

Maize fine streak virus, a New Leafhopper-Transmitted Rhabdovirus

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Accepted for publication 18 June 2002.

ABSTRACT

Redinbaugh, M. G., Seifers, D. L., Meulia, T., Abt, J. J., Anderson, R. J., Styer, W. E., Ackerman, J., Salomon, R., Houghton, W., Creamer, R., Gordon, D. T., and Hogenhout, S. A. 2002. Maize fine streak virus, a new leafhopper-transmitted rhabdovirus. *Phytopathology* 92:1167-1174.

A previously uncharacterized virus was isolated from fall-planted sweet corn (*Zea mays* L., Syngenta GSS 0966) leaves showing fine chlorotic streaks. Symptomatic plants were negative in enzyme-linked immunosorbent assay against many maize viruses, but reacted weakly with antisera to *Sorghum stunt mosaic virus* suggesting a distant relationship between the viruses. The virus was readily transmitted by vascular puncture inoculation (VPI), but not by leaf-rub inoculation. Symptoms on maize included dwarfing and fine chlorotic streaks along intermediate and small veins that developed 12 to 17 days post-VPI. The isolated virus

was bacilliform (231 ± 5 nm long and 71 ± 2 nm wide), with a knobby surface, and obvious helical structure typical of rhabdovirus morphology. Nucleorhabdovirus virions were observed by transmission electron microscopy of infected maize leaf tissue sections. Proteins unique to infected plants were observed in extracts of infected leaves, and the isolated virion contained three proteins with molecular masses 82 ± 2 , 50 ± 3 , and 32 ± 2 kDa. Preliminary sequence analysis indicated the virus had similarity to members of the family *Rhabdoviridae*. The virus was transmitted by *Graminella nigrifrons* under persistent conditions. The data indicate the virus, provisionally designated Maize fine streak virus, is a new species in the genus *Nucleorhabdovirus*.

Additional keywords: corn, emerging virus, *Maize mosaic virus*, virus disease.

Plant rhabdoviruses have complex bacilliform or bullet-shaped virions composed of RNA, protein, and lipid and are transmitted in a persistent manner by aphids, leafhoppers, planthoppers, lacebugs, or mites (17). A number of rhabdoviruses have been found to infect maize (*Zea mays* L.) worldwide. Of these, *Maize mosaic virus* (MMV) and *Wheat American striate mosaic virus* (WASMV) cause disease in North America (17). MMV was first reported in Hawaii and subsequently confirmed in Florida (5,10). However, rhabdovirus-like particles of an unidentified virus were reported in the 1970s in maize from other southeastern states (Alabama, Louisiana, Mississippi, and Texas) and Iowa and Nebraska (12).

Because the control of viral diseases requires accurate knowledge of virus properties to develop diagnostic tools and understand factors affecting virus spread, study of emerging viruses in crops is important. In this study, we report on the isolation, culture, and characterization of a previously uncharacterized maize-infecting rhabdovirus from leaf samples obtained from Decatur County in southwestern Georgia that exhibited chlorotic vein streaking. Based on symptoms incited by the virus, it has been provisionally named Maize fine streak virus (MFSV).

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Publication no. P-2002-0826-02R

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MATERIALS AND METHODS

Viral inoculum. Inoculum was prepared by grinding leaf tissue in 5 volumes of 10 mM potassium phosphate, pH 7. Initially, extracts were prepared from field-grown maize leaves that expressed streaks similar to those incited by *Maize rayado fino virus* (MRFV). Subsequently, inoculum was prepared from symptomatic 'Spirit' sweet corn leaves. Fractions from the purification procedure were used as inoculum. The Hawaiian isolate of MMV was described previously (32) and was maintained by vascular puncture inoculation (VPI) (25). Freeze-dried leaves infected with *Maize Iranian mosaic virus* (MIMV) (a gift from K. Izadpanah, Shiraz University, Iran), frozen maize leaf extracts containing *Sorghum stunt mosaic virus* (SSMV), and frozen wheat leaf extracts containing WASMV were used as positive controls for serological experiments.

Pathogen transmission. The virus was maintained in 'Spirit' sweet corn using VPI (25). Mechanical transmission of the virus by leaf-rub inoculation was tested as described for *Maize necrotic streak virus* (MNeSV) (30). For each test, 5 to 17 seedlings of maize 'Spirit' (six replications), Oh28 (eight replications), Pioneer hybrid 3379 (P3379) (two replications), *Triticum aestivum* 'Freedom' (six replications), and *Sorghum bicolor* 'Atlas' (six replications) were inoculated. Soil transmission of the pathogen was tested as previously described (30). Seven replications of 50 kernels per replicate were tested.

Vector transmission. Eight insect species that use maize as a feeding and/or developmental host were chosen to determine the vector range and transmission characteristics of the virus. The corn root aphid *Aphis maidis-radici* (Forbes), green peach aphid *Myzus persicae* (Sulzer), potato aphid *Macrosiphum euphorbiae*

(Thomas), and oat bird cherry aphid *Rhopalosiphum padi* L. from the family Aphididae; corn leafhopper *Dalbulus maidis* (DeLong and Wolcott) in the tribe Macrostelini, and black-faced leafhopper *Graminella nigrifrons* (Forbes) and painted leafhopper *Endria inimica* (Forbes) both in the tribe Deltocephalini from the family Cicadellidae; corn planthopper *Peregrinus maidis* (Ashmead) from the family Delphacidae; and, western corn rootworm *Diabrotica virgifera virgifera* (LeConte) from the family Chrysomelidae were tested. Insects were reared and transmission tests were performed as described (8,30).

Seedlings (3- to 5-day old) of maize inbred Oh28 or sweet corn hybrids ('Early Sunglow', 'Seneca Chief', or 'Spirit') were used for vector transmission. Nonpersistent transmission was tested after a 15 min starvation period using a 15 min acquisition-access period (AAP) and 24-h inoculation-access period (IAP) as described (28). For semipersistent transmission, the AAP was 2 days and the IAP 7 days (26,38). Persistent transmission involved an AAP of 7 to 14 days, followed by successive IAP of 7 days each until the insects died (3,37). After the IAP, test plants were fumigated with Vapona for 2 h to eliminate insects, and then observed for symptom development in greenhouses maintained at 28°C (day) and 22°C (night) for 28 to 35 days.

Infection of maize germ plasm. Differences in infection rates and symptom expression among maize genotypes after virus inoculation were determined by examining plants for 4 weeks under greenhouse conditions following VPI. The infection rate and area under the disease progress curve (AUDPC) was calculated for three replications of 20 kernels each (29). Significant differences among means were determined using LSD ($\alpha = 0.05$).

Maize lines Hi31 and Hi34 (36) were obtained from J. L. Brewbaker (University of Hawaii, Honolulu) and maintained in Ohio. CML202 and Tzi4 (20) were originally from CIMMYT (Zimbabwe). Pa405 (33), Oh1VI (28), Va35, Oh28, Mo17, and B73 were from our stocks. 'Spirit', VOG134, and GSS 0966 were supplied by Syngenta Seeds Inc. (Boise, ID). Pioneer hybrid 3379 (P3379) was a gift from D. Wilkinson (Pioneer Hi-Bred International, Inc., Johnston, IA), and 'Seneca Chief' was from Seminis Vegetable Seeds (Oxnard, CA).



Fig. 1. Sweet corn 'Spirit' 25 days after vascular puncture inoculation with a leaf extract from a plant infected with Maize fine streak virus.

Virus purification and characterization. MFSV was partially purified from maize leaf lamina tissue (26 to 40 days post-VPI) using procedures described by Skaf and Carroll (44) and Creamer (6). Briefly, an extract was prepared in 0.1 M sodium phosphate, pH 7.2, containing 10% sucrose and treated with activated charcoal (44). After removing the charcoal by centrifugation, the extract was filtered through polyester fiber (Fairfield Corp., Danbury, CT), layered on a 35% sucrose pad, and centrifuged at 99,000 × g for 30 min. The resulting pellet was resuspended in extraction buffer, and virions were purified on sequential 10 to 40% and 25 to 50% sucrose gradients in 0.1 M sodium phosphate, pH 7.2 (6). Purified virions were concentrated by centrifugation (105,000 × g for 3 h) and resuspended in 1 ml of 25 mM HEPES, pH 7.4, containing 0.15 M NaCl. Protein concentrations were determined using the dye binding method (39).

Electron microscopy. Purified virus samples were placed on 300-mesh formvar and carbon-coated copper grids and negatively stained using 1% phosphotungstic acid, pH 7. Samples were viewed and photographed with a Philips 201 transmission electron microscope (TEM) at 80 kV.

Ultrathin sections were prepared from infected and healthy maize leaf samples. Plant tissue was fixed in 3% glutaraldehyde, 2% paraformaldehyde, and 0.1 M sodium phosphate, pH 7.2, at 4°C for 16 h, then postfixed for 1h in 0.1 M sodium phosphate, pH 7.2, containing 2% OsO₄, dehydrated using a graded ethanol and acetone series, and embedded in Spurr's resin using a 16-h polymerization at 60°C. Ultrathin sections were counterstained with uranyl acetate and lead citrate and viewed with a Hitachi 7500 TEM.

Minipurification and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were extracted and purified using the procedure of Lane (22) as modified by Seifers et al. (43). SDS-PAGE (10% gels) was done according to Laemmli (21), and gels were stained with Commassie Brilliant Blue (CBB) (43). The analyses were conducted three times.

Proteins from purified virions were separated by SDS-PAGE on 4 to 20% Tris-HCl Ready Gels (BioRad, Richmond, CA) according to the "Ready Gels Application Guide" (Cat. No. 161-0993).

TABLE 1. Response of maize inbreds and hybrids to vascular puncture inoculation with Maize fine streak virus

Maize entry	Resistance ^w	% Infection ^x	AUDPC ^y	Severity ^z
P3379		89 a	1760 a	2
Spirit		84 a	1980 a	3
Seneca Chief		82 a	1570 ab	3
B73		80 a	1530 ab	2
Early Sunglow		79 ab	1690 a	3
Mo17	MNeSV	78 ab	1070 bc	2
Pa405	Potyviriidae	56 bc	870 cd	2
Oh28		52 c	1060 bc	3
Va35		35 cd	410 de	2
GSS 0966		25 de	310 de	3
CML202	MSV	23 def	300 de	1
Tzi4	MSV	13 def	170 e	3
VOG134		10 ef	41 e	2
Oh1VI	MCDV	1 f	2 e	1
Hi31	MMV	0 f	0 e	0
Hi34	MMV	0 f	0 e	0

^wMaize entries were resistant to the following viruses: MCDV (*Maize chlorotic dwarf virus*), MMV (*Maize mosaic virus*), MNeSV (*Maize necrotic streak virus*), and MSV (*Maize streak virus*), and the Potyviriidae including *Maize dwarf mosaic virus*, *Sugarcane mosaic virus-MDB*, and *Wheat streak mosaic virus*.

^xMean percent infection rates for three replications (20 plants per replication) are presented. Means followed by different letters were significantly different ($P > 0.05$).

^yArea under disease progress curve (AUDPC). Means followed by different letters were significantly different ($P > 0.05$).

^zThe severity rating was based on a scale of 0 (no symptoms) to 3 (severe vein streaking).

Low Range Molecular Weight Markers (BioRad) were used as standards. After electrophoresis, proteins in the gels were stained with silver (34) or CBB. The analysis was repeated nine times.

Enzyme-linked immunosorbent assay (ELISA) and western blot analyses. Two New Zealand rabbits were immunized with two injections of purified virions (0.3 or 0.75 mg of protein per injection) on day 0, and after 5 weeks (0.5 mg of protein) according to a standard protocol (13). The rabbits were bled in weeks 5, 7, 9, and 11, and antibody titer was determined using the F(ab')₂-Protein A ELISA as described (30). After 11 weeks, the animals were exsanguinated, and serum was lyophilized and stored at -20°C. For assays, leaf tissue was tested using an F(ab')₂-Protein A ELISA as described (30), except that 1 mM 2,2-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) and 0.1% H₂O₂ in 0.1 M sodium citrate buffer, pH 4.2, were used to detect horseradish peroxidase activity. The absorbances at 405 nm were measured after 20 min incubation with substrates.

For western blot analysis, leaf protein extracts (1.75 mg fresh weight) were separated on Ready Gels (BioRad) as described above, transferred to Immobilon-P membranes (Millipore, Inc., Bedford, MA), and probed with virus antisera as described (40). Antisera raised to MFSV (1:2,000 dilution), MMV (1:2,500) (11), MIMV (1:2,000) (15), SSMV (1:2,000) (6), and WASMV (1:2,000) (42) were used.

Genome characterization. Genome nucleic acid type and size were determined for nucleic acids extracted from purified virions using a modification of the procedure of Milner and Jackson (35). Briefly, purified virions were diluted 10-fold with 10 mM sodium phosphate, pH 7.2, and brought to 0.5% SDS. After incubation on ice for 45 min, the sample was layered on 10 mM EDTA, pH 7, 5.7 M CsCl and centrifuged at 100,000 × g at 10°C for 16 h. The pellet was resuspended in 500 µl of water, precipitated with sodium acetate and ethanol, and resuspended in 11 µl of water (41). The RNA concentration was determined using PicoGreen according to the manufacturer's instructions (Molecular Probes, Eugene, OR).

The isolated RNA was used for cDNA synthesis with the Superscript Choice System according to the manufacturer's instructions (Invitrogen Corp., Carlsbad, CA). The cDNAs were ligated into *Eco*RI-digested, phosphatase-treated pGEM4z (Promega Corp., Madison, WI), and transformed into *E. coli* TOP10 cells (Invitrogen Corp.). Clones carrying 1.5- (MFSV3GA) and 3-kb (MFSV6GA) inserts that hybridized with a 13-kb RNA in MFSV-infected leaves were selected for sequence analysis. Sequencing was carried out in 550- to 700-bp segments by primer walking using an ABI 3700 DNA Sequence Analyzer and Big Dye Terminator chemistry according to the supplier's instructions (PE Applied

Biosystems, Foster City, CA). Sequences were analyzed using MacPHRED-MacPHRAP (CodonCode Corp., Dedham, MA). The sequences have been deposited in GenBank with accession numbers AF518001 (MFSV3GA) and AF518002 (the 5' 1.4 kb of MFSV6GA). The translated cDNA sequences were aligned with rhabdovirus protein sequences using ClustalX (version 1.8, 45). Distances were calculated from the portion of amino acid differences, and trees were constructed using MEGA (19). Alignment gaps were removed from the aligned sequences prior to the distance calculation.

L protein sequences were obtained by translating the indicated portions of genomic sequences for the following viruses: *Bovine ephemeral fever virus* (BEFV; 9338-10871 nt of GenBank AF234533), *Infectious hematopoietic necrosis virus* (IHNV; 5785-7352 nt of GenBank NC 001652), *Mokola virus* (MOKV; 6360-7900 nt of GenBank Y09762), *Northern cereal mosaic virus* (NCMV; 7513-9044 nt of GenBank AB030277), *Rabies virus* (RABV; 6335-7875 nt of GenBank RAVMMGN), *Rice transitory yellowing virus* (RTYV; 8797-10322 nt of GenBank AB011257), *Snakehead rhabdovirus* (SHRV; 6256-7823 nt of GenBank AF147498), *Sonchus yellow net virus* (SYNV; 8189-9710 nt of GenBank L32603), *Viral hemorrhagic septicemia virus* (VHSV; 5822-7389 nt of GenBank NC 000855), *Vesicular stomatitis virus* Indiana isolate (VSVIN; 5615-7145 nt of GenBank VSVCG), and *Vesicular stomatitis virus* New Jersey isolate (VSVNJ; 896-2426 nt of GenBank M29788). *Ebola virus* (EBOV; 12492-14075 nt of GenBank NC 002549) was used as an outgroup.

N protein sequences were obtained by translating the indicated portions of the genomic sequences for the following viruses: BEFV (51-1378 nt), IHNV (175-1350 nt), *Lettuce necrotic yellows virus* (LNYV; 79-1450 nt of GenBank L30103), MOKV (49-1491 nt), NCMV (142-1437 nt), RABV (59-1482 nt), RTYV (207-1920 nt), SHRV (56-1516 nt), SYNV (47-1697 nt), VHSV (168-1422 nt), and VSVIN (64-1332 nt). EBOV (56-3026 nt) was used as the outgroup.

RESULTS

In the fall of 1999, leaf samples exhibiting chlorotic vein streaking were obtained from fields near Bainbridge (Decatur County) in southwestern Georgia. Symptoms were widespread in Syngenta Bt hybrid GSS 0966. The plants had fine chlorotic streaks along the major leaf veins similar to symptoms associated with MRFV. Symptomatic leaf material was unreactive in ELISA against antisera to MRFV, *Maize dwarf mosaic virus* (MDMV), *Sugarcane*

TABLE 2. Insects tested for transmission of Maize fine streak virus

Vector	Family	Mode ^w	No. of insects ^x	Trans ^y	n ^z
<i>Diabrotica virgifera</i>	Chrysomelidae	N	15	Neg	1
<i>Myzus persicae</i>	Aphididae	N	100	Neg	3
		P	100	Neg	2
<i>Rhopalosiphum padi</i>	Aphididae	P	50	Neg	2
<i>Peregrinus maidis</i>	Delphacidae	P	200	Neg	4
<i>Dalbulus maidis</i>	Cicadellidae	P	200	Neg	2
<i>Endria inimica</i>	Cicadellidae	S	50	Neg	2
		P	50	Neg	2
<i>Graminella nigrifrons</i>	Cicadellidae	S	200	Neg	2
		P	200	Pos	11

^wThe mode of transmission was nonpersistent (N), semipersistent (S), or persistent (P).

^x Number of insects tested per experiment.

^y Transmission was scored as positive (pos) if any test plants became symptomatic within the 28 to 35 day observation period. Neg indicates that no plants became symptomatic.

^z n = number of independent replications.

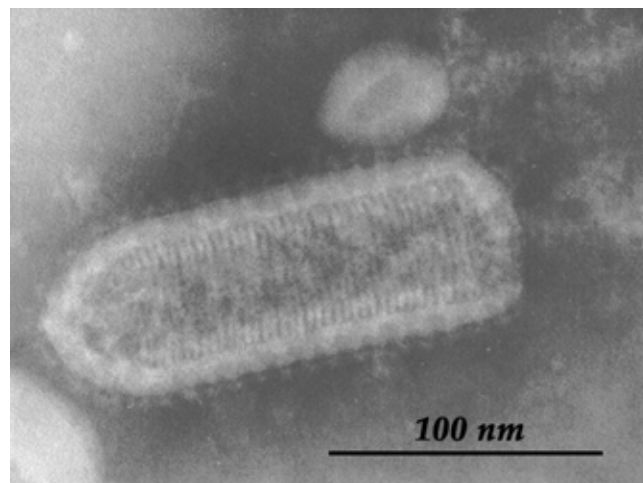


Fig. 2. Ultrastructural analysis of Maize fine streak virus (MFSV). A transmission electron micrograph of purified MFSV negatively stained with phosphotungstic acid.

maize virus-MDB (SCMV-MDB), and *Maize chlorotic dwarf virus* (MCDV), and bioassay by rub-inoculation was negative (data not shown). Similar symptoms were noted in non-Bt corn, but a much smaller percentage of plants were infected. In 2000, Bt corn was not planted in the area, but symptoms were noted on a small percentage of non-Bt corn plants from which rhabdovirus-like particles were isolated (data not shown). An extract from symptomatic leaves was used to inoculate maize 'Spirit' kernels using VPI. After 14 days, the seedlings showed symptoms similar to those on leaves used for inoculum.

Symptomatology. The first symptoms generally appeared on the second to fourth leaves 10 to 14 days post-VPI. The initial symptoms included chlorotic spots and short streaks on small veins that were unevenly distributed (data not shown). Symptoms were fully developed with continuous streaks on intermediate and small veins, on leaves four to seven, between 3 and 4 weeks post-VPI (Fig. 1). The streaks enlarged as the plants matured, such that after approximately 6 weeks post-VPI the leaves appeared white with isolated green spots. Alternatively, some plants showed a partial recovery with only scattered chlorotic spots and streaks on later leaves.

Pathogen transmission. MFSV was transmitted by VPI to several maize inbred and hybrid lines (Table 1). The virus was not mechanically transmitted by leaf-rub inoculation to maize ('Spirit', Oh28, P3379), wheat or sorghum, and no soil transmission of MFSV was observed (data not shown).

MFSV was not transmitted by any of the insects tested under nonpersistent or semipersistent conditions (Table 2). Because electron microscopy indicated the pathogen was a rhabdovirus (described below), vector transmission under persistent conditions was tested with species of the Aphididae, Delphacidae, and Cicadellidae. Transmission by the known maize rhabdovirus vectors *P. maidis* (MMV) and *Endria inimica* (WAMSV) was unsuccessful. Only the leafhopper, *G. nigrifrons*, transmitted MFSV. *G. nigrifrons* also transmitted MFSV to barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), oat (*Avena sativa*), giant foxtail (*Seteria faberi*), and rye (*Secale cereale*), but did not transmit the virus to sorghum (*Sorghum bicolor* 'Atlas') or johnson-grass (*Sorghum halpense*) (data not shown).

MFSV resistance in maize. All maize lines except Hi31 and Hi34 were infected following VPI, but at significantly different levels (Table 1). The first six entries (starting with P3379) had similar high percentage infections, while the remaining lines were infected at significantly lower levels. It should be noted that, although MFSV did not infect Hi 31 and Hi 34, their infection rates were not different from those of CML202, Tzi4, VOG134, and Oh1VI. Similar, but not identical results were seen for AUDPC

data. The severity of symptoms also varied among entries, but was different from infection levels or AUDPCs. For example, while GSS 0966 had a fairly low infection rate, viral symptoms in this hybrid were severe, but some lines with high infection rates had less severe symptoms (P3379 and B73).

Virus purification and Koch's postulates. A diffuse, optically dense band was evident after isobouyant centrifugation of extracts from symptomatic maize leaves. The yield of purified protein was $19 \pm 6 \mu\text{g/g}$ fresh weight leaf tissue (mean \pm standard error [SE], $n = 5$). The purified virus preparation contained bullet-shaped or bacilliform particles $231 \pm 5 \text{ nm}$ long and $71 \pm 2 \text{ nm}$ wide (mean \pm SE, $n = 14$) (Fig. 2). When fractions containing purified virions

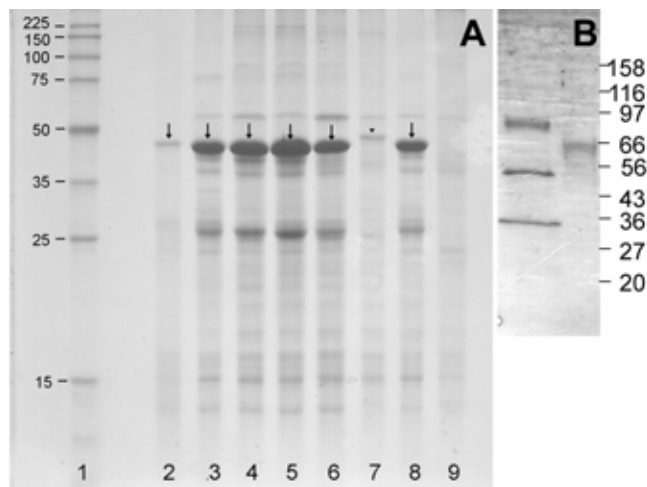


Fig. 4. Analysis of proteins associated with Maize fine streak virus (MFSV). **A**, Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of crude extracts from leaves infected with MFSV. Soluble proteins were concentrated by minipurification, separated by SDS-PAGE, and stained with Coomassie blue. Lane 1, molecular mass standards (Prosieve Protein Markers, FMC, Philadelphia, PA). The masses (kDa) of the standards are indicated. Lanes 2, 3, and 8 contain extracts of leaves infected with MFSV (2000 isolate). Lanes 5 and 6 contain extracts of leaves infected with MFSV (1999 isolate). Lane 4 contains an extract from a plant inoculated with the extract used in lane 8. Lane 7 contains an extract from a plant infected with *Maize mosaic virus* (MMV), and lane 9 contains an extract from healthy 'Spirit' corn. Vertical arrows mark position of a protein associated only with symptomatic leaves following serial transfer by vascular puncture inoculation (VPI). The arrow head (lane 7) marks location of a protein associated with MMV propagated in corn by VPI. **B**, SDS-PAGE of isolated virion proteins. Proteins from 1 μg of MFSV virions (left) and 0.15 μg of purified MMV (right) are shown. The migration of standard proteins (BioRad low range molecular weight standards) is indicated to the right of the figure.

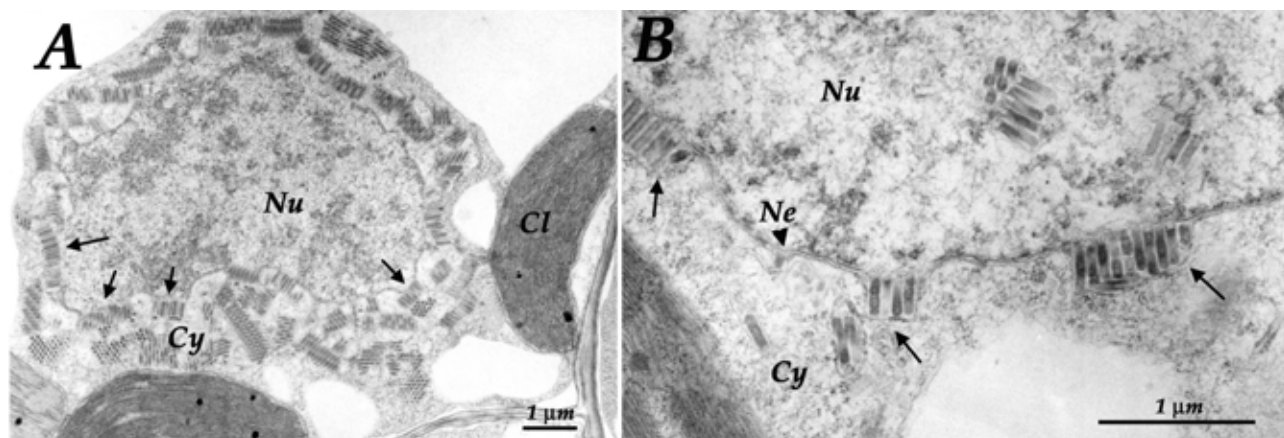


Fig. 3. Transmission electron micrographs of Maize fine streak virus infected leaves. **A** and **B** are images at two magnifications of sections through infected cells. The nucleus (Nu), nuclear envelope (Ne), cytoplasm (Cy), and chloroplasts (Cl) are indicated. The arrows indicate viral particles budding through the nuclear envelope.

were used for VPI of 'Spirit' kernels, the inoculated plants were infected at a rate of $71 \pm 7\%$ ($n = 4$). Virions of the same size and shape as the purified virus were detected in leaves of these infected plants (data not shown).

Leaves from infected plants were examined using TEM, revealing that MFSV was a nucleorhabdovirus (Fig. 3). Viral particles were present in the nucleus (Fig. 3A) and in buds from the perinuclear envelope (Fig. 3B).

Virion proteins. Similar proteins were associated with virus infection for isolates from plants collected in 1999 (Fig. 4A, lanes 5 and 6) and 2000 (Fig. 4A, lanes 2, 3, 4, and 8). The virus-associated proteins of both isolates migrated to different positions in SDS-PAGE than proteins associated with MMV (Fig. 4A, lane 7). Three major protein bands were found after SDS-PAGE of purified MFSV virions (Fig. 4B). The molecular masses of the proteins were 82 ± 2 , 50 ± 3 , and 32 ± 2 kDa (mean \pm SD, $n = 9$).

Serology. The serological relationships among maize-infecting rhabdoviruses were tested using western blot analysis (Fig. 5). Antiserum raised to purified MFSV reacted strongly with five proteins of 80, 54, 50, 36, and 32 kDa from leaves infected with MFSV. The sizes for three of the proteins correspond with those of the virion proteins seen on SDS-PAGE. The MFSV antiserum did not react with any proteins from MIMV- or MMV-infected leaves nor with any proteins from healthy maize leaves. MMV antiserum reacted strongly only with proteins from MMV-infected leaves, and that for MIMV reacted strongly only with proteins from MIMV-infected leaves. WASMV antiserum did not react with any proteins from the three viruses, indicating there was no serological relationship of WASMV to these three rhabdoviruses. In ELISA, the WASMV antiserum reacted strongly with an extract from wheat leaves infected with WASMV, but not with two different isolates of MFSV (data not shown). Antiserum to SSMV reacted with proteins in all four leaf extracts, and significantly with several proteins from MFSV-infected leaves (Fig. 5). Proteins in frozen extracts from SSMV-infected leaves also reacted with MFSV antisera, although to a lesser extent than proteins from MFSV-infected leaves (data not shown). Thus, MFSV was serologically distinct from MMV, MIMV, and WASMV, but serologically related to SSMV.

Viral genome characterization. A ribonuclease A-sensitive nucleic acid of more than 10 kb was isolated from purified MFSV (data not shown). No other RNA species was observed in nucleic acid preparations. Two cDNA clones with inserts of 1.5 kb (MFSV3GA) and 3 kb (MFSVG6A) hybridized with a large RNA species in total RNA isolated from infected plants, but not from

healthy leaves (data not shown). The open reading frame (ORF) encoded by the MFSVG3A cDNA was homologous to the N terminus of rhabdoviral L proteins (Fig. 6). The MFSVG3A ORF had the highest identity (51 to 55%) with the plant-infecting nucleorhabdovirus (SYNV and RTYV) and cytorhabdovirus (NCMV) L protein sequences. The MFSV L protein sequence was less similar (43 to 46% identity) to the mammal-infecting lyssavirus (MOKV and RABV), vesiculovirus (VSV-NJ and VSV-IN), and ephemerovirus (BEFV) sequences, and was least similar (42% identity) to the fish-infecting novirhabdoviruses (IHNV, SHRV, and VHSV). In phylogenetic analyses, the plant-infecting viruses grouped together with the nucleorhabdoviruses (MFSV, RTYV, and SYNV) forming a strongly supported group (bootstrap value = 100), and MFSV was most closely related to SYNV.

An ORF encoded by the 5' 1.4 kb of the MFSVG6A cDNA had similarity with the nucleocapsid (N) protein of rhabdoviruses (Fig. 7). The MFSV N protein had only slightly more identity (44 to 49%) with N protein sequences from plant-infecting rhabdo-

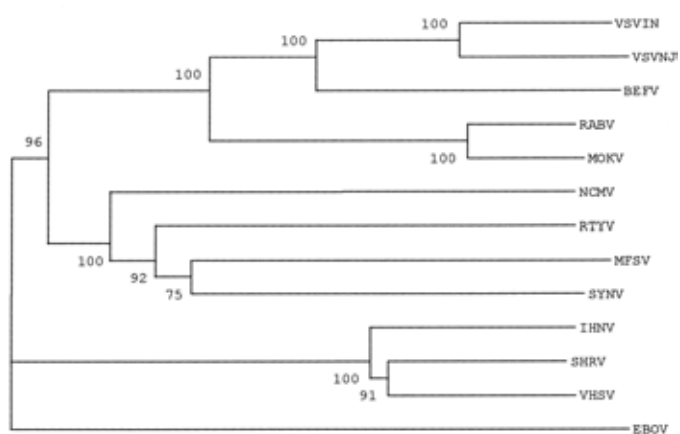


Fig. 6. Phylogenetic analysis of rhabdovirus L protein sequences. The analysis was based on the deduced amino acid sequences of 12 rhabdoviruses and used *Ebola virus* as the outgroup. The bootstrap confidence levels for 1,000 replications are indicated as percents at the branch points, and the branch lengths are proportional to the distances. The sequences compared include: *Bovine ephemeral fever virus*, *Infectious hematopoietic necrosis virus*, *Maize fine streak virus*, *Mokola virus*, *Northern cereal mosaic virus*, *Rabies virus*, *Rice transitory yellowing virus*, *Snakehead rhabdovirus*, *Sonchus yellow net virus*, *Viral hemorrhagic septicemia virus*, *Vesicular stomatitis virus* Indiana isolate, and *Vesicular stomatitis virus* New Jersey isolate.

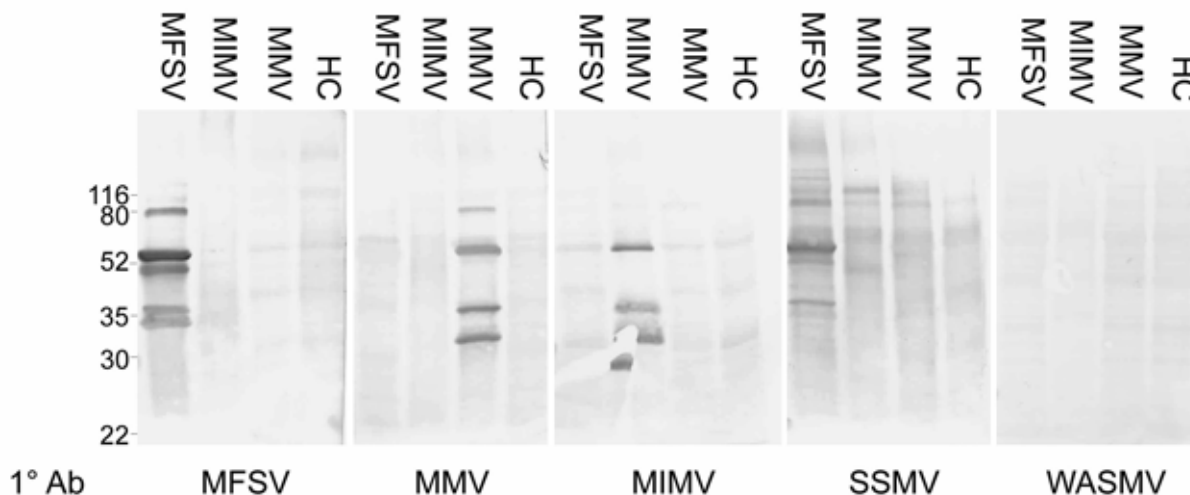


Fig. 5. Western blot analysis of Maize fine streak virus (MFSV), *Maize mosaic virus* (MMV), and *Maize Iranian mosaic virus* (MIMV) with antisera raised against maize infecting rhabdoviruses. Antisera to MFSV, MMV, MIMV, *Wheat American striate mosaic virus* (WASMV), and *Sorghum stunt mosaic virus* (SSMV) were used.

viruses than with those from rhabdoviruses infecting mammals (44 to 47%) and fish (44 to 45%). Although MFSV, RTYV, SNYV, and NCMV formed a strongly supported group in the phylogenetic analysis (Fig. 7), support for a nucleorhabdovirus group was weak (bootstrap value = 57). In addition, one plant-infecting rhabdovirus (LNYV) grouped with the fish-infecting viruses. As with the L protein analysis, the fish- and mammal-infecting viruses formed strongly supported groups.

DISCUSSION

MFSV could be propagated in maize in the absence of other pathogens, purified, and then transmitted to uninfected maize by

kernel VPI, fulfilling Koch's postulates. Virus infection incited symptoms similar to those observed in maize naturally infected in the field, and which were similar to, although milder than, those incited by MMV. The proposed name, MFSV, is based on the short chlorotic streaks and spots that occur on small veins of infected maize leaves. The symptomatology, facile transmission by VPI, transmission by *G. nigrifrons* under persistent conditions, particle morphology, and the sequence similarity to rhabdoviral L and N protein genes indicate the virus is a member of the family *Rhabdoviridae*. The presence of virus particles within the maize nucleus, and budding of virions in the perinuclear space indicate MFSV is a member of the genus *Nucleorhabdovirus*. Several other maize-infecting nucleorhabdoviruses have been described including *Cynodon chlorotic streak virus*, MMV, MIMV, SSMV, and WASMV (3,4,17,23,31,32). However, a comparison of viral properties (Table 3) suggests that MFSV is a distinct virus species.

Although MMV was reported in Florida (5,10), and MFSV was found in Georgia near the northwest border of Florida, the two viruses are serologically distinct. In addition, MFSV is transmitted by a leafhopper and MMV by a planthopper (14), and the viruses have differently sized virion proteins (Table 3). MFSV is similarly different from MIMV, and MIMV has not been detected in the Western Hemisphere (16). Both MFSV and WASMV are transmitted by leafhoppers, including *G. nigrifrons*, and both viruses are found in the United States. (42). However, WASMV has been found only in the northern plains of North America (42). In addition, WASMV virions are larger than and have differently sized virion proteins than those of MFSV (1,46), and the two viruses are serologically distinct.

The reactions of MFSV and SSMV with antisera raised against the two viruses indicate a serological relationship between these two viruses. However, in contrast to MFSV, SSMV was not transmitted by *G. nigrifrons* (7). *G. nigrifrons* did not transmit MFSV to 'Atlas' sorghum or johnsongrass, although the leafhopper uses *Sorghum* species as feeding hosts (24). Further, SSMV has not been reported outside of California (7,31). As indicated in Table 3, the sizes of virion proteins for SSMV (6) are larger than those for MFSV. While comparison of virus transmission by *G. sonora* and

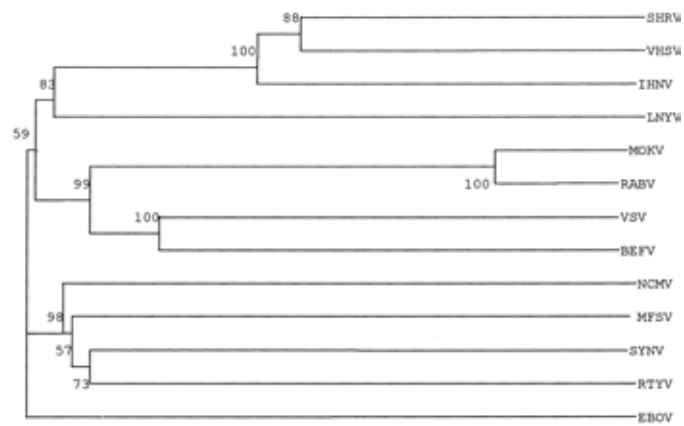


Fig. 7. Phylogenetic analysis for rhabdovirus nucleocapsid protein sequences. The analysis was based on the deduced amino acid sequences of 12 rhabdoviruses and used *Ebola virus* as the outgroup. The bootstrap confidence levels for 1,000 replications are indicated as percents at the branch points, and the branch lengths are proportional to the distances. The sequences compared include: *Bovine ephemeral fever virus*, *Infectious hematopoietic necrosis virus*, *Lettuce necrotic yellows virus*, *Maize fine streak virus*, *Mokola virus*, *Northern cereal mosaic virus*, *Rabies virus*, *Rice transitory yellowing virus*, *Snakehead rhabdovirus*, *Sonchus yellow net virus*, *Viral hemorrhagic septicemia virus*, and *Vesicular stomatitis virus* Indiana isolate.

TABLE 3. Properties of some maize infecting rhabdoviruses

Virus ^w	Vectors ^x	Nonvectors ^y	Virion size (nm)	Virion proteins (kDa)	Distribution ^z	References
MFSV	<i>Graminella nigrifrons</i>	<i>Peregrinus maidis</i> <i>Endria inimica</i> <i>Dalbulus maidis</i>	231 × 71	82 50 32	Georgia	This paper
MMV	<i>P. maidis</i>	<i>G. nigrifrons</i> <i>D. maidis</i>	225 × 68	75 54 30	Florida, Hawaii, the Caribbean, South America, Africa, and Mauritius	5,10,17
MIMV	<i>Ribautodelphax notabilis</i>	<i>P. maidis</i>	180 × 80	182 73 53 34 27 27	Iran	2,11,15,16
SSMV	<i>G. sonora</i>	<i>G. nigrifrons</i> <i>P. maidis</i>	218 × 70	91 59 36 30	California	6,7,31
WASMV	<i>E. inimica</i> <i>G. nigrifrons</i>		260 × 80	145 92 59 25	Northern plains of the United States and Canada	1,46

^w MFSV = Maize fine streak virus, MMV = *Maize mosaic virus*, MIMV = *Maize Iranian mosaic virus*, SSMV = *Sorghum stunt mosaic virus*, and WASMV = *Wheat American striate mosaic virus*.

^x Insects demonstrated to be vectors under persistent transmission conditions.

^y Insects demonstrated not to be vectors under persistent transmission conditions.

^z Areas where the virus has been reported.

G. nigrifrons would be useful to fully differentiate between MFSV and SSMV, the lack of an available isolate of SSMV prevents side-by-side comparison of the viruses. Nonetheless, the current data suggest that MFSV is a new maize-infecting rhabdovirus distinct from, but related to, SSMV.

The known vectors of maize-infecting rhabdoviruses include planthoppers and leafhoppers (Table 3). MFSV was transmitted by *G. nigrifrons*, but not by the two leafhoppers (*E. inimica* and *D. maidis*) that vector other maize-infecting rhabdoviruses, and MFSV was not transmitted by *P. maidis*, the planthopper vector of MMV. Such specificity is not unusual among maize-infecting rhabdoviruses. For example, MIMV is transmitted by *Ribautodelphax notabilis* planthoppers, but not by *P. maidis* (16). In addition, SSMV transmission is species specific, with *G. sonora* being a vector, but not *G. nigrifrons* (7). The molecular and biochemical bases for this high degree of vector specificity remain to be determined.

The preliminary sequence information indicates that MFSV is a member of the family *Rhabdoviridae*, and that the three nucleorhabdoviruses for which L protein gene sequences are available (RTYV, SYN, and MFSV) are more closely related to each other than they are to the cytorhabdovirus, NCMV. Analysis of rhabdoviral N proteins also indicated a close relationship among plant-infecting rhabdoviruses. In this analysis, groups containing the mammal-infecting and fish-infecting viruses were strongly supported, similarly to a previous analysis (9). However, the phylogenetic relationships among these groups of viruses are not clear. One problem is that there are very few complete genome sequences available for the insect- and plant-infecting rhabdoviruses. More sequence information from different groups of rhabdoviruses will be required to gain a good understanding of rhabdovirus evolution and molecular relationships between the viruses and their insect vectors.

Whenever a new virus is described, questions regarding its agronomic importance and reasons for emergence arise. In the case of MFSV, the virus has been found only in limited areas of southwestern Georgia in two fall-growing seasons, suggesting the virus is currently of limited agronomic importance. However, the maize collected in 1999 that resulted in the original isolation of MFSV was thought to be infected by SCMV-MDB. Because incorrect visual diagnosis of viral symptoms in maize could lead to underestimation of MFSV incidence, the distribution of the virus may be much greater than the areas identified in 1999 and 2000. MFSV may have emerged because of recent changes in agronomic practices for sweet corn. The virus was observed in fall-planted insect-resistant Bt sweet corn. Because the Bt-sweet corn was not sprayed to control fall armyworms and earworms, *G. nigrifrons* might have infested the fields and transmitted the virus. *G. nigrifrons* is present in most of the eastern half of the United States (18), raising the potential that MFSV may have a wide occurrence. Because maize inbreds carrying resistance to MMV showed reduced levels of MFSV infection by VPI, some of the hybrid corn grown in the southeastern United States may already carry a degree of resistance to the virus. However, more work to determine the influence of the vector on MFSV infection of maize germ plasm is required before a definitive answer about resistance can be provided. The cornbelt germ plasm tested, particularly inbreds B73 and Mo17, were easily infected by VPI and apparently carry little resistance to MFSV. The serological and molecular diagnostic tools developed in this study will be useful for determining the virus' distribution in maize and other hosts.

ACKNOWLEDGMENTS

This work is a cooperative investigation of USDA-ARS, OSU, and the OARDC, Wooster. Salaries and support provided by state and federal funds were appropriated to OSU-OARDC, Wooster. Mention of a trademark, proprietary product, trade names or commercial products in this

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