Development of Maize Dwarf Mosaic Symptoms in Eight Phytotron Environments

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ABSTRACT

Resistant and susceptible corn plants (*Zea mays* L.) were grown in eight phytotron-controlled environments, inoculated 14 days after planting with maize dwarf mosaic virus (MDMV), and observed for 31 days for symptom expression. Eighteen plants of 3 resistant entries and 148 plants of 5 susceptible entries developed symptoms. The average number of days between inoculation and symptom expression in the different environments ranged from 18 to 31 days for resistant entries, and from 4.7 to 7.3 days for susceptible entries. A significant reduction in plant height was obtained 6 days after inoculation. The most rapid expression of symptoms occurred at night temperatures of 26 C combined with day temperatures of either 26 or 30 C. Per cent plants infected and time required to show mosaic symptoms were more closely correlated with night temperatures than with day temperatures. Varied responses among resistant entries suggest that a range of evaluation environments might prove useful for identifying strains having maximum resistance. Phytopathology 60:1761-1764.

Maize dwarf mosaic virus (MDMV) in corn (*Zea mays* L.) is an important disease in many of the major corn-producing areas of the United States (3). Differences in symptom expression (primarily leaf mottling, coloration, and reduction in plant height) in different geographical areas suggest that environmental factors could be important sources of variation. Temperature is an environmental factor which varies greatly.

Tu & Ford (2) studied the effects of constant temp of 10, 15.5, 21, and 26.5 C on MDMV infection, incubation, and multiplication in Ohio W49 field corn. Symptoms appeared on infected plants after 3, 4, and 9 days' incubation at 26.5, 21, and 15.5 C, respectively. No symptoms appeared at 10 C. The percentage of plants infected increased with either higher preinoculation or higher postinoculation temp. Additional information relating to the influence of temp on symptom expression of MDMV should give a better understanding of the disease, and should assist in devising procedures for efficient selection for host resistance.

The purpose of this research was to determine the effects of differences in temp on number of days to symptom expression, intensity of leaf mottling, and reduction in plant height by comparing corn plants inoculated with MDMV with noninoculated plants in eight phytotron environments.

MATERIALS AND METHODS.—The experimental design was a split plot of seven replications with eight environments as whole plots and 16 plants as subplots (entry-inoculation combinations, i.e., 8 entries × 2 inoculation treatments) randomized within whole plots. The entries (3 resistant dent inbreds, 3 susceptible dent inbreds, 1 susceptible sweet corn hybrid, and 1 susceptible open pollinated variety) are listed in Table 1. The 2 inoculation treatments were as follows: Noninoculated and inoculated with MDMV.

Each of the seven replications in each environment consisted of the 16 subplot plants in 1-liter plastic pots on a movable truck. The 16 subplot plants were grown on the truck top in four rows of four plants each. Trucks were manually moved at 8:00 AM and 5:00 PM among four chambers for the programmed day and night temp and light regimes of the eight environments (Table 2).

For data analysis, the temp aspects of each environment were characterized in thermal units, i.e., weighted average daily temp above 10 C (Table 2). The soil medium was a mixture of 10% peat moss, 10% vermiculite, and 80% gravel plus small quantities of fertilizer and minor elements. Pots were watered as needed with modified Hoagland's solution.

Initially, three seeds were planted in each pot and kept at 22 C for 3 days. Pots were then moved into chambers with a constant temp of 24 C and 9 hr light daily for 7 days. Growth was greater than desired, so the night temp was reduced to 18 C for the next 4 days. After the seedlings became well established, excess plants were removed, leaving the desired one plant/pot.

Growth chambers were walk-in rooms 2.4 × 3.7 × 2.1 m high equipped with a combination of cool-white fluorescent and incandescent lamps in an approximate ratio of 100:30 by wattage. There were 84 T-12, 215 w, 1,500 ma fluorescent lamps with 135-degree reflectors (FR96T12/CW/1500) and 48 incandescent lamps of 100 w each. Illumination was maintained at 400 to 450 hectolux at pot level.

The leaves of each plant were counted 14 days after planting at the time of inoculation. A leaf was counted

TABLE 1. Plant size at inoculation and maize dwarf mosaic (MDMV) ratings of plants with symptoms for each of eight entries as averaged for eight phytotron environments

Entry	MDMV field classification	Plant size at inoculation[a]		MDMV symptoms[b]	
		No. leaves	Height cm	No. plants	Mottling intensity
Ga209	Resistant	3.7	15	12	2.4
Oh7B	Resistant	4.3	11	6	2.3
Tx601	Resistant	4.2	10	0	0
Mo5	Susceptible	3.4	9	33	3.8
Mp486	Susceptible	3.1	12	21	3.6
T226	Susceptible	3.0	13	22	3.6
Seneca Chief	Susceptible	3.8	14	26	3.5
Gaspe Flint	Susceptible	3.9	14	41	3.7

[a] Each value is a mean of 112 plants 14 days after planting.
[b] A total of 56 plants was inoculated for each entry. Rating scale: 0 (no symptoms) to 4 (extreme mottling).

TABLE 2. Temperature, light, and thermal units for each of eight phytotron environments and average terminal plant heights of all noninoculated plants in each environment

Environment	Temp		Light		Thermal units[a]	Terminal plant height[b]
	Day	Night	Day	Night		
	C	*C*	*hr*	*hr*	*C*	*cm*
1	30	26	9		17.5	59
2	26	26	9		16.0	56
3	30	18	9		12.5	59
4	30	18	9	3	12.5	61
5	26	18	9		11.0	55
6	26	18	9	3	11.0	62
7	30	14	9		10.0	55
8	26	14	9		8.5	63

[a] Thermal units = [(day temp × 9 hr + night temp × 15 hr)/24 hr] − 10 C.
[b] Average height of 56 noninoculated plants (7 plants × 8 entries) at termination of experiment (45 days after planting).

if it was approximately the same size as the preceding leaf. Heights of each plant were obtained 10 times during the experiment as measured from the soil surface to the intersection of the top two leaves in the whorl.

One-half the plants of each entry were inoculated with MDMV 14 days after planting. Inoculum was prepared by macerating infected leaves of the dent inbred NC7 in 0.01 M PO_4 buffer pH 7.2 using 2 ml of buffer/g of leaf tissue. Carborundum (1% 600 mesh) was added to the inoculum. The inoculation procedure was to dip the thumb and forefinger in the inoculum and pull the length of the leaf, exerting slight pressure on both sides. After the inoculation treatment was complete, all plants were placed in the appropriate environmental chambers.

Plants were examined daily and dates recorded when virus symptoms first appeared. Plants were rated for max symptoms on an integer scale from 0 (no symptoms) to 4 (extreme mottling).

RESULTS.—Plant size at inoculation, MDMV symptoms, and number of plants infected of each entry in all environments are shown in Table 1. The percentage of plants infected in the entries classified as field resistant ranged from 0 to 21%, and the percentage of infected plants in the entries classified as susceptible ranged from 38 to 73%. In the most susceptible entry, Gaspe Flint, 100% infection was obtained in two of the eight environments. Also shown in Table 1 are the average mottling intensity ratings. Susceptible entries were more intensely mottled than the resistant entries, but there were no apparent differences in mottling intensity among entries within each group.

No symptoms were noted when the plants were examined 3 days after inoculation. However, on the 4th day after inoculation, very pronounced mottling symptoms were apparent in some plants in environments 1 and 2 (the two environments with the highest night temperatures). Table 3 gives the number of days to symptom expression, mottling intensity, and number of plants infected for the three resistant entries and the five susceptible entries in each environment. The average number of days to symptom expression in susceptible entries varied from 4.7 to 7.3, whereas in the resistant entries the average number of days to symptom expression varied from 18 to 31 days. The earliest symptoms appeared in the resistant entries 14 days after inoculation, and many of the plants did not show symptoms until the end of the experiment, 31 days after inoculation.

The different environments seemed to have little effect on the intensity of mottling of susceptible entries (Table 3). A few plants of the resistant entries developed intense mottling, but the number of plants with symptoms in these entries were too few to draw definite conclusions regarding the effect of the environments on mottling intensity. The per cent plants showing symptoms in the susceptible group varied from

TABLE 3. Maize dwarf mosaic virus symptom development in three resistant and five susceptible entries in each of eight phytotron environments[a]

Environment	3 Resistant entries			5 Susceptible entries		
	No. plants	Mottling intensity	Days to symptoms	No. plants	Mottling intensity	Days to symptoms
1	6	2.7	19	26	3.5	4.7
2	5	2.2	18	21	3.5	4.7
3	1	2.0	31	14	3.4	6.3
4	1	2.0	31	21	3.7	5.9
5	1	2.0	31	20	3.8	6.7
6	0	0		20	3.8	6.8
7	3	4.0	28	11	3.8	6.5
8	1	2.0	31	13	3.9	7.3

[a] A total of 7 plants were inoculated for each entry in each environment. Mottling intensity scale: 0 (no symptoms) to 4 (extreme mottling).

31% in environment 7 to 74% in environment 1. In general, the higher percentages of infection were at the higher temp.

The differences in height between plants showing symptoms and noninoculated plants at different times during the experiment are shown in Table 4. Initial heights at inoculation (14 days after planting) were similar, but at the termination of the experiment (45 days after planting), differences ranged from 1 cm in environments 7 and 8 to 7 cm in environments 2 and 4. The first significant plant height difference (with consistent subsequent differences) was noted 6 days after inoculation in environment 2. Terminal height differences were not significant for environments 7 and 8, which had the lowest number of thermal units (10.0 and 8.5 thermal units, respectively). Terminal heights for noninoculated plants in each environment are listed in Table 2.

Correlation coefficients were determined separately for total daily thermal units, daytime thermal units, and night-time thermal units versus number of days to symptom expression, number of plants with symptoms, and differences in height between plants showing symptoms and noninoculated plants. In Table 5, it may be seen that both the number of days to symptom expression and the number of plants infected are correlated with daily thermal units, but night temp appear to have a much greater effect than day temp. On the other hand, the reduction in plant height seems to be influenced more by day temp than by night temp.

The regression of days to symptoms for the five susceptible entries on daily thermal units gave a coefficient of -0.31 ± 0.03. This coefficient indicates that the time from inoculation to symptom expression was reduced by an average of 0.31 days for each thermal unit increase.

Environments 4 and 6 had 3 hr of supplemental light during the middle of the night period. The additional light (or shorter nights) had little effect on time required for symptom expression, per cent plants showing symptoms, or intensity of mottling; but the additional light seemed to result in a greater reduction in plant height due to infection.

No red or yellow discoloration was observed in any of the plants during the experiment. At the end of the experiment, the earliest entry, Gaspe Flint, was tasseling.

DISCUSSION.—The percentage of plants that developed symptoms following inoculation was lower than expected. This may have been due in part to the fact that the plants were subjected to cool night temp for 4 days prior to inoculation. Tu & Ford (2) reported that cool preinoculation temp reduced the percentage of corn plants infected by MDMV. The percentage plants infected was also influenced by the postinoculation environments and by varieties and inbreds used.

TABLE 4. Height differences (cm) between noninoculated plants and plants with maize dwarf mosaic virus symptoms as averaged for five susceptible entries in each of eight phytotron environments[a]

Environment[b]	Days after planting									
	14[c]	17[c]	20	24	28	32	34	38	41	45
1 (30/26)	0	0	1*[d]	2	1	2	2	2	4	5*
2 (26/26)	0	0	1**	2**	3**	3*	3*	4*	5**	7**
3 (30/18)	0	−1	0	−1	2	2**	3**	3*	4*	5**
4 (30/18L)	0	0	1	3*	5**	5**	5*	5*	7*	7*
5 (26/18)	−1	0	0	0	2**	4**	4**	3*	4*	4*
6 (26/18L)	0	−1	1	1	5**	4**	4**	3**	5**	6**
7 (30/14)	0	−1	0	0	3**	3**	2*	2	0	1
8 (26/14)	−1	−1*	−1	0	1	2*	1	−2**	0	1

[a] Difference (cm) = (height of plants not inoculated) − (height of plants with symptoms); plants inoculated 14 days after planting.
[b] Temperature regime, C (day/night) indicated for each environment; L indicates 3 hr light at night (Table 2).
[c] Differences prior to symptom expression.
[d] *, ** = Significant at 5 and 1% levels of probability, respectively.

TABLE 5. Simple correlation coefficients for thermal units vs. days to symptom expression, vs. number of plants with symptoms, and vs. differences in plant height for noninoculated plants and plants showing maize dwarf mosaic virus symptoms

Thermal units	Days to symptoms	No. plants with symptoms						Differences in plant height, days after planting							
		Entry													
		4	5	6	7	8	4-8	20	24	28	32	34	38	41	45
Daily	—.97**[a]	.73*	.41	.35	.32	.32	.71	—.22	.10	—.41	—.16	.05	—.20	.24	.23
Daytime	—.29	—.29	—.29	.38	—.28	.30	—.15	.26	.84**	.00	—.12	.20	.50	.60	.67
Night time	—.92**	.84**	.50	.26	.43	.25	.77*	—.30	—.14	—.43	—.14	.00	—.34	.09	.05

[a] *, ** Significant at 5 and 1% levels, respectively; N = 8; value for 5% level, .707; for 1% level, .834.

Some of the plants in the resistant group did not show symptoms until the last day of the experiment, 31 days after inoculation. More plants in this group might have developed symptoms if observations had been continued for a longer period of time.

Most studies on the effect of temp on infection of plants by viruses, or on symptom expression, have not attempted to evaluate separately the effects of the day and night temp. This study showed that per cent plants with symptoms and the time elapsed between inoculation and expression of mosaic symptoms were both influenced more by night temp than by day temp. Growth, as measured by increase in dry wt, is influenced more by night temp than by day temp in many plants (4). Since most viruses infect vigorously growing plants more readily than slower growing plants, the night temp may have influenced infection simply by modifying the growth rate of the host. On the other hand, day temp appeared to influence plant height more. This effect may be more closely related to the metabolism of specific compounds regulating plant height than to total growth. The opt temp regime for obtaining a high percentage of infected plants and a rapid appearance of mosaic symptoms would not necessarily be the same as that for demonstrating max reduction in plant height.

Temperature regimes with 26-C night temp and either 26- or 30-C day temp gave the most rapid expression of mosaic symptoms and also a high percentage of plants infected. This is in agreement with the report by Tu & Ford (2) that a constant temp of 26.5 C resulted in a higher percentage of infected plants and a more rapid expression of symptoms than lower temp. Optimal temp for the growth of corn are 25 C at night and 30 to 35 C during the day (1). With day temp of 26 or 30 C, greater reduction in plant height occurred at night temp of 26 and 18 C than 14 C.

The resistant and susceptible entries, as previously classified by field observations, were also clearly differentiated in this study by percentage of plants developing symptoms, by time from inoculation to symptom expression, and by intensity of mottling symptoms. The resistant entries (Ga209, Oh7B, and Tx601) could not be ranked confidently for relative resistance because few plants developed symptoms and the patterns of development varied. Tx601 could be considered the most resistant because none of the plants developed symptoms. Only six plants of Oh7B developed symptoms, but symptoms appeared relatively early in only two environments (14 days, environments 1 and 2). At least one plant of Ga209 developed symptoms in seven of the eight environments; however, 21 to 31 days were necessary for symptoms to appear. These varied responses among the resistant entries indicate that a range of evaluation environments might prove useful for identifying strains having max resistance.

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