Prediction of Stewart's Wilt Disease in Single and Three-Way Crosses in Maize

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ABSTRACT

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Six inbred lines of maize (*Zea mays*)—B14A, B37, Mo17, N28, Va35, and 33-16—were used to produce the 15 possible single crosses (A × B) and the 60 possible three-way crosses [(A × B)C]. Inbred lines, single crosses, and three-way crosses were grown at two locations for 2 yr and inoculated with *Erwinia stewartii*. Disease ratings were made when 50% of the plants per plot had tasseled and again near physiological maturity. Disease ratings indicated that resistance to the pathogen was the result of additive genetic effects because the general combining ability mean square was 20 times greater than specific combining ability. Highly significant correlation coefficients were detected for observed and predicted disease ratings among the single and three-way crosses studied. A significant linear response was found for increased susceptibility to the bacterial wilt pathogen for the observed ratings with each substitution of an inbred (B14A, N28, and Va35) that was susceptible to the pathogen for a resistant one (Mo17, B37, and 33-16) in the pedigree of single or three-way crosses. Our results supported the hypothesis that disease development in three-way crosses of maize may be predicted from inbred and single-cross disease rating scores.

Additional key word: corn

Stewart's bacterial wilt, caused by *Erwinia stewartii* (E. F. Smith, 1898) Dye 1963, is an important maize (*Zea mays* L.) disease throughout the lower half of the corn belt and in the northeast (2,4). Historically, the disease has been more important on sweet corn and early maturing dent maize cultivars (6). Outbreaks of this disease generally coincide with population levels of the maize flea beetle vector *Chaetocnema pulicaria* Melsh. Mild winters favor insect buildup and disease incidence the following spring (10).

Inheritance studies were initiated in the 1930s when Stewart's wilt became epiphytotic on sweet corn. Ivanoff and Riker (6) suggested that genetic resistance to *E. stewartii* was inherited in a partially dominant manner, whereas Wellhausen (11) postulated that two completely dominant, complementary genes acted to confer resistance to the pathogen. More recent genetic studies are generally supportive of resistance to *E. stewartii* being inherited quantitatively in dent maize (2; D. R. Smith, *unpublished*). Earlier studies involving crosses among these six inbred lines have shown that resistance to *E. stewartii* is predominantly the result of additive genetic effects (2).

Maize breeders have particular interest in the additive portion of genetic variance because of its importance in genetic improvement and its predictive significance in determining characteristics of the phenotype. Methods for predicting the yield of three-way and double crosses have been useful in maize breeding programs in reducing the cost and amount of labor that would normally be involved in testing all possible combinations of lines. The purpose of this study was to determine whether Stewart's wilt reaction can be predicted for three-way crosses of maize.

MATERIALS AND METHODS

The terms "resistant" (R = rating of 1–3) and "susceptible" (S = rating of 7–9) are used throughout this article to describe the relative extent of necrotic leaf tissue observed. Three inbred lines had been previously classified as resistant (B37, Mo17, and 33-16) and three lines susceptible (B14A, N28, and Va35) to the pathogen, although inbred Va35 may be considered to have an intermediate level of resistance (2). The six inbreds were intermated to give 15 single crosses (A × B; SC), and each SC was intermated with the remaining four inbreds, respectively, to generate 60 three-way crosses [(A × B)C; TWC]. The SCs were grouped into three categories based upon the inbreds in their pedigree: two resistant, one resistant and one susceptible, and two susceptible to *E. stewartii*. The TWCs were grouped into four categories based upon the inbreds in their pedigree: three resistant, two resistant and one susceptible, one resistant and two susceptible, and three susceptible to the pathogen.

The six inbreds, 15 SCs, and 60 TWCs were planted in a randomized complete block design replicated three times in four environments (Columbia, MO, and

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Farmer City, IL, in 1977 and 1978). The single-row plots included 30 plants at a population density of about 49,000 plants per hectare. The pooled means over replications, environments, and times of rating were used in predicting Stewart's wilt disease reactions for SCs and TWCs. Griffing's (3) method 4, model I analysis for fixed genotypes was completed for the single crosses. Inbreds were not included in the diallel analysis of single crosses to remove their effect and that of inbreds vs. single crosses from the estimate of specific combining ability (SCA) variance. This permitted a clearer comparison of magnitudes of the SCA and general combining ability (GCA) variances.

A single mass-transfer isolate (GC6) of *E. stewartii* that was lypholized and used in an earlier study provided our source of inoculum (2). Although the techniques in increasing the inoculum at each location were different, the isolate used in both years and locations was from a common lypholized culture. At Columbia, the inoculum was prepared by growing lypholized cultures of the bacteria in nutrient broth in shaking Erlenmeyer flasks for 24 hr at room temperature. The inoculum was diluted to five times its original volume before inoculation. Although the original suspension used in the study was not calibrated, further experimentation with the suspension prepared in a similar manner had approximately 10^9 cells per milliliter. At Farmer City, the inoculum was increased by growing the lypholized culture on petri plates of nutrient agar that were blended together (88 plates) and adding cold, distilled water to make a final volume of 19.4 L. The wound-inoculation method used was previously described by Blanco et al (1).

Disease ratings were taken at 50% tasseling and again near physiological maturity. A descriptive rating scale was used where 1 represented the most resistant and 9 the most susceptible plants (2): 1 = no visible chlorosis surrounding inoculator perforations; 2 = localized chlorotic lesions; 3 = buff-white lesions surrounding inoculator perforations, with some necrosis; 4 = necrotic lesions beginning to coalesce; 5 = extensive coalescing and necrosis from site of inoculation to leaf tip; 6 = inoculated leaves below ear dead; 7 = two leaves above ear dead; 8 = three or more leaves above ear dead; and 9 = plant dead. Disease ratings were taken on an individual plant basis, and the plot means were used in the analyses.

The predicted Stewart's wilt disease ratings for SCs from inbred data and TWCs from SC data were calculated by using Jenkins' method B (7). Single cross AB was denoted SC_{AB} and was predicted as the average of inbreds A and B; three-way cross AB·C was denoted $TWC_{AB\cdot C}$ and was predicted as the average of SC_{AC} and SC_{BC}.

Table 1. Analysis of variance for combined Stewart's wilt disease ratings for six inbreds, 15 single crosses (SC), and 60 three-way crosses (TWC)[a]

Source	df	Mean square[b]
Environments (E)	3	690.44 **
Replications/environments	8	1.27
Genotypes (G)	80	6.31 **
TWC vs. SC and inbreds	1	1.69 NS
SC vs. inbreds	1	16.71 **
Among inbreds	5	18.09 **
Among SC	14	9.92 **
GCA	5	26.47 **
SCA	9	0.73 NS
Among TWC	59	4.36 **
Among types of TWC	5	29.65 **
Among (R × R)R[c]	2	0.92 NS
Among (R × R)S	8	3.36 NS
Among (R × S)R	17	1.32 NS
Among (R × S)S	17	2.95 NS
Among (S × S)R	8	0.80 NS
Among (S × S)S	2	0.42 NS
E × G	240	1.93 **
Pooled error A	640	0.48
Rating dates (RD)	1	1,362.36 NS
G × RD	80	0.50 NS
E × RD	3	201.71**
E × G × RD	240	0.70 **
Pooled error B	648	0.18

[a] Rated on a scale of 1–9 in which 1 represented the most resistance and 9 represented the most susceptibility to *Erwinia stewartii*; see text.
[b] * Indicates statistical significance at $P = 0.05$ and ** indicates significance at $P = 0.01$; NS indicates nonsignificance.
[c] R = a parent resistant (rating of 1–3) and S = a parent susceptible (rating of 7–9) to *E. stewartii* in crosses.

Table 2. Nonorthogonal contrasts for combined Stewart's wilt disease ratings of 15 single crosses and 60 three-way crosses[a]

Source/contrast	df	Mean square[b]
Single crosses		
R × R vs. R × S[c]	1	25.71 **
R × S vs. S × S	1	45.71 **
Three-way crosses		
(R × R)R vs. (R × R)S	1	17.48 **
(R × R)R vs. (R × S)R	1	6.86 NS
(R × S)R vs. (R × S)S	1	84.68 **
(R × S)R vs. (S × S)R	1	11.65 *
(R × S)S vs. (S × S)S	1	7.88 *
(S × S)R vs. (S × S)S	1	26.38 **
(S × S)R vs. (R × R)S	1	0.26 NS
Environment × genotype	240	1.93 **

[a] Rated on a scale of 1–9 in which 1 represented the most resistance and 9 represented the most susceptibility to *Erwinia stewartii*; see text.
[b] Indicates statistical significance at $P = 0.05$ and ** indicates significance at $P = 0.01$; NS indicates nonsignificance.
[c] R = a parent resistant (rating of 1–3) and S = a parent susceptible (rating of 7–9) to *E. stewartii* in crosses.

RESULTS

The analysis of variance for combined Stewart's wilt disease rating showed that the mean squares for environments and for environments × genotypes were highly significant, indicating that more than one environment is needed in evaluating genotypes for Stewart's wilt disease reaction (Table 1). Differences among genotypes, TWCs, SCs, and inbreds were highly significant. The GCA mean square (26.47) was highly significant ($P = 0.01$) and was approximately 20 times greater than that of SCA. The SCA mean square was not significant. Thus, additive effects were much more important than nonadditive effects in the inheritance of reaction to the Stewart's wilt pathogen in the six inbred parents evaluated. Variation within types of TWCs—(R × R)S, for instance—was not significant, whereas variation among types was highly significant. Resistant inbreds (B37, Mo17, and 33-16) contributed to low reaction ratings in SCs, and susceptible lines (B14A, N28, and Va35) contributed to higher reaction ratings in SCs.

Six types of TWCs with respect to resistant or susceptible reaction of the parents to *E. stewartii* (Table 1) were compared and found significantly different ($P = 0.01$). Differences among crosses within each type of cross were not significant (Table 1).

Nonorthogonal contrasts were used to examine the effect of substitution of an R genotype for an S genotype (Table 2). In all cases except (R × R)R vs. (R × S)R, substitution of an R for an S genotype was significant or highly significant, whether in the SCs or TWCs. The response was highly significant for substitution on the male side of the TWCs and was significant in two of three cases where substitution was for one of the two female single-cross parents. Where the male TWC parents were resistant and at least one of the female single-cross parents was resistant, the genotype of the remaining single-cross female parent did not matter [(R × R)R vs. (R × S)R].

Inbred line B14A had the highest line mean for disease rating (3.89) and positive GCA effect (0.82; $P = 0.05$), whereas 33-16 had the lowest line mean (1.85) and next to the lowest negative GCA effect (−0.36; $P = 0.05$) (Table 3). Inbred lines Mo17 and B37 had similarly significant ($P = 0.05$) negative GCA effects. The disease reaction mean of Va35 (3.28) was intermediate, as was shown by the nonsignificant GCA effect.

Using Jenkin's (7) method, we predicted B37 × 33-16 to have the lowest Stewart's wilt disease rating (1.97) of the single crosses, and it also had the lowest observed disease rating (2.49). We predicted B14A × N28 to have the highest disease rating (3.77), which it did (4.56). We predicted (Mo17 × 33-16) B37 to have the second lowest disease rating (2.52), and it had the lowest disease rating (2.27). We predicted (N28 × Va35) B14A to have the highest predicted and observed Stewart's wilt disease ratings (4.36 and 3.90, respectively).

The observed Stewart's wilt disease ratings for the three categories with different levels of resistance for SCs and the six categories for the TWCs were significantly different in the observed ratings ($P = 0.05$) (Table 4). The observed and predicted disease ratings for SCs and TWCs increased significantly ($P = 0.05$) as inbreds susceptible to the pathogen were substituted in the pedigree (Table 4). The average effect of a substitution in the observed rating of SCs was a highly significant 0.81 ($P = 0.01$). In the TWCs, each 0.25-unit increase of R germ plasm in the cross resulted in a highly significant 0.33 ($P = 0.01$) decrease in rating. A substitution of an R for an S genotype on the male side of the cross would reduce the predicted rating by 0.66, whereas a similar substitution on the female single-cross parent would result in a 0.33 rating decrease. The effect in the TWCs was greater when substitution was on the male side of the cross vs. only one of the two inbreds in the female side of the single cross. Predicted ratings were less than the observed ratings for the SCs but were uniformly higher in the TWCs. Buffering of genotypes or environmental interactions in the TWCs may explain a part of this variation.

Correlations of Stewart's wilt disease ratings among predicted and observed means and the high (S) or low (R) reaction parent in the pedigree of the 60 TWCs, 15 SCs, and all crosses (SC and TWC) for both rating dates and the ratings combined over dates were highly significant ($P = 0.01$) (Table 5). The overall observed disease ratings more closely paralleled the predicted ratings than did the observed ratings with the high or low reaction parent. There was also good agreement in the correlations between rating dates and the combined rating date. One rating date, at tasseling, would be better than at physiological maturity because of less competition from other pathogens.

DISCUSSION

Early maturing hybrids have assumed greater popularity in the southern United States because of interest in double cropping and avoidance of heat and drought stress during the grain-filling

Table 3. Stewart's wilt disease ratings and general combining ability (GCA) effect estimates for six maize inbreds combined over rating dates and environments[a]

Inbred	Disease rating	GCA effect[b]
33-16	1.85	−0.36 *
B37	2.09	−0.30 *
Mo17	2.31	−0.54 *
Va35	3.28	0.05
N28	3.64	0.43 *
B14A	3.89	0.82 *
Mean	2.84	0.00
LSD ($P = 0.05$)	0.40	0.40

[a] Rated on a scale of 1–9 in which 1 represented the most resistance and 9 represented the most susceptibility to *Erwinia stewartii*. Rating date 1 was at 50% tasseling and date 2 was at physiological maturity. Environments 1 and 2 were at Columbia, MO, and environments 3 and 4 were at Farmer City, IL.

[b] * Indicates effect is signficantly different from zero at $P = 0.05$.

Table 4. Predicted and observed Stewart's wilt disease ratings for 15 single and 60 three-way crosses constituting various combinations of parental lines having different levels of resistance to Stewart's disease[a]

Source/type of cross[b]	Fraction of resistant germ plasm in crosses	Number of crosses per type	Observed rating	Predicted rating
Single crosses				
R × R	1.0	3	2.51	2.08
R × S	0.5	9	3.20	2.85
S × S	0.0	3	4.12	3.61
LSD ($P = 0.05$ approx.)[c]			0.40	
Three-way crosses				
(R × R)R	1.0	3	2.43	2.51
(R × S)R	0.75	18	2.77	2.86
(R × R)S	0.5	9	3.00	3.20
(S × S)R	0.5	9	3.05	3.20
(R × S)S	0.25	18	3.39	3.66
(S × S)S	0.0	3	3.75	4.12
LSD ($P = 0.05$ approx.)[c]			0.32	

[a] Rated on a scale of 1–9 in which 1 represented the most resistance and 9 represented the most susceptibility to *Erwinia stewartii*; see text.

[b] R = a parent resistant (rating of 1–3) and S = a parent susceptible (rating of 7–9) to *E. stewartii* in crosses.

[c] Harmonic mean of number of crosses per type used because of imbalance.

Table 5. Correlation coefficients for Stewart's wilt disease ratings among observed and predicted means and high or low parents of single, three-way, and all crosses[a]

Means correlated	Combined over rating dates[b]	Rating date 1	Rating date 2
		Three-way crosses	
Observed and predicted	0.90**[c]	0.90**	0.85**
Observed and high parent	0.83**	0.84**	0.77**
Observed and low parent	0.83**	0.81**	0.79**
		Single crosses	
Observed and predicted	0.88**	0.90**	0.80**
Observed and high parent	0.79**	0.81**	0.66**
Observed and low parent	0.71**	0.69**	0.68**
		All crosses	
Observed and predicted	0.78**	0.86**	0.66**
Observed and high parent	0.77**	0.82**	0.66**
Observed and low parent	0.63**	0.71**	0.52**

[a] Rated on a scale of 1–9 in which 1 represented the most resistance and 9 represented the most susceptibility to *Erwinia stewartii*; see text. High refers to a rating of 7–9, and low refers to a rating of 1–3 of the specific parents in the cross.

[b] Rating date 1 was at 50% tasseling and date 2 was at physiological maturity.

[c] ** Indicates significance at $P = 0.01$.

period. With the introduction of earlier hybrids to the South, presumably Stewart's wilt disease resistance may become increasingly important to maize breeders.

Results of our study indicated that the inheritance of reaction to *E. stewartii* is predominantly additive in the SCs among the six inbreds used in this study. Thus, recurrent selection or other conventional breeding programs should be effective in increasing the level of resistance to this pathogen. In most instances, selections can be made prior to pollination and genotypes susceptible to the pathogen eliminated, enabling the breeder to make more rapid progress in increasing the gene frequency for resistance to *E. stewartii* in population improvement programs.

The practical significance of predicting Stewart's wilt disease reactions among three-way crosses remains to be seen. Although double crosses are now almost never used, three-way crosses are still quite popular in the seed industry, especially in southern maize-producing areas. Considering the ever-increasing costs involved in extensive hybrid testing programs, high predictability should reduce testing needs when considering specific three-way crosses for disease reaction.

Predicting disease reaction of genotypes is also of interest from the basic research standpoint. Additive gene action has been reported for resistance to *Exserohilum turcicum* (Pass.) Leonard and Suggs, which causes northern corn leaf blight (5); *Drechslera maydis* (Nisikado) Subram. and Jain, which causes southern corn leaf blight (8); *Colletotrichum graminicola* (Ces.) Wilson, which causes anthracnose (9); and maize dwarf mosaic virus (12). Perhaps prediction of genotypic disease reactions can be done readily for most major maize diseases. Zuber et al (12) showed earlier that double-cross disease reaction is readily predictable from single crosses in studies with maize dwarf mosaic virus.

Disease rating scales and methods for estimating yield losses continue to be a highly debated and challenging issue among plant pathologists and maize breeders. We suggest that researchers working in this area consider using genetic prediction models in evaluating or devising rating scales that equate yield loss to disease intensity. Predicted disease ratings might then be used to estimate accurately the actual yield losses in particular crosses or lines. Our data show that disease development in three-way crosses of maize may be predicted from inbred and single-cross disease rating scores using Jenkin's (7) method.

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