Identification and Serotyping of Cucumber Mosaic and Peanut Stunt Viruses from Arkansas

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ABSTRACT

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Ten isolates of cucumber mosaic virus (CMV) and two isolates of peanut stunt virus (PSV) were identified by host range, symptomatology, and serology. CMV isolates were divided into three groups according to host range and symptomatology and into two groups according to serological reactions. Comparisons with known cucumoviruses suggested that all Arkansas CMV isolates belong to the DTL serogroup and all Arkansas PSV isolates belong to the W serogroup.

The only report of cucumber mosaic virus (CMV) in Arkansas was made by Fulton (5), in which he described six isolates of CMV from spinach. Recently, Griffin et al (6) reported the presence of peanut stunt virus (PSV) in white clover and bean in Arkansas. Several virus isolates that were suspected to be cucumoviruses have been collected from various hosts throughout Arkansas. Because of their ubiquity and the importance of the diseases they cause, we have determined the host range, symptomatology, and serological relationships of these isolates.

MATERIALS AND METHODS

Viruses. Virus isolates were collected from bean, cowpea, peanut, cantaloupe, and spinach. They were propagated and maintained in the greenhouse in cowpea (*Vigna unguiculata* (L.) Walp. subsp. *unguiculata* cv. Blackeye No. 5) and tobacco (*Nicotiana tabacum* L. cv. NC 95). Known isolates of CMV and PSV used for comparison were Fulton's CMV-C and CMV-D (5), PSV-W (obtained from G. I. Mink, Prosser, WA), PSV-10 (an Arkansas isolate; J. P. Fulton and H. A. Scott, *unpublished*), PSV-V (obtained from J. M. Kaper, Beltsville, MD), CMV-Q (obtained from the American Type Culture Collection, Rockville, MD) (ATCC PV 289), and CMV-S and Marchoux's CMV-D (8) (both provided by J. M. Kaper).

Host range and symptomatology. Test plants, including bean (*Phaseolus vulgaris* L. cv. Cherokee wax), *Chenopodium quinoa* Willd., cowpea, cucumber (*Cucumis sativus* L. cv. Model), *Datura stramonium* L., Spanish peanut (*Arachis hypogaea* L.), and tobacco, were inoculated by rubbing crude sap prepared in 0.01 M phosphate buffer, pH 7.2, on Carborundum-dusted leaves with a small piece of sterile cheesecloth. Test plants were observed up to 4 wk after inoculation. When symptoms were absent, leaves of the test plants were back-inoculated onto *C. quinoa*.

Virus purification. The purification technique for CMV was a modification of Scott's method (9). For each Arkansas CMV isolate, 200 g of cowpea primary leaves harvested 10 days after inoculation were homogenized in a Waring Blendor with 300 ml of 0.5 M citrate buffer, pH 6.5, containing 0.1% sodium thioglycolate. The homogenate was filtered through cheesecloth, and the filtrate was shaken for 10 sec with 75 ml of 1:1 chloroform-butanol. The emulsion was immediately broken by centrifugation at 5,000 *g* for 10 min, after which the aqueous phase was subjected to two cycles of high-speed (78,000 *g*, 150 min) and low-speed (10,000 *g*, 10 min) centrifugation. The pellets were suspended in 0.005 M borate-0.005 M EDTA buffer, pH 9.0. Further purification was by rate-zonal density-gradient centrifugation through a sucrose gradient (0.2–0.7 M) in borate-EDTA buffer with a Beckman SW-27 rotor (27,000 rpm, 150 min). The virus band was collected and subjected to a high-speed centrifugation, and the pellet was resuspended in borate-EDTA buffer. The same method was used to purify CMV-S from Chicago Pickling cucumber and CMV-Q and Marchoux's CMV-D from *Nicotiana rustica* L.

PSV isolates were purified from *D. stramonium* leaves 2–5 wk after inoculation. The procedure followed was essentially the same as for CMV purification, except 0.3 M phosphate buffer, pH 7.6, containing 0.3% sodium thioglycolate and 0.3% sodium diethyldithiocarbamate was used for the initial extraction. After the first high-speed centrifugation, 0.01 M phosphate buffer, pH 7.6, was used for all subsequent purification steps.

Preparation of antisera. Rabbits were given a series of seven weekly intramuscular injections with purified CMV-C (total 45 mg) or PSV-10 (total 35 mg) mixed with Freund's incomplete adjuvant and were bled weekly after the eighth week. Antisera for Marchoux's CMV-D and CMV-9 were prepared by a series of five subcutaneous injections with 21 and 13 mg of the respective purified viruses. The rabbits that were injected with Marchoux's CMV-D or CMV-9 were first bled after the sixth and seventh week, respectively, after the initial injections. Antiserum for CMV-S was obtained from the American Type Culture Collection (ATCC PVAS 242a).

Immunodiffusion tests. Purified viruses at 2 mg/ml were reacted with antisera to CMV-C, PSV-10, CMV-9, CMV-S, or Marchoux's CMV-D, using an agarose medium containing 0.005 M borate-0.005 M EDTA, pH 9.0. Intragel absorption was carried out by placing sap from healthy host plants in antiserum wells 24 hr before setting up the immunodiffusion tests (10). Excess sap was removed from the wells before antisera were introduced.

RESULTS AND DISCUSSION

Host range and symptomatology. No two isolates induced identical symptoms on all seven host plants. On the basis of symptomatology, the various isolates could be placed in either the PSV group or one of three CMV groups (Table 1). All PSV isolates induced identical symptoms on bean, tobacco, cowpea, and *D. stramonium* (Table 1) but induced slightly different symptoms on cucumber, peanut, and *C. quinoa*.

The CMV isolates may be divided into symptomatological groups designated A, B, and C (Table 1). Group A consisted of severe CMV isolates, all of which were originally isolated from spinach. The most virulent isolate, CMV-C, was the only one that caused death of inoculated bean and cowpea. The CMV isolates designated CMV-23 and CMV-81 caused symptoms on most test plants that were less severe than those caused by the isolates in group A. They were the only CMV isolates that induced both local and systemic symptoms in *D. stramonium* and hence were placed in group B (Table 1).

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Table 1. Grouping of various cucumovirus isolates according to symptomatology

	Hosts													
	Phaseolus vulgaris		*Vigna unguiculata*		*Chenopodium quinoa*		*Nicotiana tabacum*		*Arachis hypogaea*		*Cucumis sativus*		*Datura stramonium*	
Virus groups	Local[a]	Syst.[a]	Local	Syst.	Local	Syst.	Local	Syst.	Local	Syst.	Local	Syst.	Local	Syst.
PSV group														
PSV-10, 11, 16, W, V	La[b]	Mo,Dis,Fil	MNLL, CLL	Mo	NLL	VN/CL/0[c]	La	Mo,Ys	La	St,Mo/Chl/MMo	La	MMo/Mo	MChl	Mo
CMV group A														
CMV-C, D, 233	NB/VN	D/Mo[d]	NB/MNLL	D/SN, Mo	NLL	0	Mo	Mo,Dis,Fil	0	0	CLL	Mo,Dis,St	NB	0
CMV group B														
CMV-23, 81	La	Mo	MNLL, CLL	Mo	NLL	0/CL,Dis[e]	CLL	Mo,Dis,Fil	0	0/La	CLL	Mo	NB	Mo
CMV group C														
CMV-EM, 61, J, CL, 14, 78, 9	La	Mo,Dis,Fil	MNLL, VN	Mo,SN	NLL	CL,Dis	La	MMo	0	0	La	MMo	0	0

[a] Local and systemic symptoms.
[b] Chl = chlorosis, CLL = chlorotic local lesions, CL = chlorotic lesions, D = death, Dis = distortion, Fil = filiform leaves, La = latent, Mo = mosaic or mottle, MMo = mild mosaic or mild mottle, MChl = mild chlorosis, MNLL = minute necrotic local lesions, NB = necrotic blotch, NLL = necrotic local lesions, St = stunting, SN = stem necrosis, VN = veinal necrosis, Ys = yellow spots, and 0 = no infection.
[c] Variations in symptoms induced by different isolates.
[d] Death of plants occurred in CMV-C infection only.
[e] Systemic symptoms on *C. quinoa* were induced by CMV-81 only.

Table 2. Serological relationships among cucumber mosaic (CMV) and peanut stunt (PSV) viruses demonstrated in Ouchterlony gel diffusion tests using antisera to CMV-C (Cs), Marchoux's CMV-D (Ds), CMV-9 (9s), CMV-S (Ss), or PSV-10 (Ps)

Cucomovirus isolates	Antisera				
	Cs	Ds	9s	Ss	Ps
Serotype CMV-I					
CMV-C	H[a]	C	S	S	W
CMV-D[b]	C	C	S	S	W
CMV-233	C	C	S	S	W
CMV-81	C	C	S	S	W
CMV-23	C	C	S	S	W
CMV-EM	C	C	S	S	W
Serogroup CMV-DTL[c]					
CMV-D[d]	C	H	S	S	W
Serotype CMV II					
CMV-61	S	S	C	S	W
CMV-J	S	S	C	S	W
CMV-CL	S	S	C	S	W
CMV-14	S	S	C	S	W
CMV-78	S	S	C	S	W
CMV-9	S	S	H	S	W
Serogroup ToRS[c]					
CMV-S	S	S	S	H	W
CMV-Q	S	S	S	C	W
Serogroup PSV-W[c]					
PSV-10	–[e]	S	–	S	H
PSV-11	–	S	–	S	C
PSV-16	–	S	–	S	C
PSV-W	–	S	–	S	S
Serogroup PSV-V[c]					
PSV-V	–	S	–	S	S

[a] Homologous (H) reactions in which precipitin bands coalesced (C) or heterologous reactions with spur (S) formation. Some heterologous reactions only formed weak precipitin bands (W) midway between the antigen and antiserum wells.
[b] Fulton's isolate (5).
[c] Devergne and Cardin (1,2).
[d] Marchoux's isolate (8).
[e] No reaction.

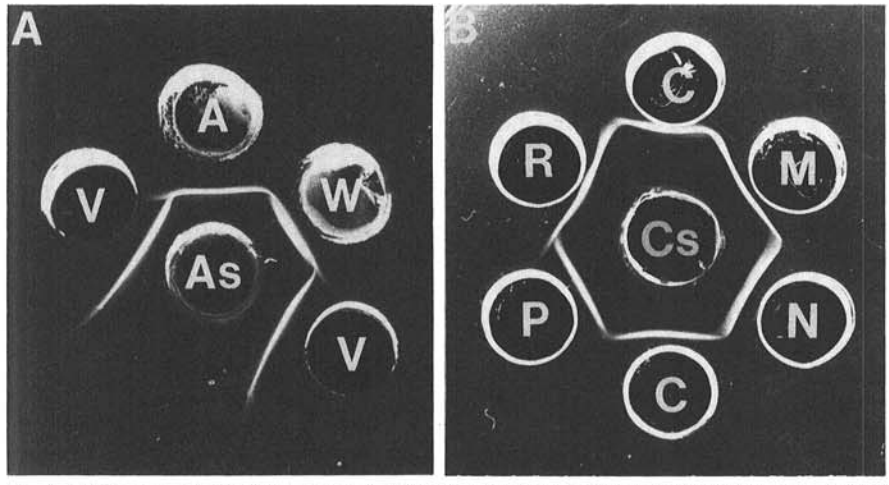

Fig. 1. Serological relationships among isolates of cucumber mosaic virus (CMV) or peanut stunt virus (PSV) determined by Ouchterlony gel diffusion tests in 0.8% agarose prepared in 0.005 M borate-0.005 M EDTA buffer, pH 9.0. **(A)** Purified virus isolates PSV-10 (A), PSV-W (W), and PSV-V (V) were reacted with antiserum to PSV-10 (As). **(B)** Purified CMV-23 (M), Fulton's CMV-D (N), CMV-78 (P), CMV-81 (R), and CMV-C (C) were reacted with antiserum to CMV-C (Cs).

Group C is composed of isolates that are characterized by the induction of mild mottling in tobacco plants, which recover with age (Table 1). All members of group C were originally isolated from legumes. All legume isolates of CMV, including CMV-81, which was placed in group B, infected *C. quinoa* both locally and systemically, whereas all other isolates infected this host only locally.

Virus purification. The methods described were successful for purification of all isolates of cucumoviruses tested. Virus yields depended on the isolate but ranged from 20 mg/100 g for Fulton's CMV-D to 50 mg/100 g for CMV-78. Yields ranging from 20 to 30 mg of virus were obtained when PSV isolates were purified from 100 g of *D. stramonium* leaves.

Immunodiffusion tests. Ouchterlony gel diffusion tests with purified viruses revealed serological differences among CMV and PSV isolates (Table 2). All CMV isolates reacted with antiserum to PSV-10, resulting in the formation of weak precipitin bands. Similarly, all five PSV isolates reacted with antisera to CMV-S or Marchoux's CMV-D, forming precipitin bands that spurred with those formed by homologous reactions. In contrast, none of the PSV isolates reacted with antisera to CMV-C or CMV-9 (Table 2). Thus, serological relatedness between CMV and PSV is dependent on the antisera and the isolates used (2,7).

Antiserum to PSV-10 reacted with all PSV isolates (Table 2; Fig. 1A). Precipitin bands of PSV-10 coalesced with precipitin bands of the other two Arkansas PSV isolates; however, PSV-10 formed a spur with PSV-V (Fig. 1A), and in some instances, an indistinct spur with PSV-W. It was also revealed that PSV-W has more antigenic determinants in common with PSV-10 than does PSV-V, since PSV-W and PSV-V formed a spur when reacted with antiserum to PSV-10. This

suggests that all Arkansas isolates belong to the PSV-W serogroup rather than the PSV-V serogroup (3).

Based on the reactions with antisera to CMV-C (Table 2; Fig. 1B) or CMV-9, the various CMV isolates are composed of two serotypes. Serotype I is made up of members of symptomatological groups A and B and CMV-EM of group C. All of these isolates formed spurs with members of serotype II, which consists of CMV-61, CMV-J, CMV-CL, CMV-14, CMV-78, and CMV-9 in group C (Table 2). They formed coalescing precipitin bands with each other against antiserum to CMV-C or CMV-9 (Table 2). On the other hand, CMV-C and Marchoux's CMV-D were serologically indistinguishable from each other since they did not form a spur when reacted with antiserum to either virus. Moreover, the reactions between antiserum to Marchoux's CMV-D and Arkansas CMV isolates were similar to those obtained with CMV-C antiserum (Table 2).

Immunodiffusion tests using antiserum to CMV-S (Table 2) revealed the formation of spurs between precipitin lines of CMV-S and CMV-C and CMV-9 and Marchoux's CMV-D but not among precipitin lines of CMV-C, CMV-9, and Marchoux's CMV-D.

Thus, despite being composed of two serotypes, all Arkansas CMV isolates have more antigenic determinants in common with one another and with Marchoux's CMV-D than with CMV-S. This suggests that all Arkansas CMV isolates described in this paper belong to the DTL serogroup rather than the ToRS serogroup of Devergne and Cardin (1,4).

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