Factors Affecting Aphid Transmission of Maize Dwarf Mosaic Virus to Corn

J. C. Tu and R. E. Ford

Postdoctoral Research Associate and Professor, respectively, Department of Botany and Plant Pathology, Iowa State University, Ames 50010. Present address of senior author, Electron Microscope Laboratory of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada.

Iowa Agriculture and Home Economics Experiment Station Journal Paper No. J-6622, Ames, Project No. 1628.

We thank T. E. Mittler, Department of Entomology, University of California, Berkeley, for the green peach aphid colony.

Accepted for publication 27 July 1971.

ABSTRACT

The transmission of maize dwarf mosaic virus (MDMV) from corn by *Myzus persicae* to corn was closely associated with MDMV concentration in situ. The transmission rate of MDMV with a dilution end point of 1/2,000 was 15% for single aphids and up to 85% for 20 aphids/plant. The maximal MDMV retention time was 25 min, identical for both single and group aphids, whether fasted or nonfasted. However, single-fasted aphids transmitted MDMV more efficiently (15%) than single nonfasted aphids (5%) shortly after (10-min) acquisition. Implications of single versus group aphid transmission of MDMV in nature are discussed.

There was no difference in susceptibility of corn to MDMV by aphid transmission when corn was grown under different temperatures when the corn was of uniform size at the time of transmission, and when transmission was done at one temperature. However, the rate of transmission of MDMV differed considerably at different temperatures if corn seedlings were grown under identical conditions except that they were incubated for 1 hr at different temperatures before transmission.

Transmission of MDMV was positively related to leaf age and MDMV concentration in vivo. MDMV concentration was lower in older leaves, resulting in decreased aphid transmission. Aphids fed on the mosaic areas of infected leaves transmitted more MDMV (13%) than those fed on nonmosaic areas (3%). Phytopathology 61: 1516-1521.

Maize dwarf mosaic virus (MDMV) is a mechanically transmissible virus and, in nature, a stylet-borne (nonpersistent) virus. Natural spread of MDMV in the field is apparently linked to its aphid vectors. Aphid transmission of MDMV has been demonstrated recently (4). Some aspects relating to the efficiency of aphid transmission need further exploration.

Transmission of MDMV could be affected by differences in activity by single aphids and by groups of aphids, and by age of the MDMV-infected plant as it influences MDMV concentration in situ in corn. Higher virus titer in the host resulted in a higher transmission rate by aphids in work with bean yellow mosaic virus (9), potato virus Y, and cucumber mosaic virus (7).

MDMV infectivity in sap from infected corn (*Zea mays* L.) leaves declines (with plant age) immediately after the peak is reached (9). Decreases in infectivity in plant saps extracted from virus-infected plant parts of different age have been demonstrated in several virus-host combinations (2, 3, 5, 6).

Temperature is a factor that might alter transmission efficiency by influencing either the vector or the host. Low temperatures generally favor virus retention by the aphid during fasting, and aphids tend to make fewer probes before making feeding penetrations at low temperatures (12). Postinoculation temperature treatments of the plants in one virus-host combination did not alter the infection percentage (10). However, little is known about the pre- or postinoculation temperature effects on the aphid transmission of MDMV.

Swenson (9) has shown that large changes in environment and nutrition are required to cause small changes in susceptibility of test plants to inoculation by aphids, and that the condition of the aphid colonies is the major source of variation.

Although aphids that fed on MDMV-diseased plants became viruliferous and transmitted MDMV to healthy corn seedlings, unexplainable differences in transmission were observed.

This study was designed to evaluate (i) the differences in acquisition period and retention period of MDMV by fasted and nonfasted aphids singly and in groups; (ii) the transmission efficiency of MDMV by aphids fed on diseased corn leaves of different ages; (iii) the transmission efficiency of MDMV by aphids fed on mosaic versus green areas of a MDMV-diseased leaf; and (iv) temperature effects on aphid transmission of MDMV

MATERIALS AND METHODS.—An Iowa isolate [Ia. 65-74 (1)] of MDMV strain A was used in this study. The aphid used was the green peach aphid (*Myzus persicae*). The aphid was propagated and maintained on young, uniform-age radish (*Raphanus sativus* L.) plants in a 21-C growth chamber programmed at a 14-hr day (1,400 ft-c) and a 10-hr night. Only fourth- and fifth-instar aphids were used in transmission experiments. The colonies were transferred to prevent crowded conditions. In fasting experiments, aphids were fasted for 2 hr, unless otherwise stated, in a beaker covered with cellophane.

MDMV source plants were W49 (*Zea mays* L. 'Ohio W49') seedlings grown in 10-cm clay pots, 5 plants/pot. Plants were maintained in a greenhouse at 24 $\pm$ 3C before inoculation. All source plants were mechanically inoculated with MDMV. Inoculum was crude sap from infected corn leaves diluted 1:1 (w/v) with 0.01 *M* neutral phosphate buffer. Inoculation was made by rubbing inoculum on leaves of the two-leaf-stage corn seedlings predusted with 600-mesh Carborundum. An equal number of plants were noninoculated controls.

Test plants were grown in steamed soil, 10 seeds/10-cm pot. They remained in the greenhouse until the test feeding unless specified. All test plants used in test feeding experiments were three-leaf-stage W49 corn seedlings. After feeding, plants were held for symptom development at 21 C in growth chambers programmed at a 10-hr day and a 14-hr night. Results were recorded 2 weeks after feeding.

Individual aphid feeding experiments were monitored with a dissecting microscope. Camel's-hair brushes were used for transferring each aphid. The duration of acquisition was between starting and ending of a probe. In group aphid-feeding tests, acquisition time was that which elapsed between placing aphids on and removing them from corn.

The relationship of aphid transmission and MDMV concentration in vivo in different leaf ages was studied by detaching infected corn leaves from various positions on the plant (9 = youngest or uppermost leaf tested in a 4-week infection; 4 = youngest or uppermost leaf tested in a 1-week infection) and feeding aphids on them for 10 min (Fig. 1). Ten aphids were then transferred to each corn test plant in the three-leaf stage. Immediately thereafter, these detached leaves were ground in 0.01 *M* phosphate buffer, pH 7.0, diluted 1:10, and also mechanically inoculated to three-leaf-stage corn seedlings for assay.

The temperature effect on the aphid transmission of MDMV was tested by two types of experiments. The first (I) was to determine whether different predisposing temperatures affected the susceptibility of corn to MDMV via aphid transmission at a uniform temperature. The second (II) was to determine the effect of temperature on efficiency of aphid transmission. For experiment I, corn was seeded in pots at different times at 15.5, 21, and 26.5 C to obtain uniform four-leaf-stage corn seedlings on the test day. All growth chambers had a 14-hr day with a fluorescent light intensity of 1,400 ft-c bench level. These test plants were set at room temperature 30 min before test aphids were placed on them. Fasted

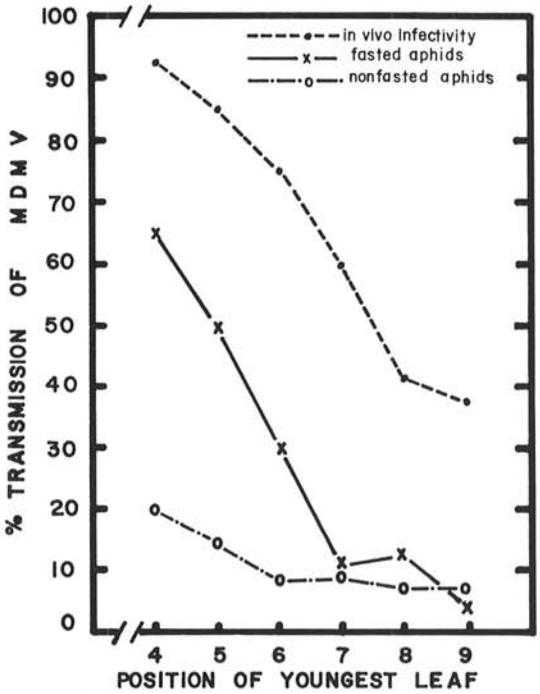

Fig. 1. A comparison of maize dwarf mosaic virus (MDMV) infectivity in vivo in corn leaves assayed by mechanical inoculation with ability of aphids to acquire and transmit MDMV at various ages of MDMV infection. When the youngest leaf was No. 4, the age of MDMV infection was about 1 week; when the youngest leaf was No. 9, the age of infection was about 4 weeks.

TABLE 1. Effect of fasting time on transmission of MDMV (maize dwarf mosaic virus) by single aphids after various acquisition times

Postacquisition fasting	Acquisition time, min[a]					
	1		5	10	15	20
	Rate of transmission[b]					
min	%		%	%	%	%
0	14	$P < .02$[c]	23	20	20	16
1	7	$P < .02$	15	16	13	10
5	6	$P \doteq .05$	12	11	8	4
10	3		5	6	4	3
15	0		4	3	1	1
20	0		1	1	1	1
25	0		1	0	0	0
30	0		0	0	0	0

[a] Aphids fed on the first fully expanded fourth leaf with systemic MDMV symptoms of early five-leaf-stage corn grown in a 21-C growth chamber. Corn was mechanically inoculated with MDMV at the two-leaf stage.

[b] Percentages were derived from a total of ca. 100 plants in two tests. Each test consisted of two replicates, 25 plants/replicate.

[c] Data were analyzed by the t-test. *P* values indicate the level of significance; all other comparisons are not significant.

aphids (groups) were allowed an acquisition time of 10 min on a fourth leaf with systemic symptoms on MDMV-infected source plants. The MDMV dilution end point in the source leaf was consistently 1:2,000. Each aphid was placed on a corn plant for assay after acquisition. For experiment II, corn plants were grown in a 21-C growth chamber to the four-leaf stage. These plants were divided into three groups and placed in three growth chambers at 15.5, 21, and 26.5 C for 1 hr before placing test aphids on them. The method of handling aphids was the same as in experiment I, except that all transmissions were done inside growth chambers.

RESULTS.–An increase of acquisition time for single aphids from 1 to 5 min increased the length of retention and transmission of MDMV. An acquisition of more than 10 min did not increase MDMV retention and transmission by aphids. Slight decreases in MDMV transmission were noted in prolonged (20 min) acquisition periods. The maximum retention of MDMV in an aphid was 25 min. The most efficient acquisition time was between 5-15 min at which the highest transmission was 20-23% (Table 1).

MDMV transmission by aphids was increased by the increase of the number of aphids per test plant. For example, when aphids had 10-min acquisition and no postacquisition fasting, MDMV transmission was 40, 68, and 85% at 5, 10, and 20 aphids/plant, respectively. The retention period remained the same, 25 min (Table 2), regardless of number of aphids involved.

Since aphids normally do not fast in nature except during migration, MDMV transmissions by fasted and

TABLE 2. Effect of fasting time on transmission of MDMV (maize dwarf mosaic virus) by groups of aphids transferred to test plants after 10 min acquisition

Postacquisition fasting	No. aphids/test plant[a]		
	5	10	20
	Rate of transmission		
min	%	%	%
0	40[b]	68	85
1	32	65	75
5	25	46	75
10	15	30	65
15	9	14	34
20	4	9	25
25	2	4	7
30	0	0	0
	NS[c]		
	S (5% level)[c]		

[a] Aphids fed on the first fully expanded fourth leaf with systemic MDMV symptoms of early five-leaf-stage corn grown in a 21-C growth chamber. Corn was mechanically inoculated with MDMV at the three-leaf stage.

[b] Percentages were derived from a total of 60 plants in two tests, three replicates/test.

[c] Data were analyzed by the F-test. S = significant; NS = not significant. No significant difference between values in columns 5 and 10 aphids/plant; all values between columns 5 and 20 aphids/plant were significantly different.

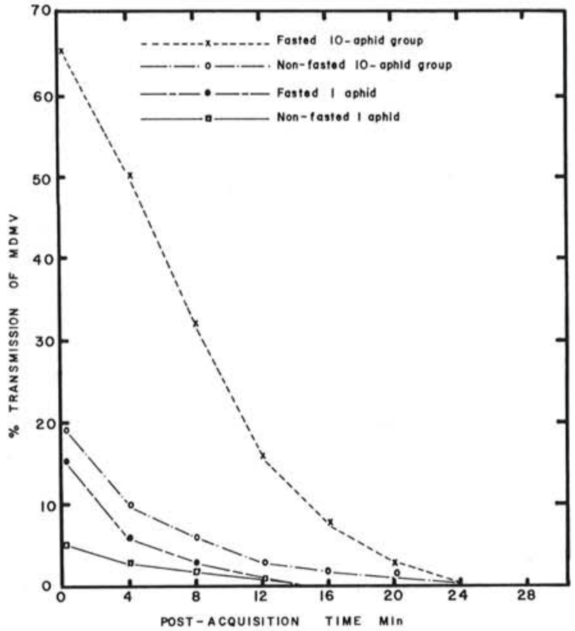

Fig. 2. Ability of fasted and nonfasted aphids to transmit MDMV (maize dwarf mosaic virus) at indicated postacquisition times. Aphids were fasted in a beaker for 1.5 hr, followed by a 10-min acquisition period. Percentages were calculated from a total of 60 plants in two tests, three replicates/test. Data were analyzed by the t-test. The only significant difference in transmission percentage between fasted and nonfasted single aphids was at 0 time ($.02 < P < .05$). All transmission percentages except 20 through 28 min were significantly different [($P < .01$) 0-12 min and ($.02 < P < .05$) 16 min] between fasted and nonfasted groups of 10 aphids.

nonfasted aphids were compared. Although fasted and nonfasted aphids retained MDMV equally long (Tables 1, 2; Fig. 2), fasted aphids were more efficient in MDMV transmission, especially shortly after acquisition (Table 1). For example, transfer of single-fasted aphids without postacquisition fasting resulted in 15% MDMV transmission, whereas only 5% of the nonfasted ones transmitted MDMV (Fig. 2). MDMV transmission percentages by fasted and nonfasted aphids were nonlinear functions of postacquisition time (Fig. 2). Thus, nonfasted or briefly fasted viruliferous aphids in mass migration could play an important role in the epiphytology of the maize dwarf mosaic disease of corn by their dissemination of MDMV.

Aphid transmission of MDMV in corn also was affected by the virus concentration in infected tissues. Aphids fed on a mosaic area versus a nonmosaic area of an infected leaf (Fig. 3) were compared for their transmission ability. Transmission was 3% and 13% from nonmosaic and mosaic areas, respectively. The same tissues were assayed by mechanical inoculation to determine virus concentration in vivo in corn. The inocula were saps obtained from mosaic and nonmosaic area and diluted 1:1 with neutral phosphate buffer (0.01 *M*) (Table 3). Nearly twice as many plants were infected

TABLE 3. Comparison of aphid and mechanical transmission of MDMV (maize dwarf mosaic virus) from mosaic and nonmosaic areas of infected leaves

Source	Aphid transmission[a]	Mechanical transmission[b]
Nonmosaic area	2/62[c] (3%)	26/48 (54%)
Mosaic area	6/48 (13%)	32/34 (94%)

[a] Aphids were fasted for 1 hr, allowed a 10 min acquisition period, and transferred, 1 aphid/test plant.
[b] Mechanical inoculation was made with 1:10 crude sap dilution in 0.01 *M* phosphate buffer, pH 7. Systemic mosaic symptoms usually appeared in the third leaf when corn seedlings were mechanically inoculated with MDMV at the one-leaf stage. The tip of the third leaf, however, usually had a wedge-shaped area which had no mosaic symptom (as shown in Fig. 1).
[c] Number of plants infected per total inoculated in two replications.

by mechanical inoculation with sap extracted from mosaic areas as with sap from nonmosaic tissues.

Further investigation was carried out to assess the relationship of the decline in infectivity in MDMV-infected corn leaves and aphid transmission of MDMV. In vivo infectivity decreased with age of MDMV infection when assayed by mechanical inoculation. MDMV transmission by fasted aphids, although much lower than mechanical transmission, was directly related to virus concentration in vivo in corn (Fig. 1). Nonfasted aphids had a lower percentage of transmission than did fasted aphids. Besides, the relationship between virus infectivity in corn and transmission by nonfasted aphids was less obvious than that of fasted ones (Fig. 1).

There were no differences in host susceptibility to aphid transmission of MDMV when corn was grown under different temperatures (15.5-26.5 C) when the assay plants were of uniform size and when they had been preconditioned 1 hr before transmission probes at room temperature. The average percentages of transmission were rather similar (13.3, 12.8, and 13.8) for plants grown at 15.5, 21, and 26.5 C, respectively (Table 4). When corn seedlings grown at 21 C were divided and moved into three growth chambers at 15.5, 21, and 26.5 C for 1 hr, and when aphid transmission experiments were made in each growth chamber, however, MDMV transmission was significantly lower (6.8%) at 15.5 than at 21 or 26.5 C (Table 5).

DISCUSSION.—We confirmed the work of Messieha (4) on the aphid transmission of MDMV, results of which are not reported here. Only those findings not previously reported for MDMV are included in this paper. The sugarcane mosaic virus strain H, which is related to MDMV, seems to be retained by some aphids longer than MDMV (4, 12). Likewise, maize mosaic virus in Yugoslavia seems to be quite similar to MDMV-A; yet *M. persicae* could transmit it after 2 hr of postacquisition fasting (11). Two other aphids were capable of transmitting maize mosaic virus after even longer fasting periods. One main difference was that the aphids in Yugoslavia acquired virus from Johnson grass, whereas in our work and that of Messieha (4), the aphids acquired the virus from corn.

The tendency for transmission efficiency of MDMV by *M. persicae* to decrease with prolonged acquisition time (Table 1) supports evidence of a reduction in transmission efficiency of potato virus Y by aphids (7) with acquisition periods up to 15 min. This reduction in transmission efficiency with increased acquisition time was seemingly amplified by increasing temperatures (7). Because MDMV is retained for only 25-30 min maximum [our data, supported by others (4)], compared with

TABLE 4. Aphid transmission of MDMV (maize dwarf mosaic virus) to corn at 21 C after corn seedlings had grown at three temperatures

Replicate	Temperature[a] 15.5		Temperature[a] 21		Temperature[a] 26.5
	Rate of transmission				
	%		%		%
I	12[b]		15		14
II	13		10		12
III	16		13		15
IV	12		13		14
Avg	13.25 ± 0.94		12.75 ± 1.02		13.75 ± 0.63
P value[c]		$.8 < P < .9$		$.4 < P < .5$	

[a] Corn plants were grown from seeding at these temperatures, and were placed in a 21-C chamber 1 hr before aphid transmission feedings at 21 C. Seeding dates were adjusted to assure uniform size seedlings from each chamber at test feeding time.
[b] Percentages were derived from ca. 50 plants/test.
[c] Data were analyzed by the t-test. *P* values showed that the differences between treatment of 15.5 and 21 C, and 21 and 26.5 C, were not significant.

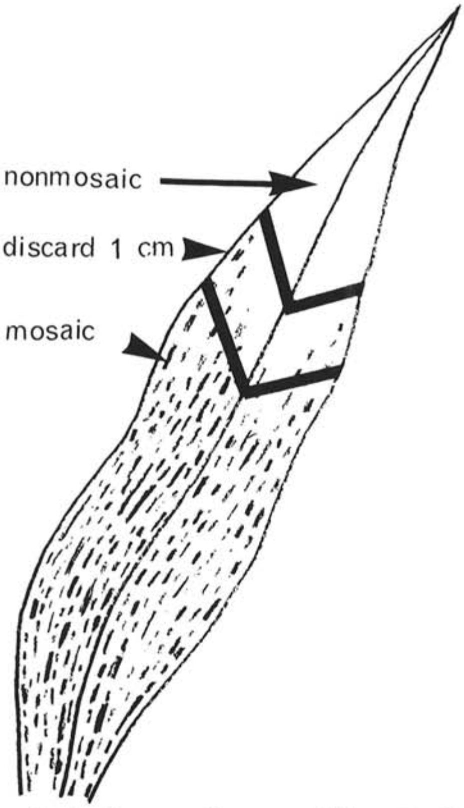

Fig. 3. A diagrammatic representation showing the mosaic and nonmosaic areas in the first systemically symptomed leaf of a maize dwarf mosaic virus (MDMV)-infected plant on which aphids were fed.

morphologically similar stylet-borne viruses (9, 11, 13) that are retained for much longer periods of time, the lability of the virus may be due to chemical breakdown, or inhibition, by saliva.

The age of the MDMV infection and plant age influenced the efficiency of MDMV transmission by aphids, probably primarily because MDMV concentrations vary at different ages of MDMV infection and in different ages of plants (12). The efficiency of aphid transmission of MDMV was generally related to virus concentration in vivo in corn leaves. Therefore, any factors (e.g., nutrition, host species, temperature, age of infection, etc.) that could affect MDMV concentration in vivo in corn probably would affect the efficiency of aphid transmission directly or indirectly.

Leaves of different ages on the same MDMV-infected corn, for example, have different virus concentrations (12). The different efficiences of *M. persicae* transmission of MDMV obtained in this study were mostly attributable to virus titer in the source plant due to age differences, as Swenson (9) found for bean yellow mosaic virus and *M. persicae*.

It is especially interesting that transmission of MDMV by nonfasted aphids (compared to fasted aphids) was least related to the virus infectivity in vivo in corn leaves (Fig. 1). We observed, however, that nonfasted aphids seemed more selective of feeding sites, and most of them settled and fed on yellower areas, whereas fasted ones most often fed where they were placed, with very little subsequent movement. Nonfasted aphids may have been attracted by the yellow mosaic areas, which had higher virus concentration than green nonmosaic areas.

The effect of preincubation temperature on corn seedlings did not alter the susceptibility to MDMV transmission by aphids, which could have been predicted from earlier work (9). Our results with MDMV corroborated Stimmann & Swenson's work (8) on the effect of preincubation temperature on the aphid transmission of cucumber mosaic virus. On the other hand, we found that aphids transmit MDMV differentially at different temperatures, especially between 15.5 and 21 C. The reason for this is not clear. Possibly, the low temperature (15.5 C) may affect the aphid feeding (probing activity) (7), or the plant may be more resistant at lower temperature. Other virus-vector combinations are similarly influenced by low temperatures (7). The time taken to begin probing and the duration of the probes are

TABLE 5. Aphid transmission of MDMV (maize dwarf mosaic virus) in controlled temperature growth chambers

	Temperature[a], C				
	15.5		21		26.5
Replicate	Rate of transmission				
	%		%		%
I	7[b]		14		15
II	6		16		14
III	9		12		10
IV	5		12		16
Avg	6.75 ± 0.66		13.50 ± 0.95		13.75 ± 1.31
P value[c]		.02 ⟨ *P* ⟨ .01		.9 ⟨ *P* ⟨ .8	

[a] Corn plants were grown at 21 C and placed at these temperatures 1 hr before aphid transmission feedings at these temperatures.

[b] Percentages were derived from ca. 50 plants/test.

[c] Data were analyzed by the t-test. *P* values showed that differences between temperature treatments of 15.5 and 21 C were significant, but not those of 21 and 26.5 C.

generally longer at lower temperatures (10).

MDMV is a stylet-borne (nonpersistent) virus with a relatively short retention period and low transmission rate. Probably for this reason, group aphid transfer improved the percentage of MDMV transmission. Our results suggest that, in nature, mass aphid migration from a diseased plant to the adjacent corn plants could spread the disease rapidly.

LITERATURE CITED

1. BUCHHOLTZ, W. F., R. E. FORD, & R. C. LAMBE. 1966. Maize dwarf mosaic virus in Iowa. Plant Dis. Reptr. 50:88.
2. CHEO, P. C., & G. S. POUND. 1952. Relation of air temperature, soil temperature, photoperiod, and light intensity to the concentration of cucumber virus I in spinach. Phytopathology 42:306-310.
3. KUHN, C. W. 1965. Decline of specific infectivity of cowpea chlorotic mottle virus in vivo. Virology 25:9-14.
4. MESSIEHA, MIMI. 1967. Aphid transmission of maize dwarf mosaic virus. Phytopathology 57:956-959.
5. ROSS, A. F. 1941. The concentration of alfalfa-mosaic virus in tobacco plants at different periods of time after inoculation. Phytopathology 31:410-420.
6. SIMONS, J. N. 1958. Titers of three nonpersistent aphid-borne viruses affecting pepper in south Florida. Phytopathology 48:265-268.
7. SIMONS, J. N. 1966. Effects of temperature and length of acquisition feeding time on transmission of nonpersistent viruses by aphids. J. Econ. Entomol. 59:1056-1062.
8. STIMMANN, M. W., & K. G. SWENSON. 1967. Aphid transmission of cucumber mosaic virus affected by temperature and age of infection in diseased plants. Phytopathology 57:1074-1076.
9. SWENSON, K. G. 1962. Bean yellow mosaic virus transmission by Myzus persicae. Aust. J. Biol. Sci. 15:468-482.
10. SYLVESTER, E. S. 1964. Some effects of temperature on the transmission of cabbage mosaic virus by Myzus persicae. J. Econ. Entomol. 57:538-544.
11. TOSIC, M., & D. SUTIC. 1968. Medusobni odnos virusa mozaika kukuruza i nekih njegovih vektora. Mikrobiologija 5:73-78.
12. TU, J. C., & R. E. FORD. 1969. Infectivity change of maize dwarf mosaic virus in vivo and in vitro. Phytopathology 59:1947-1949.
13. ZUMMO, N., & L. J. CHARPENTIER. 1964. Virus-vector relationship of sugarcane mosaic virus. I. Transmission of sugarcane mosaic virus by the brick-red sowthistle aphid (Dactynotus ambrosiae Thos.). Plant Dis. Reptr. 48:636-639.