Variation in Rust Susceptibility in Beans: Predicting Lesion Size from Leaf Developmental Stage Measured by Leaf Age, Length, and Plastochron Index

M. Shaik, T. A. Dickinson, and J. R. Steadman

First two authors, Department of Botany, Royal Ontario Museum, 100 Queen's Park, Toronto, Ontario, Canada M5S 2C6; third author, Department of Plant Pathology, University of Nebraska, Lincoln 68583-0722.

ABSTRACT

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The importance of controlling for leaf developmental stage at the time of inoculation in studies of lesion size was investigated. Significant differences were obtained between two cultivars, Pompadour Checa and Pinto 650, by regressing lesion size on leaf developmental stage at time of inoculation. Leaf developmental stage was recorded as leaf age (days from unfolding), as leaf length, and by means of a plastochron index.

Of these three variables, leaf age and plastochron index were better predictors of lesion size than leaf length. Plastochron index is a superior indicator of leaf development since it integrates chronological age and increase in size and is easier to assess. The results reported here may explain certain aspects of the field resistance of Pompadour Checa.

Additional keywords: Phaseolus vulgaris, resistance, Uromyces appendiculatus.

Rust caused by *Uromyces appendiculatus* (Pers.) Unger var. *appendiculatus* is an important disease of beans especially in the tropics. Because the pathogen exists as numerous physiological races (2,22), attempts to control rust by using specific resistance have had limited success in the past (22,23). Nonspecific resistance to rust has been suggested to occur as lower frequencies, smaller size, and slower development of disease lesions (2,6,23). The use of nonspecific resistance as a control strategy has been recommended for more than a decade (2,6,23). However, studies of nonspecific resistance remain scarce. Features of nonspecific resistance are difficult to evaluate because they are affected by factors such as the leaf developmental stage at the time of inoculation (8,14,16) and the leaf position inoculated (8,14–16).

Leaf developmental stage has been assessed as leaf age (days from unfolding) (8,16) and as leaf length (14,16) in previous studies. There are several disadvantages to using leaf age or leaf length as indicators of leaf developmental stage. Plants and their individual parts develop at different rates under different conditions (5,9). Plants (or leaves) of the same chronological age but growing under different conditions may not necessarily be

at the same developmental stage. Consequently, results based on the use of leaf age could be difficult to reproduce unless growing conditions were duplicated exactly.

Use of leaf size as an indicator of leaf developmental stage may provide more reproducible results. Preliminary studies (16) showed that uredinium size was negatively correlated with leaf length at the time of inoculation. However, leaves stop increasing in size 6 to 7 days after unfolding, although their susceptibility to rust (measured by uredinium size) does not cease to change (16). Changes in susceptibility that occur after a leaf is fully expanded cannot be related to leaf size (16).

A plastochron index for bean plants may provide a better measure of the developmental stage of the plant as a whole, and of individual leaves, than either leaf age or leaf size. The plastochron is the time period between initiation of successive leaves (1). A plastochron index is a morphological index of plant development, the units of which are plastochrons, rather than absolute time. Erickson and Michelini (7) proposed an elegant geometric model for determining plastochron ages from macroscopic measurements of emerged leaves. Their leaf plastochron index takes advantage of the observation that the exponential growth of leaves results in growth curves that are linear when a leaf dimension is plotted on a logarithmic scale

against time (7,9). If the growth curves of successive leaves plotted in this way have similar slopes (i.e., equal growth rates) and are equally spaced, then the relationships between similar triangles permit estimation of the elapsed proportion of a plastochron from length measurements of only two leaves, at a single time. This approach could be of considerable value in studies of nonspecific resistance where the single time that is of interest is that at which inoculation occurs.

It is important to define accurately, and control for, leaf developmental stage if we are to understand the reasons for the nonspecific resistance exhibited by some bean cultivars. One such cultivar is Pompadour Checa, a component of the land race Pompadour grown in the Dominican Republic. Pompadour Checa develops fewer and smaller uredinia in the field compared to the susceptible cultivar, Pinto 650, grown under the same conditions (20). Lower uredinium densities on Pompadour Checa are probably due to the dense pubescence of the leaves of this cultivar (20) as a negative correlation has been established between uredinium density and leaf hairiness (15). The reasons for smaller uredinium size, however, are not clear. Preliminary greenhouse tests were carried out by inoculating approximately 50–75% expanded primary leaves of Pompadour Checa and Pinto 650 with five collections of urediniospores, and scoring their reactions on a 1–6 grading scale (19). Both cultivars developed grade 5 to 6 uredinia (diameter ≥ 0.5 mm) (19) in response to inoculations with four of the five collections of urediniospores. The fifth collection produced grade 5 to 6 uredinia on Pinto 650, but grade 3 uredinia (diameter ≤ 0.3 mm) (19) surrounded by necrosis on Pompadour Checa (M. Shaik and J. R. Steadman, *unpublished*).

In a preliminary study (16), we established a negative correlation between leaf developmental stage at the time of inoculation and uredinium size. This study also documented differences between primary and trifoliolate leaves in the uredinium size that developed when these leaves were inoculated at a range of developmental stages. In addition, differences were found between three susceptibility parameters, uredinial area, fungal colony area, and secondary sporulating area, in the ease with which they could be measured and the cultivar differences that they demonstrated (16). From these results it is clear that controlled environment studies should employ leaves at all stages of their development, and at several positions on the stem, to understand the differences in uredinium size between Pompadour Checa and Pinto 650 that were noted in the field. Thus, the objectives of the study reported here were, first, to evaluate the differences between Pompadour Checa and Pinto 650 with respect to the three susceptibility parameters (16), by using leaves at five positions on the stem and a range of developmental stages for each leaf; and, second, to define a plastochron index for bean plants to compare its performance as an index of leaf development with leaf age and leaf size.

MATERIALS AND METHODS

Bean cultivars and rust isolate. Two bean cultivars, Pinto 650 and Pompadour Checa, were selected for this study. Pinto 650 is a susceptible cultivar that develops grade 5 to 6 uredinia with most known races of *U. appendiculatus* (19). Pompadour Checa is a component of the land race Pompadour that occurs in the Dominican Republic. Seeds of single plant selections of Pinto 650 and Pompadour Checa used in this study are designated 83-14 and 83-30, respectively. A single uredinium isolate of rust, D85SJ8-1-A, was selected for this study. This isolate was obtained from rust collections made in the Dominican Republic and was known to produce uredinia of grade 5 to 6 on primary leaves of both Pinto 650 and Pompadour Checa.

Experimental conditions. The experimental layout consisted of a randomized block design with two replications (blocks), identical to the one used for the sporulating reactions in a parallel study (17). Thus, a staggered planting schedule was used to create plants which, on the day of inoculation, were from 30 to 8 days from sowing (17). The sample size allotment (number of plants/age/cultivar/block) and the growing conditions were identical

to those outlined for the sporulating reactions of a parallel study (17). In addition, in the present study, the trifoliolate leaves were used together with the primary leaves in assessing cultivar differences. Leaves on the plants were numbered sequentially starting from the base. Thus, the two primary leaves were considered as leaf position 1, and subsequent trifoliolate leaves as leaf positions 2, 3, 4, 5, and so on. The dates of unfolding of each leaf on the main stem of each plant were recorded. Leaf unfolding is a developmental stage recognized when the two halves of leaves (primary leaves) or leaflets (trifoliolate leaves), which are folded in the bud, unfold. Leaf unfolding data for the first five leaves was summarized for each cultivar. This experiment was conducted from 8 September to 24 October 1986, in the greenhouse of the Department of Plant Pathology, University of Nebraska, Lincoln.

Growth studies. The six oldest plants (30 days at the time of inoculation) in each block were used for detailed growth studies. Because the two primary leaves on one plant are nearly identical in size, only one primary leaf on each plant was measured. The length of a leaf or a leaflet was taken as the distance, measured to the nearest millimeter, along the leaf blade from the insertion of petiole or petiolule to the leaf tip. Thus, on each of these plants the length of one primary leaf, and of the median leaflet of each trifoliolate leaf on the main stem, was recorded daily for 10 days starting from the day of unfolding, and then again on the 15th day.

Inoculation. On the day of inoculation (7 and 8 October 1986, for blocks 1 and 2, respectively), the leaf ages (days from unfolding) and lengths of primary leaves and of all median leaflets of trifoliolate leaves on the main stem were recorded for all the plants. For leaves that had not unfolded when inoculated the leaf age was assessed as 0 days. In the remainder of this paper, the terms leaf age, leaf length, leaf developmental stage, and plastochron index are used to denote stages or measurements assessed at the time of inoculation only. The term leaf length is used regardless of whether the structure being measured is a primary leaf or the median leaflet of a trifoliolate leaf. 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 of the rust isolate D85SJ8-1-A. The method of inoculation was identical to the one already described for the sporulating reactions of a parallel study (17).

Data collection. Fourteen days after inoculation, 20 randomly selected uredinia were removed from the primary leaves of each plant by cutting out approximately 4- × 4-mm leaf pieces, each containing one uredinium. Uredinia from the primary leaves of one plant were placed in one vial. Similarly, 20 uredinia collected from one trifoliolate leaf were placed in one vial. From each plant uredinia were collected from the first five leaves (primary leaves and the four subsequent trifoliolate leaves). The sample size for leaves at a given position depended on how many leaves were present at that position, on the 72 plants used for each cultivar, at the time of inoculation. On the older plants (plant age ≥ 26 days at the time of inoculation) nearly all the first leaves and a few second leaves had senesced by the time the uredinia were collected. On the younger plants (plant age ≤ 12 days at the time of inoculation) only the first leaves were present when inoculated. On plants that were between 14 and 30 days at the time of inoculation varying numbers of leaves 2, 3, 4, and 5 were present. Thus, the sample sizes for leaves at five positions for each cultivar were not equal.

The leaf pieces with uredinia were cleared and stained, and the areas of uredinia, fungal colonies, and secondary uredinia were measured using the methods described previously (17).

Data analysis. Data were analyzed by using Statistical Analysis Systems (SAS) procedures (13) and BIOM-pc programs (10) running on an MS-DOS microcomputer, and the functions and macros of the data analysis and graphics package S (3), running on the University of Toronto Department of Statistics Sun Unix system.

The 20 individual values for each leaf of the susceptibility parameters uredinial area, fungal colony area, and secondary

uredinial area were square-root transformed. This was done to reduce the curvilinearity of their relationship with the leaf development parameters (leaf age and leaf length) (18). Means were calculated for each leaf of each plant from these transformed data. These means were used in the analyses described below. Preliminary analyses were carried out to confirm that the two blocks did not differ significantly. Subsequent analyses were carried out on data pooled over the two blocks. Susceptibility parameters were plotted against leaf age and leaf length for each leaf of each cultivar. Differences between cultivar means and slopes for each leaf were tested for significance by analyses of covariance (ANCOVAs) (10,18).

Cultivar differences in secondary uredinial area could not be analyzed because secondary uredinia were infrequent on Pompadour Checa. The available data for each leaf age value were pooled by cultivar and leaf position, so as to calculate the proportion of uredinia developing secondary uredinia for each leaf position of each cultivar.

Leaf length data (transformed to $\log_e$ [ln] scale) for the plants in the growth studies were summarized, plant by plant, as a series of growth curves (Fig. 1). Calculation of a leaf plastochron index involves determining a reference length within the period during which these curves are linear. The reference length must be long enough to be longer than the smallest leaves (L_{n+1}, in the equation below) that can conveniently be measured, but short enough to maximize the number of leaves (L_n) longer than the reference length but still growing exponentially. For the cultivars studied here, a useful reference length for calculation of plastochron index values was found to be 4.5 cm (Fig. 1). ANCOVAs were carried out to test the equality of growth rates (slopes) within plants (between leaf positions 2, 3, 4, and 5) and within cultivars (between plants). Analyses of variance (ANOVAs) were carried out to test

the equality of the mean temporal separation between growth curves for leaf positions 2 and 3, leaf positions 3 and 4, and leaf positions 4 and 5, at the reference length of 4.5 cm. For each plant, the time to reach 4.5 cm was estimated for each leaf from the corresponding regression equation. From these, the three separations were obtained by subtraction. These were considered replicates in comparisons of the four leaf positions (across plants) and the six plants (for each cultivar).

Values of the plastochron index at the time of inoculation were calculated for those plants for which the necessary data were available. This calculation is

$$\text{Plastochron index value} = n + (\ln L_n(t) - \ln L_r)/(\ln L_n(t) - \ln L_{n+1}(t)),$$

where n is the leaf position of the smallest leaf whose length at the time of inoculation t, $L_n(t)$, is greater than or equal to the reference length L_r (7,9), and ln signifies taking the natural logarithm. This calculation requires that the length of the next younger leaf, L_{n+1}, also be available.

Plastochron index values were calculated in this way for leaf positions 2, 3, 4, and 5 for all plants in which the smallest leaflet (measured at the time of inoculation) was shorter than the reference length. Regressions of square-root transformed fungal colony area on leaf age, leaf length, and plastochron index for leaf positions 2 and 3 were compared on the basis of the normality of the regression residuals (11,12), the proportion of variance explained by cultivar regressions (coefficient of determination), and the extent to which significant differences in susceptibility between cultivars (slope or adjusted mean) were recovered by means of ANCOVAs.
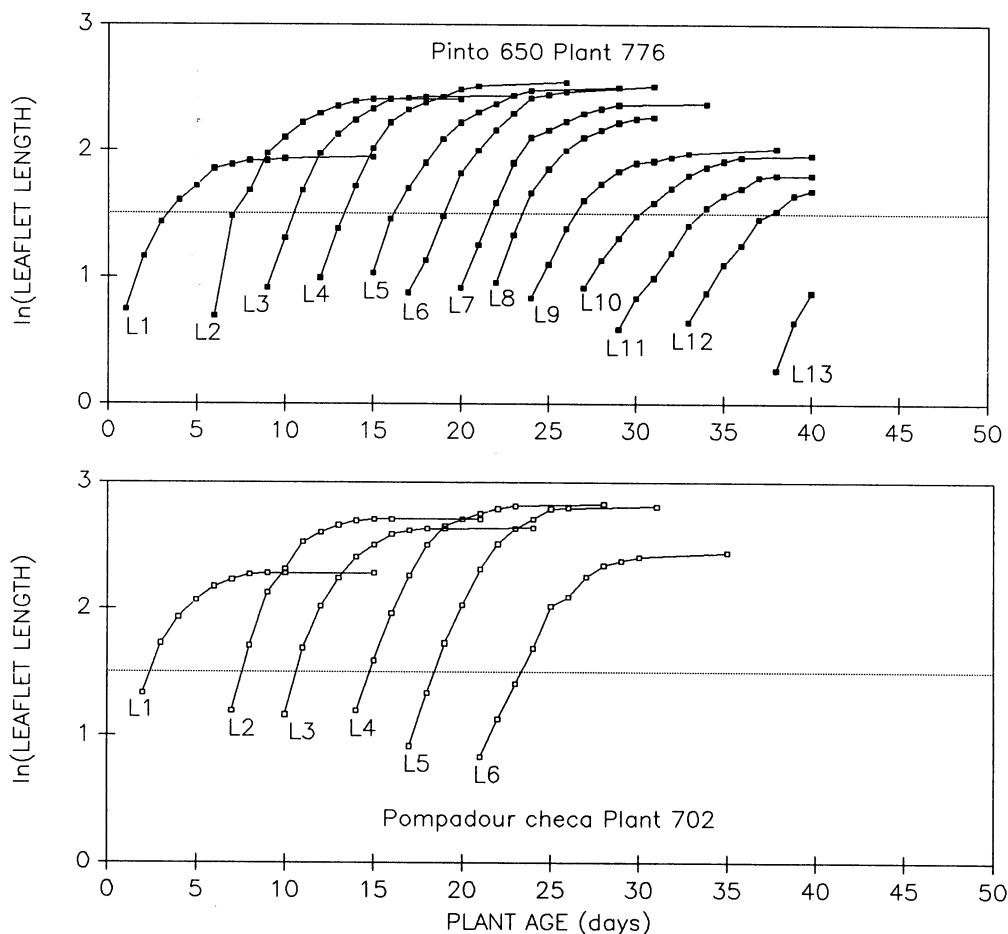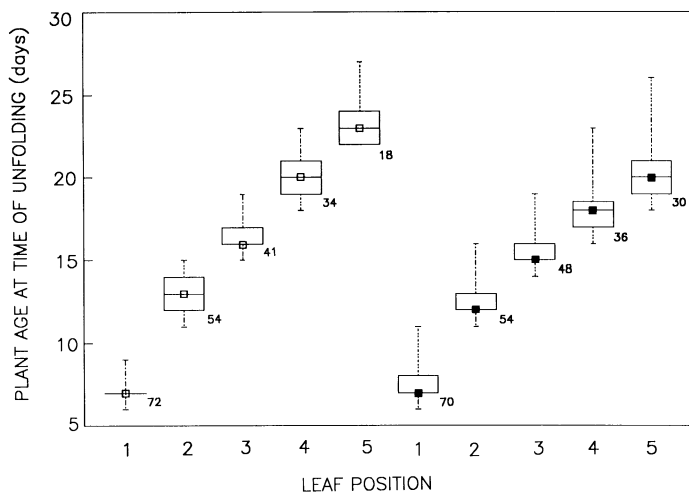

Fig. 1. Growth curves for primary leaves (L1) and the median leaflet of trifoliolate leaves (L2–L6, L2–L13) of representative plants of Pinto 650 (Plant 776; solid squares) and Pompadour Checa (Plant 702; open squares). Leaflet length (cm) is transformed to natural logarithms; the horizontal line represents the natural logarithm of the reference length of 4.5 cm.

RESULTS

Leaf growth. The plant ages at which successive leaves unfolded were not normally distributed, and increased in variability, in Pompadour Checa from leaf position 2 onward, and in Pinto 650 from leaf position 4 onward (Fig. 2). In addition, all leaf positions of Pinto 650 were more variable than the corresponding ones of Pompadour Checa (ranges, Fig. 2). In both Pinto 650 and Pompadour Checa, logarithmic plots of the lengths of median leaflets over time (Fig. 1) suggest that leaflet growth remains exponential (and hence the curves are linear, with coefficients of dete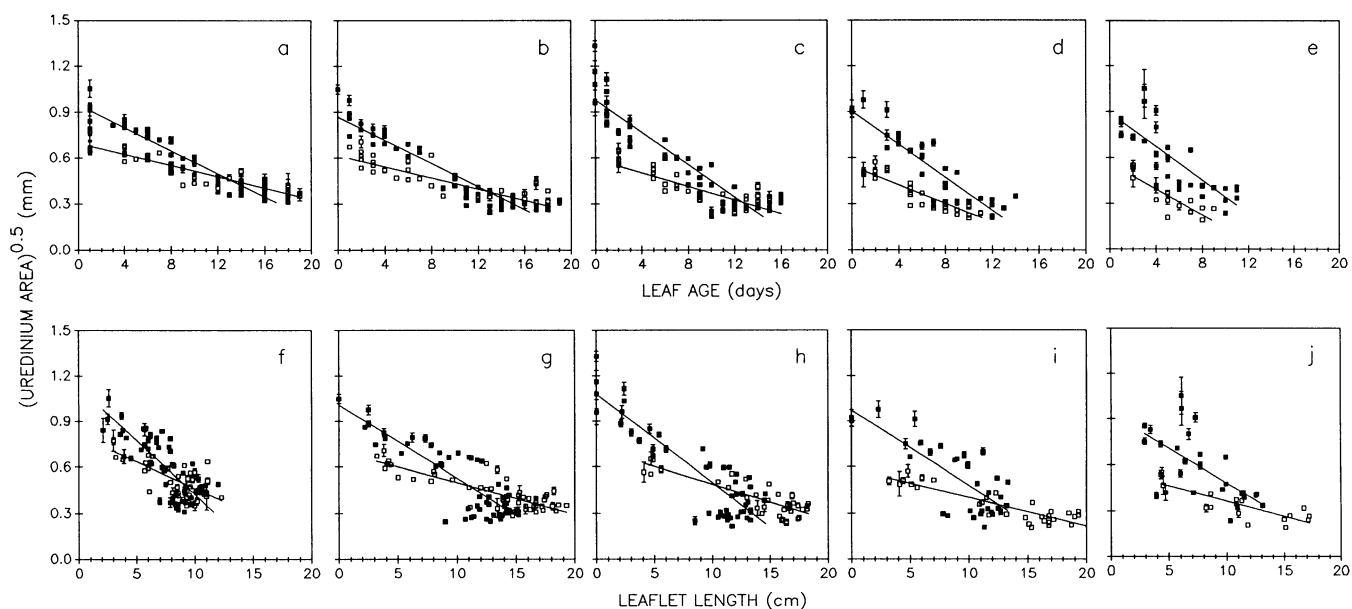rmination approaching 1.0) for at least 4 days after the lamina unfolds. For leaf positions 2 through 4 in each of six plants from each cultivar, coefficients of determination were found, with one exception (L2, for plant 776 in Fig. 1), to be

TABLE 1. Comparison of leaf age and leaf length at time of inoculation as predictors of square-root transformed uredinium area for leaf positions 1 through 5 for two bean cultivars

Leaf position	Parameter[y]	Leaf age	Leaf length
Pompadour Checa			
Leaf 1 ($N = 52$)[z]	slope	−0.018	−0.034
	intercept	0.696	0.806
	R^2	0.828	0.563
Leaf 2 ($N = 53$)	slope	−0.018	−0.021
	intercept	0.616	0.703
	R^2	0.753	0.681
Leaf 3 ($N = 38$)	slope	−0.022	−0.023
	intercept	0.592	0.719
	R^2	0.689	0.724
Leaf 4 ($N = 30$)	slope	−0.031	−0.018
	intercept	0.547	0.587
	R^2	0.843	0.801
Leaf 5 ($N = 16$)	slope	−0.042	−0.020
	intercept	0.561	0.564
	R^2	0.723	0.672
Pinto 650			
Leaf 1 ($N = 50$)	slope	−0.038	−0.070
	intercept	0.950	1.125
	R^2	0.914	0.621
Leaf 2 ($N = 50$)	slope	−0.038	−0.048
	intercept	0.870	1.101
	R^2	0.878	0.733
Leaf 3 ($N = 46$)	slope	−0.053	−0.058
	intercept	0.978	1.078
	R^2	0.845	0.792
Leaf 4 ($N = 32$)	slope	−0.054	−0.049
	intercept	0.904	0.970
	R^2	0.778	0.619
Leaf 5 ($N = 28$)	slope	−0.055	−0.045
	intercept	0.893	0.932
	R^2	0.645	0.426

[y] Regression equation parameters (Fig. 3).
[z] N = sample size (number of plants, out of 72 for each cultivar, from which leaves at a given position were obtained).

Fig. 2. Box plot (4,21) of plant ages at which successive leaves (positions 1–5) unfold, in Pompadour Checa (open squares) and Pinto 650 (solid squares). The bottom of the box represents lower quartile of the observations, while the top represents the upper quartile. The median value is indicated by the position of the square. Whiskers extend above and below each box to indicate the maximum and minimum of the observations, respectively. Numbers indicate sample sizes (numbers of plants). Asymmetry of the distributions around the medians indicates nonnormality.

Fig. 3. Comparison of leaf age at time of inoculation (a–e) and leaflet length at time of inoculation (f–j) as predictors of square-root transformed uredinium area for leaf positions 1 through 5 and bean cultivars Pinto 650 (solid squares) and Pompadour Checa (open squares) inoculated with rust isolate D85SJ8-1-A. Leaflet length is the length of one of the primary leaves for leaf position 1, and of the median leaflet for leaf positions 2 through 5. Each data point represents a mean of 20 uredinia for a single plant, while the error bars represent the standard error of the mean. Most error bars are hidden by the symbols. Slopes of cultivar regression lines (Table 1) were found to differ significantly ($P < 0.001$, except in j, where $P < 0.05$) in all but one case (e, $P = 0.37$). Testing the covariate-adjusted cultivar means for equality indicated significant differences in some (a–e, $P < 0.001$; j, $P < 0.01$) but not all cases.

in the range 0.915–0.999. Regression line slopes for leaf positions 2 through 4 were found to be homogeneous within plants and over all six plants for Pinto 650 ($P > 0.05$). With Pompadour Checa, homogeneity was demonstrated only within individual plants ($P > 0.05$). In both cultivars slopes tended to decrease at higher leaf positions (Fig. 1).

Mean separation in time between when the median leaflet at leaf positions 2, 3, and 4 reached the reference length of 4.5 cm, and when that on the next leaf (3, 4, and 5, respectively) did, were found to be homogeneous between plants but not between pairs of successive leaf positions, over these plants. In the plants of Pinto 650 plastochrons tended to decrease with increasing leaf position, while in Pompadour Checa there was a tendency in the opposite direction (Fig. 1). Thus, two of the three conditions for use of a plastochron index (exponential growth, equal growth rates, and equal plastochrons) (9) are satisfied by the first four trifoliolate leaves of the two bean cultivars studied here. As discussed below, failure to meet the third of these conditions is not seen to be of great consequence for use of the plastochron index here.

Rust susceptibility in relation to leaf developmental stage. Plots of square-root transformed uredinium or fungal colony areas against leaf age and leaf length were linear, with significantly non-zero negative slopes for each of the first five leaves of each cultivar (Tables 1 and 2). The intercepts for Pompadour Checa were lower than those for Pinto 650 in all comparisons (Tables 1 and 2). The corresponding ANCOVAs showed significant differences between cultivar slopes and covariate-adjusted means in all but one case (slopes for leaf 5) when leaf age was used as a predictor for uredinium area (Fig. 3) and fungal colony area (Fig. 4). However, while slopes were generally significantly different, the covariate-adjusted means were, in most cases, not significantly different when leaf length was used as predictor for uredinium area (Fig. 3) and fungal colony area (Fig. 4).

Plots of secondary uredinial area against leaf age and leaf length were also linear with significantly non-zero negative slopes for Pinto 650 (Fig. 5). No regression analyses could be performed for Pompadour Checa because leaves that were more than 3 days old at the time of inoculation did not develop secondary uredinia. For the same reason, statistical comparisons of the cultivars could not be made. However, the means of secondary uredinial areas

for Pompadour Checa were considerably smaller than those for Pinto 650 for comparable leaf age values (Fig. 5). Moreover, cultivar differences were evident in the proportion of uredinia developing secondaries (Fig. 6). Leaves of Pinto 650 were

TABLE 2. Comparison of leaf age and leaf length at time of inoculation as predictors of square-root transformed fungal colony area for leaf positions 1 through 5 for two bean cultivars

Leaf position	Parameter[y]	Leaf age	Leaf length
Pompadour Checa			
Leaf 1 ($N = 52$)[z]	slope	−0.093	−0.189
	intercept	2.154	2.824
	R^2	0.834	0.656
Leaf 2 ($N = 53$)	slope	−0.082	−0.096
	intercept	1.771	2.208
	R^2	0.815	0.790
Leaf 3 ($N = 38$)	slope	−0.090	−0.095
	intercept	1.626	2.147
	R^2	0.780	0.828
Leaf 4 ($N = 30$)	slope	−0.105	−0.064
	intercept	1.406	1.556
	R^2	0.874	0.857
Leaf 5 ($N = 16$)	slope	−0.103	−0.048
	intercept	1.242	1.240
	R^2	0.729	0.656
Pinto 650			
Leaf 1 ($N = 50$)	slope	−0.163	−0.328
	intercept	3.309	4.250
	R^2	0.893	0.707
Leaf 2 ($N = 50$)	slope	−0.151	−0.191
	intercept	2.915	3.453
	R^2	0.899	0.740
Leaf 3 ($N = 46$)	slope	−0.169	−0.177
	intercept	2.841	3.084
	R^2	0.884	0.753
Leaf 4 ($N = 32$)	slope	−0.173	−0.155
	intercept	2.579	2.766
	R^2	0.794	0.602
Leaf 5 ($N = 28$)	slope	−0.164	−0.137
	intercept	2.380	2.517
	R^2	0.702	0.482

[y]Regression equation parameters (Fig. 4).
[z]N = sample size (number of plants, out of 72 for each cultivar, from which leaves at a given position were obtained).
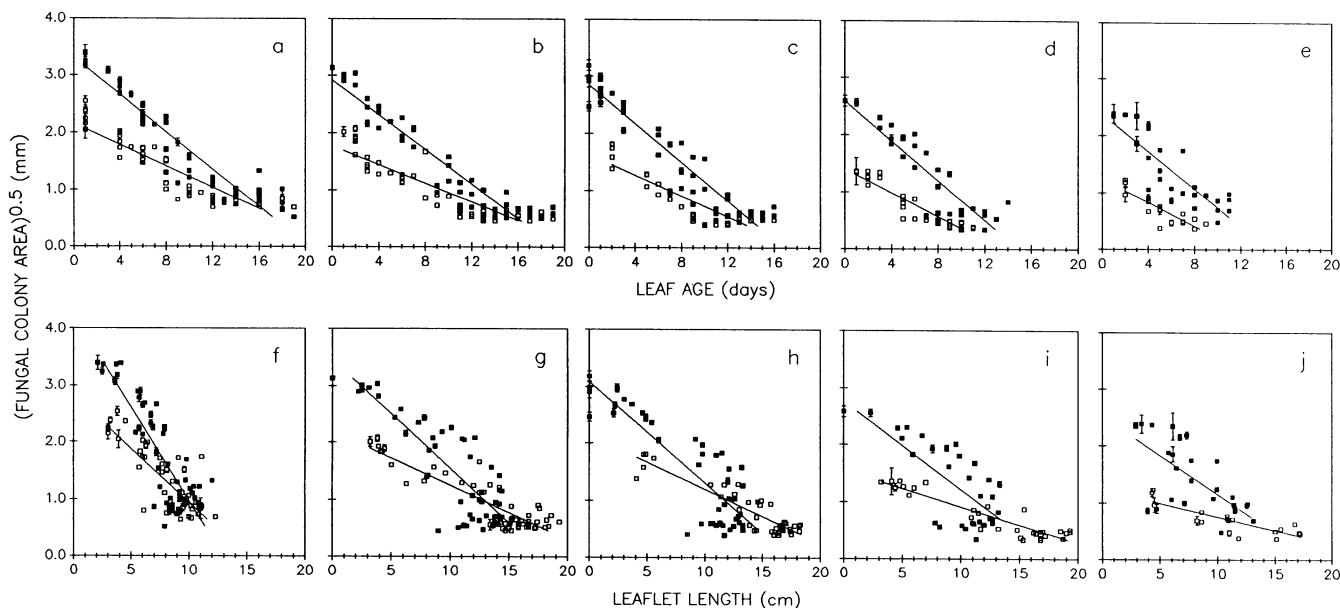

Fig. 4. Comparison of leaf age at time of inoculation (a–e) and leaflet length at time of inoculation (f–j) as predictors of square-root transformed fungal colony area for leaf positions 1 through 5 and bean cultivars Pinto 650 (solid squares) and Pompadour Checa (open squares) inoculated with rust isolate D85SJ8-1-A. Leaflet length is the length of one of the primary leaves for leaf position 1, and of the median leaflet for leaf positions 2 through 5. Each data point represents a mean of 20 uredinia for a single plant, while the error bars represent the standard error of the mean. Most error bars are hidden by the symbols. Slopes of cultivar regression lines (Table 2) were found to differ significantly ($P < 0.001$, except in j, where $P < 0.01$) in all but one case (e, $P = 0.11$). Testing the covariate-adjusted cultivar means for equality indicated significant differences in some (a–f, $P < 0.001$; i, $P < 0.05$; j, $P < 0.01$) but not all cases.

susceptible to the development of secondary uredinia for a longer time (leaf age 0 to 11) than were leaves of Pompadour Checa (leaf age $\leq$ 3; Fig. 6).

A comparison of leaf age, leaf length, and plastochron index as predictors of rust susceptibility (square-root transformed fungal colony area) indicated that the plastochron index performed almost as well as leaf age (Fig. 7, Table 3). Regression residuals were normally distributed when leaf age and plastochron index, but not leaf length (for Pinto 650), were used as independent variables (P values, Table 3). Plastochron index explained almost as much of the variance in susceptibility as did leaf age (R^2 values, Table 3). The poor performance of leaf length may be related to the presence of some aberrant leaves that stopped growing before reaching their full length (Fig. 7E). These leaflets were inoculated at between 10 and 12 days after unfolding (Fig. 7D), at plastochron index values between 4.5 and 5.5 (Fig. 7F), values in each case that fit the trend exhibited by the rest of the sample. Thus, provided plastochron index is calculated from two leaves that are growing normally, it is able to represent the developmental stage of the plant as a whole and its constituent leaves in a way that appears to be unaffected by the possibly abnormal growth of other leaves. In addition, use of plastochron index (but not leaf length) as a covariate to control for developmental stage in comparisons of cultivars preserved the differences observed when leaf age was used in the same way (Fig. 7).

DISCUSSION

By inoculating leaves at a range of developmental stages, significant differences with respect to three susceptibility parameters were demonstrated between the cultivars Pompadour Checa and Pinto 650 for all five leaves (Figs. 3–5). These cultivar differences were not revealed in our preliminary studies in which the primary leaves were inoculated when approximately 50–75% expanded, and in which the uredinia were graded rather than measured. Moreover, certain aspects of the cultivar differences were better elucidated by inoculating leaves at a range of developmental stages, as discussed below.

There are probably two reasons for the relatively small uredinium size and infrequent development of secondary uredinia on Pompadour Checa. First, this cultivar possesses resistance, which restricts uredinium size; this is seen in the intercept differ-

ences (Figs. 3 and 4). This resistance is probably conferred by as yet unknown factors operating within a leaf, after pathogen entry. Second, other factors apparently operate to limit pathogen entry into the leaf, especially in the initial stages of leaf development, as follows.

Uredinium densities are negatively correlated with the hairiness of bean leaves, the density of which increases with increasing leaf position (15). This is especially true of the leaves of Pompadour Checa (20). Leaf hairs may prevent urediniospores from reaching the leaf surface, and probably prevent the germ tubes of the rust fungus from contacting the leaf epidermis and entering the leaf (15). In early leaf developmental stages these hairs are close together and probably eliminate pathogen entry

TABLE 3. Comparison of leaf age, leaf length, and plastochron index at time of inoculation as predictors of square-root transformed fungal colony area on leaf positions 2 and 3, using as criteria the normality of the residuals (P, for testing null hypothesis of normality), and the coefficient of determination (R^2), for each linear regression (Fig. 7)

Leaf position	Parameters	Age	Length	Plasto-chron index
Pompadour Checa				
Leaf 2 ($N = 14$)[a]	slope	−0.064	−0.085	−0.210
	intercept	1.445	2.067	1.648
	P	>0.10	0.10	>0.10
	R^2	0.774	0.540	0.714
Leaf 3 ($N = 13$)	slope	−0.107	−0.083	−0.404
	intercept	1.649	1.966	2.633
	P	>0.10	>0.10	>0.10
	R^2	0.869	0.794	0.867
Pinto 650				
Leaf 2 ($N = 20$)	slope	−0.183	−0.199	−0.488
	intercept	3.079	3.663	3.409
	P	>0.10	<0.05	>0.10
	R^2	0.924	0.529	0.851
Leaf 3 ($N = 20$)	slope	−0.209	−0.153	−0.581
	intercept	3.016	3.041	4.197
	P	>0.10	<0.05	>0.10
	R^2	0.850	0.640	0.768

[a]Sample sizes (N) are subsamples of those shown in Tables 1 and 2 (see text for explanation).
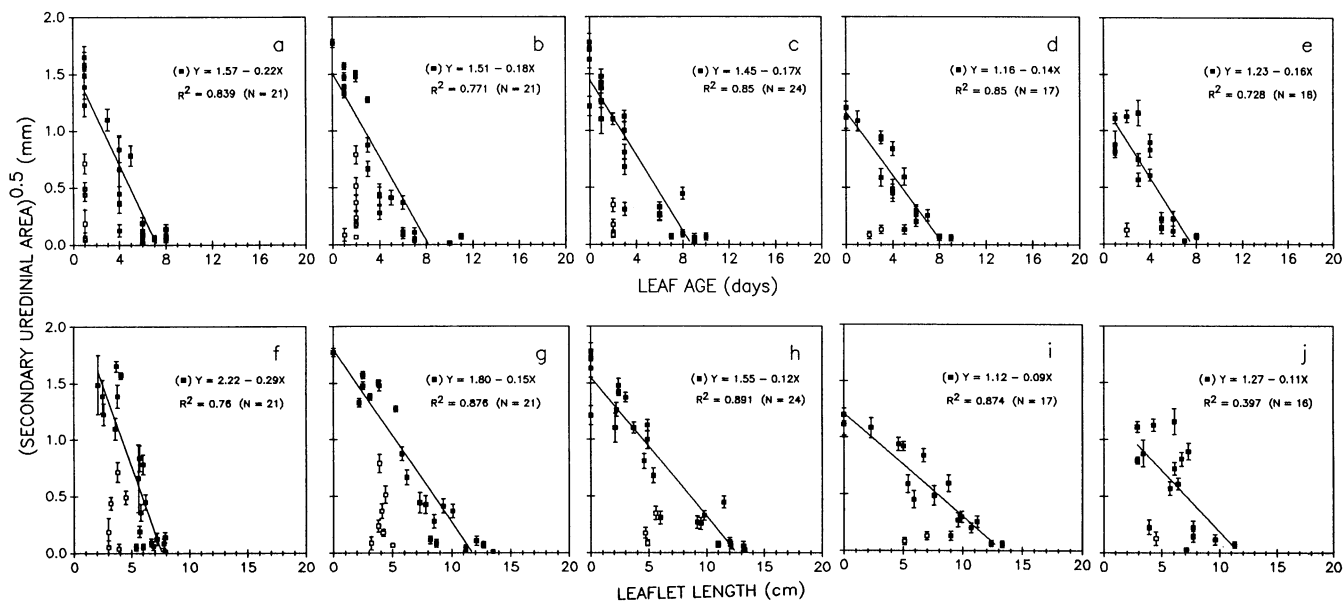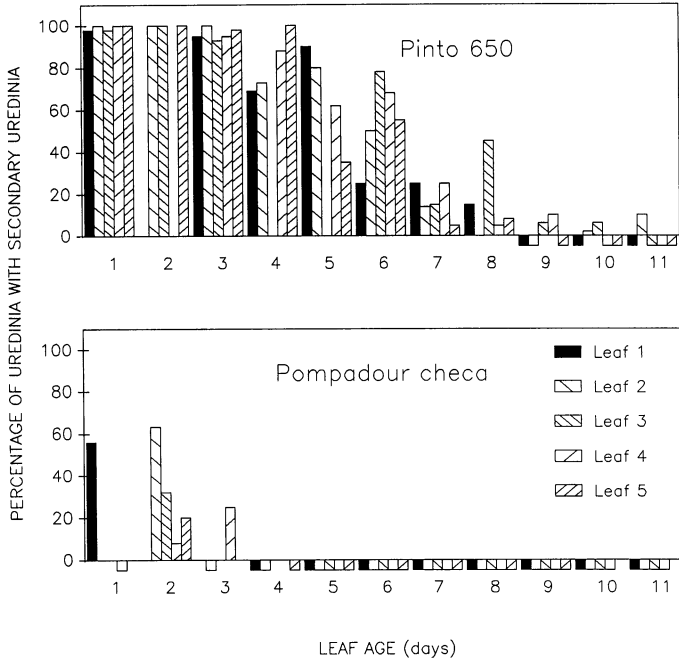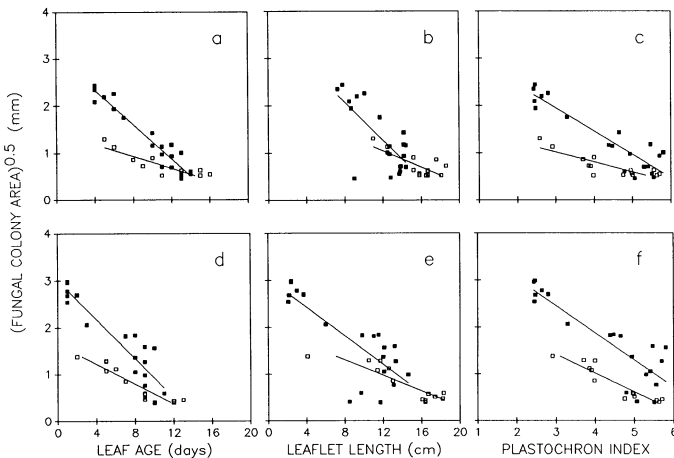

Fig. 5. Comparison of leaf age at time of inoculation (a–e) and leaflet length at time of inoculation (f–j) as predictors of square-root transformed secondary uredinial area for leaf positions 1 through 5 and bean cultivars Pinto 650 (solid squares) and Pompadour Checa (open squares) inoculated with rust isolate D85SJ8-1-A. Leaflet length is the length of one of the primary leaves for leaf position 1, and of the median leaflet for leaf positions 2 through 5. Each data point represents a mean of 20 uredinia for a single plant, while the error bars represent the standard error of the mean. Some error bars are hidden by the symbols. Sample sizes represent those plants on which secondary uredinia were observed, out of the total number of plants plotted in Figs. 3 or 4.

completely (20). This means that the leaves avoid infection at a stage when uredinia and secondary uredinia can develop to the maximum extent. This aspect of resistance is evident in the present study in the lack of data for extremely small leaf age values for Pompadour Checa, especially for leaf positions 3, 4, and 5 (Figs. 3–5). By comparison, leaves of Pinto 650, which do not possess the pubescence-related resistance (20), are receptive to rust from a day before unfolding (Figs. 3–5).

Of the three susceptibility parameters studied, the greatest

Fig. 6. The proportion of uredinia with secondary uredinia on leaves inoculated with rust isolate D85SJ8-1-A at different leaf ages, for five leaf positions and two bean cultivars. See text for explanation of calculations. Data values of 0% are represented by negative bar lengths to distinguish them from missing values.

Fig. 7. Comparison of leaf age at time of inoculation, median leaflet length at time of inoculation, and plastochron index at time of inoculation as predictors of square-root transformed fungal colony area for leaf position 2 (a–c) and 3 (d–f) and bean cultivars Pinto 650 (solid squares) and Pompadour Checa (open squares) inoculated with rust isolate D85SJ8-1-A. Each data point represents a mean of 20 uredinia for a single plant, with the same plants being represented in each of a–c and d–f. Slopes of cultivar regression lines (Table 3) were found to be significantly different in some (a and c, $P < 0.001$; d, $P < 0.01$) but not all comparisons. Where slopes were not significantly different (b, e, f), the covariate-adjusted cultivar means were tested for equality; only in f were they found to differ significantly ($P < 0.001$).

cultivar differences were reflected in secondary uredinial area. The importance of this parameter, particularly with reference to the cultivar differences, is poorly documented in earlier studies. Secondary uredinial area can be up to four times larger than uredinium area (compare Figs. 3 and 5), and hence is an important criterion for evaluating cultivar differences. Leaves of Pompadour Checa avoid the development of secondary uredinia apparently by being unreceptive to rust germ tubes during early stages of leaf development, as discussed above. Furthermore, even when the rust fungus successfully produced uredinia, secondary uredinia were smaller (Fig. 5) and less frequent (Fig. 6) on Pompadour Checa than on Pinto 650. The reasons for this are not known and may involve factors operating within a leaf, after pathogen entry.

The relationship between the size of lesions of *U. appendiculatus* and the development of bean leaves documented here suggests that more detailed studies of fungal morphogenesis are needed. Such studies should compare fungal development initiated at a range of leaf developmental stages, in order to elucidate the unknown mechanisms (referred to above) by which fungal growth is increasingly restricted as the age of leaves when inoculated increases (Figs. 3–5 and 7). Correlation of fungal development and host development in this way would likely indicate what aspects of the former are most affected by which processes of the latter and how these may vary between cultivars.

The results presented here suggest how leaf developmental stage is best quantified for use in rust studies. Plant age should not be used to describe leaf ages, in view of the great variation observed in the plant ages at which leaves at a given position unfold (Fig. 2). Square-root transformed uredinium and fungal colony area vary linearly with leaf age, more so than with leaf length (Tables 1 and 2). This is because increase in leaf size ceases to be exponential 4–6 days after unfolding (Fig. 1), and subsequently ceases altogether. However, rust susceptibility continues to decrease linearly with leaf age until 12 or more days after unfolding (Figs. 3 and 4). As a result, leaf age is a better predictor of rust susceptibility than leaf length, and so a better covariate for use in statistical control for developmental stage in cultivar or other comparisons.

Leaf age, while it is a better variable than leaf size and plant age, is extremely tedious to record for large experiments because it involves observing leaf unfoldings on each plant every day. Furthermore, leaf age (recorded as days from unfolding) is a discontinuous variable, taking only integer values. Rendering leaf age a continuous variable would be even more tedious, as it would involve attempting to record the times of day when leaf unfolding takes place. In addition, the size of leaves at the time of unfolding varies between cultivars. For example, leaves of Pompadour Checa are generally larger than those of Pinto 650 when they unfold. Thus, leaves of the two cultivars with the same leaf age value may not be at the same developmental stage. Differences in growing conditions from one experiment to another may further exaggerate discrepancies such as these, making comparisons of leaf developmental stage expressed as leaf age extremely difficult, both within and between cultivars.

Plastochron index is a better indicator of leaf developmental stage because it integrates plant chronological age and increase in leaf size, and because rust susceptibility varies linearly with plastochron index almost as much as with leaf age (Table 3, Fig. 7). Because it provides a morphologically based time scale, plastochron index describes leaf development in a way that can be invariant with seasonal differences in growing conditions (5). Plastochron index also provides a means of quantifying host plant developmental stage that requires a minimum of measurement and record-keeping. Unlike leaf age, it provides a ratio scale on which to measure host developmental stage. The use of a plastochron index does involve examination of growth curves for specific cultivars, and determination of a suitable reference length. Once these are done, however, data (length, position on stem) from only two leaves need to be recorded for each plant at the time of inoculation.

In the present study, the sample sizes for which plastochron

index could be calculated were considerably smaller than the total sample sizes (compare Table 3 with Tables 1 and 2). This was because, in the experiment reported here, only unfolded median leaflets were measured, and these frequently had reached lengths in excess of the reference length when measured. However, calculation of plastochron index requires that the younger of the two leaves measured be less than or equal to the reference length. This problem can be overcome by measuring median leaflets that have not yet unfolded. Departures from the assumption of equal plastochrons is unlikely to vitiate use of plastochron index as a descriptor of leaf development (and predictor of rust susceptibility) since the relationship of similar triangles from which the plastochron index is calculated (7,9) depends on equal slopes (growth rates) rather than on equal plastochrons. The effect of unequal plastochrons is merely to make the plastochron index relative to development rather than absolute in terms of time.

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