

Aphid Transmission of Maize Dwarf Mosaic Virus Strains

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ABSTRACT

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Rhopalosiphum maidis, *R. padi*, and *Myzus persicae* acquired maize dwarf mosaic virus (MDMV)-A and MDMV-B after probes of less than 30, 10, and 30 seconds, respectively. *Rhopalosiphum maidis*, *R. padi*, and *M. persicae* retained MDMV-A at least 90, 120, and 240 minutes, respectively; and MDMV-B at least 30, 90, and 30 minutes, respectively. The rates of loss of ability to transmit MDMV-A and MDMV-B were the same for all aphid-virus combinations studied. The efficiency of MDMV-A transmission by aphids from corn to corn, corn to Johnson-

grass, and Johnsongrass to corn did not differ significantly, indicating that both plants were equally effective as virus sources. Strain MDMV-A was retained longer (at least 90, 120, and 240 minutes by *R. maidis*, *R. padi*, and *M. persicae*, respectively) than was MDMV-B (at least 30, 90, and 30 minutes by *R. maidis*, *R. padi*, and *M. persicae*, respectively), after a 10-minute acquisition access on diseased corn leaves. Strain MDMV-B was not transmitted to Johnsongrass by the aphids tested.

Additional key words: stylet-borne viruses.

Maize dwarf mosaic virus (MDMV) is a member of the potato virus Y group of plant viruses. Its known host range is restricted to the family Gramineae (9) and includes many annual crops, small grains, and some perennial grasses, in which the virus persists through the winter in some areas (3, 4, 16). Two major strains that have many similarities have been reported (10, 13). A key distinction between the two strains is their differential host range (2, 9, 12); strain A (MDMV-A) infects Johnsongrass [*Sorghum halepense* (L.) Pers.] while strain B (MDMV-B) does not.

As with other potyviruses, MDMV is transmitted by aphids in a stylet-borne manner (4, 16). Aphids acquire the virus after short probes on infected plants, and transmit it readily to susceptible hosts. However, loss of transmission ability occurs within a short period after viruliferous aphids leave the infected plants. Nearly all the work reported on MDMV-vector relationships has dealt with one strain or different isolates of one strain of the virus (1, 4, 5, 6, 11, 16). In only a few studies was a comparison made between the two strains of the virus, or among aphid species (7, 8, 14).

In the U. S., a retention time of 30 minutes or less was reported (4, 8, 16), and in Yugoslavia up to 6 hours (14). It became of interest to resolve reported differences in MDMV retention times in aphids. We compared the

mode of transmission of MDMV-A and MDMV-B by three aphid species (*Rhopalosiphum maidis* Fitch., *R. padi* L., and *Myzus persicae* Sulzer) and report those results here. We also report results from studies on the efficiency of MDMV transmission after different periods of acquisition feeding, and especially on the aphid's ability to retain the two strains of MDMV after acquisition.

MATERIALS AND METHODS

Stock cultures of MDMV were maintained insect-free in the greenhouse in sweet corn (*Zea mays* L. 'Golden Bantam'). The Iowa isolates of MDMV-A (Ia. 65-74) and MDMV-B (ATCC-PV 53) were used in all transmission studies. Inoculations were accomplished by rubbing leaves of healthy corn plants with a pestle dipped in a homogenate of MDMV-infected leaves prepared in 10 mM phosphate buffer pH 7.0 plus about 0.1 gm of Carborundum as abrasive. Plants were inoculated at the two-leaf stage. Inoculations were done on a regular schedule so that when the youngest systemically-infected leaves were harvested and used for acquisition feeding they were almost fully expanded (9-10 days after inoculation). At this stage, the virus concentration in the youngest leaf has been found highest (15). When

Johnsongrass was used as the source of MDMV-A inoculum for retention studies, the plants were mechanically inoculated when they were in a three- or four-leaf stage. Only the youngest systemically-infected leaves were used for acquisition feeding.

Test plants used were sweet corn cultivar Golden Bantam in a two-leaf stage (5-8 days old), and Johnsongrass in a four-leaf stage. The corn was seeded in 10-cm diameter clay pots, four seeds per pot. Johnsongrass seeds were sown in metal flats and transplanted to 10-cm diameter clay pots, three plants per pot, after germination.

Three species of aphids were used in all studies, except as otherwise indicated. *Rhopalosiphum maidis* and *R. padi* were colonized on winter barley (*Hordeum vulgare* L. 'Hudson'). Cultures of *R. maidis* were maintained in a growth chamber set at 30 C day temperature and 24 C night temperature, and 14-hour day length, and those of *R. padi* in an air-conditioned greenhouse at 17.5 C. *Myzus persicae* was colonized on sugar beets and maintained in a growth chamber with a constant temperature of 21 C, and a 16-hour day length. All aphids used were the apterous form and were starved in closed glass containers for about one hour before acquisition feeding on mosaic areas of detached corn or Johnsongrass leaves systemically infected with MDMV.

In the acquisition study, feedings were observed under a stereomicroscope. Aphids were manipulated with a camel's-hair brush. In all studies, aphids were transferred to the test plants after either acquisition or postacquisition starvation. Each test plant was then caged and left overnight in the transfer room at 21 C for inoculation feeding. Inoculation feedings by *R. maidis* and *R. padi* were terminated by fumigation with Vapona for 30 minutes, and that of *M. persicae* by spraying with nicotine sulfate. After termination of inoculation feeding, plants were transferred to a greenhouse and maintained for two weeks for symptom development. The number of systemically-infected plants was recorded daily, beginning the fourth day after inoculation feeding.

Methods for each type of experiment are given in the appropriate part of the results section. The results reported here are based upon data recorded from 50 plants in each treatment group, 30 in one experiment and 20 in another.

RESULTS

Acquisition of MDMV by single aphids.—Aphids were allowed to feed on virus-infected corn leaves for 10, 30, 60, 120, and 300 seconds. Feeding was timed from when aphid stylets touched the leaf surface and started to probe into the leaf tissue, and was terminated by gently disturbing and removing the aphids after the desired period of feeding. Immediately after acquisition feeding aphids were singly transferred to healthy corn plants for inoculation feeding.

Single aphids of *R. maidis* and *M. persicae* did not transmit either MDMV-A or MDMV-B following 10-second acquisition probes. However, after 30-second acquisition probes both aphids transmitted the two strains of the virus (Fig. 1). By contrast, *R. padi* transmitted both MDMV-A and MDMV-B after 10-second acquisition probes. The optimal acquisition times

for maximum transmission rates of MDMV-A and MDMV-B, respectively, were: 120- and 30-second probes for *R. maidis*; 30- and about 60-second probes for *R. padi*; and 120- and 60-second probes for *M. persicae*. Statistical analysis by the *t*-test indicated no significant differences between the transmission percentages at different acquisition times of MDMV-A and MDMV-B by *R. maidis* and *R. padi* at $P = 0.05$. However, the percentage transmission of MDMV-A by *M. persicae* was significantly greater ($P = 0.05$) than that of MDMV-B.

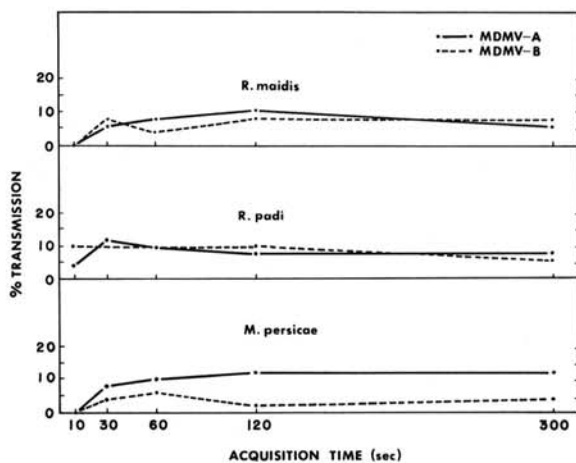


Fig. 1. Transmission rates (percentage) of two strains of maize dwarf mosaic virus, MDMV-A and MDMV-B, by single *Rhopalosiphum maidis*, *Rhopalosiphum padi*, and *Myzus persicae* after various acquisition times (seconds) on infected sweet corn. The data represent 50 plants in each treatment group, 30 in one experiment and 20 in another.

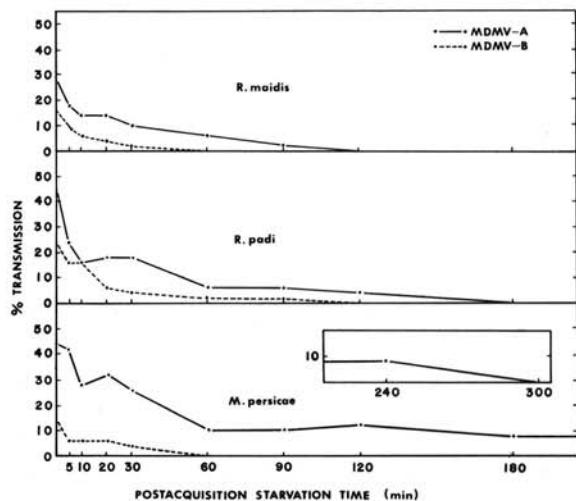


Fig. 2. Retention of two strains of maize dwarf mosaic virus, MDMV-A and MDMV-B, by groups of 10 *Rhopalosiphum maidis*, *Rhopalosiphum padi*, and *Myzus persicae* using various postacquisition starvation times after 10 minutes of acquisition on infected sweet corn. The data represent 50 plants in each treatment group, 30 in one experiment and 20 in another.

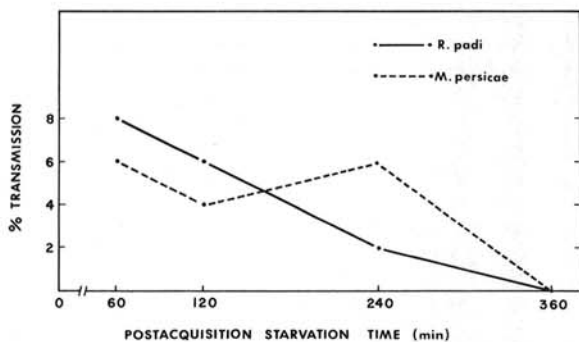


Fig. 3. Retention of maize dwarf mosaic virus strain A (MDMV-A) by groups of 10 *Rhopalosiphum padi* and *Myzus persicae* using different postacquisition starvation times after 10 minutes of acquisition on infected Johnsongrass. The data represent the 50 plants in each treatment group, 30 in one experiment and 20 in another.

Retention of MDMV acquired from corn.—Aphids were allowed acquisition access to the portions of corn leaves showing mosaic symptoms in small glass vials with plastic caps. After 10 minutes of acquisition access aphids found on leaf portions were dislodged, gently shaken into other vials and starved for 0, 5, 10, 20, 30, 60, 90, 120, and 180 minutes for *R. maidis* and *R. padi*. For *M. persicae*, starvation periods were extended to 240, or 300 minutes. After postacquisition starvation, aphids were transferred to test plants for inoculation feeding (10 aphids per plant).

The retention time following 10 minutes of acquisition of MDMV-A and MDMV-B by each species of aphid was 90 and 30 minutes for *R. maidis*, 120 and 90 minutes for *R. padi*, and 240 and 30 minutes for *M. persicae*, respectively (Fig. 2). Postacquisition starvation sharply decreased the transmission rate of MDMV for the first 20 to 30 minutes, after which time the transmission rate continued to decline much more slowly until the aphids ceased transmission. The transmission rate of MDMV-A was significantly (*t*-test) higher and longer, at both $P = 0.01$ and $P = 0.05$, than that of MDMV-B by each species of aphid.

Retention of MDMV acquired from Johnsongrass.—In this experiment only *R. padi* and *M. persicae* were used. Aphids were starved for 60, 120, 240, 360, and 480 minutes after 10 minutes of acquisition access on MDMV-A-infected Johnsongrass leaves. They were then transferred to corn test plants for inoculation feeding (10 aphids per plant).

The results of tests to determine the ability of aphids to retain and transmit MDMV-A were about the same for *R. padi* and *M. persicae* (Fig. 3). Both transmitted MDMV-A after 240 minutes of postacquisition starvation, but were unable to transmit when starved for 360 minutes.

Comparison of transmission by *R. padi* and *M. persicae* of MDMV-A and MDMV-B from corn to Johnsongrass or corn.—Aphids were transferred to test plants immediately after 10 minutes of acquisition access on mosaic corn leaves systemically infected with either MDMV-A or MDMV-B. Only *R. padi* and *M. persicae* were used in the study. Both species were not able to transmit MDMV-B from corn to Johnsongrass. Transmission rates of MDMV-A from corn to Johnsongrass and from corn to corn were about the same for both species of aphids (Table 1). Transmission rates of MDMV-A from corn to corn were higher for *M. persicae* than for *R. padi*.

DISCUSSION

Transmission rates of MDMV by *R. maidis*, *R. padi*, and *M. persicae* at different acquisition times were about the same. Transmission increased sharply with time of acquisition until maximum transmission rates were obtained after 30 to 60 seconds of acquisition probes, beyond which no further increase occurred. Transmission remained constant or decreased only slightly with increasing acquisition time. The duration of acquisition probes that result in the greatest efficiency of transmission can be defined as the optimal acquisition period. Optimal acquisition periods of MDMV by the aphids studied were probes between 30 and 60 seconds. It has been shown previously that there was a gradual reduction in transmission rates of MDMV resulting from the acquisition probes longer than the optimum duration (5), but results in this study do not support this. Messieha (4) also obtained the uniform transmission rates after the

TABLE 1. Comparison of maize dwarf mosaic virus (MDMV) transmission from corn to Johnsongrass and from corn to corn by groups of 10 *Rhopalosiphum padi* and *Myzus persicae* after 10 minutes of acquisition on infected sweet corn

MDMV strain	Transmission ^a							
	<i>R. padi</i>				<i>M. persicae</i>			
	To Johnsongrass		To corn		To Johnsongrass		To corn	
Rate	(%)	Rate	(%)	Rate	(%)	Rate	(%)	
A	4/12 ^b	33	4/14	28	5/12	42	7/12	58
B	0/21	0	2/8	25	0/20	0	2/6	33

^aAphids were fed on test plants immediately after acquisition on MDMV-infected sweet corn.

^bData represent the totals obtained from two experiments performed separately, expressed as the number of infected plants per number of plants inoculated.

probes beyond the optimal duration probes for acquisition.

Rhopalosiphum padi was more efficient in transmitting MDMV-A and MDMV-B than was *R. maidis* or *M. persicae*; *R. padi* transmitted the virus after 10-second probes, while the other two species required probes lasting between 10 and 30 seconds. This suggests that *R. padi* may have an acquisition threshold of less than 10 seconds. The precise acquisition threshold was not determined in this study since 10 seconds was the shortest time interval tested. The acquisition threshold of *M. persicae* found in this study was the same as that reported by Messieha (4). However, Shaunak and Pitre (7) found that *M. persicae* could transmit MDMV after acquisition probes of 10 seconds or less.

The three aphid species used here can retain the virus for different times after leaving the virus source. The differences existed not only among the species of aphids, but between the strains of the virus as well. The rates of loss of ability to transmit MDMV-A and MDMV-B were apparently the same for all aphid-virus combinations studied, but the main difference in retention times may be the initial amount of virus acquired. Most of the studies done on the ability of aphids to retain MDMV by using either serial transfers to healthy test plants (4, 8) or postacquisition starvation (4, 8, 16) led to the conclusion that aphids cannot retain MDMV longer than 30 minutes after they have left the virus source. However, results from two previous studies suggested that aphids could retain MDMV longer than 30 minutes (7, 14). Results from this study indicated that *R. maidis*, *R. padi*, and *M. persicae* could retain both MDMV-A and MDMV-B longer than 30 minutes after acquisition. The different results of insect transmission of MDMV from various studies can be explained by many variables, such as the source of virus isolates, experimental conditions and different techniques used in the studies, and aphid behaviors. As observed in this study, different aphid species have different feeding behaviors. *Rhopalosiphum padi* and *M. persicae* probed more readily than *R. maidis* during acquisition feeding after a period of preacquisition starvation. *Rhopalosiphum maidis* did more searching before it started to probe the leaf tissue. This search period might have some effects on the retention. After aphids had acquired the virus and the postacquisition starvation period was terminated, the aphids were transferred to the test plants where some of the aphids might feed immediately while others might not, because of the unknown nature of their feeding behaviors. This may explain the shorter retention period of the virus by *R. maidis* than with the other two species.

When Johnsongrass was used as the virus source, transmission rates and retention period obtained were about the same as those for corn. Thus, Johnsongrass is not a more effective virus source than corn. However, Messieha (4) found that transmission rate of MDMV by *M. persicae* from Johnsongrass was about twice that from

corn. Considering the relatively long retention period of aphid transmission of MDMV and the availability of Johnsongrass, aphid transmission undoubtedly plays an important role in the prevalence of MDMV-A in nature.

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