Nonrandom Distribution of Virulences Within Two Field Collections of *Uromyces appendiculatus*

J. V. Groth and E. A. Ozmon

Department of Plant Pathology, University of Minnesota, St. Paul 55108.
Accepted for publication 27 March 2002.

ABSTRACT

Groth, J. V., and Ozmon, E. A. 2002. Nonrandom distribution of virulences within two field collections of *Uromyces appendiculatus*. *Phytopathology* 92:755-761.

Two collections of urediniospores of *Uromyces appendiculatus*, each from a different commercial bean field, were characterized for associations of virulence among individuals within each collection. Four bean (*Phaseolus vulgaris*) lines with distinct, race-specific resistance to which virulence in each population was polymorphic were used to obtain measures of all six possible pairwise virulence associations for each collection. We inoculated one of the lines and collected urediniospores only from the segment of the population that was virulent on that line. This segment, when compared with nonselected collections from susceptible Pinto 111, gave a direct measure of degree of association as the change in frequency of virulence observed. Plants of the second bean line were inoculated in separate sets with both selected and unselected collections. Frequencies of virulence were estimated from the numbers of susceptible

ble-type and resistant-type infections. Reciprocals of each pairing also were made. For collection P21, all virulences were significantly associated, either positively or negatively, except one pair (in one direction of selection only); whereas, for collection M5, all virulences were significantly associated. Virulence association in P21 was shown to be the result of predominance of phenotypes with certain combinations of virulence by inoculation of the four bean lines with 10 randomly chosen single-uredinial individuals. In support of this, a large random-mated F1 population derived from each collection showed much less virulence association, with the majority of pairs of virulences showing nonsignificant changes in virulence frequency after passage through the first line. Random mating also significantly changed virulence frequency from that of the original population in all instances. Changes were in both directions, suggesting either that virulences were not all recessive, or that heterozygote frequency was sometimes above and sometimes below the Hardy-Weinberg expectation in the field populations.

Some plant pathogenic fungi have been subdivided into physiological races based on virulence and avirulence patterns on sets of host lines with different genes for resistance. For many pathogens, the utility of this means of characterizing an important trait is clear, and it has allowed more informed decisions about the use of race-specific resistance. Race identification has value especially for many of the cereal rust fungi, powdery mildews, and other biotrophic pathogens. In some cases, the dynamics of race changes have provided information about the biology of the pathogen. This is the case with stem rust of wheat, *Puccinia graminis* f. sp. *tritici*. In the central United States, the relatively stagnant nature of race changes, whereby new races appear rarely and are very similar to existing races, has verified that sexual reproduction, parasexuality, and migration are not usually operating to provide variation. In contrast, sexual reproduction is operating in the Intermountain region of the western United States, with the result that the numbers of races are higher, and virulence genes (16) and allozymes (4) are nearly randomly distributed among races. Using contingency testing, Alexander et al. (2) found that virulence genes were highly associated in isolates sampled from stem rust on wheat in the central United States, because of a small number of races and the predominance of one or two of them. The sexual population from the western United States had much smaller, but measurable, degrees of association, indicating that, while sexual reproduction was having its expected effect on gene distribution, it did not result in truly random distribution, possibly because of selec-

tion during subsequent asexual cycles via repeating uredinial infection.

The emphasis on tracking races has been applied to other rust fungi, or in other areas of the world, in cases where the biology of the pathogen is not as well understood. Where sexual reproduction is known or suspected, it is not always clear how information on race numbers, frequencies, or changes is to be used. If variation for a pathogen is high due in part to sexual reproduction, the use of single, race-specific resistance genes is not recommended, but may still be useful if other kinds of resistance are unavailable, inadequate, or if careful screening is done to select the most widely effective race-specific resistance (18).

Uromyces appendiculatus (Pers.:Pers.) Unger is a pathogen that has proven difficult to control through race-specific resistance (7,18). In many areas, but particularly in temperate areas, the rust populations that occasionally cause losses are local and dependent on teliospores for overwintering (or oversummering). Hence, they are forced to undergo sexual recombination annually. Evidence for the importance of teliospores for survival is based on rapid loss of viability of urediniospores in storage at all but the coldest storage temperatures (9,17), on the prolific production of telia by nearly all collections made in colder temperate states, and on several reports of aecia in the early season (12). These sexual populations are polymorphic for virulence and isozyme alleles (14), and are composed of many races, even in relatively small sampling areas (9,11,15,18). In the north-central states, high diversity was found when the pathogen was sampled late in the season when rust levels allowed adequate samples to be made from susceptible bean cultivars (9,15).

In sexual organisms, population structure typically is characterized by extreme phenotypic diversity with little or no association of markers. Al Kherb et al. (3) found that, in crown rust of

Corresponding author: J. V. Groth; E-mail address: jamesg@puccini.crl.umn.edu

oats (*Puccinia coronata*), the number of races resulting from sexual recombination in teliospore germination was high (in aeciospores sampled from the aecial host, buckthorn [*Rhamnus cathartica*]), but decreased rapidly with time and distance from buckthorn even though there were no known resistance genes in the oats on which the pathogen reproduced asexually via repeating uredinial generations.

The objective of this work was to determine whether virulence associations that signal reduced variability occur in two sexual field populations of *U. appendiculatus* sampled as bulk urediniospore collections late in epidemics on commercial dry beans in Minnesota.

MATERIALS AND METHODS

The pathogen. Samples of urediniospores were obtained from two locations and years in commercial beans in Minnesota. Collection P21 was made on 26 August 1982 from Pinto 111 beans in Renville County. This is the same collection used by Alexander et al. (1). Collection M5 was made in early September 1990 from Midland navy beans in Wadena County. In both cases, approximately 60 leaves were sampled with a vacuum aspirator in a single location in the field approximately 5 m in diameter. In both years, rust was heavy enough to allow approximately 100 mg of urediniospores to be gathered from several hundred uredinia on each leaf. Collections were increased in the greenhouse at 25°C using a portion of the urediniospores to heavily inoculate approximately 30 plants of Pinto 111, a cultivar that is generally susceptible to all races of rust found on commercial dry beans in Minnesota (putatively of Mesoamerican, as opposed to Andean, origin) (13). Leaves were tapped over aluminum foil at 2- to 3-day intervals, and urediniospores were dried for 48 h in a desiccator and stored at -50°C until use.

The host. Based on earlier work with isolates and bulk collections (10,15), four differential bean lines were selected for this study that contained distinct effective resistance genes, for which virulence in the collections was polymorphic, and that gave a single, observable incompatible reaction. The resistance reaction of Early Gallatin and US#3 against single isolates are each determined by independent dominant genes (6). Three of the differential lines, Early Gallatin, US#3, and 4C-69, were used for both collections, but because virulence to the fourth line, B1660, was not polymorphic in collection M5, another line, 765, was substituted for B1660 with M5.

The method. In random mating populations of any organism, genes that are not linked always will be distributed randomly among individuals if differential selection is not operating. This is the basis for the test of virulence associations using bulk urediniospore collections. For any two putatively distinct virulences, the two lines containing the respective resistance genes are inoculated serially. The first line is inoculated, and frequency of virulent and avirulent infections counted. Urediniospores are collected from this line that are mostly or entirely from the virulent fraction of the original collection. This we refer to as the selected collection or selected virulence. At the same time, a line that is universally susceptible to the population (Pinto 111) is inoculated with the original collection, and urediniospores are collected as on the differential line. This collection is the (relatively) unselected control. Next, some plants of the second line are inoculated with the virulent fraction and some with the unselected collection. If, in the rust population, there is no association of virulence to the first with that to the second line, the frequency of virulence to the second line will be identical for selected and unselected collections, because selection for virulence to the first line will yield a random sample with respect to the second virulence. Reciprocal serial inoculations of each pair of lines also were made.

Although homozygous bean lines were selected that had readily countable pustules and flecks, at times limited sporulation oc-

curred in the uredinia of the incompatible reaction, and the collecting method was modified to minimize obtaining spores from incompatible infections by scraping spores only from compatible uredinia.

Counts were made for each treatment on at least 50 plants divided among several trials, with from 50 to 100 pustules per primary leaf. Each inoculation included the selected and unselected collections simultaneously.

Virulence testing of isolates. Urediniospores were collected randomly from 12-day-old single pustules of P21 on Pinto 111 by scraping them above and below the leaf with the edge of a 00 gelatin capsule so that the spores fell into the capsule. Within a day or two, young, partially expanded primary leaves of several plants of Pinto 111 were inoculated with each isolate by adding several drops of soltrol 170 mineral oil (Chevron Phillips Chemical Company, Houston, TX) to the spores in each capsule and spraying the suspension onto the upper surface with an atomizer. After the oil had evaporated, plants were transferred to a moist chamber for 24 h at 24°C and then moved to the greenhouse. Urediniospores were collected by tapping the leaves over aluminum foil. After this increase, urediniospores of each isolate were sprayed onto several plants of each of the differentials as above. Infection types were scored 12 days after inoculation.

Random mating. Teliospores were scraped into water from several thousand telia of P21 and M5 on 4-week-old Pinto 111 leaves. Teliospores were gathered on glass wool filters using a millipore vacuum filter, air dried, and stored dry in a refrigerator at 12°C for at least 8 months. On each of six petri plates of 2% water agar, 50 mg of teliospores (approximately 10^6) were suspended in a small volume of glass-distilled water and spread evenly over the surface of the agar. Plates were incubated in a desiccator in a cool north window to provide natural daylight and long-day conditions. Approximately a week later, teliospores began to germinate. When several promycelia could be seen above the teliospores in every $\times 100$ field of the microscope, plates were suspended over 8-day-old Pinto 111 plants enclosed in clear plastic tubes pushed into the soil of the pot. Plates were left in place for approximately 5 days in the greenhouse at 22°C. Approximately 10 days later, pycnia were evident in large numbers on the primary leaves. Separately for each collection, random mating was done among pycnia of each by mixing nectar containing pycniospores with water and spreading uniformly over all leaves. Aecia appeared below most pycnia in approximately 8 days. Aeciospores were collected in bulk from about 10,000 aecia of P21 and 4,500 aecia of M5. A portion of the aeciospores was used to inoculate several pots of Pinto 111 to obtain enough urediniospores to complete all subsequent inoculations without another increase.

Statistical analysis. For testing whether changes in virulence frequency were significant, individual leaves or plants containing from approximately 50 to 200 infections were considered individual units of observation. When approximately 50 such units were obtained in several trials, they were plotted according to frequency and sample size (data not shown). If the distribution of frequencies appeared symmetrical by observation, standard deviations of untransformed frequency were obtained. Extreme outliers were excluded. Pooled standard deviations were calculated and used to determine a least significant difference, based on the *t* distribution and $\alpha = 0.05$, for each comparison. A Bartlett's test of homogeneity of variances was applied to each comparison; six comparisons (Early Gallatin to 4C-69 and 4C-69 to 765 with P21 field; Early Gallatin to 765, 765 to Early Gallatin and 765 to US#3 with M5 field; and Early Gallatin to B1660 with P21 random mated) did not have homogeneous variances. All but one of these six (765 to US#3 with M5 field) had differences that were large and significant. Although the statistical tests were compromised by heterogeneous variances, the differences in the five were large enough, with *P* values much less than 0.05, to allow them to be counted as showing significant change.

Fig. 1. Changes in virulence frequency in *Uromyces appendiculatus* collection P21 for all pairs of serial inoculations and their reciprocals on four bean lines. In each graph, squares are used where the first-named bean line is inoculated first and circles where the second-named line is inoculated first. "First" refers to the fact that the bean line is the first differential line experienced by a collection, while "second" is when the collection used is the virulent fraction from the companion line. Solid lines = the field collection, dashed lines = the random-mated population derived from P21. Least significant differences ranged from 0.44 to 2.53%.

Fig. 2. Changes in virulence frequency in *Uromyces appendiculatus* collection M5 for all pairs of serial inoculations and their reciprocals on four bean lines. In each graph, squares are used where the first-named bean line is inoculated first and circles where the second-named line is inoculated first. "First" refers to the fact that the bean line is the first differential line experienced by a collection, while "second" is when the collection used is the virulent fraction from the companion line. Solid lines = the field collection, dashed lines = the random-mated population derived from M5. Least significant differences ranged from 0.79 to 2.84%

RESULTS

Changes in nonselected virulence. In general, all serial inoculations of the two field collections except one resulted in significant positive or negative changes in virulence frequency. For P21, the six possible combinations of the four bean lines on which the collection was polymorphic are shown in Figure 1 (solid lines only). Reciprocal serial inoculations always resulted in changes in virulence that were similar in direction but not magnitude. Changes were positive except in the case of US#3 and Early Gallatin. The solid lines in Figure 2 show the changes in virulence frequency in serial inoculations of four differential bean lines with a second field collection of bean rust, M5. Only three of the bean lines are the same as for P21; line B1660 was not used with M5 because avirulence to this line was fixed in this collection. Line 765 was used instead with M5 because virulence to 765 was polymorphic in M5 (P21 was 93.3% avirulent on this line). In collection M5, as with P21, reciprocal serial inoculations resulted in similar directions of change, except in the case of 4C-69 to US#3, where no significant change in virulence frequency occurred (all other cases showed significant change). Unlike P21, most changes in virulence frequency (4 of 6) were negative with M5.

We conclude that, in these two bean rust urediniospore collections, virulences are not random with respect to one another; rather, they are often strongly associated or disassociated. We assume that the apparent lack of random association of virulences was most likely due to sampling of a finite number of sexually derived phenotypes, resulting from selection during the several asexual generations that occurred during the growing season. To test this, two things were done. First, 10 isolates of P21 were obtained by selecting single uredinia randomly from leaves of Pinto 111. Virulence patterns on the four bean lines used for P21 were determined for the 10 isolates and it was found that there were four virulence phenotypes, with 4, 3, 2, and 1 isolates of each. The pattern of virulence in the most common phenotypes (data not shown) on each pair of bean lines generally explained the direction of change but not the magnitude (i.e., whether virulences were in coupling or repulsion in common phenotypes resulted in positive or negative change in nonselected virulence) (data not shown). Second, the field collections were taken through one sexual cycle with random mating approximated by using large numbers of pycnia that were randomly fertilized. These "sexual" populations are shown in Figures 1 and 2 as dashed lines. These derived populations showed considerably reduced overall change in virulence frequency in serial inoculations compared with their parent populations (Table 1). Most (15/24) of the pairs of virulences appeared to be randomly associated in the sexual populations.

To ensure that selection on the first host line was effectively eliminating avirulent isolates, uredinia were collected from each differential bean line and the same line inoculated with the select-

ed spores. In most cases, the frequency of virulence was over 98%. In a few cases, most notably those involving the line 4C-69, the frequency of virulence was less than 95%. Virulence frequencies of less than 100% are due either to collection of a few urediniospores from avirulent (necrotic) pustules with limited sporulation in the center or to contamination.

Changes in virulence frequency due to random mating. When the two field collections were random mated, in all cases it resulted in significant changes in virulence frequency from that seen in the unselected sample (Figs. 1 and 2, the left-hand point in each plot). The direction of this change varied; in P21, the frequency of virulence went up only slightly for U.S. #3, and down for the other three lines, while in M5 it went up for all lines except Early Gallatin, where it went down (Table 2). These changes are contributed to by two phenomena. The first is that random mating brings the population close to Hardy-Weinberg equilibrium for all genes. Departures from equilibrium in the field populations appear as higher or lower frequency of heterozygotes than would be predicted by Hardy-Weinberg expectations ($2pq$, where the frequency of avirulence is p and of virulence q). For recessive virulence, which is most common for this pathogen (8), a decrease in virulence indicates that there were fewer heterozygotes in the field population than would be predicted for a random-mated population. The increase in frequency of heterozygotes that results from random mating appears as a virulence decrease, because heterozygotes are phenotypically avirulent. A virulence increase when random mating occurs likewise is the re-

TABLE 2. Change in frequency of virulence on five bean lines^a

Collection ^b	Mean change (%) ^c	Asexual change (%) ^d
P21		
Early Gallatin	-9.3	1.4
US#3	4.2	1.7
4C-69	-32.2	3.9
B1660	-8.3	0.2
M5		
Early Gallatin	-12.1	-1.4
US#3	2.8	4.1
4C-69	13.7	-0.9
765	13.9	2.9

^a Change in frequency resulting when each of two polymorphic field populations of *Uromyces appendiculatus* were taken through a sexual generation on fully susceptible Pinto 111 and a large number of pycnia random mated within each population. Asexual change is based on change of frequency of virulence when the sexually derived populations were increased asexually on Pinto 111 through urediniospores.

^b P21 was collected from Pinto 111 in 1981 in Renville County, MN; M5 was collected from Midland Navy beans in 1990 in Wadena County, MN.

^c Change in virulence frequency from random mating, mean of three measurements.

^d Change in virulence frequency from selection in asexual generations, mean of three measurements.

TABLE 1. Changes in frequency of virulence of polymorphic collections of *Uromyces appendiculatus* on four bean lines when each was inoculated with urediniospores and resulting urediniospores were collected only from susceptible pustules and then used to inoculate the second line^a

Collection	P21			M5		
	Mean change (%) ^b	Absolute change (%) ^c	Number significant ^d	Mean change (%)	Absolute change (%)	Number significant
Field collection	14.8	23.1	12/12	6.9	16.6	11/12
Random F1	3.3	6.4	5/12	-0.3	4.9	6/12
Field, 3 common lines ^e	8.1	25.0	6/6	-3.6	10.3	5/6

^a All paired combinations and their reciprocals are used to obtain percent changes shown. P21 was collected from Pinto 111 in 1981 in Renville County, MN; M5 was collected from Midland Navy beans in 1990 in Wadena County, MN.

^b Sign considered.

^c Mean change, sign ignored.

^d Proportion of cases in which a significant change in virulence frequency was found. Least significant differences ranged from 0.44 to 2.54%; t test, $\alpha = 0.05$.

^e Bean lines on which both pathogen populations were polymorphic, Early Gallatin, US#3, and 4C-69.

sult of an abundance of heterozygotes in the field population. The other phenomenon that could contribute to virulence frequency changes in random mating is selection, which certainly occurs during asexual and sexual generations (1,10). The degree to which selection influenced virulence on each bean line during a single asexual increase of the random-mated populations on Pinto 111 is shown in Table 2. In general, the magnitude of virulence change due to asexual selection was smaller than total observed change, and could not account for all of the change in virulence frequency observed after random mating.

DISCUSSION

Changes in nonselected virulence. The two collections used in these studies represent populations of *U. appendiculatus* that are sexual. All isolates in such collections produce teliospores readily and the pathogen probably does not overwinter except through teliospores. Patterns of disease on dry beans in Minnesota strongly suggest that germinating teliospores are the source of primary inoculum resulting in early buildup, and damage from bean rust has been reduced through crop rotation (J. V. Groth, *unpublished data*). This does not preclude the possibility that the late-season population contains genotypes that migrate in from populations elsewhere, especially from further south. Migration could lead to linkage disequilibrium by mixing dissimilar populations. The two collections were both made at the end of the growing season, when the fungus had been reproducing vegetatively for several generations. Either a founder population from a small number of teliospores initiated the epidemic early in the summer with a small number of phenotypes, or a subset of a larger, more variable early population apparently was able to predominate in the population. Field collections of economically important rust fungi seem to become dominated by a clonal pattern, whether they are known to be sexual, like crown rust of oats derived from basidiospore infection of the alternate host, buckthorn (3), or asexual, like stem rust of wheat in the central Great Plains of the United States, where the alternate host has been eliminated (16). A purely sexual pattern is probably rare in rust fungi of crop plants, and has been best documented in the nonrust fungal pathogen *Mycosphaerella graminicola* (anamorph *Septoria tritici*) by Chen and McDonald (5), where great diversity for molecular markers is maintained throughout the growing season. This pattern may suggest that asexual reproduction through conidia is less prevalent in *M. graminicola* than in cereal rust fungi. Bean rust falls between these two extremes, with a pattern that is influenced by both sexual and asexual reproduction.

In contrast to the field collections, bulk-mated F1 populations derived from each collection showed random association for more than half of the pairs of genes, and showed less than one-third of the absolute change of the field collections. This supports the idea that virulence association or disassociation is mostly the result of a population that is dominated by a smaller number of clonally reproducing phenotypes rather than the result of genetic linkage.

For each pair of bean lines, the direction of change that was observed was determined by whether the predominant phenotype or phenotypes contained virulence to both or only to one of the lines. If both, the virulence to the second line was increased as virulence to the first was selected out, but if the predominant selected (virulent to the first line) phenotype or phenotypes did not contain virulence to the second line, then frequency of virulence to the second line dropped. When virulences are in coupling in the predominant phenotype or phenotypes, the changes in reciprocal serial inoculations can be attributed to selection of some or all of the same phenotypes. Not so for cases where virulence frequency dropped. In these instances, selection for virulence to the first line cannot be mirrored in the reciprocal serial inoculation, because the predominant phenotypes selected in the original case would be

avirulent in the reciprocal case, resulting in their elimination. The predominant, virulent phenotypes that are selected in the reciprocal case also will be avirulent on the second line (or they would have resulted in an increase in virulence frequency originally), but they will be distinct phenotypes from those selected in the original serial inoculation.

Differences in magnitude of change in all positive cases but one (US#3 and B1660 with P21) suggest that the number of races changing frequency was not the same in reciprocal cases. Nothing can be said about the number of races involved in the case of selection for races with virulence in repulsion, because different races must be selected in these cases.

Changes in virulence frequency due to random mating. Based on the direction of virulence changes after random mating, the two collections, P21 and M5, strongly differed in the frequency of heterozygotes for virulence only for line 4C-69. For Early Gallatin virulence, both populations appeared to have fewer heterozygotes than expected with random mating. Both were close to Hardy-Weinberg expectations for virulence to U.S. #3. So, unlike some bean rust populations that we have sampled which tended to have an excess of heterozygotes (15), P21 and M5 presented a mixed picture of heterozygote frequencies, at least on the bean lines chosen.

ACKNOWLEDGMENTS

This work was supported by the Minnesota Agricultural Experiment Station.

LITERATURE CITED

- 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.
- 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.
- Al-Kherb, S. M., Roelfs, A. P., and Groth, J. V. 1987. Diversity for virulence in a sexually reproducing population of *Puccinia coronata*. *Can. J. Bot.* 65:994-998.
- Burdon, J. J., and Roelfs, A. P. 1985. The effect of sexual and asexual reproduction on the isozyme structure of populations of *Puccinia graminis*. *Phytopathology* 75:1068-1073.
- Chen, R. S., and McDonald, B. A. 1996. Sexual reproduction plays a major role in the genetic structure of populations of the fungus *Mycosphaerella graminicola*. *Genetics* 142:1119-1127.
- 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.
- Coyne, D. P., and Schuster, M. L. 1975. Genetic and breeding strategy for resistance to rust (*Uromyces phaseoli* (Reben) Wint) in beans (*Phaseolus vulgaris* L.). *Euphytica* 24:795-803.
- Groth, J. V. 1988. *Uromyces appendiculatus*, rust of Phaseolus beans. Chapter 26 in: *Advances in Plant Pathology*. D. S. Ingram and P. H. Williams, eds. Vol. 6, *Genetics of Plant Pathogenic Fungi*. G. S. Sidhu, ed. Academic Press, London.
- Groth, J. V., McCain, J. W., and Roelfs, A. P. 1995. Virulence and isozyme diversity of sexual vs. asexual collections of *Uromyces appendiculatus* (bean rust fungus). *Heredity* 75:234-242.
- Groth, J. V., and Ozmon, E. A. 1994. Contrasting the effects of asexual reproduction and random mating on changes in virulence frequency in a field collection of *Uromyces appendiculatus*. *Phytopathology* 84:566-569.
- Harter, L. L., and Zaumeyer, W. J. 1941. Differentiation of physiologic races of *Uromyces phaseoli typica* on bean. *J. Agric. Res.* 62:717-731.
- Jones, E. D. 1960. Aecial stage of bean rust found in New York State. *Plant Dis. Rep.* 44:809.
- Maclean, D. J., Braithwaite, K. S., Irwin, J. A. G., Manners, J. M., and Groth, J. V. 1995. Random amplified polymorphic DNA reveals relationships among diverse genotypes in Australian and American collections of *Uromyces appendiculatus*. *Phytopathology* 85:757-765.
- McCain, J. W., Groth, J. V., and Roelfs, A. P. 1992. Inter and Intra-popu-

- lation isozyme variation in collections from sexually reproducing populations of the bean rust fungus, *Uromyces appendiculatus*. *Mycologia* 84:329-340.
15. McCain, J. W., Ozmon, E. A., and Groth, J. V. 1990. Virulence frequency in the bean rust fungus: Comparison of phenotypic vs. genotypic polymorphism. *Plant Dis.* 74:496-501.
 16. Roelfs, A. P., and Groth, J. V. 1980. A comparison of virulence phenotypes in wheat stem rust populations reproducing sexually and asexually. *Phytopathology* 70:855-862.
 17. Schein, R. D. 1962. Storage viability of bean rust uredospores. *Phytopathology* 52:653-657.
 18. Stavely, J. R., and Pastor-Corrales, M. A. 1989. Rust. Pages 159-194 in: *Bean Production Problems in the Tropics*. H. F. Schwartz and M. A. Pastor-Corrales, eds. CIAT, Cali, Colombia.