Maize Chlorotic Mottle Virus and Crop Rotation: Effect of Sorghum on Virus Incidence

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ABSTRACT

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Subplots planted to a corn-corn sequence (1979 and 1980) contained 1.6% infection of maize chlorotic mottle virus in the surveys of 28 June–2 July 1980. The virus was not detected in the sorghum-corn sequence subplots in this period. Second and third surveys on 8 and 21 July revealed maize chlorotic mottle virus incidence of 4.7% in corn-corn and 0.2% in sorghum-corn subplots. On 18 August, virus incidence was 12.2% in corn-corn and 0.6% in sorghum-corn plots. Corn yields on sorghum-corn subplots were significantly higher ($P = 0.05$), but the increased yields could not be ascribed wholly to reduced incidence of the virus. In 1979, soil fumigation trials did not reduce maize chlorotic mottle virus infections, and yields of two corn hybrids were similar.

Additional key words: maize dwarf mosaic viruses A and B, nematodes, wheat streak mosaic virus

Maize chlorotic mottle virus (MCMV) was first described in Peru in 1973 (3,10). In 1976, Niblett and Claflin detected it in Norton County, KS (14); it has since been found in Cloud, Osborne, Phillips, Smith, and Republic counties of north central Kansas (19) and in south central Nebraska (6).

In Kansas, MCMV was found in field corn (*Zea mays* L.) along with wheat streak mosaic virus (WSMV) or maize dwarf virus (MDMV), a combination of viruses interacting synergistically to cause corn lethal necrosis disease (CLND) (14,20).

Nault et al (13) identified six beetle species of the family Chrysomelidae as vectors of MCMV. Both adults and larvae of the cereal leaf beetle (*Oulema melanopa* (L.)) (11) and the southern (*Diabrotica undecimpunctata* Mannerheim) (12), western (*D. virgifera* LeConte), and northern (*D. lonicornis* (Say)) corn rootworm beetles (S. G. Jensen, *personal communication*) transmit MCMV. Of the potential vectors, the western corn rootworm and flea beetles (*Systena frontalis* (Fab.)) are economic pests in Kansas.

MCMV has been detected in field corn before adult western corn rootworm beetles have emerged. Also, even though flea bettles and southern corn rootworm beetles overwinter as adults (12) and feeding injury on newly emerged corn plants was readily evident, MCMV was not found in those corn plants (Uyemoto, *unpublished data*). In addition, grassy weeds sampled in April–June near affected cornfields were free of MCMV (2), which suggests that they do not serve as a virus reservoir.

During the past three seasons, potted corn seedlings were placed around the perimeters of cornfields where CLND was known to be present and exposed for 1- to 2-wk intervals. Although potted plants remained free of MCMV (ie, while adult western corn rootworm beetles were absent), virus infections were usually found in field corn plants in late June. Based on the absence of a weed virus reservoir, lack of MCMV in potted bait plants, and MCMV infections in field-grown plants, we hypothesized that MCMV is initially soilborne (20).

It is now presumed that corn rootworm larvae are instrumental in transmitting MCMV to young corn plants and that virus is acquired from MCMV-infected corn residue (20). Recent evidence indicates that MCMV overwinters in infected corn residues (Uyemoto, *unpublished data*).

The study reported here was designed to determine the effects of soil fumigation and crop rotation on MCMV incidence.

MATERIALS AND METHODS

Field experiments were conducted during 1979 and 1980 growing seasons in Norton County at a site planted to continuous corn for 12 yr and containing MCMV-infected corn plants since 1979. The soil was a silt loam with a pH of 7.0. Average annual precipitation is 57.74 cm; corn is normally irrigated.

Plot design, soil fumigation, and cropping sequence. Except where experimental design required change, the plot area was managed by the grower cooperator. In late April or early May, anhydrous ammonia was applied at 202 kg/ha during seedbed preparation with a rotary tiller (FMC Sidewinder). At this time, atrazine (Ciba-Geigy) at 1.12 kg a.i./ha and Eradicane (EPTC, Stauffer Chemicals) at 1.26 kg a.i./ha were applied and immediately incorporated. No herbicides were applied in 1979. Thimet (phorate 15 g, American Cyanamid Corp.) was applied on 14 June

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1979 at 1.3 kg a.i./ha and on 5 and 15 May 1980, each at 2.7 kg a.i./ha. Corn was hilled in May and irrigated five times during each growing season. After harvest, cattle were allowed to graze the stubble.

A split-plot design with four replicates was used; each replicate was split into two ranges 15.24 m long × 13.71 m wide. On 14 May 1979, one range of each paired replicate was treated with 1,3-dichloropropene and chloropicrin (Telone C17, Dow Chemical Company) at 177.6 L/ha. Fumigant was applied with a shank applicator with shanks spaced at 0.3 m and to 0.15–0.2 m deep. Two weeks after fumigation, individual ranges were subdivided into three subplots, each with six rows (15.24 m long × 0.75 m apart). They were planted in randomized fashion with two cultivars of field corn—Pioneer 3194 (tolerant to CLND) and Pioneer 3183 (susceptible)—and a forage sorghum (*Sorghum bicolor* (L.) Moench. 'Early Sumac'). Under greenhouse conditions, Early Sumac is susceptible (systemic and symptomless) to MCMV. All seeds were hand-planted at 74,000/ha and later thinned to 61,750 plants per hectare.

In 1980, the entire plot site was planted to Pioneer 3183 corn, with eight replicates for each cropping sequence from which virus data were taken.

Tissue sampling procedures and virus assays. Whole plant samples were taken at weekly intervals from plant emergence until the nine-leaf growth stage; thereafter, only leaf collections were taken.

During 1979, five plants were randomly selected from each corn subplot. When entire corn plants were taken, tissues from the crown and roots were diced with a razor blade and ground in a mortar and pestle with 2–3 ml of 0.02 M potassium phosphate (KPO_4) buffer, pH 7.0. Leaf samples were extracted with a leaf squeezer unit with 3–5 ml of neutral 0.02 M KPO_4 buffer added to the roller bars (9). All extracts were seroassayed in double immunodiffusion (DID) plates (0.75% ionagar; 0.05 M tris-HCl, pH 7.5; 0.85% sodium chloride; and 0.02% sodium azide) against MCMV antiserum and bioassayed on wheat (*Triticum aestivum* L. 'Parker'), corn (N28Ht), and sorghum (Asgrow Bug-Off and DeKalb E59+) (19).

Extracts were hand-rubbed onto Carborundum-dusted plants (600 mesh), which were then incubated at least 2 wk in the greenhouse before being read for symptoms. All of the viruses cause a prominent mosaic on corn. MDMV strain A produces mosaic symptoms in both sorghum varieties, and B induces severe red leaf symptoms on Bug-Off sorghum. WSMV and (occasionally) MCMV cause mosaic symptoms in wheat (19).

In 1980, random sampling and processing of entire plants (40 plants per cropping sequence per week) were done until plants were at the seven- to nine-leaf stage when virus infections were evident. Leaves were collected only from plants showing mosaic symptoms. When MCMV was detected, sampling of all symptomatic plants was confined to the inner four rows and inner 12.19-m row lengths of each subplot. Leaves were extracted with the leaf squeezer and a minimal volume of tissue extraction buffer (0.01 M KPO_4, pH 7.0; 0.85% sodium chloride, 0.05% Tween-20, and 2% polyvinylpyrrolidone, mol wt 44,000).

Extracts were assayed in enzyme-linked immunosorbent assay (ELISA) (4,18). Each sample was tested against MCMV-K (18), MDMV-A, and MDMV-B antisera. On two sampling dates, extracts were also tested against WSMV antiserum (22). ELISA results were read visually or in a Hitachi Perkin-Elmer double-beam spectrophotometer (405 nm). All extracts were also tested in MCMV-DID, and several samples were bioassayed.

Final incidence of MCMV was determined during the last 2 wk of August 1979 and 1980. In 1979, 10 consecutively positioned corn plants were sampled per subplot; 60 sorghum samples were taken in a similar manner. In 1980, all symptomatic plants were collected and processed in the usual way.

Soil sampling procedures, nematode extractions, and insect egg isolations. During each growing season, six soil cores (35 cc/core) were taken from each subplot with a kick sampler and composited. Each soil sample (100 cc) was processed by the direct-centrifugal flotation method (5). Western corn rootworm eggs were trapped on a 150-μm mesh screen and nematodes on a 37-μm mesh screen. Insect eggs were poured into a specimen dish for viewing. Fractions containing nematodes were diluted to 20 ml, and 1 ml was placed on a Hawksley glass slide and examined for *Helicotylenchus, Paratylenchus, Pratylenchus, Tylenchorhynchus,* and *Xiphinema* spp.

In 1979, soil samples were taken before fumigation, at planting (2 wk after fumigation), and at weekly intervals until mid-June. June samplings were screened only for corn rootworm eggs to ascertain approximate hatching period. About 106 days after fumigation, soil and root samples were taken from each subplot for nematode analyses. Four randomly selected roots were removed and each divided into four pieces. One quarter section of each root, with a portion of the adhering soil, was composited and represented a single sample per subplot. Soil was processed for nematode extraction. To extract endoparasitic *Paratylenchus*, roots were washed, the finer roots were cut into small pieces, and 10 g was placed on a screen inside a funnel. Funnels were kept in a continuous mist chamber for 14 days. Water mist, containing nematodes, accumulated in the bottom of a clamped funnel and was collected four times. Following mist extraction, roots were dried in a forced-air dryer for 1 wk, then weighed. Nematodes were expressed as numbers per gram of dry root.

In 1980, soil samples were taken at planting time and at midseason.

Corn harvest. Ears were collected in September from the inner 7.62 m of two center rows of each subplot. In 1979, all eight ranges were harvested; in 1980, only four ranges were included. Yields were adjusted to 15.5% moisture and expressed in kilograms per hectare.

Statistical analyses. Except for yield, all data were analyzed by nonparametric tests because numbers of nematodes and of virus-infected plants were not distributed normally and did not have equal variances. Results of the fumigation trials were analyzed by the signed rank test for paired samples (16) to compare differences between fumigated and unfumigated subplots. The 1980 data were analyzed using Friedman's test (8). All yield data were analyzed by analysis of variance and Duncan's multiple range test (16) because equal variances were present.

RESULTS

Soil fumigation study, 1979. MCMV was first detected on 10 July in six plant samples, each from different corn subplots; four of the positives were from fumigated subplots. On 20 July, four samples were positive for MCMV; three were from unfumigated subplots. On 30 July, 11 MCMV infections each were found in fumigated and unfumigated subplots. At the end of August, MCMV incidence was 12.5% (fumigated) and 22.5% (unfumigated). MCMV was not found in sorghum.

Incidence of MDMV ranged up to 20% in the weekly collections; MDMV strain B was found in 75% of the MDMV-positive samples. WSMV rarely occurred in the subplots (less than 1%).

Before fumigation, untreated subplots contained an average of 0.4 rootworm eggs per 100 cc of soil; subplots to be fumigated contained 0.1 eggs per 100 cc of soil. At planting, untreated and treated subplots contained 0.3 and 0.0 eggs per 100 cc of soil, respectively. After 18 June, fewer whole eggs and more egg fragments were found, presumably because of hatching. Adult beetles were observed on 10 July but not on 28 June.

Populations of *Tylenchorhynchus* in prefumigation soil samples and *Pratylenchus* in 106 day root samples were significantly different ($P = 0.05$). For example, *Tylenchorhynchus* numbers at prefumigation were 66/100 cc of soil on fumigant-designated plots and 32 on unfumigated plots. At 106 days, roots from unfumigated plots contained significantly more *Pratylenchus* (1,176) than those from fumigated plots (731).

None of the other soil collection times or nematode species showed statistical differences ($P = 0.05$).

There were no significant differences ($P = 0.05$) in corn yields among treatments. The sorghum plants were not harvested.

Rotation study, 1980. MCMV incidence and corn yields in continuous corn-corn sequences of Pioneer 3183 or 3194 in 1979 and all Pioneer 3183 in 1980 did not differ significantly ($P = 0.05$). Hence, results stated hereafter were from a single eight-replicate corn-corn sequence. Data for the 1979 sorghum and 1980 corn (Pioneer 3183) subplots were designated under sorghum-corn subplots.

MCMV was not detected in plants assayed up to the eight-leaf stage. After the first MCMV infection was detected on 25 June, all symptomatic plants were sampled on 28 June and 2 July; sampled plants were marked with paint, and those collected on 2 July had not been sampled on 28 June. On 8 and 21 July, two additional complete samplings were done.

On 25 June, one sample from corn-corn subplots was positive for MCMV. For combined samplings from 28 June and 2 July, average MCMV incidence was 1.6% in corn-corn and 0% in sorghum-corn subplots. On 8 and 21 July, MCMV incidence was 4.7% in corn-corn subplots; in sorghum-corn subplots, the incidence was 0.2%. On 18 August, MCMV incidence was 12.2% (corn-corn) and 0.6% (sorghum-corn). On all dates, incidence of MCMV in sorghum-corn subplots was significantly lower ($P = 0.05$) than in corn-corn subplots.

In tissue extracts of field material, MCMV-DID tests confirmed 87% of the MCMV-ELISA positives, and MCMV-ELISA detected 93% of bioassay positives. Average ELISA readings were 0.8 and 0.18 for MCMV-infected and healthy extracts, respectively.

MDMV-B was first detected on 5 June. On 8 July, a total of 190 samples was positive for MDMV; 59 and 41% were identified as strains B and A, respectively, and 91 samples were doubly infected with both strains. No statistical differences ($P = 0.05$) in MDMV incidence were found in either corn-corn or sorghum-corn subplots. At the final sampling (18 August), 303 plants were positive for MDMV, and infected plants were distributed evenly throughout the plots. Average ELISA readings for samples positive for MDMV strains A and B were 0.67 and 0.80, respectively; averages for healthy samples on those respective plates were 0.12 and 0.18. In field-collected tissues, ELISA confirmed 83% (MDMV-A) and 82% (MDMV-B) of MDMV infections detected by bioassays.

WSMV was distributed evenly in the various plots, with an incidence of less than 1%.

Nematode populations did not differ statistically between cropping sequences ($P = 0.05$). Number of rootworm eggs averaged 0.5 (corn-corn) and 1.3/100 cc (sorghum-corn subplots). Adult western corn rootworm beetles were observed on 2 July but not on 28 June.

Average corn yields of 7,432 kg/ha from corn-corn subplots and 10,356 kg/ha from sorghum-corn subplots were significantly different ($P = 0.05$).

DISCUSSION

Results of the 1979 fumigation study were inconclusive. Incidence of MCMV and distribution and populations of nematodes and insect eggs, irrespective of treatment, were essentially similar. Our failure to control MCMV with fumigation may stem from ineffective fumigation, or the delay of about 3 wk in planting and seedling emergence may have offset the "necessary" biological sequence of events required for early-season virus transmissions (ie, in June).

In the early portion of the 1980 growing season, MCMV was found (1.6%) in corn-corn subplots but not in sorghum-corn subplots. Later, incidence of MCMV in corn-corn subplots rose to 5% in early July and 12% in late August, whereas sorghum-corn subplots had less than 1% infection. Assuming that overwintering and potentially viruliferous adults of southern corn rootworm or flea beetles arising outside the field would not show preference for only corn-corn sequence subplots, these results support the hypothesis that MCMV is soilborne initially—ie, the virus is transmitted to young corn plants by viruliferous, soil-inhabiting corn rootworm larvae. Based on calculated degree days necessary for egg maturation, most of the larvae hatched by early June to mid-June and remained in the larval stage 3–6 wk (7). Under those conditions, transmissions of MCMV to corn via larvae-root feedings should be expressed as systemic mosaic symptoms by late June or early July. In 1980 as well as 1978 and 1979, the first MCMV-infected plants were found on or near 25 June (Uyemoto, *unpublished data*).

In the 1980 cropping season and under more normal circumstances, we would have expected a higher virus incidence in both corn-corn and sorghum-corn subplots. At or near the time of beetle emergence from the soil, however, day temperatures in 1980 exceeded 38 C during 4–15 July and thereafter were 37–42 C until 21 July, which apparently affected beetle foraging behavior and limited the spread of virus.

The effectiveness of a sorghum cropping period in reducing the incidence of MCMV likely stems from elimination (by decomposition) of infected corn residues, a likely source of virus for larvae vectors. Also, use of crops other than corn, such as soybean, sorghum, and alfalfa (15), is recommended for rootworm larvae control in highly infested cornfields; larvae residing in such soils perish because of lack of a proper diet. Crop rotation could also affect the egg-laying behavior of female beetles, thereby reducing egg populations for the next cropping season.

During the 1980 season, we surveyed three commercial cornfields that had been rotated from corn to sorghum or soybean in 1979. These fields had been severely affected by CLND in 1978. In August 1980, no CLND was found in two of the fields and only a trace (four infected plants with symptoms confined to terminal leaves, suggesting virus transmissions at anthesis [21] by viruliferous adult beetles) in the third field. Incidence of CLND in neighboring continuous cornfields averaged 3.6%.

In 1980, corn yields from sorghum-corn plants were about 39% higher than from corn-corn plots, and grain or forage yields from the two commercial fields mentioned above, were increased 30% compared with continuous cornfields planted to the same hybrids. These increased yields cannot be attributed entirely to the reduced incidence of MCMV. Other studies (1,17) indicate that rotated plots may yield from 5 to 46% more than continuous corn plots; it is likely that beneficial effects of crop rotation contributed greatly to crop performance in our test plots. To maintain effective MCMV control, noncorn plantings may be required in alternate cropping seasons. Once corn is replanted, viruliferous beetles migrating in middle to late summer will introduce virus into a cornfield and reestablish a virus reservoir in that field.

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