Host Range and Seed-Transmission Studies of Maize Chlorotic Mottle Virus in Grasses and Corn

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ABSTRACT

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Nineteen and 15 grass species were systemic hosts for Kansas and Peru serotypes, respectively, of maize chlorotic mottle virus. Host responses between serotypes were similar, with differences primarily in latent hosts. Maize chlorotic mottle virus was not found in 230 grass samples (representing 14 species) collected near fields of infected corn, suggesting that the virus does not overwinter in grassy weeds. However, maize dwarf mosaic virus strain B and wheat streak mosaic virus were found in those grasses and corn bait plants. Maize chlorotic mottle virus was not seed transmitted in 14 inbred corn lines, five corn hybrids, *Panicum miliaceum*, *Setaria lutescens*, or *S. viridis*.

Additional key words: bait plant containers, brome mosaic virus, weed hosts

Corn lethal necrosis disease, first reported in Kansas in 1976 (9), has since appeared in severe outbreaks in north central Kansas (11) and south central Nebraska (5). The disease is caused by a combination of maize chlorotic mottle virus (MCMV) and either maize dwarf mosaic virus (MDMV) or wheat streak mosaic virus (WSMV). Yield losses in corn (*Zea mays* L.) have been estimated at 50% or more, based on artificial (12) and natural (9,11) incidences of corn lethal necrosis disease in the field.

MCMV was first described infecting corn in Peru (MCMV-P) in 1973 (3,7) and in the United States (Kansas 1 serotype, MCMV-K) in 1976 (9,11). Six chrysomelid beetle species were reported as vectors; five of these occur in Kansas (8). Eight grass hosts were reported for MCMV-P, and 50 others were nonhosts (3,7). In Kansas, hosts for MCMV-K and new hosts for MCMV-P were reported recently (2).

We conducted host range studies on many Kansas grass species in the greenhouse to identify potential reservoirs for MCMV and to compare host ranges of MCMV-K and MCMV-P. To identify potential overwintering hosts for MCMV, we assayed native grasses from collections near fields of MCMV-infected corn during several growing seasons. We also placed corn bait plants near the fields to monitor airborne insect transmittance of MCMV. Results of our efforts are reported herein.

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MATERIALS AND METHODS

Assay and inoculation. Crude buffer extracts of MCMV-infected plant tissues were rubbed onto leaves of test plants dusted with 600-mesh Carborundum and rinsed immediately with water. Crude virus extracts used in double immunodiffusion (DID) assays, bioassays, or for general inoculation were prepared by grinding infected tissue in 0.05 M potassium phosphate buffer, pH 7.0, with a mortar and pestle or a mechanical leaf squeezer (6).

Seroassays were used to identify MCMV and brome mosaic virus (BMV). Antisera of MCMV and BMV were gifts from C. L. Niblett. Plates contained 0.75% Ionagar, 0.85% sodium chloride, and 0.02% sodium azide in 0.01 M tris-hydrogen chloride, pH 7.2. Enzyme-linked immunosorbent assay (4) was also used to identify MCMV (10), WSMV (13), and strains A and B of MDMV (Uyemoto, *unpublished data*).

Bioassay plants were N28Ht (*Z. mays*), sorghum (*Sorghum bicolor* (L.) Moench 'Asgrow Bug-off' and 'DeKalb E59+'), and wheat (*Triticum aestivum* L.) (11). Host responses to virus infections, except BMV, were as described previously (11). BMV incited mosaic in all indicator plants, and killed corn.

Host range. MCMV serotypes Kansas 1 and Peru were used (9,11); MCMV-P was provided by C. L. Niblett (9). Grass seeds were obtained from the Department of Agronomy, Kansas State University, Manhattan; the U.S. Department of Agriculture, Soil Conservation Service, Plant Materials Center, Manhattan; and from a few personal collections.

Cultures of MCMV were maintained on N28Ht corn, and 7- to 14-day-old infected tissues were used as inocula sources. Grass seedlings were planted in 10-cm-diameter plastic pots, and at least 10 plants were usually tested. All experiments were conducted in the greenhouses, with temperatures from 25 to 35 C. Precautions were taken to contain the virus cultures; all soils and pots were autoclaved or washed, respectively. Inoculated and newly formed leaves of test grasses were assayed separately for MCMV by DID and bioassay on N28Ht corn. Virus-positive corn indicator plants were reassayed by MCMV-DID.

Distribution in native grasses. During the 1977, 1978, and 1979 cropping seasons, grass samples were collected from or near fields of corn infected with MCMV. Samples were maintained on ice and assayed soon after collection on a differential host range or with MCMV-DID serologic tests. Serologic assays for MCMV were conducted only with symptomatic grass tissues and corn indicator plants. Some grass collections were readily identified; others were transplanted and grown to maturity. Identifications were made using Barkley's key (1) and by personnel in the Kansas State University Herbarium.

Seed transmission. Seeds from MCMV-infected plants of 14 inbred corn lines, five corn hybrids (all corn lines inoculated at seven-leaf stage in a field plot), and three grass species (*Panicum miliaceum* L., *Setaria lutescens* (Weigel) Hubbard, and *S. viridis* (L.) Beauv.) from the host range study were germinated in the greenhouse; seedlings were assayed in a composite of 15 leaves per extract after 28–50 days of growth. Leaf tissues were extracted in 0.05 M phosphate buffer, pH 7.0 (1:1, w/v), and rubbed onto N28Ht corn. One group of 300 *P. miliaceum* seedlings was assayed in groups of 20.

Bait plants. In 1978 and 1979, self-maintained, potted corn plants were used around or in fields of MCMV-infected

corn. Containers were constructed with two polyfoam strips (10 cm wide × 2.5 cm deep × 1.3 m long) that were wrapped snugly around a clay pot (13 cm diam) and secured with binding tape. Pots were immersed in water, and the foam layer was repeatedly compressed to replace air spaces with water. Water-saturated pots were placed in a plastic bag with the edge of the bag tucked inside and slightly below the soil surface. N28Ht seedlings (7–10 days old) were transplanted into the soil, and pots were placed in pot coasters and distributed in the fields. These self-contained units permitted seedling growth and required minimal maintenance in the field for about 2 wk. A 0.95-cm-mesh, galvanized wire cage was placed over the seedlings to prevent rodent damage.

Pots were distributed and collected at about 2-wk intervals. Recovered pots were isolated in the greenhouse and disinfested with malathion. Plants were examined for virus symptoms and discarded after 3 wk if free from infection. Individual plants with virus symptoms were assayed for MCMV by both DID and bioassay.

RESULTS

Host range. Forty-four and 38 species representing 27 and 22 genera were tested as hosts for MCMV-K and MCMV-P, respectively. All hosts with systemic symptoms were the same for both virus serotypes; usually 90–100% of the plants showed systemic symptoms. MCMV was recovered in extracts of inoculated leaves of all grass species tested except *Agropyron repens* (L.) Beauv., *Elymus virginicus* L., *Festuca arundinacea* Schreb., and *Poa pratensis* L., which were immune to both serotypes, and *Cynodon dactylon* (L.) Pers., which was immune to MCMV-P but was infected locally with MCMV-K. Based on host susceptibility, these native grasses could be divided into four groups: hosts with systemic symptoms, hosts with latent infections, hosts with infection only of inoculated leaves, and hosts that were immune.

Hosts (single accessions in each) with systemic symptoms for both MCMV serotypes were *Andropogon scoparius* Michx., *Bromus japonicus* Thunb., *B. secalinus* L., *Digitaria sanguinalis* (L.) Scop., *Eragrostis trichodes* (Nutt.) Nash., *Hordeum pusillum* Nutt., *H. vulgare* L. ('Reno'), *Panicum miliaceum*, *Setaria faberi* Herm., *S. italica* (L.) Beauv., *S. lutescens*, *S. viridis*, and *T. aestivum* ('Parker' and a cultivar of durum and of soft white wheat). *H. vulgare* and *T. aestivum* exhibited systemic symptoms only occasionally under our test conditions.

Hosts that were systemically infected but symptomless for both virus serotypes were *Bromus tectorum* L. and *Sorghum bicolor* ('Redland' and 'Orange Sorgo'). Hegari (*S. bicolor*), not tested with MCMV-P, was susceptible to MCMV-K. *Bouteloua gracilis* (H.B.K.) Lag. ex. Steud., *Calamovilfa longifolia* (Hook.) Hack., *Panicum dichotomiflorum* Michx., and *Spartina pectinata* Link. were susceptible to MCMV-K, but only locally infected with MCMV-P.

Grasses with infection of inoculated leaves only for both virus serotypes were *Agropyron desertorum* (Fisch.) Schult., *A. smithii* Rydb., *Andropogon gerardi* Vitman., *Avena sativa* L. ('Wintok'), *Bouteloua curtipendula* (Michx.) Torr., *Cenchrus longispinus* (Hack.) Fern., *Echinochloa colonum* (L.) Link., *E. crus-galli* (L.) Beauv., *E. frumentacea* (Roxb.) Link., *Eleusine indica* (L.) Gaertn., *Hordeum jubatum* L., *Panicum virgatum* L., *Secale cereale* L. ('Balboa'), and *Sorghum halepense* (L.) Pers.. Grasses locally infected with MCMV-K but not tested with MCMV-P were *Avena sativa* ('Andrew,' 'Carolee,' 'Mustang,' and 'Tech'), *Bromus inermis* Leyss., *Buchloe dactyloides* (Nutt.) Engelm., *Dactylis glomerata* L., *H. vulgare* ('Clayton' and 'Wong'), *Phalaris arundinacea* L., *Sorghastrum nutans* (L.) Nash., *Sorghum bicolor* ('DeKalb BR54,' 'Sumac Sorgo,' and wild cane), *Tripsacum dactyloides* L., and *Triticum aestivum* ('Anderson,' 'Atlas,' and 'Blueboy').

Distribution in native grasses. Most samples were collected based on a mottle or mosaic in the leaves; however, some healthy-appearing collections were assayed for possible latent infections, especially *Bromus tectorum* and a few other grasses. These samples were often bioassayed in groups of 10 plants.

MCMV was not detected in 230 grass samples collected from the field in 1977 (32 collections), 1978 (16 collections), or 1979 (182 collections). However, 5, 35, and 64 samples were infected with BMV, WSMV, and MDMV-B, respectively. MDMV-A was not detected. Both WSMV and MDMV-B were isolated from *Bromus tectorum*, *Digitaria sanguinalis*, *Echinochloa crus-galli*, *Setaria viridis*, and *S. lutescens*. *Panicum dichotomiflorum* was infected with MDMV-B, and *Bromus inermis* was infected by BMV. One *B. tectorum* and two *S. viridis* samples were doubly infected by WSMV and MDMV-B. Grass samples with no detectable viruses included two collections of *B. japonicus*, two of *Cenchrus longispinus*, one of *Agropyron smithii*, two of *Elymus virginicus*, one of *Muhlenbergia bushii* Pohl, three of *H. jubatum*, and six of *Z. mays* (volunteer corn).

Seed transmission. Kernels were collected from corn inbreds A634Ht, W135R, H95, A632Ht, W117Ht, W64Ht, B73Ht, C123Ht, B14AHt, Oh43Ht, A635Ht, H49, C103, and N28Ht, and from corn hybrids Mo17 × N28, A619Ht × A632Ht, B73 × Mo16Ht, Pioneer 3183, and Prairie Valley 37S. Assays of corn seedlings (2,153 seedlings of inbreds and 1,898 of hybrids) and other grass species (842, *Panicum miliaceum*; 135, *Setaria lutescens*; and 2,676, *S. viridis*) were negative for MCMV.

Bait plants. In 1978, a high percentage of WSMV transmission to bait plants occurred during 14–26 June. Bait plants were not distributed after June 26, when MCMV-infected corn plants were detected in growers' fields.

In 1979, WSMV was transmitted to bait plants from 1 June to 20 July. A few bait plants were infected with MDMV-B throughout the growing season. Plants in one bait pot exposed for 2 wk beginning on 29 June were doubly infected by MDMV-B and MCMV. Also, an infection by MCMV occurred when bait plants were placed for 2 wk (distributed on 20 July) within a field of diseased corn. These were confirmed by MCMV-DID. In addition, enzyme-linked immunosorbent assay confirmed that six bait plants were infected by MDMV-B and 11 by WSMV.

DISCUSSION

Nineteen and 15 grass species are reported here as systemic hosts for MCMV-K and MCMV-P, respectively. *Setaria lutescens*, *Sorghum bicolor*, and *Triticum aestivum* were previously reported as hosts for MCMV-P (3,7), as were *Panicum virgatum* and *Sorghum halepense*; the latter two were not systemically infected with either virus serotype in our tests. Overall, host range responses to MCMV-K and MCMV-P inoculations were similar, with minor differences among latent hosts.

Extensive surveys for MCMV-infected grasses adjacent to or in creeks near fields of MCMV-infected corn were negative, which suggests that MCMV does not overwinter in grass hosts. Of the grasses common to all sampling sites, only *Bromus japonicus* or *B. tectorum*, both winter annuals, could serve as overwintering hosts, yet MCMV was not isolated from field-collected samples of either. All other grasses observed were either summer annuals, nonhosts, or not common to all diseased areas. We were unable to demonstrate seed transmission of MCMV with seed from infected corn inbreds or hybrids or three weed species.

The negative MCMV corn-seed transmission tests, absence of MCMV in winter annual grasses, lack of MCMV-insect transmission to bait plants early in the growing season, and persistence of MCMV in the same fields from year to year (11) suggest that MCMV overwinters in infected corn residue. We have recently recovered MCMV in corn residue collected in mid-April (Uyemoto, *unpublished data*).

The most likely source of WSMV infection in bait plants and annual grasses was maturing, cultivated wheat during June and July when wheat curl mites would have been migrating to other

hosts. Bait plants also detected early transmittance (15 May–14 June) of MDMV-B and infections in annual grasses in late July. Source of MDMV-B in the early season may be *B. tectorum*, whereas the midseason virus source may be corn. We are continuing our investigations on this weed host as a possible overseasoning host of MDMV-B.

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