

Occurrence of Cereal Chlorotic Mottle Virus in Northern Africa

B. E. L. LOCKHART, Department of Plant Pathology, University of Minnesota, St. Paul 55108/Department de Phytopathologie, Institut Agronomique et Veterinaire Hassan II, Complexe Horticole, Agadir, Morocco

ABSTRACT

Lockhart, B. E. L. 1986. Occurrence of cereal chlorotic mottle virus in northern Africa. *Plant Disease* 70:912-915.

Cereal chlorotic mottle virus (CCMV), a cicadellid-transmitted plant rhabdovirus reported previously only from eastern Australia, was identified as the causal agent of a necrotic leaf streak disease occurring on oats (*Avena sativa*) and barley (*Hordeum vulgare*) in southern Morocco. The virus occurred naturally in *Setaria verticillata*, *Agrostis semiverticillata*, and *Phalaris* sp. and was transmitted experimentally by the cicadellid leafhopper *Cicadulina bipunctata* subsp. *bipunctella*. The virus caused severe necrotic and chlorotic leaf streak symptoms in oats, durum wheat (*Triticum durum*), and barley (*Hordeum vulgare*) but did not infect sorghum (*Sorghum bicolor*), sugarcane (*Saccharum officinarum*), any of five maize (*Zea mays*) cultivars, or bermudagrass (*Cynodon dactylon*). *Oryzopsis miliacea* was a symptomless host. In negatively stained leaf-dip preparation, the virus particles measured about 220–250 × 60 nm and showed a surface pattern and helical cross-striations similar to those of Australian CCMV. The Moroccan (CCMV-M) and Australian (CCMV-A) virus isolates cross-reacted with each other's antiserum without spur formation in immunodiffusion tests, and no serological differences between the two virus isolates were detected. In enzyme immune assay (EIA), CCMV-M infection in barley was detectable by homologous immunoglobulin G (IgG) at a sap dilution in excess of 100,000-fold and provided a sensitive method for detecting CCMV infection in plants.

In early 1985, rhabdoviruslike particles were found associated with a necrotic streak disease of oats in southern Morocco. Extracts of infected plants did not react with antisera to *Cynodon chlorotic streak virus* (CCSV) (4) or barley yellow striate mosaic virus (BYSMV) (B. E. L. Lockhart, M. El Maataoui, T. W. Carroll, and A. M. Lennon, *unpublished*), two delphacid-transmitted plant rhabdoviruses occurring in wild and cultivated Gramineae in Morocco and elsewhere in the Mediterranean basin. In immune electron microscopy (IEM) experiments, the oat rhabdovirus was not trapped by either CCSV or BYSMV antisera and did not react in enzyme immune assay (EIA) with either CCSV or BYSMV IgG. In particle dimensions, and especially in appearance, the oat rhabdovirus particles resembled those of CCMV (2,3), which was reported only from Queensland, Australia. Subsequent vector transmission, host range, and serological tests revealed that the Moroccan oat rhabdovirus was either identical or closely related to Australian CCMV. In early 1986, CCMV was also

identified in volunteer barley in southern Morocco.

MATERIALS AND METHODS

Virus source. The CCMV-M isolates used in this study were obtained from naturally infected oats (*Avena sativa* L., cultivar unknown), *Setaria verticillata* (L.) P.B., and *Agrostis semiverticillata* (Forsk.) Christ. After a vector was identified, virus isolates from the three original plant sources were transmitted to and maintained in *A. semiverticillata*.

Insect transmission. No mechanical or aphid transmission tests were attempted. Transmission tests were done with five cicadellid leafhopper species and two delphacid plant hopper species that occur commonly on Gramineae in Morocco. The cicadellid leafhoppers tested were *Cicadulina bipunctata* (Melichar) subsp. *bipunctella* (Matsumura), *Aconurella prolixa* (Lethierry), *Agallia laevis* (Ribaut), *Psammotettix striatus* (Linné) and *Exitianus nanus* (Distant). The delphacid plant hoppers used were *Tonya propinqua* (Fieber) and *Laodelphax striatellus* (Fallen). All insects were raised on virus-free bermudagrass (*Cynodon dactylon* L.) grown from seed. Transmission tests were done by mass transfer. Insects were transferred to infected source plants (oats, *S. verticillata*, and *A. semiverticillata*) and allowed to feed for 2 wk. They were then caged in groups of 20–25 on young healthy test plants and allowed to feed until they died.

Partial virus purification and antiserum preparation. CCMV-M was partially purified from infected oats by a slight modification of the procedure described for CCSV (4). This involved clarification of initial leaf extracts by acidification rather than by filtration through celite or celite-charcoal pads (5), which, as in the case of CCSV, entailed much loss of virus. Fresh infected leaf tissue was extracted 1:2 (w/v) in cold 0.05 M Tris-citrate, pH 6.8, containing 0.5% Na₂SO₃ and 10% (w/v) sucrose. The extract was filtered and acidified to pH 5.5 by adding droplets of 5% (v/v) acetic acid. The acidified extract was stored at 4 C for 60–90 min and clarified by centrifugation at 10,000 g for 10 min. The virus particles were concentrated by centrifugation at 48,000 g for 45 min, and the pellets were resuspended in 0.01 M phosphate buffer, pH 7.2, containing 10% (w/v) sucrose and 0.9% (w/v) NaCl. After centrifugation at 8,000 g for 10 min, the partially purified extracts were recentrifuged at 48,000 g for 45 min and the pellets resuspended as before in 0.01 M phosphate buffer, pH 7.2, containing 10% sucrose and 0.9% NaCl. This final partially purified suspension was used for antiserum preparation. Rabbits were first given multiple-site intramuscular and subcutaneous injections of partially purified CCMV-M emulsified in Freund's complete adjuvant followed 1 mo later by a similar series of injections of virus emulsified in incomplete adjuvant. Blood was collected starting 2 wk after the second series of injections.

Serological tests. Immunodiffusion tests were done in 0.8% agarose, 0.1% NaN₃, in distilled water. Undiluted, untreated leaf sap from healthy and infected plants or partially purified extracts were used as antigens as described. For titer determination, antiserum was diluted in 5% bovine serum albumin in 0.05 M Tris-HCl, pH 7.2, containing 0.85% NaCl (6). Antisera to CCSV and BYSMV were prepared as described previously (4; B. E. L. Lockhart, M. El Maataoui, T. W. Carroll, and A. M. Lennon, *unpublished*). Antisera to CCMV-A and maize sterile stunt virus (MSSV) were provided by R. S. Greber. Antisera to isolates of maize mosaic virus (MMV) were provided by R. Lastra, R. E. Gingery, and J.-C. Autrey;

Accepted for publication 4 April 1986 (submitted for electronic processing).

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.

CCMV-A antigens were provided by R. S. Greber. Double-antibody sandwich enzyme immune assays (DAS-EIA) using alkaline phosphatase-IgG conjugate were done using standard procedure (1). Polystyrene plates were coated with 1 µg/ml purified CCMV-M IgG. Leaf samples were extracted and diluted in phosphate-buffered saline containing 0.05% (v/v) Tween 20 and 2% polyvinylpyrrolidone (PBST-PVP). Enzyme conjugate was used at a 1/1,000 or 1/2,000 dilution. Absorbances were determined spectrophotometrically at 405 nm with a Dynatech Minireader.

Electron microscopy. Leaf-dip and partially purified preparations were negatively stained with 2% sodium phosphotungstate, pH 7.0 (PTA), or 2% ammonium molybdate, pH 6.8 (AM), or positively stained with saturated uranyl formate (UF). Preparations were stained either unfixed or after fixation in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.0. In IEM tests, carbon-coated grids were coated for 15 min at 37 C with antiserum diluted 1:1,000 in 0.05 M phosphate, pH 7.0. After rinsing in the same buffer, grids were incubated at 4 C on droplets of extracts from CCMV-M-infected oat leaf tissue, rinsed with buffer, and negatively stained with PTA for electron microscopy examination.

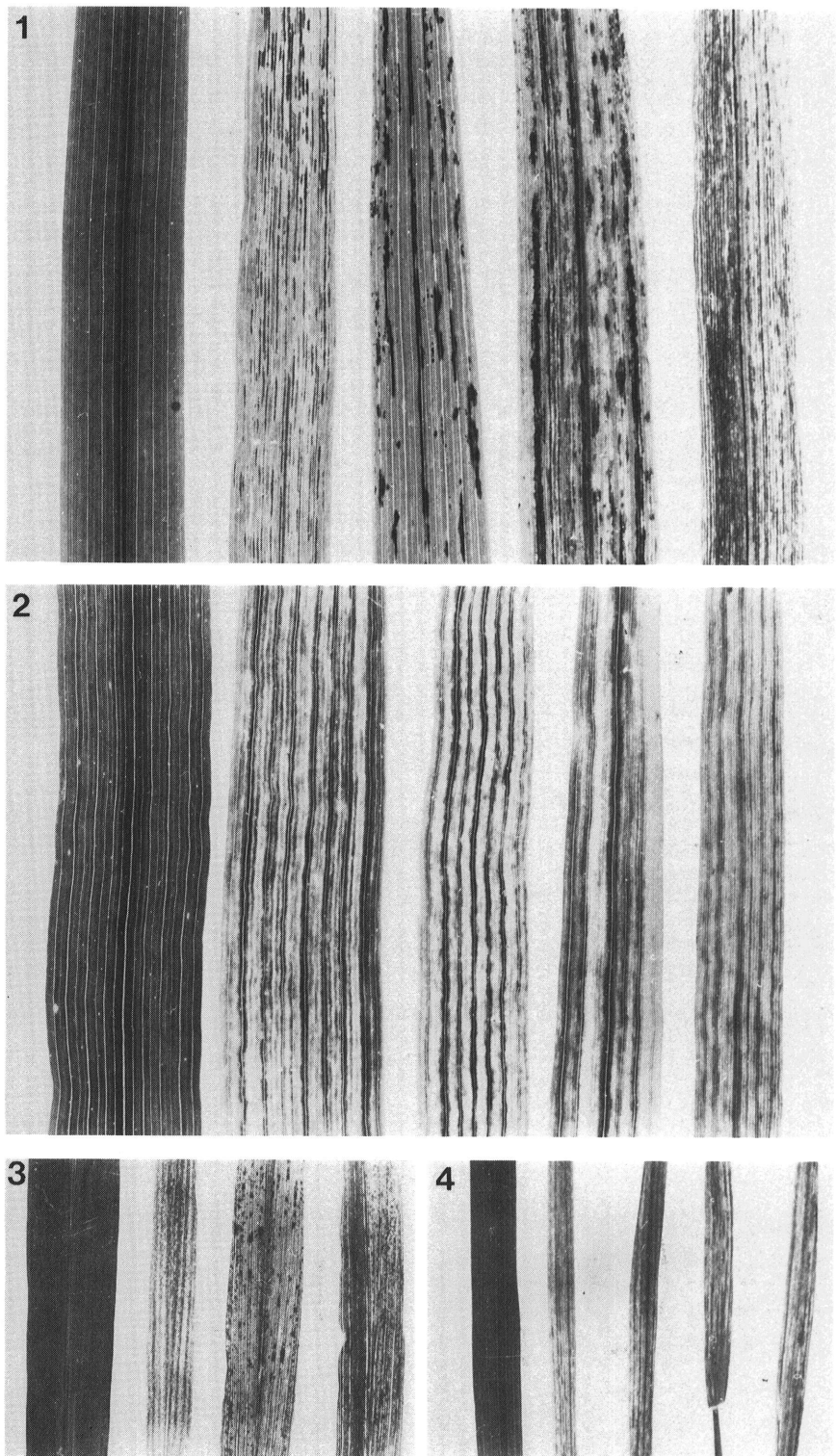
RESULTS

Field symptoms and natural occurrence. CCMV-M, which was initially found in oats, was later found to occur naturally in *S. verticillata* and *A. semiverticillata* volunteer barley and *Phalaris* sp. in several locations in southern Morocco. Symptoms in naturally infected oats and barley consisted of chlorotic and necrotic streaks of varying widths. Portions of some infected leaves became totally necrotic. Symptoms in field-infected plants were identical to those produced in greenhouse-grown plants inoculated using *C. bipunctata* subsp. *bipunctella* (Figs. 1 and 2). In oats, red necrotic stripes and streaks along leaves, laminae, and leaf sheaths sometimes closely resembled those caused by barley yellow dwarf virus (BYDV) infection, and it was at times difficult to distinguish between CCMV and BYDV symptoms in oats on the basis of casual visual observation. Necrotic streak symptoms were not observed on either naturally or experimentally infected *S. verticillata* or *A. semiverticillata*. In these hosts, symptoms consisted of broken chlorotic lines and chlorotic mottle (Figs. 3 and 4). Similar symptoms also occurred in naturally infected *Phalaris* sp. CCMV-M has so far not been found to occur naturally in wheat in Morocco.

Insect transmission, host range, and symptoms on test plants. CCMV-M was transmitted by *Cicadulina bipunctata* subsp. *bipunctella* from infected oats, *S. verticillata*, and *A. semiverticillata* to

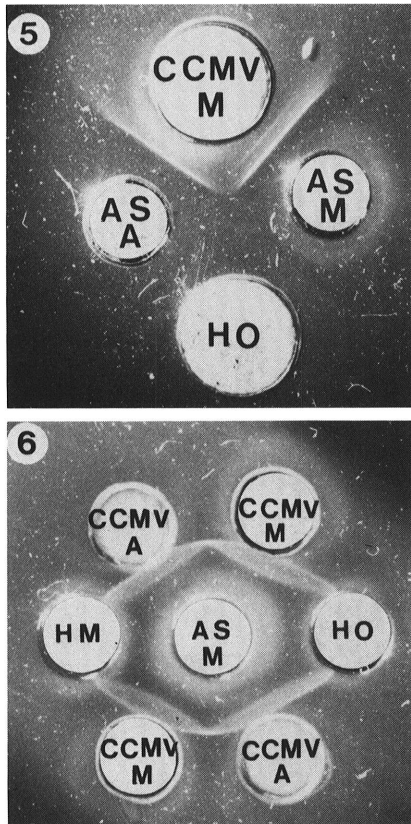
healthy oats, *S. verticillata*, *A. semiverticillata*, durum wheat (*Triticum durum* L.), and barley (*Hordeum vulgare* L. cvs. Capri and NK 38). The virus was not transmitted to sorghum (*Sorghum bicolor* L.), bermudagrass (*Cynodon dactylon* L.), sugarcane (*Saccharum*

officinatum L. cv. CP 44-101), or to any of the maize (*Zea mays* L.) cvs. LG 55, LG 11, Earliking, Jubilee, or NK 199. No symptoms appeared in any of these plants and CCMV-M was not detected in them by EIA. Symptomless infection, verified by EIA and electron microscopy,



Figs. 1-4. Symptoms of cereal chlorotic mottle virus, Moroccan isolate (CCMV-M), infection in naturally and experimentally infected host plants. (1) Symptoms of CCMV-M infection in Clintland 64 oats infected by insect transmission from *Setaria verticillata*. Healthy leaf on left. (2) Symptoms of CCMV-M infection in NK 38 barley resulting from insect transmission from *Agrostis semiverticillata*. Healthy leaf on left. (3) Symptoms of natural CCMV-M infection in *S. verticillata*. Healthy leaf on left. (4) Symptoms of natural CCMV-M infection in *A. semiverticillata*. Healthy leaf on left.

occurred in *Oryzopsis miliacea* (L.) Asch. & Schw. Symptoms on experimentally infected oats (cultivar Clintland 64) (Fig. 1), barley (cultivar Capri), *S. verticillata*, and *A. semiverticillata* were identical to those observed in naturally infected plants. Symptoms in naturally and experimentally infected barley (Fig. 2)



Figs. 5 and 6. Homologous and heterologous immunodiffusion reactions between Moroccan (CCMV-M) and Australian (CCMV-A) isolates of cereal chlorotic mottle virus (CCMV) antigens and antisera. (8) Reaction of CCMV-M antigen (undiluted infected oat sap) with CCMV-M and CCMV-A antisera. HO = undiluted healthy oat sap. (9) Reaction of CCMV-M (undiluted infected oat sap) and CCMV-A (infected maize sap) antigens with CCMV-M antiserum. HO = healthy oat sap; HM = healthy maize sap.

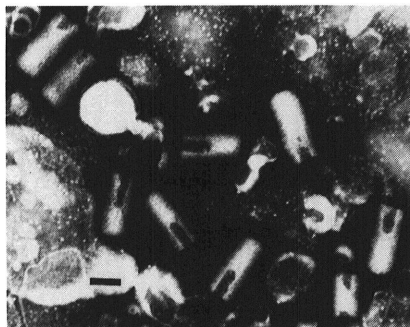


Fig. 7. Appearance of cereal chlorotic mottle virus, Moroccan isolate, particles in a negatively stained leaf-dip preparation from infected oats. Preparation was negatively stained with 2% sodium phosphotungstate, pH 7.0. Scale bar = 100 nm.

consisted of necrotic and chlorotic lines and stripes, chlorotic mottle, and reddish necrosis. Symptoms in experimentally infected durum wheat were similar to those produced in barley. *Phalaris* sp. was not used in transmission experiments.

CCMV-M was not transmitted by *A. prolixa*, *A. laevis*, *P. striatus*, *E. nanms*, *T. propinqua*, or *L. striatellus*.

Partial purification. Partially purified CCMV-M preparations contained bullet-shaped and some bacilliform particles, but such preparations were heavily contaminated by host cellular material. Partially purified preparations reacted in immunodiffusion tests with antisera to both CCMV-M and CCMV-A.

Serology. Antiserum prepared against partially purified CCMV-M had a specific reciprocal titer of 128 in immunodiffusion tests using undiluted, untreated infected oat leaf sap as antigen. One or two precipitin lines were formed. The number and intensity of such precipitin lines has been shown to be dependent on relative antigen-antibody concentrations (3,4). CCMV-M and CCMV-A cross-reacted in both heterologous combinations without evidence of spur formation (Figs. 5 and 6). No reaction was obtained in immunodiffusion tests between CCMV-M and antisera to CCSV, BYSMV, MSSV, or MMV.

In EIA, CCMV infection was readily detected in extracts of infected plants. Infected Capri barley extracts diluted

12,800-fold, 51,200-fold, and 102,400-fold gave A_{405nm} values of 0.73, 0.45, and 0.18 respectively, compared with healthy values of 0.08, 0.07, and 0.06, respectively. In EIA, CCMV-M did not react with CCSV or BYSMV IgG.

Electron microscopy. Unfixed particles of CCMV-M were stable in PTA, AM, and UF, but glutaraldehyde fixation improved particle contrast. Virus particles were numerous in leaf-dip preparations from oats (Fig. 7), barley, *S. verticillata*, and *A. semiverticillata*. Negatively stained particles showed the netlike surface appearance (Fig. 8), surface projections, and 4.5-nm helix spacing (Fig. 9) similar to those described for CCMV-A (2).

In initial IEM tests, CCMV-M particles were not trapped by CCSV or BYSMV antisera. Trapping experiments using homologous CCMV-M antiserum were not done.

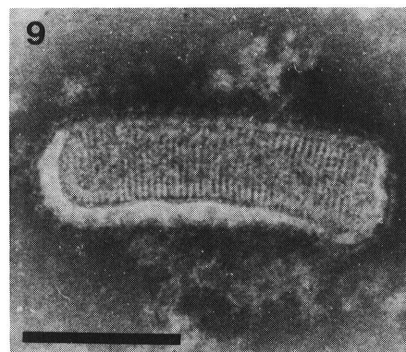
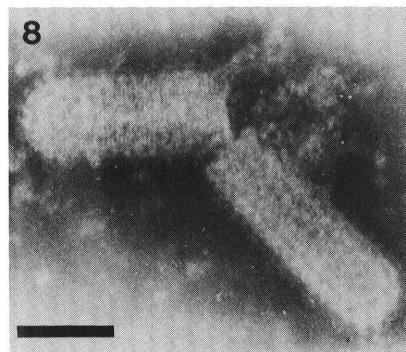
DISCUSSION

The rhabdovirus associated with the necrotic streak disease of oats in Morocco was demonstrated to be the causal agent of the disease and was identified as an isolate of CCMV either identical or closely related to CCMV-A. Oats was not reported as a natural host of CCMV-A (2). The major difference in the descriptions of CCMV-M and CCMV-A was the failure of CCMV-M to infect any of the field or sweet corn cultivars tested. Many maize cultivars are highly resistant to CCMV-A (2,3), and it is possible that a similar differential susceptibility to CCMV-M exists among maize cultivars. Inbred maize lines such as H-84, which are highly susceptible to CCMV-A (2,3), were not available for testing with CCMV-M.

Although *Cynodon dactylon*, the major grass weed in Morocco, is a host on which *C. bipunctata* subsp. *bipunctella* breeds prolifically, this plant was not a host of CCMV-M. The virus infected *Oryzopsis miliacea* (another drought-tolerant perennial species on which the vector also breeds), which may provide, in addition to *S. verticillata*, *A. semiverticillata*, and *Phalaris* sp., a reservoir of both virus and vector for infection of cultivated cereals. CCMV-M isolates from *S. verticillata* and *A. semiverticillata* did not differ in biological or serological behavior from the oat isolate. It will be of interest to determine the distribution of CCMV in the Mediterranean basin outside of Morocco.

ACKNOWLEDGMENTS

I wish to thank the Virology Section, Scottish Crop Research Institute, Invergowrie, Dundee, for use of electron microscope facilities and I. M. Roberts and Angela M. Lennon of SCRI for helpful discussion. I also thank R. S. Greber, Department of Primary Industries, Indooroopilly, Queensland, Australia, for gifts of CCMV-A antigens and antiserum. Leafhopper and planthopper species used in this study were identified by M. S. K. Ghauri and M. R.



Figs. 8 and 9. Appearance of cereal chlorotic mottle virus, Moroccan isolate, particles in negatively stained leaf-dip preparations from infected oats, showing surface projections, surface pattern, and 4.5-nm helix cross-striations. Both preparations were negatively stained with 2% sodium phosphotungstate pH 7.0. Scale bars = 100 nm.

Wilson of the Commonwealth Institute of Entomology, London, England. This research was supported by USAID/University of Minnesota Contract 608-0160-001.

LITERATURE CITED

1. Clark, M. F., and Adams, A. N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *J. Gen. Virol.* 34:475-483.
2. Greber, R. S. 1979. Cereal chlorotic mottle virus—a rhabdovirus of Graminae in Australia transmitted by *Nesoclutha pallida* (Evans). *Aust. J. Agric. Res.* 30:433-443.
3. Greber, R. S. 1982. Cereal chlorotic mottle virus. No. 251. Descriptions of Plant Viruses. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England. 4 pp.
4. Lockhart, B. E. L., Khaless, N., El Maataoui, M., and Lastra, R. 1985. *Cynodon* chlorotic streak virus, a previously undescribed plant rhabdovirus occurring in the Mediterranean area. *Phytopathology* 75:1094-1098.
5. McLean, G. D., and Francki, R. I. B. 1967. Purification of lettuce necrotic yellow virus by column chromatography on calcium phosphate gel. *Virology* 31:585-591.
6. 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. Tech. Bull.* 788. 39 pp.