Race-Nonspecific Resistance in Bean Cultivars to Races of *Uromyces appendiculatus* var. *appendiculatus* and its Correlation with Leaf Epidermal Characteristics

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ABSTRACT

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Race-nonspecific resistance of beans (*Phaseolus vulgaris*) to *Uromyces appendiculatus* var. *appendiculatus* was assessed as the number of pustules (uredosori) that developed. Five cultivars (Jamaica Red, 27R, Round Red, Miss Kelly, and Portland Red) were inoculated with three Jamaican races (J4, J10, and J15) of *U. appendiculatus*. The cultivars differed significantly in the number of pustules per square centimeter of leaf, regardless of the race or leaf position inoculated. In general, the order of cultivars from most resistant to least resistant was Jamaica Red, 27R, Round Red, Miss Kelly, and Portland Red. Mean number of pustules per square centimeter was positively correlated with mean stomatal density on the adaxial leaf surface and negatively correlated with mean hair density on both leaf surfaces. However, a number of additional factors are probably involved in the apparent relationship between epidermal characteristics and the development of fewer pustules. The feature of race-nonspecific resistance reported here appears to be independent of the race-specific resistance present in the cultivars studied. Jamaica Red, which developed fewest pustules, showed race-specific resistance to none of the Jamaican races tested. Other components of resistance, such as development of smaller or more sparsely sporulating pustules or longer latent periods, were not exhibited by cultivars that developed fewer pustules per unit leaf area.

Additional key words: horizontal resistance, vertical resistance.

Rust of beans (*Phaseolus vulgaris* L.) caused by *Uromyces appendiculatus* (Pers.) Unger var. *appendiculatus* (= *U. phaseoli* (Pers.) Wint. [7]) has been recognized as a disease of economic importance in Jamaica (12) and elsewhere (2,15,17). Race-specific resistance has been used as a means of rust control, but without permanent success because of the appearance of new races of the pathogen that are virulent on the resistance genes present. Several workers (2,6,15,17) have recommended investigation of the possible components of race-nonspecific resistance to bean rust, such as the development of fewer and smaller pustules, and longer latent periods. Studies of some of these characteristics have already been reported (2,9,10,15). However, no study to date appears to have employed more than one race of *U. appendiculatus* to evaluate the features of race-nonspecific resistance in bean cultivars.

The objectives of the study reported here were to examine the differences among five bean cultivars with respect to the number of pustules developed following inoculations with three races of *U. appendiculatus*, and to investigate the relationship between these differences and some leaf epidermal characteristics of potential importance to the infection process.

MATERIALS AND METHODS

Bean cultivars and races of *U. appendiculatus*. Five pink- to red-seeded bush-type bean cultivars, Jamaica Red, 27R, Round Red, Miss Kelly, and Portland Red were studied. These cultivars were selected for study because preliminary investigations showed that, under comparable conditions, they differed in the number of pustules developed.

The three races of *U. appendiculatus* used in this study, designated J4, J10, and J15, were among the 21 races, J1 through J21, isolated from single pustules from collections made at different sites in Jamaica. These 21 races were distinguished on the basis of differences in the reactions of U.S. differential bean cultivars (13). The uredospores of a given race required for the inoculations described below were increased and maintained on susceptible bean plants kept under isolation chambers made of muslin stretched over wooden frames.

Experimental procedure. There were nine treatments (inoculations), each involving plants of one age (12, 20, or 30 days from sowing) and one race of *U. appendiculatus* (J4, J10, or J15). These plant ages were chosen for inoculations because earlier observations indicated that on 12-, 20-, and 30-day-old plants leaves 1 (primary, unifoliate), 2, and 3 (the first two trifoliolates), respectively, were most susceptible to infection, being between 5 and 7 days from opening.

Plants for the nine inoculations were grown in as many staggered batches from January to April 1980, in a greenhouse of the Botany Department, University of the West Indies, Mona, Jamaica. For each inoculation, enough plants were grown to ensure ten comparable plants of each cultivar for inoculation. Seeds were nicked opposite the hilum with a razor blade and soaked in water for 12 hr. The day of soaking was considered as day 1 in calculating plant ages. After being soaked, the seeds were placed in petri dishes lined with moist filter paper for a further 24 hr to allow germination to take place. Each germinated seed (radicle approximately 5 mm long) was transferred to a 10-cm-diameter pot containing 450 cc of a planting mixture composed of soil and coir (4:1, v/v). The pots were labeled, arranged on greenhouse benches, and watered daily. During the experiments, daily minimum and maximum temperatures were 18–20 C and 28–30 C, respectively.

For one inoculation, ten plants of each cultivar were assembled in an inoculating room and the lengths of all leaves or leaflets present on the plants were measured to the nearest millimeter. Leaf or leaflet length was taken as the distance from the point of insertion of the blade on the petiole or petiolule to the tip of the

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blade. Immediately after these measurements were made the plants were randomly arranged in a line on a bench and inoculated as follows.

Inoculum was prepared by adding 1 mg of freshly harvested spores of a given race to 4 ml of distilled water. The volumes of inoculum required to inoculate all the leaves present on 12-, 20-, and 30-day-old plants were different since leaf number increased with plant age. No wetting agent was added to the inoculum since previous studies showed that several of these agents reduced spore germination. The inoculum was sprayed onto the leaves with an atomizer driven by an electric air pump. Both leaf surfaces of all leaves present were sprayed as uniformly as possible. The atomizer was agitated constantly to ensure uniform distribution of spores in the spray. Inoculations were performed between 1700 hours and 1800 hours. Following inoculation, plants were placed under a moist chamber for 15 hr, after which they were arranged on the benches of a greenhouse allotted to growing inoculated plants.

Fourteen days after inoculation, the number of pustules or necrotic spots that developed on each leaf or leaflet were counted on all the plants. Pustule size (grade) was assessed visually by comparing the leaves with the scale devised by Davison and Vaughan (8).

Study of leaf epidermal characteristics. To study leaf epidermal characteristics, enough plants were raised to ensure six plants of each of the five cultivars, as described already. On each plant leaves 1, 2, and 3 were sampled as follows. Eight regions, approximately 1 cm^2, on leaf 1 (four on each unifoliate leaf) and twelve on each of leaves 2 and 3 (four on each leaflet) were considered for sampling. One region at each leaf position was randomly selected and smeared with a thin layer of 4% celloidin solution on both surfaces. After the celloidin had dried, it was peeled from the leaf surfaces. In this way, celloidin peels from leaves 1, 2, and 3 were obtained when the plants were 20, 30, and 40 days old, respectively, a time when the leaves sampled were fully expanded and near senescence. On each peel the numbers of impressions of stomates and of hooked epidermal hairs were counted in six high-magnification (×200) fields of view (total area = 1.04 mm^2).

Data analysis. The data were analyzed by using subprograms of the Statistical Package for the Social Sciences (SPSS) (11) running on the CDC Cyber 170/835 installation of the University of Western Ontario Computing Centre. Earlier analyses (13) established the linear relationship between the square root of leaf or leaflet area and leaf or leaflet length, on the basis of measurements made on healthy plants of each of the five cultivars studied here. Accordingly, leaf or leaflet area at the time of inoculation was estimated from the length measurements made. Counts of pustules or necrotic spots for each leaf or leaflet were then converted to numbers per square centimeter of leaf area at the time of inoculation (PPCM).

To reduce the heterogeneity of cultivar variances PPCM values were transformed to square roots (SPCM) and logarithms (LPCM) as follows (14):

$$\text{SPCM} = \sqrt{(\text{PPCM} + 0.5)},$$

and

$$\text{LPCM} = \log_{10}(\text{PPCM} + 0.5).$$

Heterogeneity of cultivar variances for PPCM, SPCM, and LPCM in each of the nine plant-age/pathogen-race/leaf-position combinations was examined by using Cochran's test (11). Although analysis of the raw data showed that the cultivars were significantly ($P < 0.05$) heteroscedastic (14), both of the above transformations produced homogeneous variances in most cases. One-way analyses of variance were done to test the significance of cultivar differences, using whichever transformation of PPCM produced homoscedasticity. Each analysis was followed by Duncan's multiple range test to identify subsets of cultivar means that were not significantly different at $P = 0.05$.

Numbers of stomata and hairs in six fields of view on a given peel were totaled and converted to numbers per square centimeter. Densities of stomata and hairs (numbers per square centimeter of leaf surface) were square-root transformed to obtain equal cultivar variances for each leaf-position/leaf-surface combination. One-way analyses of variance of the transformed data were carried out to test the significance of differences among cultivars. Correlation analyses were done to examine the association between cultivar susceptibility to each race of *U. appendiculatus* that produced pustules on all five cultivars and each of the epidermal characteristics, using the pooled, untransformed data of leaves 1, 2, and 3.

RESULTS

All the cultivars developed pustules when inoculated with race J10 or J15. With race J4, cultivar Portland Red developed necrotic spots, Miss Kelly was symptomless, and the remaining cultivars developed pustules. Necrotic spots developed by Portland Red were of various shapes and sizes, ranging from minute flecks to large areas covering up to 25% of the leaf or leaflet area. Pustule size was larger on leaves inoculated at an earlier ontogenetic stage than on leaves inoculated later. This pattern corresponds to that described earlier for bean rust by Groth and Urs (10). The pustules produced on Miss Kelly by J10 were an exception in being extremely small (grade 3) regardless of the ontogenetic stage of the leaf at the time of inoculation.

Greatest pustule densities developed on leaves 1, 2, and 3, on 12-, 20-, and 30-day-old plants, respectively. The analyses of differences among cultivars reported here pertain to data for these leaves only, although a brief summary is given of the results for the other leaves. In all of the one-way analyses of variance that were performed, cultivars contributed significantly to the total variation in pustules per square centimeter (Table 1). In inoculations with J10 and J15, the means of Jamaica Red, 27R, and Round Red were generally smaller than those of Miss Kelly and Portland Red (Table 1). With J4, the means of Jamaica Red were significantly smaller than those of 27R and Round Red (Table 1).

Leaves 1 on 20-day-old plants and leaves 1 and 2 on 30-day-old plants were fully expanded and nearing senescence when inoculated. A few pustules appeared on these leaves as green spots, mostly without any sign of sporulation. These leaves had senesced and abcised by the time of symptom scoring. Leaves 3 and 4 on 20- and 30-day-old plants, respectively, were not more than 25% expanded when inoculated. Pustule densities on these leaves were minimal although pustule size was larger than that on any other leaves in this study. For these leaves the trends among cultivars were similar to those in Table 1, with Portland Red developing greatest pustule densities.

In general, the mean numbers of stomata per square centimeter on the adaxial surfaces of leaves of Miss Kelly and Portland Red were greater than those for Jamaica Red, 27R, and Round Red (Table 2). No particular cultivar-related trends were observed in stomatal density on the abaxial leaf surfaces. On both leaf surfaces Jamaica Red, 27R, and Round Red had greater hair densities than did either Portland Red or Miss Kelly (Table 2). Pustule densities were positively correlated with stomatal densities on the adaxial leaf surfaces and negatively correlated with hair densities on either leaf surface (Table 3).

DISCUSSION

The overall order of cultivars from most to least resistant was Jamaica Red, 27R, Round Red, Miss Kelly, and Portland Red when the resistance was measured by pustule densities. In general, Jamaica Red developed the smallest pustule densities not only in the experiments reported here but also in field trials (13). Several one-way analyses of variance performed on the data presented in Table 1 showed that there were no significant differences between J10 and J15 in the pustule densities they produced on leaves at a given position of a given cultivar (13). Pustule densities produced by J4 were significantly greater than those produced by J10 or J15 on leaves of 27R and Round Red. Several one-way analyses of variance performed on the same data showed that, in general, pustule densities on primary leaves of a given cultivar inoculated

with a given race were significantly greater than those on the trifoliolate leaves (13).

It is possible to speculate on the probable mechanisms underlying the resistance reported here in view of the significant correlations between mean pustule densities and mean stomatal or hair densities. The roles of stomatal numbers and epidermal hairs in limiting infection have already been suggested in the case of bean rust (1,10).

Because *U. appendiculatus* enters the leaves through the stomata, it has been suggested that a reduction in stomatal numbers is likely to limit infection (10). However, in view of the high stomatal densities observed in this study, it seems unlikely that differences in stomatal numbers alone could account for the differences in rust susceptibility among the cultivars. In these inoculations, approximately 100 spores were deposited per square centimeter of leaf surface. It seems unlikely that failure of a pustule to form was due to failure of a germ tube to encounter a stoma for entry since stomatal densities ranged from 3,760 to 56,890 per square centimeter of leaf surface. It has been suggested that structural features of stomata are probably involved in triggering appressorium formation (16). Possibly a reduction in numbers of stomata would lead to a reduction in the number of stomata that are structurally suitable for penetration.

Epidermal hairs may distract a germ tube, as reported by Alten (1). He found that in some instances the germ tubes "twined from one leaf hair to another like wires without touching the leaf surface" (1). In addition, epidermal hairs could limit infection in the following manner. In studies of dew formation on wheat leaves Burrage (3) found that water droplets form first on the epidermal hairs and later coalesce, forming water films on the leaf surface. It may be that a relatively unbroken water film could form more readily on bean leaves with greater hair densities than on less hairy leaves. Spores of *U. appendiculatus*, being highly unwettable, are likely to rise to the surface of the water film, the latter in turn acting as a barrier between the spores and leaf epidermis. It has been demonstrated by Wynn (16) that germ tubes of *U. appendiculatus* grow in close contact with the leaf epidermis and that contact stimuli are probably involved in triggering appressorium formation over the stoma. In view of Wynn's (16) observation for bean rust, it is plausible to suggest that water films might minimize the contacts between germ tubes and leaf epidermis, thus limiting infection by *U. appendiculatus*.

Given the extreme susceptibility of Portland Red with respect to the pustule densities it developed, the small necrotic spot counts were unexpected. Christ and Groth (5) reported that the difference between densities of necrotic spots and pustules was not significant in bean cultivars US 3 and Early Gallatin. However, these authors did not report extreme variation in the size and shape of necrotic spots on those cultivars. Such a variation observed in the case of necrotic spots on Portland Red could explain the large difference between densities of necrotic spots and pustules on this cultivar. It is possible that not all the infection sites of race J4 on Portland Red developed into visible necrotic spots. Necrosis at the cellular level would have escaped detection. Furthermore, very large necrotic spots might have resulted from coalescence of several adjacent spots. In comparison to necrotic spots, pustules were more discrete and thus were a more reliable variable for quantitative assessment.

It is interesting that the feature of race-nonspecific resistance reported here appears to be unrelated to the race-specific resistance of the cultivars studied. In separate experiments, it was found that of 11 races tested, Miss Kelly, Portland Red, Round Red, 27R, and Jamaica Red were resistant (immune or developed necrotic spots) to 4, 3, 2, 0, and 0 races, respectively, and susceptible (developed pustules) to the remaining races (13). Cultivar 27R, however, has been reported to develop resistant reactions in other studies (4). The cultivar Jamaica Red was exceptional in not showing a resistant reaction to any race, nor to any of the unisolated inocula that were tested (13). The mechanism that confers resistance manifested by small pustule densities thus appears to be independent of the race-specific resistance present in a cultivar.

TABLE 1. Untransformed mean number of pustules per square centimeter of leaf area produced by three races of *Uromyces appendiculatus* var. *appendiculatus* on leaves at three positions on plants of five bean cultivars

	Pathogen-race/Plant-age/Leaf position								
Cultivar	J4/12/1	J4/20/2	J4/30/3	J10/12/1	J10/20/2	J10/30/3	J15/12/1	J15/20/2	J15/30/3
Jamaica Red	4.7[x]b	6.0 a	3.7 a	13.0 a	4.2	4.1 b	8.4 a	1.0 a	2.0
27R	8.3 c	7.3 a	11.3 c	13.2 a	4.5	3.1 ab	10.2 a	1.3 a	3.5
Round Red	···	10.0 b	6.5 b	12.1 a	6.3	1.6 a	14.3 b	3.0 b	2.7
Miss Kelly	0[y]	0[y]	0[y]	13.1 a	8.7	6.7 c	15.8 b	6.6 c	9.2
Portland Red	2.5[z]a	5.6[z]a	4.3[z]a	24.2 b	18.1	17.8 d	25.1 c	10.4 d	10.8

[x]Each value is a mean of 20 leaves (primary leaves, position 1) or 30 leaflets (trifoliolate leaves, position 2 or 3). Where fewer leaves were available, because of senescence, procedures appropriate for unequal sample sizes were used in calculating the a posteriori comparisons. In each column, means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test. Square root or logarithmic transformations were carried out on the data in each column in order to obtain homogeneity of cultivar variances. The transformation which produced homoscedasticity was used in the one-way analysis of variance (logarithmic in data columns 1, 4, 6, 7, and 8; square root, in columns 2 and 3). In columns 5 and 9, the cultivar variances were significantly heterogeneous ($P < 0.05$) regardless of the transformation used, so that analysis of variance results are not reported for these data.

[y]Immune rust reactions, not included in the analyses.

[z]Necrotic spot reactions, included in the analyses.

TABLE 2. Untransformed mean number of stomata or hooked epidermal hairs on adaxial (AD) and abaxial (AB) leaf surfaces at three positions on plants of five bean cultivars

	Leaf 1				Leaf 2				Leaf 3			
	Stomata ($\times 10^3$/cm^2)		Hairs ($\times 10^3$/cm^2)		Stomata ($\times 10^3$/cm^2)		Hairs ($\times 10^3$/cm^2)		Stomata ($\times 10^3$/cm^2)		Hairs ($\times 10^3$/cm^2)	
Cultivar	AD	AB	AD	AB	AD	AB	AD	AB	AD	AB	AD	AB
Jamaica Red	6.8[z]a	31.7 a	0.5 a	2.2 b	5.1 a	37.0 a	1.0 c	8.1 c	3.8 a	35.2 ab	1.3 d	9.7 c
27R	7.6 a	29.9 a	0.3 a	1.7 b	6.1 a	34.8 a	0.8 bc	7.6 c	4.7 ab	39.3 b	1.3 d	8.5 c
Round Red	8.7 ab	37.5 b	0.4 a	0.8 a	6.0 a	46.1 b	0.5 b	3.3 b	5.8 bc	56.9 c	0.6 c	5.0 b
Miss Kelly	9.8 b	29.7 a	0.3 a	0.9 a	9.0 b	32.7 a	0.2 a	2.4 a	7.1 c	30.3 a	0.4 b	3.8 b
Portland Red	12.0 c	40.9 b	0.3 a	0.6 a	15.5 b	53.1 b	0.2 a	2.1 a	9.4 d	38.7 b	0.2 a	2.5 a

[z]Each entry is a mean of six replicate plants. In each column, means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test. Square-root-transformed data were used in the one-way analyses of variance because that transformation resulted in homogeneous cultivar variances.

TABLE 3. Correlation between susceptibility to *Uromyces appendiculatus* var. *appendiculatus* race J10 or J15 and four epidermal characteristics of bean leaves

	Correlation coefficients			
	STCM[a]		HCM[a]	
Race	AD[b]	AB[b]	AD	AB
J10	0.83**[c]	−0.04 NS	−0.68**	−0.76**
J15	0.71**	−0.14 NS	−0.61*	−0.78**

[a] Epidermal characteristics: STCM, mean number of stomata per square centimeter; HCM, mean number of epidermal hairs per square centimeter.
[b] Leaf surfaces: AD, adaxial; AB, abaxial.
[c] Each entry is the coefficient of correlation between means of the number of pustules per square centimeter of leaf area, produced by one race on leaves at positions 1, 2, and 3 on plants of five bean cultivars ($N = 15$ means; data in Table 1), and the means of one epidermal characteristic scored on leaves at the same positions of different plants of the same cultivars ($N = 15$ means; data in Table 2). NS, $P > 0.05$; *, $0.01 < P < 0.05$; and **, $P < 0.01$.

However, the cultivars that developed fewer pustules did not show other features of resistance such as smaller pustules or longer latent periods. In general, pustule size was influenced by the race of the pathogen and the age of leaves at the time of inoculation. However, under comparable conditions with respect to race and leaf age, smaller pustules were more often noted on cultivars with race-specific resistance to a larger number of races than on those with race-specific resistance to fewer or no races (e.g., Miss Kelly inoculated with J10 and several other examples [13]). The actual mechanism in bean cultivars which restricts pustule size, however, needs further study.

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