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ABSTRACT

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There is a dearth of information on pathogen variation within an individual field. In this study, virulence diversity of *Uromyces appendiculatus*, cause of bean rust, within individual fields was investigated. From six bean fields in the United States, Honduras, Dominican Republic, and South Africa, 380 *U. appendiculatus* isolates were differentiated into 65 virulence phenotypes on bean lines containing Andean- and Middle American-derived rust resistance genes. Race variation among bean rust isolates from different geographic regions was found, and virulence phenotypes found in fields from tropical and subtropical regions were more virulent and diverse than those found in fields from temperate regions. The variance components between fields was greater than the variance within a field based on mean disease score on 12 differentials but the variance components within a field were greater than the variances between fields based on number of virulence phenotypes. This is the first report that multiple site samples are needed to represent the fungal virulence diversity in a diseased field. In developing sampling plans, the entire cost of sampling one field is higher than the cost of taking more samples; therefore, to estimate virulence diversity variation, we recommend selecting fewer fields and collecting more samples per field.

Additional keywords: *Phaseolus vulgaris*

Bean rust, caused by *Uromyces appendiculatus* (Pers.) Link, is distributed worldwide and is one of the most economically important diseases of common bean, causing yield losses that range from 25 to 100% in susceptible cultivars (29,46). Common bean (*Phaseolus vulgaris* L.) is one of the most important leguminous crops worldwide, and it is the main source of dietary protein for millions of people in both developing and developed countries. Bean species are cultivated in a diverse range of climates, and the world's production is estimated to exceed 23 million metric tons (3). Morphological and biochemical traits divide common bean lines into two geographically distinct gene pools, Andean and Middle American, which correspond to the centers of common bean domestication (8).

Rust epidemics in common bean have been reported in many different regions of the world, and severe losses occur in tropical and subtropical climates (30,44,46). *U. appendiculatus* is known to have high virulence diversity, and this has been reported from many different regions of the world (2,4-6,15,28,29). Virulence reaction after pathogen inoculations of a set of differential bean lines or cultivars composed of different single or multiple rust resistance genes traditionally has been used to define virulence diversity of populations of *U. appendiculatus* as well as the cereal rust pathogens. Virulence diversity of *U. appendiculatus* first was reported by Harter et al. (13). The first 20 physiologic races of *U. appendiculatus* were identified in the United States in 1941 (14). Several other virulence phenotypes were identified from 1952 to 1983 (7,12,26,48) and, by 1996, over 300 races were reported from different parts of the world (29). Additional virulence diversity of the bean rust pathogen has been reported (2,17,19).

Another method of measuring diversity in fungal pathogens is through the use of molecular markers (18,22,23). Linde et al. (20) used enzymes to compare isozyme and virulence patterns of *U. appendiculatus* isolates from Central America and the United States. They found lower isozyme variation than virulence variation. Groth et al. (9) did not detect polymorphism in band patterns of five isozymes in sexual and asexual reproducing populations of *U. appendiculatus* from Minnesota. MacLean et al. (22) used random amplified polymorphic DNA (RAPD) analysis to compare Australian and American bean rust pathogen isolates. They found that most of the Australian isolates clustered together and were genetically divergent from the American isolates, suggesting coevolution of the bean rust pathogen and its host; however, they did not address virulence diversity. Sandlin et al. (37) also used RAPD analysis to separate *U. appendiculatus* isolates specifically virulent on Andean bean from nonspecific isolates from Middle American origin. Using RAPD-polymerase chain reaction (PCR) analysis, Araya et al. (2) separated isolates of *U. appendiculatus* collected from bean in South and Central America into two groups, Andean and Middle American, corresponding to the two centers of bean domestication, and a third heterogeneous group composed of both Andean and Middle American isolates. The same result was found in virulence tests, suggesting parallel evolution of the rust pathogen and its host, common bean. Although some associations between phenotypic and genotypic variation have been found, phenotypic diversity is the most relevant for use in disease management strategies.

Strategies for bean rust management include the use of fungicides, cultural practices such as crop rotation, incorporation of crop residue, adjustment of planting dates, and host resistance (30,32,35). However, because of the relatively high cost of fungicide treatments, decreasing profits, and variable effectiveness of cultural practices, more attention has been given to host resistance. Some bean cultivars with single genes for resistance to *U. appendiculatus* have been developed, but most of them were overcome by the likely appearance of races of the pathogen with new or different virulence soon after their release (30,43). The occurrence of different races of *U. appendiculatus* in the bean production regions and narrow resistance gene pool makes breeding for resistance a challenge because resistance becomes ineffective in a relatively short period of time. In fact, *U. appendiculatus* is among the most pathogenically variable plant pathogens with the most virulence diversity, and its race components in a population can vary in time and space. More than one race of bean rust can be found on a small spatial scale such as a single sample of spores from a leaf or field populations, but one race predominates (11,24,42). On a

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large spatial scale, large virulence differences among races from different countries or geographical regions have been reported (2,29). However, virulence diversity of the bean rust fungus and, in fact, other (high virulence diversity) fungi in a single field has not been studied. Most of the previous research in pathogenic variation and identification of sources of resistance against the bean rust pathogen has been based on a few leaves, with uredinia taken from each field to represent populations in a region. Considering the high number of *U. appendiculatus* races, a small sample size may not represent the diversity of this pathogen in a single field. In fact, Linde *et al.* (20) and Groth and Roelfs (10) reported that the sample size studied affects the pathogenic diversity found in pathogen population studies. In addition, pathogenic variation among bean rust isolates from a single leaf sample has been reported (7,12). Thus, whether high pathogenic diversity exists among bean rust isolates collected in different sites of the same field needs to be determined. Furthermore, it is important to develop sampling methods that more accurately determine pathogenic variability of a pathogen in a field or region.

For development of effective and durable rust resistant bean cultivars, it is necessary to determine the virulence diversity of populations of *U. appendiculatus* that exists in a region and to detect rare races. Therefore, the study of pathogenic variability of the bean rust fungus that exists within and among bean fields is a prerequisite for better understanding of the pathogenic diversity in an entire region. Thus, the objectives of this study were to (i) determine pathogenic variability of populations of *U. appendiculatus* within and among individual bean fields, (ii) compare pathogenic variability of *U. appendiculatus* among bean fields from different geographical regions, (iii) estimate variance components among and within fields, and (iv) develop sampling plans to accurately characterize bean rust for a given total cost of sampling.

MATERIALS AND METHODS

Urediniospore collection and single-uredinium isolation. Bean leaf samples infected with rust were collected in each of the six bean fields in Nebraska and Michigan, United States; Yuscaran and Tatumbla, Honduras; San Juan Valley, Dominican Republic; and Natal, South Africa (Table 1). Ten bean rust samples were collected at random in each of the six fields. Green leaves with new rust infections were collected to ensure viability of urediniospores for virulence analysis. In all, 60 samples of urediniospores were collected for this study. Each sample of urediniospores was increased on a susceptible cultivar before inoculation on bean rust differential cultivars. Each sample of urediniospores was used to inoculate 7-day-old primary leaves of a set of 12 bean differentials (Table 2) containing different rust resistance genes representing the two main regions of common bean subdomestication, Andes and Middle America, for single-uredinium isolation. A single uredinium (pustule) was isolated from a differential plant that had a mixed or single rust reaction 7 to 8 days after inoculation. The pustule was cut out of the leaf, crushed with an artist's brush moistened in Tween 20 solution, and re-inoculated on primary leaves of a new plant of the same differential line. Urediniospores produced from each single pustule were considered one rust isolate. If a differential line had a mixed rust reaction, the representative uredinium size was reisolated until a stable, uniform size was obtained. In all, 380 rust isolates were collected from single-pustule isolation procedures and the number of isolates collected per field is shown in Table 1.

Virulence tests. The same set of 12 bean differentials used for single-uredinium isolation were used in virulence tests. Rust inoculum of each of the 380 isolates was prepared by suspending 2.5 mg of urediniospores in 30 ml of Tween 20 solution (40 µl of Tween 20 per 1,000 ml of distillate water) and used to inoculate 7-day-old primary leaves of bean differentials using a hand sprayer. The inoculated plants were allowed to dry, then placed in a mist chamber at 100% relative humidity and 21 ± 1°C for 16 h. After misting, the plants were placed in a growth chamber at 22 ± 2°C for incubation. Disease reactions and uredinium diameters, were scored 14 days after inoculation using a hand lens with sizing scale. The infection grade was recorded from each differential line using the standard 1-to-6 bean rust grading scale published by Stavely at al. (44; Table 3). If two different uredinium sizes were present on the same plant differential, the reaction grade was scored according to their frequency. The inoculations and disease reaction evaluations were repeated twice.

Estimation of costs for bean rust sampling and estimation of variance components. To develop a sampling plan for characterizing bean rust, it is necessary to first consider the total costs of sampling. The total cost can be approximated by the formula total cost = $c_1n_1 + c_2n_1n_2$ where c_1 is the average cost of sampling a field, c_2 is the average cost of a sample, n_1 is the number of fields, and n_2 is the number of samples per field (41). The above formula can be rewritten as the following expression so that n_1 is a function of n_2:

$$n_1 = \frac{\text{total cost}}{c_1 + c_2 n_2} \qquad \text{(eq. 1)}$$

Given equation 1, the number of fields (n_1) to be sampled can be estimated for a given total cost of sampling, cost of sampling a field (c_1), cost of a single sample in a field (c_2), and number of samples per field (n_2).

The variance of the sample mean (for either the number of pathotypes or the mean disease score [MDS]) can be computed as

$$V(\bar{y}) = \frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_1 n_2}$$

where σ_1^2 is the variance component among fields and σ_2^2 is the variance com-

Table 1. Locations where the 380 isolates of *Uromyces appendiculatus* used in virulence tests were collected

Field location	Bean cultivar	Number of collections per field	
		Samples[a]	Isolates
Nebraska, United States	Othello	10	56
Michigan, United States	Othello	10	70
Yuscaran, Honduras	Milpero	10	67
Tatumbla, Honduras	Green bean landrace	10	44
San Juan, Dominican Republic	Arroyo Loro Negro	10	65
Natal, South Africa	Teebus	10	78

[a] Sample = one to two bean leaves with urediniospores.

Table 2. Bean differential cultivars used to separate isolates of *Uromyces appendiculatus*

Differential number	Cultivar or line[a]	Resistance gene	Gene pool
1	Early Gallatin	*Ur-4*	Andean
2	Redlands Pioneer	*Ur-13*	Andean
3	Montcalm	…	Andean
4	PC-50	*Ur-9*, *Ur-12*	Andean
5	GGW	*Ur-6*	Andean
6	PI 260418	…	Andean
7	GN 1140	*Ur-7*	Middle American
8	Aurora	*Ur-3*	Middle American
9	Mexico 309	*Ur-5*	Middle American
10	Mexico 235	*Ur-3+*	Middle American
11	CNC	…	Middle American
12	PI 181996	*Ur-11*	Middle American

[a] Bean rust differential cultivars or lines adopted in the Third International Bean Rust Workshop in South Africa in 2002 (46). GGW = Golden Gate Wax and CNC = Compuesto Negro Chimaltenango.

ponent within field (41). The best sampling plan is obtained to minimize this variance subject to total cost limitation. When sampling bean rust, the average cost of a field is usually much higher than the average cost of a sample. Therefore, it is impractical to have a large number of fields; for a given total cost, we need to find the best number of fields and samples. This can be achieved by substituting equation 1 into the variance equation. The equation can be expressed as

$$\frac{\sigma_1^2}{\frac{\text{total cost}}{c_1 + c_2 n_2}} + \frac{\sigma_2^2}{\frac{\text{total cost}}{c_1 + c_2 n_2}} * n_2$$

By taking the first derivative with respect to the parameter n_2 and setting it to zero, we can get the number of samples per field, n_2, which minimizes the variance equation. The number of samples per field can be expressed as

$$n_2 = \sqrt{\frac{c_1 \sigma_2^2}{c_2 \sigma_1^2}} \qquad \text{(eq. 2)}$$

If we substitute n_2 into the total cost function, we can get the n_1. Thus, to estimate n_1 and n_2, we need estimates of σ_1^2 and σ_2^2 based on the bean rust data.

The σ_1^2 and σ_2^2 for MDS were estimated by using analysis of variance (ANOVA) estimators using the model $y_{ijk} = \mu + R_i + F_i(R_i) + \varepsilon_{ijk}$, where y_{ijk} was the response for the kth sample in the jth field in the ith region (tropical or temperate), F_i was the random field effect nested in region with mean 0 and variance σ_1^2, R_i was the fixed region effect, and ε_{ijk} was the residual with mean 0 and variance σ_2^2. Obtaining mean squares from the analysis of variance, σ_2^2 was estimated with the mean square error, and σ_1^2 was estimated as $(MS_{F(R)} - MS_e)$, where $MS_{F(R)}$ and MS_e were the mean squares for fields within region and error, respectively, and n_2 was the number of samples per field. The σ_1^2 and σ_2^2 for the number of races per field was estimated using restricted pseudolikelihood in a generalized linear mixed model with a Poisson distribution and a log link where the linear predictor was $\mu + R_i + F_i(R_i)$, and where all terms were defined as with the ANOVA model above (38,47). For both MDS and number of races, we used likelihood ratio tests to evaluate the homogeneity of σ_1^2 and σ_2^2 across regions.

Data analysis. The 1-to-6 rust grading scale was converted to quantitative disease scores published by Mmbaga at al. (29) for statistical analysis (Table 3). Analyses of variance of rust MDSs of the 380 isolates and tests of significance were performed using SAS PROC GLM for mixed models (38) to estimate components of variance (σ_1^2 and σ_2^2) that contributed to isolate variation. Analyses of variance also were performed on rust disease scores for each of the 12 bean differentials to identify bean differentials that differentiated the 380 rust isolates, and for each of the six fields to detect variation among samples in each field.

To determine virulence diversity among the 380 rust isolates collected from different regions, the isolates were grouped into different races based on rust reaction similarity on 12 bean differential lines or cultivars: a single rust reaction change on a single differential was considered a different race. The quantitative disease scores were converted to susceptible and resistant descriptive rust reactions for pathotype identification (Table 3). A binary system, where 1 = susceptible and 0 = resistant rust reactions, was used for this analysis. The Dice coefficient in the SimQual program of NTSYS pc (version 2.0; 36) was used to compare all pairs of the 380 rust isolates. The hierarchical clustering program SAHN of NTSYS with the unweighted pair group method average (UPGMA) procedure was used to group the isolates based on rust reaction similarity. Each group of isolates was considered a unique rust race and differed from the others in rust reaction in at least one differential. The degree of virulence of each pathotype was evaluated by determining the total number of susceptible rust reactions produced by each race across all 12 bean differentials. For the number of races per field, the within- and among-field components of variance (σ_1^2 and σ_2^2) for the generalized linear mixed model as described above were estimated using SAS PROC GLIMMIX (38).

RESULTS

MDS variation. For MDSs, the variances among all fields and among fields within countries contributed most to the total variance observed among the 380 isolates. Analyses of variance of the mean quantitative disease scores (MDS) of the 380 isolates showed significant differences ($P < 0.01$) in rust reactions of isolates among all fields, among samples within fields, and among fields within countries. No significant differences were detected for MDS among the four countries (United States, Honduras, Dominican Republic, and South Africa; Table 4).

The 12 bean differentials generally produced different rust reactions to the 380 isolates. There were significant differences in rust reactions of the 380 isolates on each of the 12 differentials among samples within fields, except for Redlands Pioneer; among fields; and among fields within countries, except for Golden Gate Wax (GGW), which did not show significant variation due to fields within countries. Variance among fields within countries on Aurora was significant with $P < 0.1$. Variances among countries were significant only on GGW and Aurora ($P < 0.1$ and 0.05, respectively; *data not shown*).

Differences in MDS among samples in each field were significant in Nebraska and Yuscaran, Honduras ($P < 0.05$); and Michigan, Tatumbla, Honduras, and South Africa ($P \leq 0.1$). No significant differences were detected on MDS among samples in the Dominican Republic field (Table 5). The variances among samples in fields

Table 3. Grading scale adopted for *Uromyces appendiculatus* survey and their corresponding quantitative disease scores

Reaction type[a]	Description	Quantitative disease score[b]	Rust reaction
1	Immune, no visible symptoms	1.1	Resistant
2	Necrotic spots without sporulation	2.1	Resistant
2,3	Reaction 2 with few type 3	2.4	Resistant
3,2	Reaction 3 with few type 2	2.7	Resistant
3	Uredinia < 0.3 mm in diameter	3.1	Resistant
3,4	Reaction 3 with few type 4	3.4	Susceptible
4,3	Reaction 4 with few type 3	3.7	Susceptible
4	Uredinia 0.3 to 0.49 mm in diameter	4.1	Susceptible
4,5	Reaction 4 with few type 5	4.4	Susceptible
5,4	Reaction 5 with few type 4	4.7	Susceptible
5	Uredinia 0.5 to 0.8 mm in diameter	5.1	Susceptible
5,6	Reaction 5 with few type 6	5.4	Susceptible
6,5	Reaction 6 with few type 5	5.7	Susceptible
6	Uredinia 0.8 to 1.2 mm in diameter	6.1	Susceptible

[a] According to Stavely et al. (44).
[b] According to Mmbaga et al. (29).

Table 4. Analysis of variance of mean disease scores of 380 *Uromyces appendiculatus* isolates

Source	Type I MS	df	*F* value	$P > F$[a]
All field	3.67	5	45.8	<0.0001**
Country	4.58	3	1.73	0.39 ns
Field (country)	2.31	2	16.8	<0.0001**
Sample (field)	0.14	54	2.13	<0.0001**
Error	0.07	320	…	…

[a] Asterisks (**) = significant at $P \leq 0.01$ and ns = not significant.

from tropical areas (Honduras, Dominican Republic, and South Africa) were higher than those from fields from temperate areas (Nebraska and Michigan). Overall, significant differences in rust reaction among samples in each field were detected for most of the 12 differentials. However, rust reaction among samples on each differential was not significant in at least one of the six fields. The Middle American bean differentials Aurora and Mexico 235 had no variation (variance = 0) in rust reactions among samples in Nebraska and Michigan fields (*data not shown*).

Race variation. In all, 65 unique virulence pathotypes or races of *U. appendiculatus* were identified from cluster analysis of the 380 isolates collected in Nebraska; Michigan; Yuscaran and Tatumbla, Honduras; Dominican Republic; and South Africa based on rust reaction similarity on 12 bean differential lines or cultivars. Twenty-three races were common in at least two of the six locations. The number of unique races was 21 in Honduras, 10 in Dominican Republic, 10 in South Africa, only 1 in Nebraska, and none in Michigan. The number of unique races was higher in fields from tropical regions (Honduras, Dominican Republic, and South Africa) than in fields from temperate regions (Nebraska and Michigan, United States). The frequency of occurrence of the races varied from 2 to 71 times.

A large variation in race was found among isolates collected in individual fields. The number of unique races found in each individual bean field varied from 2 to 25 (Table 6). The highest number of races was found in a Yuscaran, Honduras field (n = 25) followed by South African (n = 23) and Dominican Republic (n = 20) fields. The Michigan field had the lowest number of races (n = 2).

Race variation among rust samples collected in different sites of the same field was detected (Table 7). The number of races in individual samples varied from one in Nebraska and Michigan fields to eight in Yuscaran, Honduras. The number of races found in individual samples from each fields are presented in Table 6. Even though some races were more frequent than others, none of the races occurred in all 10 samples of the same field except one race in the Michigan field. Moreover, none of the 10 individual samples in each field represented all the races identified in that field.

The degree of virulence of each race also was determined. None of the races produced a susceptible reaction on all 12 bean differentials. Overall, races that produced a high number of susceptible reactions across the 12 bean differentials were found in South Africa, Honduras, and Dominican Republic. In all, 25 races (38.5% of the 65 races) produced susceptible reactions on 8 to 11 differentials. The most virulent race was found in South Africa, with 11 susceptible reactions. In all, 4 races (6.2% of the 65 races) were the least virulent, with three susceptible reactions. Thirty-six races (55.4%) produced susceptible reactions on between four and seven differentials and were found in all locations.

Reaction of bean differential lines or cultivars to rust isolates. Overall, bean differentials from the Middle American gene pool showed more resistant reactions to most of the rust isolates from all locations than bean differentials from the Andean gene pool (Table 8). Line PI 181996 (*Ur-11* gene) showed a high degree of resistance to most of the isolates in all locations. None of the isolates from Nebraska, Michigan, Dominican Republic, and South Africa produced uredinia on this line. A few rust isolates grouped in pathotypes 43, 44, 45, and 46 (2% of the 380 isolates) found in Yuscaran, Honduras produced a susceptible reaction (grade 5.1) on PI 181996. All Middle American differentials except GN 1140 showed a resistant reaction to all rust isolates from Nebraska and Michigan. On the other hand, Andean bean lines showed a susceptible reaction to most of the isolates in all six locations, with the exceptions of Redlands Pioneer, which was resistant to 55 (98%) of the 56 isolates from Nebraska, and PI 260418, which was resistant to 63 (97%) of the 65 isolates from Dominican Republic (Table 7). Excluding Montcalm that was not resistant to any of the 380 isolates, all other differentials showed a resistant rust reaction to at least one isolate (Table 7) but none of these differentials was resistant to all 380 isolates.

Development of bean rust sampling plans based on costs. The estimated variance components based on MDS and number of races are shown in Table 9. The variance component among fields (σ_1^2) was greater than the variance component within field (σ_2^2) for MDS whereas the opposite occurred for the number of pathotypes.

Assume that the total funding available for a study (total cost including supplies and transportation and per diem of a person) is approximately $10,000, where the average cost of sampling another field varies between $500 and $5,000 and the average cost of a single sample is between $5 and $1,000. Using equation 2, the number of fields and number of samples per field were estimated. The required number of fields to be sampled and number of samples per field to minimize the variance of the estimate for different costs of sampling for the MDS, and the number of pathotypes given that approximately $10,000 is available for the study, are illustrated in Table 10. For both, the MDS and the number of races, σ_1^2 did not differ significantly ($P > 0.05$) across regions, nor did σ_2^2 differ significantly across regions for number of races. However, for MDS, σ_2^2 did differ significantly ($P < 0.01$) across regions, with the temperate variance being smaller than the tropical (0.011 versus 0.032); however, these differences had only a slight impact on n_1 and n_2, and the pooled estimate (0.025) was used in Table 10.

DISCUSSION

The identification of 65 unique bean rust races from 380 isolates from four countries demonstrates the high pathogenic diversity of this fungus and emphasizes the need of monitoring this variability. High pathogenic variability of the bean rust pathogen has been reported previously from different regions of the world (2,4,6,29,31,43).

Some of the 65 rust races identified in this study were unique to a country or location. Isolates from fields from tropical or subtropical countries (i.e., Honduras, Dominican Republic, and South Africa)

Table 5. Analysis of variance of mean disease scores of *Uromyces appendiculatus* isolates by field

Field[a]	MS	df	*F* value	*P* > *F*[b]
NE	0.08	9	2.77	0.011*
MI	0.03	9	1.82	0.08
YH	0.23	9	2.87	0.007**
TH	0.23	9	1.82	0.1
DR	0.18	9	1.59	0.14 ns
SA	0.10	9	1.77	0.09

[a] Fields where the rust samples were collected: NE = Nebraska, United States; MI = Michigan, United States; YH = Yuscaran, Honduras; TH = Tatumbla, Honduras; DR = Dominican Republic; and SA = South Africa.

[b] Asterisks * and ** = significant at $P \leq 0.05$ and 0.01, respectively; MI, TH, and SA = significant at $P \leq 0.10$; ns = not significant.

Table 6. Number of unique *Uromyces appendiculatus* races and total number of isolates found at each location

Location[a]	No. of unique races	Total no. of isolates
NE	7	56
MI	2	70
YH	25	67
TH	19	44
DR	20	65
SA	23	78

[a] Fields where the rust samples were collected: NE = Nebraska, United States; MI = Michigan, United States; YH = Yuscaran, Honduras; TH = Tatumbla, Honduras; DR = Dominican Republic; and SA = South Africa.

had higher virulence and a larger number of races than isolates from fields from temperate regions of the United States. High virulence diversity of the bean rust pathogen in tropical areas was reported by Stavely and Pastor-Corrales (45). Mmbaga et al. (29) also found high virulence and a large number of races in rust isolates from Honduras and Dominican Republic. This study confirms the finding of Stavely and Pastor-Corrales (45) that more virulent isolates of *U. appendiculatus* are found in Latin America and Africa than in temperate regions. The high virulence diversity found in tropical regions may explain the high rust incidence and severity reported from these regions (15,30,46).

Several factors may contribute to the high virulence and large number of bean rust races in tropical areas. Mutation and hybridization that may occur in asexual races (21) can introduce variation because the sexual stage has not been reported in these areas (45). In most tropical countries such as Honduras, Dominican Republic, and South Africa, bean cultivars are planted in multiple seasons throughout the year, allowing the occurrence of several asexual rust cycles and production of large numbers of urediniospores. Environmental factors and bean genotypes used in a region may influence the genetic composition of the bean rust pathogen populations of each region. Temperatures between 16 and 24°C with moisture (16,45), which are common in highland tropical areas or during the dry season, favor the development of rust infections and, as a result, most rust races may survive between crops. On the other hand, temperatures greater than 32°C and low relative humidity may differentially kill the rust fungus (16), reducing the amount of *U. appendiculatus* survivors. The high temperatures that occur during summer in temperate regions of the United States may affect the virulence diversity of the bean rust fungus as in the case of Nebraska, where fewer pathotypes were found in a large field than were found in small fields from tropical countries. Similarly, although *U. appendiculatus* has been reported to overwinter on the U.S. High Plains, some races may be lost due to poor survival and diversity would be affected (25). In Honduras, bean lines from Andean and Middle American origin as well as landraces are grown in proximity to wild and weedy forms of the common bean and other *Phaseolus* spp. This diversity of bean genotypes also may explain the high virulence diversity and the presence of virulent races of *U. appendiculatus* in Honduras because the pathogen interacts with a wide range of bean genotypes when compared with isolates from Nebraska and Michigan, United States, where the genetic base of cultivars is similar and wild bean lines and landraces are not present. The low race diversity (two races) found in the Michigan field may be attributed to the small size of the field sampled, late bean season, and late rust infection, and the bean rust pathogen does not cause frequent and severe epidemics in Michigan.

Although many studies report sampling races from multiple locations, sites, or populations, this is the first report of virulence diversity of a fungal plant pathogen within a field that was not represented by a single site sample. The number of unique rust races found at individual sites varied from one in Nebraska and Michigan to eight in Yuscaran, Honduras, and races identified at each site in a field did not represent all races identified in that field. This race variation among samples collected at different sites of the same field has implications on identification of sources of rust resistance if only a few leaf samples with rust are collected from one site in each field. Race variation among bean rust isolates from a single leaf sample has been reported (7,12); however, whether this diversity occurred among samples from different sites of the same field was not determined. Mutation and hybridization that may occur in *U. appendiculatus* isolates on a single bean leaf can introduce variation among isolates within a leaf as well as among rust samples collected in different sites of the same field. In addition, the race variation within a single field also may be attributed to windborne spores from other fields near or far. Spores of the bean rust pathogen from different regions can land in the same bean field, act as primary sources of inoculum, and introduce race variation among samples collected in different sites of the same field, as detected in this study. Linde et al. (20)

Table 7. Number of *Uromyces appendiculatus* races in each sample by location

	Number of races per field[a]					
Sample number	**NE**	**MI**	**YH**	**TH**	**DR**	**SA**
1	3	2	2	4	4	5
2	3	1	2	3	4	6
3	3	2	5	4	4	4
4	2	2	5	3	5	5
5	2	2	4	2	6	4
6	2	2	8	4	4	5
7	4	2	4	3	5	4
8	3	2	5	3	4	5
9	1	2	6	4	4	6
10	4	1	6	4	4	5

[a] Fields where the rust samples were collected: NE = Nebraska, United States; MI = Michigan, United States; YH = Yuscaran, Honduras; TH = Tatumbla, Honduras; DR = Dominican Republic; and SA = South Africa.

Table 8. Percentage of *Uromyces appendiculatus* isolates from Nebraska; Michigan; Yuscaran, Honduras; Tatumbla, Honduras; Dominican Republic; and South Africa producing a resistant reaction on the 12 bean differential lines or cultivars

	Resistant reactions (%)[a]					
Line or cultivar[b]	**NE**	**MI**	**YH**	**TH**	**DR**	**SA**
Early Gallatin	29	0	69	11	59	4
Redlands Pioneer	98	0	72	57	37	4
Montcalm	0	0	0	0	0	0
PC 50	77	0	69	46	60	1
GGW	0	0	16	9	55	1
PI 260418	21	81	79	7	97	40
GN 1140	57	0	43	61	29	3
Mexico 235	100	100	81	93	85	58
Mexico 309	100	100	28	77	40	77
Aurora	100	100	73	93	2	35
CNC	100	100	31	80	46	78
PI 181996	100	100	91	100	100	100

[a] Resistant reaction = grades 1.1 to 3.1 from rust-grading scale in Table 3. NE = Nebraska, United States; MI = Michigan, United States; YH = Yuscaran, Honduras; TH = Tatumbla, Honduras; DR = Dominican Republic; and SA = South Africa.

[b] GGW = Golden Gate Wax and CNC = Compuesto Negro Chimaltenango. The first six bean lines are from the Andean gene pool and the last six are from the Middle American gene pool.

Table 9. Variance components based on the mean disease score and number of races[a]

Variable	**σ_1^2**	**σ_2^2**	**No. of fields sampled**	**No. of samples per field**
Mean disease score	0.06218	0.02453	6	10
Number of races	0.00376	0.32000	6	10

[a] Variance component among and within fields = σ_1^2 and σ_2^2, respectively.

and Groth and Roelfs (10) reported that the size of the sample affects the virulence diversity identified in pathogen population studies but also did not address the issue of within field variation. Within-field variation among strains of bacterium *Clavibacter michiganense* subsp. *nebraskense* has been reported on the basis of morphology and pathogenicity (40). McNeil et al. (27) also reported within-field genetic variation among isolates of *Wheat streak mosaic virus*. The race variation among *U. appendiculatus* leaf samples collected in different sites of the same field found in this study indicates that more than one sample per field should be collected and, thus, additional time and expenses may be required. Because the main components of variation among *U. appendiculatus* isolates in this study were variances among samples within fields and among all fields, future studies are needed to determine the number of *U. appendiculatus* samples to be collected per field and the number of fields per region or country to be sampled to accurately identify the pathogenic variability of populations of *U. appendiculatus* in a region.

The MDS variances among samples in fields from tropical or subtropical areas (Honduras, Dominican Republic, and South Africa) were significantly higher than the variances in fields from temperate areas (Nebraska and Michigan), suggesting higher virulence diversity among samples within fields from tropical areas than within fields from temperate areas. The larger number of races and higher variances among rust samples found in fields from tropical areas than in fields from temperate areas suggest that more rust samples should be collected per field in tropical regions than in temperate areas.

The presence of common races in different geographic regions indicates the absence of geographic differences of *U. appendiculatus* pathotypes and may be attributed to long-distance spore dissemination by wind, as suggested by MacLean et al. (22) after finding South American rust pathotypes in Australia. Convergent evolution also might be invoked to explain this sharing of similar phenotypes in different regions (1) because similar environmental conditions for infection and the same genus and species of host are necessary.

The bean differential lines or cultivars from the Middle American gene pool predominantly showed a wider resistance to *U. appendiculatus* isolates from different geographic regions than differentials from the Andean gene pool. Resistance of the Middle American genes to a larger number of rust races than the Andean genes also was reported by Pastor-Corrales (33). However, some highly virulent isolates from Honduras, Dominican Republic, and South Africa produced susceptible reactions in some of the Middle American differential lines or cultivars. The Middle American line PI 181996 (*Ur-11* rust resistance gene) showed a high degree of resistance in all locations; only four races from Honduras were able to cause susceptible reactions on this line, but Early Gallatin (*Ur-4*) and PI 260418 from Andean origin, as well as Aurora (*Ur-3*) and Mexico 235 (*Ur-3*+) from Middle American origin, were resistant to these races. The *Ur-11* gene has been reported to be resistant to a larger number of rust races (46), and it was reported to be resistant to 89 of the 90 races maintained at the United States Department of Agriculture, Beltsville, MD (33,34). The lack of stable rust resistance in most of the 12 bean differentials to isolates from Honduras and Dominican Republic is consistent with the difficulty of finding rust resistance in these countries as reported by Mmbaga et al. (29). The Middle American genes *Ur-5* and *Ur-3* and the unnamed gene from the Andean cv. Redlands Pioneer did not show consistent resistance to *U. appendiculatus* isolates from South Africa as previously reported by Steadman et al. (46). New races of *U. appendiculatus* that overcome these genes may have emerged in South Africa in the last year or two, confirming variability in virulence of this pathogen over time and the rapid breakdown of resistance after release of resistant bean cultivars in numerous reports (34,39,43).

All the differentials from Middle American origin except GN 1140 were resistant to isolates from Nebraska and Michigan, United States, whereas most of the Andean origin bean lines were susceptible. The susceptibility of the Andean bean lines to these isolates may be due to the extensive cultivation in the United States of dry bean cultivars containing *Ur-4* and *Ur-6* Andean rust resistance genes which may share the same Andean genetic background. The high race variation and the susceptibility of the single rust-resistance genes to different races of *U. appendiculatus* indicate that breeders should consider pyramiding various single genes to produce bean cultivars with multiple gene resistance through marker-assisted selection or use of specific rust races. *U. appendiculatus* races vary in different geographic regions or countries; therefore, development of bean germplasm resistant to rust should take into account the geographical distributions of such races.

The 12 bean differential lines or cultivars containing single or multiple rust resistance genes used in the present study produced different disease reactions to *U. appendiculatus* isolates from different geographic regions and even to isolates collected in the same field. For instance,

Table 10. Required number of fields and samples per field based on mean disease score and number of races, assuming a total cost budget of $10,000[a]

Cost per[b]		Mean disease score		Number of races	
Field	**Sample**	**Fields (n_1)**	**Samples (n_2)**	**Fields (n_1)**	**Samples (n_2)**
500	5	20	7	11	93
500	10	19	5	9	66
500	20	18	4	7	47
500	50	17	2	5	30
500	100	15	2	4	21
1,000	5	10	9	7	131
1,000	10	10	7	6	93
1,000	20	10	5	5	66
1,000	50	9	3	4	42
1,000	100	9	2	3	20
1,000	200	8	2	2	21
1,000	500	7	1	2[c]	13
2,000	5	5	13	4	185
2,000	10	5	9	4	131
2,000	20	5	7	3	93
2,000	50	5	4	3	59
2,000	100	5	3	2	42
2,000	200	5	2	2[c]	27
2,000	500	4	2	2[c]	11
2,000	1,000	4	1	2[c]	5
5,000	5	2	20	2	292
5,000	10	2	15	2	146
5,000	20	2	10	2	55
5,000	50	2	7	2	93
5,000	100	2	5	2[c]	25
5,000	200	2	4	2[c]	12
5,000	500	2	2	2[c]	5
5,000	1,000	2	2	2[c]	2

[a] Actual total cost may exceed the budget due to rounding.

[b] Cost in U.S. dollars, euros, or any other currency using total costs $c, n, + c_2 n, n_2$ where c is the average cost of sampling a field, c_2 is the average cost of a sample, n is the number of fields, and n_2 is the number of samples per field.

[c] Sample sizes estimated to ensure that at least two fields are sampled, a maximum budget of $15,000, or both.

Aurora produced susceptible reactions to most of the isolates from Dominican Republic and South Africa and resistant reactions to most of the isolates from Honduras and all isolates from Nebraska and Michigan. Even though Montcalm produced susceptible reaction (grades 3.4 to 6.1) to all 380 isolates, the ANOVA detected significant differences on quantitative rust scores of this differential in some locations. Although correlation analyses to reveal the relationship between the rust resistance genes present in the 12 bean differentials were not performed, differences in rust reactions in most of the differentials were evident. Even though disease reactions of the differential cultivars do not provide precise information about the number and types of resistance genes present in each cultivar or genes shared by other cultivars, they constitute a useful tool to determine virulence diversity and aid in the identification of sources of resistance to pathogens. Thus, the 12 bean differential lines or cultivars adopted in the Third International Bean Rust Workshop (46) should be maintained for future studies of the virulence diversity of *U. appendiculatus*.

Although sample sizes were small in this study and variance component estimates may not be very precise, we can draw some reasonable conclusions about sampling plans for bean rust. When comparing the variance components based on MDS, we observe that the variance among fields (σ_1^2) is greater than the variance within field (σ_2^2) (Table 9). Thus, if disease score is considered, relatively more fields need to be sampled with fewer samples per field. On the other hand, with the number of races, the variance components within field (σ_2^2) are greater than the variances among fields (σ_1^2) (Table 9), which means that relatively more samples need to be collected in fewer fields. These observations are supported by the required number of fields (n_1) and the number of samples per field (n_2) in Table 10. Therefore, the sampling plan depends on the type of variable to be considered. If one wants to measure disease prevalence using the MDS, relatively more fields should be sampled with fewer samples per field. However, if the number of races is being considered, more samples per field should be taken and fewer fields sampled. These results should hold in general; however, they are based on the assumption of equal within-field variances in both temperate and tropical regions. With our data, this assumption was not severely violated; however, it is likely that variances will be larger in tropical regions than in temperate regions. High virulence diversity of the bean rust pathogen has been reported from tropical regions (2,4,6,29,31). In this case, an even larger number of samples per field likely would be needed in tropical regions than what is reported here.

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