Transmission of Pepper Mottle Virus From Susceptible and Resistant Pepper Cultivars

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ABSTRACT

Virus transmission by the green peach aphid, *Myzus persicae,* and by sap inoculation, was used to study the behavior of pepper mottle virus (PMV) in susceptible and resistant pepper cultivars. Both methods of transmission gave comparable results. The two susceptible cultivars, 'Early Calwonder' (EC) and breeding line '23Y', and the resistant line 'AV23Y' were equally susceptible as test hosts using either method of inoculation. The susceptible cultivars behaved similarly as virus source plants, with leaf position on the source plant, and time after inoculation, having little effect on the percentage of aphid transmission to EC. Transmissions by one aphid per test plant averaged 70%.

Virus transmission from the susceptible cultivars was high at 1-2 weeks after inoculation, and remained high during the 7-week assay period. Virus acquisition from the resistant source plants was low, and indicated reduced virus transmission. Transmission by aphids from the resistant source plants was 60% lower than from the susceptible cultivars. Greatest transmission from the resistant plants was reached 3 weeks after inoculation, with aphids acquiring virus most efficiently from the lower leaf position.

Transmission by aphids for PMV from the susceptible pepper cultivars appears to be equivalent to the transmission achieved for other viruses of the PVY-group.

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Zitter and Ozaki (9) have reported substantial differences in the amount of infection observed when susceptible and resistant pepper varieties were exposed to a natural virus complex in south Florida. The commercially grown variety of *Capsicum annuum* L. 'Early Calwonder' (EC), is susceptible to at least four naturally occurring viruses. These include two strains of tobacco etch (TEV-C and TEV-A), a common strain of potato virus Y (PVY-C), and an additional virus named pepper mottle virus (PMV) (7). In a severe epidemic year, this virus complex has caused 100% infection of pepper plants in commercial fields 6 weeks after the first diseased plants were found. A Florida pepper breeding line designated '23Y' is susceptible to only two of the four viruses (TEV-A and PMV), and this variety did not reach total infection as readily as EC in two of the three years that field spread was studied (9). Additional breeding lines obtained by crossing the Brazilian variety 'Avelar' (AV) with line 23Y (AV23Y) (8) are immune to TEV-C and PVY-C while expressing resistance to TEV-A and PMV. These cultivars exhibit few virus symptoms or apparent virus spread in the field.

Greenhouse inoculation and transmission studies by mechanical means and by aphids were conducted using PMV to verify varietal susceptibility differences observed in the field, to establish the plant-virus relationship of PMV in EC, 23Y, and AV23Y, and to determine the nature of resistance of AV and its crosses to PMV.

MATERIALS AND METHODS.—The original isolate of PMV obtained from a commercial pepper field in eastern Palm Beach County in 1971 (7) was used throughout this study. The virus was maintained in EC stock plants by aphid and mechanical inoculations.

Seeds for pepper line 23Y and the cross AV23Y (homozygous F_3 and F_4 generation) were obtained by selfing stock plants in a greenhouse.

Nonviruliferous green peach aphids (*Myzus persicae* Sulzer) were reared on caged, healthy EC plants. Late instar and adult apterae were used as vectors. Aphids were starved for 3-4 hours before being given observed and timed acquisition feeding periods. When mass aphid transfers were used, six aphids were given an acquisition period lasting 2 min before being transferred. When one aphid was used per test plant, an acquisition period of less than 1 minute and generally 15-20 seconds was found to be sufficient. Pepper test hosts used in aphid trials in both instances were generally in the two- to four-leaf stage.

Preliminary experiments to compare transmission of PMV by aphids and mechanical means from susceptible and resistant source plants were conducted using EC and AV, respectively. The virus source plants were prepared as follows. Six EC and AV seedlings in the two-leaf stage, and with the third and fourth leaves beginning to expand, were mechanically inoculated by rubbing the cotyledons and leaves 1 and 2 with a crude sap preparation of PMV. At 1, 2, 3, 4, and 5 weeks after inoculation, either inoculated or fully expanded leaves near the plant top were selected for either aphid or mechanical inoculation. Aphid inoculations were performed by mass aphid transfer using 4-8 EC test hosts. In the case of mechanical

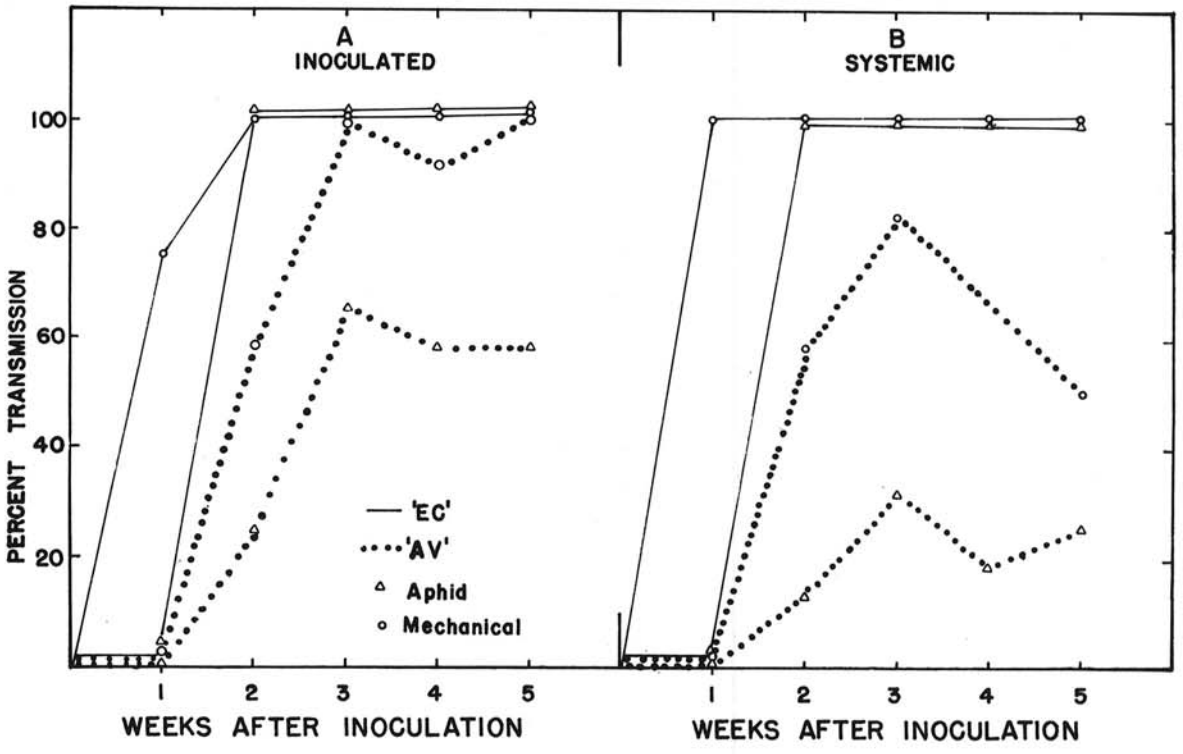

Fig. 1-(A,B). Comparison of weekly transmission of pepper mottle virus (PMV) from Early Calwonder (EC) and Avelar (AV) by mass aphid transfer and mechanical inoculation to Early Calwonder using **A)** inoculated or **B)** systemically infected leaves.

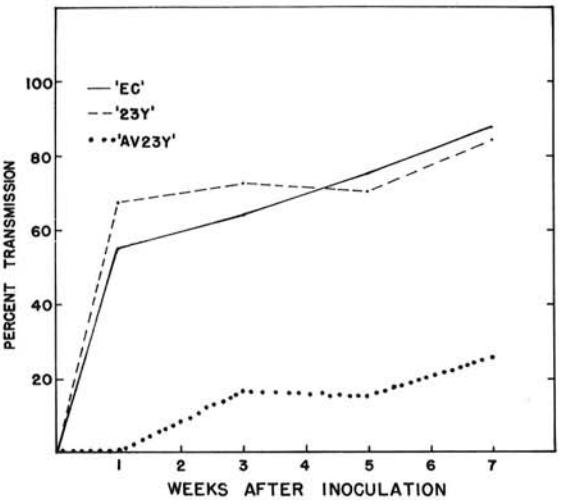

Fig. 2. Transmission of pepper mottle virus (PMV) from Early Calwonder (EC), line 23Y and line AV23Y using one aphid per Early Calwonder test host.

TABLE 1. Transmission of pepper mottle virus (PMV) from three leaf positions on AV23Y resistant plants to Early Calwonder using one aphid per plant

Weeks after inoculation	Percentage transmission[a]		
	Lower	Middle	Upper
1	0	...[b]	...
3	40	5	0
5	33	5	0
7	25	15	0

[a]Based on 20 inoculated plants each week.
[b]Leaves in this position not available.

inoculations, several leaves from the same leaf position were pooled to supply the needed tissue. Crude sap was prepared by grinding the leaves in a mortar with 1-2 ml of 0.01 M phosphate buffer, and the juice was rubbed onto four EC test plants.

In later studies, the three pepper cultivars (EC, 23Y, and AV23Y) served as both virus source and test hosts for assay 1, 3, 5, and 7 weeks. The virus source plants were prepared as before, but all transmission attempts were made using one aphid per test plant with 20 test plants used for each test. When the three pepper cultivars were used as test hosts, they were inoculated at random to insure an equal chance of infection.

When it became apparent that leaf location on the virus source plant, especially on the resistant plants, was important for virus transmission, leaves were grouped into three categories during the 7-week experiment. They were termed lower (leaves 3 and 4), middle (leaves 5, 6 and 7) and upper (leaves 8 and above). In most cases plant branching occurred above the 9th or 10th leaf by the end of the fourth week making additional leaf counting impractical. Leaves from the three positions were selected at random from four to eight stock source plants.

Symptoms of PMV were clearly evident on susceptible test host 7-10 days after inoculation, while symptom appearance in the resistant plants required 2-3 weeks. Resistant plants free of virus symptoms were indexed for virus using immunodiffusion tests, as described previously (7).

Plants were routinely sprayed with nicotine sulfate to control aphids in the greenhouse.

RESULTS.—*Transmission of PMV from EC and AV by aphid and mechanical inoculations.*—Transmission of PMV by either mechanical or mass aphid transfer gave comparable results when either inoculated (Fig. 1-A) or systemically infected leaves (Fig. 1-B) were used as the virus source. Virus transmission from EC reached a high level 2 weeks after inoculation and remained near 100% thereafter. Both mechanical and aphid inoculation methods demonstrated a gradual increase in virus transmission from AV, with a peak noted 3 weeks after inoculation from both leaf sources. Virus transmission from the inoculated leaves of AV was higher using either method of transmission.

Transmission of PMV by aphids 3 weeks after inoculation from the two cultivars showed marked differences. Transmission from EC was 34% and 69% greater than from resistant plants when either the inoculated or systemically infected leaves were assayed.

Because aphids effectively transmitted PMV, and are the natural means of spread in the field, subsequent inoculations were made using one aphid per test plant.

Effect of three virus source plants on the transmission of PMV.—Transmission of virus from EC, 23Y and AV23Y to EC test plants for four assay periods is shown in Fig. 2. Results related well with those obtained in the preliminary experiment. Virus transmissions from EC and 23Y were substantial 1 week after inoculation, and tended to increase in the ensuing weeks. Aphids appeared to acquire PMV more readily from 23Y in the initial stages of the experiment, but transmission from both varieties were similar at the end of 7 weeks. Virus transmission from AV23Y first peaked at 3 weeks, and increased slightly again at 7 weeks. Transmissions from EC were 44% higher than from the resistant plants when assayed at 3 week inoculation, and these differences increased to 62% at the end of 7 weeks.

Effect of leaf position on resistant plants on virus transmission.—In both previous experiments it was noted that symptom development in AV and AV23Y plants took 1-2 weeks longer than in the susceptible cultivars and when symptoms did appear they were most noticeable on the lower inoculated leaves. An aphid's ability to acquire virus from three leaf positions during 7 weeks, and how this related to visible symptoms, was therefore investigated. AV23Y plants infected for 1, 3, 5, and 7 weeks served as the virus source, while 20 EC test hosts were inoculated for each test using one aphid per plant. Results are shown in Table 1. Aphids failed to acquire virus from the lower leaf position at the 1 week inoculation, but virus was recovered from this position in the ensuing weeks. Virus transmissions again peaked 3 weeks after inoculation, but only in the lower leaves, and then decreased during the next 4 weeks. Virus transmission increased slightly for the middle leaf position, and no virus was acquired from the upper position during the 7-week period.

Effect of virus test host on the transmission of PMV.—The three pepper cultivars EC, 23Y, and AV23Y

were used as virus test hosts to determine their effect on virus transmission. Susceptible EC plants served as the virus source plant for assay at 1, 3, 5, and 7 weeks after inoculation. In addition, leaves from three positions on the source plants were harvested for inoculation to determine if leaf location on a susceptible source plant was as important as previously noted with AV23Y. The lower leaf position was assayed four times, the middle position three times, and the upper position twice. Thus the three cultivars were used nine times as test hosts during the 7-week period.

Except at the end of the first week, the three test hosts showed much variation in susceptibility, depending on leaf location and time after inoculation (Fig. 3). However, when the nine inoculations were averaged together, the three test hosts were very similar in relative infectibility. The two susceptible cultivars averaged 75% and 68% infection for EC and 23Y respectively, while resistant AV23Y averaged 72% infection.

Infection based on leaf position also showed variability for any given week, but when averaged together the differences were minor. The percent transmissions from the three positions on EC were 73, 68, and 75% for lower, middle, and upper leaf positions, respectively.

The percent transmission of virus from EC regardless of leaf position and test host for each inoculation period are given in Fig. 3. When the data for these four periods are added together, the transmissions averaged 70%.

Mechanical inoculation of the three test hosts with PMV from EC source plants also produced similar results. Seedlings inoculated in the three- to four-leaf stage with a 1:10 dilution (w/v) of the inoculum resulted in 29 of 30 infected plants for both EC and 23Y, and 27 of 30 for AV23Y. One notable difference was the length of incubation period for the three cultivars. Symptoms were detected after 5 days in 23Y, one day sooner than in EC, while approximately 3 weeks were needed for visual detection of infection in AV23Y.

DISCUSSION.—The importance of virus source plant and test host in determining aphid transmissibility of virus diseases has previously been noted (1, 2, 4, 6). These factors also influence the amount of virus spread in the field (4). In the present study, the three pepper cultivars were similar in terms of their relative susceptibility when used as virus test hosts, but differed when used as virus source plants.

The two susceptible plants were both better virus sources than the resistant line. This would imply higher virus availability in the susceptible varieties, and thus facilitate greater aphid acquisition of virus. Aphids acquired virus more readily from 23Y than from EC in the very early stages of infection, a fact which is consistent with the shorter incubation period noted for this cultivar. The resistant line, on the other hand, showed no recoverable virus from inoculated or systemically infected leaves until 2 weeks after inoculation with a peak of activity noted 1 week later.

Inoculation by a single aphid, or by mechanically rubbing several leaves per plant, gave similar results, as was found by Simons (3) in earlier studies.

The leaf position chosen for assay had little effect on virus transmission from susceptible plants; however, leaf position in resistant plants greatly influenced the transmission. Most virus was recovered from resistant-plant leaves selected from the lower position at the 3-week inoculation, which coincided with symptom appearance on these leaves.

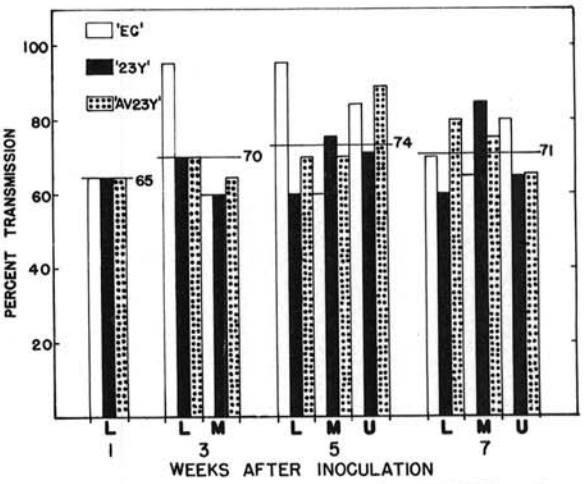

Fig. 3. Transmission of pepper mottle virus (PMV) from three general leaf positions on Early Calwonder to three pepper test hosts using one aphid per plant. Leaf positions sampled were L = lower, M = middle and U = upper. Average transmission is shown for each inoculation period.

The nature of resistance of AV and its crosses to PMV is believed to depend on reduced virus increase. This greatly reduces an aphid's ability to acquire and transmit virus, and has been shown to be an important factor in reducing within-field virus spread (9). The role of inhibitory compounds in conferring resistance of 'Italian El' (IE) to PVY has been noted previously (4, 5). The present situation differs from that case in several ways. First, our resistant line was as suceptible to PMV as EC and 23Y when used as test hosts, whereas IE was a poor test host for PVY when compared with Calwonder. Secondly, our resistant line was a very poor source for PMV compared with the two susceptible cultivars. IE, on the other hand, was as good a source for PVY as Calwonder.

The plant-virus relationship of PMV in susceptible pepper is very similar to that of PVY (3). Both are efficiently transmitted by the green peach aphid (70%) in a stylet-borne manner, and both maintain high and constant virus titers in susceptible pepper cultivars.

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