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EFFECT OF CULTURAL PRACTICES ON CONTROL OF MAIZE CHLOROTIC MOTTLE VIRUS AND BEETLE LARVAE-VIRUS TRANSMISSION STUDIES

by

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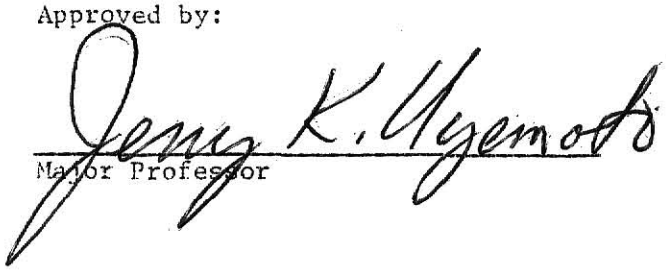

Major Professor

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EFFECT OF CROP ROTATION ON INCIDENCE OF MAIZE CHLOROTIC MOTTLE VIRUS

Nicki Jo Hutchens

ABSTRACT

HUTCHENS, N. J. 1980. Effect of Crop Rotation on Incidence of Maize Chlorotic Mottle Virus. M. S. Thesis. Kansas State University, Manhattan, Kansas.

Subplots planted to a corn-corn sequence (1979 and 1980), contained 1.6% MCMV-infections during the initial complete survey period of June 28 to July 2, 1980. No MCMV was detected in the sorghum-corn subplots in this period. Second and third complete sampling dates, viz., July 8 and 21, revealed that subplots of corn-corn and sorghum-corn contained at both surveys ca. 4.7 and 0.2% MCMV infections, respectively. On August 18, a final sampling showed MCMV infections at 12.2% (corn-corn) and 0.6% (sorghum-corn). Although corn yields on sorghum-corn subplots were significantly higher ($p=0.05$) the lower yields from corn-corn subplots could not be ascribed wholly to MCMV. However, crop rotation did reduce the incidence of MCMV; also, this practice likely contributed to the increased corn yield.

Additional key words: incidence of MDMV-A, -B, and WSMV; soil fumigation; nematodes; crop rotation.

INTRODUCTION

Maize chlorotic mottle virus (MCMV) was first described in Peru in 1973 (3, 9). In 1976, Niblett and Claflin detected MCMV in Norton County, Kansas (13), and since has been identified in Cloud, Osborne, Phillips, Smith, and Republic Counties (2, 17), all in North Central Kansas. MCMV has also been identified 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 mosaic virus (MDMV). This combination of viruses interact synergistically to cause corn lethal necrosis disease (CLND) (13).

Nault et al. (12) identified six beetle species of the family Chrysomelidae as vectors of MCMV. Both adults and larvae of the cereal leaf beetle (*Oulema melanopa* (L.)) (12), and the southern (*Diabrotica undecimpunctata* Mannerheim) (10), western (*D. virgifera* LeConte), and northern (*D. longicornis* (Say)) corn rootworm beetles (Stan Jensen, personal communication) transmit MCMV. Of these potential vectors, the western corn rootworm and the flea beetles (*Systena frontalis* (Fab.)) are economic pests in Kansas.

The identification of beetle vectors does not completely elucidate the epidemiology of MCMV, however. This is because MCMV has been detected in field corn plants prior to emergence of adult western corn rootworm beetles (Uyemoto, unpublished data). Flea beetles and southern corn rootworm beetles overwinter as adults (11) and feeding injury of newly emerged corn plants was readily evident; however, no MCMV infection was found in the corn plants (Uyemoto, unpublished data). Also, grassy weeds sampled in

April-June near affected corn fields were free of MCMV (2), which suggests that these do not serve as virus reservoir.

During the past three seasons, potted corn seedlings were placed around the perimeters of known CLND-corn fields, and exposed for 1-2 week intervals. Although potted corn plants remained free of MCMV, i.e., during absence of adult western corn rootworm beetles, virus infections were usually found in field corn plants in late June (2). Based on the above field data, viz., absences of a weed virus reservoir, lack of MCMV in potted bait plants, and MCMV infections in field grown plants, it was hypothesized that MCMV is initially soil-borne (18).

Currently, it is presumed that corn rootworm larvae are instrumental in vectoring MCMV to young corn plants, and that virus is acquired from MCMV infected corn residue (18). Recently, our evidence indicate that MCMV can overwinter in infected corn residues (Uyemoto, unpublished).

The present study was done to determine the effects of soil fumigation and crop rotation on MCMV incidence.

MATERIALS AND METHODS

Field experiments were conducted during the 1979 and 1980 growing seasons in Norton County, Kansas, at a site planted to continuous corn for 12 years and since 1976 contained MCMV infected corn plants. The soil has a silt loam texture with a pH of 7.0. The average annual precipitation at this location is 57.74 cm (22.77 inches); 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-early May, anhydrous ammonia was applied at 202 kg/ha (180 lbs./A) during seed bed preparation using a rototiller unit (FMC Sidewinder^{R}). At this time, atrazine (Ciba-Geigy) at 1.12 kg A.I./ha (1.00 lbs. A.I./A) and Eradicane (EPTC, Stauffer Chemicals) 1.26 kg A.I./ha (1.12 lbs. A.I./A) were applied and immediately incorporated. No herbicides were applied in 1979. Thimet (phorate 15g, American Cyanamid Corp.) was applied on June 14, 1979, at 1.3 kg A.I./ha (1.05 lbs. A.I./A) and May 5 and May 15, 1980, each at 2.7 kg A.I./ha (2.10 lbs. A.I./A). Corn was hilled in May and irrigated five times during each growing season. After harvest, cattle were allowed to graze on the corn stubble.

A split-plot design with four replications was used and each replicate split into two ranges of 15.24 m long x 13.71 m wide (50 x 45 ft.). On May 14, 1979, one range of each paired replicate was treated with 1,3-Dichloropropene and Chloropicrin (Telone C17) at 177.6 l/ha (19 gal/A). Fumigant was applied with a shank applicator; shanks were spaced at 0.3 m and to a soil depth of 0.15-0.20 m. Two weeks after fumigation, individual ranges were subdivided

into three subplots, each with six rows (15.24 m long x 0.75 m apart) (50 x 2.5 ft.) and planted in randomized fashion with two cultivars of field corn (*Zea mays* L., Pioneer 3194, which showed field tolerance to CLND and Pioneer 3183, which is extremely susceptible) and a forage sorghum (*Sorghum bicolor* (L.) Moench., Early Sumac). Under greenhouse conditions, Early Sumac is susceptible (systemic and latent) to MCMV. Seeds were hand planted at a rate of 74,100/ha (30,000/acre) and later thinned to 61,750 plants/ha (25,000/acre).

In 1980, the entire plot site was planted to Pioneer 3183. Since the effect of soil fumigation should not carry over in the season following treatment, the plot totaled eight replications 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 time of plant emergence and until the 9-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 (using a razor blade) and ground in a mortar and pestle with 2-3 mls of 0.02 M KPO_4 buffer, pH 7.0. However, leaf collections were extracted with a mechanical 'leaf squeezer' unit with 3-5 mls of neutral 0.02 M KPO_4 buffer added to the roller bars. These extracts were collected in disposable Kahn tubes (12 x 15 mm). All extracts were seroassayed in double immunodiffusion (DID) plates (0.75% Ionagar, 0.05 M Tris-HCl, pH 7.5, 0.85% NaCl, and 0.02% NaN_3) against MCMV antiserum and bioassayed onto a host range consisting of wheat (*Triticum aestivum* L. var 'Parker'), corn (inbred N28Ht), and sorghum (Asgrow 'Bug-off' and Dekalb 'E59+'). Extracts were hand rubbed

onto carborundum dusted plants and these were incubated for at least two weeks in the greenhouse before being read for symptoms. For diagnostic purposes, all of these viruses cause a prominent mosaic on corn, MDMV strain A cause mosaic symptoms in both sorghum varieties and B induces severe red leaf symptoms on Bug-off sorghum. WSMV and, occasionally, MCMV cause mosaic on wheat (17).

In 1980, random sampling and processing of entire plants (40 plants were collected/cropping sequence/week) were done until plants were at the 7-9 leaf stage, ca. 42 days post planting. At that time, virus infections were evident and leaves were collected from only those plants showing mosaic symptoms. When MCMV was detected, sampling of all symptomatic plants was confined to the inner 4 rows and inner 12.19 m row lengths of each subplot. Leaves were extracted with the mechanical leaf squeezer using a minimal volume of tissue extraction buffer (0.01 M KPO_4, pH 7.0, 0.85% NaCl, 0.05% Tween^R-20, and 2% Polyvinylpyrrolidine, mol wt 44,000). Extracts were assayed in enzyme-linked immunosorbent assay (ELISA) (4, 16). Each sample was tested against MCMV-K (16), MDMV-A, and MDMV-B antisera (Uyemoto, unpublished). On two sampling dates, extracts were also tested against WSMV antisera (19). All extracts were also tested in MCMV-DID. Concomitantly, several samples were inoculated onto the differential host range. ELISA results were read visually or in a Hitachi Perkin-Elmer double beam spectrophotometer (at 405 nm).

During the last two weeks of August, 1979 and 1980, final incidence of MCMV was determined. In 1979, ten consecutively positioned corn plants were sampled per subplot. Also, sixty samples of sorghum were selected 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. Soil samples were taken during each growing season. Using a kick sampler, six soil cores (35 cc/core) were taken from each subplot and composited. Each soil sample (100 cc) was processed using 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. The insect eggs were poured into a specimen dish for viewing. The nematode fractions were diluted to 20 mls and one ml was placed on a Hawksley glass slide and examined for plant parasitic *Helicotylenchus*, *Paratylenchus*, *Pratylenchus*, *Tylenchorhynchus*, and *Xiphinema* spp.

In 1979, soil samples were taken prior to fumigation, at planting (two weeks post-fumigation), and at weekly intervals until the middle of June. The June samplings were screened only for corn rootworm eggs to ascertain approximate hatching period. Also, at ca. 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 for endoparasitic *Pratylenchus*, roots were washed, the finer roots were cut into small pieces, and ten grams were 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 one week and weighed. Nematode counts were expressed as numbers per gram of dry root.

In 1980, soil samples were taken at planting time and at mid-season (see Appendix 1).

CORN HARVEST. Corn 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 kg/ha.

STATISTICAL ANALYSES. Except for yield data, all other data were analyzed using non-parametric tests because numbers of nematodes and virus infected plants were not distributed normally and did not have equal variances. Results of the fumigation trials were analyzed using the Signed Rank Test for Paired Samples (14), to compare differences between fumigated and non-fumigated subplots. The 1980 data were analyzed using Friedman's Test (8). All yield data were analyzed using Analysis of Variance and Duncan's Test (14) since equal variances were present.

RESULTS

SOIL FUMIGATION STUDY, 1979. MCMV was first detected in plant samples taken on July 10. MCMV was found in six samples, each from different corn subplots; four positives were from fumigated subplots. On July 20, four samples were positive for MCMV, three from non-fumigated subplots. By July 30, there was a total of 22 MCMV infected samples, 11 from fumigated and non-fumigated subplots each. The final sampling for MCMV, done at the end of August, showed the corn hybrids and subplots averaged 17%, i.e., Pioneer 3194 and fumigated with 12.5% and Pioneer 3183 and unfumigated with 22.5%. No MCMV was found in sorghum.

MDMV was frequently identified in tissue samples tested; incidence ranged from 0 to 20% in the various weekly collections. MDMV strain B was found in 75% of the MDMV positive collections. WSMV rarely occurred in the plot (less than 1%).

Rootworm eggs were detected at extremely low levels, so statistical analysis was not done. At prefumigation, untreated subplots averaged 0.4 egg/100 cc of soil and subplots to be fumigated averaged 0.1 egg/100 cc. At planting, untreated and treated subplots averaged 0.3 and 0.0 eggs/100 cc, respectively. After June 18, fewer whole eggs and increased numbers of egg fragments were found, presumably due to hatch. Adult beetles were first observed on July 10 (adults were absent on June 28).

Except for pre-fumigation soil samples and root samples taken at 106 days, no statistical difference in numbers of nematodes within each genus, irrespective of treatment, was found ($p=0.05$) (Table 1). However, fumigant designated ranges prior to treatment contained significantly more *Tylenchorhynchus* than control ranges ($p=0.05$). At 106 days post-planting,

roots from non-fumigated subplots had more endoparasitic *Pratylenchus*.

Yields of corn subplots averaged 8705.37 kg/ha. There were no statistical differences in corn yield among treatments (Table 2). The sorghum plants were not harvested.

CROP ROTATION STUDY, 1980. MCMV incidence and corn yields in both continuous corn-corn sequences, viz., either Pioneer 3183 or 3194 in 1979 and all Pioneer 3183 in 1980, did not differ significantly (p=0.05) (Tables 3 and 5). Hence, results stated hereafter will be from a single 8-replicated corn-corn sequence. Data for the 1979 sorghum and 1980 corn (Pioneer 3183) subplots will be designated under sorghum-corn subplots.

No MCMV was detected in plants assayed up to the 8-leaf stage. When the first MCMV infections were detected (June 25), all symptomatic plants were collected on June 28 and on July 2; sampled plants were marked with paint and those collected on July 2 were not sampled on June 28. On July 8 and 21, two additional complete samplings were done.

On June 25, one positive MCMV sample was taken from corn-corn subplots. For the combined sampling dates of June 28 and July 2, the corn-corn subplots averaged 30 (1.6%) infections and sorghum-corn subplots, zero (Table 3). On July 8 and 21, there were 94 and 88 MCMV infections (ca. 4.7%), respectively, in corn-corn subplots. In sorghum-corn subplots, 2 and 4 infections (ca. 0.2%) occurred, respectively, for each sampling date. A final sampling on August 18 showed MCMV infections averaged 235 (12.2%) on corn-corn and 11 (0.6%) on sorghum-corn subplots. On all dates, MCMV infections in sorghum-corn subplots were significantly lower (p=0.05) than in corn-corn subplots.

In tissue extracts of field material, MCMV-DID serologic tests confirmed 87% of the MCMV-ELISA positives and MCMV-ELISA detected 93% of bioassay

positives. Average ELISA readings for MCMV extracts were 0.80 and healthy samples, 0.18.

MDMV-B was first detected on June 5. On July 8, a total of 190 samples were positive for MDMV, of which 59% and 41% were identified as strains B and A, respectively. In this collection, 91 samples were doubly infected with both virus strains. No statistical differences (p=0.05) were found in the incidence of MDMV in either corn-corn subplots and sorghum-corn subplots. At the final sampling (August 18), 303 plants were positive for MDMV, and virus distribution in the various subplots was the same. Average ELISA readings for samples positive for MDMV-A and -B were 0.67 and 0.80, respectively. The average readings 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 subplots and incidence was less than 1%.

Nematode populations in the genera represented in our soil samples did not differ statistically between cropping sequences (p=0.05) (Table 4). Number of rootworm eggs averaged 0.5 egg/100 cc (corn-corn subplots) and 1.3 eggs/100 cc (sorghum-corn subplots). Adult Western Corn Rootworm beetles were observed on July 2, but not on June 28.

Corn yields in corn-corn subplots averaged 7432 kg/ha and sorghum-corn subplots, 10,356 kg/ha. These yields differed significantly (p=0.05) (Table 5).

DISCUSSION

Results of the 1979 fumigation study were inconclusive. Incidence of MCMV, distribution and populations of nematodes and insect eggs, irrespective of treatment, were essentially similar. Our failure to obtain more meaningful information with fumigation may be due to ineffectiveness of the fumigation or the delay of ca. 3 weeks in planting and seedling emergence was sufficient to offset the chronological sequence of events necessary for early season virus transmissions.

However, in the early portion of the 1980 growing season, MCMV was found (1.6%) in corn-corn subplots, but not in sorghum-corn subplots. Thereafter incidence in corn-corn subplots increased to ca. 4.7% in early July and ca. 12.2% in late August. In sorghum-corn subplots there was less than 1% infection. The difference in initial infection rates between corn-corn and sorghum-corn subplots support the thesis that MCMV is soil borne as western corn rootworm beetles were not seen until July 2 and virus transmissions by them to corn plants would require a week or more to develop symptoms.

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. However, in 1980, at or near the emergence of beetles from the soil, the day temperatures exceeded 38 C during July 4 to 15 and was 37-42 C thereafter to July 21; hence, the feeding behavior of the beetle vectors was dramatically affected.

The timing sequence of early MCMV infections support the hypothesis that larvae serve as primary vectors. Based on calculated degree days

necessary for egg maturation, most of the larvae hatched by early- to mid-June and remained in the larval stage for 3 to 6 weeks (7). Under these conditions, any MCMV transmitted via larva-root feeding in mid-June should develop systemic symptoms ca. late June or early July.

At this time, nematodes are not considered to be vectors (see Appendix 1). Also, based on the even distribution of MDMV and WSMV infections throughout the test plot, it may be concluded for these viruses that, at least two insect vectors did not show a preference for corn-corn or sorghum-corn subplots. Hence, during the early growth stages of corn plants, it is likely that potential beetle vectors viz. overwintering adults of southern corn rootworm or corn flea beetles, arising from outside the field would follow a similar migration pattern into the test plot. However, as stated earlier, the introduction of MCMV, via beetle vectors, into a corn field in May-June is remote (see Introduction section).

The effectiveness of the crop rotation in reducing the incidence of MCMV is likely due to elimination (by decomposition) of infected corn residues and hence, a source of virus for larvae vectors. Alternate crops should also discourage the egg laying behavior of female beetles, resulting in a greatly reduced population of eggs for the next cropping season.

In 1980, corn yields from sorghum-corn subplots were ca. 39% higher than corn-corn subplots. However, the increased yield cannot be attributed entirely to the reduced incidence of MCMV. Other studies indicate that rotated plots may yield from 5% to 46% (1, 15) more than continuous corn plots; it is likely that the beneficial effects of crop rotation contributed greatly to the performance in our test plots.

To effectively maintain MCMV control, non-corn plantings may be required in alternate cropping seasons. Once corn is replanted, migrating viruliferous

beetles in mid- to late-summer will introduce virus into a corn field and thereby reestablish a virus reservoir in that field.

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Table 1. Nematode counts in treated and untreated soils in 1979.

Sampling dates and treatments[b]	Nematode genera[a]				
	Helicotylenchus	*Paratylenchus*	*Pratylenchus*	*Tylenchorhynchus*	*Xiphinema*
May 14, 1979					
Fumigated	12	63	13	66[c]	0
Non-fumigated	15	43	7	32	3
May 30, 1979					
Fumigated	40	5	18	33	3
Non-fumigated	55	3	13	43	3
August 28, 1979					
Fumigated	96	91[d]	16	89	3
Non-fumigated	56	224	24	98	6
Root samples					
Fumigated			731[c]		
Non-fumigated			1,176		

a Nematode counts given as averages of 12 subplots; 100 cc soil and 1 g roots.

b Soil or root samples collected on May 14, pre-treatment; May 30, post-treatment; August 28, 106 days post-treatment. Telone C17 applied at 177.6 l/ha.

c Statistically different at the p=0.05 level; all other figures are statistically the same.

d Differences in this case were mainly due to the high number of *Paratylenchus* found in one sample.

Table 2. 1979 Corn yield data from soil fumigation plot.[a]

Crop	Treatment: Fumigated	Treatment: Non-fumigated	Average
Pioneer 3194[b]			
Rep 1	9841.32[c]	8960.31	
Rep 2	8418.51	9293.42	8973.51
Rep 3	9763.44	9331.67	
Rep 4	8273.85	7905.54	
Pioneer 3183			
Rep 1	9248.53	7589.60	
Rep 2	8841.14	8151.08	8437.23
Rep 3	8390.52	6797.88	
Rep 4	8377.59	10101.41	
Average	8894.36	8516.38	8705.37

a Corn ears, harvested from the inner 7.62 m of two center rows in each subplot.

b Yields expressed in kg/ha; moisture adjusted to 15.5%.

c All figures are statistically the same at the p=0.05 level.

Table 3. Percent of maize chlorotic mottle virus infected plants on four sampling dates in the crop rotation plot, in 1980.

	Sampling Dates							
	6/28-7/2		7/8		7/21		8/18	
1979 Crop	#MCMV	%	#MCMV	%	#MCMV	%	#MCMV	%
Corn	30	1.6	94	4.8	88	4.6	235	12.2
Sorghum	0[a]	0.0	2[a]	0.1	4[a]	0.2	11[a]	0.6

a These figures are statistically different at the p=0.05 level from corn values.

Table 4. Nematode counts in soil samples from crop rotation plot; May, 1980.[a]

	Nematode Genera[b]				
1979 crop	*Helicotylenchus*	*Paratylenchus*	*Pratylenchus*	*Tylenchorhynchus*	*Xiphinema*
Pioneer 3194	50	15	13	63	0
Pioneer 3183	20	3	3	68	3
Sorghum	38	3	13	70	3

a Nematode counts given as averages for 8 subplots; 100 cc soil.

b All figures per genus are statistically the same at the p=0.05 level.

Table 5. 1980 Corn yield data from crop rotation study.

	Range[a]				
1979 crop	2	3	4	5	Average
Corn[b]	8618.7	7465.0	6434.6	7208.6	7432.9
Sorghum	9444.9[c]	10013.2[c]	1178.6[c]	10791.0[c]	10356.9[c]

a Corn harvested from the inner 7.62 m of the two center rows in ranges 2, 3, 4, and 5.

b Corn yields average of both corn-corn subplots; yields expressed in kg/ha; moisture adjusted to 15.5%.

c Statistically different at the p=0.05 level from corn values.

APPENDIX 1

INVESTIGATING MAIZE CHLOROTIC MOTTLE VIRUS TRANSMISSION USING FIELD SOIL DEVOID OF INSECT LARVAE

Although Chrysomelid beetles will vector maize chlorotic mottle virus, the possibility of other soil inhabiting organisms acting as virus vectors was investigated. This was attempted because initial infections found in the crop rotation study suggested soil borne transmissions (4). Bockelman (1) attempted to transmit MCMV using hand picked *Xiphinema americanum*, but was unsuccessful, partially because of poor survival of the nematodes. The present study was done in a gross fashion, i.e., corn seedlings were used as bait plants and such plants were bioassayed for infection. The result of this study is reported herein.

MATERIALS AND METHODS

Soil and root samples were taken from the Almena field plot during the 1980 growing season (4). Soil samples were taken directly from the corn row using a kick sampler on July 8, 1980. Four replicated subplots/crop rotation sequence were sampled; six divets were taken at random per subplot and composited. They were then divided into 2 lots each and examined for insects. All larvae and mature insects were removed. Soil (6-300 ml units) was placed into a 10.16 cm diam. clay pot, which was placed in a larger plastic pot (19.42 cm diam.) filled with sterile soil. Five germinated corn seedlings (*Zea mays*, N28Ht) were transplanted into the field soil, insect cages were placed over the seedlings, and incubated in a controlled environment growth chamber, at 21 C and with a 12 hour light period for six weeks.

After the incubation period, plants were observed for symptoms. Leaf and root samples were ground in a mechanical 'leaf squeezer' with 1-2 mls of tissue extraction buffer (0.01 M KPO_4 pH 7.0, 0.85% NaCl, 0.05% Tween^R-20, and 2.0% polyvinylpyrrolidine, mol wt 44,000). Extracts were seroassayed in agar gel plates against MCMV antisera (7), and bioassayed onto carborundum dusted corn seedlings (N28Ht). These were incubated for 3-4 weeks and infections were confirmed by seroassay.

A second soil collection was made on July 15, 1980, and was composed of soil adhering to roots of six corn lethal necrosis disease infected plants. Soil from all six plants was composited and 300 ml placed in each of six clay pots (10.16 cm diam.). Pots and soil were handled as described previously except that corn roots were cut into small pieces, composited, and 10 g placed in each pot. A thin layer of field soil was placed over the roots before seedlings were planted and incubated for five weeks in the growth chamber.

Field collected roots were also tested for virus content. Tissue extractions were made and serial dilutions of 1/20, 1/200, 1/2000, and 1/20,000 were inoculated onto corn seedlings (N28Ht); three replications/dilution. After three weeks, individual plants were read visually and seroassayed.

For each experiment above, genera and numbers of nematodes were determined. In the first experiment, soil samples, (1-100 ml unit/subplot; 12 total) were processed using the direct centrifugal flotation method (3). Nematodes were trapped on a 37 µm mesh screen and were washed into test tubes. Nematode fractions were diluted to 20 ml and one ml aliquots were observed under the compound microscope for plant parasitic nematodes of the genera *Helicotylenchus*, *Paratylenchus*, *Pratylenchus*, *Tylenchorhynchus*, and *Xiphinema*.

In the second and at the end of both experiments, nematodes were extracted using Cobb's screening technique (2). Soil samples (2- or 3-250 ml units) were placed in a 2 l flat pan, mixed with enough water to cover the soil, and then poured through a series of screens. Nematodes were trapped on the 150 µm mesh screen and on the 37 µm mesh screen. Nematodes caught on the 37 µm mesh screen were placed in a modified Baermann funnel to remove dirt and debris (5). Nematodes were collected in tubes, allowed to settle to the bottom, and excess water removed by suction. Nematode fractions were placed in a Hawksley glass slide and examined for *X. americanum*.

At the end of each transmission, roots of bait plants were collected, stained with lactophenol/cotton blue, and observed under the compound microscope for resting structures of lower fungi (especially potential virus vectors such as *Olpidium* and *Polymyxa*).

To be certain that environmental regimes were favorable for development of MCMV symptoms, corn seedlings (N28Ht) were inoculated and placed in the same growth chamber. MCMV infected tissue was ground in a mortar and pestle (1:10 ratio with tissue extraction buffer), and serial dilutions made, viz., of 1/100, 1/1000, 1/10,000 and 1/100,000. Four pots of seedlings were leaf-inoculated with each serial dilution. Also, seedling roots were dusted with carborundum and inoculated with extracts at 1/10, 1/100, 1/1000, and 1/10,000 dilutions, with two replications/dilution. Seedlings were transplanted, incubated for three weeks, and read for symptoms.

RESULTS

For both experiments, no bait plants exhibited any virus symptoms and all were negative to bio- and sero-assays.

The infectivity of root extracts were 12.5, 28.6, 14.3, and 25%, respectively, for dilutions of 1/20, 1/200, 1/2000, and 1/20,000.

Soil samples taken July 8, showed no statistical difference ($p=0.05$) in numbers and kinds of plant parasitic nematodes from corn-corn or sorghum-corn subplots. Average populations for all genera were: Pioneer 3194--50/100 cc of soil, Pioneer 3183--23 and sorghum--56. Prior to experiment one, few *X. americanum* were found, while in the second experiment they averaged 10/100 cc of soil (pre-experimental counts). *Xiphinema* counts at the end of both experiments averaged 4 each/100 cc of soil.

No resting structures of primitive fungi were identified in seedling roots.

All extracts of infected tissue except 10^{-5} dilution were highly infectious when rubbed onto corn leaves; symptoms developed in ca. 2 weeks. At 10^{-5} dilution, only 50% infection occurred. In contrast, root inoculated seedlings were infected at only the 1/10 dilution.

DISCUSSION

No soil-related transmission occurred from field collected soil and no difference was found in the over-all nematode populations among corn-corn and sorghum-corn subplots. *X. americanum* was present at fairly low levels and their survival throughout the experiments was ca. 40% (comparing pre- and post-experimental counts from the second experiment). McGuire and Douthit (6) reported that *X. americanum* will transmit tobacco ringspot at a rate of 30%, with a single nematode per cucumber plant, indicating a good vectoring efficiency. Since all nematodes presumably, were given equal access to a source, viz., infected roots of field plants (the second

experiment), and no transmission occurred, the results suggest that nematodes are not vectors of MCMV.

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APPENDIX 2

COLEOPTILE AND ROOT TRANSMISSION STUDIES WITH MAIZE CHLOROTIC MOTTLE VIRUS

A crop rotation study recently completed (1) supports the assumption that maize chlorotic mottle virus is soil-borne (3). Although it is suspected that the western corn rootworm larvae are involved in MCMV transmission (3), their ability to do so under field conditions lacks experimental evidence.

The purpose of this series of experiments was to determine: 1) if MCMV could be transmitted to corn seedlings through root inoculations, and 2) if MCMV could be transmitted by strictly passive means in the soil.

MATERIALS AND METHODS

Corn seedlings (*Zea mays* L., N28Ht) were germinated in closed plastic boxes for 6-8 days or until seminal roots were developed and coleoptiles had emerged. Inoculum was prepared by grinding frozen MCMV infected corn tissue in the leaf squeezer at a 1:5 ratio with 0.02 M KPO_4 pH 7.0. To assess virus titer, serial dilutions of the inoculum were made using 0.02 M KPO_4 pH 7.0. These dilutions, 1/10, 1/100, 1/1000, 1/10,000 and 1/100,000 were inoculated to young corn seedlings (N28Ht). Seedlings were incubated for three weeks and infections confirmed by seroassays.

MECHANICAL INOCULATIONS. Carborundum was added to the inoculum and the coleoptiles or roots of 25 seedlings each were finger rubbed and seedlings planted in soil. Twenty-five additional seedlings were rubbed in the same

way with 0.02 M KPO_4 pH 7.0 and transplanted in a separate container.

ROOT DIP INOCULATIONS. Seedlings were germinated and inoculum was prepared as for mechanical inoculations except no carborundum was added. A portion of the radicle was cut off and the cut surface dipped in the inoculum for 30 seconds, and rinsed in distilled water for 15 seconds. Seedlings were transplanted. Also, seedlings were treated in the same manner except the radicles were dipped in 0.02 M KPO_4 buffer at pH 7.0. All were planted in a separate container according to treatment.

In another test, seedlings were germinated and a portion of the radicles were cut off. At various post-cut incubation periods, the radicle portion was dipped in inoculum for 30 seconds, rinsed under running tap water for 30 seconds, and running distilled water for five seconds. Post-cut incubation periods were 0 (radicle cut while immersed in the inoculum) and 30 seconds or 1, 15, and 30 minutes, or 1 and 2 hours. Five seedlings were used per time period. As a control, also, five seedlings with uncut radicles were dipped in the inoculum and rinsed and five seedling radicles were cut, after 30 seconds dipped in 0.02 M KPO_4 pH 7.0, and rinsed. Seedlings from each treatment were planted in separate pots.

INFECTED SAP WATERING. Eighty pots of corn seedlings (7-10 day old) were divided into four treatment groups: A--watered with tap water; B--watered with tap water plus subjected to soil disturbance; C--watered with infectious sap; and D--watered with infectious sap plus soil disturbance. Soil disturbance consisted of raking a metal spatula through the top surface (1.5 inches) of the soil immediately prior to every second or third watering.

Infective sap was obtained by grinding MCMV infected corn tissue at

a 1:10 ratio with distilled water in a Waring blender. Ten mls of tap water or infected sap were used to irrigate seedlings every 2-3 days. All pots were watered with tap water between treatments. After 4 weeks (12 watering periods and 7 times of soil disturbance) the treatments were terminated and infections confirmed by seroassays (2).

In a second series of experiments, potted plants were watered 1, 3, 5, or 12 times at two day intervals with MCMV-tissue extracts; 50 mls were used per pot. Four pots with three corn seedlings (N28Ht) were used per treatment and distilled water controls. All control pots and those no longer receiving infectious sap were watered with 50 ml of distilled water. At each watering period (including paired controls), soil disturbance, using scissors, was done. A week following the 12th treatment, the test was completed and leaf samples were seroassayed (2).

This experiment was also repeated using 1, 2, 3, or 5 times watering with infective sap.

In a third experiment, potted plants were watered with infective sap at various dilutions. Inoculum was prepared as before and these dilutions were made using distilled water: 1/10 (original inoculum), 1/100, 1/1000, 1/10,000, and 1/100,000. For each dilution, four pots containing three corn seedlings (N28Ht) were used. Six pots of distilled water controls were used for each treatment. Pots were watered every day with infective sap or distilled water. Soil disturbance, using the snipping action of scissors through the soil, was applied at every other watering. After four weeks (14 watering periods and 8 times of soil disturbance) plants were seroassayed for virus infections (2).

RESULTS

The inoculum was infective to a dilution of 1/100,000, where half of the inoculated plants developed symptoms.

MECHANICAL INOCULATION. An average of 28% of coleoptile inoculated plants (4/24, 10/25) became infected. Buffer inoculated controls (0/22, 0/24) remained healthy. With root inoculations, an average of 87% (23/25, 13/17, 14/15) were infected. However, 9% of the buffer inoculated controls (2/24, 1/15, 2/15) were also infected.

ROOT DIP INOCULATIONS. In the first test, an average of 21% of the treated plants (4/22, 5/24, 4/22) were infected with MCMV. All buffer dipped controls were healthy (0/23, 0/20, 0/22).

In the second experiment, cut roots dipped after 0 and 30 seconds, 1, 15 and 30 minutes, and 2 hours produced infections between 6 and 24% (Table 1). Cut roots dipped after 1 hour and uncut roots had 0 and 7% infections, respectively. Buffer dipped roots remained healthy.

INFECTED SAP WATERING. All plants watered with MCMV infective sap without soil disturbance and plants watered with distilled water, with or without soil disturbance remained healthy. However, 73% of the plants (11/15) watered with infective sap with soil disturbance became infected.

In the second series of tests, plants watered with infective sap for 1, 3, 5, or 12 times showed infections of, respectively, 0, 33, 27, and 55%. Controls were all healthy.

Plants watered with infective sap for 1, 2, 3, or 5 times showed infections of 0, 17, 25, and 25%, respectively. Controls were all healthy.

In the third experiment, seedlings treated with the various virus

dilutions and soil disturbance became infected, viz., 1/10--67%; 1/100--58%; 1/1000--27%; 1/10,000--0%; and 1/100,000--0%. Plants treated with distilled water were healthy.

DISCUSSION

Maize chlorotic mottle virus was easily mechanically transmitted to corn plants via rubbing the coleoptile or roots. The high rate of contamination seen in the controls for the root inoculations may be explained by the way the experiment was set up. All materials for the experiment (expressed sap and buffer inoculations) were done in a common area. Control seedlings were handled immediately following infected sap inoculation of seedlings.

When radicles were cut and immersed in MCMV inoculum, infections also occurred even following a 2 hour post cut incubation and dip. In the soil disturbance series of experiments, higher rates of infections occurred. This was likely due to more cut surfaces being exposed to infectious sap. This study also illustrated that with a 1/10 dilution, that 2 cutting-watering periods were necessary for infections to occur. In addition, a virus titer contained in a 1/1000 dilution and repeated watering and root injury were required to produce infections.

It does not appear likely that the mechanism of cutting/watering (attempting to simulate root pruning caused during cultivation) is important in the field epidemiology of MCMV. Although, infections occurred in the root dip tests, successful virus transmission on rooted plants require a fairly high concentration of virus and repeated exposure to inoculum. Therefore, at this time soil transmission of MCMV through abiotic means does not appear to be involved in the epidemiology of MCMV.

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Table 1. Root dip inoculations. Effect of various post-cut incubation periods on percent of maize chlorotic mottle virus infected plants.[a]

	Replications				
Post-cut intervals	Rep 1	Rep 2	Rep 3	Rep 4	Weighted Averages
0 seconds	0	0	20	0	6
30 seconds	0	20	0	20	11
1 minute	33	0	0	20	8
15 minutes	33	--	20	20	24
30 minutes	33	0	0	50	21
1 hour	0	0	0	0	0
2 hours	--	0	20	20	11
uncut roots	--	0	20	0	7
buffer (control)	0	0	0	0	0

a Results given in percentages.

APPENDIX 3

MAIZE CHLOROTIC MOTTLE VIRUS TRANSMISSION STUDIES USING *DIABROTICA VIRGIFERA* LARVAE

This series of experiments were attempted to transmit maize chlorotic mottle virus with western corn rootworm larvae (*Diabrotica virgifera* LeConte) by simulating conditions present in a grower's diseased field. We are assuming that MCMV infected corn debris is an over-wintering source for MCMV (2). In our tests, dried and then rehydrated MCMV infected corn tissues (*Zea mays* L., N28Ht) were used; controls included fresh infected and fresh and dried/rehydrated healthy plants. Second and third instar larvae were used in all tests.

TEST I. Both virus acquisition and transmission steps were completed in plastic pots. The acquisition access period was that time when larvae were allowed access to source tissues and before availability of healthy corn seedlings.

Six acquisition access periods were used: at 0 hours (seedlings, source tissues and larvae added together), after 8 hours, 16 hours, 1 day, 2 days, and 6 days exposure of larvae to source tissue. Source tissues included 4 week old infected or healthy N28Ht corn plants that were dried on a laboratory bench for 5 weeks. Prior to use, tissues were soaked in distilled water for 2 hours.

For each treatment and acquisition access period, 8 pots were used. Four contained MCMV infected tissue (2 each of foliar or root tissues) and 4 had healthy tissue. Pots were partially filled with sterile soil, overlayed with source tissue and five larvae/pot, and covered with soil. After

designated access periods had elapsed, eight healthy seedlings were planted/ pot. At each time period, two control pots were emptied, healthy larvae counted, and pots reassembled. As a control, eight pots were prepared as described, except no larvae were added.

The seedlings, source tissues, and larvae mixtures were incubated 4 weeks and infection confirmed by seroassays (1).

TEST II. Acquisition periods were also conducted in petri dishes. Sixty larvae were allowed access to MCMV infected dried/rehydrated tissue and 10 healthy dried/rehydrated tissue. At 24, 36, and 48 hours, larvae, 10, 10, and 7, respectively, were removed from the infected tissue. At 48 hours, 6 larvae were removed from the healthy tissue. Larvae were rinsed twice in distilled water and placed in an individual petri dish with one seedling. After 4 days, seedlings and larvae were planted in individual pots. After 4 weeks, results were assessed visually.

TEST III. Larvae were divided into 4 treatment groups: 15 larvae on MCMV infected fresh tissue; 15 on MCMV infected dried/rehydrated tissue; 10 on healthy fresh tissue; and 10 on healthy dried/rehydrated tissue. Only roots were used. A 24 hour acquisition feed was done in petri dishes. After 24 hours, larvae and acquisition material were placed in a partially filled pot, covered with soil, and two germinated corn seedlings were transplanted. Two and one pots per treatment were used for each infected and healthy source tissues, respectively. Results were read visually after 3 weeks.

TEST IV. Larvae were divided into 4 treatment groups; 35 larvae on fresh MCMV infected tissue; 10 on fresh healthy tissue; 45 on dried/rehydrated infected tissue; and 10 on dried/rehydrated healthy tissue. Before given

access to tissues, larvae were first starved for 8 hours, divided into groups of 5, and allowed a 24 hour acquisition feed on root/crown source tissues. An average of 4 larvae survived the acquisition feed period. After the acquisition feed, the source tissues were replaced with two corn seedlings for a 24 hour transmission feed. At the end of 24 hours, larvae were removed (except those too deeply imbedded) and seedlings were planted and given a 3 week incubation period. Results were determined serologically.

TEST V. With minor variations, this experiment was identical to Test IV. Larvae were starved for 9.5 hours, given a 36 hour acquisition feed and a 45 hour transmission feed. In this test, tissue was dried for 6 weeks and soaked in distilled water for 2.5 hours.

TEST VI. Larvae were given a 4 day acquisition feed on healthy or infected dried/rehydrated tissue as in Test V. After the acquisition feed, 42 larvae from the infected tissue and 10 larvae from the healthy tissue were placed in pairs in pots planted with one corn seedling. After 3 weeks, tissue samples were seroassayed.

INOCULUM POTENTIAL. For experiments I, III, IV, V, and VI, the inoculum potential of source tissues were determined. The tissue used in each experiment (foliar or root) was ground in a mortar and pestle with tissue extraction buffer (0.01 M KPO_4, pH 7.0, 0.85% NaCl, 0.05% Tween^R-20, and 2% Polyvinylpyrrolidine, mol wt 44,000) (1/10, w/v). Serial dilutions were made of 1/10 (original extract), 1/100, 1/1000, 1/10,000, and 1/100,000. Healthy tissue was used at the 1/10 dilution. Extracts were inoculated in three replications onto carborundum dusted corn seedlings (N28Ht). Experiments IV, V, and VI utilized the same source tissues and were utilized over a 1.5 week period, when the inoculum potential was determined.

RESULTS

TEST I. One corn plant from a pot that had larvae, healthy tissue, and a 2-day acquisition period was positive for MCMV. All other corn plants were healthy. An average of 3-4 larvae/pot survived the acquisition feed.

TESTS II AND III. All corn plants were healthy.

TEST IV. All plants with larvae given healthy tissue and plants with larvae given dried/rehydrated infected tissue were healthy. Three of seven surviving corn plants (larvae given fresh infected tissue) were positive; each infected plant was from a different larvae group.

TEST V. Two of nine plants (larvae given fresh infected tissue) were positive for MCMV; both infected plants were from separate larvae groups. One of five plants with larvae fed on infected dried/rehydrated material, was positive for MCMV. Control plants were healthy (larvae given healthy source tissue).

TEST VI. All plants were healthy.

INOCULUM POTENTIAL. Inoculum potential of source tissues used in tests: I. Plants inoculated with extracts of dried healthy leaves and roots were healthy. Extracts of dried infected roots and leaves were infective to a dilution of 1/100; at this dilution, 2 and 1 pots each contained infected corn plants for root and leaf extracts, respectively.

III. Healthy extracts of fresh and dried/rehydrated tissue were non-infective. Extracts of fresh MCMV infected tissue was infective at a dilution of 1/10,000 (highest dilution tested) with 50% of the inoculated plants infected. Extracts of dried/rehydrated tissue was infective up to

a dilution of 1/100 and caused 18% infection.

IV, V, VI. Plants inoculated with extracts from healthy tissue were healthy. Fresh infected tissue was infective to a dilution of 1/100,000 where 43% of the inoculated plants were infected. Dried/rehydrated infected tissue was infective to a dilution of 1/100, and 45% of the inoculated plants were infected.

DISCUSSION

A total of 246 western corn rootworm larvae survived the acquisition feed and were used to attempt transmission of MCMV from dried/rehydrated tissue; only one plant became infected. If it is assumed that MCMV was transmitted to this plant by one larva (and that the infection was not due to contamination), then there was a transmission rate of 0.4%. However, a contamination was realized in Test I.

In retrospect to results obtained in the crop rotation experiment, there was an average of 0.5 eggs/100 cc of soil in May, 1980, or a potential of 424,753 eggs in the 8 replicated corn-corn subplot (i.e., 15.24 m x 4.57 m x 0.3 m, (length x width x depth) x 8 replications). Assuming 50% larval hatch (Gerald Wilde, personal communication), there would be 212,377 larvae. During the initial incidence of MCMV (6/28-7/2) there were 30 infected plants in a corn-corn subplot series. Assuming that each infection was the result of one larva transmitting MCMV, then 0.01% of these larvae initiated transmittance of MCMV. If the above assumptions are correct, then virus transmission in the field is lower than that realized in the laboratory.

Larvae, given an acquisition feed on fresh MCMV tissue, transmitted virus to 5 plants. If these resulted from a single larva feeding, then

9% (5/56) of the larvae transmitted MCMV from fresh infected tissue. A 0-25% transmission rate was recorded in another laboratory (Stan Jensen, personal communication).

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APPENDIX 4

THE BIOLOGY OF THE NORTHERN AND WESTERN CORN ROOTWORMS: A REVIEW

The biology of the northern corn rootworm, *Diabrotica longicornis* (Say) and the western corn rootworm, *Diabrotica virgifera* LeConte is quite similar. A general life history will be given with differences indicated.

DESCRIPTION. The eggs of these rootworm species are small, white and globular and can be distinguished by the distinct chorion sculpturing (1). Larvae are white worms with a dark spot on the posterior end. When full grown, larvae are 1/2 inch long. Pupae are white and enclosed in an earthen cell (2, 11). The adults are small, ca. 1/4 inch long. Western adults are pale yellow green with black stripes down the back. Northern adults are solidly colored from pale green to yellow.

DISTRIBUTION. Both insects are endogenous to North America and are important in corn production regions of the north-central states (9). Northern corn rootworms are most abundant in the area bounded by Colorado, North Dakota, Oklahoma, and the Eastern seaboard. The western corn rootworm is found in the high plains region from the Texas panhandle to the Dakotas (2). The range of the western corn rootworm has expanded north and east since the 1950's, partly because of developed resistance to cyclodiene soil insecticides (9).

HOST RANGE AND DAMAGE. Corn rootworms generally require corn to complete their life cycle. Larvae feed on corn roots and hence, reduce absorptive function of roots and weaken the entire root system (2, 6, 9).

Adults feed on pollen, silk, and kernels of corn. Ten or more adults/ plant may clip the silks and affect pollination (9, 11). Adults also feed on pollen and fruiting structures of weeds, ornamentals, and alfalfa, especially after corn plants mature (9).

Some works indicate that larvae can complete immature stages on some graminaceous hosts other than corn (4, 5). However, sorghum is not a host because it is toxic to larvae and may cause death (3).

LIFE HISTORY. Both species overwinter as eggs, which normally require a diapause period. However, some eggs mature without exposure to a chill period. Also, some northern corn rootworm eggs survive two winters before maturing (6).

After the diapause period, the threshold soil temperature for development is 11.1 C (52° F) with 400 degree-days needed for hatching of the northern corn rootworm (7). Western corn rootworm show a similar threshold temperature with 380 degree-days needed for egg maturation (10).

Larvae of both species pass through 3 instars. Laboratory work with the western corn rootworm larvae indicate that 27-41 days and 17-24 days are consumed during the larval and pupal stages, respectively (8).

In the field, most eggs mature in June; however larvae hatch may begin in May and continue through August (2). Adults begin to emerge early in July. They remain as adults 5-6 weeks. Oviposition begins 2-2.5 weeks post-emergence (9). Most of the female's 500 eggs (average) are oviposited in August. In irrigated fields, most eggs are deposited between rows whereas in dryland fields, ovipositing occurs mostly in the rows (6, 9). Eggs are usually concentrated in the upper 15 cm of soil (2).

CONTROL. Control is aimed mainly at the larvae since these are the most damaging. Crop rotation is used to discourage ovipositing in fields. Since adults lay eggs in corn, a field planted to an alternate crop will have fewer eggs and larvae (6, 9, 11). On fields planted to continuous corn, soil insecticides (organophosphates and carbamates) are applied at planting or after corn emergence for larvae control (11). When adults are in high enough concentration to interfere with pollination, insecticides are applied (11).

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EFFECT OF CULTURAL PRACTICES ON CONTROL OF MAIZE CHLOROTIC MOTTLE VIRUS AND BEETLE LARVAE-VIRUS TRANSMISSION STUDIES

by

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ABSTRACT

Subplots planted to a corn-corn sequence (1979 and 1980), contained 1.6% MCMV infections during the initial complete survey period of June 28 to July 2, 1980. No MCMV was detected in the sorghum-corn subplots in this period. Second and third complete sampling dates, viz., July 8 and 21, revealed that subplots of corn-corn and sorghum-corn contained at both surveys ca. 4.7 and 0.2% MCMV infections, respectively. On August 18, a final sampling showed MCMV infections at 12.2 (corn-corn) and 0.6% (sorghum-corn). Although corn yields on sorghum-corn subplots were significantly higher ($p=0.05$) the lower yields could not be ascribed wholly to MCMV. However, crop rotation did reduce the incidence of MCMV; also this practice, likely, contributed to the increased corn yield.

No nematode transmission of MCMV occurred from field collected soil; however, populations and survival of *Xiphinema americanum* was not high. MCMV was easily transmitted to corn through mechanical inoculations. Potted plants watered with infected sap or cut roots dipped in infective sap resulted in low incidence of infections. It does not appear that passive soil transmission is important in MCMV epidemiology. One of 246 western corn rootworm larvae transmitted MCMV when given access to dried/rehydrated infected tissue. Larvae given an access feed on fresh infected tissue had a 9% transmission efficiency rate (5/56).