# EPIDEMIOLOGICAL STUDIES ON MAIZE CHLOROTIC MOTTLE VIRUS

by

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# Host Range Study of Maize Chlorotic Mottle Virus Strains and Incidence of Maize Dwarf Mosaic and Wheat Streak Mosaic Viruses in Native Grasses

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## ABSTRACT

BOCKELMAN, D. L. 1980. Host Range Study of Maize Chlorotic Mottle Virus and Incidence of Maize Dwarf Mosaic and Wheat Streak Mosaic Viruses in Native Grasses. M. S. Thesis. Kansas State University, Manhattan, Kansas.

Nineteen and fifteen grass species were found as systemic hosts for Kansas and Peru strains of maize chlorotic mottle virus (MCMV), respectively. Host response between strains are similar with differences found primarily in latently infected hosts. No MCMV was found in 230 grass samples, representing 14 species, collected near infested corn fields. However, maize dwarf mosaic virus, strain B and wheat streak mosaic virus were detected in annual grass species and active insect transmissions to corn bait plants were also realized.

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Corn lethal necrosis (CLN) was first reported in Kansas in 1976 (21) and since has caused severe disease outbreaks in several areas of north central Kansas (30) and south central Nebraska (6). The disease is caused by the combination of maize chlorotic mottle virus (MCMV) and either maize

dwarf mosaic virus (MDMV) or wheat streak mosaic virus (WSMV). Yield losses have been estimated at 50% or higher based on CLN incidence in the field (21,30). Symptoms of CLN occur as a bright yellow mosaic with the tips and edges of infected leaves becoming progressively necrotic, causing premature death of the plant (21,31). Plants are severely stunted with limited or no ear development.

MCMV was first described infecting corn in Peru in 1973 (4,10) and in the United States in 1976 (21). Six chrysomelid beetle species were reported as vectors of MCMV of which 3 corn rootworm species and 2 corn flea beetle species occur in Kansas (20). Attempts to transmit Peru and Kansas strains of MCMV (MCMV-P and MCMV-K, respectively) with aphids have failed (4,10,20). Eight grass hosts were reported for MCMV-P and 50 other non-hosts were tested (4,10). However, many of these species are not found in Kansas. Fourteen hosts for MCMV-K and eight new hosts for MCMV-P were recently reported (3).

In 1944, WSMV was described (17) and isolated from Kansas wheat fields as early as 1932 (16,18). The wheat curl mite, Aceria tulipae Keifer, is the vector of WSMV (24,25) and numerous grasses have been reported as hosts for the mite and virus (25). In 1962, MDMV-A was found in Ohio (11,33,34) and MDMV-B from Pennsylvania in 1966 (15), and since have been reported from many areas of the United States (7,9,14,19,26,27). Johnson grass, *Sorghum halepense* (L.) Pers., is susceptible to MDMV-A but not MDMV-B (15). Johnson grass rhizomes serve as a primary overwintering host for MDMV-A (1,12), and infested corn fields have been closely associated with nearby infected Johnson grass (1,13,23,34). It is not known how MDMV-B overwinters.

Host range studies on many Kansas grass species were conducted in the greenhouse to identify potential reservoirs for MCMV and to compare the

host ranges of MCMV-K and MCMV-P. Extensive host range studies exist for MDMV-B and will not be duplicated here (8,22,28). To identify potential overwintering hosts for MCMV and MDMV-B, native grasses were collected near MCMV infested fields throughout the corn growing season, and assayed for virus. Corn bait plants in pots were placed near fields to monitor for air-borne insect transmittance of viruses in the area. These studies were conducted to better understand the epidemiology of CLN incitants.

## MATERIALS AND METHODS

ASSAY AND INOCULATION METHODS. Plants were inoculated with virus by rubbing carborundum dusted leaves with crude extracts of virus infected tissue. A pestle or fingers were used in applying tissue extracts to leaves. Inoculated plants were rinsed immediately with water and hands were thoroughly washed with soap and water between inoculations. Crude virus extracts used in double immunodiffusion (DID) assays, bioassays, or general inoculation purposes were prepared by grinding infected tissue in a volume of 0.05 M potassium phosphate ($KPO_4$), pH 7.0 buffer with a mortar and pestle or a mechanical leaf squeezer. Plans for the leaf squeezer were designed by R. C. Hastings, H. L. Kucera, and Everett Tool, North Dakota State University, and modified as suggested by O. W. Barnett, Clemson University.

Seroassays with DID were used to detect MCMV and brome mosaic virus (BMV). Antisera of MCMV and BMV were prepared by J. K. Uyemoto and C. L. Niblett, respectively. Agar plates were composed of 0.75% Ionagar, 0.85% NaCl, 0.02% $NaN_3$, in 0.01M Tris-HCl, pH 7.2. Antisera was placed in the center well of Ionagar plates with eight surrounding wells filled with

tissue extracts. Enzyme-linked immunosorbent assay (ELISA) (5) was used to confirm the presence of MDMV-B and WSMV (29,32). Antisera preparations for ELISA were provided by J. K. Uyemoto.

Bioassays were used to detect viruses affecting corn, *Zea mays* L., by inoculating corn plants (inbred line N28Ht), or by utilizing a differential host range (30). The differential host range consisted of N28Ht corn, sorghum (*Sorghum bicolor* (L.) Moench. var. 'Asgrow Bugoff' and 'Dekalb E59+'), and wheat (*Triticum aestivum* L. var. 'Parker'). In early tests 'Redland' sorghum was used. Reactions of viruses on N28Ht corn occurred as a mosaic with MCMV, MDMV-A, MDMV-B, WSMV, and a mosaic and death of plants infected with BMV. Reactions of viruses on 'Asgrow Bugoff' sorghum were a mosaic and moderate redleaf symptoms with MDMV-A, a mosaic and severe redleaf symptoms with MDMV-B, a mosaic with BMV, and an immune response with MCMV and WSMV. Reactions of viruses on 'Dekalb E59+' sorghum were a mosaic with MDMV-A and BMV, occasional red local lesions and latent infection with MDMV-B, a latent infection with MCMV, and an immune response with WSMV. Reactions of viruses to 'Redland' sorghum were a mosaic and moderate redleaf symptoms with MDMV-A, occasional red local lesions with MDMV-B, a latent infection with MCMV, a mosaic with BMV, and an immune response with WSMV. Reactions of viruses to 'Parker' wheat were a mosaic with WSMV and BMV, a diffuse mosaic on inoculated leaves and occasionally a systemic diffuse mosaic with MCMV, and an immune response with MDMV-A and MDMV-B.

HOST RANGE STUDY. Kansas and Peru isolates of MCMV were used. Kansas isolates of MCMV were extracted from a corn field in Norton County, KS (field N1). The Peru isolate was provided by T. T. Hebert, North Carolina State University, Raleigh. Grass seeds were obtained from the Department

of Agronomy, Kansas State University, Manhattan; the United States Department of Agriculture, Soil Conservation Service, Plant Materials Center, Manhattan, KS; and from a few personal collections.

Isolates of MCMV were maintained on inbred line N28Ht corn. Seven to fourteen days after inoculation of N28Ht corn seedlings with MCMV, symptomatic tissue was ground in $KPO_4$ buffer with a mortar and pestle and utilized as the inoculum for grass seedlings. Dead leaves were removed from the plants at the time of inoculation and number of inoculated leaves were counted, except in some early tests. Grass seedlings were planted in 10 cm. diam. plastic pots. Ten or more plants were usually tested in each pot. All experiments were carried out in the greenhouse with temperatures ranging from 25° to 35° C. Time intervals between planting, inoculation, and assay varied. Most grass species were tested two or more times at different seasons with MCMV-K. Tests were repeated if there were an insufficient number of plants per pot, when inoculated leaf counts were absent, or if data varied in repeated trials.

Inoculated and newly formed grass leaves were assayed separately for MCMV by DID and bioassayed on N28Ht corn. Symptoms were recorded as they appeared. Virus positive N28Ht indicator plants were assayed for MCMV by DID, unless a previous positive serological reaction was recorded.

ASSAY AND DISTRIBUTION OF VIRUSES IN NATIVE GRASSES. During the 1977, 1978, and 1979 growing seasons, grass samples were collected within or near corn fields infested with MCMV. Samples were collected from corn fields in Republic County near Norway, KS. (fields R1 and R2), in Smith County near Gaylord, KS (fields S1 and S2), in Norton County near Almena, KS (field N1), and in Phillips County near Long Island, KS (fields P1 and P2). Samples were maintained under refrigeration and assayed, soon after collection,

on a differential host range, with MCMV serology using DID, or by both techniques. Serological assays for MCMV were conducted only with symptomatic grass tissue and N28Ht corn when infection resulted from bioassays. Identity of MDMV-B and WSMV from a representative number of samples were confirmed using ELISA. The presence of BMV was confirmed by DID. Grasses nearby MCMV infested corn fields were identified and their relative abundance and growth stage were recorded. A number of grass samples that could not be identified taxonomically during the seedling stages were potted and grown to maturity. Identifications were made using a key by T. M. Barkley (2) and by personnel in the Kansas State University Herbarium.

BAIT POT STUDIES. In 1978 and 1979, potted corn plants were placed in the grassy borders of MCMV infested corn fields. Polyfoam was tightly wrapped 5 cm. thick around clay pots (13 cm. diam.) and secured with binding tape. Pots were immersed in water and the foam layer was repeatedly compressed to replace the air spaces with water. Water-saturated pots were placed in a plastic bag, which was secured with a rubber band around the lip of the pot. The edge of the plastic bag was tucked inside the pot and slightly below the soil surface. N28Ht corn seedlings (7-10 days old) were transplanted into the soil and complete pots were placed in a pot coaster and distributed in the field. This self-contained unit permitted seedling growth and required minimal maintenance in the field for two or more weeks. To prevent rodent damage, a 3/8 in. mesh galvanized wire cage was placed over the seedlings.

Bait pots were placed by fields N1 and R1 in 1978, and by fields N1 and P1 in 1979. Pots were distributed and collected at ca. 2 week intervals. In 1978, 18 pots were placed along the border of field N1 during

each time interval and 6 pots were placed at field R1. In 1979, 8 pots were located at field N1 and 12 pots were located at field P1. Pots distributed on 29 June and 20 July 1979 at field N1 were placed ca. 20 to 40 ft. inside the corn field.

Pots recovered with seedlings were isolated in the greenhouse and sprayed with malathion. Plants were examined for virus symptoms and discarded after 3 weeks if no symptoms developed. Individual plants with virus symptoms were assayed for MCMV using DID and by bioassay, except pots exposed on 14-28 June 1978, where infected plants from individual pots were bulked for assays. A representative number of WSMV and MDMV-B infected plants were confirmed by ELISA in 1979.

## RESULTS

HOST RANGE STUDY. A total of 44 and 37 species representing 27 and 22 genera were tested as hosts for MCMV-K and MCMV-P, respectively. Results are listed in Table 1 and 2. All hosts with systemic symptoms were the same for both virus strains. Percentage of plants showing systemic symptoms was 90 to 100% with a few exceptions as indicated (Tables 1 and 2). 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 strains, and *Cynodon dactylon* (L.) Per. which was immune to MCMV-P but was locally infected with MCMV-K. Based on host susceptibility, these native grasses could be divided into 4 groups, viz., hosts with systemic symptoms, systemic latent hosts, infection of inoculated leaves only, and immune grasses.

The following grass species were hosts with systemic symptoms for both MCMV strains: *Andropogon scoparius* Michx., *Bromus japonicus* Thunb., *B. secalinus* L., *Digitaria sanguinalis* (L.) Scop., *Eragostis trichodes* (Nutt.) Nash., *Hordeum pusillum* Nutt., *H. vulgare* L. (var. 'Reno'), *Panicum miliaceum* L., *Setaria faberi* Herm., *S. italica* (L.) Beauv., *S. lutescens* (Weigel) Hubbard., *S. viridis* (L.) Beauv., and *T. aestivum* L. (var. 'Parker' and an unknown variety of durum and soft white wheat). *Hordeum vulgare* and *T. aestivum* exhibited systemic symptoms only occasionally under our test conditions.

Systemic but latent hosts for both strains included *Bromus tectorum* L., *E. trichodes*, and *S. bicolor* (var. 'Redland' and orange sorgo). Hegari (*S. bicolor*) was susceptible to MCMV-K and was not tested with MCMV-P. *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.

Grass species with infections of inoculated leaves only for both MCMV strains included *Agropyron desertorum* (Fisch.) Schult., *A. smithii* Rydb., *Andropogon gerardi* Vitman., *Avena sativa* L. (var. 'Wintok'), *Bouteloua curtipendula* (Michx.) Torr., *Cenchrus longispinus* (Hack.) Fern., *Echinochloa colonum* (L.) Link., *E. crusgalli* (L.) Beauv., *E. frumentacea* (Roxb.) Link., *Eleusine indica* (L.) Gaertn., *Hordeum jubatum* L., *Panicum virgatum* L., *Secale cereale* L. (var. 'Balboa'), *S. bicolor* (sudan grass), and *S. halepense*. Grasses locally infected with MCMV-K (and not tested with MCMV-P) included *A. sativa* (var. 'Andrew', 'Carolee', 'Mustang', and 'Tech'), *Bromus inermis* Leyss., *Buchloe dactyloides* (Nutt.) Engelm., *Dactylis glomerata* L., *H. vulgare* (var. 'Clayton' and 'Wong'), *Phalaris arundinacea* L., *Sorghastrum nutans* (L.) Nash., *S. bicolor* (var. 'Dekalb

BR54', Sumac Sorgo, and wild cane), *Tripsacum dactyloides* L., and *T. aestivum* (var. 'Anderson', 'Atlas', and 'Blueboy').

ASSAY AND DISTRIBUTION OF VIRUSES IN NATIVE GRASSES. No MCMV was detected in 230 grass samples collected from the field in 1977 (32 collections made), 1978 (16 collections), and 1979 (182 collections). However a total of 5, 35, and 64 samples were infected with BMV, WSMV, and MDMV-B, respectively (Table 3). Both WSMV and MDMV-B were recovered from *B. tectorum*, *D. sanguinalis*, *S. viridis*, *S. lutescens*, and *E. crusgalli*. *Panicum dichotomiflorum* was infected with MDMV-B and *B. inermis* was infected by BMV (Table 3). One *B. tectorum* and two *S. viridis* samples were doubly infected with WSMV and MDMV-B. Grass samples with no detectable viruses included 2 collections of *B. japonicus*, 2 of *C. longispinus*, 1 of *A. smithii*, 2 of *E. virginicus*, 1 of *Muhlenbergia bushii* Pchl. tide McGregor, 3 of *H. jubatum*, and 6 of *Z. mays* (volunteer corn). Most samples were collected based on mottle or mosaic appearance in the leaves. However, some healthy appearing collections were assayed for possible latent infections, especially in *B. tectorum* and a few other non-infected grass samples (see above). Symptomless samples were often bioassayed in groups of 10 or more plants.

Serological confirmation of MDMV-B in infected indicator plants was done by ELISA for 16 grass samples (4 of *B. tectorum*, 5 of *D. sanguinalis*, 1 of *P. dichotomiflorum*, 1 of *S. lutescens*, and 5 of *S. viridis*). These were collected in 3 counties on 5 different dates. WSMV was confirmed by ELISA for 10 samples (4 of *D. sanguinalis*, 1 of *E. crusgalli*, and 5 of *S. viridis*) collected in 3 counties on 2 different dates.

BAIT POT STUDIES. Results of 1978 studies show a large percentage of WSMV transmissions to corn bait pots from 14 to 26 June at field N1 (Table

5). Pots were not distributed after 26 June at which time MCMV infected corn plants were detected in numerous fields. In 1979, WSMV was transmitted to bait plants from 1 June to 20 July, at fields N1 and P1 (Table 5).

In 1979, a few corn bait plants were infected with MDMV-B throughout the growing season at field P1 (Table 5). A bait pot distributed on 29 June at field P1 had a double infection of MDMV-B and MCMV. Also, an infection of MCMV occurred when a bait pot was placed within field N1 on 20 July (Table 5). Six MDMV-B (at P1) and 11 WSMV (at N1 and P1) infected bait plants collected in 1979 were confirmed by ELISA; no MDMV-B was transmitted to bait pots at field N1.

Plants in bait pots placed at field N1 in 1978, from 17 April to 30 May, were severely damaged by rodents which accounts for the low recovery rate during that period. All bait plants distributed on 7 May 1979 were killed by frost. In other instances, survival of bait plants was attributed to excessive wet periods in 1979, resulting in seedling rots, and also severe grasshopper infestations.

## DISCUSSION

Nineteen and 15 grass species are reported here as systemically susceptible hosts for MCMV-K and MCMV-P, respectively. Three of these species, *S. lutescens*, *S. bicolor*, and *T. aestivum*, have previously been reported as hosts for MCMV-P (4,10) and *Panicum virgatum* and *S. halepense*, which were not systemically infected with either strain in these tests. Overall, host range responses to MCMV-K and MCMV-P inoculations were similar, with minor differences found among latent hosts.

Extensive surveys for MCMV infected grasses, adjacent to MCMV infested corn fields, were negative which suggest that MCMV does not overwinter in grass hosts. Of the grasses common to all sampling sites, only *B. japonicus* or *B. tectorum*, both winter annuals, could serve as overwintering hosts, yet no MCMV was recovered from field collected samples. All other grasses observed in MCMV infested areas were either summer annuals, non-hosts, or not common to all diseased areas. Seed transmission of MCMV was not detected in corn (3,4,10) or in *P. miliaceum* and *S. lutescens* (3).

The apparent absence of MCMV in winter annual grasses, lack of insect transmissions to bait stations early in the season, and persistence of MCMV in the same fields from year to year (30) suggests that MCMV is soil-borne in fields of continuous corn. However, further studies are needed to determine the soil-borne nature of MCMV.

The most likely source of WSMV infections in bait plants and annual grasses during June and July (Tables 4 and 5) is cultivated wheat as wheat curl mites would be migrating to other hosts when it was maturing. Bait plants also detected early transmittance (15 May-14 June) of MDMV-B (Table 5) and infections in annual grasses were found in late-July (Table 4). Source of MDMV-B in the early season is likely *B. tectorum* (see below); while mid-season virus source is corn.

During the growing season, MDMV-B was recovered from *B. tectorum, D. sanguinalis, E. crusgalli, P. dichotomiflorum, S. viridis,* and *S. lutescens.* These grasses have previously been reported as hosts in greenhouse tests (8,15,22,28), however, none have been reported infected under natural field conditions. *Panicum dichotomiflorum* is a new host for MDMV-B. Although early season bulked plant assays of *B. tectorum* were negative for MDMV-B (Table 4), such winter annuals could serve as overwintering hosts as

suggested by others (15,22). More recently, MDMV-B inoculated and healthy *B. tectorum*, transplanted in the field, survived the 1979-80 winter season. In the spring, MDMV-B was recovered only from inoculated plants and aphid colonies were present in April 1980 (Bockelman, unpublished data). This investigation is being continued.

Table 1. Reaction of grass species to the Kansas isolate of maize chlorotic mottle virus (MCMV-K).

| Scientific and Common Name of Test Plants | Stage of Growth at Inoculation[a] | Days from Planting to Inoculation[b] | Days from Inoculation to Assays[c] | Virus Symptoms[d] I[h] | Virus Symptoms[d] N[i] | Grass Serology[e] I | Grass Serology[e] N | Corn Bioassay[f] I | Corn Bioassay[f] N | Corn Serology[g] I | Corn Serology[g] N |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Agropyron desertorum* (Fisch.) Schult. | | 18 | 29 | CLL | NS | - | - | - | - | | |
| Crested Wheat grass | 3 to 4 | 33 | 56 | NS | NS | | - | | - | | |
| | 3 | 18 | 24 | CLL | NS | - | - | + | - | + | |
| | 5 to 6 | 90 | 41 | NS | NS | - | - | + | - | + | |
| | 4 | 30 | 28 | NS | NS | - | - | + | - | + | |
| *Agropyron repens* (L.) Beauv. | 4 | 36 | 36 | NS | NS | - | - | - | - | | |
| Quackgrass | | | | | | | | | | | |
| *Agropyron smithii* Rydb. | | 30 | 21 | CLL | NS | - | - | + | - | + | |
| 'Barton' Western Wheatgrass | 3 to 4 | 33 | 47 | NS | NS | | - | | - | | |
| | 2 to 3 | 22 | 37 | CLL | NS | - | - | + | - | + | |
| | 4 to 5 | 90 | 41 | CLL | NS | - | - | + | - | + | |
| *Andropogon gerardi* Vitman. | | 26 | 14 | CLL | NS | - | - | + | - | + | |
| 'Kaw' Big Bluestem | 4 | 20 | 21 | CLL | NS | - | - | + | - | + | |
| | 3 to 4 | 18 | 41 | CLL | NS | - | - | + | - | + | |
| | 5 | 30 | 29 | NS | NS | - | - | - | - | | |
| *Andropogon scoparius* Michx. | | 26 | 14 | CLL | NS | - | - | + | - | + | |
| 'Aldous' Little Bluestem | | | | | M(2/17)[j] | | + | | + | | |
| | 4 | 20 | 21 | CLL | M(3/21)[k] | - | + | - | + | | |
| | 5 | 22 | 37 | CLL | M(4/25)[k] | - | + | + | + | + | |

(Table 1 continued on next page)

Table 1 (continued)

| Scientific and Common Name of Test Plants | Stage of Growth at Inoculation[a] | Days from Planting to Inoculation[b] | Days from Inoculation to Assays[c] | Virus Symptoms[d] | | Grass Serology[e] | | Corn Bioassay[f] | | Corn Serology[g] | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | I[h] | N[i] | I | N | I | N | I | N |
| *Avena sativa* L. | | | | | | | | | | | |
| 'Andrew' Oats | 2 | 8 | 26 | NS | NS | - | - | - | - | | |
| 'Carolee' Oats | 2 | 9 | 29 | NS | NS | - | - | + | - | + | |
| 'Mustang' Oats | 2 | 9 | 21 | NS | NS | - | - | + | - | + | |
| | 2 | 9 | 29 | NS | NS | + | - | + | - | | |
| 'Tech' Oats | 2 | 9 | 29 | NS | NS | + | - | + | - | | |
| 'Wintok' Oats | 3 | 20 | 25 | NS | NS | - | - | + | - | + | |
| *Bouteloua curtipendula* (Michx.) Torr. | | 26 | 21 | CLL, CS | NS | - | - | + | - | + | |
| 'El Reno' Sideoats Grama | 4 to 5 | 20 | 13 | CLL | NS | - | - | + | - | + | |
| | 3 to 4 | 18 | 24 | CLL, CS | NS | - | - | + | - | + | |
| | 5 | 38 | 31 | NS | NS | - | - | + | - | + | |
| *Bouteloua gracilis* (HBK.) Lag. ex Steud. | | 36 | 28 | NS | NS | | - | | + | | + |
| Blue Gramagrass | 5 | 49 | 41 | NS | NS | | - | | + | | + |
| | 5 | 38 | 31 | NS | NS | - | - | + | - | + | |
| *Bromus inermis* Leyss. | | 30 | 15 | NS | NS | - | - | + | - | + | |
| Smooth Brome | 3 | 20 | 15 | CLL | NS | - | - | + | - | + | |
| *Bromus japonicus* Thunb. | 4 to 5 | 41 | 28 | DM | DM | + | - | + | + | | + |
| Japanese Brome | 3 | 20 | 35 | DM | DM | + | - | + | + | | + |
| *Bromus secalinus* L. | | 30 | 16 | DM | M | + | - | + | + | | + |
| Cheat | 3 | 20 | 15 | DM | M | + | + | + | + | | |

(Table 1 continued on next page)

Table 1 (continued)

| Scientific and Common Name of Test Plants | Stage of Growth at Inoculation[a] | Days from Planting to Inoculation[b] | Days from Inoculation to Assays[c] | Virus Symptoms[d] | | Grass Serology[e] | | Corn Bioassay[f] | | Corn Serology[g] | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | I[h] | N[i] | I | N | I | N | I | N |
| *Bromus tectorum* L. | 4 to 5 | 41 | 28 | DM | NS | + | - | + | + | | + |
| Downy Brome | 3 | 20 | 35 | DM | NS | + | - | + | + | | + |
| *Buchloe dactyloides* (Nutt.) Engelm. | 4 to 5 | 91 | 41 | CLL | NS | - | - | + | - | + | |
| Buffalo Grass | 3 | 36 | 28 | NS | NS | + | - | + | - | | |
| *Calamovilfa longifolia* (Hook.) Hack. | | 26 | 21 | CLL | NS | - | - | + | + | + | + |
| Prairie Sandreed | 4 to 5 | 33 | 57 | NS | NS | | - | | - | | |
| | 3 | 22 | 20 | CLL | NS | - | - | + | - | + | |
| *Cenchrus longispinus* (Hack.) Fern. | 2 to 3 | 12 | 25 | CLL | NS | - | - | + | - | + | |
| Sandbur | | | | | | | | | | | |
| *Cynodon dactylon* (L.) Pers. | | 26 | 21 | NS | NS | - | - | + | - | + | |
| Bermuda Grass | 6 to 8 | 25 | 31 | NS | NS | - | - | - | - | | |
| *Dactylis glomerata* L. | 3 to 4 | 25 | 26 | M | NS | - | - | + | - | + | |
| 'Sterling' Orchard Grass | | | | | | | | | | | |
| *Digitaria sanguinalis* (L.) Scop. | | 22 | 26 | CLL | DM | + | - | + | + | | + |
| Crabgrass | 5 to 6 | 30 | 19 | CLL | DM | + | + | + | + | | |
| | 4 to 6 | 44 | 25 | CLL | M | + | + | + | + | | |
| *Echinochloa colonum* (L.) Link | 4 | 18 | 15 | DM | NS | - | - | + | - | + | |
| 'Baldwin' Jungle Ricegrass | | | | | | | | | | | |
| *Echinochloa crusgalli* (L.) Beauv. | | 22 | 26 | CLL | NS | + | - | + | - | | |
| Barnyard Grass | 4 to 5 | 30 | 16 | CLL, CS | NS | - | - | + | - | + | |

(Table 1 continued on next page)

Table 1 (continued)

| Scientific and Common Name of Test Plants | Stage of Growth at Inoculation[a] | Days from Planting to Inoculation[b] | Days from Inoculation to Assays[c] | Virus Symptoms[d] I[h] | Virus Symptoms[d] N[i] | Grass Serology[e] I | Grass Serology[e] N | Corn Bioassay[f] I | Corn Bioassay[f] N | Corn Serology[g] I | Corn Serology[g] N |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Echinochloa frumentacea* (Roxb.) Link. | | 8 | 26 | NS | NS | - | - | - | - | | |
| 'Chiwapa' Japanese Millet | 4 | 18 | 24 | NS | NS | - | - | + | - | + | |
| *Eleusine indica* (L.) Gaertn. | 5 to 6 | 18 | 23 | NS | NS | - | - | + | - | + | |
| Goosegrass | 5 | 25 | 32 | NS | NS | - | - | - | - | | |
| *Elymus virginicus* L. | 9 | 53 | 36 | NS | NS | - | - | - | - | | |
| Virginia Wild Rye | | | | | | | | | | | |
| *Eragrostis trichodes* (Nutt.) Nash. | | 26 | 14 | CLL | NS | - | + | - | + | | + |
| 'Bend' Sand Lovegrass | | | | | M(1/15)[j] | | + | | + | | |
| | 4 to 5 | 20 | 21 | CLL | NS | + | - | + | + | | + |
| | 4 | 18 | 24 | CLL | NS | - | - | + | - | + | |
| | 4 to 5 | 30 | 30 | CLL | NS | + | - | + | + | | + |
| | | | | | M(3/23)[l] | | + | | + | | |
| *Festuca arundinacea* Schreb. | | 30 | 15 | NS | NS | - | - | - | - | | |
| Tall Fescue | 3 | 31 | 32 | NS | NS | - | - | - | - | | |
| *Hordeum jubatum* L. | 7 to 9 | 53 | 36 | CLL | NS | + | - | + | - | | |
| Foxtail Barley | | | | | | | | | | | |
| *Hordeum pusillum* Nutt. | 3 | 26 | 27 | M | M | + | + | + | + | | |
| Little Barley | | | | | | | | | | | |

(Table 1 continued on next page)

Table 1 (continued)

| Scientific and Common Name of Test Plants | Stage of Growth at Inoculation[a] | Days from Planting to Inoculation[b] | Days from Inoculation to Assays[c] | Virus Symptoms[d] | | Grass Serology[e] | | Corn Bioassay[f] | | Corn Serology[g] | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | I[h] | N[i] | I | N | I | N | I | N |
| *Hordeum vulgare* L. | | | | | | | | | | | |
| 'Clayton' Barley | 2 | 13 | 24 | NS | NS | - | - | + | - | + | |
| 'Reno' Barley | | 14 | 26 | CLL | NS | - | - | + | - | + | |
| | 2 | 13 | 24 | CLL | DM | + | + | + | + | | |
| 'Wong' Barley | 2 | 13 | 24 | NS | NS | + | - | + | - | | |
| *Panicum dichotomiflorum* Michx. | | 30 | 16 | CLL | NS | - | - | + | + | + | + |
| Fall Panicum | 3 to 4 | 19 | 14 | CLL | NS | - | - | + | + | + | + |
| | 4 | 18 | 24 | CLL | NS | - | - | + | - | + | |
| | 4 | 25 | 32 | CLL | NS | - | - | + | + | + | + |
| *Panicum miliaceum* L. | | 22 | 26 | CS | M | + | + | + | + | | |
| Broomcorn Millet | 5 | 30 | 16 | CS | M | + | + | + | + | | |
| *Panicum virgatum* L. | | 30 | 15 | CLL | NS | - | - | + | - | + | |
| 'Blackwell' Switchgrass | 3 | 18 | 18 | CLL | NS | + | - | + | - | | |
| | 3 | 18 | 24 | CLL | NS | + | - | + | - | | |
| *Phalaris arundinacea* L. | | 30 | 15 | NS | NS | - | - | + | - | + | |
| Reed Canary Grass | 3 to 4 | 20 | 15 | DM | NS | - | - | + | - | + | |
| *Poa pratensis* L. | 4 | 49 | 41 | NS | NS | | - | | - | | |
| Kentucky Bluegrass | 3 to 4 | 22 | 19 | NS | NS | - | - | - | - | | |
| *Secale cereale* L. | 4 | 20 | 25 | NS | NS | - | - | + | - | + | |
| 'Balboa' Rye | | | | | | | | | | | |

(Table 1 continued on next page)

Table 1 (continued)

| Scientific and Common Name of Test Plants | Stage of Growth at Inoculation[a] | Days from Planting to Inoculation[b] | Days from Inoculation to Assays[c] | Virus Symptoms[d] I[h] | Virus Symptoms[d] N[i] | Grass Serology[e] I | Grass Serology[e] N | Corn Bioassay[f] I | Corn Bioassay[f] N | Corn Serology[g] I | Corn Serology[g] N |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Setaria faberi* Herm. | | 30 | 19 | M | M | + | + | + | + | | |
| Giant Bristlegrass | 3 | 16 | 22 | M | M | | + | | + | | |
| *Setaria italica* (L.) Beauv. | 3 to 4 | 15 | 27 | CLL, CS | M | + | + | + | + | | |
| 'German strain R' Pearl Millet | | | | | | | | | | | |
| *Setaria lutescens* (Weigel) Hubbard | | 22 | 26 | CLL | M | + | + | + | + | | |
| Yellow Foxtail | 5 | 30 | 19 | CLL | M | - | - | + | + | + | + |
| | 3 | 17 | 23 | CLL | M | - | + | + | + | + | |
| *Setaria viridis* (L.) Beauv. | | 30 | 19 | CLL | M | + | - | + | + | | + |
| Green Foxtail | 5 | 18 | 24 | CLL | M | + | + | + | + | | |
| *Sorghastrum nutans* (L.) Nash. | 5 | 20 | 21 | DM | NS | - | - | + | - | + | |
| Indian Grass | | | | | | | | | | | |
| *Sorghum bicolor* (L.) Moench. | | | | | | | | | | | |
| 'Dekalb BR54' Sorghum | 3 | 13 | 24 | NS | NS | + | - | + | - | | |
| Hegari | 3 | 10 | 26 | NS | NS | - | - | + | + | + | + |
| Orange Sorgo | 3 | 10 | 26 | NS | NS | - | + | + | + | + | |
| 'Redland' Sorghum | 2 to 3 | 8 | 21 | NS | NS | - | - | + | + | + | + |
| Sumac Sorgo | 4 to 5 | 14 | 21 | NS | NS | + | - | + | - | | |
| Wildcane | | 22 | 26 | NS | NS | - | - | + | - | + | |
| | | 30 | 15 | NS | NS | - | - | + | - | + | |
| | 4 | 14 | 14 | CLL | NS | + | - | + | - | | |

(Table 1 continued on next page)

Table 1 (continued)

| Scientific and Common Name of Test Plants | Stage of Growth at Inoculation[a] | Days from Planting to Inoculation[b] | Days from Inoculation to Assays[c] | Virus Symptoms[d] | | Grass Serology[e] | | Corn Bioassay[f] | | Corn Serology[g] | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | I[h] | N[i] | I | N | I | N | I | N |
| (cont.) *Sorghum bicolor* (L.) Moench. | | | | | | | | | | | |
| Sudan Grass | 3 | 15 | 27 | CLL | NS | | - | | - | | |
| *Sorghum halepense* (L.) Pers. | | 30 | 16 | NS | NS | - | - | + | - | + | |
| Johnson Grass | 5 | 18 | 24 | NS | NS | - | - | + | - | + | |
| | 5 | 26 | 25 | NS | NS | - | - | + | - | + | |
| | 3 to 4 | 14 | 14 | NS | NS | - | - | + | - | + | |
| *Spartina pectinata* Link. | | 36 | 28 | NS | NS | | - | | + | | + |
| Prairie Cordgrass | 4 | 49 | 31 | NS | NS | | - | | + | | + |
| | 5 | 34 | 26 | CLL | NS | + | - | + | + | | + |
| *Tripsacum dactyloides* L. | 4 to 5 | 20 | 21 | M | NS | + | - | + | - | | |
| Gamagrass | | | | | | | | | | | |
| *Triticum aestivum* L. | | | | | | | | | | | |
| 'Anderson' Wheat | 2 | 9 | 21 | DM | NS | + | - | + | - | | |
| | 2 | 9 | 29 | DM | NS | + | - | + | - | | |
| 'Atlas' Wheat | 2 | 9 | 21 | DM | NS | + | - | + | - | | |
| | 2 | 9 | 29 | DM | NS | + | - | + | - | | |
| 'Blueboy' Wheat | 2 | 9 | 21 | DM | NS | + | - | + | - | | |
| | 2 | 9 | 29 | DM | NS | + | - | + | - | | |
| Durum Wheat | 2 | 10 | 25 | DM | DM | + | + | + | + | | |

(Table 1 continued on next page)

Table 1 (continued)

| Scientific and Common Name of Test Plants | Stage of Growth at Inoculation[a] | Days from Planting to Inoculation[b] | Days from Inoculation to Assays[c] | Virus Symptoms[d] I[h] | Virus Symptoms[d] N[i] | Grass Serology[e] I | Grass Serology[e] N | Corn Bioassay[f] I | Corn Bioassay[f] N | Corn Serology[g] I | Corn Serology[g] N |
|---|---|---|---|---|---|---|---|---|---|---|---|
| (cont.) *Triticum aestivum* L. | | | | | | | | | | | |
| 'Parker' Wheat | 2 | 8 | 26 | DM | DM | - | + | + | + | + | |
| | 2 | 10 | 20 | DM | NS | + | - | + | - | | |
| Soft White Wheat | 2 | 8 | 26 | DM | DM | + | + | + | + | | |

[a]The number of leaves present at the time of inoculation.

[b]The number of days from planting to inoculation.

[c]The number of days from inoculation to host serology and bioassay.

[d]Virus symptoms observed as they developed. Symbols: NS = no symptoms; CLL = chlorotic local lesions; CS = chlorotic striping; DM = diffuse mosaic; M = mosaic.

[e]Serological assay for MCMV by double immunodiffusion (DID) using test plant tissue.

[f]Bioassay of test plant tissue on N28Ht corn.

[g]Serological assay of N28Ht corn for MCMV using DID; if test plant serology was negative for MCMV and corn bioassay was positive for virus.

[h]I = Assays from inoculated leaf tissue of test plants.

[i]N = Assays of test plants *from* new leaf growth after inoculation with MCMV.

[j]Mosaic symptoms expressed 37 days after MCMV inoculation. Plants with mosaic symptoms were assayed at this time. The fraction denotes the number of plants with symptoms over the number of plants in the pot.

[k]The fraction denotes the number of plants with mosaic symptoms over the number of plants in the pot. Mosaic plants were bulked with a few symptomless plants for assays.

[l]The number of plants with mosaic symptoms over the number of plants in the pot. The new leaves of plants with mosaic symptoms were assayed separately from those with no symptoms.

Table 2. Reaction of grass species to the Peru isolate of maize chlorotic mottle virus (MCMV-P).

| Scientific and Common Name of Test Plants | Stage of Growth at Inoculation[a] | Days from Planting to Inoculation[b] | Days from Inoculation to Assays[c] | Virus Symptoms[d] | | Grass Serology[e] | | Corn Bioassay[f] | | Corn Serology[g] | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | I[h] | N[i] | I | N | I | N | I | N |
| *Agropyron desertorum* (Fisch.) Schult. | 3 | 19 | 23 | CLL | NS | + | - | + | - | | |
| Crested Wheatgrass | 5 | 32 | 28 | NS | NS | - | - | + | - | + | |
| *Agropyron repens* (L.) Beauv. | 3 | 36 | 36 | NS | NS | - | - | - | - | | |
| Quackgrass | | | | | | | | | | | |
| *Agropyron smithii* Rydb. | 4 | 32 | 31 | NS | NS | - | - | + | - | + | |
| 'Barton' Western Wheatgrass | | | | | | | | | | | |
| *Andropogon gerardi* Vitman. | 4 | 19 | 39 | CLL | NS | - | - | - | - | | |
| 'Kaw' Big Bluestem | 4 to 5 | 32 | 28 | CLL | NS | - | - | + | - | + | |
| *Andropogon scoparius* Michx. | 6 | 26 | 37 | CLL | NS | - | - | - | + | | + |
| 'Aldous' Little Bluestem | | | | | M(2/11)[l] | | + | | + | | |
| *Avena sativa* L. | 3 | 20 | 25 | NS | NS | - | - | + | - | + | |
| 'Wintok' Oats | | | | | | | | | | | |
| *Bouteloua curtipendula* (Michx.) Torr. | 3 to 4 | 19 | 23 | CLL | NS | - | - | + | - | + | |
| 'El Reno' Sideoats Grama | 5 | 39 | 29 | CLL | NS | - | - | + | - | + | |
| *Bouteloua gracilis* (HBK.) Lag. ex Steud. | 4 | 39 | 29 | NS | NS | - | - | + | - | + | |
| Blue Gramagrass | | | | | | | | | | | |
| *Bromus japonicus* Thunb. | 4 to 5 | 41 | 28 | DM | DM | + | + | + | + | | |
| Japanese Brome | 3 | 20 | 35 | DM | DM | + | - | + | + | | + |
| *Bromus secalinus* L. | 4 | 28 | 29 | DM | M | + | + | + | + | | |
| Cheat | | | | | | | | | | | |

(Table 2 continued on next page)

Table 2 (continued)

| Scientific and Common Name of Test Plants | Stage of Growth at Inoculation[a] | Days from Planting to Inoculation[b] | Days from Inoculation to Assays[c] | Virus Symptoms[d] | | Grass Serology[e] | | Corn Bioassay[f] | | Corn Serology[g] | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | I[h] | N[i] | I | N | I | N | I | N |
| *Bromus tectorum* L. | 4 to 5 | 41 | 28 | DM | NS | - | - | + | + | + | + |
| Downy Brome | 3 | 20 | 35 | DM | NS | - | - | + | + | + | + |
| *Calamovilfa longifolia* (Hook.) Hack. | 4 | 39 | 29 | NS | NS | - | - | + | - | + | |
| Prairie Sandreed | | | | | | | | | | | |
| *Cenchrus longispinus* (Hack.) Fern. | 3 to 4 | 12 | 24 | CLL | NS | - | - | + | - | + | |
| Sandbur | 4 to 5 | 27 | 25 | NS | NS | - | - | + | - | + | |
| *Cynodon dactylon* (L.) Per. | 6 to 8 | 25 | 31 | NS | NS | - | - | - | - | | |
| Bermuda Grass | | | | | | | | | | | |
| *Digitaria sanguinalis* (L.) Scop. | 4 | 29 | 26 | DM | NS | - | - | + | - | + | |
| Crabgrass | 4 to 6 | 44 | 25 | CLL | M | + | + | + | + | | |
| *Echinochloa colonum* | 3 | 27 | 25 | DM | NS | + | - | + | - | | |
| 'Baldwin' Jungle Ricegrass | | | | | | | | | | | |
| *Echinochloa crusgalli* (L.) Beauv. | 4 to 5 | 22 | 35 | NS | NS | - | - | - | - | | |
| Barnyard Grass | 4 | 27 | 25 | NS | NS | + | - | + | - | | |
| *Echinochloa frumentacea* (Roxb.) Link. | 4 | 19 | 23 | NS | NS | - | - | + | - | + | |
| 'Chiwapa' Japanese Millet | | | | | | | | | | | |
| *Eleusine indica* (L.) Gaertn. | 5 to 6 | 19 | 23 | NS | NS | - | - | + | - | + | |
| Goosegrass | 6 | 26 | 31 | NS | NS | - | - | + | - | + | |
| *Elymus virginicus* L. | 9 | 53 | 36 | NS | NS | - | - | - | - | | |
| Virginia Wild Rye | | | | | | | | | | | |

(Table 2 continued on next page)

Table 2 (continued)

| Scientific and Common Name of Test Plants | Stage of Growth at Inoculation[a] | Days from Planting to Inoculation[b] | Days from Inoculation to Assays[c] | Virus Symptoms[d] | | Grass Serology[e] | | Corn Bioassay[f] | | Corn Serology[g] | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | I[h] | N[i] | I | N | I | N | I | N |
| *Eragrostis trichodes* (Nutt.) Nash. | 4 | 19 | 23 | CLL | NS | - | - | + | + | + | + |
| 'Bend' Sand Lovegrass | 4 to 5 | 32 | 31 | CLL | M(9/15)[k] | + | - | + | + | | + |
| *Festuca arundinacea* Schreb. | 3 | 32 | 31 | NS | NS | - | - | - | - | | |
| Tall Fescue | | | | | | | | | | | |
| *Hordeum jubatum* L. | 7 to 9 | 53 | 36 | CLL | NS | + | - | + | - | | |
| Foxtail Barley | | | | | | | | | | | |
| *Hordeum pusillum* Nutt. | 3 | 26 | 27 | M | M | + | + | + | + | | |
| Little Barley | | | | | | | | | | | |
| *Hordeum vulgare* L. | 2 | 13 | 24 | CLL | DM | + | + | + | + | | |
| 'Reno' Barley | 3 | 20 | 25 | CLL | NS | + | - | + | - | | |
| *Panicum dichotomiflorum* Michx. | 4 to 5 | 19 | 23 | CLL | NS | - | - | + | - | + | |
| Fall Panicum | | | | | | | | | | | |
| *Panicum miliaceum* L. | 4 | 15 | 26 | CLL, CS | M | + | + | + | + | | |
| Broomcorn Millet | | | | | | | | | | | |
| *Panicum virgatum* L. | 3 | 19 | 23 | CLL | NS | + | - | + | - | | |
| 'Blackwell' Switchgrass | | | | | | | | | | | |
| *Poa pratensis* L. | 4 | 39 | 29 | NS | NS | - | - | - | - | | |
| Kentucky Bluegrass | | | | | | | | | | | |
| *Secale cereale* L. | 4 | 20 | 25 | NS | NS | - | - | + | - | + | |
| 'Balboa' Rye | | | | | | | | | | | |

(Table 2 continued on next page)

Table 2 (continued)

| Scientific and Common Name of Test Plant | Stage of Growth at Inoculation[a] | Days from Planting to Inoculation[b] | Days from Inoculation to Assays[c] | Virus Symptoms[d] | | Grass Serology[e] | | Corn Bioassay[f] | | Corn Serology[g] | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | I[h] | N[i] | I | N | I | N | I | N |
| *Setaria faberi* Herm. | 3 | 17 | 21 | M | M | | + | | + | | |
| Giant Bristle grass | | | | | | | | | | | |
| *Setaria italica* (L.) Beauv. | 4 | 27 | 29 | M | M | + | + | + | + | | |
| 'German strain R' Pearl Millet | | | | | | | | | | | |
| *Setaria lutescens* (Weigel) Hubbard | 3 | 17 | 21 | CLL, CS | M | - | + | + | + | + | |
| Yellow Foxtail | | | | | | | | | | | |
| *Setaria viridis* (L.) Beauv. | 5 to 6 | 19 | 23 | DM | M | + | + | + | + | | |
| Green Foxtail | | | | | | | | | | | |
| *Sorghum bicolor* (L.) Moench. | | | | | | | | | | | |
| Orange Sorgo | 3 | 10 | 24 | NS | NS | - | - | + | + | + | + |
| 'Redland' Sorghum | 2 to 3 | 7 | 20 | NS | NS | + | - | + | + | | + |
| Sudan Grass | 3 to 4 | 13 | 31 | CLL | NS | | - | | - | | |
| *Sorghum halepense* (L.) Pers. | 5 | 19 | 23 | NS | NS | - | - | + | - | + | |
| Johnson Grass | 5 | 26 | 25 | NS | NS | - | - | + | - | + | |
| *Spartina pectinata* Link. | 5 to 6 | 39 | 29 | CLL | NS | + | - | + | - | + | |
| Prairie Cordgrass | | | | | | | | | | | |
| *Triticum aestivum* L. | | | | | | | | | | | |
| Durum Wheat | 2 | 10 | 25 | CLL | DM | + | + | + | + | | |
| 'Parker' Wheat | 2 | 8 | 26 | DM | DM | + | + | + | + | | |
| | 3 | 20 | 25 | DM | DM | + | - | + | - | | |

(Table 2 continued on next page)

Table 2 (continued)

| Scientific and Common Name of Test Plant | Stage of Growth at Inoculation[a] | Days from Planting to Inoculation[b] | Days from Inoculation to Assays[c] | Virus Symptoms[d] I[h] | Virus Symptoms[d] N[i] | Grass Serology[e] I | Grass Serology[e] N | Corn Bioassay[f] I | Corn Bioassay[f] N | Corn Serology[g] I | Corn Serology[g] N |
|---|---|---|---|---|---|---|---|---|---|---|---|
| (cont.) *Triticum aestivum* L. | | | | | | | | | | | |
| Soft White Wheat | 2 | 8 | 26 | DM | DM | + | + | + | + | | |

[a]The number of leaves present at the time of inoculation.

[b]The number of days from planting to inoculation.

[c]The number of days from inoculation to host serology and bioassay.

[d]Virus symptoms observed as they developed. Symbols: NS = no symptoms; CLL = chlorotic local lesions; CS = chlorotic striping; DM = diffuse mosaic; M = mosaic.

[e]Serological assay for MCMV by double immunodiffusion (DID) using test plant tissue.

[f]Bioassay of test plant tissue on N28Ht corn.

[g]Serological assay of N28Ht corn for MCMV using DID; if test plant serology was negative for MCMV and corn bioassay was positive for virus.

[h]I = Assays from inoculated leaf tissue of test plants.

[i]N = Assays of test plants from new leaf growth after inoculation with MCMV.

[j]Mosaic symptoms expressed 37 days after MCMV inoculation. Plants with mosaic symptoms were assayed at this time. The fraction denotes the number of plants with symptoms over the number of plants in the pot.

[k]The fraction denotes the number of plants with mosaic symptoms over the number of plants in the pot. Mosaic plants were bulked with a few symptomless plants for assays.

[l]The number of plants with mosaic symptoms over the number of plants in the pot. The new leaves of plants with mosaic symptoms were assayed separately from those with no symptoms.

Table 3. Summary of virus assays of grasses collected in 1977, 1978, and 1979.

| Grasses Sampled | Number of Samples Assayed for Virus Identification[1] | | | |
|---|---|---|---|---|
| | BMV | WSMV | MDMV-B | NR |
| *Bromus inermis* | 5 | | | 18 |
| *Bromus tectorum* | | 2[2] | 23[2] | 61 |
| *Digitaria sanguinalis* | | 14 | 13 | 11 |
| *Echinochloa crusgalli* | | 1 | 1 | 1 |
| *Panicum dichotomiflorum* | | | 5 | |
| *Setaria lutescens* | | | 3 | 4 |
| *Setaria viridis* | | 18[2] | 19[2] | 17 |
| *Zea mays* (volunteer) | | | | 6 |
| Six other grass species[3] | | | | 11 |
| Totals | 5 | 35 | 64 | 129 |

[1]Virus identification by bioassays and serology (DID for BMV and MCMV infected samples; ELISA for WSMV and MDMV-B). Abbreviations: BMV = Brome mosaic virus; WSMV = wheat streak mosaic virus; MDMV-B = maize dwarf mosaic, strain B; NR = no virus detected from bioassays or MCMV serology.

[2]Three samples with double infections of WSMV and MDMV-B.

[3]Numbers and species given in the text.

Table 4. Identification of viruses by differential host range assays from the three most frequently sampled grasses in 1979, from four county locations.

| Sampling | *Bromus tectorum* L.[1] | | | | *Digitaria sanguinalis* (L.) Scop. | | | | *Setaria viridis* (L.) Beauv. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Date | Norton | Phillips | Republic | Smith | Norton | Phillips | Republic | Smith | Norton | Phillips | Republic | Smith |
| 3/21 | 4H[2] | 1H | | | | | | | | | | |
| 4/18 | | 8H | 9H | 3H | | | | | | | | |
| 5/7 | 1M,5H | 1M,1W,8H | 6H | | | | | | | | | |
| 5/16 | | 3M,1MW,1H | | | | | | | | | | |
| 6/1 | | 1M | | 2M,1H | | | | | 1W | | | |
| 6/20 | | | | | 1H | | 1H | | 6H | | | |
| 7/20 | | | | | 10W | 1M,1W | | | 9W,2H | 1M | | |
| 8/23 | | | | | 2M,1H | 3M | | 2M | 2W,1H | 3M | | 1W,2MW |
| 8/29 | | | | | | | | | | 2M | | |
| 9/12 | | 4H | | | | | | | | | | 6M |
| 9/21 | | 4H | | | | 4M | | | | 5M | | |
| 10/8 | | 13M,6H | | | | | | | | | | |

[1]Individual samples collected 4/18, 9/12, 9/21, and 10/8 from Phillips Co., and 4/18 from Smith Co. could not be differentiated in the seedling stage as *B. tectorum* L. or *B. japonicus* Thunb., however, the species observed in the fields at heading time were almost entirely *B. tectorum* L. Representative samples collected 3/21 in Norton Co. and Phillips Co., and 4/18 in Republic Co. were potted, grown in the greenhouse, and identified as *B. tectorum* L. All other samples were identified as *B. tectorum* L. from the field samples.

[2]Symbols: H = healthy sample, no virus recovered; M = maize dwarf mosaic virus strain B infected sample; W = wheat streak mosaic virus infected sample. The number preceeding the symbol indicates the number of samples in that category.

Table 5. Virus identification and frequency of virus infection in bait plants (inbred N28Ht corn) placed at two county locations in 1978 and 1979.

| | Phillips County[1] | | | | Norton County[2] | | |
|---|---|---|---|---|---|---|---|
| | | Number of Pots and Plants with Virus[4] | | | | Number of Pots and Plants with Virus[4] | |
| Period in Field | Pots Recovered[3] | MDMV-B | WSMV | MCMV | Pots Recovered[3] | WSMV | MCMV |
| 4/17/78 to 5/1/78 | | | | | 0 | | |
| 5/1/78 to 5/16/78 | | | | | 4 | | |
| 5/16/78 to 5/30/78 | | | | | 1 | | |
| 5/30/78 to 6/14/78 | | | | | 18 | | |
| 6/14/78 to 6/26/78 | | | | | 18 | 18/62 | |
| 5/7/79 to 5/16/79 | 0 | | | | 0 | | |
| 5/16/79 to 6/1/79 | 12 | 1/1 | | | 8 | | |
| 6/1/79 to 6/14/79 | 12 | 1/1 | 3/5 | | 8 | | |
| 6/14/79 to 6/29/79 | 11 | | 7/12 | | 8 | 5/6 | |
| 6/29/79 to 7/20/79 | 10 | 1/1[5] | 4/7 | 1/1[5] | 8 | 4/5 | |
| 7/20/79 to 8/7/79 | 5 | | | | 8 | | 1/1 |
| 8/23/79 to 9/12/79 | | | | | 4 | | |
| 9/21/79 to 10/8/79 | 9 | 3/5 | | | | | |

[1]Twelve bait pots, 5 to 6 plants per pot, were distributed at a Phillips Co. corn field for each time interval. No trials were attempted in 1978.

[2]Eighteen and eight bait pots, 5 to 6 plants per pot, were distributed for each time interval in 1978 and 1979, respectively, at a Norton Co. corn field.

[3]Number of pots recovered with live plants at collection time. Blank spaces indicate that no pots were distributed.

[4]The number of bait pots with symptomatic plant(s) / total number of virus infected plants. Viruses were identified by bio- and sero- assays as maize dwarf mosaic virus, strain B (MDMV-B), wheat streak mosaic virus (WSMV), or maize chlorotic mottle virus (MCMV).

[5]Represent the same sample with a double infection of MDMV-B and MCMV.

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# EPIDEMIOLOGY OF MAIZE CHLOROTIC MOTTLE VIRUS AND CORN LETHAL NECROSIS IN NORTH CENTRAL KANSAS

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## ABSTRACT

BOCKELMAN, D. L. 1980. Epidemiology of maize chlorotic mottle virus and corn lethal necrosis in north central Kansas. M.S. Thesis. Kansas State Univ., Manhattan, Kansas.

Assays and field observations of corn, *Zea mays* L., during a 3-year period, indicated that maize chlorotic mottle virus (MCMV), was prevalent in three major irrigation districts in Kansas. MCMV was initially detected in late June and infected plants were randomly distributed in the field. Maize dwarf mosaic virus, strain B was also commonly detected in June. Later in the season, as numbers of corn rootworn beetles increased, the incidence of corn lethal necrosis increased. Active virus transmissions by field collected adults of *Diabrotica virgifera* LeConte and *D. undecimpunctata* Mannerheim ranged from 0 to 24%. However, in enzyme-linked immunosorbent assay (ELISA) serologic tests, beetles with MCMV ranged from 8 to 100%. No transmission occurred with adult *Colapsis flavida* Say, even though MCMV-ELISA positives were found in extracts of several adults and a larva.

Corn lethal necrosis (CLN) is caused by a combination of two viruses, maize chlorotic mottle virus (MCMV) and maize dwarf mosaic virus (MDMV) or wheat streak mosaic virus (WSMV) (13). MCMV was first described on corn, *Zea mays* L., in Peru in 1973 (4,9) and in the United States in 1976 as a virus component of CLN (13). In 1978, MCMV and CLN were found in several areas of north central Kansas (16) and south central Nebraska (8). Disease symptoms and vectors of CLN incitants have been previously reported (4,12, 13,17).

## MATERIALS AND METHODS

VIRUS SURVEYS AND ASSAYS. Corn fields in north central Kansas were surveyed for MCMV and incidence of CLN in 1977, 1978, and 1979. Corn samples were refrigerated until bioassayed on inbred N28Ht corn plants or on a differential host range (16) comprised of inbred N28Ht corn, sorghum (*Sorghum bicolor* (L.) Moench. var. 'Asgrow Bugoff' and 'Dekalb E59+', or 'Redland'), and wheat (*Triticum aestivum* L. var. 'Parker'), seroassayed by MCMV double immunodiffusion (DID), or assayed by both methods as previously described (1).

Roots and leaves of symptomless corn plants were collected in Norton (field N1) and Phillips Counties (field P1) on 7 June, 14 June (no collections at field N1), 21 and 29 June in 1979 for ascertaining initial MCMV infections. Root and leaf tissue of 5 plants were bioassayed collectively on N28Ht corn. Leaf samples collected on 1 June at fields N1 and P1, and plants with mottled leaves collected on other dates were assayed singly.

Percentages of plants with CLN and mosaic symptoms at field N1 in 1978 were determined for 50 plants at each of two locations in the field of 15 different hybrids, planted in 12-row strips. In 1979, percentages of plants with CLN and mosaic symptoms were determined for 7 hybrids at field N1, from 2 rows approximately 373 m long. Infected plants were located within the rows by the number of paces taken. At field P1 and a corn field in Smith County (field S1), percentage of virus infections were recorded in 5 locations within each field from 2 rows approximately 46 m long. Percent virus infections were recorded in the same sites over the season and representative samples were bio- and sero-assayed for virus identification. Area of survey sites in fields were approximated by paces taken in rows. In 1977, surveys were conducted on 13 and 14 July in 4 counties and on previous dates at field N1.

TRANSMISSION AND ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) OF BEETLES, PUPAE, LARVAE, AND EGGS. In 1978 and 1979, adults of the southern (*Diabrotica undecimpunctata* Mannerheim) and western (*D. virgifera* LeConte) corn rootworm, and grape colapsis (*Colapsis flavida* Say) were collected from CLN or MCMV infested corn fields using an aspirator connected to a 500 ml Erlenmeyer flask. Beetles were refrigerated, transferred (ca. 24 hr.) to 1-2 week old N28Ht corn plants, and allowed to feed for 25-30 hr. Beetles were removed and placed in test tubes containing 0.75 ml of tissue extraction buffer (TEB) consisting of 0.01 M K/Na phosphate buffered Saline (0.085%), 2% polyvinylpyrrolidine (mol. wt. 40,000), and 0.5% Tween 20, and stored at -5°C.

For MCMV ELISA tests (7,15), individual beetles from feeding studies and other beetles collected and preserved by freezing, were thawed and

crushed with a mortar and pestle. In addition, two larvae and one grape colapsis pupa, and two corn rootworm pupae, collected in mid-July in Smith County were included. Western corn rootworm beetles collected on 30 July, 7 August, and 23 August 1979 were sexed prior to ELISA by comparing size of antennal segments (2). Healthy and MCMV infected corn tissue were extracted in TEB (1:50, w/v). Reactions in ELISA tests were read at an absorbance of 405 nm in a Gilford 250 spectrophotometer. Readings with an $A_{405}$ of 0.3 or greater were considered positive for MCMV. In 1977, groups of 10 western corn rootworm beetles were crushed in potassium phosphate buffer ($KPO_4$), pH 7.0 and assayed only by MCMV-DID serologic tests. In 1978, beetles collected in Phillips County (field P2) were used for virus transmissions and ELISA serologic tests.

In 1979, beetles were collected from the same area in field N1 on each sampling date. Beetles were also collected from fields in Smith County. The number of beetles used in transmission studies varied as follows: 1-2, 3-4, 4-5, and 3-5 beetles per pot of corn, respectively, for 10 July, 30 July, 7 August, and 23 August 1979. One beetle per corn pot was used in 1978.

Extracts of 53 beetles tested in ELISA, were selected and assayed on N28Ht corn. In addition, 14 female western corn rootworm beetles were collected on 29 August 1979 in field N1. Eggs were extracted and homogenized separately from remaining body tissues with a tissue homogenizer in 0.4 ml of TEB and seroassayed. Egg number and relative egg maturity were recorded. Controls consisting of MCMV infected corn tissue at 1:50 and 1:100 dilutions of tissue to TEB, and healthy corn tissue at a 1:50 dilution were included in ELISA.

# RESULTS

VIRUS SURVEYS AND ASSAYS. 1977 Surveys. At field N1, MCMV was detected by DID in 22 of 44 samples collected on 3 dates (28 June, 14 and 27 July). Symptoms of MCMV were first detected in corn at the 10 to 11 leaf stage on 28 June; 5 of 8 samples were positive. Two corn plants in field N1 contained WSMV. Bioassays of 14 plants, including some with CLN-like symptoms, detected only MCMV. However, 'Redland' sorghum used in these bioassays would not have detected MDMV, strain B (MDMV-B), which was later shown to be the second most commonly occurring virus in known CLN affected corn fields.

Samples collected on 13 and 14 July 1977 in 2, 3, 7, and 2 fields in Cloud, Norton, Republic, and Smith Counties, respectively, were assayed by MCMV-DID. MCMV was detected in 13 of 14 samples collected in Norton County. In three corn fields in Republic County, 16 of 31 samples were positive for MCMV, while 14 samples from four other fields were negative. On a previous sampling data (8 July), one MCMV infested corn field yielded 10 positives in 17 samples tested. One field in Smith County, had 1 MCMV positive of 4 samples tested and 7 samples from other fields were negative. No MCMV was detected in 13 samples collected in Cloud County.

1978 Surveys. At field N1, an estimated 10% of the corn plants (at the 6-7 leaf stage of growth on 14 July) had mosaic symptoms. Serologic tests and bioassays detected MCMV in 1 plant of 21 tested (14 June). On 26 June, an estimated 20 to 30% of the corn plants exhibited systemic symptoms and 8 of 56 samples were positive for MCMV. Of these indicator plants used (N28Ht corn, 'Parker' Wheat, and 'Redland' sorghum), only

corn became infected and some developed CLN (these were MCMV positive in serologic tests), with others only mosaic symptoms (and negative for MCMV) were observed. Extracts prepared from several of these plants were assayed on 'Asgrow Bugoff' resulting in redleaf symptoms. Inoculated 'Dekalb E59+' seedlings remained symptomless. These results confirmed the presence of MDMV-B (16).

At field N1, CLN symptoms were first observed on 13 July. CLN and mosaic percentages were recorded (on 13 July, 1 and 18 August) of 15 hybrids (Table 1). Leaf samples were taken on 13 July and 10 of 10 CLN plants and 54 of 148 samples with mosaic symptoms were serologically positive for MCMV. As the season progressed, increases in percentage of plants exhibiting CLN were evident in all hybrids.

1979 Surveys. Bioassays of 30 single plant samples and 22 groups of 5 plants each collected on 1, 7, and 21 June at field N1 were negative. On June 29, symptomless plants were collected and 2 of 10 extracts (each comprised of 5 plants) were infectious and transmitted MCMV to corn seedlings. In addition, 6 single symptomatic plants collected on 29 June were bioassayed and 4 MDMV-B and 2 MCMV infections were found.

On 10 July, 96 plants with mosaic symptoms were counted in six 2-row lengths of the field (373 m), and leaf samples were collected. In serologic tests (DID), 91 samples were positive for MCMV. Bioassays of 15 MCMV serologic positive samples failed to detect presence of a second virus; while 2 MCMV serologic negative samples were positive for MDMV-B. On 7 August, 29 mosaic plants were positive in MCMV serologic tests and 9 samples were bioassayed and only MCMV was found.

Percentages of virus infections were taken on 10, 20, 30 July, and 7, 29 August in several areas of field N1 (Table 2). Since MDMV-B

infections were not abundant, all plants with mosaic symptoms were considered MCMV induced. MCMV infections averaged 0.8% on 10 July and 8.8 % on 29 August. On 10 July, initial MCMV infections appeared in a random distribution in field N1 (Fig. 1). In subsequent surveys, the number of diseased plants increased and clustering of infected plants was evident (Fig. 2 and 3). Figure 4 shows increased clustering of MCMV infected plants in the same 2-rows for 5 survey periods. New infected plants were generally located near previous infections, however, some infected plants were not immediately adjacent to known infected plants sites.

At field P1, nonsymptomatic plants were collected on 1, 7, 14, and 21 June. Assays of 26 single plants and 18 groups of 5 plants each were negative. On 29 June, 14 mosaic plants were infected by MDMV-B and MCMV was found in 2 of 5 extracts prepared from 25 symptomless plants. MDMV-B infections averaged 4.7% in this field.

On 10 July at field P1, 10 of 25 plants were serologically positive for MCMV and 4 bioassayed samples contained only MCMV. Four of the serologic negative samples were infected by MDMV-B. Percent of infected plants with mosaic symptoms in the field was 9.1%. On 7 August, 2.3% had CLN symptoms. At that date symptoms of MDMV-B infections were faint. However, pronounced MCMV symptoms were observed in 8.7% of plants in the field. To confirm those MCMV infections, 39 samples were seroassayed and 38 were positive. Ten bioassayed samples failed to detect a second virus, and the single MCMV negative sample was MDMV-B infected. On 29 August, 2.0% of the plants showed CLN and 13.6% mosaic symptoms.

Incidence of CLN in field S1 on 10, 20, and 30 July, and 23 August in 1979 is included in Table 3. On 10 July, nearly all of the plants

exhibited mosaic symptoms indicative of MDMV-B. Three plants with CLN symptoms were positive for MCMV (DID) and 2 bioassays also indicated presence of MDMV-B.

Nine other fields in Phillips and Smith Counties and one field in Republic County also contained CLN. Assays of samples showed infections caused by MCMV and MDMV-B.

TRANSMISSION AND ELISA OF BEETLES, PUPAE, LARVAE, AND EGGS. In 1977, two groups of 10 corn rootworm beetles from field N1 and R1 were positive in MCMV-DID tests, and one group of 10 corn rootworm beetles from a healthy field in Republic County was negative.

In greenhouse transmission tests, one of twenty-one adult corn rootworm beetles, collected at field P2 on 14 July 1978, transmitted MCMV to N28Ht corn. Also, single beetles tested in MCMV-ELISA showed that four beetles were MCMV positive, including the single beetle that transmitted MCMV to corn. Average ELISA absorbance readings for positive and negative beetle extracts were 0.38 and 0.14, respectively. None of 11 adult corn rootworm beetles collected at field P2 on 18 August 1978 transmitted MCMV to corn, but six beetles assayed positive in ELISA. Average absorbance readings were 0.41 (positive) and 0.18 (negative). Four beetles collected from a healthy field of corn had an average absorbance reading of 0.11. Controls consisting of MCMV infected corn tissue at a 1:50 dilution averaged 0.64.

The 1979 virus transmission and ELISA test results for corn rootworm beetles collected at three locations are presented in table 3. Beetles transmitted MCMV to a total of 36 of 72 pots of corn and 1, 5, 13, and 17 pots contained 4, 3, 2, and 1 infected plants per pot, respectively. Transmission rates varied from 0 to 23.9%; this represents minimum rates

since only 1 beetle per pot was considered viruliferous. As the season progressed, the percentage of beetles with MCMV and beetle transmissions increased. Final percent MCMV infections were higher at fields S1 and S3 than at field N1 (Table 3), probably due to higher corn rootworm populations (estimated beetle populations at fields S1 and S3 were 5 per plant and less than 1 per plant at field N1). On 23 August, beetles were difficult to find at field S3 and those collected fed poorly on corn, resulting in no virus transmission to corn.

In ELISA, average absorbance readings for MCMV positive beetles collected at all locations and time periods in 1979 were 0.60 (10 July), 0.67 (20 July), 0.87 (30 July), and 0.86 (7 August); negative beetles were 0.23 (10 July), 0.15 (20 July), 0.20 (30 July), and 0.28 (7 August). All beetle samples collected on 23 August were positive and showed values at 3.18. Virus infected corn tissue (N28Ht) and healthy corn gave absorbance readings of 2.41 and 0.10, respectively, in a 1:50 (w/v) extraction. At least one beetle per pot was positive in MCMV-ELISA where beetles transmitted MCMV to corn plants. Two corn rootworm pupae were negative (0.10).

No MCMV transmission occurred with 14 grape colapsis beetles collected on four dates. However, most of these beetles did not feed well on N28Ht corn. Three grape colapsis beetles tested in ELISA were positive (0.65) and 29 were negative (0.10). Grape colapsis beetles were common in all MCMV infested fields observed in 1979 and were first observed at field P1 on 21 June. One grape colapsis larva was positive in ELISA with an absorbance reading of 0.94 while one pupa and larva was negative with 0.23 and 0.18 readings, respectively.

For collections made on 30 July and 7 August, average ELISA values

for extracts of male (m) and female (f) rootworm beetles were similar, viz., absorbance readings of 0.68 (m) and 0.79 (f), and 0.74 (m) and 0.82 (f), respectively, were realized. However, beetle collections of 23 August differed; 1.24 (m) and 3.31 (f). Percentages of female beetles collected on 30 July, 7 August, and 23 August were 71%, 59%, and 84%, respectively. No differences were found in the percentage of males and females with virus, e.g., positive males and females for collections of 30 July, 7 and 23 August were 82% (m) and 86% (f), 100% (m) and 92% (f), and 100% (m and f), respectively. No effort was made to determine if male or female beetles transmitted MCMV more efficiently.

The number of MCMV positive / negative in bioassays of 53 beetles extracts were 0/6 (0.1-0.3), 2/5 (0.3-0.4), 18/7 (0.4-0.9), and 15/0 (>0.9). Beetle extracts with ELISA values above 0.3 were infectious on N28Ht corn plants and no MCMV was transmitted with beetle extracts below 0.3 absorbance values. All symptomatic assay plants tested positive in serologic-DID tests.

None of the egg extracts were positive in ELISA, i.e., values below 0.3. However, one extract of a female beetle (minus eggs) was positive (0.69) while five other extracts were between 0.2 and 0.3. In these tests, average values for 1:50 and 1:100 dilutions of infected corn tissue were 0.34 and 0.16, respectively. A 1:50 dilution of healthy corn tissue was 0.10. Beetle eggs were found in a variety of developmental stages with many eggs being only partially developed. The average number of eggs per female found in 6 beetles with fully developed eggs was 96.

# DISCUSSION

Surveys conducted in this study and by Uyemoto, et al. (16) showed that MCMV and CLN occurred in several areas of north central Kansas, including Norton, Osborne, Phillips, Republic, and Smith Counties. Recent surveys have detected MCMV in Cloud County (Uyemoto, unpublished). In 1977, MCMV was found in several areas, but no CLN was detected, apparently due to the lack of MDMV and WSMV transmissions. In 1978, CLN was severe at field N1 and in other areas (16). Many fields were moderately to severely infected with CLN in 1979 in southern Smith and Phillips Counties, and one field in Republic County. However, in Norton County and northwestern Phillips County, MCMV was primarily found alone.

MCMV was first detected in 1977 and 1978 on 28 June and 14 June, respectively. In 1979, extensive early season corn assays at fields N1 and P1 failed to detect MCMV until 29 June. Western corn rootworm beetles were first observed on 10 July 1979 at field N1. The initial MCMV infected plants appeared randomly distributed in the field (Fig. 1 and 4). Later surveys revealed that infected plants were clustered around previous infection sites (Fig. 2, 3 and 4) suggesting secondary spread of MCMV by adult western corn rootworm beetles or, possibly, larvae. Larvae of the southern corn rootworm will transmit MCMV (10).

Increased percentages of infected plants indicate that MCMV is vectored throughout the season. Fields with greater numbers of corn rootworm beetles contained heavier MCMV infections (Table 3). Even within field N1 it appeared that MCMV infections were greater where beetles were more abundant.

It was shown that adult corn rootworm beetles migrate from corn field to corn field and to surrounding areas to feed on pollen of other plants and then return to a corn field for oviposition (3,6,14). The migratory habit of western corn rootworm beetles is exemplified by its spread to new geographical areas in the United States (5). Therefore, it is probable that MCMV will be disseminated to new areas.

Survey results did not conclusively show how initial infections of MCMV occur. However, it was suggested that corn rootworm larvae may acquire MCMV by feeding on infected corn debris (17). This potential exists since adults were not observed during the early growing season when MCMV was initially detected.

Percent transmission by beetles depended on the time and location of beetle sampling with the highest percent transmission being 23.9% on the last sampling date at field N1 (Table 3). A 16% transmission rate was reported using western and southern corn rootworms collected from the field (10). Percent transmission of MCMV in greenhouse tests have been reported as 5 to 30% for northern and western corn rootworms (10) and 24% for the western corn rootworm (12). The percent of viruliferous beetles increased during the season. However, all beetles with virus did not transmit MCMV to test plants during a 1-day transmission feeding period. The higher concentration of virus in beetles late in the growing season suggests an accumulation of MCMV particles in the beetles or may reflect a higher concentration of virus in the source plants, hence, permitting a greater dosage of virus to be acquired. MCMV was not detected in western corn rootworm eggs. The use of ELISA to monitor virus content in beetle vectors may further epidemiological studies.

No transmission occurred with *C. flavida* collected from MCMV infested fields but MCMV was detected (ELISA) in several of these beetles species, viz. adults and larva. *Colapsis flavida* is a member of the family Chrysomelidae. The larvae overwinter in the soil and emerge in early summer as adults after feeding on corn roots (11). Thus, it may be possible for MCMV to overwinter in larvae and virus infections initiated in the spring during the feeding process. However, such evidence is lacking.

Table 1. Percent of corn hybrids infected with corn lethal necrosis (CLN) and mosaic symptoms at 3 dates in a Norton Co. corn field (field N1) in 1978.

| | Date of Counts[1] | | | | | |
|---|---|---|---|---|---|---|
| | 7/13 | | | 8/1 | | 8/18 |
| Corn Hybrid | % CLN | % Mosaic | MCMV Serology[2] | % CLN | % Mosaic | % CLN |
| Prairie Valley 76S | 10 | 53 | 4/10[3] | 19 | 17 | 46 |
| Pioneer 3311 | 20 | 45 | 1/10 | 35 | 31 | 70 |
| Pioneer 3183 | 17 | 53 | 7/10 | 39 | 31 | 68 |
| Pioneer 3195 | 0 | 11 | 4/8 | 2 | 6 | 7 |
| Pioneer 3184 | 0 | 33 | 0/10 | 12 | 22 | 28 |
| Pioneer 3194 | 0 | 11 | 5/10 | 3 | 4 | 6 |
| Pioneer 3360 | 10 | 33 | 4/10 | 8 | 10 | 42 |
| DeKalb XL72AA | 18 | 36 | 5/10 | 25 | 16 | 50 |
| Bojac X56A | 4 | 41 | 1/10 | 31 | 15 | 50 |
| Bojac X69 | 0 | 18 | 6/10 | 2 | 3 | 3 |
| Bojac X28 | 6 | 28 | 2/10 | 19 | 5 | 41 |
| Bojac X577 | 0 | 31 | 1/10 | 3 | 10 | 13 |
| Bojac X5615 | 5 | 39 | 2/10 | 20 | 18 | 43 |
| Trojan T1058 | 10 | 44 | 8/10 | 18 | 15 | 27 |
| Trojan TX115 | 13 | 35 | 4/10 | 33 | 16 | 45 |
| Averages | 7.5 | 34.1 | 3.6/10 | 17.9 | 14.6 | 35.9 |

[1]Counts consist of 50 plants at 2 locations in the field (100 total plants) on 13 July, 1 August, and 18 August.

[2]Maize chlorotic mottle virus (MCMV) was determined by double immunodiffusion with plants exhibiting mild mosaic symptoms.

[3]The number of MCMV positive samples / the number of samples tested.

Table 2. Percent of maize chlorotic mottle virus (MCMV) infected plants observed at field N1 with different hybrids at 5 sampling dates in 1979.

| | Date of Counts[1] | | | | |
|---|---|---|---|---|---|
| Hybrid[2] | 7/10 | 7/20 | 7/30 | 8/7 | 8/29 |
| Pioneer 3184 (rows 14-15) | 0.1[3] | 1.4 | | | 6.1 |
| Pioneer 3090 (rows 35-36) | 0.1 | 0.5 | | | 3.2 |
| Pioneer 3090 (rows 41-42) | 0.8 | 2.3 | 4.5 | 5.6 | 9.5 |
| Pioneer 3195 (rows 47-48) | 0.6 | 1.6 | | | 7.6 |
| Pioneer 3183 (rows 59-60) | 1.1 | 2.5 | 4.4 | | 10.8 |
| Pioneer 3382 (rows 71-72) | | | | 12.9 | |
| Pioneer 3194 (rows 89-90) | 1.2 | | | | 15.8[4] |
| Pioneer 3195 (rows 119-120) | 1.7 | | | | |
| Pioneer 3184 (rows 169-170) | 1.4 | | | | |
| Pioneer 3184 (rows 219-220) | 0.0 | | | | |
| Averages | 0.8 | 1.7 | 4.5 | 9.3 | 8.8 |

[1]Counts were taken in 2 rows, each of approximately 373 m (1224 ft) length.

[2]Most hybrids were planted in 12 row strips.

[3]Percentage of MCMV infected plants in 2 rows.

[4]Only one row counted for that hybrid at that date.

Table 3. Percentage of corn rootworm beetles with maize chlorotic mottle virus (MCMV) and those that transmitted MCMV, and incidence of corn lethal necrosis (CLN) or MCMV at three beetle collection sites in 1979.

| | Field S1 | | | | | Field S3 | | | | | Field N1 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MCMV ELISA | | MCMV Transmission | | | MCMV ELISA | | MCMV Transmission | | | MCMV ELISA | | MCMV Transmission | | |
| Date Collected | No.[1] | %[2] | No.[1] | %[2] | % CLN in Field[3] | No.[1] | %[2] | No.[1] | %[2] | % CLN in Field[3] | No.[1] | %[2] | No.[1] | %[2] | % MCMV in Field[3] |
| 7/10 | 5 | 40 | 5 | 0 | 1.5 | | | | | | 26 | 34.6 | 18 | 5.6 | 0.8 |
| 7/20 | 50 | 8 | | | 2.6 | | | | | | 47 | 70.2 | | | 2.2 |
| 7/30 | 23 | 100 | | | 7.6 | 39 | 94.9 | 33 | 9.4 | 30.0 | 36 | 61.1 | 35 | 8.1 | 3.4 |
| 8/7 | | | | | | 45 | 91.1 | 45 | 17.8 | | 40 | 97.5 | 40 | 12.5 | 4.9 |
| 8/23 | | | | | 24.1 | 8 | 100 | 10 | 0.0 | 49.1 | 67 | 100 | 67 | 23.9 | 9.2 |

[1]Number of southern and western corn rootworms tested in MCMV enzyme-linked immunosorbant assay (ELISA) and MCMV transmission tests. Only 7 southern corn rootworms were included in tests.

[2]Percentage of beetles positive for MCMV ELISA (absorbance readings of > 0.3) and percentage transmitting MCMV to inbred N28Ht corn in the greenhouse.

[3]Percentage of CLN or MCMV in the field where beetles were collected.

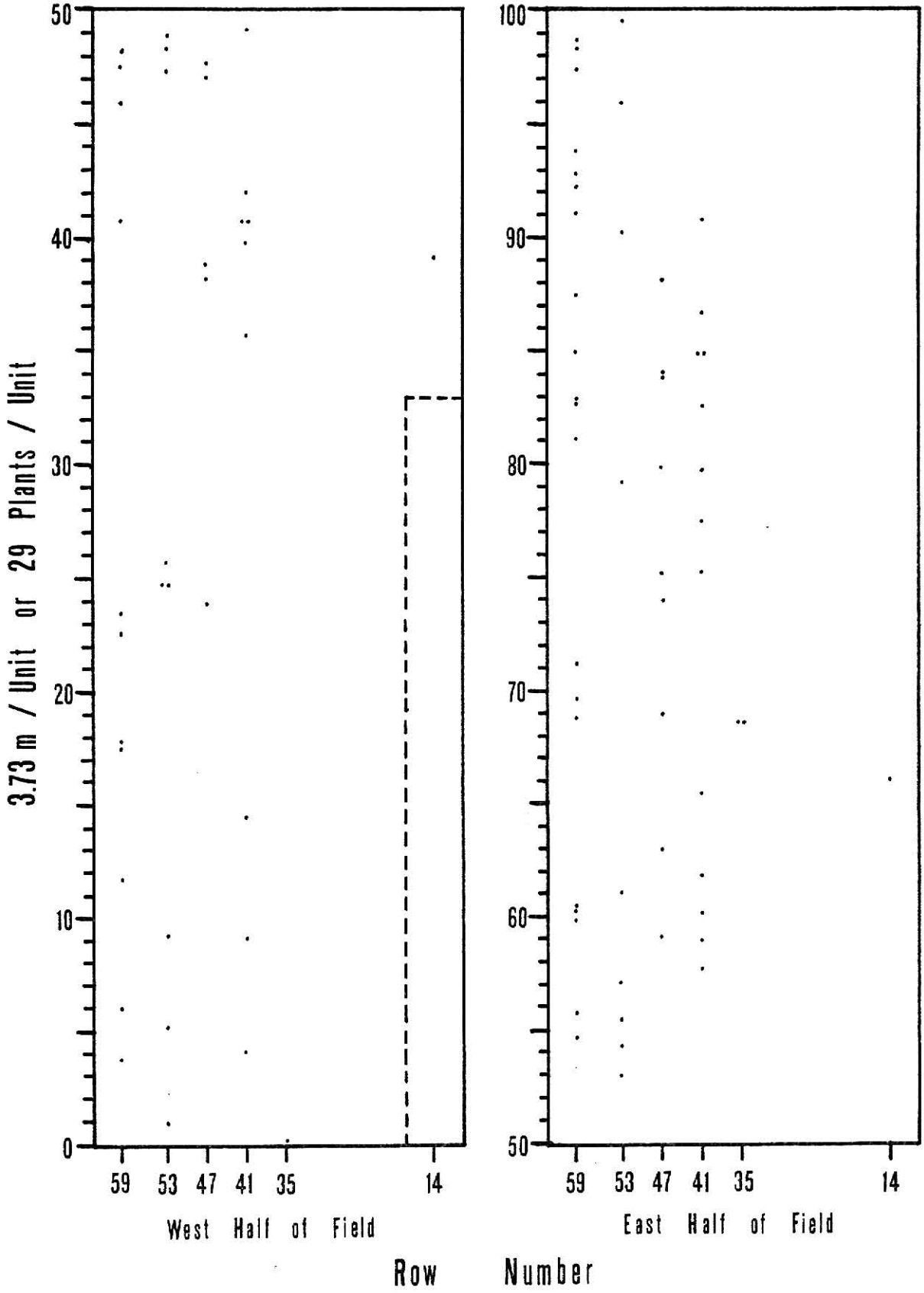


Fig. 1. Distribution of MCMV infected corn plants on 10 July 1979 at field N1 in six 2-row areas, designated by a single row number. One dot represents one infected plant and horizontally adjacent dots indicate other infected plants in the same 0.73 m area. The area enclosed by dotted lines was not surveyed.

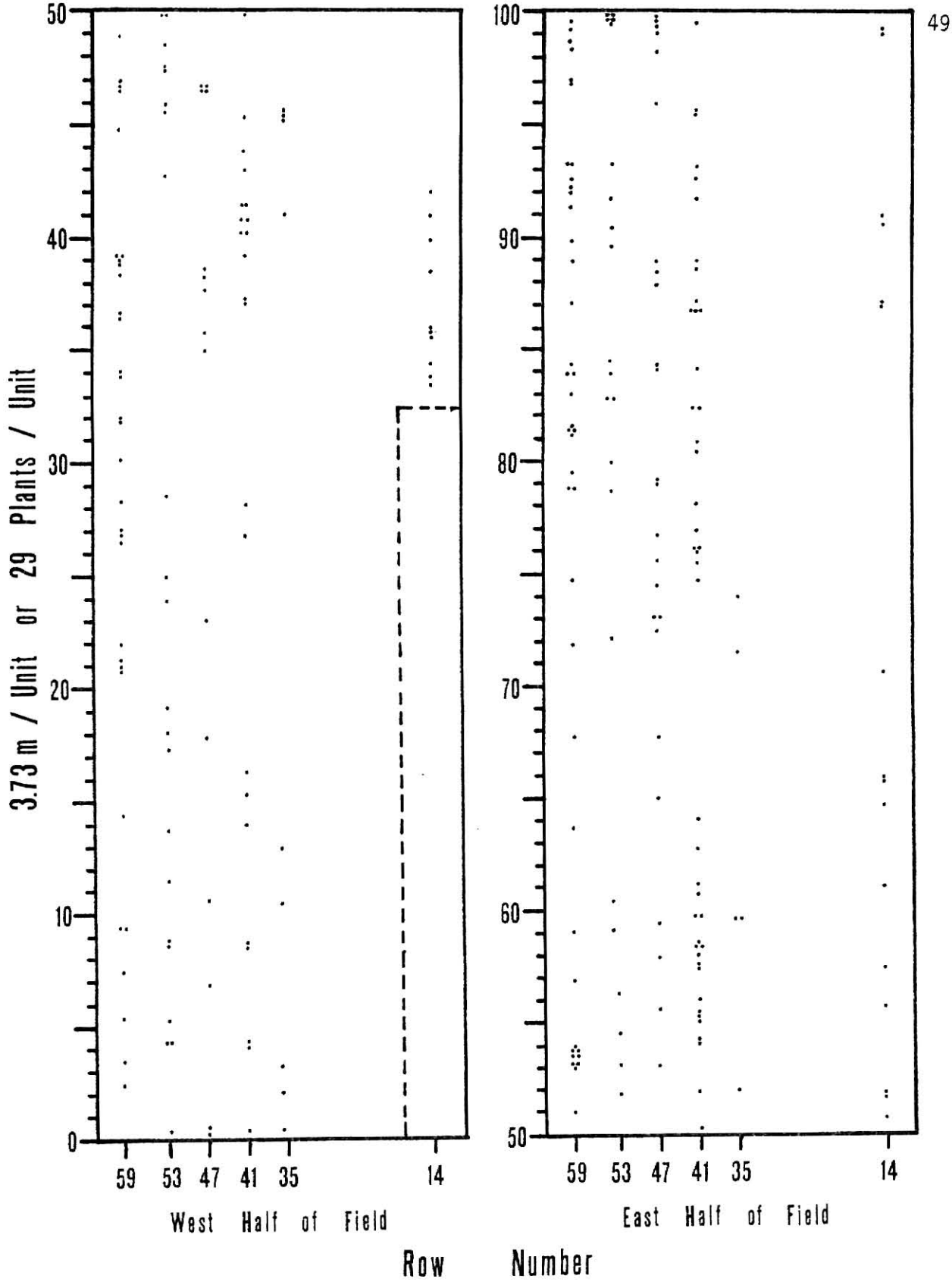


Fig. 2. Distribution of MCMV infected corn plants on 20 July 1979 at field N1 in six 2-row areas, designated by a single row number. One dot represents one infected plant and horizontally adjacent dots indicate other infected plants in the same 0.73 m area. The area enclosed by dotted lines was not surveyed.

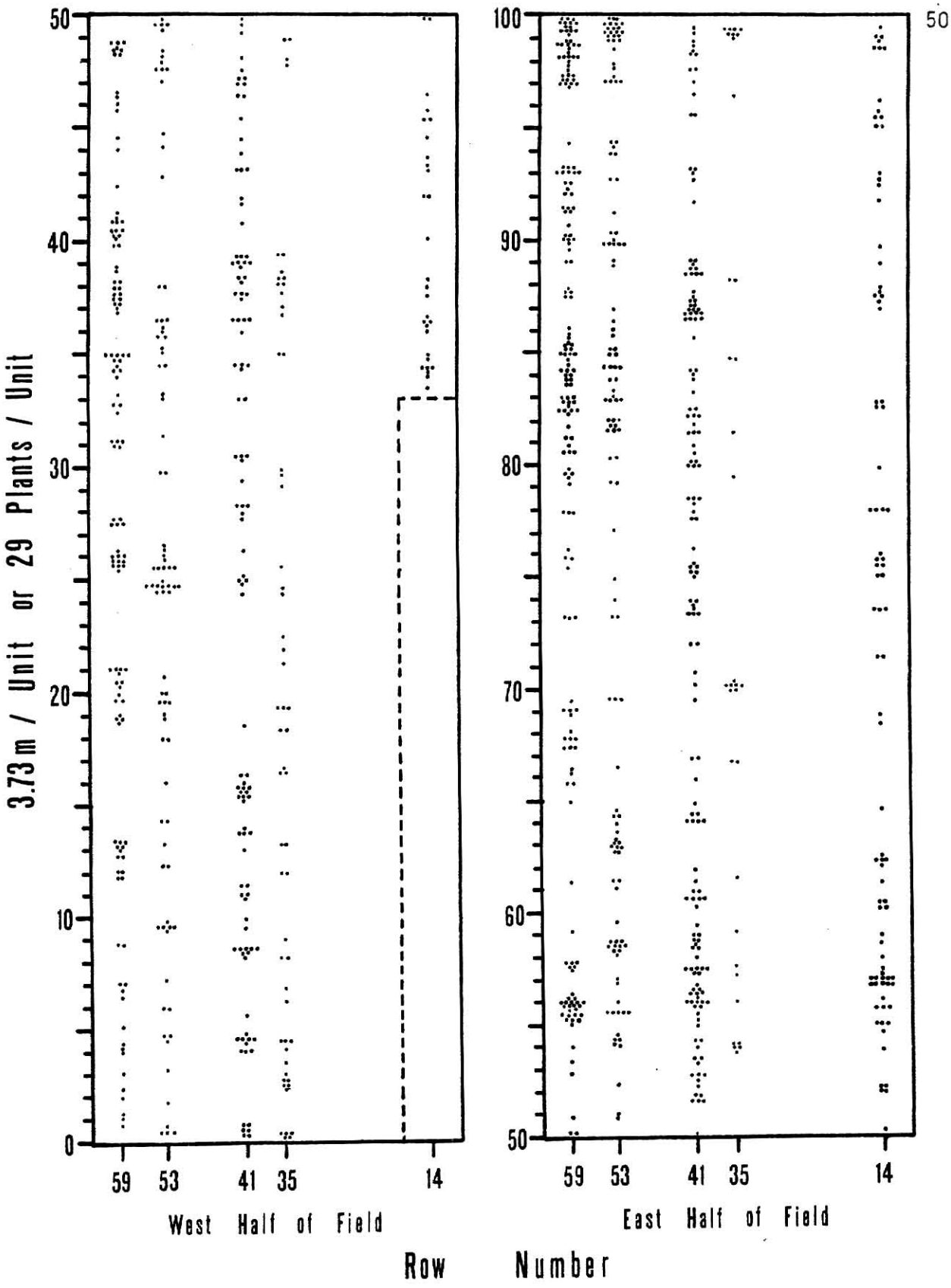


Fig. 3. Distribution of MCMV infected corn plants on 29 August 1979 at field N1 in six 2-row areas, designated by a single row number. One dot represents one infected plant and horizontally adjacent dots indicate other infected plants in the same 0.73 m area. The area enclosed by dotted lines was not surveyed.

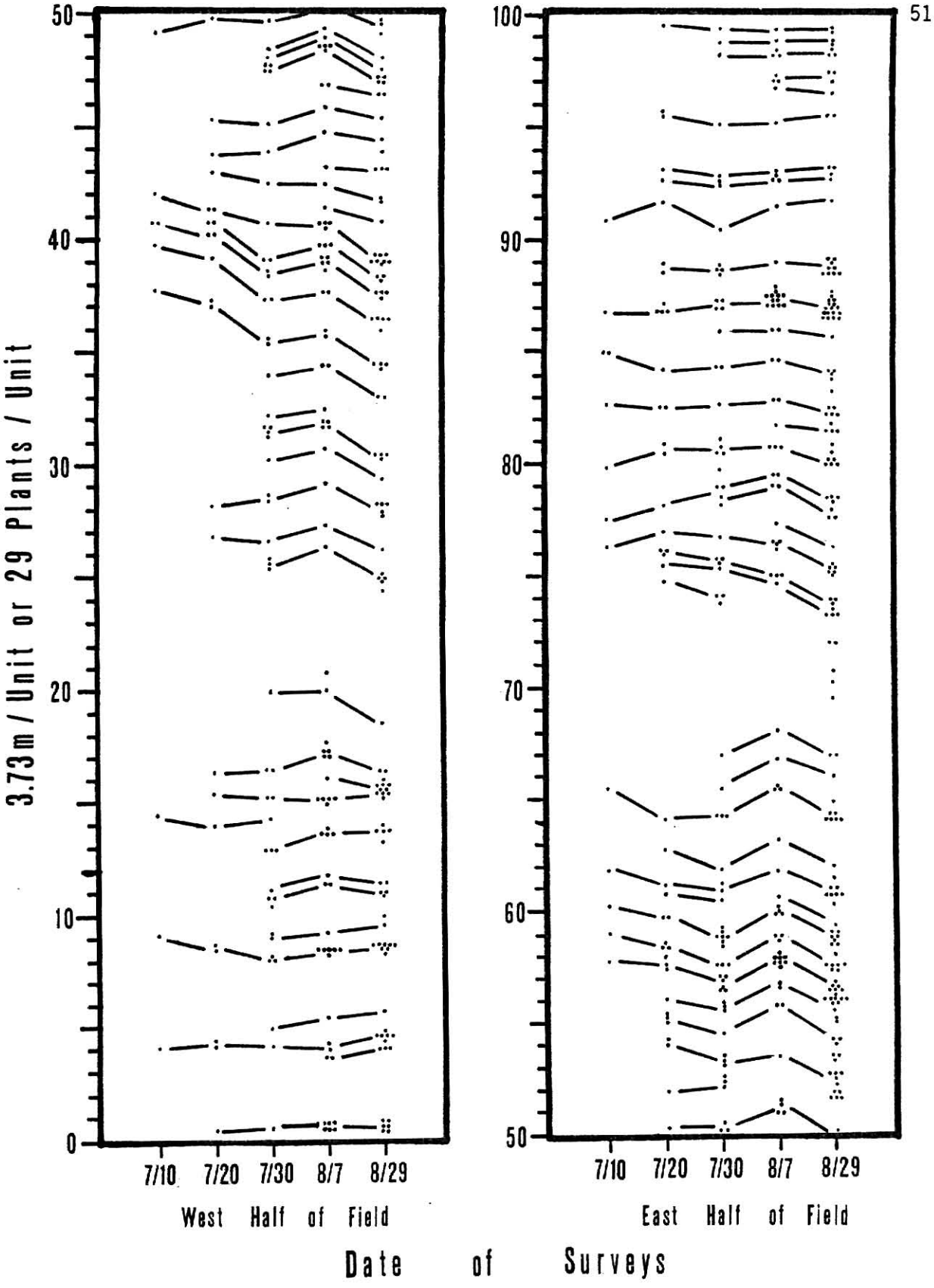


Fig. 4. Distribution of maize chlorotic mottle virus infected corn plants in rows 41-42 of Pioneer 3090, on 5 dates at field N1 in 1979. One dot represents one infected plant and horizontally adjacent dots indicate other infected plants in the same 0.73 m area.

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# APPENDIX 1

## Incidence and Distribution of Maize Dwarf Mosaic Virus and Wheat Streak Mosaic Virus in Native Grasses

Maize dwarf mosaic virus, strain B (MDMV-B) was first detected (7 May 1979) in *Bromus tectorum* L. growing at edges of fields P1 and N1 and recovered (16 May) from *B. tectorum* collected in two Phillips County locations. Infected plants exhibited a mottle in the boot to early heading stage of growth. Repeated MDMV-B isolations from *B. tectorum* were made from collections at field P1 and field S2 on 1 June (Table 4, p. 27). *Bromus tectorum* matured at all locations by 29 June. In 1978, MDMV-B was recovered from *B. tectorum* on 30 May at field N1.

On 29 August 1979, a large population of *B. tectorum* had germinated (at field P1) and developed to the 1-2 leaf stage. However, lack of rainfall during September killed (by 8 October) most of those plants. A few small groups of plants, which survived under trees, were infected with MDMV-B. Virus was recovered from plants at 3-4 leaf stage (Table 4, p. 27). Observations made on 18 December 1979 showed that newly germinated seedlings, found under plant debris, were in the 1-2 leaf growth stage.

Annual grasses, including *Cenchrus longispinus* (Hack.) Fern., *Digitaria sanguinalis* (L.) Scop., *Eragrostis cilianensis* (All.) Link., *Echinochloa crusgalli* (L.) Beauv., *Panicum capillare* L., and *Setaria viridis* (L.) Beauv., were first observed on 1 June 1979 in open areas at field N1 with 2-4 leaves emerged. Plants were identified at maturity from transplants grown in the greenhouse. Colonies of corn leaf aphids, *Rhopalosiphum maidis* Fitch., were observed on these grasses, however, no

virus symptoms developed on any plants in the greenhouse. *Digitaria sanguinalis*, *S. viridis*, and *Panicum dichotomiflorum* Michx. were growing in protected areas, as late as 8 October at field P1.

In 1979 at field N1, wheat streak mosaic virus (WSMV) was first detected in *S. viridis* from one sample collected on 1 June and subsequent collections on 20 July revealed that many (ca. 60%) *D. sanguinalis* and *S. viridis* plants were infected (table 4, p. 27). As the growth rate of those grasses began to decline, most WSMV symptoms had faded by 23 August. However, on the same date symptomatic *S. viridis* plants were collected at fields N1 and S1 and WSMV was recovered by bioassays. Also, one *E. crusgalli* sample was infected with WSMV collected at field N1. WSMV infections were detected on 1 *D. sanguinalis* and 2 *S. viridis* samples collected at field N1 on 8 August 1978. Two *D. sanguinalis* and one *S. viridis* sample collected at field R1 in the first 2 weeks of July 1977 were WSMV infected.

Incidence of MDMV-B increased in *D. sanguinalis* and *S. viridis* in 1979 at field P1 from 20 July until 21 September (Table 4, p. 27). Symptoms were observed as late as 8 October on surviving *D. sanguinalis* and *S. viridis*. In 1979, MDMV-B was also abundant at Smith County. However, limited MDMV-B infections occurred in grasses at field N1 (Table 4, p. 27). MDMV-B isolates were collected from 5 *P. dichotomiflorum* samples at field P1 (29 July and 21 August). Other MDMV-B isolations were obtained from 3 *Setaria lutescens* (Weigel) Hubbard samples collected on 23 August and 12 September 1979 from field S2, and from 1 *E. crusgalli* sample collected on 29 June. In 1978, MDMV-B was isolated from 1 *D. sanguinalis* sample collected on 1 August at field N1.

Grasses identified at all seven sampling locations were *Bromus inermis* Leyss., *Bromus japonicus* Thunb. or *B. tectorum*, *C. longispinus*, *D. sanguinalis*, *E. crusgalli*, *S. lutescens*, and *S. viridis*. The most common grass species at all locations was *B. inermis*, covering much of the roadsides and field borders, with *B. tectorum* and some *B. japonicus* growing in patches in those areas. *Digitaria sanguinalis* and *S. viridis* were very common, especially in disturbed areas around fields, as well as *C. longispinus* and some *S. lutescens* and *E. crusgalli*. Grasses in less abundance, found in at least one sampling site, were *Agropyron smithii* Rydb., *Andropogon gerardi* Vitman., *Elymus virginicus* L., *E. cilianensis*, *Hordeum jubatum* L., *Hordeum pusillum* Nutt., *Leptochloa fascicularis* (Lan.) Gray, *Muhlenbergia bushii* Pchl. tide McGregor, *P. capillare*, *P. dichotomiflorum*, *Poa pratensis* L., *Secale cereale* L., and *Sporobolus cryptandrus* (Torr.) Gray.

# APPENDIX 2

## Seed-borne Transmission Studies of Maize Cholorotic Mottle Virus

Seed-borne virus transmission of maize chlorotic mottle virus (MCMV) was studied. Seeds of 14 inbred corn lines (*Zea mays* L.), 5 corn hybrids, and 3 grass species (*Panicum miliaceum* L., *Setaria lutescens* (Weigel) Hubbard, and *S. viridis* (L.) Beauv.) were collected from MCMV infected plants. Seeds were germinated in 30 by 56 cm flats in the greenhouse and seedlings were assayed after 28 to 50 days. Leaf tissues of 15 plants were composited and extracted in 0.05M $KPO_4$, pH 7.0 buffer (1:1 w/v) and rubbed onto carborundum dusted leaves of N28Ht corn. Plants with mottle symptoms were assayed singly. One group of 300 *P. miliaceum* seedlings were assayed in groups of 20. Kernals were collected from corn inbreds: A634Ht, W135R, H95, A632Ht, W117Ht, W64AHt, B73Ht, C123Ht, B14AHt, Oh43Ht, A635Ht, H49, C103, and N28, and corn hybrids: Mo17 x N28, A619Ht x A632Ht, B73 x Mo17Ht, 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, *P. miliaceum;* 135, *S. lutescens;* and 2,676, *S. viridis*) were all negative for MCMV.

# APPENDIX 3

## Maize Chlorotic Mottle Virus Transmission Studies with Soil Collected from Infested Fields

Soil collected from eight different fields infested with maize chlorotic mottle virus (MCMV) were potted in 15 cm diam. plastic pots into which five, 1-2 week old N28Ht inbred corn (*Zea mays* L.) seedlings were transplanted. In August of 1977, 6 pots of soil were collected from 2 infected corn fields. In 1979, a total of 62 pots of soil were collected beginning in late March to late July. Soil samples were collected with a T-soil probe or kick sampler to a 30 cm soil depth in a 10 m diam. area, except those collected in July 1979, where soil was collected around roots of plants infected with MCMV.

In these bait experiments, only one plant became MCMV infected (from soil collected in a Smith County corn field on 20 July). Symptoms appeared 18 days after transplanting and the plant was positive in MCMV serologic tests. The infectious soil was extracted for nematodes and corn rootworm larvae by the screening plus Baermann funnel method (1) and modified as described in Appendix #4. Small roots and debris collected on a 710 micron mesh sieve were also put on Baermann funnels. A total of 35 *Xiphinema americanum* Cobb and 38 corn rootworm larvae were extracted from the contents of the infested pot. Data are inconclusive to determine the mode of infection.

# APPENDIX 4

## Maize Chlorotic Mottle Virus Transmission Studies Using *Xiphinema americanum* Cobb

Attempts were made to transmit maize chlorotic mottle virus (MCMV) by *Xiphinema americanum* Cobb collected from virus infested and healthy fields. Live *X. americanum* were extracted from the soil by the screening plus Baermann funnel (SBF) method (1). The SBF process was modified by screening soil suspensions through 710, 149, 74, and 45 micron mesh sieves; materials on all sieves, except the 710 micron mesh, were washed into a 200 ml beaker. The beaker was covered with a Kemwipe and inverted on a 320 micron mesh sieve which was placed in a 123 mm funnel in a mist chamber. Nematodes were drawn off 24 to 48 hrs. later.

In experiment #1, *X. americanum* were obtained from a sorghum-soybean rotated field at Ashland research farm, Manhattan, KS. A total of 3,450 live *X. americanum* were hand picked from SBF extractions and diluted in water to obtain 25 nematodes per ml. Two ml of suspended nematodes, about 50 *X. americanum*, were applied to each 3 week old, MCMV infected N28Ht corn plant (*Zea mays* L.). Nematodes were applied by pushing the tip of an Eppendorf pipet into the root zone, depositing the nematodes, and covering the hole. A total of 69 N28Ht corn plants in 14 pots were inoculated with *X. americanum*.

Nematodes were allowed to feed on MCMV infected corn for 12 days at which time they were extracted from pots by SBF using only 250 and 74 micron mesh sieves. Only 167 *X. americanum* were obtained and diluted in 170 ml of water. One ml of suspended nematodes were placed on 17, two week

old N28Ht corn plants in 3 pots to obtain about 10 *X. americanum* per plant. Plants (assayed on N28Ht corn after 3 weeks) were observed for virus symptoms for 5 weeks.

Test plants in this experiment were grown in sterilized sand in 10 cm diam. plastic pots, under high pressure sodium lucolux lamps and multi-vapor lamps with a 16 hr. photoperiod at room temperature (23 - 28°C). Plants were watered daily to keep as constant a moisture level as possible and fertilized with a solution of balanced nutrients regularly.

Experiment #2 was conducted with *X. americanum* collected on 19 April 1979 from a corn field infested the previous year with MCMV, located in Norton County (field N1). A total of 83 live *X. americanum* were extracted by SBF and placed on 19 healthy, week old N28Ht corn seedlings. Corn was placed in a Percival growth chamber at 27°C with a 16 hr. photoperiod for 5 weeks.

No MCMV transmission occurred with *X. americanum* in either experiment. In experiment #1 a 95% loss of *X. americanum* occurred between acquisition and feeding periods.

# APPENDIX 5

## Soil Extractions for *Longidorus* and *Xiphinema* in Maize Chlorotic Mottle Virus Infested Fields

In the fall of 1978, several *Longidorus* spp. were isolated from a field near Almena, KS in Norton County (field N1). Since *Longidorus* spp. are known to vector viruses (2) and are rarely found in corn fields in other areas of the state (O. J. Dickerson and L. D. Lash, personnel communication), soil samples from other MCMV infested fields were obtained in 1979 to isolate *Longidorus*.

A total of 163 soil samples were processed for nematodes by direct centrifugal-flotation (DCF) or the screening plus Baermann funnel (SBF) method (1), modified as described in appendix #4. In DCF and SBF procedures, 100 cc and about 250 cc of soil were processed for each extraction, respectively. Samples were taken from 9 MCMV infested corn fields in 4 different geographical locations. Soil samples were collected using a T-soil probe or a kick sampler and bulking soil from 10 probes taken in a 10 m diam. sampling area. Soil samples taken in 3 fields in July were collected by digging up the roots and surrounding soil of MCMV infected plants (54 extractions made).

Five *Longidorus* spp. were isolated from 90 extractions from soil collected at field N1 and none were found in other fields sampled. Apparently, *Longidorus* only occurs in small numbers at field N1 or the sampling or extraction methods used were not efficient for *Longidorus* recovery. *Xiphinema americanum* Cobb, also a known virus vector (2), was detected in all fields sampled and appeared in relatively high numbers in some areas of

the fields; numbers of *X. americanum* ranged from 0 to 70 / 100 cc of soil, however, 10-30 / 100 cc of soil was common and an average number for all quantitative counts was 5 / 100 cc of soil.

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# EPIDEMIOLOGICAL STUDIES ON MAIZE CHLOROTIC MOTTLE VIRUS

by

DONALD L. BOCKELMAN

B. S. Kansas State University, 1976

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AN ABSTRACT OF A MASTER'S THESIS

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# ABSTRACT

Nineteen and fifteen grass species were found as systemic hosts for Kansas and Peru strains of maize chlorotic mottle virus (MCMV), respectively. Host response between strains are similar with differences found primarily in latently infected hosts. No MCMV was found in 230 grass samples, representing 14 species, collected near infested corn fields. However, maize dwarf mosaic virus, strain B and wheat streak mosaic virus were detected in annual grass species and active insect transmissions to corn bait plants were also realized.

Assays and field observations of corn, *Zea mays* L., during a 3-year period, indicated that maize chlorotic mottle virus (MCMV), was prevalent in three major irrigation districts in Kansas. MCMV was initially detected in late June and infected plants were randomly distributed in the field. Maize dwarf mosaic virus, strain B was also commonly detected in June. Later in the season, as numbers of rootworm beetles increased, the incidence of corn lethal necrosis increased. Active virus transmissions by field collected adults of *Diabrotica virgifera* LeConte and *D. undecimpunctata* Mannerheim ranged from 0 to 24%. However, in enzyme-linked immunosorbent assay (ELISA) serologic tests, beetles with MCMV ranged from 8 to 100%. No transmission occurred with adult *Colaspis flavida* Say, even though MCMV-ELISA positives were found in extracts of several adults and a larva.

Seed transmission of MCMV did not occur in 14 inbred corn lines, 5 corn hybrids, *Panicum miliaceum* L., *Setaria lutescens* (Weigel) Hubbard, and *S. viridis* (L.) Beauv. *Xiphinema americanum* Cobb did not transmit

MCMV to corn in laboratory experiments, however, nematode survival was poor. Nematode extractions from MCMV infested corn fields detected *X. americanum* in relatively high numbers in some field areas and *Longidorus* spp, were detected in one field.