

## The Effect of Leaf Developmental Stage on the Variation of Resistant and Susceptible Reactions of *Phaseolus vulgaris* to *Uromyces appendiculatus*

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### ABSTRACT

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The effect of leaf developmental stage of beans (*Phaseolus vulgaris*) at the time of inoculation on the expression of various reactions to rust (*Uromyces appendiculatus*) was investigated. The areas of uredinia, fungal colonies, and secondary uredinia were negatively correlated with leaf age or leaf length at the time of inoculation, in the most susceptible reaction (large uredinia). These three susceptibility parameters were all positively correlated with each other. In the reaction of smaller uredinia surrounded by necrosis, the percentage of uredinia surrounded by necrosis and fungal colony areas were negatively correlated, whereas uredinial area was

positively correlated with leaf developmental stage at the time of inoculation. The effect of leaf developmental stage on uredinial area in this reaction was thus the opposite of that observed for large uredinia. However, the covariate (leaf age)-adjusted means of uredinial and fungal colony areas were significantly lower in the smaller uredinial reaction than those in the large uredinial reaction. In highly resistant reactions (immunity and necrotic spots), leaf age effects were not apparent. Based on these results, several recommendations are made for studying resistance manifested by small uredinia.

One of the most destructive fungal diseases of beans (*Phaseolus vulgaris* L.) is bean rust, caused by *Uromyces appendiculatus* (Pers.) Unger var. *appendiculatus* (= *U. phaseoli* (Pers.) Wint.). The search for resistance is complicated because the pathogen exists as numerous races (1,7,17,19). Race specific resistance used in commercial cultivars has been overcome by the pathogen in the past (1,2,17,19,21). Nonspecific resistance has been recommended as an alternative control strategy (1,2,21). Nonspecific resistance is believed to be manifested by lower frequencies, smaller size, and slower development of uredinia (1,2). Although these features of resistance may also be affected by the race of the pathogen, certain cultivars exhibit these features consistently under field conditions.

It is difficult to evaluate the components of nonspecific resistance because their expression is affected by experimental conditions. For example, it has been noted that uredinial size decreases with increasing leaf age or plant age at the time of inoculation (6-8,12,20,22). The evaluation of leaf age effects is based on visual assessments of uredinial size in some studies (7,12,20), uredinial diameter (6,8), or area (22) measurements in others.

Groth and Urs (6) assessed the diameters of uredinia developing on leaves inoculated when 15% expanded and when almost completely expanded. The diameters of uredinia on the older leaves were 16% of those on younger leaves (6).

Imhoff et al (8) measured total lesion diameters and sporulation of uredinia developing on the first trifoliolate leaves inoculated 11 and 25 days after plant emergence. Lesion areas and sporulation were lower on leaves that were older at the time of inoculation (8).

Wei (20) found that in the most susceptible reaction, manifested by large uredinia, although uredinial size decreased with increasing leaf age at the time of inoculation, the decrease in size was not sufficient to cause a change in the uredinial grade. In these studies (20), the leaf age effects were more apparent in the reaction characterized by small uredinia surrounded by necrosis. However, Wei (20) did not report the statistical significance of the variation

in the small uredinial reaction due to leaf age.

Zulu and Wheeler (22) found that the mean uredinial areas on primary leaves inoculated at four different plant ages were not significantly different, whereas the largest mean on trifoliolate leaves was significantly different from three smaller means. Although the authors (22) reported a visual decrease in uredinial size according to increasing plant age at the time of inoculation, no regression analyses were attempted.

If resistance manifested by small uredinia is to be incorporated into bean cultivars it is important that variation in uredinial size due to leaf age effects be thoroughly understood. This requires proper experimental design, measurement, and statistical analysis. Our preliminary studies (14,15) indicate highly significant correlations between uredinial area and leaf developmental stage at the time of inoculation. In addition, the areas of fungal colonies and secondary uredinia were also found to be correlated with leaf age at the time of inoculation (15).

Studies of variation in uredinial size and other types of rust reactions (immunity and necrotic spots) are also important in another area of bean rust research, namely, the identification of races with subsequent virulence/resistance analyses. Races are identified by inoculating a set of differential cultivars and grading their reactions on a 0-10 (7) or 1-5 (3) or, more recently, on a 1-6 (17) grading scale. The lower two grades of all of these scales denote immunity and necrotic spots without uredinia and the higher grades denote uredinia of increasing diameters. Implicit in these scales is the assumption that each grade describes the interaction between a particular cultivar and a race of the fungus. The effect of leaf age at the time of inoculation on the expression of various grades with reference to race distinctions has not been assessed. The objective of this study is, therefore, to quantify the effect of leaf age at the time of inoculation on uredinial size and on the expression of immunity and necrotic spots.

### MATERIALS AND METHODS

**Sporulating reactions.** Two types of sporulating reactions were studied. One of these was the large uredinial reaction, which is considered as susceptible and evaluated as grade 5-6 on the most

recent grading scale (17). The other reaction was small uredinia surrounded by necrosis. This reaction is considered as resistant and evaluated as grade 2-3 on the 1-6 scale (17). Two bean cultivars, Pinto 650 and Pompadour Checa, and a single uredinial isolate of rust, 83HG25-7-C-A, were selected. This rust isolate, obtained from rust spore collections from the Dominican Republic, was known to produce large uredinia (grade 5-6) on Pinto 650 and small uredinia surrounded by necrosis (grade 2-3) on Pompadour Checa. Pinto 650 is a standard susceptible that develops grade 5-6 uredinia with nearly all the known races of *U. appendiculatus* (17). Pompadour Checa is a component of a land race of *P. vulgaris* occurring in the Dominican Republic. Several races of the fungus produce large uredinia, while others produce small uredinia surrounded by necrosis, on Pompadour Checa (M. Shaik and J. R. Steadman, unpublished). However, in the field, Pompadour Checa develops little rust, probably resisting the disease by means of nonspecific mechanisms (18). Seeds of single plant selections of Pinto 650 and Pompadour Checa used in this study are designated 83-14 and 83-30, respectively.

A staggered planting schedule was used to create plants of different ages. Seeds were nicked and sown (three per pot) in 15-cm-diameter pots filled with a soil/vermiculite/peat moss mixture (4:1:1, v/v/v). The seedlings were thinned to one per pot as soon as they emerged. A randomized block design with two replications (blocks) was used. Each block had 12 treatments, each of which had plants belonging to one age. Thus, the treatments consisted of plants that were 30, 28, 26, 24, 22, 20, 18, 16, 14, 12, 10, and 8 days old on the day of inoculation (sowing day is considered as day 1 in calculating plant ages). Each treatment had six plants (one plant per pot), three of Pinto 650 and three of Pompadour Checa. The treatments within a block and the six plants in a treatment were randomized on a greenhouse bench. The two blocks, which were placed on two adjacent benches of a greenhouse, were also staggered, with block one 1 day older than block two. This was done to enable measuring and inoculating all leaves of the 72 plants in a block in 1 day, and to be able to fit these plants into one large mist chamber. The greenhouse temperature was  $24 \pm 3$  C. No artificial light was used. This experiment was conducted from 16 September to 31 October 1986.

The dates of unfolding of primary leaves on every plant were recorded. On the day of inoculation (16 and 17 October, for blocks one and two, respectively) the leaf ages (days from unfolding) and lengths of primary leaves were recorded for all the plants. Because the two primary leaves on any one plant are nearly identical in size, only one primary leaf on each plant was measured. The length of a leaf was taken as the distance, measured to the nearest millimeter, along the leaf blade from the insertion of petiole to the leaf tip. In the remainder of this paper, the terms leaf age, leaf length, and leaf developmental stage are used to denote measurements or stages assessed at the time of inoculation only. After the measurements, the plants were arranged in a line on a bench, in the same random order as on the greenhouse bench, and inoculated with urediniospores. Inoculum was prepared by adding freshly harvested urediniospores of isolate 83HG25-7-C-A to tap water at the rate of 5 mg to 150 ml of water. No wetting agents were added to the inoculum. A total of 1.2 L of the inoculum was used to spray, as uniformly as possible, both surfaces of all the leaves present on the 72 plants of one block. The inoculum was sprayed using a pressurized propellant (Fisher Chemicals). In our preliminary studies (14,15), it was established that the above concentration of inoculum (5 mg of spores per 150 ml of water) and the method of application (spraying) produced uredinia far apart from one another to facilitate the removal of individual uredinia as described below. It was also found (14,15) that this method of inoculation eliminated the crowding of uredinia that results in reduction of their size (4). Immediately after the inoculation, the plants were placed in a mist chamber (100% RH and 17-20 C) for 20 hr, after which they were removed and placed on the greenhouse bench.

Fourteen days after inoculation, 20 randomly selected uredinia were removed from the primary leaves of each plant by cutting out approximately  $4 \times 4$  mm leaf pieces each containing one uredinium. Uredinia from the primary leaves of one plant were placed in one vial. The leaf pieces were cleared and stained using a modified procedure of McBryde (9). Approximately 5 ml of chloral hydrate in water (2:1, w/v) was added to each vial containing the leaf pieces. The leaf pieces were left in this solution up to 2 days to clear the leaf pigments. The leaf pieces were then stained for 4-6 hr in 0.5 ml of 2% acid fuchsin in 70% ETOH, 5 ml of the chloral hydrate solution, and 3 ml of 90% ETOH. The leaf pieces were destained in the chloral hydrate solution, mounted in lactophenol, and observed under the microscope.

Acid fuchsin stains the fungal mycelium pink, while the mesophyll remains relatively colorless. The areas of primary and secondary uredinia on the abaxial surface of the leaf and of the fungal colony in the mesophyll were measured. Area of the fungal colony was the area of the mesophyll occupied by the mycelium, and included the primary and secondary uredinial areas. In the cases of uredinia surrounded by necrosis, the fungal colony area includes the necrotic area. By means of a camera lucida attached to a microscope the image of a uredinium was projected onto a digitizing tablet (Jandel Scientific, Sausalito, CA). The outlines of the three areas were traced by means of an LED cursor and converted to area measurements using Sigma Scan software (Jandel Scientific) running on an MS-DOS microcomputer. In addition to the area measurements on Pompadour Checa (small uredinial reaction), the number of uredinia, of the 20 sampled, surrounded by necrosis was counted. Secondary uredinial formation was rare on Pompadour Checa, and, therefore, this variable was not measured on this cultivar.

The data were analyzed with a series of Statistical Analysis Systems (SAS) procedures (11) and BIOM-pc programs (10) running on an MS-DOS microcomputer. To reduce the slight curvilinearity present in the raw data, the individual values for uredinial area, fungal colony area, and secondary uredinial area were square root transformed (16). Means of the transformed variables were calculated for primary leaves of each plant from the 20 uredinia. Percentage of uredinia surrounded by necrosis was calculated for each plant of Pompadour Checa. These means and percentages were used first to confirm that the two blocks did not differ significantly. Subsequently, the following analyses were performed on data pooled over the two blocks.

A series of simple regression analyses were carried out in all combinations using leaf age and leaf length as independent variables and uredinial, secondary uredinial, and fungal colony areas, and the percentage of uredinia surrounded by necrosis as dependent variables for each cultivar. The differences between cultivar means and slopes were tested by analyses of covariance (ANCOVAs) (10). Correlations between and among the susceptibility parameters and leaf developmental indices (leaf age and leaf length) were calculated.

**Nonsporulating reactions.** The effect of leaf age on the expression of immunity and necrotic spots was investigated in three inoculations. In each of these inoculations, there were four treatments, consisting of plants that were 26, 20, 16, and 8 days old at the time of inoculation. The different plant ages were created by a staggered planting schedule. The treatments were randomized, and the plants within each treatment (three per cultivar), were also randomly arranged on a greenhouse bench. The method of inoculation was the same as described above for the sporulating reactions, but the scoring of symptoms was different. Only visual observations of symptom expression were made. Notes were made on the presence or absence and size of necrotic spots. No measurements were made and hence no statistical analyses were done.

With the above experimental design, three cultivars, Compuesto Negro Chemaltenango (CNC), Golden Gate Wax (GGW), and Pinto 650, were inoculated with D85SJ3-1 to which CNC and GGW were known to be immune. Two cultivars, Mexico 309 and Pinto 650, were inoculated with D85SJ2-1, an isolate to which

Mexico 309 was known to be immune. Lastly, two cultivars, Olathe and Pinto 650, were inoculated with US85NP11-1, which was known to produce necrotic spots on Olathe. In all three inoculations the susceptible check, Pinto 650, was included for comparison as well as to confirm the viability of urediniospores and the success of the inoculations.

## RESULTS

**Sporulating reactions.** At the time of inoculation, the staggered planting schedule produced plants ranging in age from 8 to 30 days from sowing. These plants had primary leaves that were 1–24 days old (days from unfolding) when inoculated and that were 14 days older when uredinia were harvested. Many of the older leaves (leaf age >20) had senesced and dropped off by the time of uredinial collection. All the analyses presented in this report used the data of primary leaves whose leaf age was between 1 to 20 when inoculated because only for these were complete data available.

On Pompadour Checa, which developed the small uredinial reaction, leaves with youngest leaf age values had large necrotic areas with minute uredinia in their centers (Fig. 1). The pink-stained mycelium was visible within the necrotic area and, in most cases, had grown beyond the necrotic region (Figs. 2 and 3), sometimes producing secondary uredinia (Fig. 2). At older leaf age values, very few or none of the uredinia were surrounded by necrosis (Fig. 4). Pinto 650 developed large uredinia, and the difference in uredinial size on this cultivar due to increasing leaf age is shown for comparison (Figs. 5 and 6). Uredinia on Pinto 650, regardless of size, dehisced and liberated viable spores by the time the uredinia were harvested. On Pompadour Checa, uredinial dehiscence and viability of spores from younger leaves (leaf age <10) was similar to that on Pinto 650. On older leaves (leaf age >10), however, the uredinia were abnormal in that they often did not dehisce to liberate spores and the latter were not as viable as those developing on younger leaves.

On Pinto 650, uredinial area, secondary uredinial area, and fungal colony area were positively correlated with one another and negatively correlated with leaf age and leaf length (Table 1). All these correlations were highly significant (Table 1). On Pompadour Checa, the uredinial area was negatively correlated with fungal colony area and positively correlated with leaf age and leaf length, the opposite of the trends on Pinto 650 (Table 1). Correlations between fungal colony area and leaf age and leaf length on Pompadour Checa were negative, as in the case of Pinto 650 (Table 1). Percentage of uredinia surrounded by necrosis was negatively correlated with leaf age and leaf length (Table 1).

TABLE 1. Correlations between and among leaf developmental indices (leaf age and leaf length<sup>a</sup>) and rust susceptibility parameters (SUA, SFCA, SSEC, and PERC<sup>b</sup>) for two bean cultivars, Pinto 650 and Pompadour Checa (sample size  $N = 52$  plants per cultivar)

	LLTI	SUA	SFCA	SSEC	PERC
Pinto 650					
LATI	0.861*** <sup>c</sup>	-0.940***	-0.957***	-0.954***	...
LLTI		-0.884***	-0.889***	-0.879***	...
SUA			0.981***	0.966***	...
SFCA				0.993***	...
Pompadour Checa					
LATI	0.795***	0.423**	-0.817***	...	-0.928***
LLTI		0.730***	-0.827***	...	-0.843***
SUA			-0.409**	...	-0.497***
SFCA				...	-0.837***

<sup>a</sup>LATI and LLTI are leaf age and leaf length, respectively, at the time of inoculation.

<sup>b</sup>SUA, SFCA, and SSEC stand for means of square root transformed uredinial area, fungal colony area, and secondary uredinial area, respectively, and, PERC for percentage of uredinia surrounded by necrosis.

<sup>c</sup>\*\*\* = 0.01 >  $P$  > 0.001. \*\*\* =  $P$  < 0.001.

Simple regressions (Figs. 7 and 8) demonstrated the relationship between the susceptibility parameters and leaf age and leaf length that were revealed in the correlation analyses. Susceptibility measured by uredinial size and related variables was negatively related with leaf developmental stage at the time of inoculation. The exception to this trend was in the small uredinial reaction on Pompadour Checa where uredinial area was positively related with leaf length but not linearly related to leaf age (Fig. 8). Coefficients of determination ( $R^2$ ) were higher when leaf age was used as a predictor, than when leaf length was used (Figs. 7 and 8E and F), except for fungal colony area on Pompadour Checa (Fig. 8C and D).

The results of ANCOVAs revealed highly significant ( $P < 0.001$ ) differences between the slopes and adjusted means of the two cultivars in the uredinial areas and fungal colony areas (Fig. 7A–D compared with Fig. 8A–D, respectively).

**Nonsporulating reactions.** The effect of leaf age on the expression of immunity and necrotic spots was minimal. In the case of immune reactions, i.e., CNC and GGW inoculated with D85SJ3-1 and Mexico 309 with D85SJ2-1, the primary leaves on all plants were symptomless as expected. However, on the trifoliolate leaves there were small necrotic flecks visible to the naked eye. In the necrotic spot reaction on Olathe inoculated with US85NP11-1 the effect of leaf age or leaf position was not apparent. Occasionally, on extremely young leaves (leaf age = 1), the size of the spots was larger than on older leaves.

## DISCUSSION

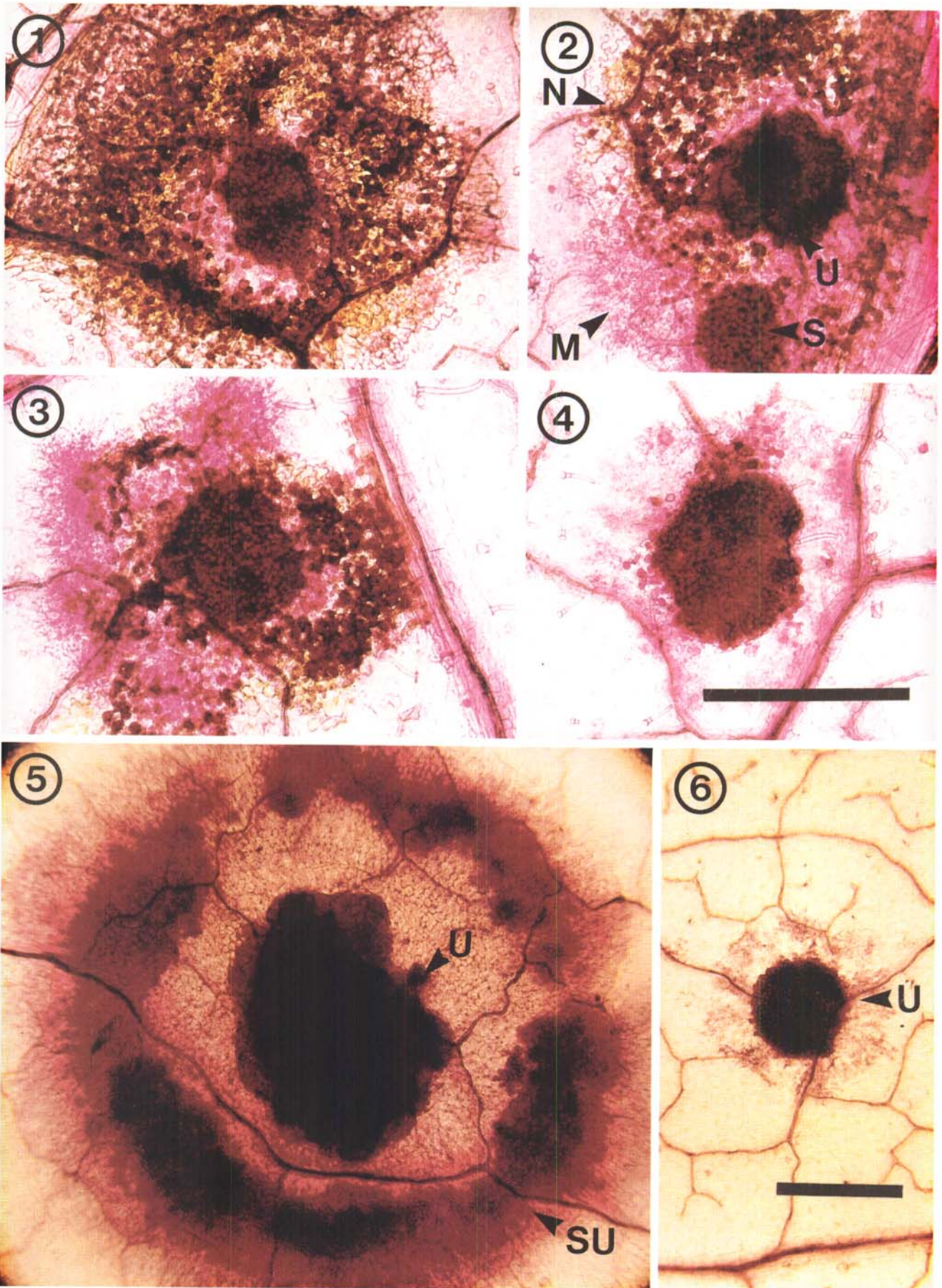
**The structure of bean rust uredinia.** The size of bean rust uredinia has long been used as a measure of susceptibility. Traditionally (3,7,17), the size of uredinia has been graded according to diameter. The architecture of uredinia should be taken into account, however, to find a variable that would best describe susceptibility. In most cases, uredinia and secondary uredinia sporulate on both surfaces of the leaves. The uredinial areas on the two leaf surfaces are nearly equal in most cases. In other cases, although highly correlated, the abaxial uredinial area is slightly greater than the adaxial one (M. Shaik and J. R. Steadman, *unpublished*).

Epidemiologically, it is the number of spores produced in the uredinia that is of most importance in the spread of disease. However, measuring the quantity of spores produced in individual uredinia would be extremely tedious. Uredinial size is an alternate indicator of susceptibility. In the past, the areas of uredinia have been estimated from their diameters. The present study shows that while smaller uredinia are circular in shape, the larger ones are not (Figs. 1–6). Diameter measurements are thus not necessarily appropriate for all bean rust uredinia. Fungal colony area, which is circular for most uredinia (Figs. 1–6), is more suitable for diameter measurements.

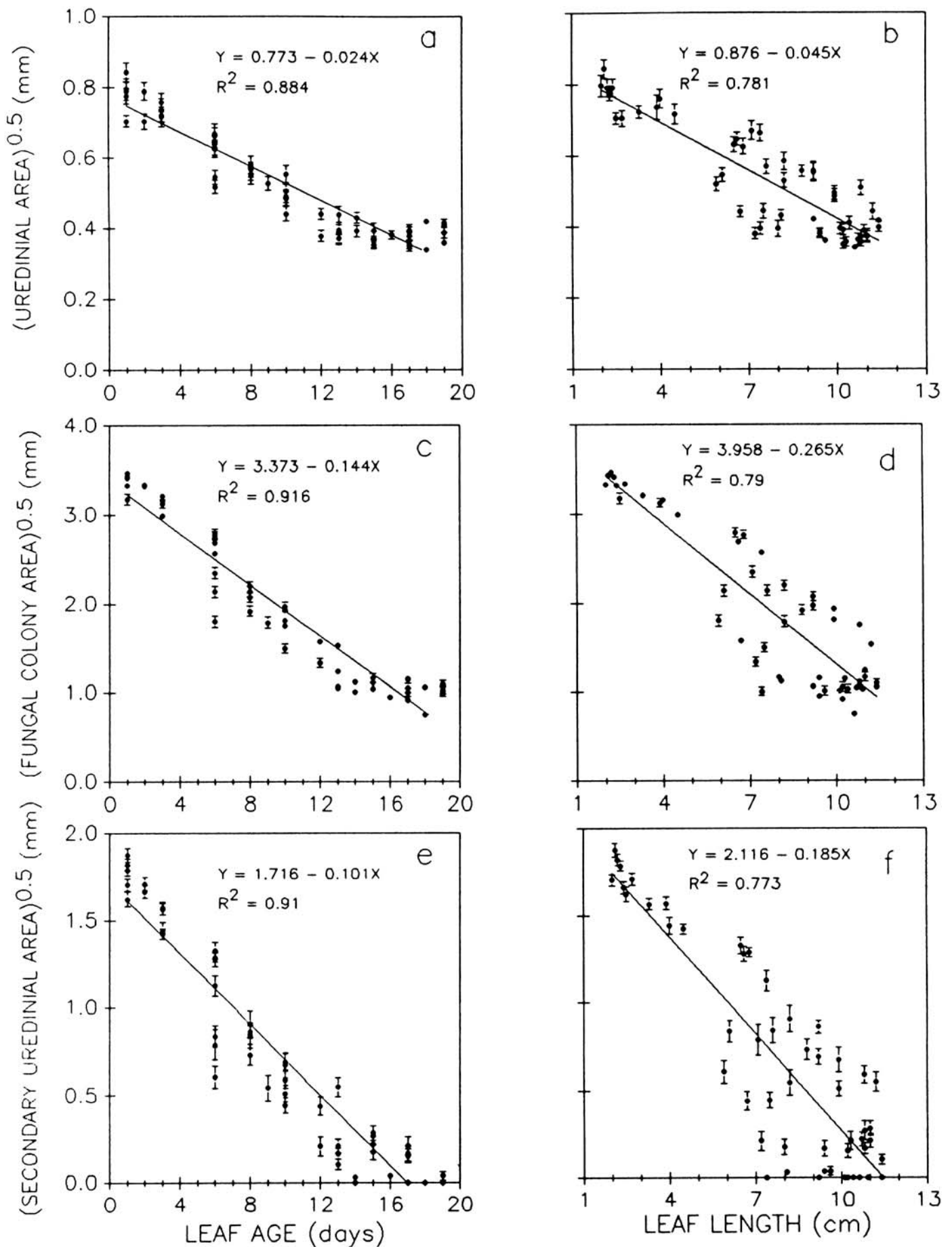
Measuring the fungal colony as a susceptibility parameter has the following advantages. First, it can be easily stained and measured accurately. Second, since fungal colonies are circular, their areas are proportional to diameter measurements. Third, the fungal colony area exists within the mesophyll only, and not, as is the case with uredinia, on both the leaf surfaces.

Of the three susceptibility parameters, secondary uredinial area is the most tedious to measure because it exhibits most irregular shapes. It develops as a nearly complete ring around large uredinia (Fig. 5). This ring is broken into several units for smaller uredinia while it is altogether absent for smallest ones (Fig. 6). Nevertheless, secondary uredinial area should be of greater epidemiological significance than uredinial area because the former can be up to four times larger than the latter (Fig. 7). Detailed studies (13) have shown that cultivar differences in secondary uredinial formation can be demonstrated merely by counting the number of uredinia surrounded by secondary uredinia.

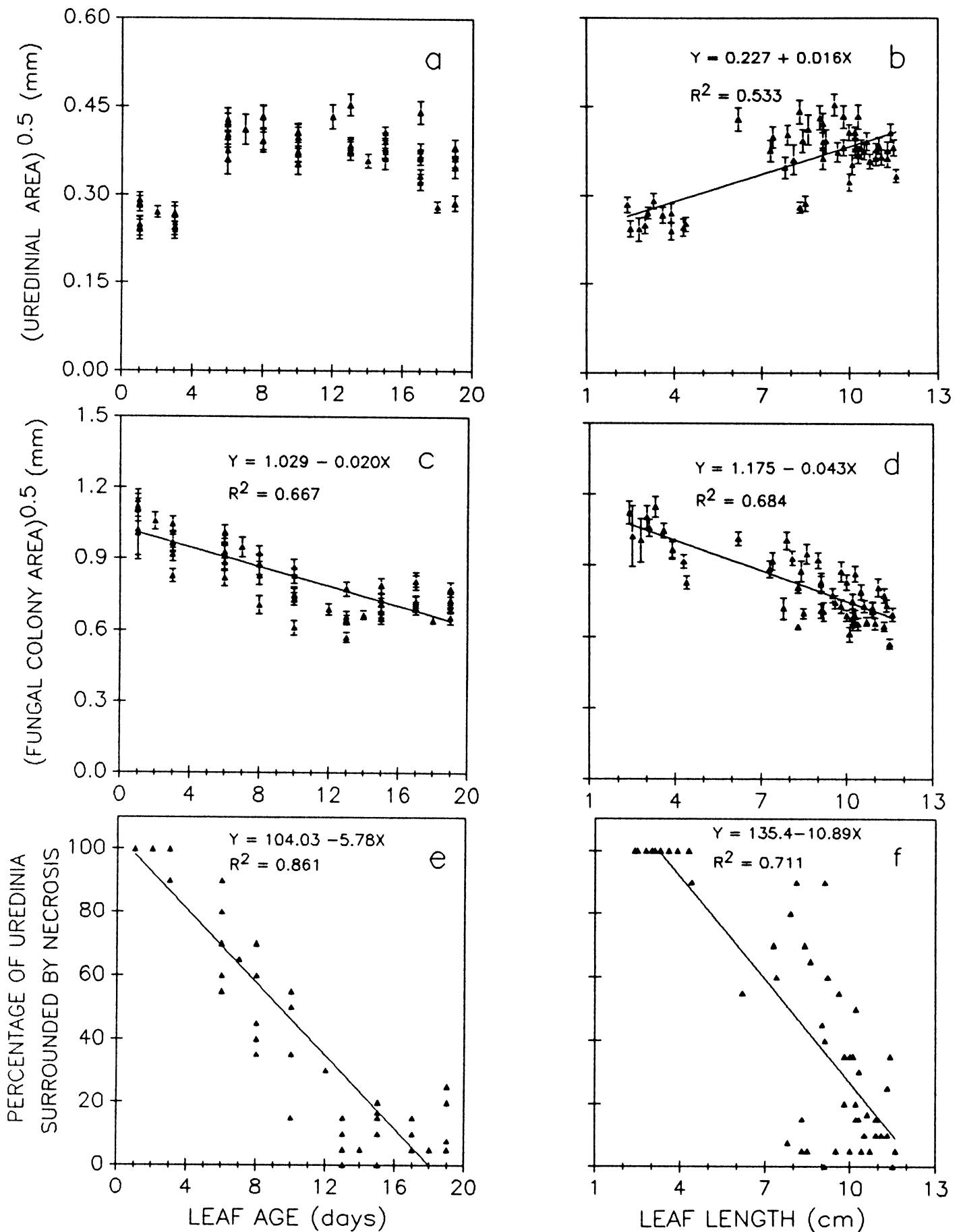
There are two important structural differences between small and large uredinia. First, secondary uredinial formation is rare in the small uredinial reaction. Secondly, uredinial and fungal colony areas are positively correlated for large uredinia and



**Figs. 1-6.** Photomicrographs of uredinia from a small uredinial reaction on cultivar Pompadour Checa (Figs. 1-4) and large uredinial reaction on Pinto 650 (Figs. 5 and 6). The uredinia were obtained from leaves that were 3 (Figs. 1 and 5), 6 (Fig. 2), 8 (Fig. 3), 16 (Fig. 4), and 20 days (Fig. 6) old when inoculated. N = necrosis, U = uredinium, S & SU = secondary uredinium, and M = mycelium. Scale bars (0.5 mm) are shown in Figure 4 for Figures 1-4 and in Figure 6 for Figures 5 and 6.



**Fig. 7.** Square root transformed uredinial area, fungal colony area, and secondary uredinial area regressed on leaf age and leaf length at the time of inoculation for the large uredinial reaction of primary leaves of bean cultivar Pinto 650. Each data point represents a mean of 20 uredinia for a single plant (sample size  $N = 52$  plants), while error bars represent the standard error of the mean. Most error bars are hidden by the symbols. There are several superimposed data points in all the graphs.



**Fig. 8.** Square root transformed uredinial and fungal colony areas and percentage of uredinia surrounded by necrosis regressed on leaf age and leaf length at the time of inoculation for small uredinial reaction of primary leaves of bean cultivar Pompadour Checa. Each data point represents a mean of 20 uredinia for a single plant (sample size  $N = 52$  plants), while error bars represent the standard error of the mean. Some error bars are hidden by the symbols. The percentages of uredinia surrounded by necrosis are also calculated from the same 20 uredinia for single plants. There are several superimposed data points in all the graphs.

negatively correlated for small uredinia (Table 1). The latter difference is probably related to the presence of necrosis in the small uredinial reaction. Necrosis appears to limit uredinial area but not fungal colony area (Figs. 1-4).

**The effect of leaf developmental stage on susceptibility.** There is a highly significant negative correlation between susceptibility as measured by uredinial area, fungal colony area, or secondary uredinial area and the developmental stage of the leaves at the time of inoculation for the susceptible reaction on Pinto 650. These results are consistent with those of a preliminary study with the same isolate and cultivar (15). In an earlier study (20), this relationship was not revealed, probably because uredinial size was estimated from their diameter-grades rather than from actual measurements. In another study (22), the lack of significant difference between the means of areas of uredinia developing on plants inoculated at four different ages was probably due to the small sample size used, i.e., only seven uredinia were measured on each leaf. The present study shows that on individual leaves variation in uredinial size is considerable. As a result, large sample sizes are necessary to document the significance of the difference in uredinial size.

Because the extent of necrosis is greater when leaves are inoculated at earlier developmental stages, and because necrosis apparently limits uredinial size, correlations between uredinial size and leaf age (and leaf length) were positive in Pompadour Checa in contrast to Pinto 650. It should be emphasized that in this reaction, the fungus produces uredinia despite the surrounding necrosis. The necrosis develops 2-3 days after inoculation, while uredinia appear in its center about 5 days after inoculation. Therefore, it would appear that the initial defense reaction of the host does not suppress fungal growth and sporulation completely. As infection occurs at increasingly older leaf ages, necrosis is less frequent and less extensive and uredinial size is not similarly limited (Figs. 2-4). In a susceptible reaction, there is no necrosis, and uredinial size reaches its maximum development determined by leaf age alone (Figs. 5 and 6).

In general, the development of necrosis in relation to leaf age in the small uredinial reaction on Pompadour Checa was similar to that previously reported by Wei (20). However, in Wei's study (20), up to 55% of disease lesions on younger leaves were necrotic spots without any uredinia. In the present study, all the lesions examined had uredinia. Uredinia surrounded by necrotic areas were especially small, and their presence in some cases could only be confirmed under the microscope. It is possible that this difference between Wei's (20) study and the present one is due to the use of different cultivars or a different pathogenic race.

The developmental stage of leaves at the time of inoculation was assessed as leaf age and leaf length. Of the two, leaf age is the better predictor of susceptibility in all but one of five comparisons of  $R^2$  values (Figs. 7 and 8). This is because leaf age varies continuously, whereas leaf length ceases to increase about 5 to 6 days after unfolding (13). As a result, leaf length can account for susceptibility changes in rapidly expanding, but not fully expanded, leaves. However, chronological age is not necessarily the best descriptor of leaf developmental stage (5), and an alternative is described elsewhere (13).

The effect of leaf developmental stage on uredinial size has been demonstrated repeatedly for bean rust, although large sample sizes are necessary to obtain statistically significant results. The present study has documented the statistical significance found in preliminary studies (14,15) with the same isolate/cultivar combinations. Similar results have been obtained in another inoculation involving primary and trifoliolate leaves (13). Based on these studies, the following recommendations can be made for two areas of bean rust research, namely identification of races with virulence analysis, and evaluation of resistance manifested by uredinial size.

**Identification of races.** The present study shows that immunity and necrotic spots are constant for a given race/cultivar combination regardless of plant age or leaf age at the time of inoculation. Uredinial size, on the other hand, varies with leaf developmental stage at the time of inoculation. Races are identified

by inoculating plants of differential cultivars at a given age (3,7,17). The leaves on identical-aged plants, however, are rarely at the same developmental stage (13). As a result, observed variation in uredinial grades might well be due to differences in leaf developmental stages at the time of inoculation. Race distinctions based on uredinial grade differences alone could thus be questioned. The present study indicates that, ideally, the difference between races in the size of uredinia they produce on a given cultivar should be evaluated on the basis of covariate (leaf age)-adjusted means.

**Evaluation of resistance.** The leaf developmental stage at the time of inoculation must be taken into account when evaluating the differences in the uredinial size between the cultivars. Resistance manifested by small uredinia has been recommended for more than a decade (1,2,21). However, methods to incorporate leaf age into the experiments evaluating uredinial size have not yet been developed. We suggest that cultivars developing small uredinia consistently under field conditions be selected for detailed studies. Preliminary cultivar screenings can be done in the greenhouse by inoculations performed at one leaf age, preferably within 6 days after unfolding. Detailed evaluations of cultivar differences, however, must be done by inoculating leaves at a range of developmental stages. Areas or diameters of the fungal colonies should be measured to investigate the cultivar differences. Cultivars can be compared by means of ANCOVA, testing first whether the relationships between susceptibility and leaf age differ (heterogeneity of slopes) and second, whether the covariate (leaf age)-adjusted means differ significantly.

A variable that has not been given sufficient importance in previous studies is the secondary uredinial formation. Secondary uredinial area can be up to four times greater than the primary uredinial area (Fig. 7). As already discussed, this variable is extremely tedious to measure. However, cultivar differences in the proportion of uredinia developing secondary uredinia on leaves inoculated at different developmental stages would be easier to assess (13). This would be an invaluable variable in selecting resistance that limits sporulation.

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