Studies on the Reason for Differential Transmissibility of Cauliflower Mosaic Virus Isolates by Aphids

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Kentucky Agricultural Experiment Station Journal Series Paper No. 72-11-164.

Accepted for publication 25 January 1973.

ABSTRACT

Lack of aphid transmissibility of certain isolates of cauliflower mosaic virus (ClMV) could not be attributed to low virus concentration in entire leaves or in epidermal cells. A nontransmissible isolate had the same distribution and location in plant cells (determined by electron microscopy) as did transmissible isolates, and did not differ from the transmissible isolates in the number and distribution of inclusion bodies (determined by light microscopy). Virus particles of a nontransmissible isolate migrated at a rate intermediate to that of two transmissible isolates in agarose-acrylamide gel electrophoresis.

The normally nontransmissible isolates could be transmitted by aphids from plants which were also infected with a transmissible isolate, or by aphids which had previously probed plants infected with a transmissible isolate. The results suggest that a factor necessary for aphid transmission of ClMV is present in leaves of plants infected with transmissible isolates that is not present in leaves infected with nontransmissible isolates. This factor may be acquired by aphids which probe leaves infected with transmissible isolates, subsequently enabling them to transmit the normally nontransmissible isolates.

Phytopathology 63:910-914.

Factors responsible for differences in aphid transmissibility were studied with five isolates of cauliflower mosaic virus (ClMV). This virus was chosen because (i) isolates were found which differed in aphid transmissibility, (ii) the relatively large size of the virus makes it easily identifiable by electron microscopy of infected cells, and (iii) the virus may be readily purified, thus making physical-chemical and in vitro transmission studies feasible. Some of the data have appeared in an abstract (4).

MATERIALS AND METHODS.—Aphids, *Myzus persicae* (Sulz.) were reared on 'Tendergreen' mustard plants (*Brassica pervirdis* Bailey) in a growth chamber at 24 C under 14-hr light period and were transferred to fresh plants every week.

The Campbell, CM 1841, Cabbage B, KK, and New York 8153 isolates of ClMV were supplied by R. J. Shepherd, University of California, Davis. All isolates were maintained in desiccated leaf pieces at 2 C to avoid possible intermixing of isolates. Ten to 15 days before each test, mustard plants to be used as virus source plants for transmission experiments were inoculated from the stock cultures. Mustard seedlings in the cotyledonary stage were used as test plants in transmission tests.

Aphid transmission tests.—The source of virus for aphid transmission tests was either (i) intact young leaves with fully developed systemic symptoms, (ii) leaf disks cut from such leaves, or (iii) epidermis stripped from systemically infected leaves. The techniques described by Normand & Pirone (7) were used for manipulation of the source tissue, aphids, and test plants, except as otherwise noted.

Infectivity assays.—The whole young leaves, leaf

disks, or epidermal strips which had been used as virus sources for aphid transmission tests were assayed for virus concentration by the dilution end point method. Leaf disks 8 mm in diameter or epidermal strips weighing 0.05 g were triturated in 0.5 ml of distilled water. A series of 10-fold dilutions was then made and assayed on young mustard plants. Inoculum was applied with a cheesecloth pad to leaves which had been dusted with 600-mesh Carborundum.

Electron microscopy.—Small pieces of leaf tissue and epidermal strips from systemically infected mustard plants which had been used for aphid transmission and infectivity assay tests were fixed with 6% glutaraldehyde in 0.1 M potassium phosphate buffer (pH 7.5) for 2 hr at room temperature, and washed with the phosphate buffer. The specimens were postfixed with 2% osmium tetroxide in 0.1 M potassium phosphate (pH 7.5) for 3 hr at room temperature. After fixation, specimens were dehydrated in increasing concentrations of ethanol followed by propylene oxide, and embedded in Epon 812. Thin sections were double-stained with uranyl acetate and lead citrate.

Light microscopy of inclusion bodies.—Epidermis was stripped from infected leaves and stained with 1% Phloxine B in 1% NaCl. Inclusion bodies stained red and were easily distinguishable.

Electrophoretic mobility.—The virus isolates were propagated in mustard and purified by the method of Pirone et al. (9). Agarose-acrylamide gels were prepared by techniques similar to those described by Peacock & Dingman (8). Four stock solutions were used in the preparation of the gels (i) 20% acrylamide monomer solution, made by adding 19 g of acrylamide and 1 g of N, N-methylenebisacrylamide to 100 ml of water; (ii) 6.4% dimethylaminopropionitrile in water; (iii) 1.6% ammonium persulfate in water; (iv) 0.02 M potassium phosphate buffer (pH 7.5).

The gel was prepared by adding 80 ml of distilled water to 0.8 g agarose. The mixture was stirred vigorously and refluxed at 100 C for 15 min and then cooled to 40 C. Buffer (11.3 ml), dimethylaminopropionitrile (7 ml), and acrylamide monomer solution (11.3 ml) were mixed and warmed to 35 C. The agarose and acrylamide solutions were mixed, the temperature was adjusted to 35 C, and 4.5 ml of 1.6% ammonium persulfate was added. The completed gel was mixed and poured rapidly into glass columns. The gel column was approximately 0.5 X 5.0 cm. No stacking gel was used. Potassium phosphate buffer (0.02 M, pH 7.5) was used in the electrode compartments.

Virus samples in 0.02 M potassium phosphate buffer pH 7.5 were mixed with 10% sucrose which contained enough bromphenol blue to give a visible color. A 50-μl aliquot which contained 10.5 μg virus was ten layered on the gel column. The upper (sample) end of the column was connected to the negative terminal of the power supply.

All gel columns were purified by pre-electrophoresis for 30 min. For separation of the different virus isolates, electrophoresis was performed at 4 mA per column for 85 min at room temperature. The gels were then stained with 0.1% aniline blue black in 7% acetic acid and destained in 7% acetic acid.

RESULTS.—*Aphid transmission from plants.*—To study the differences in efficiency of transmission of five ClMV isolates, aphids were allowed to probe leaf disks cut from mustard plants systemically infected with each of the five isolates. The Cabbage B and New York 8153 isolates were readily transmitted, the KK isolate was transmitted less efficiently, and the Campbell and CM 1841 isolates were not transmitted at all (Table 1). To determine whether the virus concentration or time after inoculation might affect transmissibility by aphids, mustard plants at the five or six leaf stage were mechanically inoculated with the ClMV isolates. Aphids were allowed to probe leaf disks cut from the first leaf which showed fully developed systemic symptoms. A transmission test was conducted every three days for 15 days. The virus concentration in the leaf disks was determined by dilution end point assay immediately after each transmission test.

TABLE 1. Transmissibility of five isolates of cauliflower mosaic virus acquired by *Myzus persicae* from infected mustard leaves

Expt. No.	Virus isolate: Campbell	CM 1841	Cabbage B	KK	New York 8153
1	0/24[a]	0/24	8/24	0/24	5/24
2	0/24	0/24	6/24	2/24	
3	0/24	0/24	1/24	0/24	4/24
Total	0/72	0/72	15/72	2/72	9/48

[a]Numerator = number of plants infected; denominator = number of test plants used. One aphid was placed on each test plant.

Although there was some variation in transmission levels among experiments, the results definitely and consistently showed that the Campbell isolate was not transmitted by *M. persicae*. The titer of the Campbell isolate, as measured by dilution end point infectivity assays, was at least as high as that of the Cabbage B and New York 8153 isolates. Results of a typical experiment are shown in Table 2. No aphid transmission of the CM 1841 isolate was obtained, and the transmission level of the KK isolate was again intermediate. Infectivity assays indicated that the titers of these isolates in systemically infected leaves were lower than those of the other three isolates. Based on these findings, the Campbell, Cabbage B, and New York 8153 isolates were chosen for further study.

Concentration of virus in epidermal tissue.—To investigate the possibility that a low virus concentration in epidermal cells may have been responsible for lack of transmission of the Campbell

TABLE 2. Comparison of aphid transmission level and virus titer of three isolates of cauliflower mosaic virus from infected leaves

Isolate	Day after inoculation	Transmission level	Dilution end[b] point
Campbell	10	0/24[a]	10^{-3}
	13	0/24	10^{-3}
	16	0/24	10^{-3}
	19	0/24	10^{-2}
	22	0/24	10^{-2}
	Total =	0/120	
Cabbage B	10	8/24	10^{-1}
	13	8/24	10^{-2}
	16	1/24	10^{-1}
	19	3/24	10^{-2}
	22	0/24	10^{-2}
	Total =	20/120	
New York 8153	10	6/24	10^{-1}
	13	4/24	10^{-2}
	16	0/24	10^{-2}
	19	1/24	10^{-2}
	22	1/24	10^{-2}
	Total =	12/120	

[a]Numerator = number of plants infected; denominator = number of plants used. One aphid was placed on each test plant.

[b]Highest dilution producing infection on mustard plants. Leaf disks from which aphids had acquired virus were used to prepare the extracts. Each dilution was mechanically inoculated to 24 mustard plants.

isolate, aphids were allowed to probe epidermal strips from infected leaves. At the completion of each aphid transmission test, the epidermal tissues were subjected to dilution end point assay. The nontransmissible Campbell isolate was present in concentrations similar to, if not higher than, that of the two aphid transmissible isolates, Cabbage B and New York 8153 (Table 3). The results of aphid transmission tests from epidermal tissue were similar to those from whole leaf disks.

Form and location of virus in cells.—The overall concentration of virus in the tissue, as measured by assays of tissue homogenates, does not necessarily reflect the concentration at the site at which the aphids acquire the virus. Thus, one possible explanation for the differences in transmissibilities of isolates of ClMV might be that differences in the location of the virus isolates in the infected cells affect their availability to aphids. Leaf tissues infected with the Cabbage B, New York 8153, and Campbell isolates were examined under the electron microscope. The virus particles of all three isolates were circular in profile, with a uniform diameter about 500 nm and were present mainly in electron-dense inclusion bodies. These inclusion bodies were always located within the cytoplasm near to the cell wall. Inclusion bodies were present in most types of leaf tissues, but were particularly conspicuous in the palisade and spongy parenchyma, and to some extent in the epidermal cells. Occasionally, free virus particles were observed scattered in the ground cytoplasm. Electron micrographs depicting the intracellular appearance and location of cauliflower mosaic virus have been published by others (1, 6). No virus-like particles were found in the nuclei, chloroplasts, or mitochondria. We could find no evidence of differences in the location or distribution among these three isolates within cells or tissues.

Inclusion bodies produced by the Cabbage B, Campbell, and New York 8153 isolates were also observed by light microscopy. We could find no differences in the number or distribution of inclusions in epidermal cells of leaves infected with these isolates.

Electrophoretic mobility of virus.—One possible explanation for the lack of aphid transmissibility of the Campbell isolate might be that the net charge of the virus particle is not suitable for combination with aphid stylets. Since the Cabbage B, New York 8153, and Campbell isolates were found to have the same size and shape, disk electrophoresis was used to determine whether the net charge of the particles differed.

TABLE 3. Comparison of aphid transmission level and virus titer of three isolates of cauliflower mosaic virus from infected epidermal strips

Isolate	Expt. no.	Transmission level	Dilution end[b] point
Campbell	1	0/24[a]	10^{-3}
	2	0/24	10^{-3}
	3	0/24	10^{-3}
	4	0/24	10^{-3}
	5	0/24	10^{-3}
	Total =	0/120	
Cabbage B	1	1/24	10^{-3}
	2	1/24	10^{-2}
	3	9/24	10^{-3}
	4	4/24	10^{-3}
	5	6/24	10^{-2}
	Total=	21/120	
New York 8153	1	1/24	10^{-3}
	2	1/24	10^{-2}
	3	4/24	10^{-3}
	4	6/24	10^{-2}
	5	6/24	10^{-3}
	Total=	18/120	

[a]Numerator = number of plants infected; denominator = number of plants used. One aphid was placed on each test plant.

[b]Highest dilution producing infection on mustard plants. Epidermal strips from which aphids had acquired virus were used to prepare the extracts.

When these three isolates were submitted to electrophoresis on agarose-acrylamide gel, each isolate migrated as a single band completely separated from the other two. (Fig. 1). Mobility of the non-transmissible strain was intermediate between that of the two transmissible strains; R_F values, the ratio of distance moved by the sample to the distance moved by the tracking dye, were 0.44 for Cabbage B, 0.39 for Campbell, and 0.34 for the New York 8153 isolate.

Transmission of virus acquired by consecutive probes.—Potato aucuba mosaic virus (PAMV) and potato virus C (PVC) can only be transmitted by *M. persicae* from plants also infected with the transmissible potato virus Y (PVY) or from singly-infected plants by aphids which have first probed plants infected with PVY (2). The possibility that this type of relationship might also exist with ClMV isolates was studied next.

Mustard plants were doubly inoculated with the Cabbage B and Campbell isolates. When systemic symptoms developed, aphids were allowed to probe the infected leaves and were then transferred to test plants. When test plants developed symptoms, they were assayed on *Datura stramonium*, a plant which produces local lesions when inoculated with any of these ClMV isolates except Cabbage B, which produces no symptoms (5). Thus test plants were known to have been infected by the Campbell isolate if extracts of these plants produced local lesions on *D. stramonium*. Twenty-three of 144 test plants were found to be infected with the Campbell isolate (Table 4).

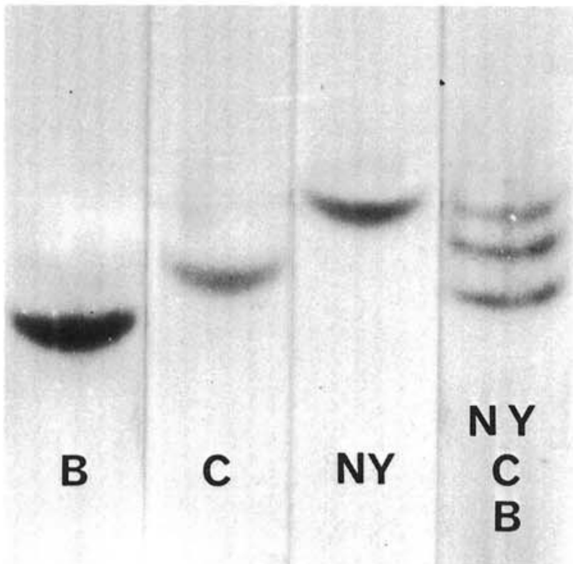

Fig. 1. Relative electrophoretic mobility of three cauliflower mosaic virus isolates in agarose-acrylamide gels in 0.02 M potassium phosphate buffer (pH 7.5). Migration was from the top of the column, which was attached to the negative terminal. B = Cabbage B; C = Campbell; NY = New York 8153.

TABLE 4. Effect of the normally-transmissible Cabbage B isolate of cauliflower mosaic virus (ClMV) upon aphid transmission of the normally-nontransmissible Campbell isolate.

Probing[a] sequence	Total no. of plants infected	No. of plants infected with the Campbell isolate
Campbell and Cabbage B (doubly-infected plants)	27/144[b]	23/144
Cabbage B then Campbell	28/100	26/100
Campbell then Cabbage B	15/100	0/100
Healthy plants then Campbell	0/100	0/100
Campbell only	0/100	0/100
Cabbage B only	10/100	0/100

[a]Aphids allowed to acquire virus in 30-sec probes made on plants infected with indicated isolate in indicated sequence.

[b]Numerator = number of plants infected; denominator = number of plants used. Two aphids were placed on each test plant.

Double infection was not required for transmission. Consecutive acquisition probe tests were made, in which the aphids were allowed to probe first into one virus source and then into another. The virus sources used were also tested on *D. stramonium* plants before and after each test to ascertain that they were not contaminated with other isolates. When the aphids were allowed to first probe into leaves infected only with the Cabbage B isolate and then into leaves infected with the Campbell isolate, the latter was transmitted as efficiently as it was from leaves infected with both isolates, but it was not transmitted when the order of probing was reversed; nor was it transmitted by aphids which had first probed healthy mustard leaves (Table 4).

Consecutive acquisition probe experiments with the CM 1841 isolate yielded similar results, although less extensive tests were made. Increased transmission of the KK isolate was obtained with aphids which had previously probed Cabbage B-infected leaves. These data are shown in Table 5.

DISCUSSION.—Several of the theories which have been proposed to explain lack of aphid transmissibility of virus strains or isolates were directly tested in this study. Lack of transmissibility of CM 1841 could have been due to a low concentration of virus, based on preliminary tests, and KK was aphid-transmissible at a very low level. Therefore these isolates were not used in the detailed tests. Lack of transmissibility of the Campbell isolate could not be attributed to a low virus concentration based on infectivity assays. Nor could lack of transmission be attributed to concentration or distribution in infected cells, based on electron or light microscopy; these were similar for the Campbell and the transmissible isolates. The fact that the

TABLE 5. Effect of prior probing on Cabbage B infected leaves on aphid transmission of the CM 1841 and KK isolates

Probing[a] sequence	Total no. of plants infected	No. plants infected with CM 1841	No. plants infected with KK
CM 1841 only	0/100[b]	0/100	
Cabbage B, then CM 1841	8/100	5/100	
KK only	2/100		2/100
Cabbage B, then KK	12/100		11/100
Cabbage B only	9/100		

[a]Aphids allowed to acquire virus in 30 sec probes made on indicated plants in indicated sequence.

[b]Numerator = number of plants infected; denominator = number of plants used. One aphid was placed on each test plant.

Campbell isolate had an electrophoretic mobility intermediate between that of two transmissible isolates indicates that transmissibility cannot be dependent upon a net charge more negative or more positive than that of transmissible isolates.

The results of the consecutive probe tests with ClMV are similar to those obtained by Kassanis and Govier with viruses of another group (3). They found that the nontransmissible PAMV and PVC could be transmitted by aphids which had previously probed plants infected with any of a number of transmissible polyviruses. ClMV is morphologically and biochemically distinct from these viruses and thus the requirement of a "transmission factor" may be a general phenomenon which regulates aphid transmissibility of plant viruses. Our data are compatible with a hypothesis that a factor necessary for aphid transmission of ClMV is present in leaves of plants infected with transmissible isolates and is not present in leaves infected with nontransmissible isolates. This factor may be acquired by aphids which probe leaves infected with transmissible isolates; this enables the aphids to subsequently transmit the normally nontransmissible isolates.

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