Optimum Conditions for Studies of Maize Dwarf Mosaic Virus Strains A and B in Sorghum

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ABSTRACT

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Optimum conditions for the study of maize dwarf mosaic virus strains A and B (MDMV-A and MDMV-B) in sorghum are 1) use of 1:10 and 1:2 dilutions of inoculum (MDMV-A and MDMV-B, respectively); 2) source plants inoculated 10 days before use; 3) an incubation temperature of 25 C; and 4) inoculation of the first leaf 6 days after plant emergence, of the second leaf 2 days later, and of the third leaf at least 14 days after plant emergence.

Maize dwarf mosaic of sorghum (*Sorghum bicolor* (L.) Moench), caused by maize dwarf mosaic virus (MDMV), was first reported on sorghum in the mid-1960s and is now recognized as an important virus disease of sorghum in the United States and other countries (5,8,10). The physical characteristics of the virus, symptoms of the disease, vectors, strains of the virus, and overwintering hosts have been investigated (1–3,6,7,9,10).

Little information is available concerning controlled conditions necessary for the study of this host-pathogen interaction. Therefore, studies were conducted to determine the appropriate incubation period, inoculum dilution, effect of incubation temperature on systemic symptom expression, and the effect of leaf age at inoculation time on mechanical transmission of MDMV. This report concerns conditions found to be optimal for further studies of MDMV strains A and B (MDMV-A and MDMV-B) in sorghum.

MATERIALS AND METHODS

Plant and virus maintenance. MDMV-A was obtained from R. E. Ford (Iowa State University) and maintained in G499GBR (Funks) sorghum seedlings. MDMV-B was supplied by J. K. Uyemoto (Kansas State University) and maintained in seedlings of Bugoff (Asgrow). Sorghum used to produce inoculum of each strain was seeded into soil-filled flats (30 × 70 cm) and inoculated 6 days after seedling emergence.

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Inoculum used for greenhouse and growth chamber experiments was prepared by triturating whole, infected sorghum plants (10–14 days after inoculation) in a blender for 1 min in a 0.02 M potassium phosphate buffer, pH 6.9 (1 g of tissue per 10 ml). The triturate was filtered through four layers of cheesecloth and 1 g of abrasive (Crystolon flour B, 600-mesh, Norton Co., Worchester, MA) was added to each 100 ml of filtrate.

Unless otherwise stated, plants in all experiments were inoculated as described by Martin (4) with a DeVilbiss No. 152 atomizer (4.2 kg/cm^2 air pressure) held 1–2 cm from the plants and grown in a greenhouse at 27 ± 3 C.

Sorghum hybrids G499GBR for MDMV-A and Bugoff for MDMV-B were used in these studies because previous work in this laboratory had shown them to be susceptible and to produce distinct symptoms in response to the respective virus strain.

Inoculum dilution studies. Inoculum for dilution studies was prepared for both virus strains by macerating 10-day-old infected seedlings at an initial dilution of 1:1 (w/v) in 0.02 M potassium phosphate buffer, pH 6.9. The 1:1 dilution was then diluted further to make 1:2, 1:5, 1:20, 1:30, 1:40, 1:50, 1:60, 1:70, 1:80, 1:90, 1:100, 1:150, 1:200, 1:300, 1:400, and 1:1,000 dilutions. Two hundred milliliters of each dilution was used to inoculate 100 six-day-old seedlings of the appropriate variety. Inoculated plants were incubated in the greenhouse at 27 ± 3 C for 10 days and the number of systemically infected plants was recorded.

Time of maximum virus concentration in source plants. Sorghum seedlings used to determine the optimum incubation period for inoculum production were inoculated 6 days after planting and harvested 2, 4, 6, 8, 10, 12, 14, and 16 days later. A 1:10 (w/v) dilution of plant material (whole plants) from each harvest date was used to inoculate 200 sorghum seedlings and the number of systemically infected plants was recorded after 10 days.

Effects of temperature on systemic symptom development. One hundred seedlings of each sorghum hybrid were inoculated 6 days after planting with the appropriate virus strain and incubated at 15, 20, 25, and 30 C in growth chambers (Warren/Sherer Model EL38-15) with a 12-hr photoperiod of fluorescent light (5,400 lux). Control plants, inoculated with buffer and abrasive only, were maintained in the same growth chambers. Beginning 6 days after inoculation, the numbers of plants with systemic symptoms were recorded at 2-day intervals.

Effects of leaf age on symptom development. Sorghum was planted on three consecutive days for each inoculation date for each leaf to be inoculated. Pots were thinned to five plants with uniform leaf lengths before inoculation. The first leaf of five plants per pot, with five replicate pots, was inoculated with a 1:10 concentration of the appropriate virus by the finger-thumb method 2, 4, 6, 8, 10, 12, and 14 days after emergence. The same procedure was used to inoculate the second leaf on a corresponding set of plants as for the third leaf on an equal number of plants. Infected plants were recorded 14 days after inoculation.

Experimental replication and statistical analysis. All experiments were repeated three times, with four replicates per experiment unless otherwise stated. Data from each replicate were pooled for comparison of treatments. Values obtained were subjected to the analysis of variance or standard deviations of the means were plotted directly on the figures. When significant treatment effects were found, individual means were compared by the Student-Newman-Keuls multiple range test. All percentage data were arc sine transformed before statistical analysis. Control plants maintained under conditions identical to the inoculated plants developed no symptoms in any experiment.

RESULTS AND DISCUSSION

The 1:1 inoculum dilution for both MDMV-A and MDMV-B resulted in more infected plants than all other dilutions (Table 1). For MDMV-A, however, this dilution did not prove significantly different from the 1:2, 1:5, and 1:10 dilutions, and for MDMV-B, the 1:1 was not different from the 1:2 but was different from the 1:5 and 1:10

dilutions. Table 1 also shows that as the inoculum became less concentrated, the numbers of plants infected with MDMV-A or MDMV-B generally decreased. These results indicate that a 1:10 dilution of inoculum, derived from source plants inoculated 10 days earlier, provides the optimum dilution for infection of sorghum seedlings by MDMV-A, whereas a 1:2 dilution of inoculum is optimal for MDMV-B.

Table 1. Percentages of sorghum plants infected by maize dwarf mosaic virus strains A (MDMV-A) and B (MDMV-B) at various inoculum dilutions[w]

Dilution	Percent infection[x]	
	MDMV-A	MDMV-B
1:1	73 a[y]	72 a
1:2	70 ab	67 ab
1:5	67 abc	64 bc
1:10	64 abcd	60 bcd
1:20	54 e	50 ef
1:30	51 ef	51 e
1:40	44 fg	46 efgh
1:50	43 fgh	47 efg
1:60	38 ghij	41 ghij
1:70	43 fgh	37 ghijkl
1:80	37 ghij	39 ghijk
1:90	38 ghi	42 efghi
1:100	33 ghijkl	35 ijklm
1:150	32 ghijklm	32 ijklmn
1:200	28 ijklmn	29 lmno
1:300	22 no	22 p
1:400	21 nop	16 pq
1:1000	12 q	13 qr
Control[z]	0 r	0 s

[w] Average values obtained from three experiments.
[x] Percentages in table were arc sine transformed.
[y] Means within a column not followed by the same letter differ significantly according to the Student-Newman-Keuls multiple range test ($P < 0.05$).
[z] Control consisted of plants inoculated with buffer containing an abrasive.

Table 2. Percentages of sorghum plants infected by maize dwarf mosaic virus strains A (MDMV-A) or B (MDMV-B) from inocula prepared at different times after inoculation of source plants[x]

Days after inoculation of source plants	Percent infection[y]	
	MDMV-A	MDMV-B
2	0 l[z]	9 l
4	29 defghijk	43 bcdefghijk
6	54 abc	46 bcdefghijk
8	55 ab	53 abcdef
10	59 a	65 a
12	44 abcdef	56 abcd
14	43 abcdefgh	60 ab
16	46 abcde	51 abcdefg
18	45 abcdefg	51 abcdefgh
20	48 abcd	56 abcde
22	42 abc	48 abc
24	38 bcdefghij	57 abc

[x] Average values obtained from 12 replicates in three experiments.
[y] Percentages in table were arc sine transformed.
[z] Means within a column not followed by the same letter differ significantly according to the Student-Newman-Keuls multiple range test ($P < 0.05$).

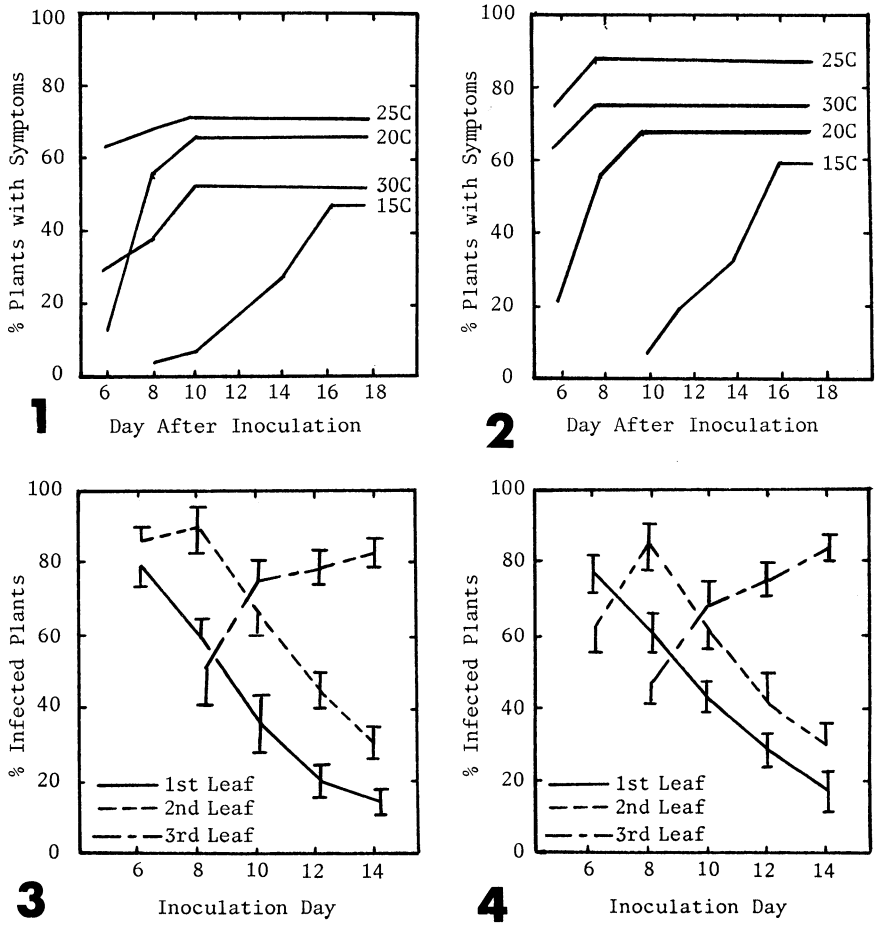
MDMV-A and MDMV-B inoculum prepared from source plants inoculated 10 days earlier gave the highest percentage of infected plants (Table 2). With MDMV-A-infected plants, this percentage (59%) did not differ significantly from those obtained from inocula extracted earlier or later (from 6 to 22 days postinoculation). With MDMV-B-infected plants, only inocula made 2, 4, and 6 days after inoculation of source plants gave statistically significant decreases in infection.

The most rapid expression of symptoms occurred at 25 C in both MDMV-A- and MDMV-B-infected sorghum, with the slowest symptom expression at 15 C (Figs. 1 and 2). Symptoms were not detected in MDMV-B-infected sorghum at 15 C until 10 days after inoculation, when leaf necrosis (red leaf reaction) was observed (7). At this temperature, there was no mosaic reaction as was observed at 20, 25, and 30 C.

Statistical analysis of MDMV-A-infected sorghum indicated no significant difference between percentage of infected plants at 25 and 20 C. However, the percentage of infected plants at 20 C proved to be significantly different from those at 30 and 15 C. Also, the percentage of infected plants at 30 C was significantly different from that at 15 C. With MDMV-B-infected sorghum, there were no differences between percentages of infected plants at 30 and 20 C or between those at 20 and 15 C.

These results indicate that under conditions used, the optimum temperature for symptom development in the sorghum varieties used when infected with MDMV-A or MDMV-B is 25 C. It should be noted, however, that no statistical difference was observed in MDMV-A-infected sorghum between 25 and 20 C.

Highest disease incidence for MDMV-A- and MDMV-B-infected sorghum occurred when the first leaf was inoculated 6 days and the second leaf 8 days after seedling emergence, and for the third leaf, numbers of infected plants increased through day 14, the last day inoculations were made (Figs. 3 and 4).



Figs. 1-4. (1) Average percentages of Funks G499GBR sorghum plants developing maize dwarf (MDM) symptoms when incubated at 15, 20, 25, and 30 C after inoculation with maize dwarf mosaic virus (MDMV) strain A. **(2)** Average percentages of Asgrow Bugoff sorghum plants developing MDM symptoms when incubated at 15, 20, 25, and 30 C after inoculation with MDMV strain B. **(3)** Average percentages of Funks G499GBR sorghum plants developing MDM symptoms when the first, second, and third leaves were inoculated with MDMV-A 6, 8, 10, 12, and 14 days after planting. Data points are bracketed by the standard deviations of the means. **(4)** Average percentages of Asgrow Bugoff sorghum plants developing MDM symptoms when the first, second, and third leaves were inoculated with MDMV-B 6, 8, 10, 12, and 14 days after planting. Data points are bracketed by the standard deviations of the means.

The results of these experiments provide information concerning conditions necessary to further study MDMV-A and MDMV-B in sorghum.

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