New Diseases and Epidemics

Identification of a New Strain of Maize Dwarf Mosaic Virus

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ABSTRACT

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A maize virus isolate from Texas induced symptoms on maize (*Zea mays*) resembling those incited by maize dwarf mosaic virus strain A (MDMV-A). The host range of this mechanically transmitted isolate was confined to the Gramineae and was similar to that of MDMV-A, except that it infected oats (*Avena sativa*). Maize inbreds CG1, CI 44, Pa32, and Pa405 were immune to infection. Properties of this virus in Oh28 maize sap were dilution end point, 10^{-3} to 10^{-4}; longevity in vitro, 2–3 days at room temperature; and thermal inactivation point, 55–60 C. The virus was transmitted from maize to maize nonpersistently by *Rhopalosiphum maidis*. It was not seed-transmitted in johnsongrass (*Sorghum halepense*), oats, or Oh28 maize. Infective virus was recovered from diseased tissues stored 17 mo at 3 and −23 C. The virus had flexuous, rod-shaped particles 690–800 nm long. Pinwheel and bundle inclusions, but no laminated aggregates, were observed in the cytoplasm of infected cells. The virus was partially purified by a protocol that featured 0.5 M potassium phosphate buffer (pH 7.0) at all steps, chloroform clarification, and high-speed and sucrose density gradient centrifugation. Isolate sedimentation characteristics matched those of MDMV-A in rate-zonal centrifuged sucrose density gradients. No serological relationships were established between this isolate and Ohio isolates of MDMV-A and MDMV-B when tested with antisera to the two strains by agar gel double-diffusion, microprecipitin, or double-antibody sandwich enzyme-linked immunosorbent assay. The isolate, considered a new strain of MDMV, was designated the oat-infecting or MDMV-O strain.

In 1976, two maize (*Zea mays* L.) leaf samples with viruslike symptoms were collected from a field near Corpus Christi, TX (5). Maize dwarf mosaic virus strain A (MDMV-A) was detected in one (D. T. Gordon, *unpublished*). The remaining sample was infected with a virus distinct from MDMV-A or other known U.S. maize viruses, as determined by host range and serology. However, this virus, temporarily designated the Texas virus isolate (TVI), had hosts with one notable exception, symptomatology, and particle morphology similar to that of MDMV-A (5). Therefore, a study was initiated to determine its properties and to establish relationships to other maize viruses. This report demonstrates that TVI is a new strain of MDMV that differs principally in host range and serological properties.

MATERIALS AND METHODS

Virus isolates and maintenance. Ohio MDMV-A and MDMV-B were propagated in maize inbred Oh28 and sorghum (*Sorghum bicolor* (L.) Moench cv. Sart), whereas TVI was propagated in Oh28 maize and Garland oats (*Avena sativa* L.).

Inoculation and infectivity assay. Inoculum for TVI increase and host range studies was prepared by grinding infected leaves of Garland oats in 0.01 M potassium phosphate buffer, pH 7.0 (1:10 w/v), with mortar and pestle. Test plant leaves were rub-inoculated with extracts containing 600-mesh Carborundum. Bioassay plants were Garland oats and Oh28 maize unless stated otherwise. Buffer-rubbed controls were included in bioassays.

Host range and properties in sap. Test plants were greenhouse-grown in a steam-sterilized soil mixture, illuminated 12–16 hr with natural and supplemental fluorescent light per 24 hr, maintained daily at 16–36 C, and fertilized and sprayed with insecticide and miticide each week. Tests were replicated two or more times. Ratings for infection were made 14–30 days after inoculations. Known susceptible plants were included in each inoculation to test infectivity of inoculum used in host range studies. Attempts were made to recover virus from inoculated and uninoculated young leaves of plants that were symptomless and from those that showed local lesions by back-inoculations to Oh28 maize.

Dilution end point, longevity in vitro, and thermal inactivation point were determined in three replicates of each test for TVI in sap from infected Oh28 maize inoculated 7–10 days before assay. Sap dilutions for estimating dilution end point were prepared with 0.01 M potassium phosphate buffer, pH 7.0; room temperature (about 20 C) was used for longevity in vitro estimates; and sap aliquots (1 ml) were exposed to various water bath temperatures for 10 min to obtain the thermal inactivation point. All preparations were assayed for infectivity on about 20 Oh28 maize seedlings.

Electron microscopy. TVI particles for leaf-dip preparations were obtained from two infected Oh28 maize plants 6 days after inoculation and visualized in 3% phosphotungstic acid, pH 7.0, with a Philips 201 transmission electron microscope (Philips Electronic Instruments, Inc., Mahwah, NJ). The microscope was routinely calibrated at ×10,000 and ×45,000 using fragments of a 2,160-line-per-nanometer, crossed-lines grating replica (Ernest F. Fullam, Inc., Schenectady, NY).

Oh28 maize leaf tissue collected 7 days after inoculation was prepared for transmission electron microscopy by fixation with 3% glutaraldehyde, 2% paraformaldehyde, and 1.5% acrolein in a 0.1 M collidine buffer, pH 7.3. Specimens were postfixed with 2% osmium tetroxide in the collidine buffer and stained in 1% uranyl acetate. Specimens were dehydrated sequentially in an ethanol and acetone series and embedded in Spurr's medium (18). Steps preceding the acetone series were conducted at 3 C and subsequent steps, at room temperature. Sections 50–100 nm thick were placed on Formvar-coated grids, poststained with 0.5% uranyl acetate and 0.1% lead citrate, and examined with the electron microscope; 20–25 sections each of healthy and infected tissue were examined.

Purification and rate-zonal density gradient centrifugation assay. The field-collected leaf sample was ground with a pestle in a mortar containing 0.5 M potassium phosphate buffer, pH 7.0, plus 0.1% thioglycollic acid (1 g of tissue per 1 ml of buffer). The crude extract was filtered through cheesecloth, and one-half volume of chloroform was added to the extract. The extract was emulsified and the aqueous phase separated by low-

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speed centrifugation (10,000 rpm for 10 min). The latter was centrifuged in a Beckman Type 40 rotor (Beckman Instruments Inc., Palo Alto, CA) at 40,000 rpm for 1 hr in a Beckman Model L ultracentrifuge. Pelleted virus was suspended in 0.5 M potassium phosphate buffer, pH 7.0, to give a 25-fold increase in concentration above the virus concentration in infected tissue. Virus in suspension was further purified by rate-zonal density gradient centrifugation on linear sucrose density gradients prepared from 0.9, 1.0, 1.0, and 0.5 ml of 35, 25, 15, and 5% sucrose, respectively, in 0.5 M potassium phosphate buffer, pH 7.0, in Beckman SW 56 rotor tubes. Gradients were allowed to diffuse overnight at 3–4 C and warmed to room temperature before 0.1 ml of each partially purified preparation was layered onto a gradient. The SW 56 rotor tubes were centrifuged in a Beckman L2-50 ultracentrifuge at 45,000 rpm for a total of 5.4×10^{10} rad^2/sec (about 40 min) at 20 C. Centrifuged gradients were fractionated with an ISCO Model 640 density gradient fractionator and scanned at 254 nm with an ISCO Model UA-5 ultraviolet absorbance monitor (Instrumentation Specialties Co., Lincoln, NB). As virus controls, partially purified MDMV-A and maize chlorotic dwarf virus (MCDV) were similarly obtained and centrifuged in separate gradients.

Serological assay. *Agar gel double-diffusion assay.* For this assay, 0.1 ml of each partially purified virus was mixed with 0.1 ml of 5% pyrrolidine and placed in a microwell of a petri dish containing 1% Ionagar No. 2 (Colab Laboratories, Inc., Chicago Heights, IL) dissolved in 0.2 M potassium phosphate buffer, pH 7.0, plus 0.1% sodium azide (4). Petri dishes were incubated at room temperature (about 20 C), and observations of precipitin bands were made 24 and 48 hr after addition of samples.

Antiserum to MDMV-A was diluted 1:2 with physiologically buffered saline (PBS) (0.01 M sodium phosphate buffer, pH 7.0, plus 0.15 M NaCl) and placed in the central well; partially purified preparations, including MDMV-A, were placed in peripheral wells. Each test was replicated three times. Antisera to MDMV-A and MDMV-B (used in the microprecipitin assay) were prepared as described previously (9,24).

Microprecipitin assay. TVI was extracted from infected leaves of Oh28 maize in PBS (1 g of tissue per 2 ml of PBS), and twofold dilutions (1:4–1:16) were prepared with PBS. These, along with the extract, were tested in the microprecipitin assay. Extracts of MDMV-A- and MDMV-B-infected Sart sorghum and healthy maize, respectively, served as positive and negative controls. MDMV-A and MDMV-B antisera were used at a 1:8 dilution. The microprecipitin assay was conducted as modified (24) with plates incubated overnight at 4 C, and reactions were recorded the following morning.

Enzyme-linked immunosorbent assay (ELISA). The double-antibody sandwich ELISA was performed as described previously (10), except absorbances were measured at 405 nm ($A_{405\ mn}$) with a Bio-Tek EIA Reader (model EL307, Bio-Tek Instruments, Inc., Burlington, VT) without adding the 10% diethanolamine. Reactions were terminated 2.5 hr after addition of substrate. Controls included extracts from MDMV-A- and MDMV-B-infected Sart sorghum, healthy maize and sorghum, and PBS with Tween 20 (PBS-T) (0.15 M NaCl, 0.02 M sodium phosphate, and 0.02% sodium azide, pH 7.4, plus 0.05% polyoxyethylene sorbitan monolaurate [Tween 20]). Extracts from TVI-infected maize and sorghum served as test samples. For the latter, differences of mean absorbance values greater than three standard deviations from the negative control means were scored positive for virus. For tests with MDMV-A antibodies, the negative controls were PBS-T, MDMV-B-infected sorghum, and healthy maize and sorghum preparations, and for those with MDMV-B antibodies, PBS-T, MDMV-A- infected sorghum, and healthy maize and sorghum. Test samples were replicated twice and extracts of each were added to two wells, whereas control samples were not replicated. Extracts of the latter and PBS-T were added to two wells each.

Seed transmission. Seeds of johnsongrass (*Sorghum halepense* (L.) Pers.), Oh28 maize, and Garland oats were produced in a greenhouse. Plants for seed production were given supplemental lighting with either fluorescent or high-intensity (1,000W) metal-halide lamps. Seed was collected from inoculated and buffer-rubbed plants, dried in a forced-air oven at 35 C for 4–5 days, and stored at 3 C until sown. Plants were rated for symptoms 14–35 days after seedling emergence. Symptomless johnsongrass seedlings were assayed using Oh28 maize to detect latent infections.

Vector transmission. Aphid colonies of *Rhopalosiphum maidis* (Fitch), *R. padi* (L.), and *Schizaphis graminum* (Rondani) were reared on *Hordeum vulgare* L. The virus source was Oh28 maize that had been mechanically inoculated 1–2 wk earlier with TVI that had been previously transmitted by aphids in seven consecutive transfers from maize to maize. Apterous aphids, starved 2–3 hr, were given a 10-min or 18-hr acquisition access period (AAP) on detached healthy or infected Oh28 maize leaves that had their cut ends immersed in water. Exposed aphids were transferred to Oh28 maize seedlings (10 aphids per plant) for an inoculation access period (IAP) of 24 hr. Exposed plants were sprayed with insecticide and rated for symptoms 7–14 days post-IAP.

Virus preservation. Infected leaves were diced into pieces 4–5 mm^2, dried over anhydrous calcium sulfate or lyophilized, and stored at −23 and 3 C. Lyophilized tissue was stored under vacuum. Frozen leaves were stored in plastic bags at −23 C. Preserved tissue was assayed for TVI infectivity using Oh28 maize, with inoculum prepared in 0.01 M phosphate buffer, pH 7.0, at a 1:50 (w/v) dilution for dried or lyophilized tissue or 1:5 (w/v) for frozen tissue.

RESULTS

Host range, symptomatology, and properties in sap. The following species expressed symptoms after inoculation (number of plants with symptoms per number inoculated, representing the sum of two or more replicates, in parentheses). Susceptible species were *Avena fatua* L. isolates (sender's designation) #565 (3/37) and #1223 (5/22); *A. sativa* cultivars or plant introductions CI 9170 (2/19), Cherokee (1/29), Chief 5720 (10/64), Garland (55/66), Lang (1/41), Larry (3/31), Marathon (31/50), Moore (1/11), Nodaway 70 (4/32), Richland (6/44), and Wright (28/34); *Digitaria ischaemum* (Schreb.) Schreb. ex Muhl. (20/111); *Echinochloa crusgalli* (L.) Beauv. (29/55); *Eleusine coracana* Gaertn. (33/41); *E. indica* (L.) Gaertn. (11/57); *Eragrostis cilianensis* (All.) Lutati (49/60); *Panicum miliaceum* L. (46/47); *Pennisetum typhoideum* (L.) Rich. (23/71); *Setaria italica* (L.) Beauv. (37/67); *S. viridis* (L.) Beauv. (53/70); *Sorghum bicolor* cvs. Atlas (50/55) and Rio (40/57); *S. halepense* (35/49); *S. sudanense* (Piper) Stapf. (26/57); *Zea diploperennis* Iltis, Doebley, & Guzman (50/56); *Z. luxurians* (Durieu) Bird (7/13); *Z. mays* inbreds A554 (24/50), A619 (49/55), A635 (36/54), B54 (12/43), B73 (60/71), C103 (33/37), C123 (44/49), CI 66 (39/43), GA209 (13/50), H95 (60/65), Ky61:2334 (61/65), M14 (5/38), Mo17 (69/75), Mo18W (23/23), N6J (108/110), N7A (9/72), Oh28 (50/50), Oh41 (27/27), Oh43 (42/51), Oh51A (42/62), Oh509A (54/60), Oh545 (44/45), Oh551 (56/57), Oh562 (21/61), Oh563 (14/32), T111 (36/37), T232 (29/41), Va26 (56/60), Va35 (35/40), W64A (29/53), W117 (54/59), and W153R (38/42); *Z. mays* subsp. *mexicana* (Schrad.) Iltis (59/64); *Z. mays* subsp. *parviglumis* Iltis & Doebley (15/32); and *Z. perennis* (Hitch.) Reeves & Mangelsd. (1/67). *Panicum capillare* L. expressed local lesions on inoculated leaves (11/59), but no virus was recovered; no other symptom was observed on this species. TVI produced local lesions on inbred B54 but failed to spread systemically.

The following plant species were not susceptible (no symptoms and no recoveries of virus by back-assays) to TVI (numbers of inoculated plants in parentheses): *Agropyron repens* (L.) Beauv. (*51*); *Allium cepa* L. (*40*); *Andropogon gerardii* Vitman (*50*); *A.*

scoparius Michx. (*34*); *Avena sativa* cv. Ogle (*12*); *Brassica rapa* L. (*49*); *Bromus inermis* Leyss. (*107*); *B. japonicus* Thunb. (*72*); *B. secalinus* L. (*60*); *Chenopodium amaranticolor* L. (*67*); *C. quinoa* L. (*54*); *Citrullus vulgaris* L. (*79*); *Cucumis sativus* L. (*47*); *Cynodon dactylon* (L.) Pers. (*57*); *Dactylis glomerata* L. (*51*); *Datura stramonium* L. (*63*); *Eragrostis curvula* (Schrad.) Nees (*73*); *Festuca arundinacea* Schreb. (*65*); *Glycine max* (L.) Merr. (*54*); *Gomphrena globosa* L. (*30*); *Hordeum vulgare* (*72*); *Lolium perenne* L. (*50*); *Lycopersicon esculentum* L. (*53*); *Medicago sativa* L. (*59*); *Nicotiana tabacum* L. cvs. F_2C_1 (*57*) and Samsun (*57*); *Oryza sativa* L. cvs. Bluebelle (*104*), Bluebonnet 50 (*109*), Calora (*72*), Nato (*63*), and Saturn (*71*); *Oryzopsis miliacea* (L.) Benth. (*90*); *Panicum virgatum* L. (*36*); *Phalaris arundinacea* L. (*80*); *Phaseolus vulgaris* L. (*55*); *Phleum pratense* L. (*56*); *Pisum sativum* L. (*69*); *Poa pratensis* L. (*113*); *Secale cereale* L. (*67*); *Sorghastrum nutans* (L.) Nash (*38*); *Triticum aestivum* (*119*); *Vigna sinensis* (L.) Endl. (*54*); *Z. mays* inbreds CG1 (*51*), CI 44 (*74*), Pa32 (*27*), and Pa405 (*49*).

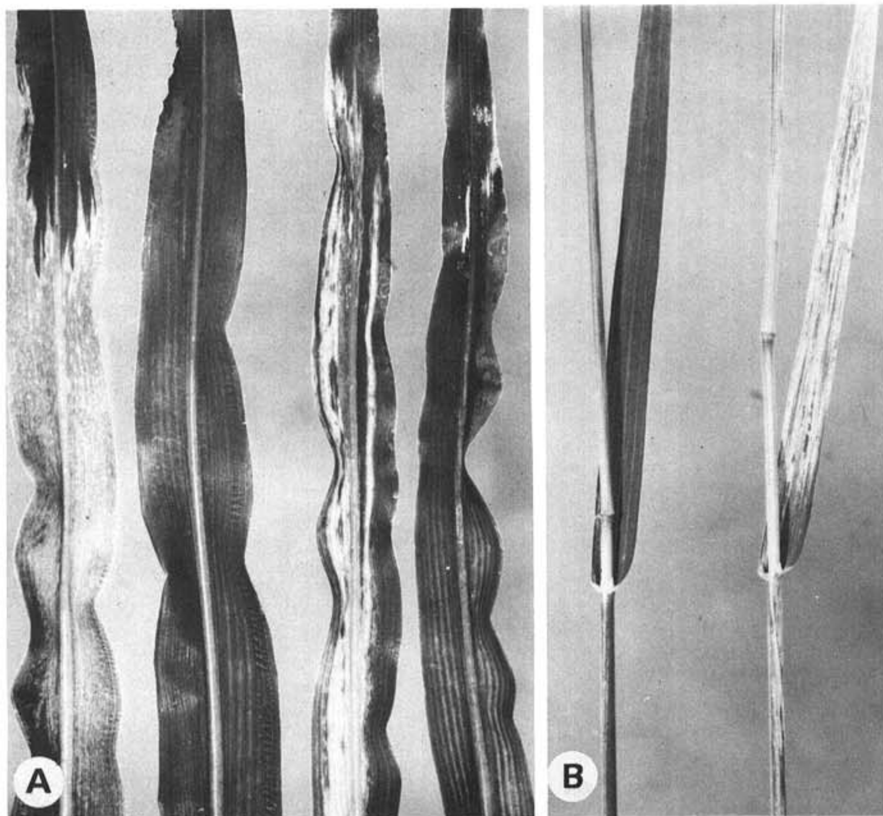

Fig. 1. Symptoms on Oh28 maize (*Zea mays*) and Garland oats (*Avena sativa*) infected by the Texas virus isolate. **(A)** Infected uninoculated and inoculated blades of maize: first and third from left, respectively. Healthy: second and fourth blades. **(B)** Spindle-shaped chlorotic and necrotic lesions on a systemically infected blade of oats on right. Healthy blade on left.

Symptoms on susceptible plants were usually expressed within 10 days of inoculation. Chlorotic spots or spindle-shaped lesions were commonly observed on inoculated leaves of most selected maize inbreds. Similar symptoms appeared on systemically infected maize leaves that emerged after inoculation, and subsequently, spots and spindle-shaped lesions merged to form chlorotic streaks and mosaic patterns (Fig. 1A). In contrast, on most cultivars or introductions of *Avena sativa*, a mild chlorotic mottle appeared initially on younger, systemically infected leaves. Spindle-shaped, necrotic lesions that sometimes covered the surfaces of leaves subsequently appeared on some cultivars (Fig. 1B).

Properties in sap for the isolate in the

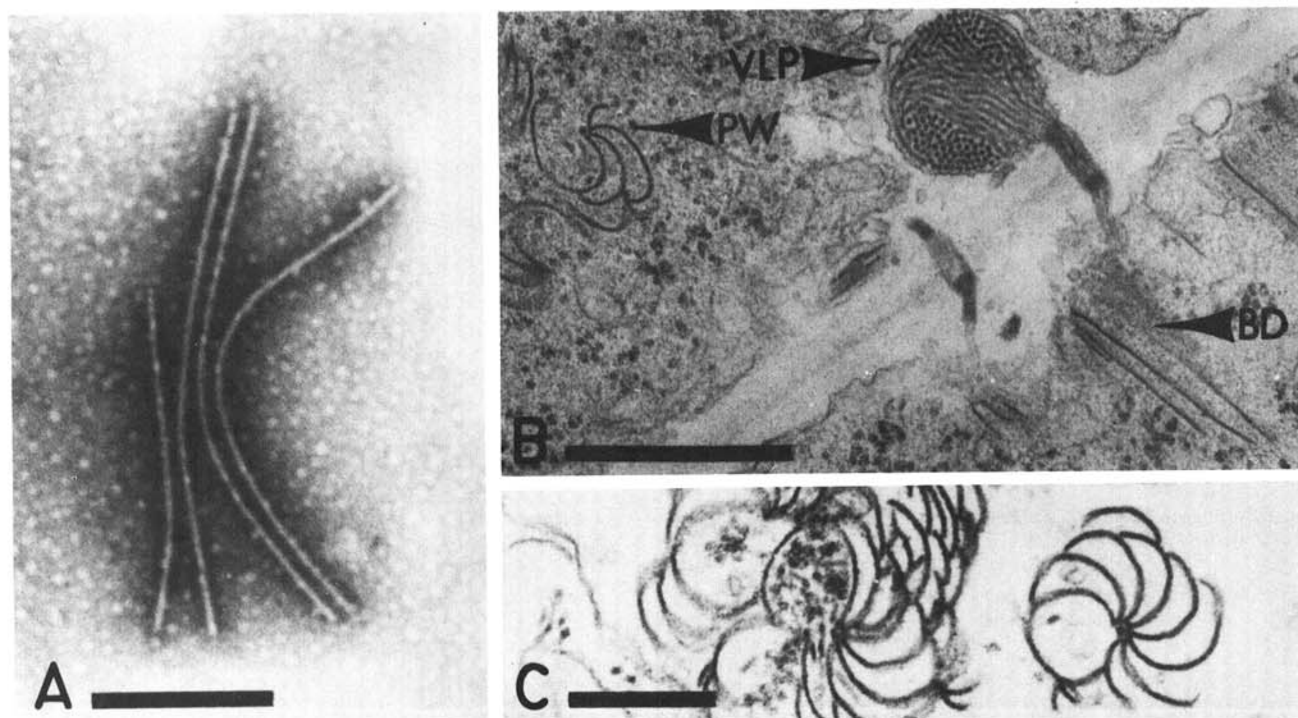

Fig. 2. Electron micrographs of maize (*Zea mays*) leaf tissue infected with Texas virus isolate (TVI). **(A)** Flexuous, rod-shaped TVI particles stained with phosphotungstic acid. Scale bar = 0.4 μm. **(B)** Membrane-bound aggregate of viruslike particles (VLP) with bundle (BD) and pinwheel (PW) inclusions near plasmodesmata. Scale bar = 1 μm. C. Close-up of pinwheel inclusions. Scale bar = 0.5 μm.

three replicates were dilution end point, 10^{-3} to 10^{-4}; longevity in vitro, 2–3 days; and thermal inactivation point, 55–60 C.

Electron microscopy. Flexuous, rod-shaped particles were observed in leaf dips from infected but not healthy leaves. No central core was observed for phosphotungstic acid-stained particles (Fig. 2A). The median particle length was 722 nm (number [n] of particles measured = 17), and the mean length was 801 ± 114 nm (95% confidence interval). In comparison, the median and mean particle lengths for MDMV-A examined at the same time were 707 and 949 ± 150 nm (n = 29), respectively.

Bundle, pinwheel, and tubular inclusions, along with membrane-delimited masses of viruslike particles, were observed in thin-sections of cells of infected (Fig. 2B,C) but not healthy leaf tissue. Bundle inclusions were associated with the plasmalemma and plasmodesmata of infected cells. These inclusions were presumably associated with TVI since infectious TVI was recovered from infected tissue, whereas neither infectious virus nor inclusions were associated with buffer-rubbed leaves.

Rate-zonal density gradient centrifugation assay. TVI banded at the same position as MDMV-A in centrifuged gradients but at a higher position than MCDV (Fig. 3). Ultraviolet absorbance profiles for centrifuged gradients containing TVI and MDMV-A showed that both gradients contained a prominent upper, slower-sedimenting band and a smaller, somewhat faster-sedimenting band. Both bands examined by transmission electron microscopy were found to contain flexuous, viruslike particles.

Serological assays. In the agar gel double-diffusion assay (Fig. 4), a precipitin band appeared between wells containing MDMV-A antiserum and MDMV-A but not TVI in three replicates.

In the microprecipitin assay, TVI did not react with either MDMV-A or MDMV-B antiserum, whereas both antisera reacted with heterologous (MDMV-A or MDMV-B) and homologous antigens (Table 1). Healthy maize extracts did not react with either antiserum.

In ELISA, TVI-infected leaf extracts from maize and sorghum were negative for virus in tests with both MDMV-A and MDMV-B antibodies. For the MDMV-A antibodies, the mean ($\bar{x}$) $A_{405\ nm}$ value for the TVI samples was 0.04 with a standard deviation (SD) of 0.01; the number of wells (n) = 8. Values for the healthy controls for these antibodies were: $\bar{x}$ = 0.03 and SD = 0.01 (n = 8). For the MDMV-A-positive controls, $\bar{x}$ = 0.40 and SD = 0.05 (n = 2). For the MDMV-B antibodies, $\bar{x}$ for TVI wells = 0.05 and SD = 0.03 (n = 8); and for the healthy control wells, $\bar{x}$ = 0.06 and SD = 0.02 (n = 8). For the MDMV-B-positive controls, $\bar{x}$ = 2.0 and SD = 0.0 (n = 2). Only the homologous antigen for both antibodies gave a yellow coloration after the addition of substrate; wells containing antibodies to MDMV-A visually rated a 3, and to MDMV-B, a 7. The visual ratings were on a scale of 0 (no coloration) to 7 (maximum coloration). There was no visible reaction between antibodies to MDMV-A and the MDMV-B antigen

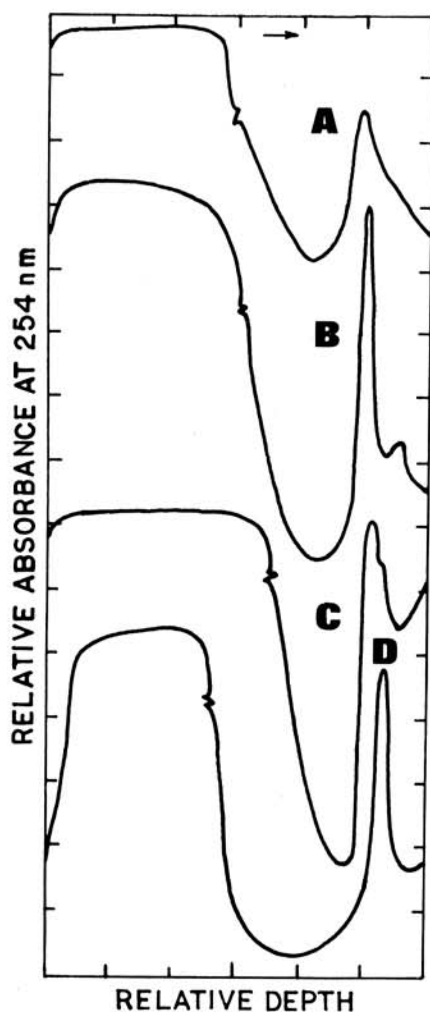

Fig. 3. Ultraviolet absorbance profiles **(A–D)** of centrifuged sucrose density gradients containing: **(A)** Texas virus isolate (TVI), **(B)** Texas isolate of maize dwarf mosaic virus strain A (MDMV-A) from a maize sample collected in the same field as TVI, **(C)** Ohio isolate of MDMV-A, and **(D)** maize chlorotic dwarf virus. All viruses were partially purified, concentrated 25-fold, suspended in 0.5 M potassium phosphate buffer (pH 7.0), and centrifuged on sucrose density gradients. Gradients were scanned at 254 nm. Arrow indicates direction of sedimentation.

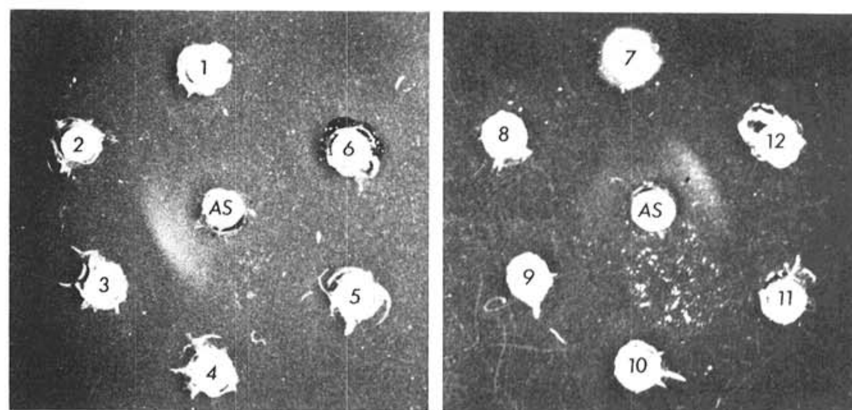

Fig. 4. Microimmune agar gel double-diffusion assay of partially purified preparations from field-collected maize leaf samples and of maize dwarf mosaic virus strain A (MDMV-A) and maize chlorotic dwarf virus (MCDV). Preparations (0.1 ml) were mixed with an equal volume of 5% pyrrolidine and placed in the peripheral wells. MDMV-A antiserum was placed in the central wells. Preparations in peripheral wells: 2 = MCDV, 3 = MDMV-A, 1 and 4–6 = field samples not involved in this study, 7 = Texas virus isolate (TVI), 8–11 = additional field samples not involved in this study, and 12 = MDMV-A from the same field as TVI.

Table 1. Microprecipitin assay of Texas virus isolate (TVI) with antisera to maize dwarf mosaic virus (MDMV) strains A and B

	Antiserum dilution[b]									
	MDMV-A					MDMV-B				
Antigen[a]	1:2	1:4	1:8	1:16	PBS[c]	1:2	1:4	1:8	1:16	PBS
TVI	–[d]	–	–	–	–	–	–	–	–	–
MDMV-A	+	+	+	+	–	+	+	+	+	–
MDMV-B	tr	tr	tr	tr	–	+	+	+	+	–
Healthy maize	–	–	–	–	–	–	–	–	–	–

[a]The antigens were leaf extracts prepared in physiologicaly buffered saline, pH 7.0 (PBS). For each antigen tested, a PBS control was also tested with each antiserum dilution, and none of these controls showed a reaction.

[b]Antiserum dilutions were made with PBS. The antisera were not cross-absorbed with the heterologous antigen.

[c]PBS is a control without antiserum.

[d]– = No reaction, + = flocculent precipitate, and tr = small flocculent precipitate.

($\bar{x}$ = 0.02, SD = 0.01, n = 2) or between MDMV-B antibodies and MDMV-A ($\bar{x}$ = 0.05, SD = 0.03, n = 2).

Seed transmission. No viruslike symptoms were observed on plants grown from seed of infected Garland oats (10,690 seedlings), johnsongrass (2,061 seedlings), and Oh28 maize (1,320 seedlings). Back-assays of johnsongrass using maize were negative.

Vector transmission. *R. maidis* transmitted TVI nonpersistently to two of 102 Oh28 maize test plants exposed to aphids after a 10-min AAP, whereas *R. padi* and *S. graminum*, tested on 102 and 100 plants, respectively, failed to transmit the virus. Aphids provided an 18-hr AAP did not transmit the virus; numbers of test plants per aphid species were 13 *R. maidis*, 5 *R. padi*, and 11 *S. graminum*.

Virus preservation. Infectious virus was recovered from all samples stored for 17 mo except from frozen oat leaves (Table 2).

DISCUSSION

The host range of TVI was confined to the Gramineae and did not differ substantially from that of MDMV-A (syn. johnsongrass strain of sugarcane mosaic virus [SCMV-Jg]) as previously summarized (14), except for the infection of wild and cultivated oats. Assuming that TVI is a new strain of MDMV, this difference expands the economic host range of MDMV to a third major U.S. grain, oats; the other two are maize and sorghum, but apparently unlike oats, both suffer significant crop losses from MDMV infection (5). Since completing this study, Gates (3) reported the existence of Canadian MDMV isolates that infected oats as well as maize and johnsongrass. These isolates were symptomless in oats and barley (*H. vulgare*); however, the latter was infrequently infected. TVI produced symptoms in oats in our tests but did not infect barley and thus appears different from these Canadian isolates.

Among entries from 13 genera of the Pooideae (festucoid) subfamily, only *Avena* spp. were susceptible and only Ogle oats among the 12 cultivars tested was immune. The finding that Pooideae grasses were less susceptible to TVI than test grasses from other subfamilies (Table 3) has been noted for other MDMV strains (14). The susceptibility of johnsongrass to TVI distinguished this virus from MDMV-B and most SCMV strains (5). However, strains A, C, D, E, and F of MDMV infect johnsongrass but not oats and thus can be differentiated from TVI by the latter host.

Although most maize inbreds tested were susceptible to TVI, four (CG1, CI 44, Pa32, and Pa405) were immune. Among these, only Pa32 differentiated TVI from MDMV-A and MDMV-B, both of which induced chlorotic spots and streaks on uninoculated leaves (L. L. McDaniel, *unpublished*).

Particle morphology and median length of TVI were indistinguishable from those for the California and Ohio MDMV isolates reported by Shepherd (15) and Bancroft et al (1), respectively.

The presence of pinwheel and bundle but not laminate-aggregate inclusions indicated that TVI belongs in subdivision I of Edwardson's (2) potyvirus classification. MDMV strains A, D, E, and F and SCMV-Jg also belong in this subdivision, whereas MDMV-B and SCMV strains A, D, E, and H do not (2). Association of bundle inclusions with plasmalemma, as we observed for TVI-infected cells, also has been reported in cells infected with MDMV-A and MDMV-B and SCMV-H (11).

TVI was partially purified by a protocol used previously to purify several MDMV strains (6). This protocol combines features not previously reported by other authors (21).

The rate-zonal centrifugation banding position and ultraviolet absorbance profile of TVI matched those of the MDMV-A control and resembled those of several MDMV strains (6).

TVI is distinctive among U.S. MDMV isolates in its lack of serological reactivity with antisera to MDMV-A or MDMV-B. Other U.S. johnsongrass-infecting isolates reacted relatively strongly with antiserum to MDMV-A or to SCMV-Jg, indicating a close serological relationship (1,12,13,15,22–24). Also, antisera not cross-absorbed with the heterologous viral antigen (MDMV-A, MDMV-B, or SCMV) are cross-reactive with the respective heterologous antigen (Table 1; 13,17,22,24). TVI did not react with antiserum to either MDMV strain and thus is probably serologically unrelated to SCMV strains. To our knowledge, only the Australian johnsongrass-infecting MDMV or SCMV isolates are unreactive to MDMV-A antiserum (19) and thus not closely related serologically to SCMV (16). Although TVI resembles these isolates, to our knowledge the infectivity of the Australian isolates to oats has not been reported. Isolates of johnsongrass-infecting MDMV or SCMV from other foreign countries have clear serological relationships to U.S. MDMV-A or SCMV-Jg isolates or to other SCMV isolates occurring in the country (7,20).

Failure to detect seed transmission of TVI in maize corresponded with a similar report for the Ohio isolate of MDMV-A (1), although others have usually detected a low rate of MDMV-A transmission through maize seed (5).

The low aphid transmission efficiency of TVI by *R. maidis* may have resulted from continuous mechanical transmission of TVI for 3–4 yr before these tests. However, seven serial aphid transmissions of TVI from maize to maize before this study failed to select a more efficient aphid-transmitted isolate. The efficiency of aphid transmission of TVI was less than that reported previously for MDMV-A; the latter was transmitted with efficiencies of 4, 4, and 14%, respectively, by *R. maidis, R. padi*, and *S. graminum* (8). Absence or suboptimal amounts of a potyvirus helper factor in TVI inoculum acquired by aphids may

Table 2. Preservation of Texas virus isolate in infected tissues of three host species

Infected host tissue	Storage		Storage time (mo)		
	Temp. (C)	Method	1	9	17
Sorghum bicolor (cv. Atlas)	−23	L[a]	10/13[b]	16/18	14/14
	−23	CD	15/15	17/20	15/15
	−23	F	16/16	8/19	1/13
	3	L	12/13	10/17	10/18
	3	CD	12/13	5/17	11/16
Avena sativa (cv. Garland)	−23	L	18/18	14/19	11/14
	−23	CD	12/12	9/20	14/14
	−23	F	12/15	9/18	0/17
	3	L	16/17	5/17	4/17
	3	CD	8/12	2/18	1/13
Zea mays (inbred Oh28)	−23	L	15/15	11/15	14/14
	−23	CD	12/12	18/18	13/13
	−23	F	10/13	14/18	1/15
	3	L	15/15	20/20	17/17
	3	CD	12/14	15/16	16/18

[a]L = lyophilization, CD = chemical drying over anhydrous calcium sulfate, and F = freezing.
[b]Number of Oh28 maize assay plants infected/total number of plants inoculated.

Table 3. Host distribution of Texas virus isolate by Gramineae subfamilies

Subfamily	Species[a]	Genera[b]
Pooideae (Festucoideae)	2/16	1/13
Eragrostioideae (Chloridiodeae)	3/5	2/3
Panicoideae		
Tribe: Paniceae	7/8	5/5
Andropogoneae	7/10	2/4

[a]Number of species susceptible/total number of species tested.
[b]Number of genera susceptible/total number of genera tested.

have contributed to poor transmission.

In conclusion, TVI more closely resembled johnsongrass-infecting strains of MDMV (i.e., strains A, C, D, E, and F) and SCMV-Jg in terms of host range and other properties reported in our study than non-johnsongrass-infecting MDMV (strain B) and SCMV (strains A through M, excluding Jg) strains. Lack of a serological reaction between TVI and MDMV-A and the ability of TVI to infect oats suggests that TVI is distinct from the other reported strains of MDMV and SCMV. We propose that TVI be designated the oat-infecting strain of MDMV or MDMV-O.

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