Effectiveness of a Disease Index System in Evaluating Corn for Resistance to Maize Dwarf Mosaic Virus

C. W. Kuhn and T. H. Smith

Department of Plant Pathology and Plant Genetics, University of Georgia, Athens, GA 30602.
The research was supported in part by CSRS-ARS Research Agreement No. 316-15-50.
Accepted for publication 21 September 1976.

ABSTRACT

KUHN, C. W., and T. H. SMITH. 1977. Effectiveness of a disease index system in evaluating corn for resistance to maize dwarf mosaic virus. Phytopathology 67: 288-291.

A disease index system was developed under greenhouse conditions to evaluate corn for resistance to maize dwarf mosaic virus (MDMV). The maize dwarf mosaic index was based on the percentage of plants that develop symptoms and time of symptom appearance after inoculation. When 550 corn lines were evaluated, three distinct disease categories were observed: resistant, intermediate, and susceptible. Index values were reproducible and significantly different between the categories. More important, the index values were related to incidence of disease and to yield of diseased plants in the field. With resistant corn lines, fewer plants became infected naturally, and yield of individually diseased plants was reduced less than with intermediate or susceptible lines. The relative MDMV infectivity levels in corn hybrids also were related to disease index values. Maize dwarf mosaic virus was detected earliest in susceptible hybrids and was present in higher concentrations than in more resistant hybrids.

Additional key words: assay for MDMV infectivity.

Resistance in corn (*Zea mays* L.) to maize dwarf mosaic virus (MDMV) is difficult to evaluate. In 7 yr of testing in Georgia, no immunity to MDMV has been found. It has been apparent, however, that some commercial hybrids and inbred lines are more resistant than others because of their ability to produce greater yields under severe maize dwarf mosaic epidemic conditions. Unfortunately, field evaluations are less than desirable because of the presence of maize chlorotic dwarf virus (MCDV) in nearly all fields with maize dwarf mosaic. Corn plants frequently become infected with both viruses, and the maize chlorotic dwarf symptoms tend to obscure maize dwarf mosaic symptoms, particularly after the midpoint of the growing season. Preliminary results (1) with an index evaluating system in a greenhouse test were promising. Therefore, tests were designed in this study to relate the index system to maize dwarf mosaic incidence in the field, yield losses in the field, and the relative concentration of MDMV in diseased plants.

MATERIALS AND METHODS

Virus.—The virus used in this study was a johnsongrass isolate of MDMV. It probably should be identified as strain A, but no efforts were made to further refine the identification as was done by Louie and Knoke (3). Several field isolates of MDMV were obtained during the course of the study (7 yr), and the reaction of corn hybrids was similar regardless of the isolate.

Index system.—An MDMV index system was introduced previously (1) and further refined in this study. In a standard test, five plants of each corn line were grown in each of five 10-cm diameter pots (25 plants total) containing a clay-loam: sand: vermiculite mixture (2:1:1, v/v). Test plants were mechanically inoculated with sap from maize dwarf mosaic-diseased plants 8-10 days after planting and were fertilized 14 days later. Plants with symptoms were counted and removed at 6, 11, 16, and 28 days after inoculation. Index values were determined as follows: maize dwarf mosaic disease index = $4W + 3X + 2Y + Z$ where W, X, Y, and Z equal total percentage of plants with symptoms by 6, 11, 16, and 28 days, respectively. For example, if five plants of the total of 25 had symptoms and were removed at each observation, then the index = $(4 \times 20\%) + (3 \times 40\%) + (2 \times 60\%) + (1 \times 80\%) = 400$.

Field studies.—Effect of natural infection of maize dwarf mosaic was studied in a field with johnsongrass [*Sorgum halepense* (L.) Pers.] and a 5-yr history of having both maize dwarf mosaic and maize chlorotic dwarf. The planting, cultural procedures, insecticide application, and identification of the two causal agents were described previously (2). Incidence of maize dwarf mosaic was determined by observing approximately 19% of 3,500 plants in each of four replications in 1974 and 17% of 1,500 plants in each of five replications in 1975.

In general, yield studies with virus diseases are difficult to conduct because of the difficulty in preventing control plants from becoming infected. To overcome this problem, individual maize dwarf mosaic-diseased plants in the field described above were tagged 75 days after planting. In a previous study in the same field (2), it was established that most corn became infected within 75 days; very few infections occurred after that time. Ears from the tagged, maize dwarf mosaic-diseased plants

were compared with ears from adjacent, healthy plants.

Effect of artificial inoculation with maize dwarf mosaic was studied in a field with no johnsongrass and no history of maize dwarf mosaic or maize chlorotic dwarf. Approximately 44 plants each of 10 hybrids (four replications of 11 plants each) were mechanically inoculated twice, once at 2 wk and again at 3 wk after planting. Observations for diseased plants were made throughout the growing season, but no additional plants showed symptoms later than 30 days after inoculation.

Virus assay method.—Sweet corn, *Zea mays* var. *saccharata* (Sturtev.) Bailey 'Golden Cross Bantam' was used to test for the presence of MDMV and to assay systemically for relative infectivity. For assays, sap from corn lines was serially diluted in twofold steps from 1/5 to 1/5,120. Ten to 16 sweet corn plants were inoculated with each dilution, and counts of diseased plants were made 14 days later. Estimation of differences between treatments was based on dilution end point and the dilution at which approximately 50% of the plants became infected.

RESULTS

Disease index system.—Maize dwarf mosaic disease index values have been determined for approximately 500 commercial corn hybrids and 50 corn inbred lines during the last 7 yr. In general, three disease categories were identified (Fig. 1). In the susceptible category, maize dwarf mosaic symptoms developed on 90-100% of the plants by 6 days after inoculation, and nearly all were diseased by 11 days. In the resistant category, few or no diseased plants were observed by 11 days, and the final percentage of plants with symptoms ranged from 10-40%. When plants in the resistant category were maintained and observed beyond 28 days, less than 10% developed symptoms, and MDMV could not be isolated from symptomless plants. Although susceptible and resistant categories were relatively easy to identify, the intermediate category was variable and probably continuous between the two extremes. In general, 5-20% of the plants had symptoms at 6 days and additional plants were diseased at each of the later reading dates.

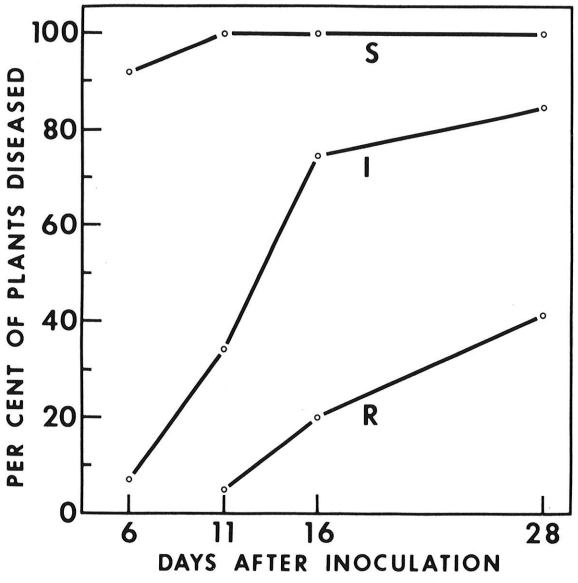

Fig. 1. Symptom development in commercial corn hybrids resistant (R), intermediate in reaction (I), and susceptible (S) to maize dwarf mosaic virus. These curves represent the means of 550 corn lines.

Evaluation of specific corn lines.—Field observations of the percent maize dwarf mosaic-diseased plants and yields from commercial hybrids indicated that the maize dwarf mosaic disease index values are related to resistance and susceptibility as follows: (i) resistant, 0-350; (ii) intermediate, 351-650; and (iii) susceptible, 651-1,000. Precise index values must be used with caution, particularly where categories may overlap. Ten commercial hybrids and one sweet corn line (used as a susceptible control) were evaluated in a series of four tests. The range of index values for each corn line was relatively small (Table 1); only two of 44 index values deviated from the category values, and the deviations were only 11 and 14 units.

The reliability and reproducibility of the disease index

TABLE 1. Relative maize dwarf mosaic index values for eleven corn lines

Corn line	Disease index value[a]		Disease classification
	Range	Average	
Funk's G-4808	15-91	52 A[b]	Highly resistant
Pioneer 3147	131-312	229 B	Resistant
P-A-G 644 W	157-361	250 B	Resistant
Pioneer 3145	218-364	289 B	Resistant
Funk's G-5757	417-552	499 C	Intermediate
Pioneer 3009	419-634	529 C	Intermediate
DeKalb 1214	712-870	791 D	Susceptible
Pioneer 511A	762-964	882 DE	Susceptible
Coker 77	785-963	885 DE	Susceptible
Funk's G-795W-1	723-1,000	895 DE	Susceptible
Golden Cross Bantam	1,000-1,000	1,000 E	Susceptible

[a]Maize dwarf mosaic virus indexes were determined from the equation: Index = 4W + 3X + 2Y + Z in which W, X, Y, and Z equal the total percentage of plants with symptoms by 6, 11, 16, and 28 days, respectively, after inoculation. The index values were determined in four tests in the greenhouse with all 11 hybrids in each test; each test represented a replication.

[b]Treatment means followed by different capital letters in the same column are significantly different, $P = 0.05$, according to Duncan's multiple range test.

TABLE 2. Incidence of maize dwarf mosaic virus in commercial corn hybrids under field conditions

Brand name	Hybrid number	Natural infection		Artificial inoculation (%)
		1974 (%)	1975 (%)	
Pioneer	3147	. .	2.6 A	2.3 A
Pioneer	3009	3.6 A[a]	2.4 A	22.5 B
DeKalb	1214	9.4 B	14.6 B	68.5 C

[a]Treatment means followed by different capital letters in the same column are significantly different, $P = 0.05$, according to Duncan's multiple range test.

values were substantiated by statistical analysis (Table 1). Funk's G-4808 was in a category by itself; its very high degree of resistance has not been observed in any other commercial hybrid and in only one inbred line, Tx601, in Georgia tests.

Each index evaluation of corn lines was made with a single inoculation and with Golden Cross Bantam and Funk's G-4808 as controls; the test was considered valid if the index values of the controls were as expected. In one study, additional inoculations were made at 6, 24, and 30 hr after the initial one. This caused the index values to be increased by an average of 135 units: Funk's G-4808, 52-182; Pioneer 3147, 229-360; Pioneer 3145, 289-362; Funk's G-5757, 499-677; and Pioneer 3009, 529-695.

Based on the maize dwarf mosaic index values, 15, 54, and 31% of 87 commercial hybrids were rated resistant, intermediate, and suceptible, respectively, in 1975. For commercial hybrids submitted for testing by ten corn seed companies, the number of resistant hybrids has increased about 50% and the number of susceptible ones has decreased about 40% during the last 5 yr. This suggests that seed companies have produced hybrids with resistance to MDMV.

TABLE 3. Yield comparisons of healthy and maize dwarf mosaic-diseased plants of three corn hybrids

Brand name and hybrid no.	Index value[a]	Yield per plant (g)		Yield loss (%)
		Healthy	Diseased	
Pioneer 3147	229	194.8	143.7[b]	26
Pioneer 3009	529	228.9	126.1	45
DeKalb 1214	791	172.4	78.5	54

[a]Maize dwarf mosaic virus indexes were determined from the equation: Index = 4W + 3X + 2Y + Z in which W, X, Y, and Z equal the total percentage of plants with symptoms by 6, 11, 16, and 28 days, respectively, after inoculation.

[b]The yield of diseased plants of each hybrid grown under field conditions was significantly decreased, $P = 0.01$.

Field tests.—The incidence of maize dwarf mosaic in three corn hybrids with different degrees of susceptibility was tested under field conditions. When exposed to a mild natural infection, the most susceptible hybrid, DeKalb 1214 (index value = 791), had significantly higher levels of maize dwarf mosaic than the more resistant ones, Pioneer 3147 (index value = 229) and Pioneer 3009 (index value = 529) (Table 2). Under this relatively low level of natural infection, no difference in incidence was detected between Pioneer 3147 and Pioneer 3009. In another field study in which the three hybrids were mechanically inoculated, however, distinct and significant differences were observed (Table 2). It was much more difficult to get infection by MDMV in the field than in the greenhouse. The inefficiency of a field inoculation had been noted in other years, but in this test, the degree of inefficiency was strongly related to the degree of resistance based on maize dwarf mosaic index values. When the ten hybrids shown in Table 1 were compared, 3% (5 of 176) of the plants of hybrids rated resistant, 22% (20 of 88) of those rated intermediate, and 56% (98 of 176) of those rated susceptible became diseased with maize dwarf mosaic.

The yield of each of the three test hybrids was significantly reduced by MDMV; but, more important, the percent yield loss was directly related to the maize dwarf mosaic index values (Table 3).

Infectivity related to disease index values.—A time-course study was conducted to determine the relative MDMV infectivity in three hybrids with different degrees of susceptibility. Infectious MDMV could be isolated from the most susceptible hybrid DeKalb 1214 at 2 days after inoculation, but not from the two more-resistant hybrids (Table 4). Infectious virus first was found in Pioneer 3147 and Pioneer 3009 at 4 days after inoculation. At all harvest times, more MDMV was found in DeKalb 1214 than in the other hybrids. During the peak infectivity period (6 and 10 days), DeKalb 1214 had approximately twice as much infective virus as Pioneer 3009 and two to 16 times as much as Pioneer 3147 (Table 4). This estimation of differences in infectivity

TABLE 4. Relative maize dwarf mosaic virus infectivity in three corn hybrids from 2 to 10 days after inoculation

Brand name and hybrid no.	Days after inoculation	Plants infected[a]	Dilution end point[b]	Dilution with approximately 50% infectivity
Pioneer 3147	2	0/165	0	. . .
Pioneer 3009	2	0/165	0	. . .
DeKalb 1214	2	2/165	1/40	. . .
Pioneer 3147	4	2/176	1/5	. . .
Pioneer 3009	4	2/176	1/5	. . .
DeKalb 1214	4	27/176	1/320	1/10
Pioneer 3147	6	36/154	1/80	1/40
Pioneer 3009	6	90/154	1/640	1/320
DeKalb 1214	6	102/154	1/1,280	1/640
Pioneer 3147	10	40/110	1/160	1/80
Pioneer 3009	10	46/110	1/640	1/160
DeKalb 1214	10	57/110	1/1,280	1/160

[a]A series of 11 twofold dilutions were made at each assay date (1/5, 1/10,—1/5,120). The number of plants at assay dates of 2, 4, 6, and 10 days after inoculation were 15, 16, 14, and 10, respectively.

[b]Last dilution at which at least one maize dwarf mosaic-diseased plant was observed.

levels is based on dilution end point and the dilution which causes approximately 50% infectivity. Pioneer 3009 had approximately two to eight times more infective virus than Pioneer 3147 at the 6- and 10-day assay dates.

In the assays described above (Table 4), test plants of the three hybrids were selected at random without regard to symptomatology. When only plants with symptoms were selected for assay at 17 and 25 days after inoculation, the infectivity level of MDMV was similar for DeKalb 1214, Pioneer 3009, and Pioneer 3147.

DISCUSSION

An evaluation system utilizing a disease index proved to be reliable in measuring varying degrees of resistance to MDMV in corn hybrids. The system is simple and can be conducted under controlled conditions (no interference from other diseases) within 4-5 wk. The standard procedure was to evaluate for diseased plants at 6, 11, 16, and 28 days after inoculation. The timing could be varied as follows without substantially altering the index values: 6-7 days, 10-12 days, 15-17 days, and 21-28 days. Although some resistant hybrids can be detected by the failure of some plants to become infected after mechanical inoculation, the index values provide more precise information which can be used to distinguish degrees of resistance.

The basic property of the virus-host relationship which allows the evaluation system to work is the length of the incubation period (time from inoculation to symptom appearance). The incubation period was short (6-10 days) for susceptible hybrids and relatively long (16-28 days) for resistant ones. The relationship between incubation period and resistance to MDMV was noted previously by Scott et al. (7) and Thompson and Hebert (9) when they tested inbred corn lines.

From an economic standpoint, yield is a better indicator of resistance than index values or the total number of plants which become infected following mechanical inoculation. The MDMV index values were directly related to the loss in yield caused by MDMV (Table 3). Furthermore, field observations over a period of 7 yr support the relationship between the index values and yield. For example, Pioneer 511A is tolerant to maize chlorotic dwarf (1) and susceptible to maize dwarf mosaic (index value = 876), and yield was severely reduced in fields with a high incidence of maize dwarf mosaic (C. W. Kuhn, *unpublished*). In this study, the yield losses were higher than anticipated, particularly for the resistant hybrid (Pioneer 3147). Three major symptoms were observed on the three test hybrids: (i) mosaic; (ii) slightly shortened plants; and (iii) smaller ears with missing kernels. The latter two symptoms were progressively more severe from Pioneer 3147 to Pioneer 3009 to DeKalb 1214. Previously, Scott and Nelson (6) demonstrated a 40% loss for susceptible corn inbred lines, and Pitre (5) found that maize dwarf mosaic caused a 23% yield loss for Pioneer 309B, a hybrid which was rated intermediate in resistance by the index system (1). A comparison of yield losses from one location to another is complicated by the existence of several isolates or strains of MDMV (3).

Resistance in corn to MDMV involves at least three factors. First, it is difficult to establish infection in resistant hybrids. About 50% of the plants do not become infected by mechanical inoculation. This probably reflects the chances of escape rather than variations in resistance in the population. This phenomenon was observed both when plants were inoculated artifically by mechanical means and naturally by aphids (4); it may be related to the type of resistance to curly top virus noted in tomatoes (8). Second, the length of the incubation period is longer in resistant hybrids than in susceptible ones. This type of resistance probably is more important in relation to the virus effect on plant metabolism and growth than to the availability and quantity of inoculum, since MDMV does not seem to be transmitted readily from corn to corn in the field (C. W. Kuhn *unpublished*). Third, the pattern of synthesis of MDMV varied with degree of resistance. The amount of infectious MDMV was related to the disease index; less virus was produced in hybrids with lower index values. The differences among hybrids were striking when total populations of plants were considered during the first 10 days after inoculation (Table 4). At later times of 17 and 25 days, the diseased plants of both resistant and susceptible hybrids had similar quantities of infectious virus. However, the data are difficult to interpret because the overall level of infectious virus in the susceptible hybrid had declined 2- to 3- fold by that time, and it was probably at a peak level in the resistant hybrid.

LITERATURE CITED

1. KUHN, C. W., and M. D. JELLUM. 1970. Evaluations for resistance to corn stunt and maize dwarf mosaic diseases in corn. Ga. Agric. Exp. Stn. Res. Bull. 82. 37 p.
2. KUHN, C. W., M. D. JELLUM, and J. N. ALL. 1975. Effect of carbofuran treatment on corn yield, maize chlorotic dwarf and maize dwarf mosaic virus diseases, and leafhopper populations. Phytopathology 65:1017-1020.
3. LOUIE, R., and J. K. KNOKE. 1975. Strains of maize dwarf mosaic virus. Plant Dis. Rep. 59:518-522.
4. MESSIEHA, M. 1967. Aphid transmission of maize dwarf mosaic virus. Phytopathology 57:956-959.
5. PITRE, H. N. 1967. Reaction of a dent corn hybrid to the Mississippi isolate of corn stunt virus and to a mechanically transmissible mosaic virus isolate found on johnsongrass: notes on disease epidemiology. Plant Dis. Rep. 51:1044-1048.
6. SCOTT, G. E., and L. R. NELSON. 1972. Effectiveness of resistance in maize to maize dwarf mosaic. Agron. J. 64:319-320.
7. SCOTT, G. E., E. E. ROSENKRANZ, and L. R. NELSON. 1969. Host reaction to maize dwarf mosaic virus from Mississippi and Ohio. Plant Dis. Rep. 53:933-935.
8. THOMAS, P. E., and M. W. MARTIN. 1972. Characterization of a factor of resistance in curly top virus-resistant tomatoes. Phytopathology 62:954-958.
9. THOMPSON, D. L., and T. T. HEBERT. 1970. Development of maize dwarf mosaic symptoms in eight phytotron environments. Phytopathology 60:1761-1764.