# Determination of Cucumber Mosaic Virus Titer in Muskmelon by Enzyme-linked Immunosorbent Assay and Correlation with Aphid Transmission

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**ABSTRACT**

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Percentage transmission by individual *Aphis gossypii* and spectrophotometric readings in indirect enzyme-linked immunosorbent assay (ELISA) were used to estimate cucumber mosaic virus (CMV) titers in muskmelon plants over a 7-wk period. Results indicated a high correlation between the two techniques with two distinct CMV isolates. One week after inoculation, the frequency of aphid transmission of CMV-243 (virulent isolate) was significantly higher than that of CMV-241 (mild isolate), and ELISA absorbance readings at 405 nm were significantly higher for isolate CMV-243. The apparent rapid increase of CMV-243 titer 1 wk after infection could provide one explanation for the difference in spread of the two CMV isolates observed in the field. Similar tests were conducted weekly, comparing mixed infections of CMV-243 and watermelon mosaic virus strain 2 (WMV-2) to single infections of each virus. There was no apparent difference in levels of aphid transmission or ELISA values from plants infected with the viruses singly or with mixed infections.

A survey of the viruses that affect cucurbit crops in three areas of New York State was made in 1980 and 1981 (1). The survey results indicated that cucumber mosaic virus (CMV) and watermelon mosaic virus strain 2 (WMV-2) were the major cucurbit viruses in the state. Both viruses occurred throughout the state in approximately equal proportions and, depending upon when plants were sampled, frequently occurred as mixed infections. The spread of CMV on a muskmelon farm in Washington County (upper Hudson River Valley of New York) appeared to be unusually rapid when compared to the spread of CMV on a mixed vegetable farm 24 km away.

The main factors that affect secondary virus spread in a susceptible crop, other than environment, are the number of vectors present, their vectoring habits, the inherent transmissibility of the virus strain, and the virus titer in the plant (7). Virus titer in a plant may be governed by either the virus strain involved (5), the presence of other viruses (6), host susceptibility (9), or a combination of these factors. Virus titer in a given host has been directly correlated with the efficiency of aphid transmission and the number of local lesions produced on an appropriate host (6,8,9,11). The purpose of this study is to report on the use of enzyme-linked immunosorbent assay (ELISA) and aphid transmission by *Aphis gossypii* (Glover) to characterize the behavior of two CMV isolates and to determine the influence of WMV-2 in mixed infections with CMV on aphid-transmission of CMV. A preliminary report of this study has appeared (2).

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## MATERIALS AND METHODS

Several CMV isolates were collected from each of two farms in Washington County, New York, during the summer of 1980. One isolate was chosen at random from each location for use in further experiments. The isolates selected did not differ from other isolates from the same location in terms of symptom severity in muskmelon (*Cucumis melo* L. 'Saticoy') and zucchini (*Cucurbita pepo* L. 'Zucchini Elite'). Isolate CMV-243 came from a muskmelon farm with a long history of losses to virus diseases and from where infected plants could be found before the end of June. Rapid virus spread occurred in these fields during July, resulting in nearly complete infection by mid-August. Isolate CMV-241 came from a nearby farm and closely typified CMV isolates in other locations in the state by first appearing in July and spreading at a slower rate. One WMV-2 isolate was collected on the farm from which CMV-243 was obtained and was selected for further experimentation in the same manner as the CMV isolates.

The relative titers of CMV-243 and CMV-241 were compared in four trials. In each trial a Saticoy muskmelon source plant in the primary leaf stage was inoculated via aphids with the appropriate isolates. Each source plant was maintained in an environmental control chamber (with a 14-hr photoperiod, a 24/18 C day/night temperature, and 78/68 day/night relative humidity) and sampled weekly for 7 wk following inoculation by excision of one expanded leaf from near the growing point of the plant. Relative virus concentration in that leaf was then estimated by single aphid transmission efficiency and indirect ELISA, both performed at the sampling time.

To test for aphid transmission efficiency, *A. gossypii* reared in an insectary room on *Cucumis sativus* L. 'Marketmore 76' under continuous light at 25 C were fasted for 4 hr before being placed in groups of five on each source plant leaf. For each group of aphids, a single aphid was observed to probe continuously for 45–60 sec; then it was transferred to a Saticoy muskmelon test plant (one aphid per test plant). The procedure was repeated until 20 test plants were inoculated for each test interval (for example, a total of 80 test plants were aphid-inoculated during the four trials for each weekly interval and for each CMV isolate). The test plants were 16 days old (in the primary leaf stage) and were grown in environmental chambers using conditions previously described. Following a 15-min transmission feeding

period, the test plants were fumigated and placed in the greenhouse for observation. Percentage transmission to the Saticoy test plants was recorded visually 3 wk after inoculation.

Following aphid transmission, the same excised source plant leaves were ground individually in a mortar and pestle with 1:10 w/v dilution of sodium carbonate buffer, pH 9.8. The macerate was centrifuged at 7,000 rpm for 10 min and the supernatant saved. A dilution series of 1:10, 1:100, 1:1,000, and 1:10,000 was made with the supernatant serving as the 1:10 dilution, and 200 $\mu$l of each dilution was dispensed into each of three wells of round-bottom microtiter ELISA plates (Dynatech Laboratories, Inc., Alexandria, VA). Wells containing healthy tissue, coating buffer, and an appropriate standard were included. Standards used were purified CMV extracts obtained from D. Gonsalves, New York State Agricultural Experiment Station, Geneva, and reconstituted freeze-dried WMV-2 infected tissue, obtained from D. Purcifull, University of Florida, Gainesville.

The indirect ELISA procedure described by Voller, et al was followed (10). The immunoglobulin G (IgG) concentration used was 1 $\mu$g/ml and the conjugate concentration was a 1:1,000 dilution of the antirabbit IgG alkaline phosphatase conjugate (Sigma Chemical Co., P.O. Box 14508, St. Louis, MO). The substrate was allowed to react for 25 min, and then 50 $\mu$l of 3 *M* NaOH was added to each well to stop the reaction. Absorbance values were measured at 405 nm with a Microelisa Auto Reader (Dynatech, Alexandria, VA).

The relative titers for CMV-243 and WMV-2 in single and mixed infections were compared using the procedure described above. Two 6-wk long trials were performed. A single aphid was observed to probe on the source plant leaf for 20–30 sec, and this was repeated until 20 test plants were inoculated. Percentage aphid transmission for single infections of CMV and WMV-2 to Saticoy test plants was noted visually 3 wk after inoculation. Percentage aphid transmission for mixed virus infections to the test plants was determined by indexing each test plant by indirect ELISA 3 wk after inoculation.

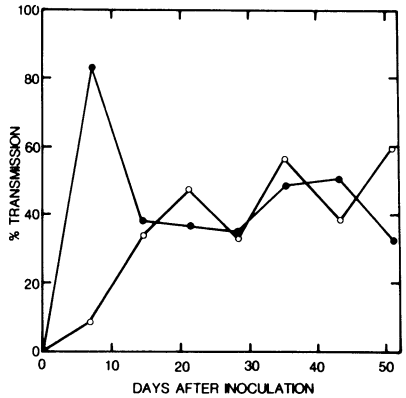


**Fig. 1.** Comparison of the transmission by *A. gossypii* of CMV-243 (●) and CMV-241 (○).

## RESULTS

**CMV transmission studies.** The average percent transmission efficiency from the four trials comparing the aphid transmission of CMV-243 with CMV-241 are plotted vs. sampling day in Fig. 1. CMV-243 was transmitted by 83.75% of the aphids 1 wk after inoculation, whereas CMV-241 was transmitted by only 8.75% of the aphids at the same time period. The difference is significant at the 0.1% level when analyzed with Student's *t* test. An analysis of variance indicates that there is no significant difference in percentage transmission between the two isolates at any of the remaining six sampling times, with both isolates averaging about 40% transmission. A significant difference was found ($P = 0.01$) between the percentage transmission at week 1 vs. weeks 2–7 for both isolates.

The ELISA readings obtained for CMV-243 and CMV-241 over the 7-wk period appear in Fig. 2. Each data point represents the average ELISA reading from four source plant leaves tested in 3 wells each. In analyzing these data, it was found that using the raw ELISA readings was adequate in obtaining the correlation coefficient *r* values. No appreciable increase in these values was obtained when ELISA readings that had been corrected using the purified CMV standards were used. This suggests that there was less variation than expected between ELISA tests conducted at different times. The 1:10 dilution of plant sap was used for these calculations because it gave readings within the linear absorbance range, and provided more consistent readings at low virus concentrations than at the other dilutions. The ELISA readings and the arcsin transformation of the percentage transmission were correlated to give correlation coefficients of 0.78 and 0.74 for CMV-243 and CMV-241, respectively. These correlatives are significant at greater than the 0.1% level. The correlative coefficients (*r*) were obtained using the following formula to correct for weekly variations using the purified CMV standard:

$$^{r}AES = \frac{^{r}AE - (^{r}SA)(^{r}SE)}{\sqrt{(1 - r^2_{SA})(1 - r^2_{SE})}}$$

where A = average weekly aphid transmission efficiency, E = average weekly ELISA reading, and S = average weekly standard ELISA reading.

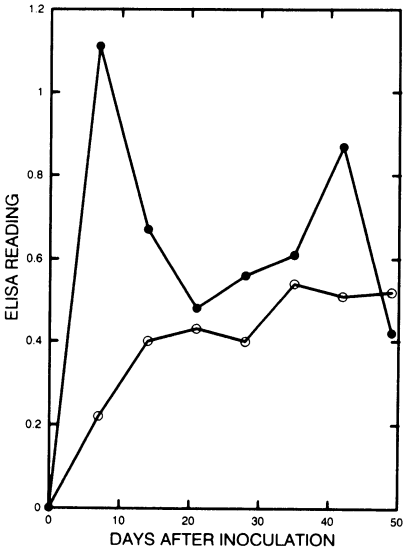


**Fig. 2.** Average ELISA readings (absorbance at 405 nm) obtained with CMV-243 (●) and CMV-241 (○) following the relative virus titer over a 7-wk period.

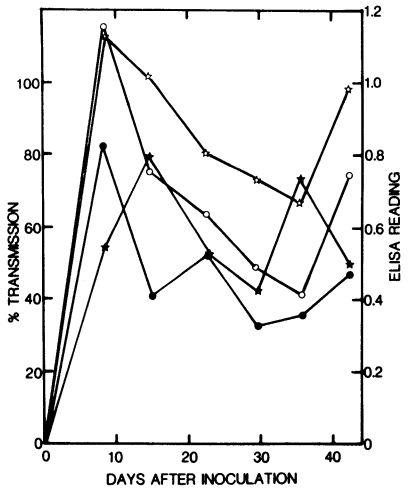


**Fig. 3.** Average percentage transmission by *A. gossypii* of CMV-243 in single (●) and mixed (★) infection with WMV-2, along with the corresponding ELISA readings from single (○) and mixed (☆) infection.

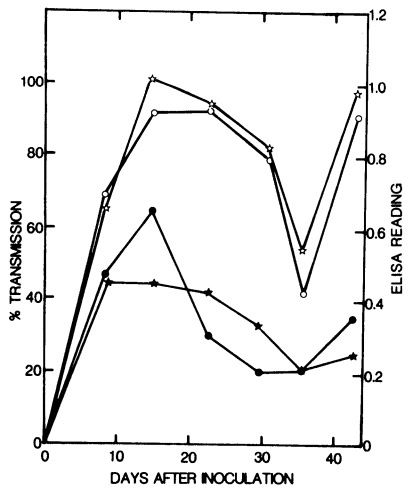


**Fig. 4.** Average percentage transmission by *A. gossypii* of WMV-2 in single (●) and mixed (★) infection with CMV-243, along with the corresponding ELISA readings from single (○) and mixed (☆) infection.

**Time of symptom development for the CMV isolates.** Efficient aphid transmission of CMV-243 1 wk after inoculation and high ELISA values clearly distinguished it as a "virulent" isolate. This rapid increase in titer of CMV-243 was also reflected in the time of symptom development of plants infected with this isolate. Only 12 days were required for all CMV-243-infected test plants to express symptoms, compared to 18 days for all test plants inoculated with CMV-241 to develop symptoms.

**Mixed infection titer studies.** Average percentage transmission from two trials comparing the transmission of CMV-243 alone and in mixed infection with WMV-2, along with corresponding ELISA readings, are plotted in Fig. 3. The same information for WMV-2 is presented in Fig. 4. In the case of aphid transmission of CMV there was a 1-wk delay in achieving maximum transmission in mixed vs. singly-infected plants, followed by a sharp decline in transmission in both cases. The highest ELISA readings occurred at the 1-wk sampling date but were still high at the second week. When differences in aphid transmission and ELISA values from single and mixed infections were analyzed on the same sampling date, no significant difference was found. Aphid transmission of WMV-2 was greatest in singly-infected plants at the 2-wk sampling and corresponded with the highest ELISA readings. No statistical difference was found in transmission and ELISA values between single and mixed infections for WMV-2.

The average correlation coefficients between the ELISA readings and the arcsin transformation of the percentage aphid transmission were as follows: CMV alone, 0.848; CMV from the mixed infection with WMV-2, 0.009: WMV-2 alone, 0.515; WMV-2 from the mixed infection with CMV, 0.085. The correlation for CMV alone is significant at the 1% level. The correlation for WMV-2 alone is significant only at the 20% level, and there is no correlation between ELISA and aphid transmission for either virus in a mixed infection. These correlation coefficients were calculated using the formula described above and using freeze-dried, reconstituted WMV-2 infected tissue as the WMV-2 standard.

## DISCUSSION

The farm from which CMV-243 originated has been used for muskmelon production for nearly 60 yr, and currently about 10 ha of melons are grown. The second farm has been devoted to mixed vegetable production during most of the same period. The preferential selection of a particularly efficiently transmitted isolate like CMV-243 could be a contributing factor to the almost annual early loss of melon production due to virus diseases for the former farm.

The inherent differences between the two isolates was also apparent in the shortened time for symptom expression for CMV-243. The behavior of CMV-243 in muskmelon appears to be unique when compared with CMV isolates studied in other virus-host interactions, because it achieves a high relative virus concentration and aphid transmissibility just 1 wk after inoculation. Most isolates react more like CMV-241, showing a gradual increase in virus titer and reaching a peak concentration of infective virus in certain leaves of crops like pepper, bean, and celery with the titer not maintained in these or subsequently infected leaves (6,8,11).

Mixed infections of CMV and WMV-2 had a minimal affect on the transmissibility of either virus and probably would not be a significant factor affecting the degree of field spread in either case. An earlier study involving inoculation of cucurbits with CMV and WMV-2, either singly or in combination, showed no evidence for synergism or interference between these viruses in greenhouse or field experiments (3). The most apparent affect on CMV transmission in a mixed infection with WMV-2 was a 1-wk delay in achieving maximum aphid transmission. A similar response was observed previously when CMV titers in celery were studied in singly or mixed infected plants with celery mosaic virus (11). The lack of a good correlation between ELISA and transmission of CMV from mixed infections may be explained in part by comparing the results of only two trials but may just as easily result from reduced opportunity for single aphids to acquire CMV from mixed infections.

Aphid transmission and spectrophotometric ELISA readings for both CMV isolates were highly correlated. This suggests that in this particular virus-host interaction, both techniques were useful for estimating relative virus concentration. It appears that the efficient transmission of CMV-243 is due to a rapid increase of virus titer in the plant in as little as 1 wk after inoculation, and may not be due to inherently higher aphid transmissibility of the virus itself. This latter point would have to be examined in separate studies.

ELISA readings and aphid transmission were not as highly correlated for WMV-2 as CMV, although the general trends were similar. This may indicate that the relative virus concentration in the plant is not as important a factor for aphid transmission of WMV-2.

With the apparent close correlation between ELISA readings and the efficiency of aphid transmissions of CMV, the potential exists for the use of ELISA in the search for CMV resistance in muskmelon. Lecoq, et al (4) have demonstrated that certain "resistant" muskmelon accessions are less efficient virus sources than others, possibly because of a lower virus titer. ELISA may make it possible to screen for such resistance efficiently on a large scale.


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