Identification of Slow-Rusting Resistance to *Puccinia polysora* in Maize Inbreds and Single Crosses

B. A. BAILEY, Research Assistant, WOLFGANG SCHUH, Research Assistant, and R. A. FREDERIKSEN, Professor, Department of Plant Pathology and Microbiology, and A. J. BOCKHOLT, Associate Professor, and J. D. SMITH, Professor, Department of Soil and Crop Science, Texas Agricultural Experiment Station, College Station 77843

ABSTRACT

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Epiphytotics of southern rust occurred in the southern United States in 1972, 1973, and 1974, raising concern over the relative susceptibility of the maize germ plasm in the United States to *Puccinia polysora*. Slow rusting is a common form of resistance to many rust diseases, but slow rusting has not been evaluated in the maize/*P. polysora* interaction. For this reason, tests were designed to evaluate the slow-rusting characters of 23 single crosses and 33 inbreds in 1983 and 1984. The area under the disease progress curve (AUDPC) was calculated for each entry, using weekly assessments of pustule density. Individual weekly assessments of pustule density were also analyzed to compare the relative effectiveness of the two methods for identifying slow rusting. Significant differences were observed among both inbreds and single crosses for AUDPC and for pustule density. The maize inbreds and single crosses evaluated showed considerable variation for the slow-rusting trait. Rank correlations between years were higher for AUDPC than for pustule density, although rank correlations over years between pustule density and AUDPC were all high. The indication is that weekly assessments, if correctly timed, are as effective for identifying slow rusting as AUDPC, although they do not provide the details that can be gained by the multiple assessments used in calculating AUDPC.

Puccinia polysora Underw., causal agent of southern rust of maize (12), is common throughout the southern United States, developing rapidly during warm humid weather. No alternate host is known for *P. polysora*, and urediospores constitute both primary and secondary inoculum. Because *P. polysora* does not overwinter in the continental United States, inoculum must be introduced annually from areas in tropical America where host species live throughout the year.

Southern rust epiphytotics in the southern United States in 1972, 1973, and 1974 emphasized the potential of *P. polysora* to reduce yield (3). Rodriguez-Ardon et al (11) measured yield losses caused by *P. polysora* of 4, 23, and 45% in three biweekly plantings in 1976 and 1978. Single-gene forms of resistance to *P. polysora* have been identified (3,14), but races of *P. polysora* exist (9) that limit their usefulness.

In Africa in the 1950s, general forms of resistance in maize to *P. polysora* developed in the many local open-pollinated populations that were effective in controlling rust epiphytotics that single-gene forms of resistance failed to control (10). Slow rusting, a general form of resistance that results in a reduced rate of disease development, has been identified in several crop/rust interactions (8). Expression of the slow-rusting response is dependent on time and levels of initial inoculum; thus, reasonably uniform inoculum levels should be established throughout test plots for efficient field evaluation.

There is no information in the literature on slow-rusting resistance of maize to *P. polysora*. The objectives of our experiments were to develop a useful field procedure for measuring slow-rusting resistance in maize and to use the procedure to evaluate maize inbreds and single crosses. Vanderplank's (15) procedure for estimating area under the disease progress curve (AUDPC) was selected as our primary evaluation procedure. This procedure requires multiple assessments, however, and a simpler method would be preferable for field data collection. Thus, we also

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analyzed the weekly assessments of pustule density and compared these results with those obtained by the AUDPC procedure. It should be noted that some modifications of field techniques were made during these experiments because we considered improvement of the field procedure more important than the critical comparisons over years of the materials being evaluated. The information obtained related to relative resistance of inbred lines, however, and the single-cross hybrids tested were of interest to us and, presumably, may be of interest to others.

MATERIALS AND METHODS

Twenty-three maize single crosses and 33 maize inbreds were evaluated for slow-rusting resistance in field tests in 1983 and 1984. The field tests were located at the Texas A&M Agricultural Experiment Station Research Farm near College Station.

The 1983 test of inbred lines consisted of four replicates of 33 entries in a completely randomized design. Each plot consisted of three rows 6.1 m long by 1.02 m wide. Standard inbred lines were included in the test as controls. Fifty seeds were planted to each row on 1 April. On 26 May, when plants were at the six- to seven-leaf stage, the first plant on the windward (south) end of each row was inoculated in the whorl by hypodermic injection with 2 ml of a urediospore/water suspension (3×10^4 spores per milliliter). Spore concentration was determined with a hemacytometer. Inoculum was collected in the field the year preceding each test, stored in liquid nitrogen, and increased in the greenhouse on susceptible inbreds Va35, CI66, and Tx5855 for use in tests in 1983 and 1984.

In 1984, the 33 entries were planted on 4 April. Rows were reduced to 3 m long, and two Tx5855 spreader plants were added to the windward (south) end of each row. On 23 May, 2 ml of a urediospore suspension (4×10^4 spores per milliliter) were injected into the whorl of one spreader plant and the first plant of each inbred in the row. Plants were at the nine-leaf stage when inoculated.

The 1983 single-cross test consisted of 23 entries planted to one-row plots. Four standard single crosses (Mo17 × B73, Tx601 × CI66, CI64 × CI66, and K55 × CI66) were included in the test as controls along with 19 commercial entries. Three-row plots were used in 1984. The remaining details of the single-cross test experimental design were the same as those of the inbred test in their respective years.

Disease was assessed at weekly intervals using a modified James scale (5) for uredial density. Disease assessments were taken on the plant next to the inoculated entry plant in the middle row of each plot by rating rust on the leaf above the top ear and on the two leaves below the top ear. In 1983, five weekly disease assessments were made from 23 June to 31 July, using a scale of 0–50%. However, leaves of highly susceptible maize lines became necrotic because of disease development, leading to false disease assessments, during the later periods. Thus, in 1984, a scale of 0–25% was used, and four weekly assessments were taken from 21 June to 11 July.

AUDPC was computed by a simplified version of the Fortran IV subroutine AREA and the associated subroutine INTEG used by Johnson and Wilcoxson (6). Details on how AREA estimates areas can be found in Bevington (1).

The AUDPC values were calculated for each leaf, then averaged over the three leaves per plant to give an overall plant rating. The plant AUDPC values were transformed to natural logarithms for analysis because of heterogeneity of variances among entries. Analyses of variance (ANOVA) were also carried out on each of the last three weekly assessments of pustule density for each test. The final assessments of each year were analyzed together as were each of the preceding two. Pustule densities for individual plants were determined in a manner similar to AUDPC for each weekly assessment. Heterogeneity of variance was also observed for pustule density. Because plot values for pustule density often fell between 0 and 1, 1 was added to each value before taking the natural log, which eliminated all problems with negative logs. Both inbred and single-cross tests were analyzed over years with a two-way factorial ANOVA. Because this analysis confounds all changes in field plot design with year effects, both year and entries were considered to be fixed variates. The LSD (0.05) was calculated for mean comparisons.

A series of Spearman rank order correlations (13) were computed to evaluate the relative efficiency of individual assessments and AUDPC in identifying slow rusting. First, rank correlations were calculated between the entry means of each year for weekly assessments of pustule density and AUDPC. Next, rank correlations were calculated between the means of each weekly assessment and AUDPC over years.

RESULTS AND DISCUSSION

The year × line interaction for AUDPC was not significant ($P = 0.05$) in either test (Table 1). The rank correlation coefficient between years (Table 2) for AUDPC was 0.910 for the inbred test and 0.753 for the single-cross test, which indicates that most of the entries were ranked consistently in both years in both tests. The greater stability of response indicated in the inbred test may have resulted from the wider range in AUDPC values among inbreds than in single crosses (Tables 3 and 4). However, the single-row plots used in the 1983 single-cross test also could have affected the determination of rank attributable to

Table 1. Analysis of variance for the last three weekly assessments[a] of pustule density and AUDPC values of inbreds and single crosses

			Mean squares			
Test	Source	df	A1	A2	A3	AUDPC
Inbred	Line	32	2.423**[b]	3.018**	5.887**	12.969**
	Year	1	75.287**	115.153**	35.507**	150.207**
	Line × year	32	0.735**	0.652**	0.301	0.580
	Error	198	0.325	0.291	0.325	0.604
Single cross	Line	22	0.312**	0.677**	1.258**	2.911**
	Year	1	0.539*	5.544**	15.741**	10.516**
	Line × year	22	0.089	0.208	0.209	0.500
	Error	138	0.107	0.189	0.273	0.620

[a] The last assessments made each year were analyzed together (A3) as were the preceding two (A1 and A2).
[b] * = Significant at $P = 0.05$ and ** = significant at $P = 0.005$.

Table 2. Spearman rank correlation coefficients involving weekly assessments and AUDPC

Test	Evaluation procedure	Between years[a]	With AUDPC[b]
Inbred	1	0.847**[c]	0.961**
	2	0.863**	0.976**
	3	0.835**	0.982**
	AUDPC	0.910**	...
Single cross	1	0.341	0.862**
	2	0.332	0.942**
	3	0.623*	0.967**
	AUDPC	0.753**	...

[a] Rank correlations were calculated between years for each evaluation procedure to provide a comparison of their repeatabilities.
[b] Rank correlations were calculated between means over years for weekly assessments and AUDPC.
[c] The probability of a greater R (Ho:RHO = 0) is * = $P < 0.05$ and ** = $P < 0.0001$.

interplot interference. Vanderplank (15) stressed the importance of controlling interplot interference when evaluating general forms of resistance.

The year effect on AUDPC was highly significant ($P = 0.005$) in both tests. Average temperatures for April and May were 3 C higher in 1984 than in 1983. June and July temperatures averaged 1 C higher in 1984 than 1983. Average AUDPC in the inbred tests was much higher in the 1983 ($\bar{x} = 4.42$) than in 1984 ($\bar{x} = 2.92$), but most of the difference can be attributed to changes in scale between years and the longer assessment period in 1983. The single-cross test had a lower average AUDPC in 1983 ($\bar{x} = 2.87$) than in 1984 ($\bar{x} = 3.35$). Visual observations indicated that disease development progressed more rapidly in the 1984 single-cross test. The change in scale between years had little effect on single-cross assessments, because ratings were seldom higher than 25% for single crosses. The increase in average AUDPC in the 1984 single-cross test may be the result of several factors including climate differences, changes in design, or increased inoculum concentrations.

Differences among entries for AUDPC were highly significant ($P = 0.005$) in both tests (Table 1), but separation according to LSD (0.05) portrays an overlapping series of groupings (Tables 3 and 4). These results are similar to those found by Kim and Brewbaker (7) when studying the maize/*P. sorghi* interaction. Several standard inbreds typify the range in slow-rusting resistance found among the lines evaluated. Tx5855, B73, CI64, and Tx601 represent a wide range in backgrounds (4). Tx5855 (Lancaster Surecrop and Surecropper) has consistently shown high susceptibility to *P. polysora*. B73 (Iowa SSS C5 sel) has been an important inbred for breeders for many years and is moderately susceptible. CI64 (Pride of Saline) is moderately resistant, and Tx601 (Yellow Tuxpan) is highly resistant. The performances of these lines were consistent in a study involving over 100 inbreds in south central Texas and Nigeria in 1985 (B. A. Bailey and S. K. Kim, *unpublished*).

There is a clustering of the single-cross AUDPCs (Table 4) around 3.22 ($e^x = 25$). Although the change in experimental design of the single-cross test in 1984 may have contributed to this clustering effect, the most probable cause relates to the reduced range in AUDPC values observed in the single-cross test. Single crosses seem to have buffering capacity attributable to hybrid vigor. AUDPC values for the most susceptible hybrids evaluated fell within the intermediate range of the inbred test. The control single crosses fell in the middle of the range of slow-rusting responses found among the commercial entries. Tx601 × CI66, CI64 × CI66, and Mol7 × B73 are intermediate to the performance of the two individual inbreds involved, which is similar to results found for the barley/*P. hordei* (6) and maize/*P. sorghi* (7) interactions. This could suggest similar genetic control of the slow-rusting resistance.

The ANOVA for pustule density (Table 1) in the inbred and single-cross tests all indicate highly significant differences exist among the entrees. The first two weekly assessments of pustule density in the inbred test also show significant year × line interactions. AUDPC did not show significant year × line interactions in either test. The ranking of pustule density means over years (Tables 3 and 4) show a high correlation (Table 2) with AUDPC in both tests. The rank correlations between means of each year for pustule density were lower than for AUDPC (Table 2). The first two assessments of pustule density in the single-cross test show a low rank correlation between years. Much of this discrepancy is probably due to the small range in values found in the first two assessments. The differences among entries were small early in the disease development and more subject to error in assessment. The remaining rank correlations between years for the single-cross and inbred tests are high. Generally, weekly assessments did a good job in evaluating slow rusting.

The major difficulty with individual disease assessments is timing. The differences between slow and fast rusting lines only become evident as the epidemic proceeds. Small differences between lines for slow-rusting character may be identifiable for only short periods of time. If disease assessments are not made during this time period, the identification of these small differences may be lost. Perhaps a solution to this problem is to use AUDPC to assess disease development in a set of checks. Once the checks show the range of disease development wanted, the disease assessments can be made on the remainder of the test. This would allow the investigator to evaluate lines at about the same point in an epidemic each year.

If properly timed, individual assessments of pustule density can identify slow-rusting response with much the same efficiency as AUDPC. The AUDPC appears to give a more stable assessment of slow rusting, being less affected by time of assessment and environmental

Table 3. Development of southern rust on 33 maize inbreds as measured by three weekly assessments for pustule density[a] and AUDPC[b]

	Weekly assessment[c]			
Inbred	**1**	**2**	**3**	**AUDPC[c]**
Tx704	2.26	2.74	3.47	5.86
Tx29A	1.67	2.16	3.21	5.35
Tx5855	1.61	1.79	3.13	5.07
Tx82	1.29	1.78	3.16	5.03
Tx81	1.48	1.86	2.77	4.88
Ga209	1.52	1.60	2.77	4.85
Tx703	1.23	1.67	2.70	4.74
Tx71	1.46	1.80	2.69	4.74
Tx403	1.27	1.73	2.58	4.65
Tx84	1.18	1.54	2.60	4.58
B73	0.99	1.39	2.54	4.49
Tx83	1.14	1.56	2.49	4.46
Tx702	1.23	1.44	1.95	4.14
Tx61m	1.09	1.30	2.15	4.10
Tx710	1.00	1.33	1.92	4.01
Tx585	0.82	1.07	1.99	4.00
Ky228	1.35	1.52	1.84	3.94
Tx91	0.92	0.99	2.09	3.93
Tx90	0.76	1.07	2.05	3.75
CI66A	0.68	1.08	1.68	3.57
Tx303	0.65	1.00	1.49	3.43
Tx97	0.60	0.81	1.57	3.20
CI66	0.28	0.64	1.76	3.19
Tx705	0.34	0.64	1.71	3.13
Tx95	0.43	0.70	1.47	2.82
Tx602	0.35	0.91	1.37	2.74
CI64	0.46	0.59	1.12	2.63
B84	0.42	0.62	1.03	2.49
Mo17	0.21	0.49	0.58	1.69
Tx601Y	0.20	0.29	0.67	1.64
Tx706	0.15	0.31	0.72	1.63
Tx94	0.09	0.18	0.51	1.30
Tx601w2	0.03	0.17	0.19	0.71
Mean	0.88	1.17	1.94	3.66
LSD (0.05)	0.79	0.75	0.79	1.08

[a] The last three weekly assessments for pustule density in 1983 and 1984 were analyzed over years and are presented as 1, 2, and 3, with 3 being the final evaluation each year.

[b] Based on summary of four replicates per year and five and four evaluations of pustule density in 1983 and 1984, respectively.

[c] All means presented represent the natural logs of plot values.

Table 4. Development of southern rust on 23 maize single crosses as measured by three weekly assessments[a] for pustule density and AUDPC[b]

Single cross	Weekly assessment[c] 1	2	3	AUDPC[c]
G4522	0.62	1.17	2.51	4.15
Sx379	0.75	1.20	2.34	4.09
955	0.63	1.04	2.28	3.96
7005X	0.48	1.01	2.18	3.83
Sx17A	0.40	0.70	2.03	3.57
Sx351	0.27	0.59	2.02	3.54
G4507	0.24	0.60	1.79	3.40
Sx373	0.47	0.81	1.77	3.37
G4733	0.25	0.66	1.73	3.29
7008X	0.37	0.68	1.63	3.23
G4589	0.34	0.65	1.66	3.18
K55 × CI66	0.19	0.54	1.64	3.18
Mo17 × B73	0.24	0.61	1.64	3.12
7007A	0.11	0.46	1.75	3.10
Tx601 × CI66	0.45	0.59	1.62	2.72
CI64 × CI66	0.16	0.40	1.38	2.72
6014X	0.16	0.40	1.41	2.71
G4779W	0.13	0.36	1.37	2.68
G4689	0.21	0.34	1.37	2.67
8990	0.04	0.19	1.27	2.36
9797	0.09	0.35	1.20	2.27
644W	0.14	0.27	1.20	2.08
Sx70W	0.13	0.17	1.00	1.96
Mean	0.29	0.60	1.69	3.09
LSD (0.05)	0.46	0.61	0.74	1.10

[a] The last three weekly assessments for pustule density in 1983 and 1984 were analyzed over years and are presented as 1, 2, and 3, with 3 being the final evaluation each year.
[b] Based on summary of four replicates per year and five and four evaluations of pustule density in 1983 and 1984, respectively.
[c] All means presented represent the natural logs of plot values.

fluctuations. AUDPC also has the potential advantage of showing changes in slow-rusting response as the plant maturity changes, which individual ratings would not identify.

In both tests, it is apparent that considerable variability existed among entries. "Slow rusting" is a relative term, and most entries showed this response to a greater or lesser degree. The slow-rusting response is dependent on limiting the rate of fungal growth without stopping it, and entries that developed practically no rust on the upper leaves had 5% or more of the lower leaves covered with rust. The observation that hybrids were generally more resistant than inbreds also suggests that the slow-rusting response may be affected by the overall vigor of the plant. The technique presented, though continually being refined during testing, provided data that allowed identification of slow-rusting entries and was consistent over 2 yr.

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