

Comparison of Isolates of Panicum Mosaic Virus from St. Augustinegrass and Centipedegrass

G. E. HOLCOMB, TZY-YIN ZOE LIU, and K. S. DERRICK, Department of Plant Pathology and Crop Physiology, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge 70803

ABSTRACT

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Several St. Augustine decline (SAD) isolates of panicum mosaic virus were compared with isolates of a virus that causes a recently described disease of centipedegrass, centipedegrass mosaic (CGM). The SAD and CGM virus isolates appeared to represent several virus strains based on serological and relative electrophoretic mobility properties, and they usually contained a satellite virus. Based on these properties and the presence of a satellite virus, isolates from centipedegrass could not be distinguished from those infecting St. Augustinegrass. Mechanical transmission of isolates between the two grasses was more difficult than transmission to the same grass. One CGM isolate was mechanically transmitted from centipedegrass to St. Augustinegrass and produced a typical mosaic, but the serological and electrophoretic properties of the virus in St. Augustinegrass were different from those of the original isolate in centipedegrass. CGM and SAD virus isolates with similar properties were obtained from a lawn mixed with centipedegrass planted from seed and St. Augustinegrass that was vegetatively propagated. No satellite virus was detected in one of the SAD isolates, but it produced a typical mosaic in St. Augustinegrass and could not be distinguished by symptomatology from satellite-containing SAD isolates under greenhouse conditions.

Additional keywords: *Eremochloa ophiuroides*, *Stenotaphrum secundatum*, turfgrasses, virus diseases

St. Augustine decline (SAD), a disease of St. Augustinegrass (*Stenotaphrum secundatum* (Walt.) Kuntze), is caused by a virus that was named St. Augustine decline virus (SADV). SAD was first observed by Toler et al (17) in the Lower Rio Grande Valley of Texas and Mexico in 1967, and was subsequently reported in Louisiana in 1972, Arkansas in 1982, and South Carolina in 1988 (3,5,8). Several years after the initial reports and naming of SADV, the virus was found to be serologically related to panicum mosaic virus (PMV) (6,10,13), which occurs in Kansas on the native range grass *Panicum virgatum* L. SADV is, therefore, more correctly referred to as a strain or isolate of PMV but, because the economically important host is St. Augustinegrass, the name SADV is frequently used. The host ranges of PMV and SADV are restricted to the Gramineae and include many symptomless hosts

(11,13,15,16). The type strain of PMV causes mild mosaic symptoms on many grasses but has not been found occurring naturally on economic crop species. SADV, however, causes significant

economic losses in St. Augustinegrass (11,17). Molinia streak virus, which is related to PMV, occurs in Germany on the perennial grass *Molinia caerulea* (L.) Moench (14).

A virus disease of centipedegrass, *Eremochloa ophiuroides* (Munro) Hack., was reported by Holcomb in 1984 (7). The disease was first observed during a survey for SAD in Hammond, LA, and was named centipedegrass mosaic (CGM). Isolates of this virus will be referred to as centipedegrass mosaic virus (CGMV) in this report rather than isolates, strains, or variants of PMV. Preliminary results with agar gel diffusion serology indicated the virus is related to PMV and SADV (7). However, centipedegrass was reported not to be a host of SADV in earlier efforts to infect it by mechanical inoculation (11).

PMV and SADV usually have a satellite virus associated with them and they differ from the satellite virus in particle size, RNA content, base composition, capsid protein molecular weight, and amino acid composition (2).

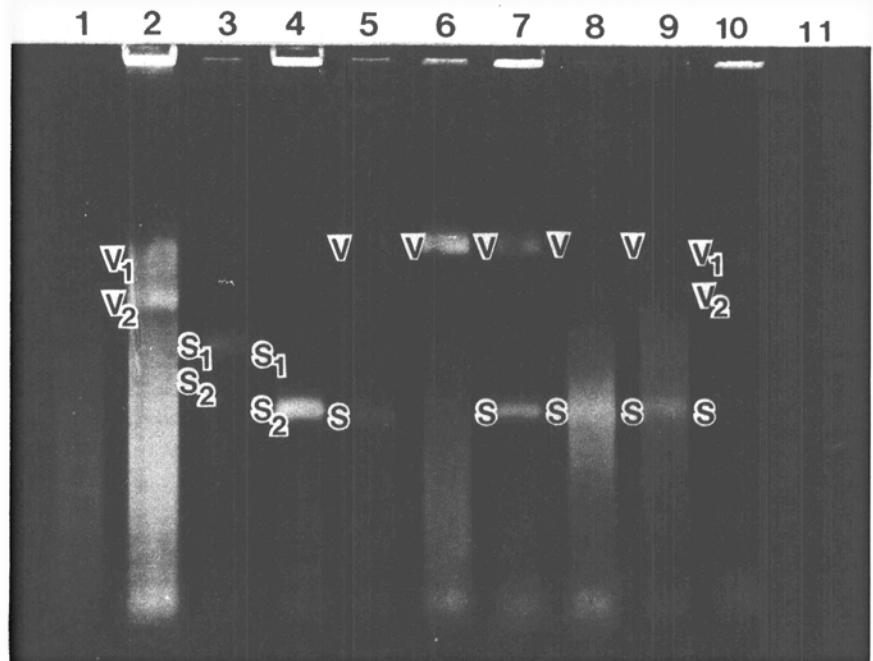


Fig. 1. Agarose gel electrophoresis of partially purified preparations from healthy grasses, St. Augustinegrass infected with St. Augustine decline virus (SADV) isolates, and centipedegrass infected with centipedegrass mosaic virus (CGMV) isolates (see text for description of individual virus isolates). 1, Healthy St. Augustinegrass; 2, SADV-1; 3, SADV-2; 4, SADV-3; 5, SADV-4; 6, SADV-5; 7, SADV-BR; 8, CGMV-BR; 9, CGMV-H; 10, CGMV-HSt; and 11, healthy centipedegrass. Electrophoretic forms of the virus coat protein are marked V, V₁, and V₂. Electrophoretic forms of the satellite virus capsid protein are marked S, S₁, and S₂.

Present address of second author: Department of Physiology, Baylor College of Medicine, Houston, TX 77030. Present address of third author: University of Florida, Institute of Food and Agricultural Sciences, Citrus Research and Education Center, Lake Alfred 33850.

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Values of about 18,500 and 30,000 daltons were determined for the capsid proteins of the satellite virus and PMV, respectively (2). PMV particles are isometric, 25–30 nm in diameter, and have a sedimentation coefficient of 109 S. The particles contain a 28 S single-stranded RNA that is infective (1,12). Satellite virus particles are isometric, 15–18 nm in diameter, have a sedimentation coefficient of 42 S, and contain a 14 S single-stranded RNA that is not infective (1,12). A sedimentation coefficient of 102 S was reported for an SADV isolate (10). This study was undertaken to

further compare several isolates of SADV and CGMV.

MATERIALS AND METHODS

Virus. Five isolates of SADV that had been collected at various sites in Louisiana and maintained in the greenhouse for several years were designated SADV 1–5. An isolate of CGMV collected from Hammond, LA, was designated CGMV-H when maintained in centipedegrass. The CGMV-H isolate was designated CGMV-HSt following mechanical transmission to St. Augustinegrass. Isolates designated SADV-BR (from St. Augustinegrass) and CGMV-BR (from centipedegrass) came from the same lawn in Baton Rouge, LA.

All SADV isolates were maintained in St. Augustinegrass and CGMV isolates were maintained in centipedegrass, except CGMV-HSt, which was maintained in St. Augustinegrass.

Partial purification of viruses and agarose gel electrophoresis. Isolates of SADV and CGMV in St. Augustinegrass or centipedegrass were partially purified up to the gradient centrifugation step, as described by Buzen et al (2). The preparations were further purified by electrophoresis on 0.7% agarose gels in 0.04 M Tris-acetate, 0.002 M EDTA, pH 8.0, at 60 V (constant voltage) for 3 hr at room temperature. The gels were stained with 0.5 µg/ml of ethidium bromide overnight at 4 C and examined with an ultraviolet transilluminator.

Protein analysis. Bands that were suspected to contain virus particles were cut from the agarose gels and boiled with 0.8 ml of treatment buffer (stacking gel buffer with 1% sodium dodecyl sulfate [SDS], 2% 2-mercaptoethanol, and 20% glycerol) for 5 min with frequent shaking. The samples were subjected to SDS-polyacrylamide gel electrophoresis (PAGE) on 12% gels (9) and were stained with Coomassie Blue. The following proteins, with molecular weights given in paren-

theses, were used as standards in the PAGE analysis of viral proteins: bovine albumin (66K), egg albumin (45K), glyceraldehyde-3-phosphate dehydrogenase (36K), carbonic anhydrase (29K), trypsin inhibitor (20.1K), and α-lactalbumin (14.2K).

Serology. An antiserum to a Kansas isolate of PMV was provided by C. L. Niblett of the University of Florida, Gainesville (13). Two antisera to Texas isolates of SADV were provided by R. W. Toler of Texas A&M University, College Station (10). Crude plant extracts containing the Louisiana isolates of SADV and CGMV were clarified by centrifugation for 1 min at 10,000 rpm and assayed by serologically specific electron microscopy (SEM), as previously described (4). The attached virus particles were given a negative stain using 2.0% aqueous uranyl acetate. Agar gel double-diffusion tests were done in

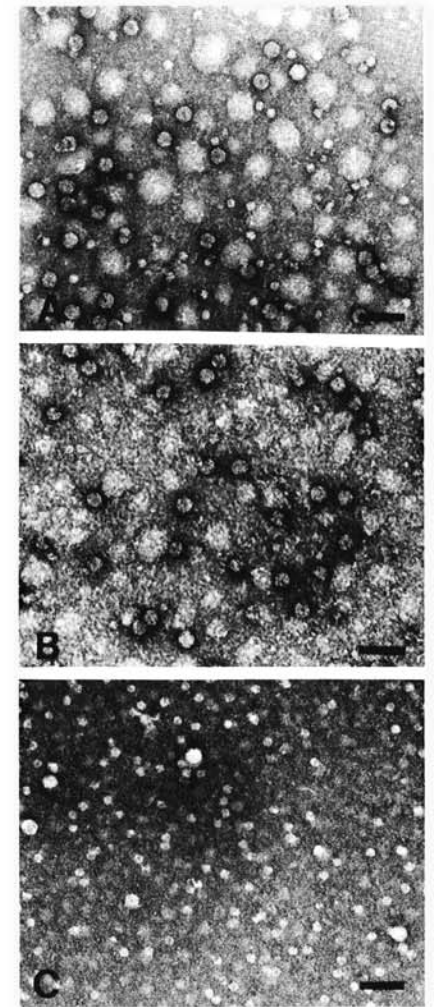


Fig. 3. Electron micrographs of serologically specific electron microscopy assays of a St. Augustine decline virus (SADV) isolate on a grid prepared using (A) antiserum to panicum mosaic virus that also contained antibodies to the satellite virus, (B) antiserum to SADV, and (C) antiserum to SADV that reacted strongly with the satellite virus. Scale bars represent 80 nm.

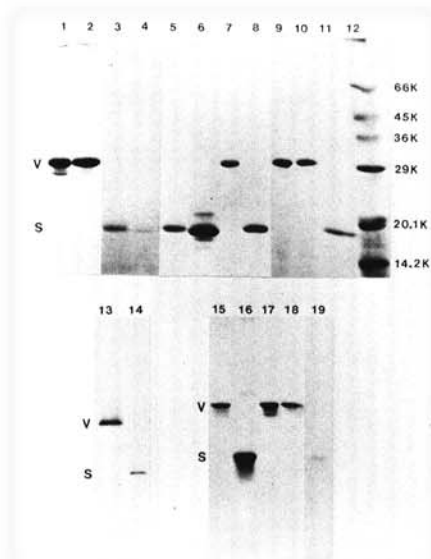


Fig. 2. Polyacrylamide gel electrophoresis of proteins from bands taken from the agarose gel shown in Figure 1. Agarose gel lane numbers and band designation: 1, lane 2, V₁; 2, lane 2, V₂; 3, lane 3, S₁; 4, lane 3, S₂; 5, lane 4, S₁; 6, lane 4, S₂; 7, lane 5, V; 8, lane 5, S; 9, lane 6, V; 10, lane 7, V; 11, lane 7, S; 12, standards; 13, lane 8, V; 14, lane 8, S; 15, lane 9, V; 16, lane 9, S; 17, lane 10, V₁; 18, lane 10, V₂; 19, lane 10, S. Virus capsid protein marked V. Satellite virus capsid protein marked S.

Table 1. Detection of St. Augustine decline virus (SADV), centipedegrass mosaic virus (CGMV), and satellite virus particles^a by serologically specific electron microscopy using different antisera

Virus isolate	Antiserum to PMV ^b		Antiserum 1 to SADV ^c		Antiserum 2 to SADV ^d	
	Virus	Satellite	Virus	Satellite	Virus	Satellite
SADV-1	+	+	+	–	+	+
SADV-2	+	+	+	–	+	+
SADV-3	+	+	+	–	+	+
SADV-4	+	+	+	–	+	+
SADV-5	+	–	+	–	+	–
SADV-BR	+	+	+	–	+	+
CGMV-BR	+	+	+	–	+	+
CGMV-Hm	+	+	+	–	+	+
CGMV-HSt	+	+	+	–	+	+
Healthy St. Augustinegrass	–	–	–	–	–	–
Healthy centipedegrass	–	–	–	–	–	–

^aFrom crude extracts clarified at 10,000 rpm.

^bAntiserum to panicum mosaic virus that reacted to both virus and satellite.

^cAntiserum to SADV that did not appear to react with satellite.

^dAntiserum to SADV that reacted with both virus and satellite.

0.5% Ionagar containing 0.15 M NaCl and 0.02% Na₂S₂O₃.

RESULTS

Analysis of partially purified preparations of the various isolates by agarose gel electrophoresis revealed two to four bands from SADV- and CGMV-infected leaves that were not observed in comparable preparations from healthy leaves (Fig. 1). All bands that were visible, some of which are very faint in the photograph (Fig. 1), were excised and analyzed for proteins by SDS-PAGE (Fig. 2). The major protein detected in the slower migrating bands (marked with a V or V₁ and V₂ when two electrophoretic forms were detected) had a relative molecular mass of about 30 kDa, as estimated from relative electrophoretic mobility. This was in agreement with the reported molecular weight of PMV capsid protein (2) and was taken as evidence that the slower migrating bands contained virus particles. Likewise, a 18.5-kDa protein was associated with the faster migrating bands on the agarose gel; these bands are marked S or S₁ and S₂ and are considered to be a satellite virus. Our value of 18.5 kDa is in reasonable agreement with the reported value of about 16 kDa for the capsid protein of PMV satellite virus (2).

The virus isolates were compared by SSEM using the following three antisera: 1) an antiserum to PMV that also contained antibodies to the satellite virus, 2) an antiserum to SADV that did not appear to react with the satellite virus, and 3) an antiserum to SADV that reacted strongly with the satellite virus. Typical results using the three antisera are shown in Figure 3. Virus particles were detected in all of the isolates (Table 1) (including those that did not give a visible virus band on agarose gels [Fig. 1]) with each of the three antisera. Satellite virus particles were detected in all of the isolates (Table 1) except SADV-5, which did not give a visible band for the satellite virus on the agarose gel.

All of the isolates, with the exception of SADV-5, gave a visible precipitin line with the antiserum to PMV when tested by agar diffusion serology (Fig. 4). Because SADV-5 does not have a satellite virus (Fig. 1, Table 1), the precipitin lines observed in Figure 4 may be due to a satellite virus. The two isolates that had two electrophoretic forms (SADV-1 and CGMV-HSt) in Figure 1 appeared heterologous (based on spur formation) when compared with the other isolates (Fig. 4). The two isolates that had two electrophoretic forms of the satellite virus (SADV-2 and SADV-3), and all the remaining isolates, appeared to be homologous (Fig. 4).

DISCUSSION

The various isolates of PMV represent a diversity of serotypes and electro-

phoretic forms (2,6). Based on serology, electrophoretic mobility, presence of a satellite virus, and the relative molecular mass of the virus and satellite virus coat proteins, the isolates causing the recently described mosaic disease of centipedegrass could not be distinguished from isolates of SADV. CGMV is, therefore, a member of the PMV/SADV group. The physical and serological properties of the viruses in the group, which vary considerably, do not correlate with their biological properties.

Due to a limited host range, comparisons of the biological properties of isolates of viruses in the group are limited. All isolates cause a persistent and indistinguishable mosaic on their natural hosts. But some isolates obviously differ in that they are more easily transmitted by sap inoculations to their natural host than to another grass. Transmissions between the same grass are very efficient, but transmission between grasses can be very low. Before finding natural infections, centipedegrass was thought to be immune to SADV and PMV (11; *authors' unpublished observations*). In repeated trials in this study there was only one transmission of CGMV to St. Augustinegrass by sap inoculation, which resulted in an isolate with different serological and electrophoretic properties.

An apparent natural spread of SADV into centipedegrass was observed. The lawn that was the source of SADV-BR and CGMV-BR used in this study was established with St. Augustinegrass planted vegetatively and centipedegrass planted from seed. No symptoms of virus infection were observed for several years. Subsequently, a mosaic was observed in the St. Augustinegrass that quickly spread throughout the lawn. It was several more years before infected centipedegrass was observed in one area of the lawn. The virus now appears to be spreading rapidly in this centipedegrass. There are no known natural vectors of PMV or SADV; SADV is apparently spread by mowing machines. It would appear that the above case of natural spread of SADV into centipedegrass was a rare event because the lawn was mowed repeatedly over a period of years during which time SADV was moving readily in the St. Augustinegrass. It should be pointed out that there are instances where St. Augustinegrass lawns have become infected without known exposure to infected grass through mowing or vegetative propagation, which leads to speculation that there may be a vector of the virus. Therefore, it is possible that the initial infection of the centipedegrass was from a source other than the infected St. Augustinegrass in the lawn.

There is some evidence to suggest that some SADV and CGMV isolates are mixtures of virus strains. The apparent change with mechanical transmission of CGMV-H to CGMV-HSt was likely due

to selective transmission of a strain into St. Augustinegrass that was not predominant in centipedegrass when the assay was made. In addition, the ratio of different strains to each other and the ratio of the virus to the satellite virus may vary with environmental conditions. The titer of the virus or satellite virus does not appear to affect symptom expression. SADV-2 and SADV-3 did not give a visible virus band on agarose gel electrophoresis, but virus particles were detected by SSEM (which is considerably more sensitive), and the isolates caused a typical mosaic in St. Augustinegrass. The titer of the satellite virus in CGMV-HSt appeared to be low, but again the isolate produced a typical mosaic in St. Augustinegrass. A satellite virus was not detected by agarose gel electrophoresis or SSEM in isolate SADV-5. This apparent absence of a satellite virus did not affect symptom expression, in that the isolate caused a typical mosaic in St. Augustinegrass.

The effect that SADV infections have on St. Augustinegrass can be a mosaic that persists for years and is barely noticeable (the homeowner may not even be aware the lawn is infected). In other cases, the grass in the entire lawn may be killed. This is attributed to lethal strains of the virus or to abiotic and biotic stresses that further weaken the virus-

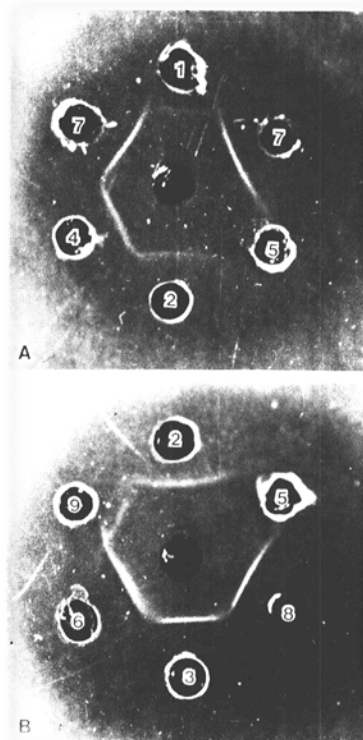


Fig. 4. Agar gel double-diffusion plates with antiserum to panicum mosaic virus in the center well. St. Augustine decline virus (SADV) and centipedegrass mosaic virus (CGMV) in wells as follows: 1, SADV-1; 2, SADV-2; 3, SADV-3; 4, SADV-4; 5, SADV-5; 6, SADV-BR; 7, CGMV-BR; 8, CGMV-H; and 9, CGMV-HSt.

infected grass. Efforts to duplicate the lethal effect under controlled conditions have not been successful. Isolates that proved to be lethal in lawns have been maintained for years under greenhouse conditions where they only cause a persistent mosaic and cannot be distinguished from nonlethal isolates. Additional experiments should be done to determine if there are lethal strains of SADV that may be predominant in the grass in lawns that are dying but that, for some reason, do not persist under greenhouse conditions.

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