Inheritance of Resistance to Tar Spot Complex in Maize

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ABSTRACT

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Tar spot complex (TSC) of maize can cause serious yield losses in Latin American countries. *Phyllachora maydis* is the most important pathogen associated with TSC. Little is known about the genetics of resistance to this disease. This study was carried out to elucidate the inheritance of resistance to *P. maydis* found in S_2 lines from a CIMMYT (International Maize and Wheat Improvement Center) maize population. Segregation and diallel analyses were performed in three environments. In the segregation analysis, each of three resistant lines (P_R) was crossed with a susceptible one (P_S) and the following generations obtained: F_1, F_2, F_3, and the backcrosses of F_1 to P_R and P_S. Expected segregation ratios for resistance and susceptibility, based on hypothetical genetic models, were compared with experimental data through a chi-square test. A joint-scaling test also was performed on one set. Results indicated the presence of a single dominant gene controlling the resistance in at least two of the three parental lines evaluated. The diallel analysis evaluated the reaction to *P. maydis* in eight parental lines. Significant general and specific combining ability effects ($P < 0.01$) were associated with resistance. This is the first published study on the genetics of resistance to TSC.

Additional keywords: Coniothyrium phyllachorae, Monographella maydis.

Tar spot complex (TSC) is a poorly understood foliar disease of maize (*Zea mays* L.). It is induced by the pathogen *Phyllachora maydis* Maubl. However, at least two other organisms can be found in TSC lesions: *Monographella maydis* Müller & Samuels and *Coniothyrium phyllachorae* Maubl. (10–12,17,19). The fungus *M. maydis* is commonly found on the surface of maize leaves, generally without any apparent reaction by the plant. It is only in association with *P. maydis* that *M. maydis* becomes pathogenic and highly virulent (19). The host reaction to each organism can be easily distinguished. *P. maydis* induces small (1–2 mm in diameter), round, dark lesions; *M. maydis* causes a brown, elliptic, necrotic halo surrounding each *P. maydis* lesion. The actual effect of *C. phyllachorae* is not completely known, but it seems to be a hyperparasite of *P. maydis* and *M. maydis* (10,12). However, *C. phyllachorae* is not found as frequently in TSC lesions as the other two organisms.

P. maydis is an obligate parasite (21), and *M. maydis* requires its presence to become pathogenic (19). There is no known technique for the in vitro culture of *P. maydis* nor for its effective artificial inoculation. Field studies with TSC depend on the natural occurrence of this disease, which is unpredictable and sporadic.

Disease development generally starts at flowering time on the lower leaves and moves rapidly up the plant. The development of *P. maydis* lesions is frequently followed by *M. maydis* lesions, which together increase the amount of leaf area affected. Under favorable environmental conditions, leaves from susceptible genotypes can be completely blighted 3–4 wk after flowering. The presence of a toxin produced by *P. maydis* has been associated with the rapid killing of plant tissue (12).

TSC has been reported in many Latin American countries (2,3,11,14,15). Its occurrence is common in moderately cool and humid, tropical or subtropical, mountainous areas (15), that is, in climates where northern corn leaf blight (caused by *Exserohilum turcicum* (Pass.) Leonard & Suggs) develops readily. Little has been published on the genetics of resistance to TSC. However, several maize populations with increased resistance to TSC have been selected at the CIMMYT (International Maize and Wheat Improvement Center) tropical station in Poza Rica, Mexico, where the disease occasionally develops during the winter cycle. The nature of this resistance has not been studied. The objectives of our study were to confirm the resistance to *P. maydis* in several S_2 maize lines selected during the 1987–1988 winter cycle at Poza Rica and to study the inheritance of resistance in those selected lines.

MATERIALS AND METHODS

During the winter of 1987–1988, TSC was very severe at Poza Rica (50 m above sea level, 20° 30′ N) and caused the early death of many maize germplasm. A tropical, late maturing, white dent maize population under improvement for general agronomic traits provided the material for this study. Because of the high TSC severity, it was possible to clearly identify families that had been more (or less) severely affected than the population mean. Several S_2 lines (obtained after two consecutive selfings) were chosen as the parental lines of two different studies.

Experiment 1. Three sets of crosses (A, B, and C), using three resistant lines and three susceptible lines, were selected for this study (Table 1). Each set was defined by a resistant and a susceptible S_2 parent that had been classified according to the reaction to TSC during the 1987–1988 winter. During the summer of 1988, the three sets, each containing a resistant and a susceptible parent, and their respective F_1s (produced during the 1987 summer) and F_2s (obtained during the 1987–1988 winter) were planted and crosses or sibbings were made, thus obtaining the following generations: P_R (resistant parent), P_S (susceptible parent), F_1, F_2, F_3 (sibbing of F_2), BC_{PR} (backcross of F_1 to resistant parent), and BC_{PS} (backcross of F_1 to susceptible parent). Each generation was obtained from no less than five crosses or sibbings. Seed from reciprocal crosses was mixed together.

The seven generations of sets A and B were planted at Poza Rica during the winter of 1988–1989. Each set was planted in a randomized complete block design with three replications at two different planting dates 4 wk apart. Nonsegregating generations (P_R, P_S, and F_1) were planted in single-row plots, and segregating generations were planted in two-row plots. Rows were 5 m long with 21 hills and 25 cm between hills. Plots were overplanted with two seeds per hill and later thinned to one plant per hill. Sets A and B also were planted along with set C in an out-of-station trial at Xicotepec de Juárez (1,200 m above sea level, 20° 17′ N), Mexico, in April 1989. Maize planted in this area is commonly affected by the disease. Soil fertility was

variable, and only partial weed and insect control were obtained. Two replications were planted at this location, following the same procedures as for the trials at Poza Rica.

Evaluation of the reaction to TSC. Preliminary studies indicated that disease severity in the plants was regular enough to justify evaluating only one leaf per plant (as long as leaves were at an equivalent developmental stage). The distribution of lesions along the leaves, in contrast, was more irregular. Therefore subsequent evaluations were performed on the entire length of one leaf per plant. Because as many as 4,000 lesions of *P. maydis* can be found on a single leaf, the evaluations were made relatively early when the number of lesions was large enough to differentiate susceptible and resistant phenotypes, but not so large as to make counting difficult and therefore unreliable. It is considered that the variable number of lesions may have an error margin of 10–15%. Depending on TSC development, the evaluations were made either on the ear leaf or on the first or second leaf below the ear, beginning 2 wk before flowering until flowering time.

The total number of lesions per leaf is somewhat biased because of differences in leaf size among the generations included—parental lines had considerably smaller leaves than the full vigor families. Therefore, lesion density rather than total number of lesions per leaf was used for analyses. Lesion density was obtained by dividing the total number of lesions per leaf by the mean area (dm^2) of the leaf selected for evaluation. The mean leaf area was the average of 10 randomly selected plants per row from each replication. Leaf area was calculated by multiplying leaf length by its width (at the widest possible sector). Further analyses were performed on lesion density rather than on total number of lesions per leaf to enhance precision.

Approximately 4 wk after flowering, a second evaluation on every plant from all the trials at Poza Rica and Xicotepec de Juárez was made by using the following 0 to 5 rating scale: 0 = Immune or highly resistant. No or very few lesions, all on leaves below the ear. Percentage of leaf area affected 0–2%. 1 = Very resistant. Some lesions on leaves below the ear. Two to 10% of leaf area affected. 2 = Resistant. Many lesions on leaves below the ear, some necrotic areas, but most of leaf area still green. Some lesions above the ear. Ten to 25% of leaf area affected. 3 = Moderately susceptible. Most of leaf area below ear necrotic, many lesions above ear coalescing. Percentage of leaf area affected 25–50%. 4 = Susceptible. No green tissue on leaves below the ear. Considerable leaf area above the ear dead. Between 50 and 80% of leaf area blighted. 5 = Very susceptible. Dead plant or with small leaf area still green. More than 80% of leaf area affected.

Data analysis of experiment 1. Two different analyses were made with the data from experiment 1: a segregation analysis and a joint-scaling test. Segregation of resistant and susceptible plants was obtained for each generation. Chi-square tests of goodness of fit between the expected and observed segregation ratios were calculated. Expected frequencies were obtained from hypothetical genetic models (dominant or recessive single gene, complementary genes, etc.). Yate's correction for continuity was used in the chi-square tests (23). The separation between resistant and susceptible classes in the case of lesion density was done by taking into account the range of variation of parental lines. The dividing point was the density of lesions that classified all plants from resistant and susceptible parents as resistant or susceptible phenotypes, respectively. Because disease development was different in the three trials evaluated and ratings were taken for each set at relatively different plant growth or disease development stages, the dividing point of each data set was different. Ratings 0 to 2 were resistant phenotypes; ratings 3 to 5 were susceptible.

A joint-scaling test on the generation means, as proposed by Mather and Jinks (16), was performed on lesion density data from set A. Since variances within each generation were not equal, the analysis had to be done on weighted means. The weight used was the inverse of the variance of the mean of each particular generation (16). This analysis allows the estimation of the following genetic parameters: midparent point (m), additive ([d]), and dominance ([h]) effects. In performing this analysis, the usual assumptions for the procedure were made (16).

Experiment 2. A set of eight S_2 parents, selected for the reaction to TSC during the 1987–1988 winter, was chosen for this study (Table 1). Three of the parents (1, 2, and 3) were classified as resistant to TSC, three as susceptible (4, 5, and 6), and the remaining two parents (7 and 8) as intermediate. Parents 1 and 3 were the resistant parents used in crosses A and C of experiment 1, respectively. In the summer of 1988, remnant seeds of the eight lines were planted and crosses made to obtain a diallel set as described by Griffing (4) in his Method 4. Reciprocal crosses were mixed together, thus providing a final set of 28 F_1 crosses. Each F_1 came from at least five crosses. The 28 F_1 crosses, with their respective parental lines, were planted together with experiment 1 following the same criteria for number of replications, planting dates, experimental design, and locations. Some of the parental lines were not planted in all replicates at Poza Rica, and no parental line was included at Xicotepec de Juárez because of lack of seed. Each family was planted in a single-row plot, following the same criteria as for experiment 1.

Data analysis of experiment 2. Analyses of variance (Proc GLM of SAS) were performed on mean rating and mean number of *P. maydis* lesions (20). Mean ratings were obtained from three environments (two planting dates at Poza Rica and Xicotepec de Juárez), whereas the mean number of lesions (average of 10 plants per row) were obtained only from the two planting dates at Poza Rica. General combining ability (GCA) and specific combining ability (SCA) effects for the parents and crosses, respectively, were estimated by following Griffing's Model 1 (4). The two planting dates at Poza Rica were considered as two different environments, and the environmental effects were considered fixed. The proper error terms were used in the analyses of variance as indicated by McIntosh (18).

Further analysis of the data was different for the two variables. Ratings were analyzed with Griffing's Method 4 (4) using a complete set of F_1 crosses without reciprocals or parental lines and the data from the three environments. The variable number of lesions per leaf was analyzed as described by Hayman (6,7). This analysis (equivalent to Griffing's Method 2) requires not only the F_1s but also the parental lines. Because the trial at Xicotepec de Juárez did not include the parental lines and the number of lesions was not estimated at this location, only data from the two planting dates at Poza Rica were used for this analysis.

Hayman's method includes the construction of a Vr/Wr graph, where Vr is the variance of each array (all of the progeny of a given parent) and Wr is the covariance between parents and their progeny. For a better understanding of the results from the Vr/Wr graph analysis, the standardized deviations of the parental measurements (Yr) and the order of dominance of the parents [$Wr + Vr$] were plotted (13). Depending on the position of each parent in this plot, they can be classified as dominant or recessive and resistant or susceptible. This classification is relevant only to the parents involved in the analysis. Normal assumptions for Hayman's method were made (7). To check the validity of the assumptions made, the t test as described by Singh and Chandhary (22) was performed. The validity of the assumptions made is also supported if the regression of Wr on Vr yields

TABLE 1. Pedigree of the parents used in experiments 1 and 2 of this study

Pedigree	Experiment 1	Experiment 2
PR87A-216-36-1	Resistant parent of set A	Parent 1
PR87A-216-74-1	Susceptible parent of set A	Parent 4
PR87A-216-113-1	Resistant parent of set B	
PR87A-218-297-1	Susceptible parent of set B	
PR86B-5477-291	Resistant parent of set C	Parent 3
PR86B-5477-170	Susceptible parent of set C	Parent 5
PR87A-218-333-2		Parent 2
PR86B-5024-94		Parent 6
PR86B-5024-1		Parent 7
PR86B-5024-12		Parent 8

a regression coefficient (b) that is not significantly different from one.

RESULTS

Disease development at Poza Rica was adequate for the two planting dates. At Xicotepec de Juárez a severe wind storm lodged most of the materials about 2 wk before flowering time. Disease onset at this location was late because of a dry and warm spring. However, by the end of the season *P. maydis* had developed, but not as severely as at Poza Rica. Interestingly, *M. maydis* developed very little at this location.

Experiment 1: Segregation analysis. Results within sets A and B were similar in the three environments evaluated. Most of the differences among these environments were due to the timing of evaluation related to disease development. Because no significant genotype-by-environment interaction was found, data were pooled across locations (within each set of crosses) for the segregation analysis. The hypothetical genetic model for the inheritance of resistance to *P. maydis* presented was that of a single dominant gene. The expected segregation ratios for each generation, therefore, were based on that assumption (Table 2).

Set A of crosses showed a good fit between observed and expected frequencies of resistant and susceptible plants for the F_2 and the F_3 generations. The presence of a susceptible plant in P_R, F_1, and BC_{PR} and a resistant plant in P_S can be due to seed mixture, volunteers, or misratings. There were too many resistant plants in the BC_{PS}, and the chi-square values were statistically significant, indicating a poor fit with the expected segregation. Segregation patterns for each generation from set A can be observed in Figure 1. The separation of the distributions of P_R and P_S (between ratings 2 and 3) coincided with the separation of resistant and susceptible classes. The frequency distributions of P_R and F_1 overlap, illustrating the strong dominance by P_R (Fig. 1A). F_2 and F_3 distributions were similar and suggested a bimodal shape that separated the 75% resistant plants from the 25% susceptible ones (Fig. 1B). A frequency peak was observed for the susceptible phenotypes of both generations at rating 4. Most of the plants from the BC_{PR} were resistant, and a frequency peak for the susceptible phenotypes also was observed at rating 4 in the BC_{PS} (Fig. 1C).

Set B showed more differences between expected and observed segregations (Table 2). The results from both parents were acceptable. In the F_1 and BC_{PR}, however, there were 36 and 29 susceptible plants (based on lesion density), respectively, whose presence was unexpected and difficult to explain. The chi-square tests of F_2 also were significant. The disagreements between observed and expected segregations were more serious for the variable rating. The lack of fit between observed and expected frequencies cannot be reasonably explained by seed mixtures or misratings. It is important to emphasize that, even though chi-square values were significant for some generations of set B, the differences between resistant and susceptible phenotypes were even more distinct than in set A. For instance, the ratios of mean density of lesions from susceptible plants/mean density of lesions from resistant plants averaged 7.88 and 36.21 for sets A and B, respectively, suggesting that the resistance of set B was relatively higher than that of set A when compared with the mean susceptible phenotype of each set. The typical phenotypic difference between two susceptible and two resistant plants from the BC_{PS}, 6 wk after flowering, is illustrated in Figure 2A. For this photograph, the plants were taken out of the row for better illustration.

There was excellent agreement between observed and expected segregation frequencies for set C (Table 2). Chi-square values were not significant and the only disagreements with the theoretical model, based on lesion density data, were the presence of one and three susceptible plants in the P_R and F_1 generations and three resistant plants in P_S. Similarly, the ratings revealed the unexpected presence of five resistant and two susceptible plants in the P_S and F_1, respectively. Apparently the degree of resistance present in set C is of the same effectiveness as that in set B, where most resistant plants tended to have a near-immune reaction (the mean rating of resistant plants for sets B and C were 0.40 and 0.37, respectively). Data supporting the conclusions for set C came from only one location where disease severity was not as high as in Poza Rica and the precision of the experiment was affected by severe lodging. However, the same parent was used in the diallel analysis (parent 3), which was evaluated under

TABLE 2. Expected and observed ratios, chi-square tests, means of densities of lesions per leaf, and disease rating means from experiment 1[a]

Family[b]	Expected ratios[c]	Mean lesion density				Mean rating			
		Observed ratios	χ^2[d]	R	S	Observed ratios	χ^2	R	S
Set A									
P_R	1:0	126:1	...	2.1	18.7	125:1	...	0.3	3.0
P_S	0:1	1:95	...	10.3	59.4	1:95	...	2.0	4.7
F_1	1:0	157:1	...	3.1	17.8	154:1	...	0.6	3.0
F_2	3:1	226:79	0.1	2.8	24.7	224:75	0.0	0.6	3.7
F_3	3:1	195:74	0.8	3.6	28.5	191:70	0.5	0.7	4.0
BC_{PR}	1:0	304:1	...	1.6	21.3	301:1	...	0.3	3.0
BC_{PS}	1:1	189:125	12.6**	3.6	42.1	173:123	8.1**	0.8	3.9
Set B									
P_R	1:0	138:2	...	1.1	28.6	131:7	...	0.1	3.5
P_S	0:1	2:136	...	0.9	65.8	2:136	...	2.0	3.4
F_1	1:0	126:36	?	1.7	48.0	102:61	?	0.1	4.2
F_2	3:1	205:103	11:3**	1.4	58.4	179:126	42.4**	0.2	4.5
F_3	3:1	229:82	0.3	1.0	56.1	213:91	3.7	0.1	4.4
BC_{PR}	1:0	285:29	?	0.9	48.3	264:46	?	0.1	4.0
BC_{PS}	1:1	166:160	0.1	2.6	49.3	144:180	3.8	0.2	4.5
Set C									
P_R	1:0	27:1	...	0.2	3.9	28:0	0.0	0.1	...
P_S	0:1	3:32	...	1.3	26.3	5:30	...	1.5	4.1
F_1	1:0	27:3	...	0.3	6.1	28:2	...	0.2	3.0
F_2	3:1	63:14	1.6	0.3	7.3	65:12	3.2	0.2	3.4
F_3	3:1	60:14	1.2	0.2	7.0	60:14	1.2	0.1	3.2
BC_{PR}	1:0	72:0	0.0	0.2	...	72:0	0.0	0.1	...
BC_{PS}	1:1	38:36	0.0	0.3	18.3	44:30	2.3	0.4	3.9

[a] Based on a 0 to 5 scale from experiment 1.
[b] Data were combined from three different environments for sets A and B. Data for set C originated from Xicotepec de Juárez.
[c] Ratios are resistant (R):susceptible (S). Expected ratios based on the assumption of a single dominant gene.
[d] When the expected frequency for one of the classes is zero, the chi-square test cannot be performed. ** Indicates significant at $P = 0.01$.

the three environments used in this experiment. Diallel analysis results supported the conclusions from the segregation analysis for set C. The mean lesion density of susceptible plants for set C tended to be higher in those generations where there were many susceptible plants (i.e., P_S and BC_{PS}) compared with those from generations with few susceptible plants (F_2 and F_3). This also was true for sets A and B at Xicotepec de Juárez, but because data for these sets have been pooled across locations, it cannot be clearly appreciated in Table 2.

Experiment 1: Joint-scaling test. The results for lesion density data from set A are presented individually for each of the three environments considered (Table 3). The analyses were performed individually to avoid pooling data with different generation means and variances. The two theoretical models for the Poza Rica data agreed well with the conclusions from the segregation analysis: the dominance/additive ratios $[h]/[d]$, close to one, suggest that a near complete dominance is exerted by the resistant parent (negative sign for h). Because neither chi-square value was significant, the observed variation in the mean of each generation can be satisfactorily explained by the three parameters of the models found for each planting date. Analysis of the original data from Xicotepec de Juárez yielded a significant chi-square value. Therefore, some of the transformations suggested by Mather and Jinks (16) were performed on the data in search of a model that would fit. Both the logarithmic and square-root transformations resulted in models that satisfactorily fit the experimental data (chi-square values not significant). In Table 3, only the results from the logarithmic transformation are presented. The joint-scaling test on set B was not done because of the serious disagreement between observed and expected segregations mentioned above. This disagreement suggested that one of the assumptions for this kind of analysis, homozygosity of both parents, may have been violated. The test was not done on data from set C because two replications at only one location were considered small for this kind of analysis.

Finally, during the preliminary studies, the proportion of *P. maydis* lesions surrounded by halos induced by *M. maydis* was found to be the same in resistant and susceptible plants (data not presented). In other words, the resistance evaluated in this experiment was only acting against *P. maydis* and had no direct effect on the development of *M. maydis*. However, because the presence of *P. maydis* is required for *M. maydis* to develop (19), any resistance to the former will have an indirect effect on the latter.

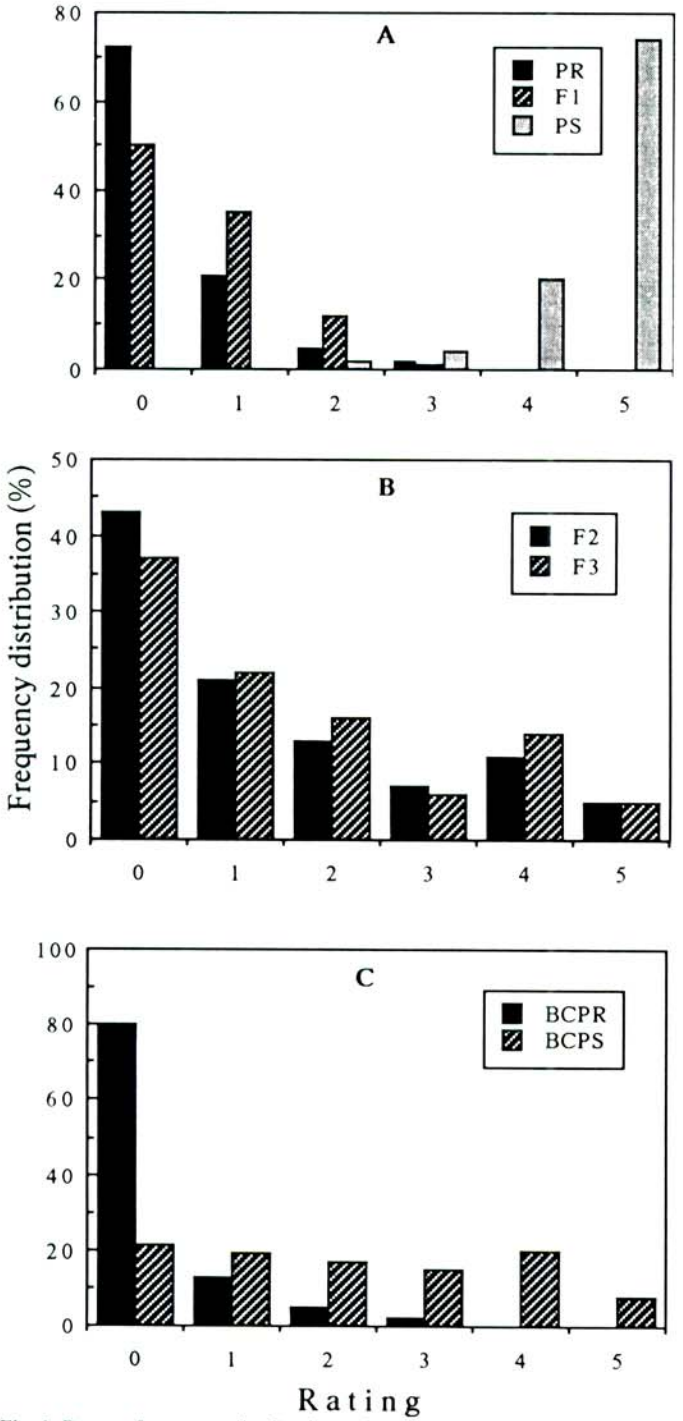

Fig. 1. Percent frequency distributions of ratings for the seven generations evaluated in set A of experiment 1. **A,** P_R, F_1, and P_S; **B,** F_2 and F_3; **C,** BC_{PR} and BC_{PS}. Classes 0, 1, and 2 were resistant, whereas classes 3, 4, and 5 were susceptible. Data from three environments were pooled.

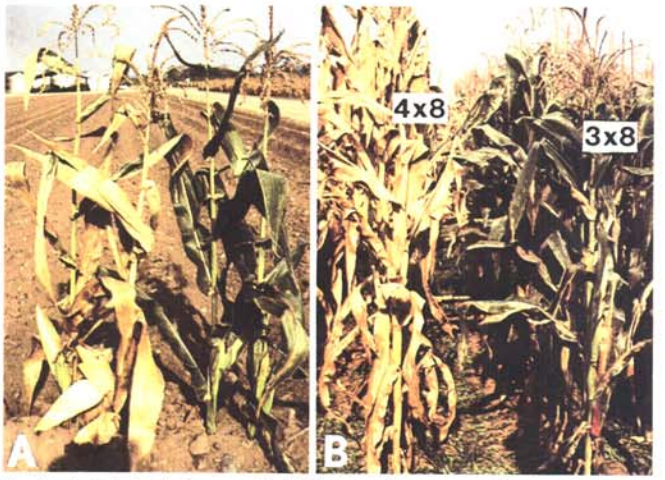

Fig. 2. A, Typical phenotypic difference between resistant (right) and susceptible (left) plants taken from the backcross to the susceptible parent of set B from experiment 1. **B,** Reaction to tar spot complex (TSC) of two F_1 crosses from the diallel study. Both crosses have in common the susceptible parent 8. Differential reaction to TSC is due to the second parent of each cross: the susceptible parent 4 for the F_1 on the left and the resistant parent 3 for the F_1 on the right.

TABLE 3. Genetic parameters and their standard errors from the joint-scaling test for lesion density of set A from experiment 1

Parameter[a]	Poza Rica		Xicotepec de Juárez[b]
	First planting	Second planting	
m	4.1 + 1.4	29.4 + 6.1	1.73 + 0.06
$[d]$	3.9 + 1.4	26.6 + 6.2	0.90 + 0.06
$[h]$	−3.6 + 1.5	−25.4 + 7.8	−0.72 + 0.12
$[h]/[d]$	0.93	0.96	0.80
Chi-square (4 df)	1.53 ($P = 0.82$)	1.86 ($P = 0.76$)	2.40 ($P = 0.66$)

[a] m = Midparent point, $[d]$ = additive effects, and $[h]$ = dominance effects.

[b] Analysis performed on the logarithmic transformation of the original data.

Experiment 2: Diallel analysis. The ANOVA results for ratings and number of lesions are presented in Table 4. The analysis indicated significant ($P < 0.01$) environmental effects. Because the estimation of components of variance is not appropriate for Model I (5), only the general and specific combining ability effects were estimated. GCA and SCA effects, as well as their interaction with the environments, were highly significant for both variables. The relative magnitudes of GCA and SCA mean squares suggest a greater importance for additive gene effects in resistance to *P. maydis*. Because genotype-by-environment interaction did not result in many ranking crossovers, further analyses were performed on the pooled data across locations.

The mean rating and mean number of *P. maydis* lesions for the eight parental lines and their 28 F_1 crosses are presented in Table 5. Values for GCA and SCA also are summarized in this table. The highly negative GCA values obtained for the first three parents for both variables indicate a high degree of resistance, particularly in parents 2 and 3. There was substantial variation in the GCA values for the remaining parents, although all were positive. The last two parents (thought to be intermediate) did show an intermediate performance when their GCA values were compared with those from susceptible parents, such as 4 and 5. Parent 6, originally classified as susceptible, showed the lowest GCA among the nonresistant parents, including those originally classified as intermediate.

TABLE 4. Analyses of variance for combining ability for ratings (three environments) and number of *Phyllachora maydis* lesions per leaf (two environments)

	Variable[a]			
	Rating		Number of lesions	
Source	df	MS	df	MS
Environments	2	35.82**[b]	1	2,120,832**
Replications (environments)	5	0.05	4	6,284
Crosses	27	24.19**	35	829,486**
GCA[c]	7	10.24**	7	3,365,806**
SCA[c]	20	0.53**	28	195,406**
Environments × crosses	54	0.99**	35	100,921**
Error	135	0.07	122	5,767

[a] Ratings were taken from the F_1 crosses only. Number of lesions per leaf were estimated on the F_1s and the eight parental lines. df = Degrees of freedom, MS = mean squares.
[b] **, Significant at $P = 0.01$.
[c] GCA = General combining ability, SCA = specific combining ability.

Significant SCA effects also were found. The only negative SCA values observed were between the crosses of resistant parents (1, 2, or 3) and susceptible ones (4 to 8), indicating that strong dominant effects for resistance were present in these parents. A low rating or number of lesions per leaf was observed not only in parents 1, 2, and 3 per se, but also in their respective progenies, regardless of the genotype of the second parent. The dominance and degree of resistance conferred by resistant parents is illustrated by the photograph in Figure 2B (taken about 6 wk after flowering); any cross involving at least one resistant parent showed a high resistance to *P. maydis* (and therefore to *M. maydis*). The differences observed in this photograph were not due to differences in the maturities of the materials evaluated because they flower at approximately the same time.

The standardized deviation graph (*Wr* + *Vr* on *Yr*) for the variable number of lesions from the two planting dates at Poza Rica is presented in Figure 3. The test for the validity of the assumptions made for this analysis was not significant ($t = 0.41$, $P > 0.50$). Also, the regression of *Wr* on *Vr* did not yield a coefficient significantly different from one ($b = 0.927 \pm 0.07$), indicating that there was no apparent violation of the assumptions made. The separation between resistant/dominant types and susceptible/recessive ones is apparent in this figure. Resistant parents 1, 2, and 3 are located in the lower left quadrant, whereas the nonresistant genotypes are in the upper right one. Parent 6 shows its intermediate level of resistance by appearing close to the origin of this plot. The conclusions derived from the graphic analysis agree with those arising from the diallel analyses for both variables presented in Table 5.

The strong dominance of the resistant parents, apparently due to the presence of a major gene (as suggested by the segregation analysis in Experiment 1), probably had a masking effect on other important information related to the genetics of resistance to *P. maydis*. A partial analysis of the five nonresistant parents therefore was performed. The standardized graph is shown in Figure 4. Parents 6, 7, and 8 are located at the left of the *Wr*/*Vr* axis, which means higher resistance (obviously only relative to the genotypes included in the analysis), whereas parents 4 and 5 are on the right, indicating their higher susceptibility. The vertical positioning of each point is indicative of the dominance present in the respective parental line. Parent 6 showed the highest degree of dominance, parent 8 was intermediate, and parents 4, 5, and 7 were recessive. It is interesting to note the recessive resistance apparently present in parent 7. This conclusion also is supported by data from Table 5. As a line per se, parent 7 had the lowest

TABLE 5. Diallel analysis of ratings[a] and number of lesions of *Phyllachora maydis* for eight parental lines and their 28 F_1 crosses

Parent	1	2	3	4	5	6	7	8	GCA[b]
1	1.3 (24)[c]	0.1 (6)	0.0 (0)	2.5 (236)	2.8 (255)	1.1 (33)	1.5 (73)	1.7 (59)	−0.59 (−234)
2	0.6 (194)	0.6 (8)	0.0 (1)	0.6 (53)	0.8 (18)	0.5 (8)	0.4 (12)	0.3 (8)	−1.75 (−293)
3	0.8 (197)	1.9 (256)	0.1 (3)	0.2 (1)	0.1 (1)	0.3 (22)	0.1 (1)	0.0 (1)	−2.07 (−301)
4	0.1 (−121)	−0.7 (−245)	−0.8 (−289)	3.6 (1,038)	4.4 (106)	4.0 (682)	4.0 (889)	4.2 (764)	1.10 (253)
5	0.3 (−96)	−0.5 (−275)	−0.9 (−283)	0.2 (218)	3.7 (1,083)	4.0 (663)	4.1 (872)	4.0 (861)	1.19 (247)
6	−0.9 (−145)	−0.3 (−1,126)	−0.1 (−88)	0.3 (17)	0.3 (4)	3.3 (701)	3.8 (604)	3.5 (543)	0.67 (74)
7	−0.5 (−162)	−0.5 (−165)	−0.5 (−167)	0.3 (167)	0.3 (156)	0.5 (61)	3.1 (646)	3.8 (713)	0.74 (131)
8	−0.4 (−168)	−0.6 (−160)	−0.6 (−159)	0.5 (50)	0.3 (153)	0.2 (8)	0.4 (121)	3.2 (728)	0.71 (123)

[a] Ratings were based on a 0 (healthy) to 5 (dead) scale on eight parental lines (diagonal) and their 28 F_1 crosses (above diagonal).
[b] GCA (*gi*) and SCA (*sij*) effects are presented in the right column and below the diagonal, respectively.
[c] Figures within parentheses are the same parameters for the number of lesions. SE (*gi*) = 0.036, SE (*sij*) = 0.08, SE (*gi* − *gj*) = 0.134, SE (*sij* − *sik*) = 0.122, and SE (*sij* − *skl*) = 0.110 for variable rating. SE (*gi*) = 21.03, and SE (*sij*) = 64.5 for variable number of lesions.

rating and mean lesion number of all the parents found to be resistant in the partial analysis (parents 6, 7, and 8). Its progeny, however, had the highest mean lesion number among the same lines.

The estimation of other genetic parameters for the complete and partial set of parents is presented in Table 6. All of these parameters were highly significant for the complete set. *D* and *H1* are the estimates of additive and dominance effects, respectively, and $\sqrt{(H1/D)}$ estimates the mean degree of dominance. For the complete set, this latter figure was 0.85, but in the partial set it was reduced (as expected) to 0.56. This reduction arises from the exclusion of the three resistant (highly dominant) parents. Similarly, *h2* values (dominance effects as an algebraic sum over all loci in heterozygous phase in all crosses) became nonsignificant when the resistant parents were not considered in the analysis. Narrow sense heritability estimates were very high and ranged from 0.94 in the full set to 0.83 in the partial set. The negative sign for *F* suggests that dominant alleles are less frequent than recessive ones, irrespective of whether or not dominant alleles have increasing or decreasing effects (22). Finally, the correlation between (*Wr* + *Vr*) and mean *Yr* was 0.98 in the full set. The sign and magnitude of this correlation coefficient is additional evidence that resistance is dominant (i.e., genes increasing the number of lesions are recessive). The exclusion of the resistant parents in the partial set significantly reduced the magnitude of the correlation coefficient to 0.55.

DISCUSSION

The results obtained clearly confirm a high degree of resistance against *P. maydis* in the resistant parents evaluated. The genetic control of the resistance in sets A and C of experiment 1 appears to be highly dominant and simple. High resistance was assumed because of the resistant phenotype of F_1 generations and because of the near unity of the [*h*]/[*d*] ratios from the joint-scaling tests of set A. The segregation analysis suggested the presence of a single dominant gene, and the simplest possible quantitative model of set A adequately explained most of the variation observed.

The resistance in set B was close to immunity and apparently dominant, but its inheritance was not definitively determined. Lack of homozygosity of the resistant parent can explain some of the disagreements between the expected and observed results of the segregation analysis in set B (too many susceptible plants in the F_1, F_2, and BC_{PR} generations). Lack of homozygosity is likely to occur at many loci, including the locus for resistance, in S_2 lines. The good fits observed in the F_3 from set B can be explained by the way this generation was obtained. F_3s were produced by sibbing F_2s planted during the 1987–1988 winter as part of the ongoing improvement of the population. At that time, some selection was exerted on the full-sib families for resistance to TSC. Therefore, the F_2 families from which the F_3 generations were obtained were probably not random samples but, somehow, biased in favor of resistance. It is possible that all F_1 plants used to obtain the BC_{PS} were, by chance, carrying the resistance gene. This can explain why the segregations of the BC_{PS} generation did not have a significant chi-square value.

It is not clear why so many resistant plants were observed in the BC_{PS} of set A. The results suggest that either F_1 plants were homozygous for the resistance gene (only possible if some plants from the susceptible parent carried it), or that some contamination occurred during the pollination or seed preparation of BC_{PS}. In spite of this disagreement (it should be stressed that the disagreement in the BC_{PS} was because of the presence of too many resistant plants), the segregations obtained strongly supported the hypothesis of a single dominant gene controlling the resistance present in set A. This hypothesis also is adequate for data from set C, which did not show any serious disagreement

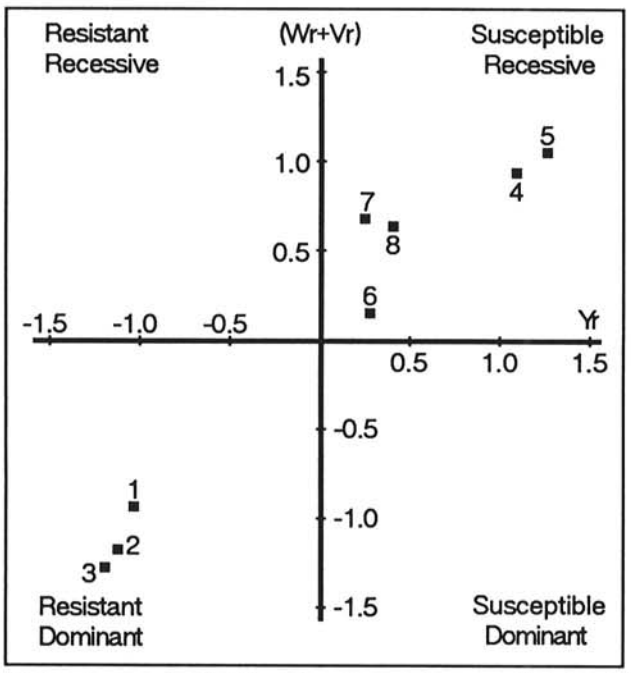

Fig. 3. Graph of standardized deviations for an eight-parent diallel analysis of the number of *Phyllachora maydis* lesions with eight parents.

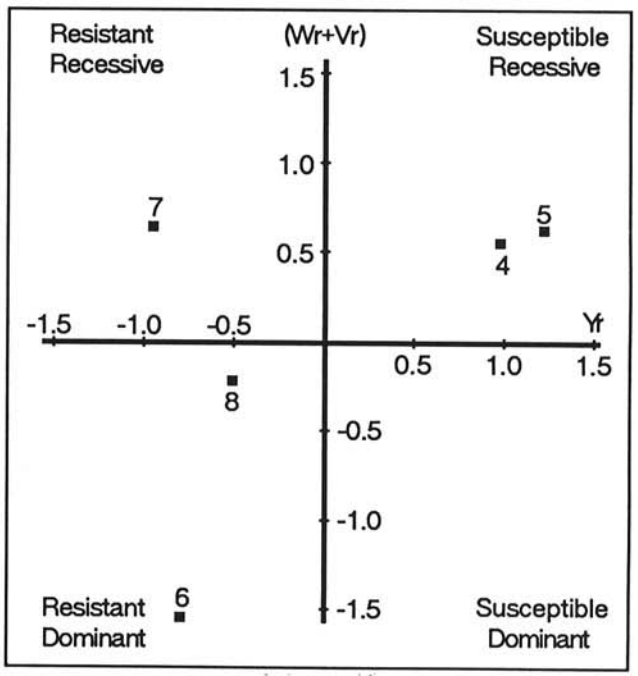

Fig. 4. Graph of standardized deviations for diallel analysis of the number of *Phyllachora maydis* lesions for the partial set with five parents.

TABLE 6. Genetic components of variation for resistance to *Phyllachora maydis* of an eight-parent diallel set and a partial set that included only the five nonresistant parents

Parameter[a]	Complete set	Partial set
D	164,897 ± 4,206	31,942 ± 1,838
F	−72,296 ± 9,982	−11,800 ± 4,362
H1	120,097 ± 9,711	9,862 ± 4,243
H2	113,437 ± 8,449	4,864 ± 3,692
h2	94,005 ± 5,666	−3,043 ± 2,476
E	7,676 ± 1,408	4,793 ± 615

[a] *D* = Component of variation due to additive gene effects, *F* = mean of covariance of additive and dominance effects over the arrays, *H1* = component of variation due to dominant gene effects, *H2* = *H1* [1 − (*u* − *v*)2] where *u* and *v* are the proportion of positive and negative genes in the parents and where *u* + *v* = 1, *h2* = dominance effects (as an algebraic sum over all loci in heterozygous phase in all crosses), and *E* = component of variation due to environmental effects.

between the expected and observed segregations, and was further confirmed by the diallel results (parent 3). The bimodal shape of the frequency distribution of ratings in F_2, F_3, and BC_{PS} of set A (Fig. 1B and C) and the differences shown in Figure 2A suggested a categorical separation among the plants evaluated, and it was another indication of the presence of a major gene controlling resistance to this pathogen.

The joint-scaling test on data from set A in experiment 1 complements the information derived from the segregation analyses. It was presented to show the agreement between two dissimilar ways of analyzing the data and to provide some evidence of the accuracy that a quantitative model can achieve. The analysis agrees with the hypothesis of a single dominant gene controlling the resistance of P_R from set A. This kind of analysis does not require or depend on any arbitrary separation between resistant and susceptible phenotypes. Data from Xicotepec de Juárez had to be transformed for the model to fit the experimental data. The logarithmic and square-root transformations yielded models with nonsignificant chi-square values. This would suggest a multiplicative gene action, rather than strictly additive effects, suggesting that more than one gene may be involved in the genetic control of resistance to TSC. It must be pointed out, however, that data from this location were not as reliable as those from the two plantings at Poza Rica.

The apparent differences in the degree of resistance between the resistant parent of set A and those from sets B and C (also supported by the diallel analysis) indicate that either these are different sources of resistance located at different loci or that significant effects of their genetic backgrounds are affecting the final expression of the resistance controlled by the same major gene.

At Xicotepec de Juárez, susceptible plants from generations with many such phenotypes (i.e., P_S and BC_{PS}) had a higher mean density of lesions per leaf and ratings than those from generations with fewer numbers of susceptible plants (i.e., F_2 and F_3). It is feasible that these differences were due to a higher inoculum buildup in those generations with a large number of susceptible plants. The inoculum effect could be observed under mild disease severity, such as that of Xicotepec de Juárez, but may be undetectable under more severe conditions such as those from Poza Rica.

The diallel analysis also provided evidence of dominant (or nonadditive) gene effects: significant SCA mean squares ($P<0.01$, Table 4), negative SCA estimates for crosses of a resistant parent with a susceptible line (Table 5), large estimates for *H1* and *h2* in Table 6, and the correlation coefficient between ($Wr + Vr$) and mean of *Yr* close to one. Furthermore, there was a clear indication that dominance was nearly complete: $\sqrt{(H1/D)}=0.85$. It can be deduced that most of this dominance is exerted by the resistant parents because their exclusion from the analysis reduced the dominance effects to nonsignificance (Table 6). The high levels and dominance of the resistance observed in parent 3 (based on data from three different environments) further confirmed the results from set C in experiment 1 (based only on data from Xicotepec de Juárez). Parent 1 showed an intermediate level of resistance in the diallel analysis, agreeing with the results for the same line from experiment 1 (set A).

The diallel study provided evidence of the existence of significant additive effects as well: significant GCA mean squares ($P < 0.01$, Table 4) and large *D* values (Table 6). In fact, the relative magnitude of GCA and SCA mean squares suggested that additive effects were more important than nonadditive effects. The resistance based on additive gene action is not only a useful source of resistance per se, but it also can be a backup system for the major gene(s) should new pathogenic races arise (1). This kind of resistance can be effectively handled through recurrent selection methods. The presence of populations with resistance to TSC among CIMMYT's materials is empirical evidence that recurrent selection methods can, in fact, improve levels of resistance to this disease.

It has to be recognized that inferences from the results of the diallel study are restricted to the population represented by the eight parental lines evaluated (Griffing's Model 1). Even if the assumed model were Model 2, the number of parents used (less than 10) would make inferences (either statistical or genetic) about population parameters based on the components of variance found inappropriate (8). However, the population from which the lines for this study were selected had many other families with resistant, susceptible, or intermediate reaction to TSC similar to those observed in the lines tested (data not presented). The three resistant parents are a random sample of the many resistant lines observed in the original population, as are the susceptible and intermediate parents. Therefore, the parents used are considered as representative of the different types of reaction to *P. maydis* found in the original population. The fact that different types of resistance (dominant and additive) were found among the lines evaluated suggests that these types of resistance are likely to be found in other maize materials as well.

There was no evidence that the assumptions made for the Hayman's diallel analysis have been violated. It is likely, however, that at least one of these assumptions had not been fulfilled—homozygosity of parental lines. It is unlikely that all loci related to resistance to *P. maydis* were homozygous, because the parental lines were only at the S_2 level. The effect of this violation (overestimation of additive effects) was not large enough to be detected by the tests used to check the validity of assumptions.

The results from lesion density analyses agreed with those based on a 0 to 5 scale, although chi-square values in the segregation analysis were generally higher for the latter. Lesion density evaluations have the advantage of higher precision than visual ratings, but they had to be obtained relatively early in the season, when differences between resistant and susceptible phenotypes were not maximized. Ratings, in contrast, could be taken late in the season, when phenotypic differences between resistant and susceptible plants were at their maximum. Nevertheless, ratings had the disadvantages of lower precision and the potentially confounding effect of other diseases (*Bipolaris maydis* (Nisik.) Shoemaker and *E. turcicum*). Taking into consideration the amount of time involved in estimating lesion densities, the use of a rating scale was found to be more efficient and is, therefore, advisable for further studies on the disease.

TSC starts with *P. maydis* but can be more devastating in the presence of a second pathogen (*M. maydis*) because of a synergistic effect. Because *P. maydis* is apparently required for the development of *M. maydis* (11,12,19), any source of resistance against the former should also be effective against the latter. It is not known if there are specific sources of resistance against this secondary pathogen. Plants severely affected by *P. maydis* but still relatively free of infection by *M. maydis* have been observed in Mexico and Ecuador (H. Ceballos, *personal observation*). This would suggest that there is some specific resistance against the latter pathogen and/or that environmental requirements of the two pathogens are different. The area most commonly affected by TSC corresponds more with subtropical or midaltitude environments than with truly tropical ones. It is necessary to introduce sources of resistance against *P. maydis* into subtropical populations because these are the materials most likely to face the devastating effects of this disease.

The monogenic resistance to *P. maydis* found has some advantages: it can be easily transferred from one source to another through backcrossing, and because its expression in several cases was close to immunity, its recognition in the field is almost immediate. However, this type of resistance is frequently overcome by new biotypes of the pathogen with different pathogenicity. Ideally both monogenic and polygenic resistance should be combined to provide durable and effective protection against *P. maydis* and TSC.

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