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Biology and Control of Maize Chlorotic Mottle Virus

Maize chlorotic mottle virus (MCMV) (Fig. 1), first isolated from diseased corn plants (*Zea mays* L.) in Peru and later characterized at Raleigh, North Carolina (2), remained an oddity until 1976 when it was recovered from a cornfield in north-central Kansas (8).

As is often the case in science, the discovery and identification of MCMV in Kansas were serendipitous. In July 1976, a county agent asked Kansas State University pathologists to assist with a disease in a cornfield in Almena, Kansas. During the site visit, the disease was diagnosed as caused by maize dwarf mosaic virus (MDMV). Shortly thereafter, a Kansas State extension pathologist was asked whether MDMV killed corn plants. The disease problem in the Almena cornfield had worsened dramatically. Corn plants showed leaf chlorosis and tissue necrosis beginning at leaf margins (Fig. 2), with stunting and early death (Fig. 3). Corn yield was later estimated at 628 kg/ha. The condition was termed corn lethal necrosis (CLN) (8).

During a second site visit, it became apparent that this was a new virus disease of corn, not one caused by abiotic factors, pests, or fungal or bacterial pathogens. With the assistance of a plant virologist, three separate viruses were sap-transmitted to host-range indicator plants from extracts of diseased tissues. Two were identified as MDMV and wheat streak mosaic virus (WSMV). Those two, inoculated either together or separately, did not cause the severe symptoms found in the Almena field. But either virus in combination with the third, unknown virus reproduced the new syndrome.

A clue to the identity of the critical virus came in March 1977 when a visitor from North Carolina State University inquired about the brilliantly yellowed corn plants he saw during a greenhouse tour. They were similar to those he had seen at Raleigh. That led to an exchange of antisera and virus cultures, which, when compared directly, proved that the unknown virus was serologically related to the Peruvian isolate of MCMV. At the same time, the Kansas virus isolate was sent to Wooster, Ohio, where the vector relationship with chrysomelid beetles was established (7). On 1 October 1977, I joined the Kansas State faculty to continue investigations with MCMV and the CLN disease complex.

Virus Distribution

MCMV now is endemic in Peru (6) and in north-central Kansas and south-central Nebraska, where affected fields are located in small river valleys and irrigation districts (Fig. 4). It was identified once in the High Plains of Texas (4) and Brazil (D. T. Gordon, *personal communication*). In Kansas, MCMV has been detected annually since 1976 and widespread outbreaks of CLN and heavy crop losses occurred during 1976, 1978, and 1980. In those epidemics, conditions favoring spread of MDMV and WSMV apparently favored additional infections with MCMV.

Because MCMV was new to our corn belt, we launched a concentrated effort 4 years ago to unravel some more of the viral etiology. Although we already knew that virus persisted in certain fields cropped continuously to corn, that commercial dent corn varieties and inbreds lacked resistance genes, and that adults and larvae of several chrysomelid species were efficient vectors, more information was required before effective control measures could be formulated.

Host Range, Seed Transmission

Several grass species native to Kansas were sap-inoculated with isolates of MCMV from Kansas and Peru. Depending on the virus isolate used, we found 15–19 species were systemic hosts for MCMV. Seeds of infected *Panicum miliaceum, Setaria lutescens, S. viridis*, and corn inbreds and hybrids were harvested and sown in sterile soil, and

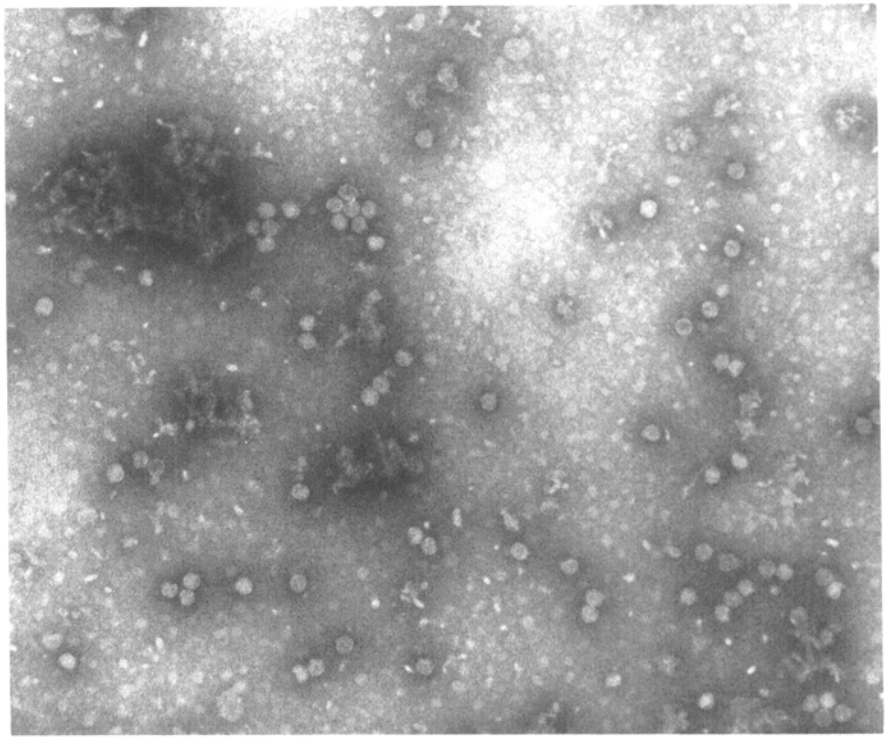

Fig. 1. Electron micrograph of maize chlorotic mottle virus particles, about 30 nm in diameter.

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plants 28–50 days old were bioassayed onto corn indicator plants. Collectively, virus was not recovered from 3,653 weeds or 4,051 corn plants (1), which indicated that MCMV is not seed-transmitted.

Epidemiology

Field surveys for MCMV were done during spring seasons of 1978 to 1980. Before corn was planted and throughout the growing season, we sampled grassy weeds along edges of known diseased fields and nearby creeks on a weekly basis. Potted corn seedlings (Fig. 5) were distributed in those arenas, to monitor for adult beetles, such as the corn flea beetle (*Chaetocnema pulicaria*) and the southern corn rootworm (*Diabrotica undecimpunctata*), that may have acquired MCMV while overwintering in the winter annual and perennial grasses, many of which could serve as potential virus reservoirs (1).

Although we frequently recovered MDMV and WSMV in grassy weeds and corn bait plants, none contained MCMV. This pattern of virus recovery held for May and early June of each survey season. We also collected corn plants in commercial fields during that period and again recovered MDMV and WSMV but not MCMV. Many of the corn plants had corn flea beetle damage on the lowest leaves, indicating that those potential virus vectors from outside cornfields had not transmitted MCMV while feeding on corn plants during the seedling growth stage. But in late June, MCMV infections appeared in cornfields. That made us consider the possibility that MCMV was initially transmitted through the soil via viruliferous larvae of the western corn rootworm (*D. virgifera*) (Fig. 6A), instead of entering the field from the outside each year. These insect larvae were prime candidates because we failed to demonstrate soil transmission of MCMV to corn bait plants sown in diseased field soils devoid of or artificially freed from rootworm larvae. Soil samples were collected throughout the year and contained plant-parasitic nematodes, including *Xiphinema americanum*, and roots of infected corn plants that may harbor potential fungal vectors.

We theorized the mechanism of soil transmission of MCMV as follows: Newly hatched larvae would, in the absence of fresh corn roots, forage on infected crop residues. Virus would be acquired and later transmitted by larvae feeding on developing corn roots.

In Kansas, more than half the overwintered eggs of our most abundant candidate vector, the western corn rootworm, are expected to hatch by mid-June (5). So if virus transmissions occurred then, the aboveground symptoms should appear after 10–14 days' incubation. During 1978–1981, MCMV infection was indeed detected around 25 June (J. K. Uyemoto, *unpublished*), which is consistent with the chronological events proposed for soilborne beetle transmission.

In preliminary experiments, laboratory-reared western corn rootworm larvae fed and tunneled into previously dried and then rehydrated corn roots and at least once transmitted MCMV. Also, corn residues collected from a diseased field in April 1980 contained infective MCMV (J. K. Uyemoto, *unpublished*). Both findings indicate that larvae forage on nonsucculent tissues and that MCMV can survive, in nature, in infected crop residues.

Also, MCMV was recovered from both field-collected *Setaria* sp. and potted corn plants during July and August. Presumably, virus was transmitted by viruliferous beetles from a diseased cornfield. During their life cycle, adults of the western corn rootworm (Fig. 6B) begin to emerge from field soil about 1 July. They can acquire MCMV then while foraging on diseased corn plants (Fig. 6C) and later transmit it to nearby plants or plants in neighboring fields.

Control

Crop rotation. The evidence led us to initiate a field study in 1979 to see if rotation with a nonhost would help control MCMV (9). Briefly, the plot contained eight randomized replications of one susceptible and one tolerant corn

Fig. 2. Symptoms of maize chlorotic mottle virus infection include leaf chlorosis and tissue necrosis beginning at leaf edges.

Fig. 3. A stunted, MCMV-infected plant near death.

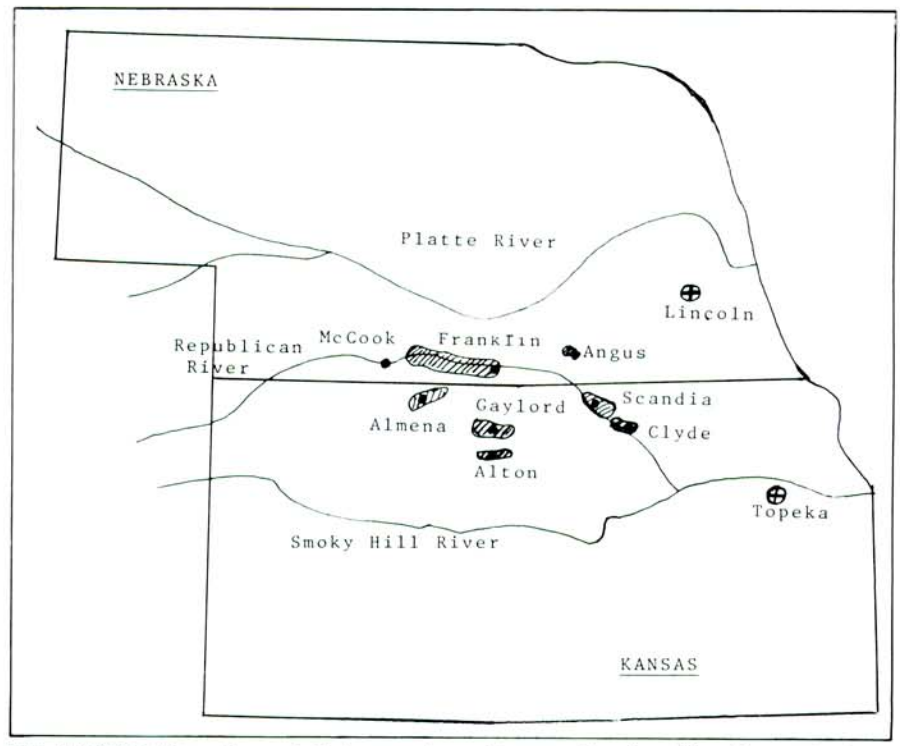

Fig. 4. Distribution of corn lethal necrosis and hence of maize chlorotic mottle virus (shaded areas) in Nebraska and Kansas in 1980.

Fig. 5. Corn seedlings in a self-contained planter. Foam strips (2-10 cm wide) are wrapped around a clay pot, bound with tape, then saturated with water and enclosed in a plastic bag. Water seeps through the pot to irrigate the plants. The wire cage is to minimize rodent damage to the plants.

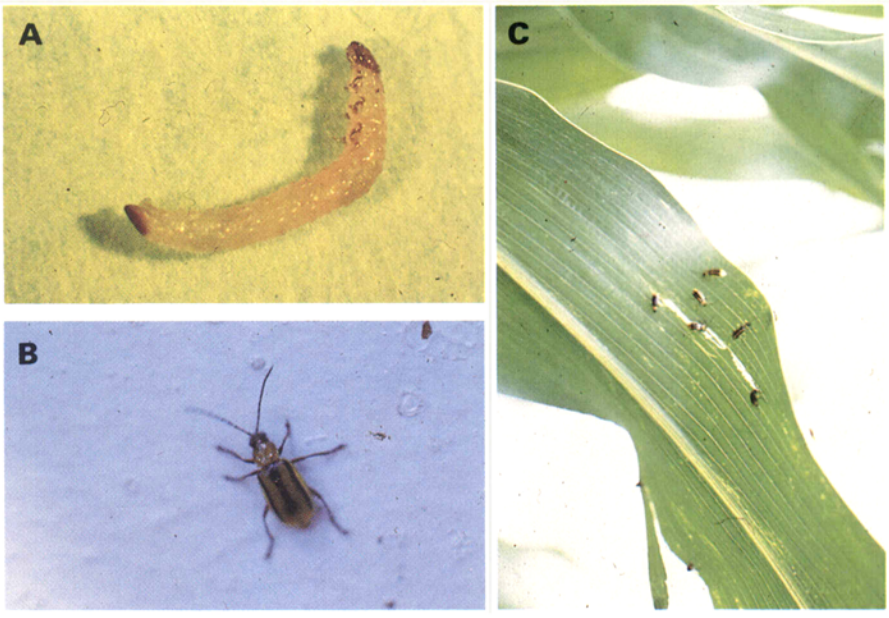

Fig. 6. Western corn rootworm (*Diabrotica virgifera*): (A) Larva, about 13 mm long. (B) Adult, about 6 mm long. (C) Group of adults feeding on a corn leaf.

hybrid and a forage sorghum (*Sorghum bicolor*). In 1980, the entire site was planted with the susceptible corn hybrid, and corn plants were sampled weekly for MCMV.

In 1979, the incidence of MCMV among different corn hybrids was statistically the same (av. 17.5%, $P = 0.05$). Sorghum subplots remained free from MCMV, even though the variety used was virus-susceptible. In 1980, MCMV was detected on 25 June, when corn plants were about 8 weeks old. Immediately thereafter and through mid-August, we sampled all symptomatic plants periodically. In plots cropped continuously to corn, MCMV incidence began at 1.6% and rose to 12.2%, whereas in plots cropped with sorghum and then with corn, virus recoveries increased from zero to 0.6% infections. Differences between cropping sequences were statistically significant ($P = 0.05$) (9).

An alternate crop might eliminate early season MCMV infections by letting infected corn residues, a potential virus reservoir, decompose. Crop rotation with sorghum or soybean in three large commercial fields the year after the 1978 CLN epidemic (10) resulted in two fields free from mosaic plants and one with only a trace of CLN, indicating presence of MCMV. Because disease symptoms were confined to terminal leaves in the third field, MCMV transmissions most probably were at anthesis by adult beetles rather than earlier in the season by rootworm larvae (11). Also, surveys of three other nearby fields planted continuously to corn showed 1–7% of the plants infected with CLN.

Other cultural practices. After the 1979 harvest, we selected several plot sites to determine the effects of fall plowing and removing stalk crowns on virus incidence the next season. At each site, treated and control plots were established in a randomized complete block design with four replications. Corn was planted in May 1980, and the sites were surveyed weekly for CLN. The number of plants showing CLN at four test sites averaged 9, 11, and 13, respectively, for removing stalk crowns, fall plowing, and control. Differences were not statistically significant.

Discussion

Based on the spread patterns of CLN (an epidemic in north-central Kansas in 1976 and 2 years later in south-central Nebraska) and the widespread use of genetically similar, disease-susceptible corn hybrids for several seasons before the first reported outbreak, MCMV is assumed to have been recently introduced into the United States. How, we do not know.

Eventual spread of the virus into neighboring river valleys (eg, Platte and Missouri rivers) appears likely, but the rate of advance depends on the availability of an immediate virus source and migrational behavior of viruliferous beetles.

Our studies indicate that crop rotation effectively controlled MCMV. But other cultural practices tested, ie, fall plowing to hasten tissue decomposition and removing residue, did not provide similar control.

Although susceptible winter annual grasses were common in the diseased areas, they apparently are not a source of MCMV. Corn plants in bait pots and commercial fields also were free from MCMV during the first 8 weeks or so into the growing season. MCMV was not seedborne in weed or corn hosts. Tests for transovarial passage of virus were made with beetle eggs from soils of MCMV-infected fields during October. These were negative, but MCMV appeared annually in late June in field-grown corn plants.

We do not yet have enough data to prove conclusively that a soilborne phase exists for MCMV, but accumulated evidence—that western corn rootworm larvae transmit MCMV (3), that virus persists under corn monoculture, and that MCMV is economically controlled by crop rotation—supports the idea that virus infections are initiated in the manner described. More work with laboratory-reared beetle larvae is planned to elucidate their involvement in MCMV soil transmissions.

We also are directing research toward understanding the relationship between soil moisture and survival and transmission of virus to corn plants. In Kansas, CLN epidemics followed years with above-normal rainfall, ie, 1975, 1977, and 1979. Also, in laboratory tests, MCMV infectivity was drastically reduced (in some instances eliminated) when infected tissues were dried 3–4 weeks at ambient temperatures. Field observations also suggest a moisture-virus survival relationship. Certain cornfields contain MCMV-infected plants annually, while other cornfields, despite being severely diseased one season, may or may not contain virus infections the next season. In those instances, soil maps show that soils in repeatedly diseased fields are silt to silty clay loam (available water capacity rating of 0.56 cm moisture 2.54 cm soil), while the other cornfields contain sandy loam soils (available water capacity rating of 0.36). Virus survival in corn tissues—and perhaps viability of beetle eggs in soil—may be attainable only in soils of high water-holding capacities, which can maintain infected crop residues, such as crown and root, in a proper state of hydration and preserve virus particles.

We are continuing investigations in this area.

Acknowledgments

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