Transmission of Maize Chlorotic Mottle Virus by Chrysomelid Beetles

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ABSTRACT

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Maize chlorotic mottle virus (MCMV) is transmitted by six species of beetles belonging to the family Chrysomelidae: the cereal leaf beetle (*Oulema melanopa*), the corn flea beetle (*Chaetocnema pulicaria*), the flea beetle (*Systena frontalis*), the southern corn rootworm (*Diabrotica undecimpunctata*), the northern corn rootworm (*D. longicornis*), and the western corn rootworm (*D. virgifera*). Both larvae and adults of *O. melanopa* are vectors; adults transmit virus for 6 days after acquisition. Crushed adult *D. virgifera* collected from infected fields in Kansas reacted positively with MCMV antiserum. Two other insects with mandibulate mouth parts, the Japanese beetle (*Popillia japonica*) and larvae of the black cutworm (*Agrotis ipsilon*), failed to transmit MCMV as did three aphid species, and a leafhopper, a planthopper, and a whitefly species.

Additional key words: Graminella nigrifrons, Myzus persicae, Peregrinus maidis, Rhopalosiphum padi, Schizaphis graminum, Trialeuroides vaporariorum, maize.

Maize chlorotic mottle virus (MCMV) was described first in Peru where it causes losses of 10-15% in floury and sweet corn cultivars (5, 6). Recently, MCMV was discovered in Kansas where in combination with maize dwarf mosaic or wheat streak mosaic virus it causes corn lethal necrosis (15).

In order to understand the epidemiology of MCMV, the insect vector species must be identified. Attempts to transmit a Peruvian strain of MCMV with the aphids *Rhopalosiphum maidis* (Fitch), *Aphis gossypii* Glover, and *Myzus persicae* (Sulzer) or the leafhopper *Dalbulus maidis* (DeLong & Wolcott) failed (5, 6).

We report the transmission of MCMV by six chrysomelid beetle species. They include the cereal leaf beetle, two corn flea beetle species, and three corn rootworm species which are serious economic pests of small grains or corn. The potentials of these species as vectors of MCMV in the field is discussed.

MATERIALS AND METHODS

Fourteen insect species were tested as vectors of MCMV. Three aphid species, *Rhopalosiphum padi* (L.), *Schizaphis graminum* (Rondani), and *M. persicae* have been maintained for several years at the OARDC on oats, oats, and turnip, respectively. The leafhopper, *Graminella nigrifrons* (Forbes), and planthopper, *Peregrinus maidis* (Ashmead), also were reared at the OARDC on oats and corn, respectively. The whitefly, *Trialeuroides vaporariorum* (Westwood) was collected from tobacco in an OARDC greenhouse. The Japanese beetle, *Popillia japonica* Newman, was collected in North Carolina and supplied by T. L. Ladd, USDA-ARS Japanese Beetle Laboratory, Wooster, OH. Laboratory-reared second and third instar larvae of the black cutworm, *Agrotis ipsilon* (Hufnagel), which were provided by R. Murray, Dept. of Entomology, OARDC. The cereal leaf beetle, *Oulema melanopa* (L.), was field-collected in Wayne and Holmes counties in Ohio and laboratory-reared specimens were provided by S. G. Wellso of the Department of Entomology, Michigan State University, East Lansing. The corn flea beetle, *Chaetocnema pulicaria* Melsheimer, was collected from field corn in Wayne County, Ohio, and also was obtained from B. D. Barry, USDA-ARS, Columbia, MO. The flea beetle, *Systena frontalis* (Fab.) was collected by B. Drees from corn in Tuscarawas County, OH. Field-collected northern corn rootworm, *Diabrotica longicornis* (Say), and western corn rootworm, *D. virgifera* LeConte, were supplied by A. J. Keaster, Department of Entomology, University of Missouri, Columbia. Additional laboratory-reared *D. virgifera* and laboratory-reared southern corn rootworms, *D. undecimpunctata* Mannerheim, were supplied by D. G. Davis, ARS-USDA, Brookings, SD.

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Field-collected and laboratory-reared insects were assayed on corn test seedlings for 24 hr prior to use in vector studies. None of the assay plants developed symptoms of virus infection indicating that the insects initially were free of MCMV.

The Kansas strain of MCMV used in these studies was obtained from naturally infected corn near Almena, KS. The culture was freed from contamination with other viruses by passage through wheat, density gradient centrifugation, and disc electrophoresis (15). The virus was maintained in Aristogold Bantam Evergreen sweet corn. Plants inoculated 10-20 days previously were used as the virus source for vectors. During the acquisition access period, incubation period, or inoculation access period, insects were confined to source plants by Plexiglas® tube cages or dacron organdy-covered metal-frame cages. Test plants were placed in a walk-in growth chamber programmed for a 16-hr, 25 C day with 37,600 lux (3,500 ft-c) of light and an 8-hr, 18 C night. Plants were observed daily for symptom development. Final results were recorded 14 to 20 days after exposure to test insects.

To confirm virus transmission to corn seedlings, microprecipitin (MP) tests were conducted with leaf extracts from two test corn plants for each beetle species tested. Leaf extracts from MCMV source plants and healthy corn plants were used as controls. Extracts were prepared from test corn leaves showing symptoms and healthy plant leaves by grinding tissue with a pestle in a mortar containing 0.1 M potassium phosphate, pH 7.0, at 2.0 ml of buffer per gram of leaf tissue. The crude extract was filtered through two layers of cheesecloth and centrifuged for 10 min at 12,000 *g*. The supernatant fraction was diluted (three twofold dilutions) in physiologically buffered saline (PBS; 0.15M NaCl + 0.01 M potassium phosphate, pH 7.0) used as the diluent. The clarified extract plus the three dilutions were tested with a 1:64 dilution of MCMV antiserum (15) diluted in PBS. This antiserum dilution gave the optimum titer for virus in diluted corn leaf extracts. The MP test was slightly modified from that described by Ball (3). Serological plates were incubated overnight at about 10 C, and results recorded the next morning.

Adult *D. virgifera* were collected from Kansas corn fields. The beetles were crushed in a small amount of 0.05 M tris-HCl buffer, pH 7.5 and assayed by agar double diffusion tests in 0.75% Ionagar No. 2 containing 0.05 M Tris-HCl, pH 7.5, 0.85% NaCl, and 0.02% NaN_3.

RESULTS AND DISCUSSION

All six species of chrysomelid beetles including both larval and adult forms of *O. melanopa,* transmitted MCMV (Table 1). All plants tested serologically after transmission reacted positively with MCMV antiserum, whereas healthy plant extracts showed no reaction. Thus, MP tests confirmed MCMV infections as determined by symptomatology. In addition, two groups of 10 adult *D.*

TABLE 1. Insect species tested as vectors for maize chlorotic mottle virus

Insect species	Acquisition access period	Inoculation access period	Insects tested (no.)	Plants infected/ plants tested (no.)	Transmission rate
Coleoptera					
Oulema melanopa (adults)	1-4 days	1-4 days	100	39/100	.390
O. melanopa (larvae)	4 days	3 days	42	38/42	.905
Chaetocnema pulicaria	2 days	3 days	215	10/43	.052[a]
Systena frontalis	2-3 days	2-3 days	175	4/35	.024[a]
Diabrotica virgifera	3 days	1 day	190	23/38	.170[a]
Diabrotica longicornis	3 days	2 days	95	10/19	.139[a]
Diabrotica undecimpunctata	3 days	2 days	105	12/21	.156[a]
Popillia japonica	1 day	1 day	55	0/18	0
Lepidoptera					
Agrotis ipsilon	1 day	1 day	50	0/50	0
Homoptera					
Rhopalosiphum padi	5-10 min	1 hr	240	0/16	0
R. padi	2 days	2 days	500	0/18	0
R. padi	2 days[b]	7 days	300	0/16	0
Myzus persicae	5-10 min	1 hr	240	0/12	0
M. persicae	2 days	2 days	500	0/13	0
M. persicae	2 days[b]	7 days	200	0/16	0
Schizaphis graminum	5-10 min	1 hr	400	0/20	0
S. graminum	2 days	2 days	360	0/12	0
Trialeuroides vaporariorum	2 days	2 days	240	0/24	0
Peregrinus maidis	2 days[c]	7 days	120	0/12	0
Graminella nigrifrons	2 days	3 days	100	0/18	0
G. nigrifrons	2 days[c]	7 days	100	0/18	0

[a]Estimated values for transmission by single insects determined by the formula $Y = 1 - \sqrt[n]{1-T}$, where: T = experimental transmission rate; Y = transmission rate for one insect/plant; n = number insects per test plant (=5).

[b]A 7-day incubation period was provided before aphids were placed on test plants.

[c]A 21-day incubation period was provided before insects were placed on test plants.

virgifera from Kansas cornfields infested with MCMV reacted positively with MCMV antiserum in agar double diffusion tests, whereas beetles from noninfested fields showed no reaction.

The two other insect species with mandibulate mouthparts, *P. japonica* and *A. ipsilon* failed to transmit MCMV as did all six homopteran species. Acquisition and inoculation access periods as well as incubation periods were adjusted to test for nonpersistent, semipersistent, and persistent transmission by aphids. Similarly, these periods were adjusted to test for semipersistent and persistent transmission by the leafhopper, *G. nigrifrons,* and to test for persistent transmission by the planthopper, *P. maidis.* With the exception of the whitefly, *T. vaporariorum,* the homopteran species tested are vectors of maize viruses. The aphid species transmit maize dwarf mosaic virus (12), *P. maidis* transmits maize mosaic virus (11), maize stripe virus, and maize line virus (10), and *G. nigrifrons* transmits maize chlorotic dwarf virus (14) and maize rayado fino virus (4).

Maize chlorotic mottle virus is the third plant virus known to be vectored by *O. melanopa.* This species also transmits cocksfoot mottle virus (CMV) (17) and phleum mottle virus (PMV) (7). The transmission pattern of MCMV by *O. melanopa* (Fig. 1) resembles that of CMV and PMV (1). The viruses are transmitted soon after acquisition and most vectors lose their ability to transmit virus after 1 wk. Unlike PMV and CMV (7, 17), MCMV is transmitted more efficiently by larvae than by adults.

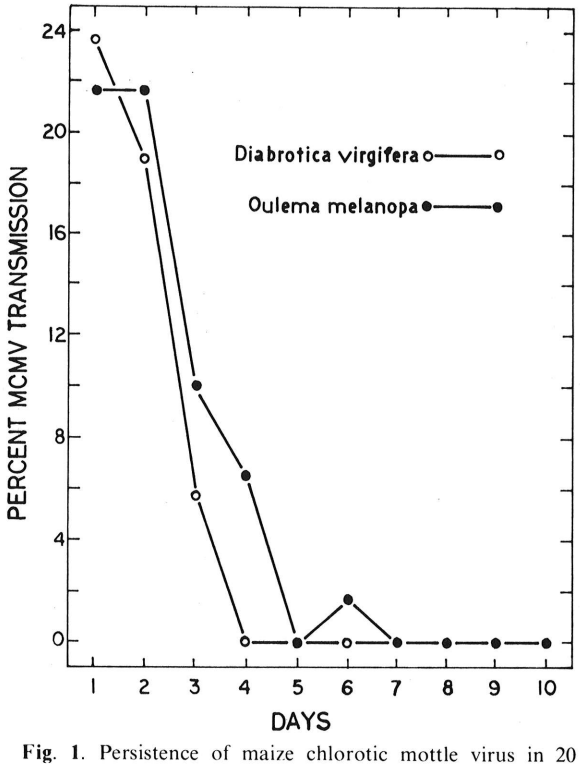

Fig. 1. Persistence of maize chlorotic mottle virus in 20 *Diabrotica virgifera* and 60 *Oulema melanopa* following a 24-hr acquisition acess period. Single beetles were transferred daily for 10 days.

Larvae of *O. melanopa* lose the ability to transmit following pupation and adult eclosion. In a test where 18 of 20 larvae transmitted MCMV, none of 15 adults which developed from a similar group of larvae transmitted virus. This is the first report of the transmission of a plant virus by *C. pulicaria* and *S. frontalis.* The *Diabrotica* species previously have been reported to be vectors of squash mosaic virus (9, 18).

These six MCMV vector species belong to three separate chrysomelid subfamilies; *O. melanopa* to the Criocerinae, the flea beetles to the Halticinae, and the *Diabrotica* spp. to the Galerucinae. Selman (16) lists beetles from three chrysomelid subfamilies as vectors of turnip yellow mosaic virus and three other plant viruses transmitted by beetles from two subfamilies. It is likely that other chrysomelid species can transmit MCMV, since there is little apparent intrafamily specificity in their transmission of viruses.

Of the six chrysomelid vector species, only *O. melanopa* does not occur in Kansas (2). We would anticipate that *C. pulicaria* and *D. undecimpunctata* are responsible for initial spread of the virus in Kansas since adults of both species overwinter and appear in the field early in the corn-growing season (13). It seems unlikely that a virus with such short persistence in its vectors would overwinter in either beetle species. Rather they probably acquire MCMV from an overwintering weed host and then transmit it to corn. *Diabrotica virgifera* and *D. longicornis* overwinter in the egg stage and do not appear as adults in the corn field until the growing season is well advanced (8). These species may be responsible for secondary spread of the virus in corn. Identification of insects which vector and do not vector MCMV will now permit more systematic epidemiological studies on both MCMV and the corn lethal necrosis disease.

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