies on four nonselected bean lines. Pathogen virulence frequencies changed over the course of the experiment on all four differential lines; virulence to US#3 increased while virulence to Early Gallatin, Roma, and B1349 decreased (Fig. 1). Combining data from replicate plants, changes in ratios of pustules to flecks were found to be highly significant by using 2 × 3 contingency table analyses (18), each with two degrees of freedom (US#3, $G = 269.88$; Early Gallatin, $G = 1{,}242.62$; Roma, $G = 1{,}452.64$; and B1349, $G = 150.64$). Selection for or against virulence did not occur during urediniospore storage in the ultrafreezer. Virulence frequencies of the original collection made 10 mo earlier were in the same range as the values obtained at the end of the experiment (percent virulence for each cultivar before/after ultrafreezer storage were: US#3, 57.0/53.8; Early Gallatin, 30.3/33.0; Roma, 39.5/37.4; and B1349, 18.6/12.8 [see also Fig. 1]).

DISCUSSION

The experimental population, after being maintained asexually for five generations on Slimgreen, showed only slight evidence of improvement in characters related to pathogen fitness on Slimgreen. Differences among generations in pathogenicity on Pinto 111, the source of the experimental population, were also minimal. If anything, however, the experimental process had opposite effects on spore production on Slimgreen and Pinto 111, suggesting that ability to reproduce on these cultivars may be negatively correlated. In contrast to the small changes documented for pathogenicity on Slimgreen and Pinto 111, virulence frequencies on the four differential lines changed dramatically over the course of the experiment. It is clear, therefore, that the genetic composition of the fungal population was altered by the experimental process.

The absence of improvement in components of pathogen reproduction on Slimgreen is not necessarily surprising. The selection regime used was conservative; selection was dependent on the pathogen genetic variation present in the original field population (and mutation) due to the absence of the sexual cycle. Further, all pustules were allowed to contribute progeny to the succeeding generation in proportion to their relative size; a more severe selection regime would have only selected urediniospores collected from pustules at the large extreme of a size distribution. Although our procedure reduced the probability of selecting superior genotypes, it also made the experiment more realistic since *U. appendiculatus* does have a prolonged period of asexual reproduction during the summer and, in nature, truncation

TABLE 1. Mean pustule diameters on three dates for *Uromyces appendiculatus* on cultivars Slimgreen and Pinto 111 after inoculation with the original urediniospore population and with populations maintained on Slimgreen for two and five asexual generations

Bean cultivar and asexual generation of the pathogen	Pustule diameter (mm) at postinoculation: day 11	day 14	day 31
Slimgreen			
Original	0.53 (0.01)[a]	0.61 (0.01)	2.68 (0.06)
2nd	0.52 (0.01)	0.64 (0.01)	2.61 (0.07)
5th	0.53 (0.01)	0.62 (0.02)	2.83 (0.06)
Pinto 111			
Original	0.69 (0.02)	0.91 (0.02)	3.72 (0.11)
2nd	0.69 (0.02)	0.99 (0.02)	4.07 (0.10)
5th	0.69 (0.01)	0.96 (0.02)	3.83 (0.07)

[a] Numbers in parentheses are standard errors of the accompanying mean.

TABLE 2. Mean weight of urediniospores per pustule for *Uromyces appendiculatus* on bean cultivars Slimgreen and Pinto 111 after inoculation with the original urediniospore population and with populations maintained on Slimgreen for two and five asexual generations

Asexual generation of the pathogen	Urediniospores/pustule (mg)		Ratio of
	Slimgreen	Pinto 111	Slimgreen/Pinto 111
Original	0.136 (0.009)[a]	0.910 (0.232)	0.149
2nd	0.160 (0.009)	0.990 (0.180)	0.162
5th	0.152 (0.009)	0.792 (0.104)	0.192

[a] Numbers in parentheses are standard errors of the accompanying mean.

selection for spore production does not occur. It is also possible that improvement in characters affecting pathogenicity on Slimgreen did occur, but was not detected. Accurate collection and quantification of urediniospore production is particularly difficult. Testing all generations might have allowed better detection of trends, but would have necessitated reduced sample size per generation.

Changes in virulence frequency for the four differential lines are probably due to genetic changes resulting from the experimental procedure. Although we cannot rule out random drift as an explanation, we feel it is not likely for three reasons. First, we began the experiment with a genetically diverse population despite the pathogen's asexual summer reproduction (data from the original generation show it is polymorphic for virulence for four lines (Fig. 1); work on similar populations (10) also recorded high levels of variation). Secondly, we maintained relatively large population sizes per generation (>400) and, thirdly, changes in virulence frequencies followed clear trends (Fig. 1). There is also no evidence for selection for or against virulence during storage of the populations in the ultrafreezer. Since we determined virulence frequencies for all generations in an experiment performed on 1 day, differences in virulence cannot be attributed to effects of environmental variation changing the phenotypic expression of the

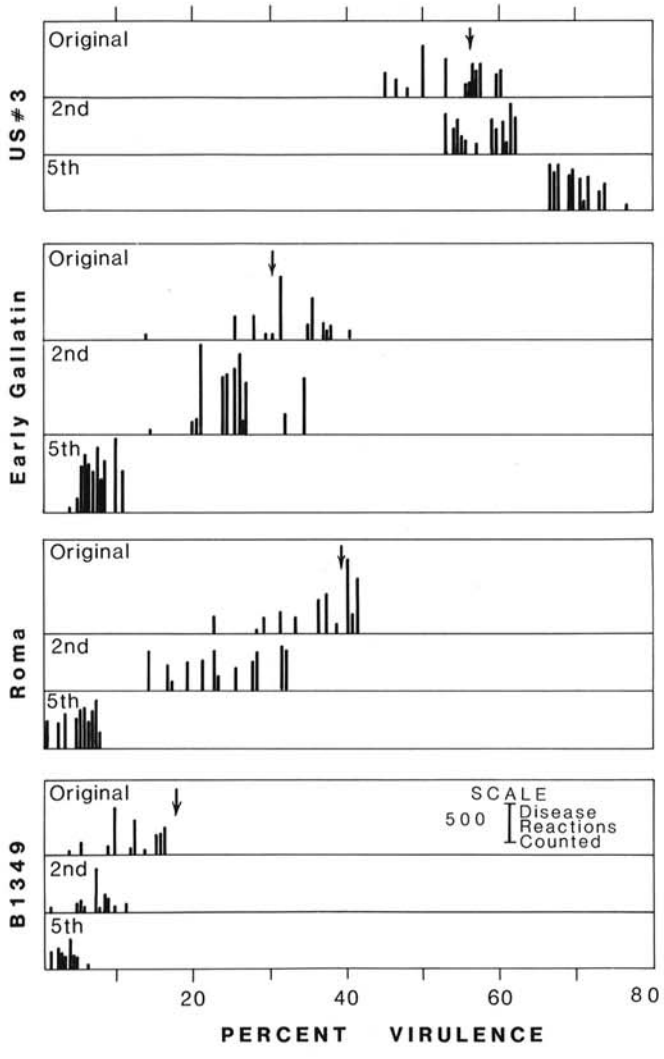

Fig. 1. Frequency of phenotypes virulent on four differential bean lines for the original urediniospore population of *Uromyces appendiculatus* and for populations maintained on Slimgreen for two and five asexual generations. Each vertical line refers to a virulence frequency measurement made on one plant; the length of the line reflects the total number of pustules and flecks counted per plant. The arrow indicates the frequency of virulent phenotypes present in the original population 10 mo before the selection experiment.

disease reaction. Although breeding lineages are difficult to document, we know of no evidence that Slimgreen is closely related to any of the other four lines. Clearly, the pathogen population responds differently to Slimgreen (uniform, small non-necrotic pustules) compared to its reaction on the other four lines (differential reactions: either pustule or necrotic fleck).

Apparently, the changes in virulence frequencies on the four differential lines under these experimental conditions were, therefore, either correlated responses (via pleiotropy or linkage) to the selection that occurred in the experiment or were due to the superior fitness of genotypes with particular combinations of these virulence genes. It is not obvious, however, whether the experiment selected for improved survival and reproduction specifically on Slimgreen or for an increased ability to survive and reproduce in the greenhouse environment. Another possibility is that the greenhouse environment changed in a consistent manner over the five generations and that pathogen genotypes responded differentially to the new conditions. The genetic basis for these results cannot be explored further with the data available. It is clear, however, that the frequency of genes affecting virulence on these four lines may be dependent on factors other than whether or not the pathogen population has been exposed to the respective host resistance. It is particularly interesting that virulence frequencies on all four lines changed and that both positive (US#3) and negative (Roma, Early Gallatin, and B1349) responses occurred.

Better understanding of the genetic correlations between traits affecting virulence to different cultivars may explain why genes for "unnecessary" virulence persist in pathogen populations. With the possible exception of Early Gallatin, these four lines have only been grown in small amounts in Minnesota (Early Gallatin is popular in Wisconsin; approximately 800 ha (2,000 acres) are grown in southeastern Minnesota 167 km from the field collection site). The original pathogen collection (one generation removed from the field) was, however, polymorphic for virulence on all lines, with virulence frequencies of 15–55%. Similar results have been found for other midwestern populations of *U. appendiculatus* (10). If the relative fitness of a genotype virulent on these lines varies under different combinations of cultivar and climatic conditions, fixation of these genes would be less probable.

This study was designed to explore the theoretical possibility that correlations between virulence to different cultivars may exist, not to determine which resistance genes in beans might be combined for effective rust control. However, in this experiment, individuals that were virulent on both US#3 and on either of the lines Roma, Early Gallatin, or B1349 apparently had lower levels of survival and/or reproduction than other individuals. More studies are needed to understand the genetic basis for these results. Attention should be concentrated on US#3 and Early Gallatin, since host and pathogen genes affecting rust reactions have been identified (5,6).

The short generation cycle of fungal populations and their microscopic size make them ideal organisms for performing genetic experiments. Selection experiments (15) and analysis of genetic variation among progeny groups (7,13) have already improved our understanding of the heritability of traits affecting virulence. Evolutionary biologists have realized, however, that correlations between traits may largely determine the response or lack of response observed with selection (2,8 [pages 300 and 303–312]). When isolates of *Puccinia graminis* f. sp. *tritici* that are virulent on commonly grown wheat cultivars are found only at very low frequencies in race surveys (16) it would seem that these genotypes are unable to initiate epidemics. Negative correlations between virulence and traits affecting propagule survival and reproduction may be responsible. Through use of genetic experiments, especially in conjunction with detailed surveys of the genetic composition of natural pathogen populations, we can increase our knowledge of the genetic constraints acting on plant pathogens and thus may improve our ability to control disease.

LITERATURE CITED

1. Alexander, H. M., Roelfs, A. P., and Groth, J. V. 1984. Pathogenicity associations in *Puccinia graminis* f. sp. *tritici* in the United States.

Phytopathology 74:1161-1166.
2. Arnold, S. J. 1981. Behavioral variation in natural populations. I. Phenotypic, genetic and environmental correlations between chemoreceptive responses to prey in the garter snake, *Thamnophis elegans*. Evolution 35:489-509.
3. Browder, L. E., and Eversmeyer, M. G. 1977. Pathogenicity associations in *Puccinia recondita tritici*. Phytopathology 67:766-771.
4. Brown, A. W. A. 1958. Insecticide resistance in arthropods. Pages 29-32 and 133-154. World Health Organization. Geneva, Switzerland.
5. Christ, B. J., and Groth, J. V. 1982. Inheritance of virulence to three bean cultivars in three isolates of the bean rust pathogen. Phytopathology 72:767-770.
6. Christ, B. J., and Groth, J. V. 1982. Inheritance of resistance in three cultivars of beans to the bean rust pathogen and the interaction of virulence and resistance genes. Phytopathology 72:771-773.
7. Emara, Y. A., and Sidhu, G. 1974. Polygenic inheritance of aggressiveness in *Ustilago hordei*. Heredity 32:219-224.
8. Falconer, D. S. 1981. Introduction to quantitative genetics. Longmans. London.
9. Gould, F. 1979. Rapid host evolution in a population of the phytophagous mite *Tetranychus urticae* Koch. Evolution 33:791-802.
10. Groth, J. V., and Roelfs, A. P. 1982. Genetic diversity for virulence in bean rust collections. (Abstr.) Phytopathology 72:982.
11. Groth, J. V., and Shrum, R. D. 1977. Virulence in Minnesota and Wisconsin bean rust collections. Plant Dis. Rep. 61:756-760.
12. Harter, L. L., and Zaumeyer, W. J. 1941. Differentiation of physiologic races of *Uromyces phaseoli typica* on bean. J. Agric. Res. 62:717-731.
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. Kiyosawa, S. 1980. On the virulence analysis of pathogen race frequencies. Ann. Phytopathol. Soc. Jpn. 46:582-592.
15. Leonard, K. J. 1969. Selection in heterogeneous populations of *Puccinia graminis* f. sp. *avenae*. Phytopathology 59:1851-1857.
16. Roelfs, A. P., Long, D. L., and Casper, D. H. 1983. Races of *Puccinia graminis* f. sp. *tritici* in the United States and Mexico in 1981. Plant Dis. 67:82-84.
17. Schein, R. D. 1964. Design, performance, and use of a quantitative inoculator. Phytopathology 54:509-513.
18. Sokal, R. R., and Rohlf, F. J. 1981. Pages 731-765 in: Biometry. 2nd edition. W. H. Freeman and Co., San Francisco.
19. Vanderplank, J. E. 1975. Pages 152-157 in: Principles of Plant Infection. Academic Press, New York.
20. Vanderplank, J. E. 1982. Pages 7-42 in: Host-Pathogen Interactions in Plant Disease. Academic Press, New York.
21. Wolfe, M. S., Barrett, J. A., Shattock, R. C., Shaw, D. S., and Whitbread, R. 1976. Phenotype-phenotype analysis: Field application of the gene-for-gene hypothesis in host-pathogen relations. Ann. Appl. Biol. 82:369-374.
22. Wolfe, M. S., and Knott, D. R. 1982. Populations of plant pathogens: Some constraints on analysis of variation in pathogenicity. Plant Pathol. (Lond.) 31:79-90.
23. Yarwood, C. E. 1961. Uredospore production by *Uromyces phaseoli*. Phytopathology 51:22-27.