

Severe Maize Chlorotic Dwarf Disease Caused by Double Infection with Mild Virus Strains

R. E. Gingery and L. R. Nault

Research chemist and adjunct professor, Corn and Soybean Research Unit, USDA-Agricultural Research Service (ARS), Department of Plant Pathology, and professor, Department of Entomology and Department of Plant Pathology, The Ohio State University (OSU), Ohio Agricultural Research and Development Center (OARDC), Wooster 44691.

Cooperative investigation of USDA-ARS and OSU-OARDC. We thank W. E. Styer and T. G. Lanker for technical assistance and D. T. Gordon for antiserum to MCDV.

Salaries and research support were provided by state and federal funds appropriated to the USDA-ARS and OSU-OARDC. Submitted as Journal Article 272-89 by the OSU-OARDC.

The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

Accepted for publication 11 January 1990 (submitted for electronic processing).

ABSTRACT

Gingery, R. E., and Nault, L. R. 1990. Severe maize chlorotic dwarf disease caused by double infection of mild virus strains. *Phytopathology* 80:687-691.

Severe symptoms thought to be caused by maize chlorotic dwarf virus (MCDV) in the field have not been consistently reproduced by isolates of the virus in the greenhouse. To determine if severe symptoms could be caused by multiple infections of mild MCDV strains, six MCDV isolates causing mild symptoms were separated from naturally infected johnsongrass (*Sorghum halepense*), the overwintering host, and then coinoculated into corn (*Zea mays*) in various combinations. One of the isolates, MCDV-M1, caused mild symptoms by itself, but severe symptoms in combination with any of the other mild isolates or the type strain. MCDV-M1 was serologically related to but distinct from the other isolates

and the type strain, and two of the three capsid proteins from MCDV-M1 were larger than those of the other isolates and type strain. The other isolates were all indistinguishable from the type strain. Virus isolated from plants with severe symptoms after double inoculation with MCDV-M1 and another of the isolates gave a capsid protein pattern expected for a combination of both viruses. We conclude that MCDV-M1 is a strain of MCDV and that severe symptoms resulted from a synergistic effect involving MCDV-M1 and one of the other isolates. In a field survey, seven of 11 severely diseased corn plants and seven of 10 johnsongrass plants were doubly infected with MCDV-M1 and the type strain.

Maize chlorotic dwarf virus (MCDV) causes an important disease in corn (*Zea mays* L.) in the southeastern United States (5) within the region of overlap of the distributions of its vector, the black-faced leafhopper, *Graminella nigrifrons* (Forbes), and its overwintering host, the perennial weed, johnsongrass (*Sorghum halepense* (L.) Pers.). Symptoms on infected corn plants in the field include stunting, leaf discoloration (reddening and yellowing), leaf tearing, and a diagnostic chlorosis of the tertiary leaf veins (veinbanding) (2,5,9,15). Leaf discoloration and plant stunting are correlated with MCDV infection, but are not diagnostic (5), that is, plants infected with maize chlorotic dwarf are frequently stunted and discolored, but sometimes they are not.

It is puzzling that the severe syndrome, characterized by marked stunting, leaf discoloration, and leaf tearing, usually attenuates during serial transfer in the greenhouse to a mild syndrome, plants with veinbanding and some mottling. Perhaps the serial transfer protocol used, in which transfers are often made before severe symptoms have a chance to be expressed, selects against the more severe symptoms (4), or it may be that a second agent is frequently part of the disease in the field that is somehow lost during greenhouse transfers. Stable strains causing unusually bright veinbanding and stunting have been identified that do not attenuate during serial transfer and are thus different from the severe syndrome studied here (7).

Circumstantial evidence for a second agent was provided by the so-called "segregating-severe" transmission of MCDV (L. R. Nault, unpublished results), in which *G. nigrifrons* leafhoppers were fed on field-collected MCDV-infected corn or johnsongrass and then on corn test plants. The test plants subsequently segregated into two distinct symptom classes: those with severe symptoms (stunting, leaf tearing, leaf discoloration, and

veinbanding), and those with mild symptoms (veinbanding and mottling). Later transmission from those test plants with severe symptoms again resulted in plants with severe or mild symptoms, but transmissions from plants with mild symptoms resulted in plants with mild symptoms only. Also, the larger the number of vectors used in inoculations from plants with severe symptoms, the greater the frequency of severe symptoms among test plants. The simplest explanation for this phenomenon is that simultaneous infection by two agents was required for induction of severe symptoms and that infection with either agent alone resulted in mild or no symptoms. Because of the similarities between MCDV and the rice tungro spherical virus (RTSV), we first speculated that the severe maize chlorotic dwarf syndrome might resemble rice tungro, a disease caused by the synergistic combination of RTSV and the rice tungro bacilliform virus, a separate kind of virus (6). However, we were unable to identify the putative second agent, bacilliform or otherwise, from MCDV-infected plants (R. E. Gingery, unpublished results).

We then hypothesized that the severe syndrome might be caused not by two readily distinguishable agents, but by two strains of MCDV acting synergistically. To test this, MCDV isolates causing mild symptoms were separated from severely diseased corn plants and then coinoculated to see if any combinations resulted in severe symptoms. One of the isolates (MCDV-M1), in combination with any of the others or the type isolate, did produce severe stunting, leaf discoloration, and leaf tearing. This isolate was serologically distinct from the others and had coat proteins of different molecular weight. This paper describes the effects of double infection involving MCDV-M1 and other MCDV isolates and strains, some of the properties of MCDV-M1, and the results of field surveys for MCDV-M1.

MATERIALS AND METHODS

Virus strains and isolates. During June 1988, johnsongrass rhizomes were collected randomly, 12 from one field and four

from another, near the Ohio River, about 8 km west of Portsmouth, OH. The first field was uncultivated and weedy, the second had been planted partly to field corn, partly to sweet corn. Maize chlorotic dwarf symptoms were present in both kinds of corn with severe stunting particularly apparent in a few of the sweet corn plants.

The rhizomes were taken to Wooster and planted in 15-cm pots in the greenhouse. Three weeks later, when shoots were about 30 cm tall, each pot was placed separately in an insect rearing cage (3). About 60 laboratory-reared adult *G. nigrifrons* were introduced into each cage and given a 3-day acquisition-access period (AAP) on the shoots. The leafhoppers were then placed in a cage with two pots (two plants per 10-cm pot) of Aristogold Bantam Evergreen (= Aristogold) sweet corn and three pots (two plants per 10-cm pot) of Oh28 inbred field corn for a 3-day inoculation-access period (IAP), after which the plants were sprayed with insecticide and placed in the greenhouse. Ten days later, the plants were transplanted singly to 15-cm pots and held for symptom development.

Field survey. Plants were collected from two private sweet corn plantings near Portsmouth, OH, on 4 August 1989. Thirteen plants were selected that showed marked stunting and leaf reddening and were designated severely diseased. Thirteen control plants were randomly selected from among those of typical height and normal leaf coloration. Virus was purified from leaf tissue (10 g) of each plant and the capsid proteins analyzed on 10% polyacrylamide gels following the procedures described below. The survey plants were also assayed for MCDV and maize dwarf mosaic virus (MDMV) by enzyme-linked immunosorbent assay (ELISA).

Virus purification. MCDV-infected leaves were ground in 0.3 M potassium phosphate buffer, pH 7.0, containing 0.5% 2-mercaptoethanol (1 g of tissue per 5 ml of buffer) and purified by the procedure of Hunt et al (7) except that the first high-speed pellet was resuspended in 0.3 M potassium phosphate buffer, pH 7.0, clarified with 1/4 volume CHCl_3 , and layered directly onto 10–40% sucrose density gradients. Virus was recovered from gradient fractions by high-speed centrifugation and the pellets were resuspended in 0.01 M potassium phosphate, 0.01 M ethylenediaminetetraacetic acid, pH 7.0. The amount of virus recovered was estimated with the BCA protein assay (Pierce Chemical Co., Rockford, IL).

Protein electrophoresis. Viral proteins were separated on 10 or 12-1/2% vertical slab polyacrylamide gels using the Laemmli system (8) and silver stained. All staining steps were done at room temperature on a rotating platform shaker. For staining, gels were fixed in 20% 2-propanol, 10% trichloroacetic acid for 30 min, rinsed with distilled water, and oxidized for 15 min in 3.2 mM HNO_3 containing 0.1% potassium dichromate. Gels were then rinsed three times for 10 min each or until all yellow color was gone from the gel. Rinsed gels were treated with AgNO_3 (1.9 g/L) for 30 min and then rinsed in water for at least 2 min. Developer was freshly prepared by adding 100 μl of formaldehyde to 200 ml of 0.28 M Na_2CO_3 . Part of the developer was poured over the gel and swirled by hand for 1–2 min until a yellowish-brown precipitate formed. The solution was then replaced with the remaining developer and incubation continued until the desired staining intensity was achieved (usually 5–15 min). Development was stopped by placing the gel in 5% acetic acid. Gels were photographed with an MP-4 Land Camera (Polaroid Corp., Cambridge, MA).

Serological tests. An indirect ELISA was used with $\text{F}(\text{ab}')_2$ fragments for trapping and biotinylated Protein A-streptavidin conjugated with horseradish peroxidase for detection. A previously described polyclonal antiserum to MCDV-type was used (5) and $\text{F}(\text{ab}')_2$ fragments and IgG were prepared as described by McDaniel and Gordon (10). Wells in the polystyrene microtiter "U" plates (Dynatech Laboratories, Inc., Alexandria, VA) were coated with 50 μl of 1 $\mu\text{g}/\text{ml}$ $\text{F}(\text{ab}')_2$ fragments for 2 hr at room temperature and blocked with 1% BSA in TBS-T (25 mM Tris-HCl, 0.15 M NaCl, pH 8.0, plus 0.05% Tween-20 [Sigma Chemical Co., St. Louis, MO]). After this and all subsequent steps, the

plates were washed three times with TBS-T. Antigen was added and the plates incubated overnight at 4 C. IgG (0.1 ml of 5 $\mu\text{g}/\text{ml}$ in TBS-T) was added and incubated for 2 hr at 37 C. Bound antibodies were then detected by adding 0.1 ml of biotinylated Protein A (Amersham Corp., Arlington Heights, IL) diluted 1:1,500 with TBS-T (2 hr at room temperature) followed by 0.1 ml of horseradish peroxidase-streptavidin (Amersham Corp.) diluted 1:1,000 with TBS-T (2 hr at room temperature). Substrate (0.1 ml of 0.1 M citric acid, 1 mM 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), pH 4.2, plus 0.1% H_2O_2) was then added, and color development followed at room temperature with a Model EL309 Automated Microplate Reader (Bio-Tek Instruments, Inc., Winooski, VT). The reactions were stopped with 5% SDS when the $A_{405\text{nm}}$ of the homologous antibody-antigen mixtures reached 1.0.

For Western blots, polyvinylidene difluoride (PVDF) membranes (Immobilon-P) (Millipore Corp., Bedford, MA) were wet in methanol, rinsed first in water, and then in 192 mM glycine, 25 mM Tris, 20% methanol, the buffer recommended for electrophoretic transfer in the Hoefer TE-42 transfer apparatus (Hoefer Scientific Instruments, San Francisco, CA). Proteins were transferred from polyacrylamide gels onto membranes for 18 hr at 40 V. After transfer, membranes were blocked with 5% nonfat dry milk (NFD) in TBS (0.9% NaCl, 20 mM Tris-HCl, pH 7.4) for 1 hr and then given three 5-min rinses in TBS containing 0.1% NFD. Blocked membranes were incubated 2 hr with primary antibody in TBS containing 1% NFD, rinsed three times as above, and incubated 1 hr with alkaline phosphatase-conjugated goat anti-rabbit IgG (1:2,000 dilution) in TBS containing 0.05% Tween 20. Membranes were then rinsed four times and developed with a nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (NBT/BCIP) solution prepared by combining 10 ml of NBT solution (0.2 mg/ml of NBT in 100 mM Tris-HCl, 5 mM MgCl_2 , 5 μM ZnCl_2) with 100 μl of BCIP solution (2 mg of BCIP in 100 μl of dimethyl sulfoxide). Color development was stopped by rinsing in distilled water. For total protein staining, membranes were placed in 0.1% amido black, 40% methanol, 10% acetic acid for 3–5 min, and then destained with several changes of 40% methanol, 10% acetic acid.

TABLE 1. Distribution of maize chlorotic dwarf symptom classes on Aristogold Evergreen Bantam sweet corn test plants inoculated by *Graminella nigrifrons* that had fed on johnsongrass shoots^a

Field no.	Johnsongrass sample no.	No. of test plants with:		
		Mild symptoms ^b	Severe symptoms ^c	No symptoms
1	1	7	3	0
1	2	6	4	0
1	3	3	7	0
1	4	3	7	0
1	5	6	4	0
1	6	3	6	1
1	7	3	7	0
1	8	3	7	0
1	9	2	8	0
1	10	6	4	0
1	11	10	0	0
1	12	4	6	0
2	1	5	5	0
2	2	4	6	0
2	3	3	7	0
2	4	10	0	0

^aAbout 60 *G. nigrifrons* were given a 3-day acquisition-access period on johnsongrass shoots and a 3-day inoculation-access period on 10 corn test seedlings (2 plants per pot). Plants were placed in the greenhouse, transplanted to 15-cm pots after 10 days and rated for symptoms 15 days later.

^bMild symptoms = veinbanding, slight stunting, and slight whorl chlorosis.

^cSevere symptoms = veinbanding, pronounced stunting, and splitting and twisting of leaves.

RESULTS

Selection of MCDV isolates. One month after leafhopper transmission from the collected johnsongrass plants to corn test plants, two symptom classes became evident: 1) mild—veinbanding, some chlorotic mottling, and slight stunting; and 2) severe—marked stunting, splitting, twisting, and intense chlorosis at the margins of the youngest leaves, reddening of the older leaves, and veinbanding. Symptoms were particularly severe in Aristogold Bantam Evergreen sweet corn. Table 1 shows the distribution of severe and mild symptoms among test plants.

Two of the Aristogold sweet corn plants showing severe symptoms from johnsongrass sample 3 from field 2 were selected for a second series of transfers using 10 leafhoppers per plant compared to inoculations with one leafhopper per plant.

To test the hypothesis that severe symptoms are caused by multiple infections of MCDV strains that separately cause mild symptoms, we selected the eight plants after single leafhopper transmission that had mild symptoms (see Table 2) and designated these plants as being infected with mild isolates M1 through M8. To confirm that these isolates caused no severe symptoms, about 160 *G. nigrifrons* were placed on each of these plants for a 24-hr AAP. Each of these vector groups were then given access to 22 test Aristogold seedlings for a 3-day IAP. After 3 wk, the plants were rated for symptoms and the above transmission repeated from these test plants to 22 new ones. Some test plants in two of the groups showed severe symptoms and those two isolates (M4 and M7) were discarded. To confirm that severe

TABLE 2. Symptom response on Aristogold Bantam Evergreen sweet corn after transfer from severely maize chlorotic dwarf diseased sweet corn with 1 or 10 *Graminella nigrifrons* per test plant^a

No. of leafhoppers per test plant	Symptoms on test plants		
	Mild ^b	Severe ^c	None
1	8/50 (16%) ^d	12/50 (24%) ^d	30/50 ^e (60%)
10	4/20 (20%) ^d	15/20 (75%) ^d	1/20 ^e (5%)

^aLeafhoppers were given a 24-hr acquisition-access period on two severely diseased corn plants that had received virus from johnsongrass plant no. 3 collected from field no. 2 and then given a 24-hr inoculation-access period on Aristogold Evergreen Bantam sweet corn seedlings. Symptoms were rated 3 wk after inoculation.

^bMild symptoms = veinbanding, slight stunting, and slight whorl chlorosis.

^cSevere symptoms = veinbanding, pronounced stunting, and splitting and twisting of leaves.

^dNumerator = number of plants expressing symptoms; denominator = total number of plants inoculated.

^eNumerator = number of symptomless plants; denominator = total number of plants inoculated.

TABLE 3. Symptoms produced on Aristogold Evergreen Bantam sweet corn following inoculation with different combinations of mild isolates of maize chlorotic dwarf virus

Isolates inoculated ^a	Symptoms on test plants	
	Mild ^b	Severe ^c
M1 + M2	2/6 ^d	4/6 ^d
M1 + M8	0/6	6/6
M6 + M8	6/6	0/6
M1 + M2 + M3 + M5 + M6 + M8	5/12	7/12
"Isolate 10"	0/5	5/5

^a*Graminella nigrifrons* were given a 48-hr acquisition-access period on corn plants infected with the various isolates and then placed on Aristogold Evergreen Bantam sweet corn test seedlings at the rate of 10 *G. nigrifrons* per isolate per plant and given a 48-hr inoculation-access period. Symptoms were rated 3 wk after inoculation.

^bMild symptoms = veinbanding, slight stunting, and slight whorl chlorosis.

^cSevere symptoms = veinbanding, pronounced stunting, and splitting and twisting of leaves.

^dNumerator = number of plants expressing symptoms; denominator = total number of plants inoculated.

symptoms could be expressed under the conditions of the above transmissions, control transfers from the original johnsongrass plant or from corn with severe symptoms after transmissions from this plant were made using 10 leafhoppers per test plant. In all such transfers, some plants developed severe symptoms. For convenience, plants showing severe symptoms after such transfers were said to be infected with "isolate 10."

Recombination of isolates. Corn test plants were inoculated simultaneously with various combinations of the mild isolates to see if severe symptoms could be recovered. The results of some of these combined infections are shown in Table 3. Various other combinations, including all possible double infections, were tested, and in all cases, except for isolate 10, only combinations that included isolate M1 induced severe symptoms (see next paragraph).

To quantify the apparent synergism between isolate M1 and the other isolates, the heights of singly and doubly inoculated plants were measured. Results with isolates M1 and M8 are shown in Figure 1. Results with isolate M1 and the other isolates were similar. Of 41 plants doubly inoculated with isolates M1 and either M2, M3, M5, or M6, 18 plants expressed severe symptoms and had a mean height (\pm the standard error) of 0.450 ± 0.026 m; the remaining 23 plants expressed mild symptoms and were 1.069 ± 0.038 m tall. Of 25 plants doubly inoculated with combinations of isolates M2, M3, M5, and M6, all had mild symptoms and their mean height was 1.163 ± 0.038 m. The 25 plants singly inoculated with isolates M2, M3, M5, or M6 all showed mild symptoms and had a mean height of 1.176 ± 0.025 m. The healthy and isolate 10 controls were those shown in Figure 1.

Thus, synergistic height reduction and severe symptom expression were seen only when isolate M1 was inoculated in combination with another isolate. Interestingly, isolate M1 alone stunted less and caused milder veinbanding symptoms than any of the other isolates. In fact, plants infected with isolate M1 were not statistically shorter than healthy control plants, whereas plants infected with any of the other isolates alone had about a 20% height reduction. The combination of isolate M1 and any of the other isolates reduced height by about 70% when severe symptoms were expressed, about the same as that caused by isolate 10. Not all plants doubly inoculated with isolate M1 and one of the other isolates became severely stunted. Some expressed only

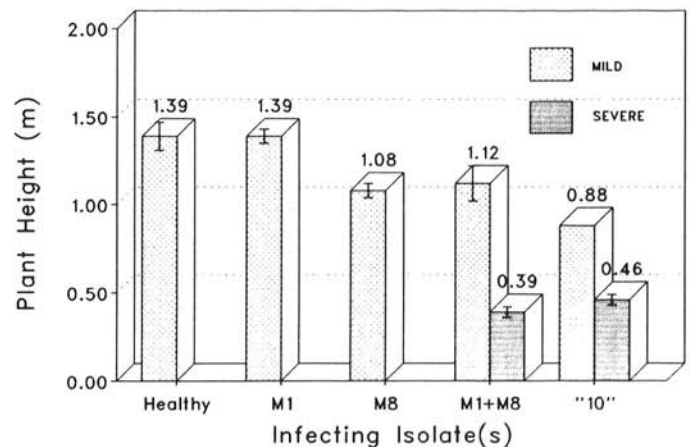


Fig. 1. Height of Aristogold Evergreen Bantam sweet corn plants inoculated with mild isolates of maize chlorotic dwarf virus. *Graminella nigrifrons* were given a 48-hr acquisition-access period on corn plants infected with the various isolates and then placed on Aristogold Evergreen Bantam sweet corn test seedlings at the rate of 10 *G. nigrifrons* per isolate per plant for a 48-hr inoculation-access period. Plants were grown in soil in 35-cm pots to maturity. Plant height was the distance from soil level to the base of the uppermost leaf. Mild symptoms included veinbanding, slight stunting, and slight whorl chlorosis. Severe symptoms included veinbanding, pronounced stunting, splitting and twisting of youngest leaves, and reddening of older leaves. Standard error bars are shown. Numbers of plants in the various classes were: healthy, 1; M1, 13; M8, 18; M1 + M8 (mild), 4; M1 + M4 (severe), 14; "isolate 10" (mild), 1; and "isolate 10" (severe), 11.

mild symptoms and may have been singly infected with one or the other of the isolates.

Electrophoresis of capsid proteins. The migration of capsid proteins in SDS-polyacrylamide gel electrophoresis revealed differences between isolates M1 and M5 (Fig. 2). The proteins were designated CP1, CP2, and CP3, in order of increasing mobility (decreasing molecular weight). CP1 from both isolates migrated at about the same rates. CP2 from isolate M1 was 2,000–2,200 Da larger and CP3 from isolate M1 was 500–900 Da larger than the corresponding proteins from isolate M5. On some gels, CP3 from isolate M1 was not clearly resolved from the CP3s of other isolates. The other mild isolates (M2, M3, M6, and M8) and the MCDV type strain had capsid protein patterns indistinguishable from that of isolate M5 (data not shown).

To determine if the difference in protein pattern could be used to identify plants doubly infected with isolate M1 and one of the other isolates, virus was isolated from plants singly inoculated with isolates M1 and M5 and from plants doubly inoculated with both. The capsid protein pattern of virus purified from plants with severe symptoms was that expected from a mixture of the two isolates (Fig. 2).

Antigenicity of capsid proteins. Even though there was no obvious difference in the molecular weights of the CP1s from isolates M1 and M8, there appeared to be no serological relationship between these two proteins. In Western blots, polyclonal antiserum to the type strain of MCDV reacted with CP2 and CP3, but not CP1 of isolate M1 (Fig. 3). Isolate M1 also reacted with MCDV-type antiserum in ELISA of intact virions, but to a lesser extent than did the MCDV-type and the M5 isolate (Fig. 4).

Field survey for isolate M1. With the discovery that severe disease could be caused by multiple infections involving isolate M1, we were curious as to the extent of such double infections among severely diseased plants in the field. Of the 13 severely diseased sweet corn plants collected, 11 contained MCDV as determined by ELISA and showed MCDV-like capsid protein patterns. The capsid protein patterns of virus from seven of the plants had four bands, which is consistent with double infection of isolate M1 and MCDV-type taking into account that the CP3s in isolate M1 and MCDV-type control lanes were not resolved by these gels; virus from the remaining four plants had three bands in the MCDV-type pattern (see Fig. 2). Only one of the controls (plants showing no stunting or leaf discoloration) was positive for MCDV; virus from that plant gave an M1-type capsid protein pattern. All of the plants except for one of the controls were also infected with maize dwarf mosaic virus (MDMV) as determined by ELISA.

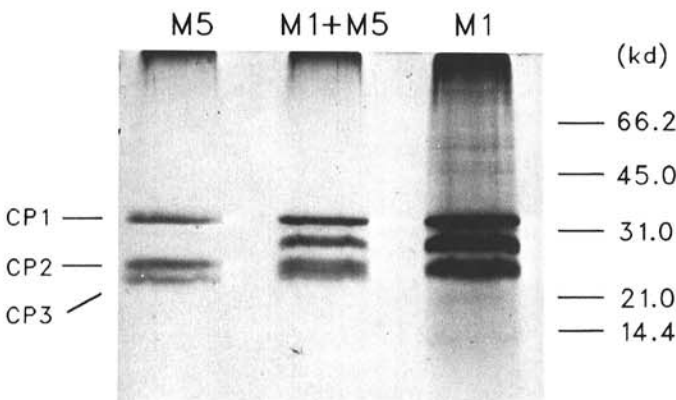


Fig. 2. Capsid proteins of maize chlorotic dwarf virus isolated from corn infected with isolates M1, M5, and a double infection of isolates M1 and M5. The capsid proteins of virus purified from plants infected with isolates M5, M1, or a mixture of the two were run on 10% polyacrylamide gels and silver stained. The protein molecular weight standards used were: bovine serum albumin (66.2 kDa), ovalbumin (45.0 kDa), carbonic anhydrase (31.0 kDa), soybean trypsin inhibitor (21.0 kDa), and lysozyme (14.4 kDa).

To obtain information about the distribution of MCDV-M1 in johnsongrass, 10 of the original johnsongrass plants collected in 1988 were assayed by capsid protein electrophoresis. To do this, virus was transmitted from these johnsongrass plants to groups of Aristogold sweet corn seedlings. Virus was then purified from the Aristogold plants that showed severe symptoms. Such plants from seven of the 10 groups showed the four bands indicative of double infection (results not shown).

DISCUSSION

The above results showed that a synergistic effect leading to severe symptoms resulted after coinfection of corn with MCDV isolate M1 and any of the other isolates tested. Each of the isolates alone caused only mild symptoms with isolate M1 causing the mildest of all.

Because isolate M1 was distinguishable from the other isolates serologically and physically, the question arises as to whether isolate M1 should be considered a strain of MCDV or a separate virus. The answer to this depends on one's perspective, because the point where two diverging isolates of a virus cease to be

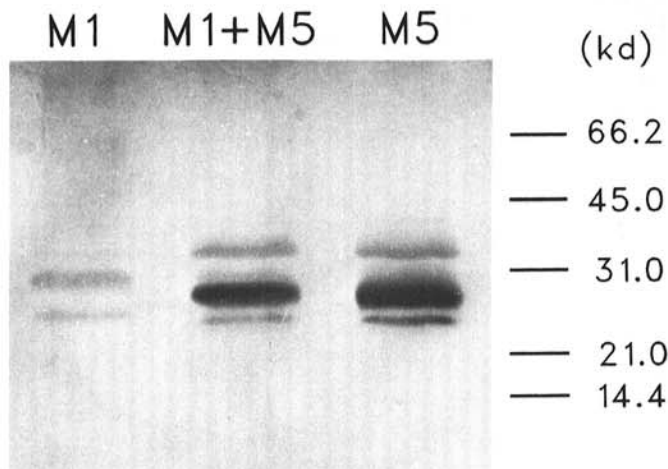


Fig. 3. Western blot analysis of maize chlorotic dwarf virus capsid proteins isolated from corn singly infected with isolates M1 or M5, and doubly infected with both. Capsid proteins from partially purified virus were electrophoresed on 10% polyacrylamide gels, blotted to polyvinylidene difluoride membranes and probed with polyclonal antiserum against the type strain of MCDV. The protein molecular weight standards used were: bovine serum albumin (66.2 kDa), ovalbumin (45.0 kDa), carbonic anhydrase (31.0 kDa), soybean trypsin inhibitor (21.0 kDa), and lysozyme (14.4 kDa).

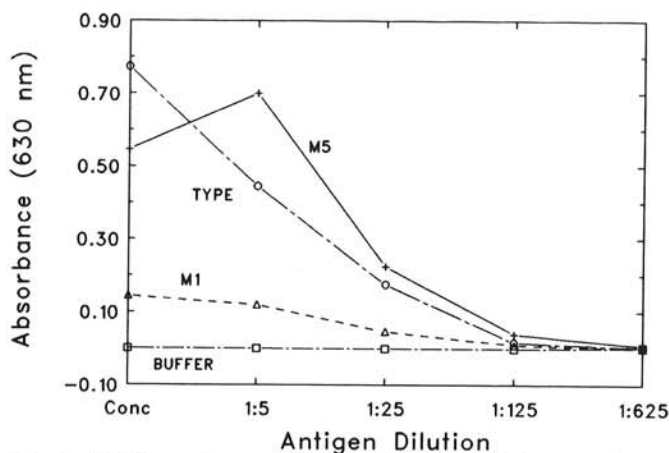


Fig. 4. ELISA reactions on dilution series of MCDV-type strain, and the M1 and M5 isolates. Purified virus preparations were adjusted to the same concentration based on a BCA protein assay and fivefold dilution series were assayed by ELISA. Development was allowed to proceed until the optical density was approximately 1.0 in the darkest wells.

strains and become separate viruses is arbitrary. In our opinion, isolate M1 was sufficiently closely related to the type strain of MCDV to be designated an MCDV strain. We based this conclusion on the observations that both isolate M1 and the type strain: 1) were serologically related; 2) were transmitted by *G. nigrifrons* with interchangeable helper components (J. R. Creamer, *personal communication*); 3) produced the diagnostic veinbanding symptom; and 4) were indistinguishable morphologically (both had 30 nm isometric particles).

To our knowledge, this is the first report of synergism between two strains of the same virus. A number of synergistic relationships in plant virology have been reported previously, but all involve two different viruses. For instance, maize chlorotic mottle virus and either MDMV or wheat streak mosaic virus combine to produce the severe corn lethal necrosis disease (12), rice tungro spherical virus and rice tungro bacilliform virus together produce rice tungro disease (6), and cucumber mosaic virus infection enhances the multiplication of zucchini yellow mosaic virus (13). Double infections of the barley yellow dwarf virus isolates RPV and MAV cause marked increase in symptom severity over either alone (1). Although the nomenclature suggests that this may be a case of synergism among virus strains, it is probably correct to consider RPV and MAV different viruses (14).

Although the survey conducted as part of this work was limited, the results suggested that mixed infections of MCDV-M1 and MCDV-type were common in severely diseased corn plants and johnsongrass plants. If future surveys confirm that MCDV-M1 is indeed widespread, then efforts to control it would be warranted. Because of the synergistic effect between MCDV-M1 and MCDV-type, resistance to either may afford adequate protection, even if the plant were susceptible to the other. However, despite considerable screening, no resistance in maize to the type strain has been identified, making a search for resistance to MCDV-M1 even more compelling. Resistance to MCDV has been observed in the wild perennial teosinte (*Zea*) relatives of maize (11). The results also raise the possibility that the severe syndrome may have other causes as well, such as the combined infection of MCDV-type and MDMV.

Synergism between MCDV-M1 and the other MCDV strains provides a likely explanation for the "segregating-severe" phenomenon in that severe symptoms result from multiple infections of MCDV-M1 and a second MCDV strain, and mild symptoms result from single infections by either isolate. The attenuation of greenhouse isolates can be explained as a

perpetuation of the single infections that result from the above segregation.

LITERATURE CITED

- Baltenberger, D. E., Ohm, H. W., and Foster, J. E. 1987. Reactions of oat, barley, and wheat to infection with barley yellow dwarf virus isolates. *Crop Sci.* 27:195-198.
- Bradfute, O. E., Louie, R., and Knoke, J. K. 1972. Isometric viruslike particles in maize with stunt symptoms. (Abstr.) *Phytopathology* 62:748.
- D'Arcy, C. J., and Nault, L. R. 1982. Insect transmission of plant viruses and mycoplasma-like and rickettsialike organisms. *Plant Dis.* 66:99-104.
- Gingery, R. E., Gordon, D. T., Nault, L. R., and Bradfute, O. E. 1981. Maize chlorotic dwarf virus. Pages 19-32 in: *Handbook of Plant Virus Infections—Comparative Diagnosis*. E. Kurstak, ed. Elsevier/North-Holland Biomedical Press, Amsterdam. 943 pp.
- Gordon, D. T., and Nault, L. R. 1977. Involvement of maize chlorotic dwarf virus and other agents in stunting diseases of *Zea mays* in the United States. *Phytopathology* 67:27-36.
- Hibino, H., Roehchan, M., and Sudarisman, S. 1978. Association of two types of virus particles with penyakit habang (tungro disease) of rice in Indonesia. *Phytopathology* 68:1412-1416.
- Hunt, R. E., Nault, L. R., and Gingery, R. E. 1988. Evidence for infectivity of maize chlorotic dwarf virus and for a helper component in its leafhopper transmission. *Phytopathology* 78:499-504.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. (London) *Nature* 227:680-684.
- Louie, R., Knoke, J. K., and Gordon, D. T. 1974. Epiphytotics of maize dwarf mosaic and maize chlorotic dwarf diseases in Ohio. *Phytopathology* 64:1455-1459.
- McDaniel, L. L., and Gordon, D. T. 1989. Characterization of the oat-infecting strain of maize dwarf mosaic virus. *Phytopathology* 79:113-120.
- Nault, L. R., Gordon, D. T., Damsteegt, V. D., and Iltis, H. H. 1982. Response of annual and perennial teosintes (*Zea*) to six maize viruses. *Plant Dis.* 66:61-62.
- Niblett, C. L., and Claffin, L. E. 1978. Corn lethal necrosis—a new virus disease of corn in Kansas. *Plant Dis. Rep.* 62:15-19.
- Poolpol, P., and Inouye, T. 1986. Enhancement of cucumber mosaic virus multiplication by zucchini yellow mosaic virus in doubly infected cucumber plants. *Ann. Phytopathol. Soc. Jpn.* 52:22-30.
- Rochow, W. F. 1979. Field variants of barley yellow dwarf virus: Detection and fluctuation during twenty years. *Phytopathology* 69:655-660.
- Rosenkranz, E. 1969. A new leafhopper-transmissible corn stunt disease agent in Ohio. *Phytopathology* 59:1344-1346.