Evaluation of Components of Partial Resistance to Oat Crown Rust Using Digital Image Analysis

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ABSTRACT

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Digital image analysis is an objective and nondestructive method potentially capable of providing accurate and precise estimates of disease resistance components. This study was conducted to quantify components of partial resistance to crown rust through the analysis of sequential digital images of inoculated leaves of adult oat plants, and to compare components found in two sources of resistance unrelated genetically. Uredinium density, relative infection frequency, latent period, days to first pustule appearance, uredinium size, and disease progress rates were assessed on three oat lines (RS-line 3W-C2R-9-3b, MN-841801, Starter) in two greenhouse experiments. Resistant lines had fewer and smaller uredinia, and these appeared later than in the susceptible check. Relative infection frequency, latent period, and uredinium size were equally important components in the expression of the partial resistance to crown rust, and the two sources of resistance could not be differentiated by any of the variables studied. The analysis of sequential digital images of diseased leaves produced precise estimates of partial resistance components and disease progress rates.

Additional keywords: *Avena sativa*, host resistance, *Puccinia coronata*

Crown rust, caused by the fungus *Puccinia coronata* Cda. f. sp. *avenae* Eriks., is the most widespread and damaging disease of cultivated oat (*Avena sativa* L.) (45). Breeding for complete race-specific resistance has been the primary disease control measure (45). However, *P. coronata* is highly variable in virulence and rapidly evolves new pathotypes that render this resistance ineffective (9,45). Partial resistance is a form of incomplete resistance characterized by a reduced rate of epidemic development despite a susceptible or high infection type (33). This type of resistance is considered to be largely race nonspecific, inherited polygenically, and potentially more durable (30).

The expression of partial resistance as a reduced rate of disease development is the cumulative result of differences in one or more of the phases of the infection cycle (29), and the resolution of partial resistance into its distinct components is called components analysis (49). Infection efficiency, latent period, infectious period, and spore production are the main resistance components examined in epidemics caused by polycyclic biotrophic leaf pathogens (29). Infection efficiency or receptivity is the percentage of spores applied that result in sporulating lesions; latent period is the time from infection to the initiation of spore production; infectious period is the average number of days during which a lesion sporulates; and urediniospore production is the number of spores produced per unit area of affected tissue. Measuring and comparing the relative contribution of the different resistance components enhances our understanding of partial resistance and aids in the development of better plant improvement and selection schemes. Furthermore, well-characterized lines with high values for different resistance components could be crossed to increase the level of partial resistance (27,40).

Disease resistance components are usually estimated using monocyclic-infection greenhouse experiments. Disease development in the greenhouse differs from the more complex polycyclic field epidemics, but the increased control over sources of variation and experimental error allow the study of a single infection cycle in detail. Greenhouse experiments provide conditions for a uniform application of a constant number of genetically uniform urediniospores and guarantee that no additional spores will interfere with the disease assessments. The 22 reports of resistance components to leaf rusts in small grains discussed in this paper used monocyclic-infection greenhouse experiments to estimate components. In five studies (14,23,27,28,42), greenhouse assessments were compared with resistance components estimated in the field.

Previous reports indicate that several components explain the observed differences in partial resistance to oat crown rust between resistant and susceptible cultivars. Heagle and Moore (13) studied the components involved in the expression of resistance to oat crown rust at the adult plant stage. Cultivars with partial resistance had lower relative infection frequencies, longer latent periods, smaller uredinia, and produced fewer urediniospores per uredinium. Brière and Kushalappa (6) evaluated infection efficiency, latent period, and uredinium size at the seedling stage using inoculation with a single crown rust isolate under controlled conditions. In their study, the three components were equally responsible for the observed differences in partial resistance. Brake and Irwin (5) also assessed resistance components to oat crown rust at the seedling stage. Although partial resistance was associated with long latent period, small uredinium size, and reduced urediniospore production, infection efficiency appeared to be the component that best differentiated cultivars. There were very high correlations between latent period and partial resistance components in barley (*Hordeum vulgare* L.) to leaf rust (caused by *Puccinia hordei* Otth.) (28,32,33), suggesting that the latent period might be the most important component in this pathosystem. Shaner et al. (43) estimated the contribution of the different partial resistance components in wheat (*Triticum aestivum* L.) to leaf rust (caused by *Puccinia triticina* Eriks.). Partial resistance observed in the field was positively associated with long latent period, small uredinium size, and reduced urediniospore production, whereas no significant difference in infection efficiency was detected between resistant and susceptible cultivars.

Visual estimation is the most commonly used method to assess disease severity. It is an easy, economical, and very efficient

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* The *e*-**X**tra logo stands for "electronic extra" and indicates that Figure 1 appears in color in the online edition.

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screening technique for routine disease assessment evaluations (25,47). Most, if not all, reported estimates of partial resistance components have also been obtained by visual examination, despite the recognized limitations associated with the use of this assessment method for detailed studies. Visual assessment of disease resistance components is laborious, time-consuming, prone to operator bias and subjectivity, and fairly imprecise and inaccurate (26,36,44).

Disease severity and partial resistance components may also be quantitatively assessed by digital image analysis. Digital image analysis is a nondestructive and noninvasive method capable of acquiring, processing, and analyzing information from digital images (25,37). Digital image analysis is proposed as a valid approach for applications that require objective, accurate, and precise estimates of quantitative variables (25). Modern image acquiring equipment and image analysis software offer the possibility of collecting hundreds of digital images per hour that are later analyzed with a high degree of automation. The image collection speed for this study was 150 digital images of leaves per hour, and the analysis of these 150 images took 2 h.

The objectives of this study were to quantify the components of partial resistance to crown rust through the analysis of sequential digital images of inoculated leaves of adult oat plants, and to compare partial resistance components found in two genetically unrelated sources of resistance.

MATERIALS AND METHODS

Oat cultivars or lines. Three oat lines were evaluated in two greenhouse experiments. One line (RS-line 3W-C2R-9-3b) was selected from an adapted and high-yielding recurrent selection population after the second cycle of selection for partial resistance to crown rust. This recurrent selection population was created in 1970 and selected for increased grain yield for four cycles. Payne et al. (34) described this population in detail and provided additional information regarding the selection procedures used. The 21 parents of the fourth cycle were then crossed to the cultivars Ogle and Starter, and the seven highest yielding Ogle and Starter crosses were selected and crossed with the cultivar Hazel and experimental line IL83-8037 (46). This opened recurrent selection population was then subjected to two rapid cycles of recurrent selection for partial resistance to crown rust. A detailed description of the selection procedure followed for improving the resistance to crown rust can be found in Díaz-Lago et al. (11). The second oat line evaluated is MN-841801, an experimental line developed at the University of Minnesota as part of an oat improvement program created to enhance crown rust resistance by combining different types of resistance from a variety of sources. MN-841801 has effective adult plant resistance to oat crown rust (20). The cultivar Starter, rated highly susceptible to crown rust in field tests at St. Paul, MN (20), was included as the susceptible check.

Experiments. Two greenhouse experiments were conducted to evaluate components of partial resistance to oat crown rust in the three oat lines and two leaf types (flag leaf and flag-1 leaf). Within each experiment, the three by two factorial treatment design was arranged as a split-plot design with five complete replicates. Oat lines were whole plots and leaf types were subplots. The whole plot was a 15-cm-diameter pot with two adult plants, and the corresponding flag or flag-1 leaves were subplots. The three lines were sown on several dates to obtain plants at the same stage of development at the time of inoculation. Plants were reduced to a single mature culm prior to inoculation to facilitate manipulation of plants during the inoculation and image collection processes. Oat plants were grown in steam-sterilized soil mix (6 parts sand, 5 parts soil, and 2 parts composted manure), and natural daylight was supplemented by sodium vapor lamps (400 W) arranged directly above the plants. Artificial illumination was limited to 12 h during the first 3 weeks and increased to 16 h afterward. The temperature in the greenhouse varied between 15 and 27°C. The same experimental design and procedures were used in both experiments; the experiments were conducted at different times.

Inoculation. Fully expanded flag and flag-1 leaves of adult oat plants were inoculated with a single-uredinium *P. coronata* isolate (92MNB-181) virulent on the three oat lines when inoculated at the seedling stage. Urediniospores, previously stored in liquid nitrogen, of the selected isolate were given a heat treatment of 10 min at 40°C and suspended in a light mineral oil (Soltrol 170, Chevron Phillips Chemical Company LP, Houston, TX) carrier medium. Oat leaves were fixed to a metal mesh with magnets, and the rust atomizer was attached to the quantitative inoculation device of Andres and Wilcoxson (3). The same atomizer was used for all leaves within each experiment to avoid variation between rust atomizers. Inoculation dates were 16 and 30 March 2000 for the first and second experiments, respectively. Inoculated plants were placed in a dew chamber overnight at 18°C and returned to the greenhouse the next morning.

Collection and analysis of digital images. Digital images of leaves were acquired every 1 or 2 days from the adaxial surfaces of flag and flag-1 leaves starting 7 days after inoculation. The last set of images was collected 19 days after inoculation. Images were obtained using a standard digital camera (Olympus D-600L, Olympus America Inc., Melville, NY) in macro focus mode and with a close-up filter (Hoya +4 lens filter, THK Photo Products Inc., Long Beach, CA). A 1,000 W mercury vapor light was utilized to supplement natural light on cloudy days. Images were temporarily stored in multiple removable and reusable image storage cards. The distance from the camera to the leaf was fixed at 15 cm. To collect sequential images from the same area of the leaf, a mark was made on each leaf before taking the first set of pictures. Images were stored in JPEG format. This format uses a compression algorithm that reduces image file size by 10 without significant degradation in image quality. The average image size was 300 KB and contained a leaf section of 10 cm^2.

The 850 images collected in the two experiments were analyzed using the image analysis software Image-Pro Plus (Image-Pro Plus, Version 4.1, Media Cybernetics, L. P., Silver Spring, MD). No preprocessing techniques were used to enhance the definition of image objects. Digital image analysis uses color segmentation to separate objects into classes. The Image Pro-Plus software has two color separation methods: one based on the color histogram and another that uses a color-cube. The color-cube method was used to create two color-range files using a random set of images. These files contain the color segmentation definitions for the specific color classes to be measured and counted. The first file defined four classes: green, orange-red-brown, dark brown-black, and intense white, and was used to measure areas. The green color class represented the healthy leaf area, orange-red-brown colors corresponded to sporulating leaf area, dark brown-black indicated area covered by telia, and intense white colors were the consequence of light reflections or natural nonpathogenic leaf spots. Chlorotic leaf area was found by subtracting the previous four classes from the total area being analyzed.

The Image Pro Plus Spatial Calibration tool was used to measure the size in pixels of a reference line of known length to obtain area estimates in square centimeters. The second color-range file had only the orange-red-brown and the dark-brown-black classes, and was used to count the number of uredinia and telia automatically. More accurate counts were obtained by creating classes with a confined color spectrum. As a result, the color-ranges of the two classes in the second file were not as wide as in the first file. Four segmentation and measurement options were enabled in the second color-range file to further increase precision and accuracy of counts: ‘Smoothing = 30’, ‘Convex Hull’, ‘4-connect’, and ‘Fill Holes’. ‘Smoothing = 30’ and ‘Convex Hull’ create a smooth perimeter for irregularly shaped objects, ‘4-connect’ separates objects that are only connected by a corner pixel, and ‘Fill Holes’ excludes from the count any objects that are embedded within other objects.

The color segmentation process operates upon the entire active image; hence, the first step to analyze a particular image, once the color-range files have been created, was the isolation of the leaf or area of interest from the rest of the image by using the Freeform area of interest selection tool. Color segmentation was then applied to the area of interest, and the objects of the different color classes measured and counted automatically. Finally, the measurements were transferred to an Excel spreadsheet (Microsoft, Redmond, WA).

Statistical analysis of data. The area and number data produced by the analysis of the digital images of leaves were processed to create four variables: healthy leaf area (%), sporulating leaf area (%), uredinium density (No.·cm^{-2}), and uredinium size (mm^2). The area and number of telia were zero for almost all leaves studied and were therefore not analyzed. Analyses of variance of the four variables were performed by experiment and date to test the significance of the two main effects and one interaction of the three oat lines by two leaf types factorial treatment design using the GLM procedure of the SAS software package (Ver. 8.1, SAS Institute, Cary, NC). The main effect of leaf type was rarely significant ($P < 0.05$), and the interaction of line by leaf type was never significant ($P < 0.05$) (Table 1). As a consequence, only the oat line treatments were considered for the combined analysis of the two experiments and for the regression analysis of the four variables versus time. The original split-plot experimental design was analyzed as a randomized complete block design with three oat line treatments and five replicates using the whole plot means.

Separate regression equations were fit for each experiment because the image sampling dates of the two greenhouse trials did not coincide. Visual inspection of the observed data versus time plots suggested the pertinence of testing curvilinear and sigmoid models. Four regression models were fit and compared using the regression procedure of SAS: the straight line and quadratic linear models and the logistic and Gompertz nonlinear models. Oat lines differed in the maximum value attained at the end of the evaluation period. When the maximum (K) varies between treatments, Campbell and Madden (8) suggest considering it as an additional regression parameter, and the linearizing transformations become $\ln[y/(K - y)]$ for the logistic model and $-\ln[-\ln(y/K)]$for the Gompertz model. Values for K were estimated for each oat line by experiment combination as the maximum value among the means by date. Since the logarithm of zero or a negative number is undefined, values of the response variable equal to or greater than K cannot be transformed. This restriction produces a biased loss of data points in the later sampling dates. To avoid this problem and to use a uniform data set among the models being compared, all regressions were fit using the means by date.

Although the coefficients of determination (R^2) associated with the linear regressions can be used to compare the four models, these should be interpreted with caution. When a regression is fit using means by date, the pure error sum of squares is zero and, as a result, R^2 only measures the lack of fit (10). Additional criteria used for selecting the best fitting model were the F statistic testing the significance of the regression equation, the visual inspection of observed and predicted values versus time, and residual versus predictor plots. The quadratic linear model fitted the healthy leaf area data satisfactorily, the Gompertz model was the most appropriate for sporulating leaf area and uredinium density, and a straight line was adequate for uredinium size. Linear regression parameter estimates were compared using two-tailed t tests at $P = 0.05$. The standard error of the difference was calculated as the square root of the sum of the estimated variances of the two estimated parameters and the degrees of freedom as $\mathrm{df} = n_1 + n_2 - (2p)$, where n_1 and n_2 are the number of observations for the two progress curves and p is the number of parameters in each model (8). When sigmoid disease progress curves are linearized employing the variable maximum version of the transformation equation, the slope or relative progress rate has limited application, and curves should be compared using the absolute rate of progress, estimated as $r{\cdot}K$ (8). The standard error of the product of the two parameter estimates was calculated as: $\mathrm{SE} = \sqrt{\{[(r_G)^2 + (\mathrm{SE}_{rG})^2]\,[(K)^2 + (\mathrm{SE}_K)^2] - (r_G\,K)^2\}}$ (24).

Two additional components of disease resistance were estimated using the regression equations of uredinium density versus time: latent period and days to first uredinium appearance. The latent period is the time newly infected tissue takes to become infectious (48), and was estimated as the number of days from inoculation to uredinium density = 0.5 K. Since the Gompertz linearizing transformation is $-\ln[-\ln(y/K)]$, latent period is days to uredinium density transformed = 0.367. Similarly, days to first uredinium appearance was estimated based on a standard idealized leaf size of

Table 1. Summary of three analyses of variance of healthy leaf area (HLA), sporulating leaf area (SLA), uredinium density (UD), and uredinium size (US) for two leaf types, and three oat lines in two experiments, 15 days after inoculation

Experiment and source of variation	df	HLA (%)		SLA (%)		UD (No.·cm^2)		US (mm^2)	
		MS[x]	P[y]	MS	P	MS	P	MS[z]	P
Experiment 1									
Replication	4	200.8		4.78		80.5		16.7	
Line	2	3,557.2	< 0.001	278.51	< 0.001	1,701.1	< 0.001	944.2	< 0.001
Error (a)	8	200.8		2.58		54.5		40.2	
Leaf type	1	111.9	0.269	11.19	0.045	153.0	0.092	7.3	0.669
Line × leaf type	2	13.2	0.854	3.16	0.278	22.6	0.618	32.4	0.451
Error (b)	11	82.6		2.19		44.8		37.9	
Experiment 2									
Replication	4	124.2		8.18		45.6		10.4	
Line	2	3,631.1	0.001	274.68	< 0.001	1,688.9	< 0.001	1,090.0	< 0.001
Error (a)	8	156.1		11.95		74.2		22.3	
Leaf type	1	19.3	0.639	0.23	0.762	2.9	0.779	0.0	1
Line × leaf type	2	154.1	0.199	1.03	0.663	26.7	0.489	10.0	0.783
Error (b)	12	83.2		2.42		35.1		40.0	
Combined analysis of the two experiments									
Experiment	1	1,776.9	0.011	23.21	0.088	612.3	0.014	12.6	0.339
Replication (experiment)	8	161.6		6.17		62.6		12.2	
Line	2	7,320.5	< 0.001	560.05	< 0.001	3,463.9	< 0.001	2,042.7	< 0.001
Experiment × line	2	4.7	0.961	0.89	0.808	23.9	0.618	8.5	0.775
Error (b)	45	116.5		4.17		49.2		33.1	

[x] Mean square derived from the type III sum of squares.

[y] Probability values for the corresponding F test.

[z] Mean square × 10^4.

$20\ \text{cm}^2$ as the number of days from inoculation to uredinium density = $1{\cdot}20\ \text{cm}^{-2}$. The standard error of an estimated value of the predictor was calculated as: $SE = \sqrt{\{[(1/r_G)^2\ (SE_{Int})^2] + \{[(A - Int)/(r_G)^2]^2\ (SE_{rG})^2\}\}}$, where Int = intercept, $A = 0.367$ for latent period, and $A = -\text{Ln}[-\text{Ln}\ (0.05/K)]$ for days to first uredinium appearance (16).

Observation of the uredinium density versus time plot for the susceptible check Starter suggested a sigmoid progress curve reaching K around day 15 after inoculation followed by an abrupt increase in the number of uredinia. The inspection of multiple image sequences attributed this increase to the formation of rings of secondary uredinia surrounding primary pustules (Fig. 1). These rings of secondary uredinia are produced by hyphae that spread radially from the primary infection unit by growing across the intercellular spaces (2,39). Therefore, the uredinium density and uredinium size progress curves of the susceptible check Starter were fit ignoring the data collected after day 15 to avoid the confounding effect created by the appearance of secondary uredinia on the number and size of primary pustules.

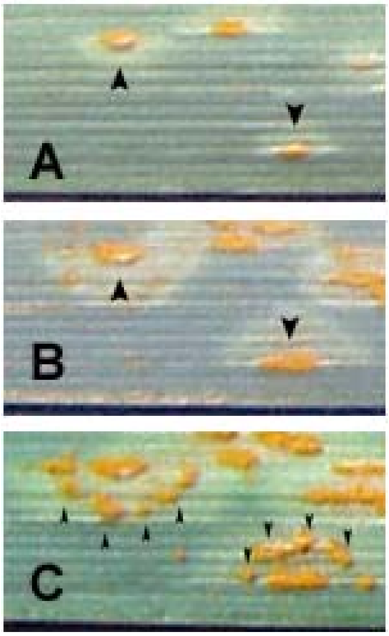

Fig. 1. Images of a leaf of the susceptible check Starter at **A,** 11, **B,** 15, and **C,** 19 days after crown rust inoculation showing the appearance of rings of secondary uredinia after day 15.

RESULTS

Sequential digital images of inoculated leaves of adult plants were analyzed to estimate healthy leaf area, sporulating leaf area, uredinium density, and uredinium size. The analyses of variance performed by experiment and date for each of these four variables measured using digital image analysis showed that the oat line main effect was always highly significant, while leaf type (flag or flag-1) was rarely significant ($P < 0.05$) and the interaction of oat line by leaf type was never significant ($P < 0.05$). The combined analysis of the two greenhouse experiments indicated that the oat line by experiment interaction was never significant ($P < 0.05$) and justified the comparison of line means across experiments. Summaries of the analyses of variance for day 15 after inoculation are presented in Table 1.

Means of the partially resistant lines MN-841801 and RS-line were similar for the four variables studied but differed significantly ($P < 0.05$) from those of the susceptible check Starter (Table 2). At 15 days after inoculation, Starter had more than 50% of its total inoculated leaf area affected by crown rust disease, whereas MN-841801 and RS-line had only about 20% affected. At this time, the two lines with partial resistance had one-fourth of the number of uredinia of Starter, and their uredinia were about half the size (Table 2).

Differences in healthy leaf area, sporulating leaf area, uredinium density, and uredinium size between resistant and susceptible lines also were observed earlier in the infection cycle, and these differences were all reflected by the corresponding progress curves (Fig. 2). Quadratic linear models fit the healthy leaf area data satisfactorily (Fig. 2A). MN-841801 and RS-line had negative quadratic terms, and Starter had positive ones in both experiments (Table 3). Starter's positive quadratic terms are a result of the diminishing rate of reduction in healthy leaf area when this variable is under 50% (Fig. 2A). The healthy leaf area of MN-841801 and RS-line was always above 50%.

The leaf area covered by uredinia (Fig. 2B) is the product of uredinium density (Fig. 2C) and uredinium size (Fig. 2D). The Gompertz model was the most appropriate for describing the change in sporulating leaf area (Fig. 2B and Table 4) and uredinium density (Fig. 2C and Table 5). This model fits sigmoid disease progress curves that approach the inflection point of the slope early, before $y = 0.5\ K$ (8). A straight regression line was adequate for uredinium size (Fig. 2D and Table 6).

The components analysis of epidemics caused by polycyclic biotrophic leaf pathogens identifies four main resistance components: infection efficiency, latent period, infectious period, and urediniospore production (29). Infection efficiency, the ratio of the number of uredinia formed to the number of spores applied, was analyzed indirectly by studying the increase in density of uredinia (Table 5). Latent period and the related measure days to first uredinium appearance were estimated using the Gompertz transformed uredinium density versus time linear regressions (Table 5). Infectious period was not studied, and urediniospore production was evaluated indirectly by measuring uredinium size (Table 6).

Components of disease resistance. Uredinium density: the average maximum number of uredinia per square centimeter was 13.8 for RS-line, 14.8 for MN-841801, and 30.6 for the susceptible check Starter (Table 5). Latent period: estimated average latent periods were 14.3 days for RS-line, 14.4 days for MN-841801, and 9.7 days for Starter (Table 5). Days to first uredinium appearance: the average days to first uredinium appearance was 9.0 days for RS-line, 9.7 days for MN-841801, and 6.7 days for Starter (Table 5). Uredinium size: estimated uredinium sizes 15 days after inoculation were $0.17\ \text{mm}^2$ for RS-line, $0.20\ \text{mm}^2$ for MN-841801, and $0.36\ \text{mm}^2$ for Starter (Table 2).

Disease progress rates. Sigmoid-shaped progress curves were found for two variables: sporulating leaf area and uredinium density (Fig. 2B and C). Van der Plank (48) suggested linearizing transformations to produce regression lines with a constant slope or infection rate for sigmoid-shaped progress curves of disease epidemics. The sporulating leaf area and uredinium density curves of the three oat lines and two experiments had variable maxima (Tables 4

Table 2. Observed means for healthy leaf area (HLA), sporulating leaf area (SLA), uredinium density (UD), and uredinium size (US) 15 days after inoculation for three oat lines in the combined analysis of two experiments

	HLA[x]		SLA[x]		UD		US	
Cultivar/line	**(%)**	**(SE[y])**	**(%)**	**(SE)**	**(No.·cm⁻²)**	**(SE)**	**(mm²)**	**(SE)**
RS-line	79.2 a[z]	(2.41)	1.5 a	(0.46)	8.7 a	(1.57)	0.17 a	(0.013)
MN-841801	79.7 a	(2.41)	1.4 a	(0.46)	7.1 a	(1.57)	0.20 a	(0.013)
Starter	45.6 b	(2.49)	10.8 b	(0.47)	31.1 b	(1.62)	0.36 b	(0.013)

[x] HLA + SLA + chlorotic leaf area + area covered by telia = 100.

[y] Standard error.

[z] Values within each column followed by the same letter are not significantly different at $P = 0.05$ according to two-tailed tests of pairwise differences with no adjustment for multiple comparisons.

and 5), and to obtain adequate regression fits the maximum (*K*) was considered as an additional parameter as was previously suggested by Campbell and Madden (8). The Gompertz linearizing transformation produced the best statistical fits among the models tested. The Gompertz model has frequently been the most appropriate because many sigmoid-shaped disease progress curves are asymmetrical about the point of inflection (4,22,35).

The slope of the linearized form or infection rate (*r*) was proposed by Van der Plank (48) as a valid parameter to compare disease progress curves. However, when the maximum (*K*) is variable, progress curves should be compared using $r \cdot K$ as a measure of the absolute rate (8). Relative and absolute disease progress rates for the sporulating leaf area are presented in Table 4.

DISCUSSION

Uredinium density. Absolute pustule density values obtained in inoculated greenhouse experiments are difficult to compare because these vary greatly depending on the amount of inoculum applied (7,23) and other known and unknown environmental conditions (20,31). Therefore, uredinium density values are frequently reported as a percentage of the value obtained by the susceptible check and referred to as the relative infection frequency. Average relative infection frequencies using Starter as the susceptible check were 44 and 49% for RS-line and MN-841801, respectively. Leonard (20) studied the adult plant resistance to crown rust in line MN-841801 and reported a relative infection frequency of 7% using Starter as the susceptible check. Comparable relative infection frequencies were observed by Luke et al., who estimated a 54% (23) and a 10% relative infection frequency (21) of crown rust for cultivar Red Rustproof. These and three other reports (5,13,18) show that relative infection frequency, although variable, is an important component of partial resistance to oat crown rust. Similar relative infection frequencies (≅50%) were observed in barley lines with partial resistance to leaf rust (15,29,31). Low relative infection frequencies (21 to 50%) against leaf rust were also found for wheat lines carrying *Lr34* adult plant resistance gene alone or in combination with *Lr13* (12,17,38). However, infection frequency has not been a very important resistance component to leaf rust in wheat slow-rusting lines, where estimated relative infection frequencies varied from 70 to 84% (19,27,42,43).

Latent period. The mean latent period of the two lines with partial resistance was 14.4 days, 48% (4.7 days) longer than the latent period of Starter (Table 5). Brière and Kushalappa (6) reported an average latent period at the seedling stage of 14.5 days for the most resistant oat line tested. This latent period was 2.3 days longer than

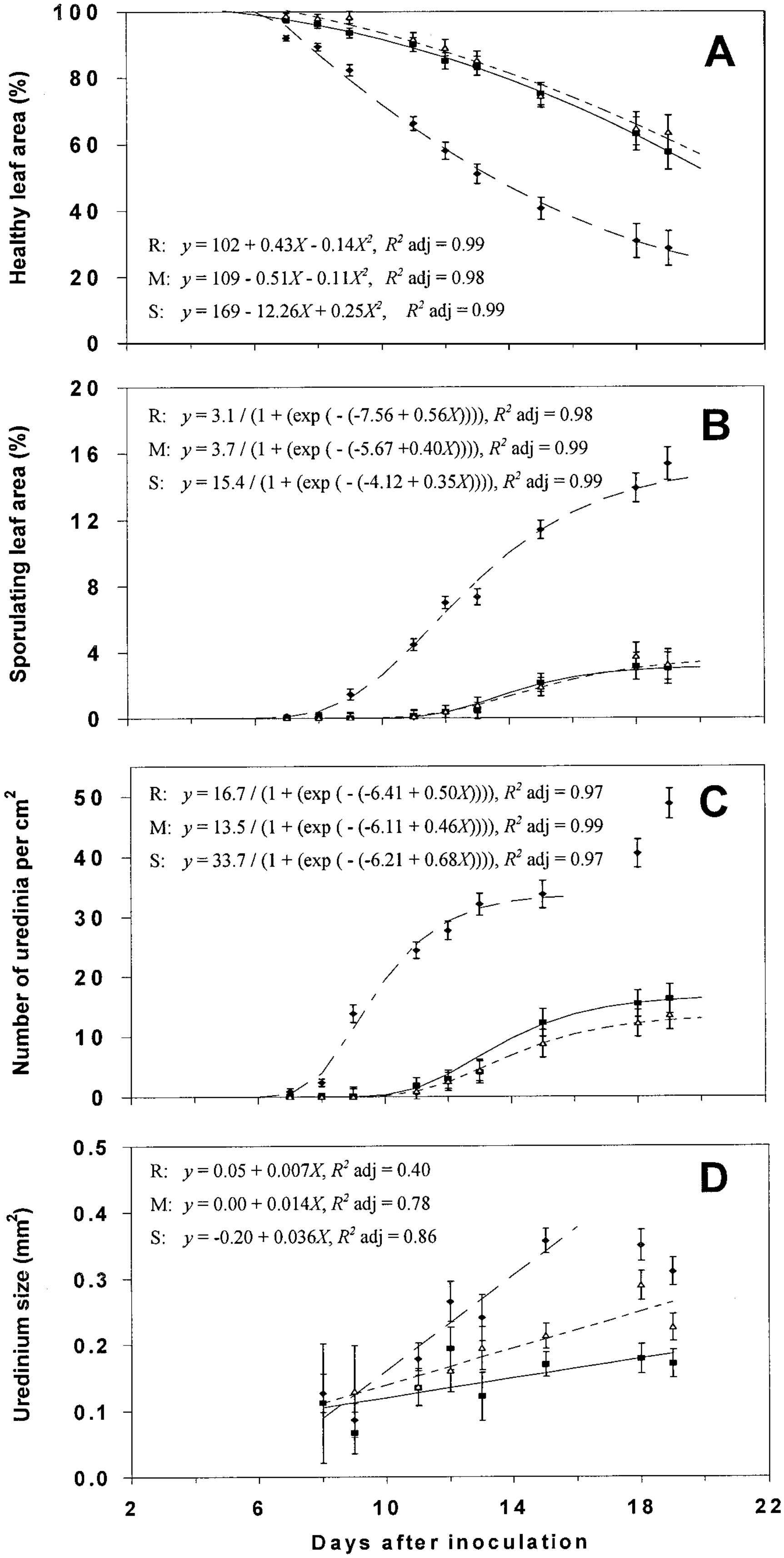

Fig. 2. Progress curves for **A,** healthy leaf area, **B,** sporulating leaf area, **C,** uredinium density, and **D,** uredinium size, in experiment 1. Solid square = RS-line (R), open triangle = MN-841801 (M), and solid diamond = Starter (S). Points represent observed means, and bars denote standard errors. The adjusted R^2 values presented in **B** and **C** were estimated for the linearized form of the corresponding nonlinear model.

the latent period of the susceptible check. A 7-day difference in latent period was observed between inoculated adult plants of the slow-rusting cultivar Red Rustproof and the susceptible cultivar Fulghum (21). Latent period is a very important component of partial resistance to leaf rust in barley and wheat as well. Differences in latent period between barley lines with high partial resistance and susceptible checks varied between 3.7 and 7.8 days (15,28,32). These differences ranged from 1.5 to 4.6 days for wheat slow-rusting lines (14,19,27,42,43). The leaf rust susceptible wheat and barley checks studied in all these experiments had latent periods shorter than the crown rust check Starter had in this study (6.5 to 8.6 versus 9.7 days).

Table 3. Linear regression parameter estimates for the progress curve of healthy leaf area (%) of three oat lines in two experiments

Experiment and cultivar/line	Linear regression parameter estimates^y		
	Intercept (SE)	L term (SE)	Q term (SE)
Experiment 1			
RS-line	101.7 a^z (2.8)	0.43 a (0.46)	–0.14 a (0.02)
MN-841801	109.2 a (8.3)	–0.51 a (1.36)	–0.11 a (0.05)
Starter	169.0 b (9.3)	–12.26 b (1.53)	0.25 b (0.06)
Experiment 2			
RS-line	100.2 a (7.4)	1.08 a (1.14)	–0.16 a (0.04)
MN-841801	99.5 a (4.7)	1.10 a (0.71)	–0.15 a (0.02)
Starter	167.5 b (9.1)	–11.60 b (1.38)	0.24 b (0.05)

[y] SE: standard error, L term: linear term, Q term: quadratic term.

[z] Values within each column and experiment followed by the same letter are not significantly different at $P = 0.05$ according to two-tailed tests of pairwise differences with no adjustment for multiple comparisons.

Table 4. Linear regression (LR) parameter estimates for the progress curve of the transformed (Gompertz linearizing transformation) sporulating leaf area (%), observed maximum (*K*) sporulating leaf area values, and absolute rate of progress of three oat lines in two experiments

Experiment and cultivar/line	LR parameter estimates		Maximum (*K*)	Absolute rate
	Intercept (SE^x)	r_G^{y} (SE)	(%) (SE)	$r_G \cdot K$ (SE)
Experiment 1				
RS-line	–7.6 a^z (0.63)	0.56 a (0.04)	3.1 a (0.82)	1.76 a (0.48)
MN-841801	–5.7 a (0.06)	0.40 b (0.01)	3.7 a (0.82)	1.49 a (0.33)
Starter	–4.1 b (0.14)	0.35 c (0.01)	15.4 b (0.97)	5.45 b (0.39)
Experiment 2				
RS-line	–6.0 a (0.43)	0.44 a (0.03)	3.0 a (0.60)	1.32 a (0.41)
MN-841801	–7.1 a (0.67)	0.50 a (0.05)	3.8 a (0.59)	1.88 a (0.46)
Starter	–5.2 a (0.57)	0.46 a (0.05)	13.1 b (0.99)	6.03 b (0.78)

[x] Standard error.

[y] Slope of the linearized form of the Gompertz model and the relative rate of infection.

[z] Values within each column and experiment followed by the same letter are not significantly different at $P = 0.05$ according to two-tailed tests of pairwise differences with no adjustment for multiple comparisons.

Days to first uredinium appearance. Epidemics caused by polycyclic biotrophic fungal pathogens result from the concatenation of multiple infection cycles (50). The very first sporulation marks the beginning of a new cycle that overlaps with the preceding cycle during the remainder of the infectious period of the preceding cycle. Therefore, the number of days from inoculation to first uredinium appearance is a relevant epidemiological variable. The susceptible check initiated sporulation 2.5 days earlier than the lines with high partial resistance, and these figures indicate that half of the difference in latent period between resistant and susceptible oat lines could be attributed to a delay in the appearance of the first uredinium. Luke et al. (21) observed a delay in days to first uredinium appearance of 2.0 days in adult plants of an oat line with high partial resistance to crown rust, and Brake and Irwin (5) report a difference of 1.2 days in days to first uredinium appearance between oat seedlings of resistant and susceptible cultivars.

Uredinium size. The average uredinium size of the two lines with high partial resistance 15 days after inoculation was half the size of the susceptible check, despite the expected lower interuredinial competition that should result from having only 25% as many uredinia by day 15 (Table 2). Brake and Irwin (5) measured crown rust uredinium sizes at the seedling stage and found that uredinia of lines with partial resistance were 32% smaller. Heagle and Moore (13) also found significant differences in uredinium size between a line with high partial resistance and the susceptible check. Uredinium size is also an important resistance component in wheat leaf rust. Uredinia of wheat slow-rusting lines were 45% smaller than the average uredinium size of the susceptible check on three occasions (27,42,43), and not significantly different on one occasion (19). Lines of wheat culti-

Table 5. Linear regression (LR) parameter estimates for the progress curve of the transformed (Gompertz linearizing transformation) uredinium density (No.·cm-2), observed maximum (K) uredinium density values, absolute rate of progress, latent period, and days to first uredinium appearance (DFUA) for three oat lines in two experiments

Experiment and cultivar/line	LR parameter estimates		Maximum (*K*)	Absolute rate	Latent period	DFUA^t
	Intercept (SE^u)	r_G^{v} (SE)	(No.·cm^{-2}) (SE)	$r_G \cdot K$ (SE^w)	(days) (SE^x)	(days) (SE^y)
Experiment 1						
RS-line	–6.4 a^z (0.54)	0.50 ab (0.04)	16.7 a (3.04)	8.35 a (1.67)	13.5 a (1.55)	9.3 ab (1.32)
MN-841801	–6.1 a (0.08)	0.46 a (0.01)	13.5 a (3.00)	6.21 a (1.39)	14.0 a (0.24)	9.5 a (0.20)
Starter	–6.2 a (0.59)	0.68 b (0.06)	33.7 b (2.30)	22.92 b (2.49)	9.7 b (1.19)	6.4 b (1.02)
Experiment 2						
RS-line	–4.5 a (0.18)	0.33 a (0.01)	10.8 a (1.40)	3.56 a (0.48)	15.0 a (0.80)	8.7 a (0.64)
MN-841801	–6.0 a (0.49)	0.43 a (0.03)	16.0 a (1.66)	6.88 b (0.89)	14.8 a (1.63)	9.9 a (1.38)
Starter	–7.8 a (0.95)	0.86 b (0.09)	27.4 b (1.91)	23.56 c (3.05)	9.6 b (1.53)	7.0 a (1.35)

[t] Days to first uredinium appearance based on a standard idealized leaf size of 20 cm^2.

[u] Standard error.

[v] Slope of the linearized form of the Gompertz model and the relative rate of infection.

[w] $\mathrm{SE} = \sqrt{}\{[(r_G)^2 + (\mathrm{SE}_{rG})^2]\,[(K)^2 + (\mathrm{SE}_K)^2] - (r_G\,K)^2\}$ (24).

[x] $\mathrm{SE} = \sqrt{}\{[(1/r_G)^2\,(\mathrm{SE}_{\mathrm{Int}})^2] + \{[(0.367 - \mathrm{Int})/(r_G)^2]^2\,(\mathrm{SE}_{rG})^2\}\}$ (16), Int: intercept, $0.367 = -\mathrm{Ln}[-\mathrm{Ln}(0.5)]$.

[y] $\mathrm{SE} = \sqrt{}\{[(1/r_G)^2\,(\mathrm{SE}_{\mathrm{Int}})^2] + \{[(A - \mathrm{Int})/(r_G)^2]^2\,(\mathrm{SE}_{rG})^2\}\}$ (16), Int: intercept, $A = -\mathrm{Ln}[-\mathrm{Ln}(0.05/K)]$.

[z] Values within each column and experiment followed by the same letter are not significantly different at $P = 0.05$ according to two-tailed tests of pairwise differences with no adjustment for multiple comparisons.

var Thatcher carrying *Lr34* had uredinium sizes 30 to 50% smaller than the average for the original leaf rust susceptible cultivar (12,17).

Uredinium size differences may result from a delay in the appearance of uredinia, a lower rate of uredinium growth, or a combination of both mechanisms. The significant differences in latent period and days to first uredinium appearance between resistant and susceptible oat lines observed in this study (Table 5) were discussed previously. Average growth rates of uredinia are presented in Table 6. Uredinia in resistant lines grew at a rate of 0.011 $\mathrm{mm}^2\cdot\mathrm{day}^{-1}$ and in Starter at 0.037 $\mathrm{mm}^2\cdot\mathrm{day}^{-1}$. Similar significant uredinium growth rate differences have been reported for wheat leaf rust. Shaner (41) estimated a rate of 0.02 $\mathrm{mm}^2\cdot\mathrm{day}^{-1}$ for slow-rusting line and 0.05 $\mathrm{mm}^2\cdot\mathrm{day}^{-1}$ for the susceptible check, and concluded that growth rate of uredinia could be a component of slow-rusting resistance in wheat leaf rust.

In summary, RS-line and MN-841801 had fewer and smaller uredinia, and these appeared later than in the susceptible check Starter. These three components of partial resistance were equally important. Relative infection frequencies were approximately 50%, latent periods were almost 50% longer, and average uredinium sizes were 50% smaller. Our results support those of three previous studies (5,6,13), indicating that the three resistance components evaluated play a significant role in the expression of partial resistance against oat crown rust.

Disease progress rates. The observed differences between oat lines in the relative progress rate (r_G) were small and not consistent across experiments. Significant ($P <$ 0.05) differences between the two resistant lines and the susceptible check were detected in the absolute rates, and these could be attributed to variation in the sporulating leaf area maxima (Table 4). The average reduction in the absolute disease progress rate from the susceptible check to the two resistant lines was 72%. For polycyclic field epidemics, Poyntz and Hyde (35) report a 36% reduction in the Gompertz infection rate of three slow-rusting wheat lines, while Adhikari et al. (1) observed a 60% reduction in the Gompertz infection rate of three lines of rice (*Oryza sativa*) with partial resistance to bacterial blight (caused by *Xanthomonas oryzae* pv. *oryzae*). Relative and absolute uredinium density progress rates are presented in Table 5. RS-line and MN-841801 had similar relative rates, and these were 45 and 42% smaller than Starter's progress rate, respectively. These differences in the relative rate of progress, combined with the variation in maximum uredinium densities, produced an average 73% reduction in the absolute rate of progress of the two lines with high partial resistance (Table 5).

Uredinium density and the associated measures relative infection frequency, latent period and days to first pustule appearance, uredinium size, and disease progress rates were all involved in the explanation of the crown rust resistance found in RS-line and MN-841801. Although the two sources of resistance could not be differentiated statistically by any of the variables studied, the inspection of data tendencies suggested that MN-841801 had fewer uredinia of larger size compared with RS-line (Fig. 2C and D, Table 2). These results confirm and describe the effective adult plant resistance of MN-841801 reported previously by Leonard (20) and indicate that the adapted high-yielding recurrent selection population has the potential for providing oat lines with high levels of partial resistance.

Our study demonstrated the usefulness of digital image analysis as an objective and nondestructive method capable of quantitatively assessing disease resistance components on sequential digital images of inoculated adult plant leaves. Furthermore, the standard errors of digitally measured traits indicate that all resistance components were estimated with relatively high precision. Stored digital images can be analyzed, shared, and used to answer questions not evident at the time of the experiment. Confirmation of the appearance of secondary uredinia in the susceptible check 15 days after inoculation was possible because the image sequences of diseased leaves were still available. Visual estimation of disease incidence and severity is often easy, economical, and time efficient and should be the preferred screening technique for routine disease assessment evaluations. However, the virtues of digital image analysis outlined here suggest that it might be an appropriate procedure for detailed epidemiological research experiments, a valid phenotyping tool for quantitative resistance loci detection studies, and a suitable instrument to better characterize parental lines in crop improvement programs.

Table 6. Linear regression (LR) parameter estimates for the progress curve of uredinium size (mm^2) of three oat lines in two experiments

Experiment and cultivar/line	LR parameter estimates	
	Intercept (SE[y])	Slope (SE)
Experiment 1		
RS-line	0.05 a[z] (0.043)	0.007 a (0.0031)
MN-841801	0.00 ab (0.042)	0.014 a (0.0030)
Starter	–0.20 b (0.073)	0.036 b (0.0063)
Experiment 2		
RS-line	0.03 a (0.051)	0.012 a (0.0034)
MN-841801	0.06 a (0.052)	0.009 a (0.0032)
Starter	–0.16 a (0.114)	0.037 b (0.0098)

[y] Standard error.

[z] Values within each column and experiment followed by the same letter are not significantly different at $P = 0.05$ according to two-tailed tests of pairwise differences with no adjustment for multiple comparisons.

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