Comparative Study of Five Mosaic Virus Isolates Infecting Corn, Johnson Grass, and Sorghum in the United States

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ABSTRACT

Five mosaic virus isolates infecting corn (*Zea mays*), Johnson grass (*Sorghum halepense*), or sweet sorghum (*S. bicolor*) from California, Virginia, Frankfort and Quicksand, Kentucky, and Mississippi, were related serologically on the basis of their reaction with maize dwarf mosaic virus strain A (MDMV-A) antiserum. They were distinct from each other in their effect on plant growth and severity of leaf symptoms on several sweet sorghum cultivars. The Frankfort, Ky., isolate generally produced the most severe leaf chlorosis on cultivars. The Virginia isolate caused a distinctive purplish-black discoloration of leaves of Rio sorghum. The Virginia and Quicksand isolates caused severe stunting of Rio sorghum with frequent killing of the growing points. The California, Quicksand, and Virginia isolates stunted Sugar Drip and Hay Grazer sorghums. Dilution end points distinguished the California, Mississippi, and Virginia isolates, while the thermal death points distinguished the Frankfort and Quicksand isolates. The five isolates were distinct from known strains of sugarcane mosaic virus (SCMV) based on infectivity in Johnson grass and reactivity with MDMV-A antiserum cross-absorbed so that it no longer reacted with strains A, B, D, E, and H of SCMV. Phytopathology 61:389-394.

Mosaic incited by the sugarcane mosaic virus (SCMV) (3), although present on corn (*Zea mays* L.) and sweet sorghum (*Sorghum bicolor* [L.] Moench) for many years in southern USA, has caused little concern on these crops. It generally has been confined to the vicinity of mosaic-infected sugarcane (*Saccharum officinarum* L.), which harbors the virus from year to year. With the exception of two reports (1, 15), each concerning a single infected plant, SCMV has not been found in or transmitted to Johnson grass. Recently, a widespread occurrence of a mosaic disease of corn and sorghum has been reported from areas of the United States where sugarcane has never been grown (2, 4, 5, 9, 14, 16, 20, 27, 28, 31). The virus causing this disease has been transmitted to sugarcane seedlings (7, 20) and plants grown from stem cuttings (10, 23). This mosaic virus readily infects Johnson grass (2, 20, 22, 29). Serological data (2, 20, 22, 25, 26, 29) indicate that it is related to SCMV. Size and shape of particles (2, 11, 20, 22, 25) approximate those of SCMV.

Reported effects of this mosaic virus on the growth of corn in different areas of the United States vary from severe stunting and top necrosis of infected plants (20, 29) to no effect on plant height (19). Nomenclature of mosaic viruses infecting corn and Johnson grass has not been uniform. Names used include (i) maize dwarf mosaic virus (MDMV) (29); (ii) Johnson grass strain of SCMV (13); (iii) Johnson grass mosaic virus (19); and (iv) sugarcane mosaic virus (9). Recently, a corn virus isolate which does not infect Johnson grass but which is related serologically to the virus that infects this host has been reported from northern USA (17). Published host ranges of many of these mosaic virus isolates are similar, indicating relationship (1, 2, 6, 8, 10, 21, 22).

Therefore, we conducted experiments to study the relationship among several mosaic virus isolates infecting corn, Johnson grass, and sorghum from different areas of the USA.

MATERIALS AND METHODS.—Mosaic virus isolates were obtained from five sources in the United States (Table 1). Each isolate was maintained in sweet sorghum cultivar, Sart. Plants for studies of symptoms and physical properties were kept in screened cubicles in a screened greenhouse under quarantine conditions at the Plant Industry Station, Beltsville, Md. For serological studies, plants infected with different isolates were kept in separate greenhouse rooms at The Ohio Agricultural Research and Development Center, Wooster, Ohio. Sorghum and corn cultivars and Johnson grass were inoculated by dusting the upper surface of the youngest expanded leaf with Carborundum and gently rubbing with the forefinger moistened with inoculum. Sugarcane plants from cuttings were inoculated by the Matz method (18). Inoculum was prepared by macerating 1 g of diseased leaves in 10 ml of water and filtering through cheesecloth. In some tests, 1 g of diseased tissue was macerated in 3 ml of water. Test plants were corn (Moews 98W, Pioneer 309B, Golden Cross Bantam), Johnson grass, sweet sorghum (Mer. 64-6, Planter, Rio, Sart, Sugar Drip, Williams), forage sorghum (Hay Grazer), and sugarcane (C.P. 31-294, C.P. 31-588, and seedling crosses 67-91 and 67-240). Twenty-five plants in the three-leaf stage (5 plants/4-inch pot) of each cultivar of corn and sorghum and Johnson grass were inoculated per treatment, except in one experiment where 15 plants (3 plants/pot) were inoculated. With sugarcane, 10 vegetative plants (1 plant/pot) were inoculated per treatment. Test plants were examined for symptoms 2 through 6 weeks after inoculation. Percentages of plants with symptoms and type of symptoms were generally recorded 6 weeks

TABLE 1. Sources of mosaic isolates from corn, Johnson grass, and sorghum tested at Beltsville, Maryland

Source	Host	Collector	Isolate designation
State College, Miss.	Sweet sorghum	N. Zummo	Mississippi
Frankfort, Ky.	Johnson grass	J. Shane	Frankfort
Quicksand, Ky.	Johnson grass	J. Shane	Quicksand
Virginia[a]	Corn	V. D. Dahmsteeg	Virginia
Berkeley, Calif.[b]	Corn	J. H. Freitag	California

[a] This isolate transmitted to sugarcane by A. G. Gillaspie (10, 23).

[b] This isolate identified as the Johnson grass strain of the sugarcane mosaic virus by J. H. Freitag.

after inoculation. Symptoms described were apparent generally by 14 days after inoculation.

Determinations of dilution end points were made using aliquots of freshly expressed juice from leaves of Sart diluted 1:10, 1:100, 1:1,000 and 1:10,000 with distilled water. Forty-five plants of Sart were inoculated per dilution. Determinations of thermal inactivation point were made using 5 ml of juice placed in 13 mm-diam glass tubes with walls 0.9 mm thick and immersed in a continuously agitated water bath for 10 min at 50, 51, 52, 53, 54, 55, 56, 57, or 58 C. Infectivity was determined on Sart (40 plants/treatment).

Serology.—For the testing of viral antigens, 1 g of diseased leaves of Oh28 corn was ground with mortar and pestle in 2 ml of 0.01 M phosphate buffer containing 0.14 M NaCl, pH 7.0 (PBS). The extract was pressed from the pulp through cheesecloth and centrifuged at 10,000 rpm for 10 min in the SS 34 rotor of the Sorvall RC-2B centrifuge. The supernatant fraction was used as viral antigen at dilutions of 1:3, 1:6, and 1:12 based on fresh wt of tissue. Purification and preparation of antiserum of MDMV strain A (MDMV-A, ATCC-PV55) were accomplished following a modification of a procedure used for the Johnson grass strain of SCMV (22). The titer for MDMV-A antigen, when the homologous antigen was used in microprecipitin tests, was 1:256. Prior to testing, antiserum was cross-absorbed with an extract, prepared as described in the previous section, containing 13B viral antigen (12, 30). Equal volumes of antiserum and extract were mixed and incubated at room temperature for 2 hr. The precipitate was removed by centrifugation of the mixture at 10,000 rpm for 10 min. The supernatant fraction was used in a second cross-absorption of the antiserum with the heterologous antigen. The supernatant fraction from the second cross-absorption was diluted with PBS to give a dilution of 1:8, the dilution used in all tests. This dilution was optimum for detection of virus in extracts. The microprecipitin test was used to determine serological relationships. Test droplets in plates were incubated at room temperature for 1 to 4 hr and then overnight at 4-6 C before reactions were recorded. Tests were made on three different occasions.

RESULTS.—*Hosts infected by mosaic isolates.*—There was considerable variation in the percentage of infection obtained with individual virus isolates on cultivars of corn and sorghum and Johnson grass (Table 2). There was no red discoloration of infected leaves of Williams and Mer. 64-6 as described for field plants in earlier work (31). Symptomless infection occurred on C.P. 31-294 inoculated with Virginia and Quicksand isolates (Table 2) as shown by the typical mosaic symptoms which developed in Sart following inoculation with extracts from C.P. 31-294. No infec-

TABLE 2. Percentage mosaic infection in various host cultivars after inoculation with each of five mosaic virus isolates

Host	No. tests	Isolates				
		California	Frankfort	Quicksand	Mississippi	Virginia
		% plants infected				
Corn						
Golden Cross Bantam	1	100	100	96	100	91
Moews 98W	4	80	96	90	93	84
Pioneer 309B	3	84	92	99	90	95
Sorghum						
Hay Grazer	3	84	81	76	73	85
Johnson grass	2	69	90	29	75	48
Mer. 64-6	2	59	98	80	58	63
Planter	3	64	87	80	69	67
Rio	2	96	54	74	54	70
Sart	2	82	98	80	93	86
Sugar Drip	2	58	76	78	54	64
Williams	1	29	75	35	37	25
Sugarcane						
C. P. 31-294	2	0	0	21[a]	0	20[a]
C. P. 31-588	2	0	0	0	0	0
Cross 67-91	2	26	4	30	30	39
Cross 67-240	1	0	0	25	0	0

[a] These plants did not show mosaic symptoms, but when juice from these plants was inoculated to healthy Sart plants, typical symptoms appeared.

tion occurred with the California, Frankfort, and Mississippi isolates on sugarcane from cuttings, as plants were symptomless and extracts were noninfectious on Sart. All isolates produced symptoms on sugarcane seedlings of cross 67-91.

Effect of mosaic isolates on plant growth.—Differentiation of the virus isolates was possible on the basis of their effect on growth of the sweet sorghum cultivars, Rio, Sugar Drip, and Hay Grazer. Stunting was determined by comparing mosaic-infected plants with inoculated plants without symptoms.

Marked differences in plant height in Rio resulted from infection with the different isolates (Fig. 1). The Virginia and Quicksand isolates severely stunted plants and caused necrosis of all or most of the terminal growing points. The Mississippi isolate also severely stunted Rio, but caused less killing of terminal shoots. This isolate reduced leaf length, but not width as other isolates did. Furthermore, whorls of infected plants were expanded and appeared cuplike. The California and Frankfort isolates only moderately stunted Rio. The latter also caused some killing of terminal growing points.

Effects on growth of Sugar Drip and Hay Grazer sorghum were not as striking as on Rio (Fig. 1). The Virginia, California, and Quicksand isolates stunted both varieties, but no terminal growing points were killed. The Frankfort and Mississippi isolates had no stunting effect.

The five isolates did not stunt or cause necrosis of terminals on the remaining sorghum and corn cultivars or Johnson grass, all of which showed mosaic symptoms.

Effect of virus isolates on leaf symptom development.—The pattern of leaf chlorosis produced by the isolates on individual cultivars varied from a mild chlorosis or mottle to a regular pattern of discrete yellowish to whitish lesions. Some of the most striking differences in response to the five isolates were shown by Rio (Fig. 2). The California and Quicksand isolates caused a severe or coarse yellow mottle with distinct yellow or green streaks. The latter appeared as green islands with the Quicksand isolate. The Mississippi isolate also caused white streaks with green islands, but symptoms were less severe. A distinctive, purplish-black coloration of leaves resulted from infection by the Virginia isolate. Also, light yellow streaks usually restricted to one side at the leaf base were characteristic. Symptoms induced by the Frankfort isolate were

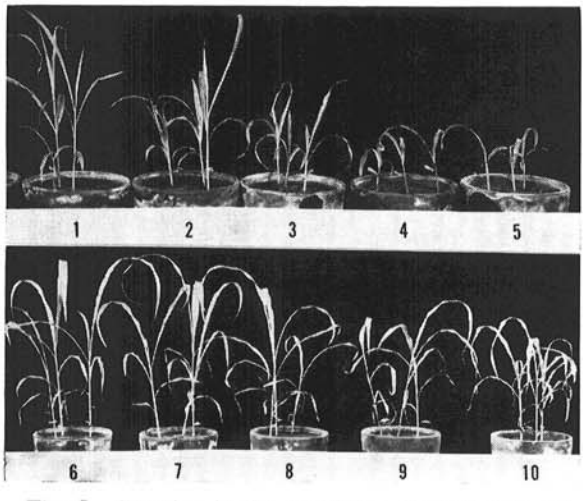

Fig. 1. Reaction in the greenhouse of two sweet sorghum cultivars to infection with mosaic isolates from 5 different areas in the USA. (Above) Rio: 1 = Frankfort; 2 = California; 3 = Mississippi; 4 = Virginia; 5 = Quicksand. (Below) Sugar Drip: 6 = Mississippi; 7 = Frankfort; 8 = Quicksand; 9 = California; 10 = Virginia.

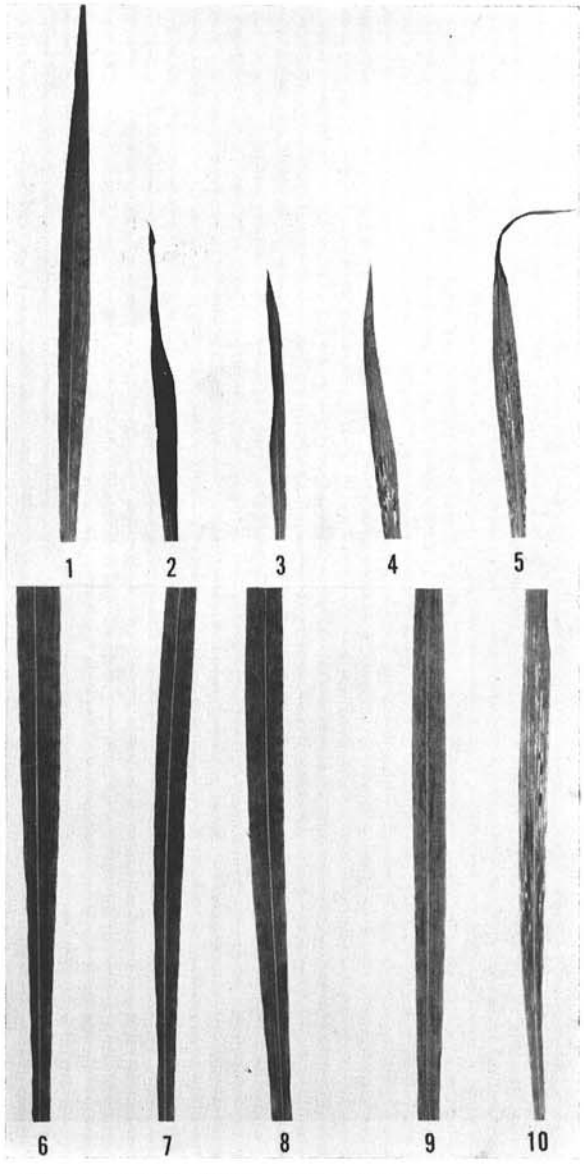

Fig. 2. Leaves of two sweet sorghum cultivars showing mosaic symptoms after inoculation with 5 virus isolates. (Above) Rio: 1 = Frankfort; 2 = Virginia; 3 = Mississippi; 4 = Quicksand; 5 = California. (Below) Sugar Drip: 6 = Mississippi; 7 = Frankfort; 8 = Quicksand; 9 = California; 10 = Virginia.

white streaks with blunt ends and limited necrotic areas within them which extended over most of the leaf. Isolates caused mild (Frankfort) to severe (remaining isolates) stunting of leaves of Rio.

The pattern of leaf chlorosis was less pronounced on Sugar Drip than on Rio, and little stunting occurred (Fig. 2). The Virginia isolate caused the most severe leaf symptoms, with discrete white streaks, some with blunt ends, and scattered green islands. The Mississippi, Frankfort, and Quicksand isolates produced a mild chlorotic mottle, with scattered green streaks and infrequent veinbanding. The California isolate produced a pronounced chlorotic mottle with distinct green streaks and dashes that frequently had blunt ends.

On Pioneer 309B corn, the Virginia and Frankfort isolates produced discrete white streaks with blunt ends, particularly with the latter isolate (Fig. 3). Also with this isolate, streaks were delineated by veins. The Quicksand isolate caused short, indistinct, and generally irregular white streaks and some veinbanding. The California isolate produced mildly chlorotic interveinal streaks with broken edges. Some streaks appeared as elongated ringspots.

On Moews 98W corn, the Frankfort isolate, which in general caused the most severe leaf chlorosis pattern on the different hosts, produced numerous sharply delineated white streaks on corn (Fig. 3). These ranged from short to long, usually with square or blunt ends; in some instances they coalesced. The Virginia isolate produced indefinite, long, thin streaks and dashes that were green or white. With the Quicksand isolate, streaks were scattered, indistinct, white, and of variable length. The California isolate also caused indistinct streaks of variable length, but these were located between veins. Streaks were green, particularly at the leaf base, or chlorotic. With the Mississippi isolate, streaks were faint, white, and infrequent.

Physical properties.—Dilution end point (DEP)

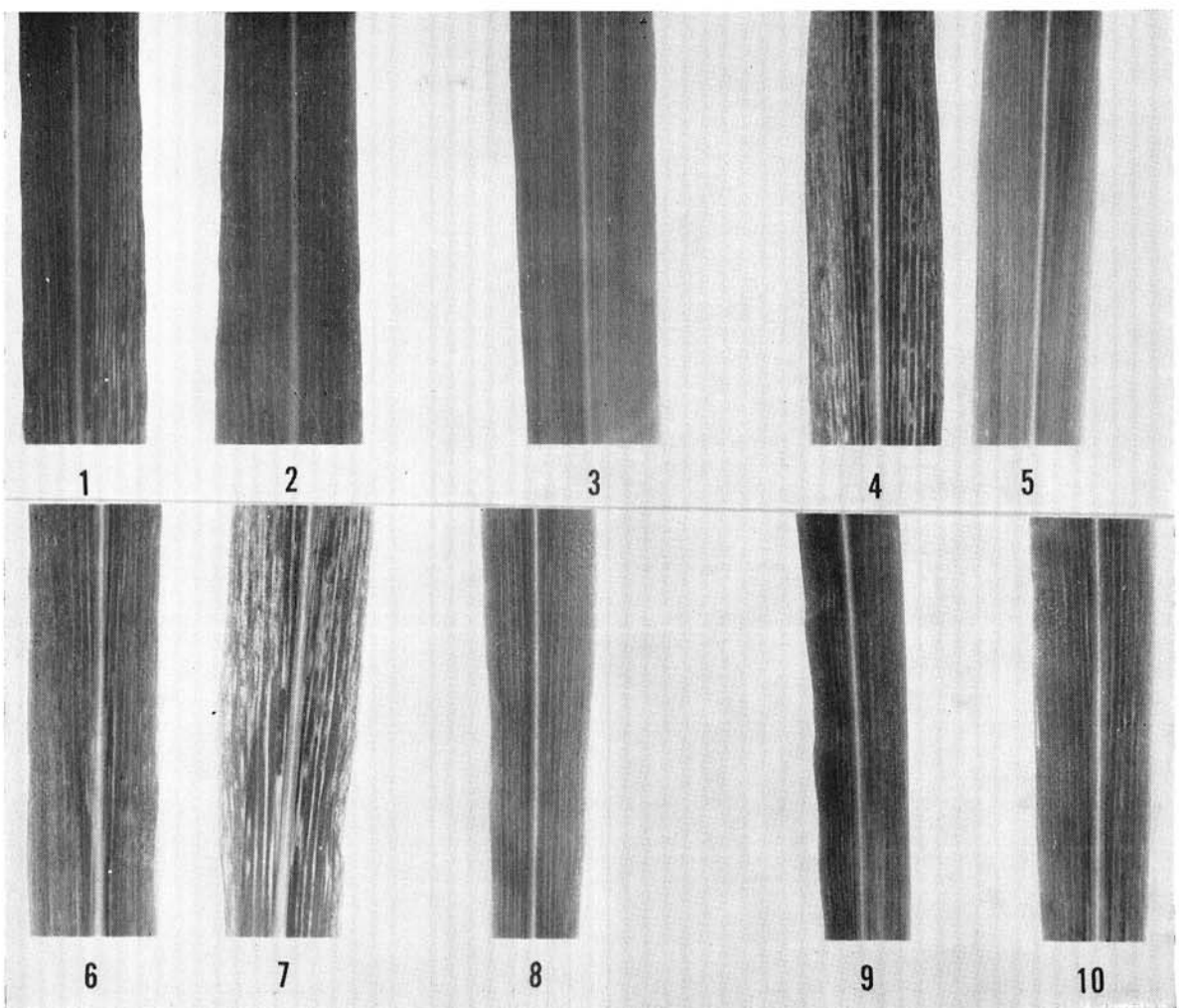

Fig. 3. Leaves of two corn cultivars showing mosaic symptoms after inoculation with 5 virus isolates. (Above) Pioneer 309B: 1 = Virginia; 2 = California; 3 = Quicksand; 4 = Frankfort; 5 = Mississippi. (Below) Moews: 6 = Virginia; 7 = Frankfort; 8 = Quicksand; 9 = California; 10 = Mississippi.

TABLE 3. Dilution end point and thermal death point of five mosaic virus isolates from corn, sorghum, etc., based on percentage infection on inoculations of plants of the sweet sorghum variety Sart

Isolate	Dilution				C								
	1:10	1:100	1:1,000	1:10,000	50	51	52	53	54	55	56	57	58
Virginia	19	11	3	0	40	33	10	11	40	13	0	0	5
Frankfort	21	13	3	0	90	60	60	60	60		10	3	0
California	29	7	0	0	50	30	30	0	22	0	0	0	0
Quicksand	4	3	4	0	70	30	20	40	45	13	3	3	3
Mississippi	14	4	0	4	70	20	20	40	20	13	3	3	5

tests showed that the California isolate was infective at a dilution of 1:10, but not at 1:100 (Table 3). Frankfort and Quicksand isolates were infective at 1:100 but not at 1:1,000. The Virginia isolate was infective at 1:1,000 but not at 1:10,000. With the Mississippi isolate, no infection was obtained at a dilution of 1:1,000; but infection resulted from a 1:10,000 dilution in one plant in each of two tests.

The Virginia, Quicksand, and Mississippi isolates remained infective when exposed to 58 C for 10 min. The Frankfort isolate remained infective when exposed to 56 C, but not at 58 C. The California isolate remained infective at temperatures to 54 C.

Serology.—The MDMV-A antiserum after cross-absorption failed to react with the heterologous antigen (Table 4). Without prior cross-absorption, MDMV-A antiserum reacted weakly with MDMV-B and SCMV strains, preventing certain identification of MDMV-A. All five isolates were related in reacting with the cross-absorbed MDMV-A antiserum.

DISCUSSION.—We believe that the five virus isolates are all isolates of MDMV-A (29), a member of the sugarcane mosaic virus group. Although we did not get infection with symptoms on vegetatively propagated sugarcane, seedlings of cross 67-91 showed typical mosaic symptoms with each of the five isolates. The host response, physical properties, and serological data further support this relationship with SCMV. Furthermore, the Virginia isolate (10) and other isolates (7, 20) have been transmitted to sugarcane. We found that these MDMV isolates can be readily separated from the known strains of SCMV (24) because the MDMV isolates readily infect Johnson grass and react strongly with MDMV-A antiserum, which after cross-absorption as described in this paper no longer reacts with any known strain of SCMV. Our results show that marked diversity exists among isolates of MDMV-A.

The origin of these mosaic virus isolates is of more than cursory interest. It may be reasoned, on the one hand, that they have existed in nature for some time but were not observed until recently. This is rather difficult to accept in light of the interest among plant pathologists in virus problems of corn and sorghum and the economic effect of mosaic viruses on these hosts in recent years. On the other hand, it seems more probable that these isolates are mutants that have become localized in certain areas. Even within one area, we may expect to find several variants. The documented variability of SCMV leads us to expect the continuing appearance of new distinct isolates or strains. This apparent high level of variation will present problems in designating the variants and defining what are major and minor differences; i.e., what kind of difference is needed to justify designating a new variant as a new strain.

Since we expect to find variation in serological properties, stability, ease of purification, aphid transmission efficiency, etc., among virus isolates of the SCMV group, probably all these criteria should be investigated before making a decision to call an isolate a new strain. Until an isolate is so characterized, it is reasonable to place new isolates with the recognized strain of SCMV or MDMV-A, whichever they most closely resemble. Principles for differentiation and nomenclature for new strains need to be developed.

TABLE 4. Serological relationship of the five mosaic virus isolates from different areas in the USA to maize dwarf mosaic virus strain A (MDMV-A) using microprecipitin tests

Viral antigen	MDMV-A[a] reaction with antiserum
Virginia	+[b]
Frankfort	+
California	+
Quicksand	+
Mississippi	+
MDMV-A	+
13B	—
Healthy corn extract	—

[a] Antiserum was cross-absorbed twice with the 13B viral antigen to remove cross reacting antibodies. Antiserum was diluted 1:8 for microprecipitin tests.

[b] + = the presence of a flocculent type precipitate in two or three of the antigen dilutions. These dilutions were 1:3, 1:6, and 1:12 with respect to the fresh weight of infected Oh28 corn leaves.

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