Properties of Bermuda Grass Etched-Line Virus, a New Leafhopper-Transmitted Virus Related to Maize Rayado Fino and Oat Blue Dwarf Viruses

B. E. L. Lockhart, Nezha Khaless, Angela M. Lennon, and Mohammed El Maatauoi

Department of Plant Pathology, University of Minnesota/Institut Agronomique et Veterinaire Hassan II, B.P. 438, Agadir, Morocco; Laboratorie de Controle des Semences, INRA, Rabat, Morocco; Scottish Crop Research Institute, Invergowrie, Dundee, Scotland; and Laboratoire de Virologie, INRA, Rabat, Morocco.

We wish to thank M. S. K. Ghauri and M. R. Wilson of the Commonwealth Institute of Entomology, London, England, for identification of some of the leafhoppers and planthoppers used in this study. We also wish to thank E. W. Kitajima, M. T. Lin, Judith Chamberlain, E. E. Banttari, R. Koenig, R. Gamez, and R. E. Gingery for conducting serological tests and for gifts of antigens and antisera.

This research was supported by funds from USAID/University of Minnesota Contract 608-0160-001.

Accepted for publication 17 June 1985.

ABSTRACT

Lockhart, B. E. L., Khaless, N., Lennon, A. M., and El Maataoui, M. 1985. Properties of Bermuda grass etched-line virus, a new leafhopper-transmitted virus related to maize rayado fino and oat blue dwarf viruses. Phytopathology 75:1258-1262.

A previously undescribed virus occurring in Bermuda grass (*Cynodon dactylon*) and Johnson grass (*Sorghum halepense*) in Morocco had isometric particles approximately 28 nm in diameter. The virus was transmitted by the cicadellid leafhopper (*Aconurella prolixa*) but not mechanically, by aphids, or through soil. The virus caused white etched-line symptoms on Bermuda grass and narrow chlorotic streaks on Johnson grass. It also infected maize, wheat, and oats but not barley or sugarcane. The virus sedimented as a top, 57S, protein component and a bottom, 118S, nucleoprotein component. Both components contained a single capsid protein with a molecular mass of 26.5–27.0 kdaltons as determined by SDS-polyacrylamide gel electrophoresis. The virus, named Bermuda grass etched-line virus (BELV), was related serologically to both maize rayado fino virus (MRFV) and oat blue dwarf virus (OBDV). Properties shared with OBDV and MRFV suggest that BELV should be assigned to the OBDV-MRFV group of plant viruses.

Bermuda grass (*Cynodon dactylon* [L.] Pers.) is the most prevalent grass weed in Morocco and occurs ubiquitously in and around cultivated fields of cereals, maize (*Zea mays* L.), and other crops. Bermuda grass is commonly infected by barley yellow dwarf virus (BYDV), and undescribed rhabdovirus, and a mycoplasmalike organism (MLO); the latter produces symptoms similar to those of Bermuda grass yellow leaf disease (2) (Lockhart and Khaless, *unpublished*). In 1983, symptoms not previously seen on Bermuda grass were observed in several locations in northern and western Morocco. Symptoms on leaves consisted of white etched lines and spots (Fig. 1), and plants were noticeably stunted. Leaf symptoms resembled surface scarification caused by tetranychid mite feeding. When negatively stained preparations of plants showing virus symptoms were examined by electron microscopy, numerous well-contrasted, isometric, viruslike particles were observed; such particles were absent in similar preparations from apparently healthy plants. Repeated attempts to transmit the virus mechanically to healthy *Cynodon* and to a range of other test plants were unsuccessful. The virus was named Bermuda grass etched-line virus (BELV).

MATERIALS AND METHODS

Virus source and culture. The original culture of BELV was field collected from infected plants of *C. dactylon* that were verified to be not infected by any mechanically transmissible viruses and to contain no viruslike particles other than 28-nm spheres. Further, these plants showed no trace of MLO infection. The source plants were maintained in the greenhouse. After identification of the leafhopper vector, the virus was transmitted to healthy *C. dactylon* grown from seed and subsequently maintained in these plants.

Mechanical, aphid, and soil transmission tests. Inoculum for mechanical transmission tests was prepared by grinding young, infected leaves of *C. dactylon* in cold 1% K_2HPO_4 containing 0.2% 2-mercaptoethanol and the crude extract was used to inoculate Carborundum-dusted leaves of test plants.

Aphid transmission tests were done with three aphid species that occur commonly on Bermuda grass: *Rhopalosiphum maidis* Fitch, *R. padi* L., and *Rungsia maydis* Pass. The two *Rhopalosiphum* spp. were raised on barley (*Hordeum vulgare* L.), and *R. maidis* on Bermuda grass. After a preacquisition fast of 30 min, nonviruliferous, apterous adults of each species were allowed a 20-min acquisition access period (AAP) on BELV-infected *C. dactylon*. Aphids were then transferred in lots of 15 to pots of healthy seedlings of *C. dactylon* and allowed to feed for 4 days before being killed by an insecticide.

Soil transmission tests were done by planting healthy seedlings of *C. dactylon* in untreated field soil collected from infected patches of *C. dactylon* and containing infected plants of the latter treated by foliar insecticidal application to reduce the risk of aerial virus transmission.

Transmission by leafhoppers and planthoppers. The following cicadellid leafhoppers were collected on uninfected *C. dactylon* in various areas of Morocco and raised on healthy *C. dactylon*. *Exitianus capicola* (Stål), *Psammotettix* sp., *Balclutha saltuella* (Kirschbaum), *Cicadulina bipunctata* (Melichar) ssp. *bipunctella* (Matsumura), and *Aconurella prolixa* (Lethierry). The delphacid planthopper *Toya propinqua* (Fieber) was collected and similarly raised.

All transmission tests were done by mass transfer and no attempt was made to determine the minimum AAP, latent period, or relative efficiency of transmission by individual insects or by different sexes or developmental stages. Insects were caged on BELV-infected *C. dactylon* for 5 days and then caged in groups of 20–30 on 15–20 healthy seedlings of *C. dactylon* or of other test plants. Insects fed on test plants until their demise. Test plants were kept under observation in the greenhouse for up to 9 mo.

Purification and properties. The following procedure was used to purify BELV from infected *C. dactylon* collected in the field or infected in the greenhouse by leafhopper transmission. Leaf tissue was homogenized in three volumes (w/v) of cold 0.1 M sodium citrate, pH 6.5, containing 0.2% mercaptoethanol. The extract was

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

filtered and the pH lowered to 5.0 by dropwise addition of glacial acetic acid. The acidified sap was kept at 4 C for 1–2 hr, after which the pH was readjusted to 6.5 with 3 N NaOH. The virus was obtained from the crude extract by two cycles of differential centrifugation of 12,100 *g* for 10 min and 139,000 *g* for 2 hr, followed by rate-zonal density gradient centrifugation for 2 hr at 107,000 *g* on 7–28% linear sucrose gradients. Virus pellets were resuspended in 0.1 M sodium citrate, pH 6.5, which was also used for preparation of sucrose gradients.

The sedimentation coefficient of purified virus was estimated by the linear-log density gradient centrifugation method of Brakke and Van Pelt (4), using squash mosaic (SqMV), tomato mosaic (ToMV), and tomato bushy stunt (TBSV) viruses as standards.

Capsid protein molecular weight determination was done by SDS-polyacrylamide gel electrophoresis (PAGE) in 7.5% polyacrylamide gels in 0.1 M sodium phosphate, pH 7.0 (13). Low-molecular-weight protein markers were obtained from Pharmacia Fine Chemicals AB, Uppsala, Sweden. Gels were electrophoresed for 1 hr and then, following sample application, for 7 hr at 150 V (constant voltage). Gels were stained with Coomassie blue-methanol-acetic acid and destained with methanol-acetic acid.

Electron microscopy. Leaf-dip and purified virus preparations were stained with 1.5% sodium phosphotungstate, pH 7.0 (PTA), for electron microscopic examination.

Serology. Antisera against separated centrifugal components of BELV were prepared in rabbits by an initial intravenous injection followed 1 wk later by an intramuscular injection of virus emulsified in Freund's incomplete adjuvant. This was followed 2 wk later by a subcutaneous injection of emulsified virus, and after another 2 wk by two intravenous injections spaced 4 days apart. Blood was collected from the animals 1 wk after the final intravenous injection.

Double-diffusion serological tests were in 0.85% agarose gels prepared in distilled water containing 0.1% NaN_3. To standardize conditions for comparative immunodiffusion tests, 3 ml of agarose was added to 5-cm-diameter petri dishes to give a gel height of approximately 2 mm. Reactant wells were 3 mm in diameter, and the distance between central and peripheral wells was 2.5 mm. Wells were charged with 15 μl samples of antiserum or antigen. In comparative immunodiffusion tests BELV was used in undiluted sap from infected Bermuda grass. Isolates of MRFV from the United States (MRFV-US) (8) and Costa Rica (MRFV-CR) (7) as well as a Minnesota isolate of OBDV (1) were used as partially purified suspensions following a single cycle of high-speed ultracentrifugation. The MRFV isolates were prepared from infected maize (R. E. Gingery and R. Gamez, *personal communication*), and OBDV from infected oats. Healthy controls consisted of undiluted sap from uninfected Bermuda grass, and of healthy maize and oat extracts prepared in the same manner as for the respective virus isolates. The diluent for antiserum titer determinations was 5% bovine serum albumin in 0.05 M tris-HCl, pH 7.2, containing 0.85% NaCl and 0.1% NaN_3 (12).

Enzyme immune assay (EIA) was done according to standard procedures (6). Polystrene plates were coated wtih 1.0 μg of purified γ-globulin per milliliter and samples were diluted 1:10 (w/v) in PBS/Tween-20 and the alkaline phosphatase conjugate diluted 1:500 were sequentially applied. Absorbances were determined spectrophotometrically at 405 nm.

RESULTS

Mechanical, aphid, and soil transmission. Repeated attempts to mechanically transmit BELV from infected *C. dactylon* to healthy *C. dactylon*, maize, wheat (*Triticum aestivum* L.), barley, and oats (*Avena sativa* L.) were unsuccessful. No symptoms appeared on healthy *C. dactylon* seedlings grown in untreated field soil containing infected plants. The virus was not transmitted from infected to healthy *Cynodon* by *R. maidis, R. padi*, or *R. maidis*. *C. dactylon* test plants were observed for symptoms in the greenhouse for 9 mo before final symptom ratings were made.

Distribution and natural occurrence. The virus was found in Bermuda grass in widely separated locations in northwestern and north-central Morocco. It also occurred on Johnson grass (*Sorghum halepense* L.) growing in the vicinity of infected Bermuda grass. Symptoms on Johnson grass consisted of narrow broken lines and streaks, differing somewhat from those observed on Bermuda grass. Numerous isometric particles were found in negatively stained leaf dip preparations of infected Johnson grass, and the virus was identified serologically by both immunodiffusion and EIA tests with BELV antiserum.

Transmission tests with leafhoppers and planthoppers. The virus was transmitted from infected to healthy *C. dactylon* by *A. prolixa* but not by *E. capicola, Psammotettix* sp., *B. saltuella, C. bipunctata* spp. *bipunctuella*, or *T. propinqua*.

Host range and symptoms. The virus was transmitted by *A. prolixa* from infected Bermuda grass to healthy Bermuda grass; maize cultivars Earliking, Extra Early Sweet, LG11, and LG55; durum wheat (*T. durum* L.) cultivar Haj Moline; and oat cultivar AV 459. Symptoms on Bermuda grass were identical to those observed in the field (Fig. 1). Foliar symptoms on all four maize cultivars consisted of narrow broken lines and short streaks (Fig. 2), similar to symptoms induced by maize rayado fino virus (MRFV). Infected plants were stunted. Symptoms in durum wheat consisted of marked stunting, thickening of leaf veins, and slight leaf deformation. No symptoms were observed in oat cultivar AV 459, but the virus was detected by EIA. EIA also confirmed infection of maize and wheat. No symptoms were produced on and no virus detected by EIA from inoculated barley or sugarcane (*Saccharum officinarum* L.) cultivar CP 44-101.

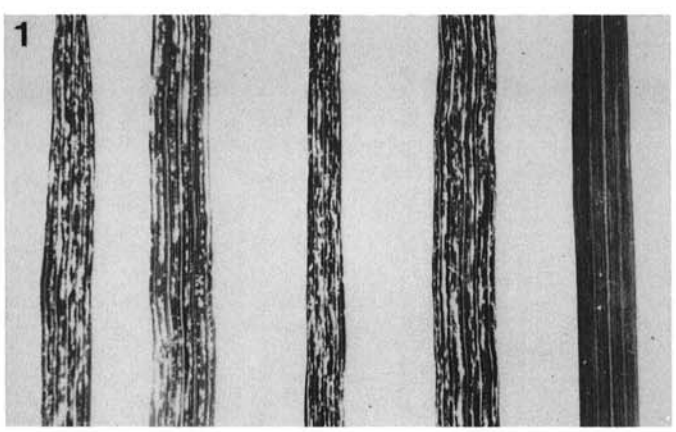


Fig. 1. Symptoms of Bermuda grass etched-line virus (BELV) infection on naturally infected Bermuda grass (*Cynodon dactylon*). Identical symptoms were produced experimentally on this host by leafhopper-transmitted BELV. Healthy leaf is at extreme right.

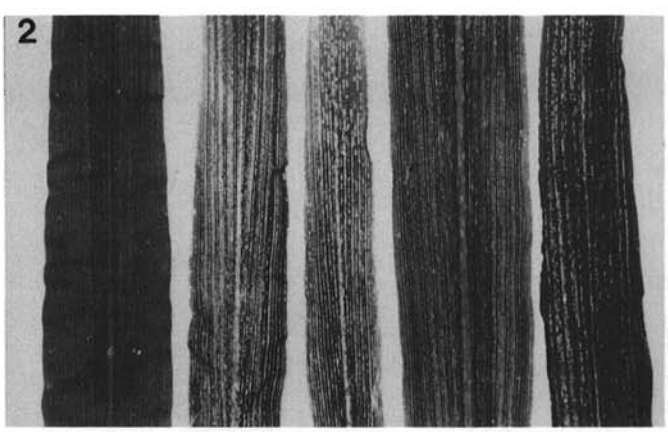


Fig. 2. Symptoms of Bermuda grass etched-line virus (BELV) infection on sweet corn (*Zea mays* 'Earliking'). The virus was transmitted to this host from infected *Cynodon dactylon* by the cicadellid leafhopper, *Aconurella prolixa*. Healthy leaf at left.

Purification and properties. The virus occurred in high concentration in infected *C. dactylon* from which it was easily purified by the procedure outlined above. Substantially less virus was obtained with procedures employing chloroform or butanol for clarification of initial extracts. Precipitation of virus by polyethylene glycol (PEG, MW 6000) resulted in final preparations highly contaminated with host material. Purified virus was stable in citrate buffer, pH 6.5, at molarities between 0.01 and 0.1 but was degraded extensively when stored overnight at 4 C in the same buffer at pH 7.5. Purified virus collected from centrifuged sucrose gradients had a 260/280 ultraviolet (UV) absorption ratio of 1.40–1.44.

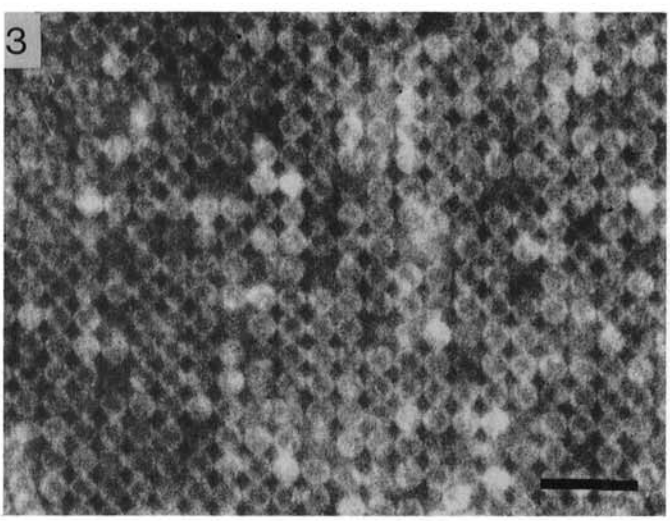
In rate-zonal density gradient centrifugation purified virus sedimented as two UV-absorbing components. The top component had a UV-absorption spectrum characteristic of protein, with a maximum at 280 nm, a minimum at 260 nm, and a 260/280 ratio of 0.77. The absorption spectrum of the faster-sedimenting bottom component was characteristic of a nucleoprotein, with a maximum at 260 nm, a well-defined minimum at 240 nm, and a 260/280 ratio of 1.57.

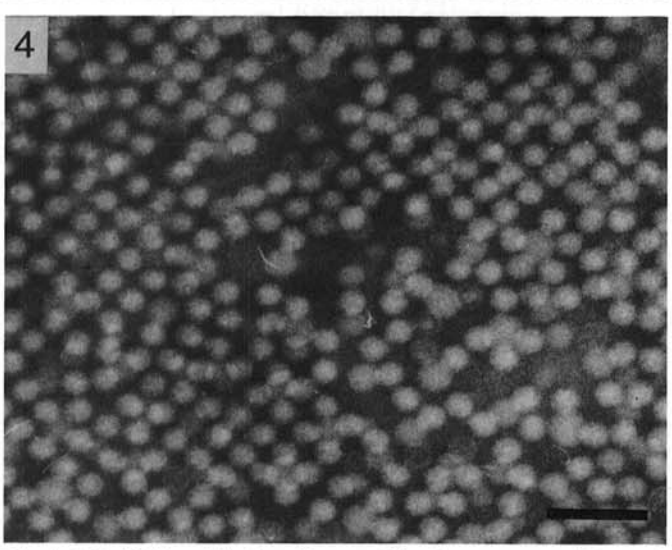
In initial experiments to estimate the sedimentation coefficient, top and bottom components co-sedimented with the corresponding components of SqMV. Mixtures of BELV and SqMV, containing 2.0 $OD_{260\ nm}$ units of each virus, sedimented to three UV-absorbing zones, representing the co-sedimenting top and bottom components of each virus and the middle, 95S, component of SqMV. Based on the reported sedimentation coefficients for SqMV components (5), the estimated values for the top and bottom components of BELV were, respectively, about 57S and 118S.

Capsid protein molecular weights were determined for separated top and bottom components. Both components contained a single migrating protein species of similar molecular mass, estimated at 26.5–27 kdaltons (Fig. 5).

Electron microscopy. The virus was stable in PTA and no disruption or distortion of particles was observed. Numerous virus particles were observed in leaf-dip preparations. Purified top component particles (Fig. 3) were penetrated by PTA, whereas the bottom component was not (Fig. 4). Both types of particle were approximately 28 nm in diameter.

Serology. Antisera prepared against separated top and bottom components had homologous titers of 1/512–1/1,024 in immunodiffusion tests with undiluted sap from infected *C. dactylon* as antigen. The batch of BELV antiserum used in tests with MRFV and OBDV had an initial healthy reciprocal antibody titer of 4, and was cross-adsorbed with sap of healthy *Cynodon* before being used. No serological differences were detected between top and bottom components in reciprocal tests with these antisera. Also, no serological relationship was found between BELV and any of the following viruses: maize chlorotic dwarf, maize chlorotic mottle (antisera supplied by R. E. Gingery), cocksfoot mottle, phleum mottle, holcus transitory mottle, brome stem-leaf mottle, cocksfoot mild mosaic, Cynosurus mottle, and Festuca mottle (J. Chamberlain, *personal communication*). In separate tests (R. Koenig, *personal communication*) no serological relationship was found between BELV and 13 tymoviruses. The Moroccan virus reacted with antiserum to MRFV isolates from the United States (antiserum and homologous antigen, as a partially purified preparation, supplied by R. E. Gingery), from Costa Rica (antiserum and homologous antigen, as a partially purified preparation, provided by R. Gamez), and from Brazil (E. Kitajima and M. Lin, *personal communication*). Spur formation occurred in all reciprocal combinations between the MRFV isolates and BELV. Both BELV and the U.S. MRFV isolate reacted with antiserum to OBDV (antiserum provided by E. E. Banttari) (Figs. 6–8). No reaction occurred between any dilution of OBDV antiserum and extracts from healthy Bermuda grass, maize, or oat leaves. As supplied, the Costa Rican MRFV antigen did not react in immunodiffusion tests with OBDV antiserum, an apparent lack of serological relationship that has been reported previously (7). Both BELV and OBDV reacted with MRFV-US and MRFV-CR antisera (Table 1). Spur formation occurred in both cases (Figs. 9–11). The results of immunodiffusion tests with BELV, OBDV, and the MRFV isolates and their respective antisera are summarized in Table 1.





Figs. 3–4. Purified top (Fig. 3) and bottom (Fig. 4) components of Bermuda grass etched-line virus (BELV) negatively stained with 1.5% sodium phosphotungstate (PTA), pH 7.0. Scale bar represents 100 nm.

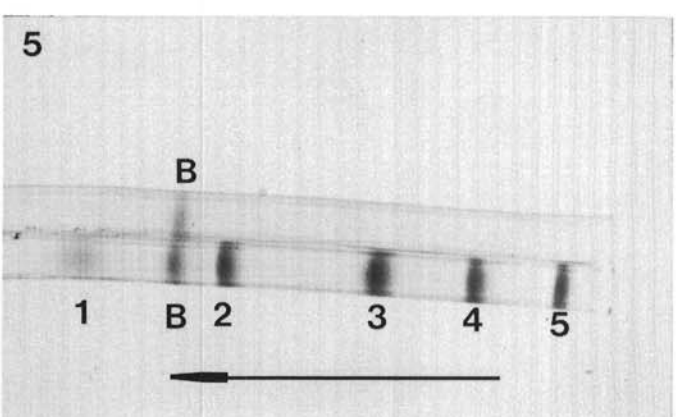


Fig. 5. Estimation of capsid protein molecular mass of Bermuda grass etched-line virus (BELV) by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Molecular mass markers, with their respective masses given in daltons, are, from left to right: 1 = trypsin inhibitor (20,000); 2 = carbonic anhydrase (30,000); 3 = ovalbumin (43,000); 4 = albumin (67,000); and 5 = phosphorylase b (94,000). B = BELV. At top, BELV alone. At bottom, BELV plus markers.

BELV could be readily detected by EIA in *Cynodon*, maize, wheat, and oats. In DAS-EIA of healthy and infected plant sap at a 1/10 dilution, the following spectrophotometric readings were obtained: infected *Cynodon*, 2.00; infected maize, 2.00; infected wheat, 2.00; infected oats, 0.13. Healthy *Cynodon*, maize, wheat, and oats gave readings of 0.00–0.01. These figures are the averaged values of six replicates per sample, and the instrument was blanked with phosphate-buffered saline containing Tween-20 (PBST) (6).

DISCUSSION

We have shown that BELV is related serologically to MRFV and that symptoms produced by both viruses on maize are similar. However, we did not test both viruses on the same range of maize cultivars and hence no conclusions can be drawn about similarities of symptoms or cultivar susceptibility. The host range of MRFV is restricted to maize and closely related genera and does not include oats, wheat, or Johnson grass (7,11), all of which were infected by BELV.

The results of immunodiffusion tests indicated distinct serological differences between BELV and the MRFV isolates tested. Serological differences have also been found between different MRFV isolates from Central and South America (7).

BELV contains a single coat protein with a molecular mass of 26.5–27.0 kdaltons whereas the U.S. MRFV isolate contains two capsid proteins of MW 22.4 and 25.6 kdaltons present in a ratio of 3:1 (8). In contrast, the Costa Rican MRFV has a single 20.5-kdalton capsid protein (7). These apparent constitutive differences may represent actual genomic differences between these viruses or may reflect differences in methodology used.

The serological relationship between OBDV and BELV was closer than that between OBDV and MRFV. Although previously (7) the Costa Rican MRFV was found to be serologically unrelated to OBDV, a distant serological relationship was found between the MRFV isolate from the United States and OBDV in microprecipitation tests (8). The degree of serological difference between these viruses may be due in part to differences in antigen concentration and serological technique. OBDV occurs in low concentration and is confined to the phloem in plant tissue. In contrast, BELV occurs in high concentration in plant extracts.

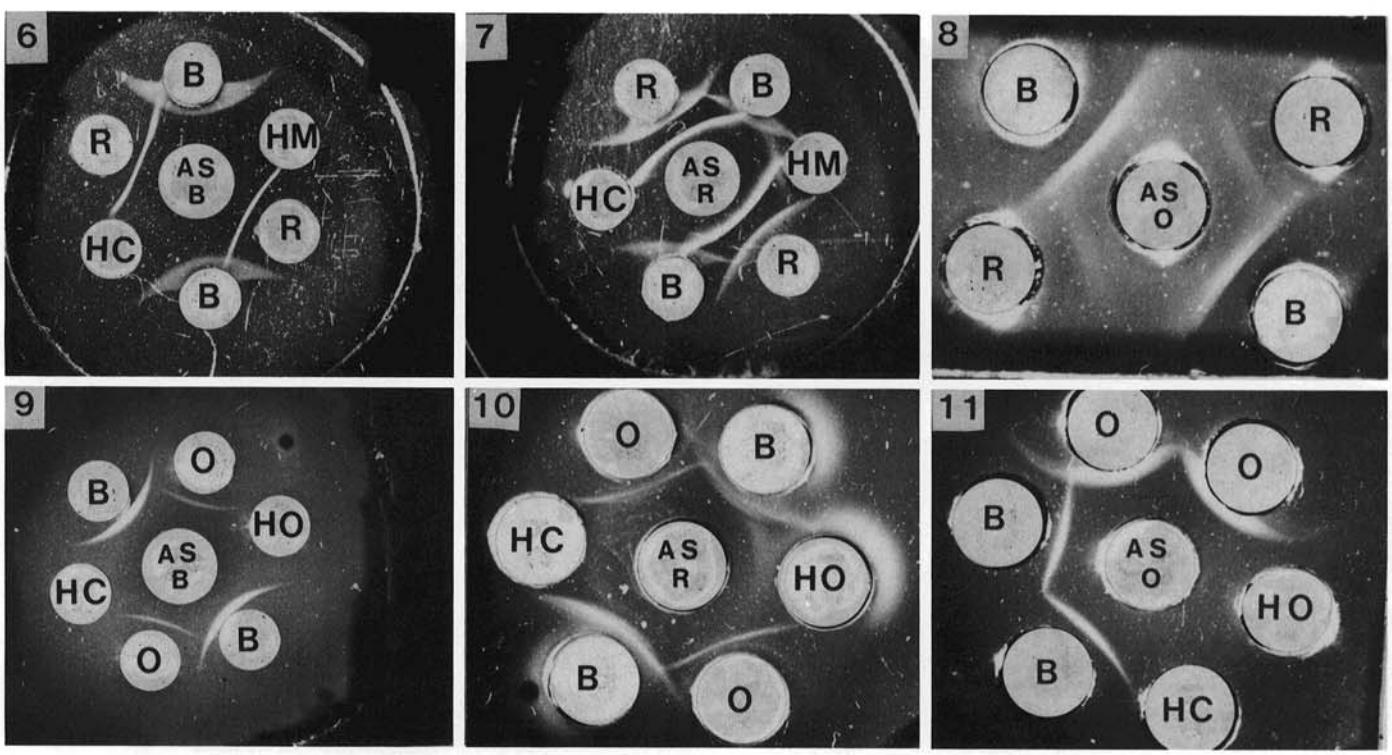
Based on the properties described above, BELV appears to differ sufficiently from MRFV to justify its designation as a distinct plant virus. OBDV, MRFV, and BELV appear related and the similarity of their biological, serological, and morphological properties suggests their classification within the same group. Results of the immunodiffusion tests presented in Table 1 and in Figs. 6–11 clearly demonstrate serological relationships between BELV, MRFV, and OBDV. However, caution must be exercised in using the reciprocal titer end points in Table 1 to assess the degree of relatedness between these viruses since antigen concentrations were not determined or controlled.

To date, MRFV has not been reported outside of the Americas (7,8) and the occurrence in Mediterranean Africa of a closely related leafhopper-transmitted virus is noteworthy for understanding the geographical distribution of these related viruses. African cereal streak virus, a phloem-limited isometric virus with a host range that includes wheat, oats, barley, and rice

TABLE 1. Summary of reciprocal immunodiffusion tests of Bermuda grass etched-line virus (BELV), isolates of maize rayado fino virus (MRFV) from Costa Rica (MRFV-CR) and the United States (MRFV-US), oat blue dwarf virus (OBDV), and their respective antisera

	Antiserum			
Antigen	BELV	MRFV-US	MRFV-CR	OBDV
BELV	1,024[a]	16	4	16
MRFV-US	32	64	32	2
MRFV-CR	32	64	64	(−)
OBDV	128	16	4	512

[a] Numbers represent reciprocal of highest antiserum dilution giving a positive reaction and the minus (−) no reaction.



Figs. 6–11. Immunodiffusion tests between Bermuda grass etched-line virus (BELV), maize rayado fino virus (U.S. isolate, MRFV-US), oat blue dwarf virus (OBDV), and their respective antisera in homologous and heterologous combinations. **Figs. 6–8** show reactions of BELV and MRFV-US with antisera to BELV, MRFV-US, and OBDV, respectively. **Figs. 9–11** show reactions of BELV and OBDV with antisera to BELV, MRFV-US, and OBDV, respectively. Legend: B = BELV antigen in undiluted sap of *Cynodon dactylon*; R = MRFV-US antigen in partially purified extract from maize; O = OBDV antigen in partially purified extract from oats; HC = sap of healthy *C. dactylon*; HM = healthy maize extract; HO = healthy oat extract; AS-B = BELV antiserum; AS-R = MRFV-US antiserum; and AS-O = OBDV antiserum. Immunodiffusion tests were done in 0.85% agarose, 0.1% NaN_3, in distilled water.

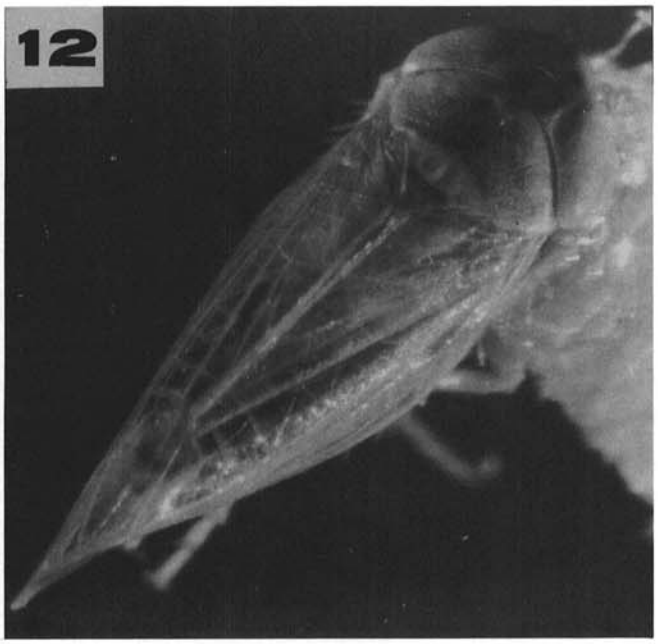


Fig. 12. Dorsal view of *Aconurella prolixa*, cicadellid vector of Bermuda grass etched-line virus (×32).

(*Oryza sativa* L.) does not infect maize (9) and is transmitted by a delphacid planthopper, *T. catilina* (Fennah), rather than a leafhopper.

BELV can cause a potentially damaging disease of wheat. Bermuda grass, the natural inoculum reservoir of the virus, occurs ubiquitously in fields of wheat and other cereals in Morocco. The eventual economic importance of BELV will probably be related to the size of vector populations. Although *A. prolixa* was shown experimentally to transmit BELV, it has not been demonstrated as a natural vector. Surveys have not been conducted to determine the occurrence of BELV in susceptible cereals and maize in Morocco.

LITERATURE CITED

1. Banttari, E. E., and Zeyen, R. J. 1973. Oat blue dwarf virus. No. 123 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst., Assoc. Appl. Biologists, Kew, Surrey, England. 4 pp.
2. Bar-Joseph, M., Zelcer, A., and Loebenstein, G. 1975. Association of mycoplasmalike organisms with bermudagrass yellow leaf. Phytopathology 65:640-641.
3. Bock, K. R. 1974. Maize streak virus. No. 133 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst., Assoc. Appl. Biologists, Kew, Surrey, England. 4 pp.
4. Brakke, M. K., and Van Pelt, N. 1970. Linear-log sucrose gradients for estimating sedimentation coefficients of plant viruses and nucleic acids. Anal. Biochem. 38:56-64.
5. Campbell, R. N. 1971. Squash mosaic virus. No. 43 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst., Assoc. Appl. Biologists, Kew, Surrey, England. 4 pp.
6. Clark, M. F., and Adams, A. M. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475-483.
7. Gamez, R. 1980. Maize rayado fino virus. No. 220 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst., Assoc. Appl. Biologists, Kew, Surrey, England. 4 pp.
8. Gingery, R. E., Gordon, D. T., and Nault, L. R. 1982. Purification and properties of an isolate of maize rayado fino virus from the United States. Phytopathology 72:1313-1318.
9. Harder, D. E., and Bakker, W. 1973. African cereal streak, a new disease of cereals in East Africa. Phytopathology 63:1407-1411.
10. Lockhart, B. E. L., and Elyamani, M. 1982. Virus and viruslike diseases of maize in Morocco. Pages 127-129 in: D. T. Gordon, J. K. Knoke, L. R. Nault, and R. M. Ritter, eds. Proc. Int. Maize Virus Dis. Colloq. and Workshop, August 1982. Ohio State University, O.A.R.D.C., Wooster, OH. 261 pp.
11. Nault, L. R., Gingery, R. E., and Gordon, D. T. 1980. Leafhopper transmission and host range of maize rayado fino virus. Phytopathology 70:709-712.
12. Purcifull, D. E., and Batchelor, B. L. 1977. Immunodiffusion tests with sodium dodecyl sulfate (SDS)-treated plant viruses and plant viral inclusions. Fla. Agric. Exp. Stn. Bull. 788 (tech.). 39 pp.
13. Weber, K., and Osborn, M. 1969. The reliability of molecular weight determinations by dodecylsulphate-polyacrylamide gel electrophoresis. J. Biol. Chem. 244:4406-4412.