Maize Chlorotic Mottle and Maize Dwarf Mosaic Viruses: Effect of Single and Double Inoculations on Symptomatology and Yield

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ABSTRACT

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Three corn hybrids (*Zea mays* L. A619 × A632, B73 × FR16, and Mo17 × B73) were planted in replicated blocks and spray-inoculated at the 3-, 7-, and 14-leaf stages. Inocula consisted of maize chlorotic mottle virus (MCMV), maize dwarf mosaic virus strain A (MDMV-A), and a combination of the two. Single virus infections caused systemic mottle or mosaic leaf patterns and reduced grain yields. Symptoms on all hybrids inoculated with the virus combination, at 3- and/or 7-leaf stages, consisted of a systemic chlorotic mottle, tissue necrosis beginning at leaf margins and progressing to the midvein, stunting, and premature death of plants. Chlorosis and necrosis of terminal leaves developed in plants at the 14-leaf stage inoculated with MCMV and MCMV plus MDMV-A. In general, early virus infections significantly reduced corn yields; losses were heaviest on plants receiving the virus combination.

Corn lethal necrosis disease (CLND) is caused by two viruses infecting a common corn plant (*Zea mays* L.) (1). A combination of maize chlorotic mottle (MCMV) and maize dwarf mosaic (MDMV) viruses or MCMV and wheat streak mosaic virus (WSMV), but not MDMV plus WSMV, will cause CLND.

In the field, we observed two types of CLND symptoms—terminal leaf necrosis of tasselled plants or leaf chlorosis and necrosis, plant stunting, and death. We reproduced these symptoms and determined their effects on yields; results of a 2-yr study are reported.

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MATERIALS AND METHODS

Procedures. MCMV, Kansas serotype 1 (5) and MDMV strain A (MDMV-A; culture obtained from D. T. Gordon, Wooster, OH) were increased separately in N28Ht corn seedlings. Virus inocula were prepared as described (6). Briefly, 14- to 21-day infected tissues were triturated in buffer (0.05 M potassium phosphate, KPO4, pH 7.0 for MCMV; 0.05 M sodium citrate, pH 7.5 for MDMV) containing 0.1% 2-mercaptoethanol. Extracts were filtered through cheesecloth, held on ice, and used within 24 hr. When a mixture of viruses was required, a 2X virus extract was made (at 2X, tissue to buffer ratio, w/v, was 1:50 for MCMV and 1:5 for MDMV) and an equal proportion of each was mixed just before use.

Inocula containing Carborundum (600 mesh, 12 g/L) were applied with a DeVilbiss spray gun (Model EGA-502; DeVilbiss Company, Somerset, PA 15501), which was held close to or in contact with surfaces of terminal leaves of corn plants at 3-, 7- and 14- (collared) leaf stages. Air pressure supplied by CO_2 cylinders was 5.62 kg/cm^2 (80 psi). Such leaf stages typically occurred 25, 42, and 56–62 days after planting.

Table 1. Effect of virus inoculum and time of inoculation on plant height and percent infection

Virus[c]	Leaf stage	Height (m)[a]			Virus recovered[b]					
					A619 × A632		B73 × FR16		Mo17 × B73	
		A619 × A632	B73 × FR16	Mo17 × B73	MCMV	MDMV	MCMV	MDMV	MCMV	MDMV
MCMV	3	2.0 a	1.5 bc	2.0 ab	100	10	100	10	100	0
	7	2.1 a	1.8 ab	2.1 ab	100	0	100	10	100	0
	14	2.1 a	2.0 a	2.3 ab	20	0	50	50[d]	20	50[d]
MDMV-A	3	1.8 ab	1.5 bc	2.0 ab	30	100	0	100	40	100
	7	2.0 a	2.0 a	2.3 ab	20	90	0	90	0	50
	14	2.0 a	2.1 a	2.2 ab	60	0	100	0	40	30
MCMV plus MDMV-A	3	1.5 b	1.2 c	1.9 b	100	100	100	100	100	80
	7	1.4 c	1.8 ab	2.1 ab	100	30	100	70	100	10
	14	2.0 a	2.0 a	2.3 ab	40	40	60	30	70	30
Control	3	2.0 a	2.3 a	2.4 a	0	0	0	10	20	0
	7	2.1 a	2.0 a	2.4 a	0	0	0	10	0	0
	14	2.1 a	2.0 a	2.4 a	30	0	90	0	30	20[d]

[a] Each value is the mean for 10 plants measured from brace root to collar of terminal leaf. In each column, means followed by the same letter are not significantly different ($P = 0.05$), according to Duncan's new multiple range test.
[b] Each value is the percent infection by maize chlorotic mottle virus (MCMV) and maize dwarf mosaic virus strain A (MDMV) in 10 plants from two replicated plots for each hybrid, treatment, and inoculated leaf stage.
[c] Inocula sprayed onto corn plants.
[d] Percent infection by MDMV strain B.

For bioassays and seroassays of corn plants in 1979, we sampled 10 terminal leaves 27–46 days after inoculation (five consecutive plants in a row from two replicated blocks were sampled). Each sample was crushed in 0.01 M KPO4 buffer containing 0.85% sodium chloride, tested in immunodouble-diffusion agar plates against MCMV antiserum (5), and assayed on seedlings of N28Ht corn and Asgrow Bugoff sorghum (*Sorghum bicolor* Moench). All symptomatic indicator corn plants were retested by MCMV-serologic test, and MDMV-A and -B were identified on sorghum indicators (6) and by enzyme-linked immunosorbent assays (4; J. K. Uyemoto, *unpublished*).

Plot design and location. Three corn hybrids of two maturity groups (105 days, A619 × A632; 118 days, B73 × FR16 and Mo17 × B73) were planted in four replicated plots in a split-split plot design. Each hybrid, planted in two rows 7.6 m long and 0.75 m apart, had a final population of 56,810 plants per hectare; 12 m of row was harvested for each hybrid, treatment, and replication. Corn ears were harvested after physiological maturity (mid-September to early October), grain yields were adjusted to 15.5% moisture, and data were analyzed statistically.

In 1978 and 1979, the plots were at the Rocky Ford Research Farm, Manhattan, KS, and at the Kansas State University Irrigated Station, Scandia, KS, respectively. Each plot site was treated with 224 kg/ha of N and 21.6 kg/ha of P; at Scandia, carbofuran (10 G) was banded (15 cm wide) over the seed bed at 1.1 kg (a.i.)/ha to control corn rootworm larvae.

RESULTS

Symptomology. Leaf chlorosis developed 21 days after inoculation in all plants of all hybrids inoculated at the 3- and 7-leaf stages with MCMV and MCMV plus MDMV-A. Plants infected with MDMV-A had light and dark green leaf mottling. Later, plants inoculated with MCMV plus MDMV-A showed leaf necrosis and plant stunting. By August, moribund plants were evident. Ears were lacking, deformed, or only partially filled, and husk leaves senesced prematurely.

Plants inoculated at the 14-leaf stage continued to develop new terminal growth, which extended 30–45 cm above the point of inoculation. Some plants inoculated with MDMV-A developed mild mosaic symptoms on terminal leaves. With MCMV and the virus combination, the new growth showed severe leaf chlorosis and/or necrosis, but ears were normal in size.

Depending on virus inocula and stage of infection, plant height was also affected. On 4 September 1979, height of plant from the brace roots to the collar of the terminal leaf was determined for a total of 10 plants for each treatment. With B73 × FR16, heights of all plants inoculated at the 3-leaf stage differed significantly from heights of uninoculated control plants; height was reduced 35–48% (Table 1). Inoculation with MCMV plus MDMV-A also caused significant stunting of Mo17 × B73 plants inoculated at the 3-leaf stage and of A619 × A632 plants inoculated at the 3- and 7-leaf stages.

Efficacy of inoculation. Bioassays and seroassays confirmed that MCMV, alone or combined with MDMV-A, was transmitted to all plants inoculated at the 3- and 7-leaf stages (Table 1). MCMV infection ranged from 20 to 70% in plants inoculated at the 14-leaf stage. With MDMV-A, all plants (except Mo17 × B73) at the 3-leaf stage inoculated with the virus mixture became infected. The incidence of MDMV-A infection in plants inoculated at 7- and 14-leaf stages ranged from 0 to 90%.

Some intraplot spread of both viruses and natural spread of MDMV-B occurred. Because assays showed that virus contaminations apparently were heaviest at or soon after anthesis, however, such virus transmissions did not affect corn yields significantly (Table 2).

Effect of viruses on yield. Table 2 shows average yields. Significant reductions in grain yields resulted from inoculations only at the earliest leaf stages of plant development: MCMV on A619 × A632 (at 7-leaf stage) and B73 × FR16 (at 3- and 7-leaf stages), and MDMV-A on B73 × FR16 and Mo17 × B73 (both at 3-leaf stage), and virus mixture on all three hybrids (at 3- and 7-leaf stages). The remaining treatments did not differ significantly from uninoculated controls.

Table 2. Effect of virus inoculum and time of inoculation on corn yields[a]

Virus[b]	Leaf stage	Yield (kg/ha)[a] of hybrid		
		A619 × A632	B73 × FR16	Mo17 × B73
MCMV	3	4683.1 bcd	2718.2 d	5944.9 bc
	7	3961.2 de	2699.4 d	5869.6 bc
	14	4789.8 bcd	4287.6 bc	6522.4 ab
MDMV-A	3	4620.3 bcd	2887.7 d	4789.8 c
	7	4488.5 cd	4268.8 bc	5951.2 bc
	14	5624.7 ab	4946.8 abc	7024.6 ab
MCMV plus MDMV-A	3	1713.8 f	1462.7 e	4545.0 c
	7	3013.2 e	2002.6 de	4821.2 c
	14	5210.4 abc	4067.9 c	5656.1 bc
Control	3	5561.9 abc	4965.6 abc	7432.7 a
	7	6057.9 a	5128.8 ab	6390.6 ab
	14	5279.5 abc	5838.2 a	7765.4 a

[a] Averages of 1978 and 1979 yields. In each column, means followed by the same letter are not significantly different ($P = 0.05$), according to Duncan's new multiple range test.
[b] Maize chlorotic mottle virus (MCMV), maize dwarf mosaic virus strain A (MDMV-A); inocula were sprayed onto corn plants.

DISCUSSION

The symptoms of disease in the field were correlated with the stage of plant growth at the time of virus inoculation. Double virus infections at the 7-leaf stage or earlier caused chlorosis and necrosis of leaf tissues, resulted in small and deformed ears, and induced stunting and premature death of plants. At the 14-leaf stage, infections by MCMV and MCMV plus MDMV-A caused severe chlorosis and/or necrosis of terminal leaves; ears were normal in size, but kernel quality (test weight) was reduced.

Plant height was reduced significantly only in a few virus-hybrid combinations; however, grain yields in several treatments were significantly lower than those of comparable controls. Although the relationship between time of infection and corn yield for MCMV was not reported previously, yield losses (attributed to CLND) of 50–90% have been estimated (1,5). Also, our results with MDMV-A alone agree with previous reports that early inoculation (3- to 9-leaf stage) causes yield losses (2,3).

In a CLND test program at Kansas State University, we have arbitrarily spray-inoculated plants of commercial corn hybrids at the 7-leaf stage and found some moderately tolerant hybrids (L. E. Claflin, *unpublished*). Based on inoculation efficiencies and yield results, it appears that the selected growth stage is ideal for such CLND-hybrid corn evaluations.

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