Widespread Occurrence of Centipedegrass Mosaic in South Carolina

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ABSTRACT

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Isolates of panicum mosaic virus (PMV) cause St. Augustinegrass decline disease (SADD) and centipedegrass mosaic disease (CGMD). SADD is widespread in Texas and occurs in scattered locations in Louisiana, Arkansas, and Mississippi. CGMD was first observed in Louisiana in 1984 but subsequent reports have been rare. Over a 2-yr period, CGMD was found in numerous lawns in 12 counties throughout South Carolina and in at least one location in Georgia, northern Louisiana, Mississippi, and North Carolina. SADD was detected in only two counties in South Carolina. Two isolates of PMV that cause CGMD (PMV-C) were consistently transmitted by standard sap inoculation techniques to centipedegrass but erratically to Texas Common St. Augustinegrass. Symptom development was more rapid when temperatures were between 22 and 30 C than between 13 and 20 C. The PMV-C isolates were also transmitted by clipping centipedegrass with shears that had been used previously to clip infected PMV-C centipedegrass. Nimblewill was identified as an additional host of PMV-C. It does not appear that PMV-C significantly impairs growth of centipedegrass in lawns or in a greenhouse environment.

Additional keywords: *Eremochloa ophiuroides*, *Muhlenbergia schreberi*, *Stenotaphrum secundatum*

Isolates of panicum mosaic virus (PMV) are the cause of St. Augustinegrass decline disease (SADD) in Texas (7), Louisiana (4), Arkansas (1), and Mississippi (12) and centipedegrass mosaic disease (CGMD) in Louisiana (3). Subsequent reports on occurrence of CGMD have been rare. The incidence of SADD in infected lawns can range from trace amounts to 100% (1,4,7,12). A mild chlorotic mottling of leaf blades is often followed by a general chlorosis and growth reduction within a few months to a year. Necrosis of leaves and stolons can occur within 3 yr after initial symptoms are observed. In other situations, SADD can persist for years, causing only mild mosaic and little noticeable damage to the lawn (5). Little is known about the effect of isolates of PMV virus that cause CGMD (PMV-C) on the vigor of the host. Isolates of PMV that cause SADD (PMV-S) and CGMD (PMV-C) can be transmitted efficiently by rubbing expressed sap from infected plants to their respective uninfected host plants (1,4-6,11,12). Spread by lawn mower blades accounts for some of the distribution of these virus diseases in nature (5,6,8,12). No insect vectors of PMV have been reported.

Chlorotic spots and blotches were observed on centipedegrass, *Eremochloa ophiuroides* (Munro) Hack., at the Clemson University Horticulture Garden in September 1987. Similar symptoms were observed on centipedegrass and St. Augustinegrass (*Stenotaphrum secundatum* (Walter) Kuntze) specimens submitted to the Clemson University Plant Problem Clinic from Charleston, SC, a few weeks later. PMV was detected in these samples by serological assays.

The objectives of this study were to determine the occurrence and distribution of CGMD and SADD in South Carolina, the effects of PMV-C and -S on centipedegrass, and if cutting shears transmit PMV. An abstract on a portion of this research has been published (2).

MATERIALS AND METHODS

To determine the occurrence and distribution of CGMD and SADD in lawns in South Carolina, symptomatic samples were collected by Clemson Cooperative Extension personnel throughout the state. Samples of 5- to 10-cm-diameter grass plugs were placed in plastic bags and submitted to the clinic. In addition, acquisition trips to several counties were made to collect from symptomatic lawns throughout South Carolina and other southeastern states.

Serological assays were conducted on sap extracted from leaves of all samples. Uninfected St. Augustinegrass and St.

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Augustinegrass infected with the Texas isolate of PMV-S (PMV-S Tx) and antiserum to PMV-S Tx were provided by R. W. Toler of Texas A&M University. These plants were maintained as negative and positive controls for serological tests and as a source of PMV-S Tx. Serological tests were performed by enzyme-linked immunosorbent assay (ELISA) (9) with coating antibody at 2.5 μg/ml and alkaline phosphatase conjugated antibody at 0.625 μg/ml. PMV-C isolates were from symptomatic centipedegrass samples collected in Charleston (PMV-C Ch) and Clemson (PMV-C Cl), which were serologically positive for PMV.

Assay plants were either grown from seed or collected from turfgrass research plots at Clemson University. St. Augustinegrass plugs collected from the field were assayed serologically for PMV and those plugs that were negative were used in inoculation studies. Seed of foxtail millet, *Setaria italica* (L.) Beauv. 'German Strain R', and centipedegrass were sown in 10-cm-diameter clay pots which contained a peat moss, sand, and vermiculite mixture (pH 6.5) amended with 14-14-14 slow-release fertilizer (Osmocote) at the rate of 85 g per 2×10^{-2} m^3 of soil mixture. St. Augustinegrass plugs were planted in the same type of medium.

Inoculum was prepared by grinding uninfected leaves or leaves infected with PMV-S or PMV-C with a mortar and pestle in 0.03 M sodium phosphate buffer, pH 7.5. Leaves of assay plants were dusted with 600-mesh corundum and rubbed with cheesecloth dipped in the extracted sap. Millet and centipedegrass were planted 10–14 days and 5–6 mo, respectively, before being inoculated. St. Augustinegrass plugs were grown in the greenhouse for at least 1 mo before inoculation.

In a growth chamber experiment, four pots each of St. Augustinegrass, centipedegrass, and millet were inoculated with sap extracted from St. Augustinegrass infected with PMV-S Tx, centipedegrass infected with PMV-C Cl, and uninfected St. Augustinegrass. The plants were placed in growth chambers (11,000 lux, fluorescent/incandescent light, 12 hr), half where the day/night temperatures averaged 28 and 22 C, respectively, and the other half with 20 and 13 C, respectively. Plants were examined for symptoms 2, 4, and 8 wk after inoculation. After 8 wk, St. Augustinegrass and centipedegrass plants were rated on a 1–7 scale based on the percent leaf surface with chlorotic spots and blotches. Immediately after the final evaluations, selected leaves from all pots were assayed by ELISA.

In a greenhouse experiment, centipedegrass infected with PMV-C Ch was used in addition to PMV-S Tx and PMV-C Cl. Seven pots of Texas Common St. Augustinegrass were inoculated with each isolate from centipedegrass and four pots of St. Augustinegrass were inoculated with the PMV-S Tx. Seven weeks after inoculation, all symptomless inoculated plants were reinoculated with sap from young symptomatic millet leaves that had been previously inoculated with the same virus isolate. Plants were maintained in a greenhouse where temperatures varied from 20 to 30 C.

In a transmission trial, grass clipping shears were used to inoculate plants. Clippers were dipped in a trisodium phosphate suspension, washed in tap water, and used to cut centipedegrass infected with PMV-C Cl. They were then used to cut six uninfected centipedegrass plants. The procedure was repeated using healthy centipedegrass as well as centipedegrass infected with PMV-C Ch to inoculate six and three additional centipedegrass plants, respectively. All plants were assayed by ELISA 8 wk after inoculation. Temperatures in the greenhouse varied from 20 to 32 C during the course of the study.

RESULTS

CGMD was identified by ELISA in 12 counties of South Carolina (Fig. 1). More than 80% of the 40 specimens submitted to the clinic with suspected CGMD were positive for PMV by ELISA. The CGMD was observed and PMV verified by ELISA in approximately 30% of 50 centipedegrass lawns randomly visited in more extensive surveys in Charleston and Beaufort counties over an 18-mo period. In one lawn in Charleston County, affected areas ranged in size from 1 to 10 m. The irregular diseased patches were easily detected because of a general off-color or yellowing but the grass did not appear to be stunted. Chlorotic spots and blotches were obvious on the blades (Fig. 2).

Symptoms were not as noticeable in the summer of 1989 as they were in 1988. In most lawns, CGMD symptoms were found only after close examination of individual leaves. In South Carolina, symptoms were first noticed in early spring within 6 wk after the grass initiated new growth. CGMD was also detected in three lawns in Columbus, MS, in a centipedegrass research plot in Griffin, GA, in four counties in North Carolina, and in northern Louisiana. The locations in North Carolina were primarily commercial landscapes in Jacksonville, Kinston, Williamston, and Wilmington counties. ELISA confirmed the presence of PMV in the sites with CGMD.

Mosaic symptoms developed more slowly in the 13–20 C chamber than in the 22–30 C chamber. Two weeks after inoculation, the only mosaic symptoms observed in the lower temperature chamber were on the millet inoculated with the PMV-S Tx and PMV-C Cl isolates. In the 22–30 C chamber, mosaic symptoms were present on the St. Augustinegrass inoculated with PMV-S Tx as well as on the millet inoculated with PMV-S Tx and PMV-C Cl. The inoculated millet plants were stunted compared with the controls. No symptoms were observed on centipedegrass.

Four weeks after inoculation, distinct mosaic symptoms were present on the St. Augustinegrass and millet plants inoculated with PMV-S Tx at 13–20 C. Only mild mosaic symptoms were

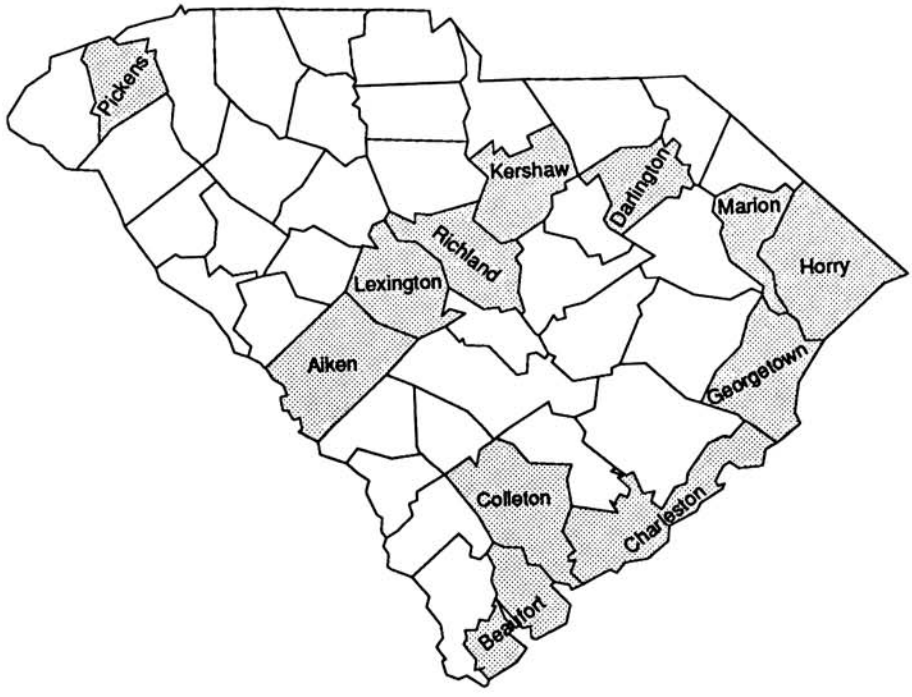

Fig. 1. South Carolina counties in which centipedegrass mosaic was confirmed.

observed on the centipedegrass inoculated with the PMV-S Tx and PMV-C Cl isolates. Symptoms on millet inoculated with the PMV-C Cl were not as pronounced as symptoms on millet inoculated with PMV-S Tx. At 22–30 C, distinct mosaic symptoms were observed on all plants inoculated with PMV-S Tx. Good symptoms were present on the centipedegrass and millet plants inoculated with PMV-C Cl. Millet plants inoculated with both isolates were stunted compared with the checks. Because of rapid vertical growth of the plants over the 4-wk period, the plants did not remain erect. Serological tests of all inoculated millet plants were positive for PMV and the plants were discarded. Chlorotic spots were observed on young leaves of one St. Augustinegrass plant inoculated with the PMV-C Cl isolate, and PMV infection was confirmed by ELISA.

Estimations of percent leaf surfaces exhibiting mosaic symptoms were made 8 wk after inoculation (Table 1). Disease indices were higher on St. Augustinegrass and centipedegrass inoculated with PMV-S Tx and maintained in the 22–30 C chamber than on similar plants maintained in the 13–20 C chamber (Table 1). Symptom development was greater on St. Augustinegrass than on centipedegrass inoculated with PMV-S Tx. On centipedegrass inoculated with PMV-C Cl, symptoms developed on less than 5% of the leaf surfaces of plants maintained at 13–20 C compared with 26–75% of leaf surfaces of plants maintained at 22–30 C. Symptoms did not develop on St. Augustinegrass plants inoculated with the PMV-C Cl and maintained at 13–20 C, but a few symptoms developed on one plant maintained at 22–30 C. No symptoms developed on the other inoculated plants. All uninoculated plants remained free of symptoms during the study. Serological assays for PMV were negative on all symptomless plants in the study and positive for all that exhibited mosaic symptoms.

In the greenhouse study in which St. Augustinegrass was inoculated with PMV-C Ch, PMV-C Cl, and PMV-S Tx, symptoms developed within 6 wk on one of seven, two of seven, and two of four plants, respectively. Symptoms on plants inoculated with the PMV-C isolates were restricted to one to three leaves per plant and flecking was the only symptom present. Chlorotic spots and blotches were observed on the two St. Augustinegrass plants which developed symptoms after inoculation with PMV-S Tx. After a second inoculation of all plants with sap from millet leaves infected with the respective isolates, symptoms developed on the other two plants inoculated with the PMV-S Tx isolate that had not exhibited symptoms after the initial inoculation. No additional symptom development was observed 6 wk after plants were reinoculated with the PMV-C isolates.

PMV-C isolates were transmitted from infected to uninfected centipedegrass via contaminated clippers. PMV-C Cl and PMV-C Ch were transmitted to four of six and three of six plants, respectively. Symptoms appeared within 6 wk after inoculation. ELISA results were positive for all plants that exhibited symptoms and negative for those that did not.

DISCUSSION

CGMD is widespread in South Carolina. Detection was common after the initial confirmation and the disease symptoms became familiar. Careful observation is necessary to find the disease, because it is not noticeably detrimental to the growth of centipedegrass. Close inspection of grass blades for mosaic symptoms at less than three sites was required to detect the disease at most locations.

After CGMD was confirmed in one or more locations in counties throughout the state to document that the disease was widespread, additional collections were not made on a regular basis. However, based on more extensive surveys in Charleston and Beaufort counties, more than 30% of the lawns in residential and commercial landscapes exhibited symptoms. CGMD was observed in North Carolina, Georgia, Mississippi, and northern Louisiana after examining only a few plantings. Therefore, it is likely that CGMD also occurs commonly in these states, but it has not been previously confirmed (except in southern Louisiana) because of the apparent lack of detrimental effects.

SADD was detected in two lawns, one in Charleston and one in Columbia, in September 1987. Symptoms were not severe and were detected primarily because close observations were being made for CGMD in the same general areas. Advanced symptoms of SADD were not noted over the next 2 yr, thus, it was presumed that a nonlethal strain of PMV-S was involved or that environmental conditions were not appropriate for the lethal disease phase. SADD was not detected in any other areas of South Carolina.

Inoculations of PMV-S and PMV-C isolates on St. Augustinegrass, centipedegrass, and millet supported previous work which found that the incubation period of PMV was temperature dependent (10). Dale and McDaniel (1) also observed symptoms on St. Augustinegrass within 15 days after inoculation when the temperature was maintained at 25 C. Ratings of percent leaf surfaces with symptoms 8 wk after inoculation also demonstrated the effect of temperature on symptom development.

Symptoms could be detected within 6 wk after centipedegrass initiated new growth in the spring by closely examining leaf blades. Symptoms were more pronounced and easier to detect later. This was probably associated with the increase in temperatures. However, temperatures of 30 C and above may not favor rapid disease development. Holcomb et al (4) found that when temperatures as high as 35 C occurred in the greenhouse, incubation periods of 40–60 days were required for symptoms to develop after St. Augustinegrass was inoculated with a PMV-S isolate.

Various cultivars of proso millet (*Panicum miliaceum* L.) react differently

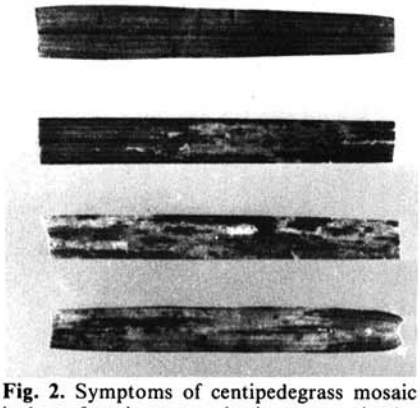

Fig. 2. Symptoms of centipedegrass mosaic isolate of panicum mosaic virus on centipedegrass. Some leaf blades have very mild symptoms (top) whereas others have large chlorotic blotches and ring patterns.

Table 1. Percent leaf surfaces of St. Augustinegrass and centipedegrass exhibiting mosaic symptoms 8 wk after inoculation of two pots each with two isolates at two temperature regimes

	St. Augustinegrass		Centipedegrass	
Isolate[a]	13–20 C	22–30 C	13–20 C	22–30 C
PMV-S Tx	5[b]	7	4	5
	4	6	2	5
PMV-C Cl	1	1	2	6
	1	3	2	5
Control	1	1	1	1
	1	1	1	1

[a] PMV-S Tx = panicum mosaic virus isolate from Texas that causes St. Augustinegrass decline disease (SADD); PMV-C Cl = panicum mosaic virus isolate from Clemson, SC, that causes centipedegrass mosaic disease.

[b] Percent leaf surface ratings are based on a 1–7 scale where 1 = no symptoms, 2 = symptoms on 1–5, 3 = 6–10, 4 = 11–25, 5 = 26–50, 6 = 51–75, and 7 = 76–100%.

to isolates of PMV-S. Plants inoculated with PMV-S from Arkansas, Texas, and Mississippi died within 5 wk after inoculation (1,7,12). Turghai proso millet inoculated with an isolate from Louisiana developed symptoms in 14–21 days but the necrotic reaction did not follow (4). Foxtail millet (*S. italica*) has been a reliable indicator host; PMV isolates from Texas, Arkansas, and South Carolina all cause a readily detectable mosaic in this species (1,6).

McCoy et al (7) reported that no symptoms developed after inoculation of centipedegrass with a PMV-S isolate from Texas. Research a few years later indicated that symptoms developed 14–28 days after mechanical inoculation of centipedegrass with a PMV-S isolate (11). However, mottling was less intense and symptom development was slower in centipedegrass than in St. Augustinegrass. Similar results were obtained in this study with different temperature regimes.

In an earlier study (5), there was only one transmission of PMV-C to common St. Augustinegrass by sap inoculation in repeated trials. This transmission resulted in an isolate with different serological and electrophoretic properties. We were unable to transmit the PMV-C Cl and PMV-C Ch isolates to the St. Augustinegrass cultivars Seville and Raleigh in preliminary studies (R. A. Haygood and O. W. Barnett, *unpublished*) and only to four of 18 Texas Common St. Augustinegrass plants in this study. When symptoms did develop, they were generally restricted to one or only a few inoculated leaves and flecking was the primary symptom observed.

During the earlier stages of this study, it was noted that nimblewill, *Muhlenbergia schreberi* J. F. Gmelin, was growing in several pots with centipedegrass collected in the field. Symptoms developed on nimblewill in pots in which grass was inoculated with PMV-C Cl but not on uninoculated nimbleweed. Serological assays of symptomatic leaves detected the presence of PMV. This is the first known report on susceptibility of nimblewill to an isolate of PMV.

Transmissions of the PMV-S Tx to Texas Common St. Augustinegrass and the PMV-C Cl and PMV-C Ch to centipedegrass were very efficient by mechanical sap inoculation techniques. Other researchers have achieved good success with transmissions of PMV-S isolates to St. Augustinegrass, and it has been presumed that spread in nature occurs primarily via lawn mowers (5,6,8,12) because there are no known insect vectors. This study supports the theory that PMV is spread by lawn mowers because PMV-C could be transmitted effectively from infected to healthy centipedegrass with clippers.

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