Purification and Properties of an Isolate of Maize Rayado Fino Virus from the United States

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ABSTRACT

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An isolate of maize rayado fino virus from infected corn in the United States (MRFV-US) was purified by chloroform clarification, rate-zonal centrifugation, and isopycnic banding in CsCl. Yields were usually 30–80 μg of virus per gram of tissue. A noninfectious top component (47S) containing no nucleic acid, and an infectious bottom component (124S) containing a single RNA species (2.4×10^6 daltons) were separated by rate-zonal centrifugation. The RNA was single-stranded, comprised 26% of the bottom component by weight, and contained 31.4% Cp, 23.1% Gp, 16.8% Ap, and 28.7% Up. The top and bottom components banded isopycnically in CsCl at densities of 1.265 and 1.425 g/ml, respectively. Two proteins (MWs 25,600 and 22,350) were found in each component in the same ratio, about three molecules of the lighter protein per molecule of the heavier. Partially purified MRFV-US had a thermal inactivation point between 90 and 100 C, dilution end point between 10^{-4} and 10^{-5}, and longevity in vitro between 9 and 11 wk. Infectivity of partially purified preparations was preserved by lyophilization. The cryptogram for the bottom component is R/1:2.4/26:S/S:S/Au. Enzyme-linked immunosorbent assay was used to detect MRFV in individual *Dalbulus maidis* leafhopper vectors and to show that the amount of virus in leafhoppers declined between 0 and 7 days, increased between 7 and 14 days, and remained high through 21 days after acquisition, indicating that MRFV multiplied in this vector. Properties of MRFV-US were similar to those of the Central and South American isolates.

Maize rayado fino virus (MRFV) was first described by Gámez (9) in 1969 from Costa Rica and has since been found in Mexico and several other Central and South American countries (10–12,23,24,28,30). It was identified in the United States in 1976 from Texas and in 1977 from Florida (2,3). Isolates from those sources have been designated MRFV-US. The corn leafhopper, *Dalbulus maidis* (Delong & Wolcott), transmits MRFV in nature (9,10) and MRFV has been transmitted experimentally by several other leafhopper species (31). One of them, *Graminella nigrifrons* (Forbes), is abundant throughout the Corn Belt in the United States (35), whereas *D. maidis* is not. The probability for spread of MRFV-US into this region remains unknown, but the only barrier may be the lack of an overwintering host (31). The occurrence of MRFV in the southern United States, its potential for northward spread, and the important economic losses it has caused in other American countries, make it important to characterize the MRFV-US and compare it with the Latin American isolates.

In this paper we report the purification and characterization of the MRFV-US. Its properties were similar to those of the other described isolates. Abstracts pertaining to parts of this work have been published (17,19).

MATERIALS AND METHODS

Virus. The MRFV isolate used in this study was recovered from maize collected in 1976 from the Rio Grande Valley of Texas and identified by Bradfute et al (3). It was propagated in various lines of sweet and dent corn by serial transmission with *D. maidis*. Leafhoppers were reared as previously described (31).

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Purification. Leaf tissue was ground in a blender with 0.5 M potassium phosphate, pH 7.0, containing either 0.1% thioglycollic acid or 0.5% 2-mercaptoethanol (2-ME) (1 g tissue per 3 ml of buffer) at room temperature. The extract was clarified by emulsification with one-third to one-half volume $CHCl_3$, and the virus was concentrated from the clarified extract by high-speed centrifugation (35,000 rpm, 2 hr, 10 C, Beckman Type 35 rotor [Beckman Instruments Inc., Palo Alto, CA 94304]). Virus pellets were suspended in 0.5 M potassium phosphate, pH 7.0. The virus was further purified by rate-zonal sedimentation through 10–40% sucrose density gradient columns prepared in 0.5 M potassium phosphate, pH 7.0, and centrifuged for $20{,}000 \times 10^7$ rad^2/sec (~3.5 hr) at 40,000 rpm and 10 C in the Beckman SW 41 Ti rotor. The fast (bottom component) and slow (top component) virus zones were collected by using an ISCO (Instrumentation Specialties Co., Lincoln, NB 68505) fractionating system; adjusted to densities of 1.42 and 1.26 g/ml, respectively, with solid CsCl; and isopycnically banded by centrifugation at 36,000 rpm and 10 C for 60–65 hr in an SW 41 Ti rotor. Virus zones were collected and stored at 4 C.

To estimate the amount of virus in gradient zones, a standard curve relating zone area and the amount of bottom component was constructed from the areas of known amounts of bottom component centrifuged in gradients.

Sedimentation rate. The sedimentation rate of MRFV was determined by a method similar to that described by Brakke and Van Pelt (5). Cucumber mosaic virus (CMV) was used as a reference particle in linear-log sucrose density gradient columns constructed for particles of density 1.3 g/ml at 14 C in the Beckman SW 41 Ti rotor (22). CMV was isolated from cucumber (*Cucumis sativus* L.) (D. T. Gordon, *unpublished*), and purified from F_2C_1 tobacco (*Nicotiana tabacum* L.) by the method of Lot et al (26). The sedimentation coefficient ($s_{20,w}$) of this isolate was determined to be 95S in a Beckman model E analytical ultracentrifuge (D. T. Gordon, *unpublished*). CMV and MRFV-bottom were centrifuged for three successive intervals of about 3.1×10^7 rad^2/sec at 41,000

rpm. After each interval, two gradients were removed from the rotor. Positions of the virus bands were located by ultraviolet absorption scans of the gradients and the $s_{20,w}$ of MRFV-bottom was estimated (see Results).

Thermal inactivation point, dilution end point, and longevity in vitro. For these tests, virus was partially purified by grinding MRFV-infected leaf tissue in a VirTis 45 homogenizer (VirTis Co., Inc., Gardiner, NY 12525) in 0.5 M potassium phosphate plus 0.5% 2-ME, pH 7.0 (3 ml/g of tissue), clarifying with $CHCl_3$, concentrating by centrifugation (40,000 rpm, 2 hr, Beckman Type 42.1 rotor), and resuspending it in 0.1 M HEPES, pH 7.0 (one-tenth of the original extract volume).

For thermal inactivation point determinations, aliquots of partially purified virus preparations were held in a water bath at various temperatures for 10 min, cooled in ice, and injected into *D. maidis*. For longevity in vitro tests, virus preparations were at room temperature (23–27 C) or in a refrigerator (4–5 C). At various times after purification, aliquots were passed through a 0.22-μm Millipore filter to remove any deleterious microorganisms and injected. To estimate the dilution end point, various dilutions (in 0.1 M HEPES, pH 7.0) of partially purified preparations were injected. For injection, leafhoppers were lightly anesthetized with CO_2, held on a microscope stage by vacuum, and injected with ~0.025 μl of inoculum by using mechanically pulled glass needles. Injected leafhoppers were maintained on maize plants for 10–12 days to allow the virus latent period in the vector to elapse and then singly transferred to test plants for a 3- to 4-day inoculation access period. Test plants were held in a greenhouse and final results were recorded after 14 days.

Buoyant density. Virus zones from rate-zonal gradients were dialyzed against 0.5 M potassium phosphate, pH 7.0, to remove sucrose, adjusted to the desired densities by adding solid CsCl, and centrifuged for 60–65 hr at 43,000 rpm in the Beckman SW 50.1 rotor at 20 C. The centrifuged gradients were fractionated into 0.4-ml fractions and the densities of individual fractions in the vicinity of the virus zones were determined by weighing 50-μl aliquots in a micropipette previously calibrated with water. Virus density was considered to be equal to the gradient density at the peak of virus absorbance.

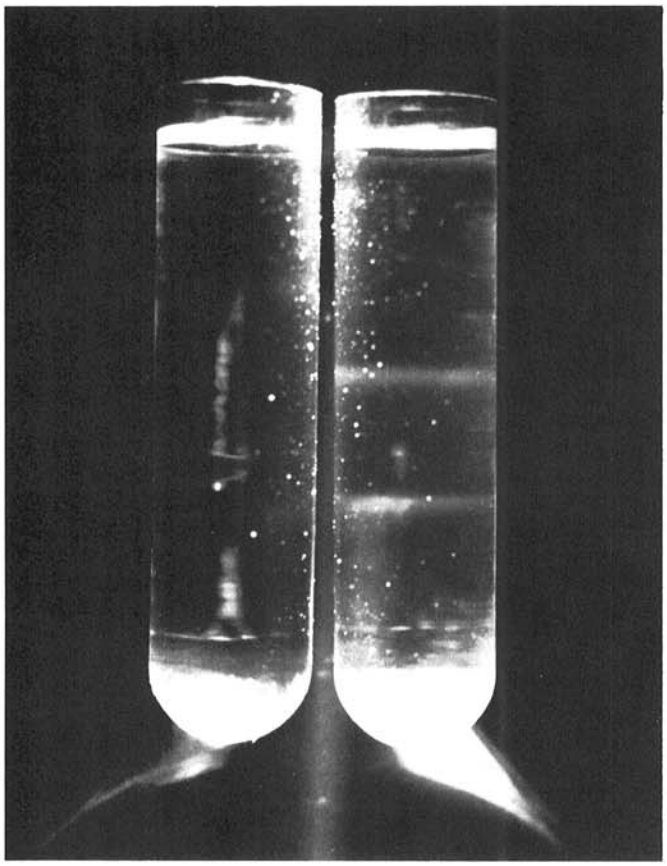

Fig. 1. Rate-zonal sedimentation of maize rayado fino virus (MRFV). Clarified extracts of healthy and MRFV-infected tissue (4 g of each) were concentrated 10-fold by high-speed centrifugation, and the concentrated preparations were layered on 10–40% linear sucrose gradients in the Beckman SW 50.1 rotor. Centrifugation was for $16{,}000 \times 10^7$ rad^2/sec at 40,000 rpm (about 2.5 hr) at 10 C. Infected (right) and healthy (left).

Molecular weight of MRFV-RNA. RNA was released from virions by heating for 5 min at 60 C in 0.1 M sodium phosphate, 0.001 M EDTA and 200 μg of bentonite per milliliter, pH 7.0 (4). The mixture was then cooled quickly in ice. The RNA was treated with formaldehyde and run on formaldehyde-containing linear-log sucrose gradients using ribosomal RNA of *Escherichia coli* and tobacco mosaic virus (TMV) RNA as markers (16).

Molecular weights of capsid proteins. Protein was released from virions by boiling for 1 min in 0.01 M sodium phosphate, 1% sodium dodecyl sulfate (SDS), 1% 2-ME, pH 8.0. Reference proteins were similarly treated. Electrophoresis was done as described by Weber and Osborn (36) except that 7.5% polyacrylamide gels (in 7.6-cm [3-in.] tubes) were used and gel tops were formed by trimming gels to 6 cm. Stained gels were scanned in the ISCO model 659 gel scanner.

Nucleotide ratios. Purified MRFV-bottom component was heated in 0.4 M NaOH for 24 hr at 37 C to hydrolyze the RNA. Nucleotides were separated by descending paper chromatography and the base ratios were determined (16).

Extinction coefficient. Purified virus preparations (4 ml) were dialyzed against distilled water (>10 changes of 3 L each over a period of 1 wk) to remove buffer salts. The extinction coefficient was calculated after measuring the absorbance (260 nm) of the dialyzed solution and the mass of virus remaining after aliquots of this solution were lyophilized.

Antiserum production, antibody preparation, and serological tests. Antiserum to MRFV was produced, the γ-globulin purified and conjugated with alkaline phosphatase, and the EIA performed as previously described (30).

Time course of MRFV concentration in *D. maidis*. Second-instar *D. maidis* were placed on MRFV-infected corn plants for a 2-day virus acquisition access period (AAP) and then sequentially transferred to healthy holding plants (corn) every 3–4 days until 21 days post-AAP. This schedule eliminated the possibility that leafhoppers inoculated and reacquired MRFV from holding plants. At 0, 7, 14, and 21 days post-AAP, approximately 60 leafhoppers were ground individually in glass tissue homogenizers with Teflon pestles in 0.3 ml of 0.15 M NaCl, 0.02 M sodium phosphate (pH 7.4) containing 0.02% sodium azide and 0.5% Tween-20. Aliquots (0.1 ml) were then transferred to wells in microtiter plates for EIA. An equal number of leafhoppers not given an AAP were taken from healthy corn and similarly assayed.

RESULTS

Purification. A slower-sedimenting top component and a faster-sedimenting bottom component were observed after rate-zonal centrifugation of preparations from MRFV-infected plants (Fig. 1). Electron microscopy of purified top and bottom components revealed large numbers of isometric particles (Fig. 2). The phosphotungstic acid stain penetrated most top component particles, but none of the bottom component particles. When injected into *D. maidis*, purified bottom component was infectious, but purified top component was not.

Virus recovery rates from propagative tissue increased up to about 4 wk after inoculation and remained fairly constant for at least 3 or 4 wk longer. Freezing tissue prior to purification had no apparent effect on virus recovery. Yields were usually between 30 and 80 μg of virus (bottom component) per gram of tissue, as calculated from virus zone areas on scans of centrifuged gradients.

MRFV was successfully concentrated from clarified extracts by recovering the precipitate obtained after a 1-hr incubation with 10% polyethylene glycol (MW 6000) plus 6% KCl at room temperature, but recovery was higher if the virus was concentrated

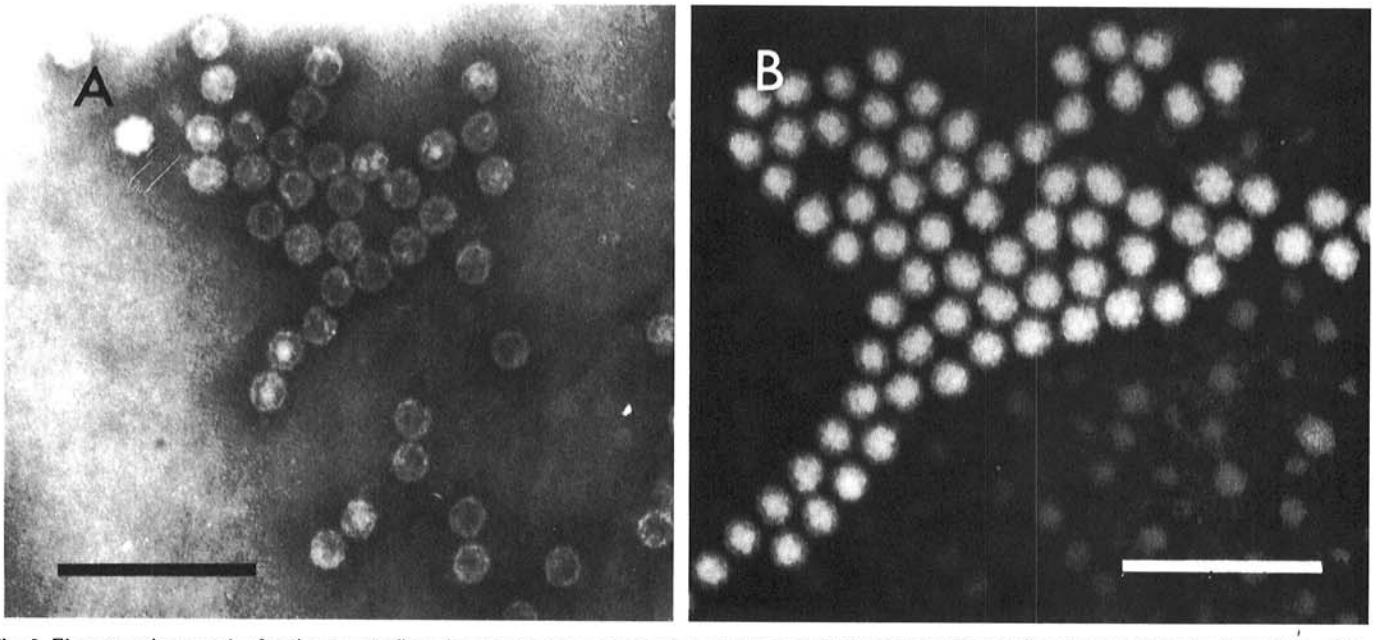

Fig. 2. Electron micrograph of maize rayado fino virus top and bottom components. Components were purified by isopycnic banding in CsCl followed by dialysis against 0.5 M potassium phosphate, pH 7.0. Components were stained with neutral potassium phosphotungstic acid. Bar represents 100 nm. **A,** Top component; **B,** Bottom component.

by high-speed centrifugation.

Viral properties. To estimate the $s_{20,w}$ of MRFV, purified bottom component and the CMV standard were centrifuged together in 16 different linear-log sucrose density gradient columns. The equation relating the depth to which CMV had sedimented and the centrifugal field (cf) was linear with a correlation coefficient of 0.9993. This equation was used to calculate the cf required to sediment CMV to the observed depth of MRFV-bottom in each gradient (called cf-calculated). The $s_{20,w}$ for MRFV-bottom was then calculated by the equation

$$\frac{\text{cf-actual}}{s_{20,w}\,(\text{CMV})} = \frac{\text{cf-calculated}}{s_{20,w}\,(\text{MRFV})}$$

and found to be 124 ± 5S.

The above procedure was checked with a TMV strain whose $s_{20,w}$ had been determined by analytical centrifugation to be 189S (D. T. Gordon, *unpublished*). The value obtained was 188S.

The sedimentation coefficient of MRFV-top was estimated to be 47 ± 2S using MRFV-bottom as the reference particle in four similar determinations.

MRFV top and bottom had buoyant densities of 1.265 and 1.425 g/ml, respectively, in CsCl buffered with 0.5 M potassium phosphate, pH 7.0.

Nucleic acid. Purified bottom component reacted positively with orcinol (34) but not diphenylamine (6), indicating an RNA genome. Top component did not react with either.

The average molar percentages of nucleotides in MRFV-RNA from four separate determinations were 31.4 ± 0.8% Cp, 23.1 ± 1.3% Gp, 16.8 ± 1.3% Ap, and 28.7 ± 0.8% Up. No species with R_f values different from authentic Ap, Gp, Cp, or Up were detected.

Three different lyophilized preparations of bottom component averaged 2.49 ± 0.08% phosphorus by weight (29). The phosphorus content of MRFV-RNA based on the above nucleotide ratios was 9.75% by weight. From these data, MRFV was calculated to contain 25.5 ± 0.8% RNA by weight. The top component showed no phosphorus in similar analyses.

The molecular weight of MRFV-RNA was estimated to be 2.4 ± 0.1 × 10^6 under formaldehyde-denaturing conditions. MRFV-RNA is probably single-stranded because the nucleotides were not found in ratios typical for double-stranded RNA and because the sedimentation rate was slowed 35–40% after formalinization.

Protein. Polyacrylamide gel electrophoresis of SDS-denatured MRFV components revealed two protein species in the ratio of about three molecules of the lighter protein per molecule of the heavier for both top and bottom as determined by measuring the peak areas (Fig. 3). The molecular weights were, respectively, 22,200 ± 200 and 25,500 ± 200 for the bottom component, and 22,500 ± 400 and 25,700 ± 500 for the top component. These values were statistically the same, and the protein constituents of both components were probably identical.

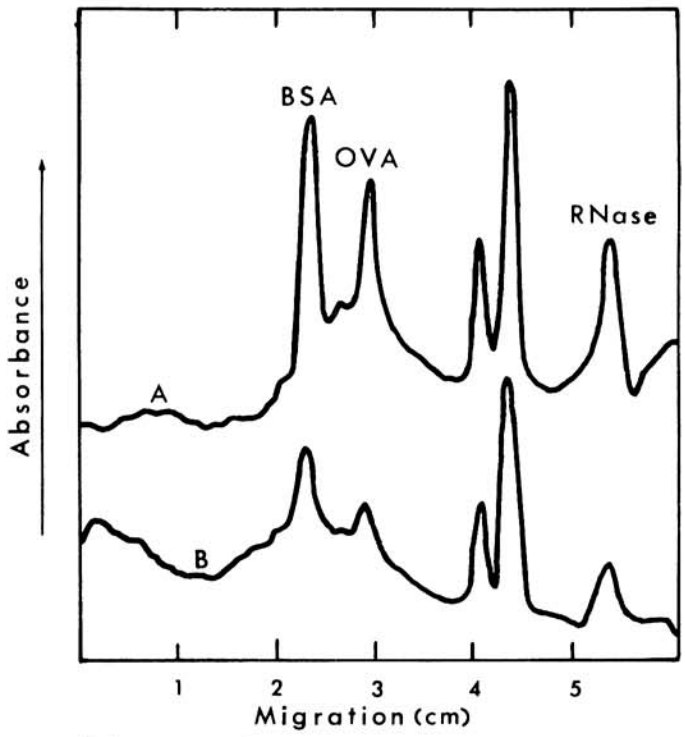

Fig. 3. SDS-polyacrylamide gel electrophoresis of proteins from maize rayado fino virus (MRFV) top and bottom components. Electrophoresis was on 7.5% gels for ~2 hr at 5 ma per gel. **A,** MRFV-top and a mixture of bovine serum albumin (BSA) (MW 68,000), ovalbumin (OVA) (MW 43,000), and pancreatic ribonuclease (RNase) (MW 13,700). **B,** MRFV-bottom component and a mixture of BSA, OVA, and RNase.

Other properties of MRFV. The extinction coefficients ($E^{0.1\%}_{260\ nm}$) of MRFV components were estimated to be 0.91 ± 0.06 cm^2/mg (top) and 6.2 ± 0.2 cm^2/mg (bottom). Extinction coefficients could not be determined by measuring the optical densities of suspensions of known weights of virus because all solvents tested, except the 30% H_2SO_4 used for phosphorus determinations, failed to suspend lyophilized preparations.

Ultraviolet absorbance profiles of MRFV top and bottom components were typical of a protein and nucleoprotein, respectively (Fig. 4). For top, the absorbance maximum and minimum were 278 ± 1 nm and 249 ± 1 nm, respectively, and for bottom, 259 ± 1 nm and 239 ± 1 nm, respectively. The 280/260 absorbance ratios were 0.58 ± 0.02 (bottom) and 1.72 ± 0.06 (top). There was a tyrosine-tryptophan shoulder evident at 288-290 nm for the top component.

Treatments affecting MRFV-US infectivity. The thermal inactivation point was between 90 and 100 C, the dilution end point was between 10^{-4} and 10^{-5}, and the longevity in vitro was between 9 and 11 wk at both 4–5 C and 23–27 C (Table 1). Neither freezing and thawing nor lyophilization reduced infectivity. Twenty-nine of 62 (47%) leafhoppers injected with frozen-and-thawed partially purified MRFV transmitted virus compared to 42 of 90 (47%) injected with a nonfrozen control portion and 37 of 77 (48%) injected with partially purified MRFV, which had been lyophilized and reconstituted 1 wk later. In a second experiment, 43 of 80 (54%) leafhoppers injected with a lyophilized preparation reconstituted after 1 wk transmitted the virus compared to 55 of 98 (56%) injected immediately after purification with the nonlyophilized control. The infectivity of another lyophilized aliquot stored for 5 mo was slightly reduced; 45 of 92 (49%) injected leafhoppers were vectors.

TABLE 1. Thermal inactivation point, dilution end point, and longevity in vitro of maize rayado fino virus[a]

	Temperature (C)						
Thermal inactivation point	50	60	70	80	90	100	Check[b]
	59/121[c]	38/113	40/135	15/117	5/152	0/148	0/116

	Dilution							
Dilution end point	0	10^{-2}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	Check
	17/68	20/59	8/71	0/51	0/72	0/72	0/73	0/63

	Weeks							
Longevity in vitro	0	1	3	5	7	9	11	Check
at: 23–27 C	13/43	18/50	19/52	8/42	11/42	1/42	0/46	0/50
4–5 C	14/55	30/48	25/54	10/44	15/45	4/34	0/26	0/50

[a] Virus was extracted from infected corn leaves with 0.5 M potassium phosphate, 0.5% 2-mercaptoethanol, pH 7.0, clarified with $CHCl_3$, concentrated by centrifugation, and resuspended in 1/10 the original extract volume of 0.1 M HEPES, pH 7.0.

[b] Uninjected control leafhoppers from stock colony.

[c] Approximately 0.025 μl of treated extracts were injected into young adult *Dalbulus maidis*. Following a 10–12 day incubation period, leafhoppers were placed singly on corn test plants for a 3–4 day inoculation access period. Results were recorded 14 days later. The numerator is the number of test plants infected with MRFV; the denominator is the number of plants exposed to injected leafhoppers.

Multiplication of MRFV in *D. maidis*. The mean EIA absorbance values of test leafhoppers assayed at 0, 7, 14, and 21 days post-AAP were all significantly greater than those of control leafhoppers not given an AAP (Table 2), indicating that MRFV was detected in test leafhoppers at all assay times. To determine if the amount of MRFV in *D. maidis* changed with time after AAP, we compared the differences in the mean absorbances between test and control leafhoppers (θ_i) for $i = 0$, 7, 14, and 21. Comparisons were θ_0 with θ_7, θ_7 with θ_{14}, and θ_{14} with θ_{21}. The differences between the means of test and control populations were compared rather than the means of the test populations because the control population means were not homogeneous for the four assay times.

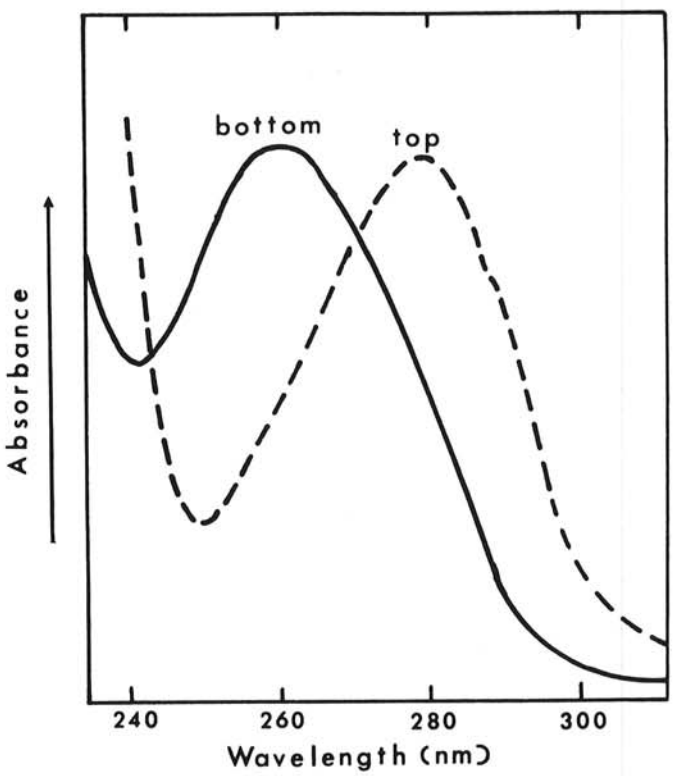

Fig. 4. Absorption spectra of purified maize rayado fino virus top and bottom components suspended in 0.5 M potassium phosphate, pH 7.0. Scanning was done in a Beckman Acta V Spectrophotometer.

TABLE 2. Pooled *t*-test analysis of absorbance values from enzyme-linked immunosorbent assays (EIA) of *Dalbulus maidis* sampled at 0, 7, 14, and 21 days after either a 2-day acquisition access period (AAP) on maize rayado fino virus-infected maize (test population) or no AAP (control population)

	Days post-AAP							
	0 days		7 days		14 days		21 days	
	Test	Control	Test	Control	Test	Control	Test	Control
Number of leafhoppers	60	58	60	60	60	49	60	59
Mean EIA absorbance[a]	69.6	37.8	55.2	43.5	149.1	110.6	89.9	40.1
Standard deviation	61.1	5.0	13.0	5.55	89.8	21.7	75.4	7.29
$\hat{\theta}$[b]	31.8		11.7		38.5		49.8	
T statistic	−3.945		−6.474		−2.926		−4.743	
Significance probability	0.001		0.000		0.002		0.000	

[a] Absorbance values × 1,000.

[b] $\hat{\theta}$ = mean absorbance of test population minus mean absorbance of control population.

To limit the type I error to 0.05 for these comparisons, an α value of 0.017 for individual comparisons was chosen. The $100(1-\alpha)\%$ confidence intervals for $(\theta_0-\theta_7)$, $(\theta_7-\theta_{14})$, and $(\theta_{14}-\theta_{21})$ were 2.71, 37.23; −52.5, −0.858; and −41.2, 24.6, respectively. These intervals indicated that θ_0 was significantly greater than θ_7 and that θ_7 was significantly lower than θ_{14}. However, although the statistic $\hat{\theta}_{14}$ was lower than the statistic $\hat{\theta}_{21}$, there was insufficient evidence to conclude that the difference was significant.

Among individual leafhopper extracts assayed at 0, 7, 14, and 21 days post-AAP, 24, 12, 15, and 21, respectively (each per 60 assayed), each had absorbances greater than the respective control mean by more than three times the standard deviation. At 0 days, these greater values ranged between 3.6 and 73.6 times the standard deviation; at 7 days, between 3.2 and 12 times; at 14 days, between 3.2 and 19.2 times; and at 21 days, between 3.3 and 62 times. Because of these high values, the distribution of absorbances for the test populations were clearly skewed (*unpublished*), whereas the distributions of absorbances for the control populations were approximately normal. Thus, it was evident that MRFV was readily detected in some, but not all, individual test leafhoppers.

DISCUSSION

There is reasonably good agreement between the physical properties of MRFV-US and the Costa Rican isolate (Table 3). These data, together with previously reported similarities in host range (10,31), characteristics of transmission (18,31), and antigenicity (3), indicate a close relationship between the two isolates.

We report a higher thermal inactivation point and longer longevity in vitro for MRFV-US than did Martínez-López (27) for a Colombian isolate. Our isolate may have been more stable, but we cannot rule out that this apparent greater stability was due to the higher purity of our preparations and to our bioassay method that involved needle injection of leafhoppers. Martínez-López used membrane feeding of crude preparations.

The cryptogram (21) for MRFV (bottom), based on our characterization, is R/1:2.4/26:S/S:S/Au. This compares to R/1:2.0/(33-36):S/S:S/Ve/Au reported for the bottom component of the Costa Rican isolate (13,25). MRFV does not appear to belong to any plant virus group thus far described, and Gámez (13) reported that MRFV failed to react with antisera to more than 50 other small isometric plant viruses. As Gámez et al (14) stated, MRFV has characteristics similar to the picornaviruses of vertebrates.

The persistent transmission of MRFV by *D. maidis* (10) and the long latent period between virus acquisition and transmission (9,10,18,31) suggest that MRFV multiplies in this vector. We obtained more direct evidence for this by measuring the amount of virus in leafhoppers at various times after acquisition. The MRFV concentration in *D. maidis* was relatively high immediately after a 2-day AAP, low at 7 days post-AAP, and again high at 14 and 21 days post-AAP. We interpret the decrease at 7 days to represent a loss of acquired virus, possibly by excretion with honeydew or degradation within the leafhoppers. The increase at 14 and 21 days suggests MRFV multiplication in the vector. Rivera et al (32) independently concluded that MRFV replicates in *D. maidis*, based on EIA and fluorescent antibody tests and on detection of virus in situ. We are unaware of any other evidence obtained by EIA for virus replication in leafhoppers or planthoppers or of EIA for detection of virus in this group of vectors. However, EIA has been used to detect virus in aphid vectors (7,8,15), although it was sometimes necessary to combine several aphids before virus could be detected. Our results indicate that EIA is sufficiently sensitive to detect MRFV in individual *D. maidis*. This should prove valuable, particularly in virus epidemiological studies. Such an application of EIA has been reported for detection of citrus stubborn spiroplasma (*Spiroplasma citri*) in individual insects collected in citrus orchards (20).

Oat blue dwarf virus (OBDV) has biological and physical properties similar to those of MRFV. OBDV has a small, isometric particle, is persistently transmitted, and replicates in its leafhopper vector, *Macrosteles fascifrons* (1). However, Gámez (13) found no serological relationship between OBDV and MRFV. We also found no relationship between them in reciprocal agar-gel double-diffusion tests but did detect a distant serological relationship by a microprecipitin assay (R. E. Gingery and J. H. Hill, *unpublished*). We agree with Leon and Gámez (25) who have proposed that OBDV and MRFV are distinct members of a new group of plant viruses.

Most obligately vectored viruses are maintained by serial transfer in plants. Preservation of infectivity after lyophilization of partially purified preparations allows long-term storage of MRFV. Thus, MRFV joins barley yellow dwarf virus (33) as a non-sap-transmissible virus that can be preserved and stored in the American Type Culture Collection.

TABLE 3. Comparison of Costa Rican and United States isolates of maize rayado fino virus (MRFV)

Property	MRFV	
	Costa Rica[a]	United States
Bouyant density in CsCl:		
Bottom component	1.46 g/ml	1.425 g/ml
Top component	1.28 g/ml	1.265 g/ml
Sedimentation rate:		
Bottom component	120 ± 1S	124 ± 2S
Top component	54 ± 4S	47 ± 2S
Particle diameter	31.5–33 nm	22–27 nm[b]
RNA:		
Molecular weight	2.0×10^6	2.4×10^6
% of particle weight	33–36	26
Protein	One main component: MW 21,000	Main component: MW 22,350 Minor component: MW 25,600

[a] Data for MRFV-Costa Rica are from Gámez (13) and Leon and Gámez (25).
[b] (3).

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