Concentration of Maize Dwarf Mosaic Virus in Susceptible and Resistant Corn Hybrids

R. K. Jones and S. A. Tolin

Instructor and Assistant Professor, respectively, Department of Plant Pathology and Physiology, Virginia Polytechnic Institute and State University, Blacksburg 24061. Present address of senior author: Plant Pathology Department, North Carolina State University, Raleigh 27607.

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ABSTRACT

The concentration of maize dwarf mosaic virus (MDMV), assayed by analytical sucrose density-gradient centrifugation of clarified sap, varied with time after inoculation, leaf position, environmental conditions, and the corn hybrid used as the host. In the susceptible hybrid, Hy × C103, which was grown in the greenhouse, MDMV concentration was 20-25 μg/g fresh wt from 9 through 18 days after inoculation and declined gradually thereafter. Susceptible Golden Cross Bantam grown under the same conditions contained 20-25 μg MDMV/g from day 7 through day 10, but almost no virus after 10 days. Virus concentration in field-grown Hy × C103 and DeKalb 805A, mechanically inoculated with an artist's airbrush, reached a maximum of over 40 μg/g in 4-5 weeks after inoculation, and remained above 20 μg/g for 6-12 weeks. A resistant corn hybrid, T8 × 07B, showed systemic mosaic symptoms when grown in the greenhouse in the winter, but symptoms initially appeared 5-6 days later than in susceptible hybrids. Virus concentration in T8 × 07B ca. 10 days after inoculation was equivalent to that in the susceptible corn. During the summer, in both the greenhouse and field, T8 × 07B showed only occasional longitudinal bands of chlorotic tissue in otherwise normal, dark green leaves. A high concentration of MDMV was extracted from the chlorotic bands but not from the dark green part of the same leaves. The results suggest that the mechanism of resistance in T8 × 07B is not against virus infection or multiplication, but is against movement of the virus in the host.

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The most successful means of controlling maize dwarf mosaic virus (MDMV) has been through the development of resistant corn hybrids, but little has been done to determine the type of resistance expressed by these hybrids. Resistant hybrids often become infected upon inoculation in the greenhouse, but symptom appearance is usually delayed (9, 15). The time of symptom appearance has been correlated with field resistance of commercial hybrids (9). Tu & Ford (23) compared the relative virus concentration in the susceptible Seneca Chief and the resistant Illinois A corn by dilution end point assays and found that the inoculated leaves of both hybrids contained about the same amount of infectious MDMV, but systemic infection occurred in the susceptible hybrid only. They concluded that Illinois A has a genetic resistance that prevents virus movement within the plant.

A necessary step in studying any virus-host interaction is a means of measuring the amount of virus in the plant by a rapid and accurate assay. Lack of such an assay has presented difficulties to workers investigating MDMV. Systemic dilution end point assays have been used to obtain relative levels of MDMV in relation to environmental and other factors (8, 19, 20, 21, 22, 23), but such assays are rather laborious and insensitive. Local lesions are formed by MDMV on certain sorghum hybrids (16), but have not been useful for quantitative assays because of their low number and tendency to become confluent.

Virus assays have been developed which measure the amount of virus nucleoprotein in clarified sap rather than infectious virus. Brakke & Ball (5) measured wheat streak mosaic virus concentration by density-gradient centrifugation and photometric scanning. This method was more sensitive than previous systemic infectivity assays (3) and required less time.

The present study was undertaken to develop a rapid and accurate assay to measure the amount of MDMV in infected corn plants. The assay was then utilized to compare the concentration of MDMV in different corn hybrids grown under greenhouse and field conditions, and to measure MDMV extracted from susceptible and resistant corn hybrids. A preliminary report of this work has been published (7).

MATERIALS AND METHODS.–The MDMV culture used in these studies was isolated from naturally infected corn plants in Nelson County, Va. Its host range indicated it to be strain A (Johnson grass strain), as reported by Roane & Tolin (13). A stock culture of the virus had been maintained in the greenhouse for 3-4 years by mechanical transfer in *Zea mays* L. 'Golden Cross Bantam', 'Hy × C103', or 'DeKalb 805A' grown in 4- to 5-inch pots. Inoculations were made either by rubbing leaves, previously dusted with 600-mesh Carborundum, with inoculum prepared by grinding infected tissue in an equal volume of 0.01 M sodium phosphate, pH 7.0, or by spraying inoculum on leaves by means of an artist's airbrush (Thayer & Chandler, Inc., Chicago, Ill., Model C). Air pressure was maintained at 120 psi in the greenhouse and at 80-100 psi in field inoculations. For airbrush inoculations, infected tissue was ground in 0.1 M sodium citrate containing 0.1% mercaptoethanol (R. W. Toler, *personal communication*). The homogenate was strained through cheesecloth and diluted with the same solution to a final concentration of 1:10 (w/v), and

1 g of Carborundum per 100 ml of inoculum was incorporated. Corn seedlings were inoculated at the one- to two-leaf stage in the greenhouse or at the two- to four-leaf stage in the field. Field studies were conducted in the Blacksburg area, where MDMV and Johnson grass do not occur naturally.

MDMV was extracted from systemically infected corn leaves without the midrib by homogenizing the leaves in a volume of cold 0.1 M sodium citrate, containing 0.5% mercaptoethanol, equal to the weight of the tissue. Concentration determinations from greenhouse-grown plants were made from 2 g of fresh leaf tissue ground in a cold mortar and pestle, whereas those from field-grown tissue were made from 100 g of fresh tissue ground in a cold Waring Blendor. In both cases, leaves were collected from several plants, midribs were removed to facilitate grinding, and leaf blades were cut into ca. 1-inch pieces. The homogenate was strained through cheesecloth, then centrifuged for 10 min at 10,000 *g*. The volume of the supernatant was measured, emulsified with one-half volume of chloroform, and centrifuged for 5 min at 4,000 *g* to separate the emulsion. The aqueous, upper phase was collected and centrifuged for 15 min at 12,000 *g*. A 2-ml aliquot of the supernatant, or clarified sap, representing 1.50-1.75 g of tissue was layered on a sucrose density-gradient column and centrifuged for 3 hr at 27,000 rpm in the SW 27 rotor of the Beckman L2-65B ultracentrifuge. The tissue extract was kept refrigerated or on ice throughout the procedure. The sucrose gradients were made by layering 6, 9, 9, and 10 ml of solutions containing 100, 200, 300, and 400 g sucrose/liter, respectively, into 1 X 3½ inch cellulose nitrate tubes. The sucrose was dissolved in distilled H_2O; citrate buffer (0.5 M sodium citrate adjusted to pH 7.0 with citric acid) was added to make a final concentration of 0.005 M citrate. Gradients were allowed to stand overnight at 4 C before centrifugation.

Centrifuged gradients were scanned photometrically by the method of Brakke (4) with an ISCO (Instrumentation Specialties Co., Lincoln, Nebr.) ultraviolet analyzer adjusted to give a full scale

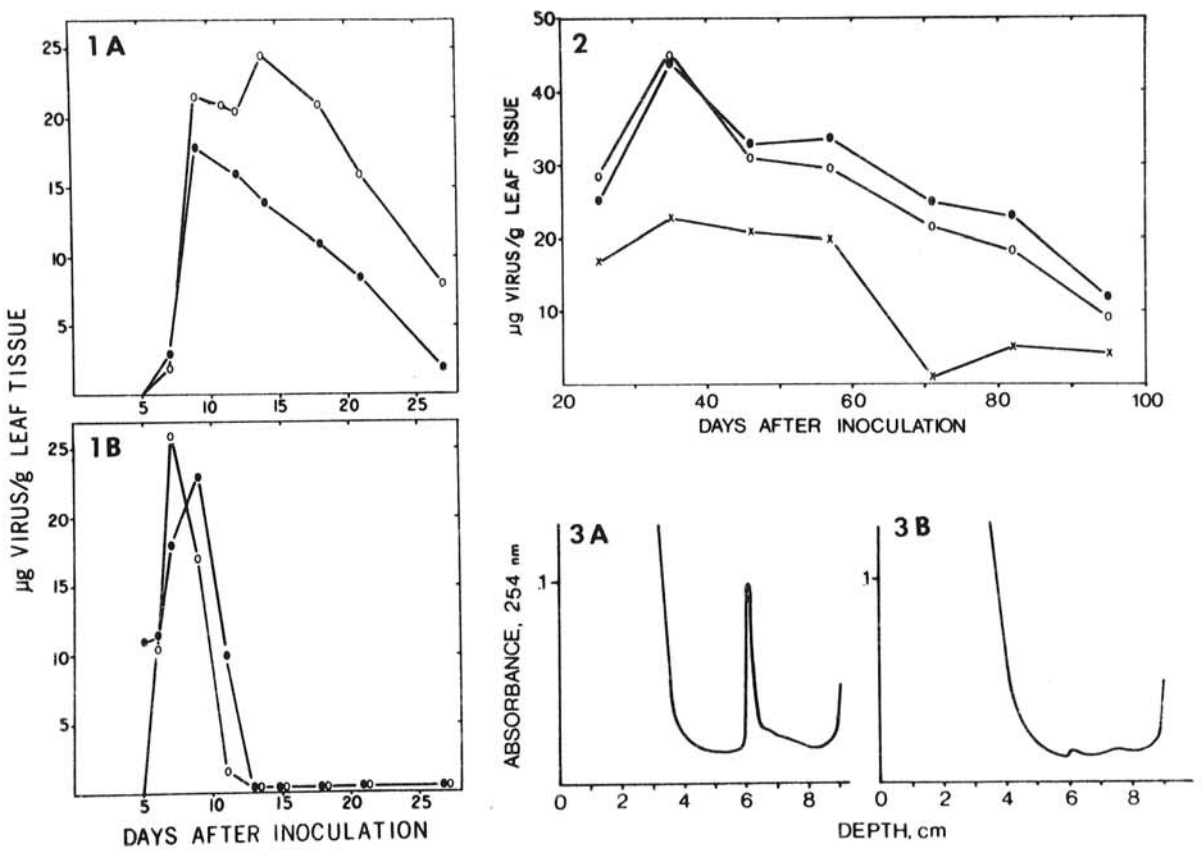

Fig. 1-3. 1) Concentration of maize dwarf mosaic virus (MDMV) in the third (-○-) and fourth (-●-) leaves of (A) Hy X C103 and (B) Golden Cross Bantam for 28 days after inoculation in the greenhouse. Concentrations were determined by density-gradient analysis of clarified sap representing 1.5 g of leaf tissue from several plants. 2) Concentration of MDMV in Hy X C103 (-○-), DeKalb 805A (-●-), and Golden Cross Bantam (-X-) for 95 days after inoculation in the field in 1970. Concentrations were determined by density-gradient analysis of a 2-ml aliquot of clarified sap, representing 1.5 g of tissue, from a 100-g sample of leaf tissue. 3) Density-gradient scanning patterns of a 2-ml aliquot, representing 1.5 g leaf tissue, of clarified sap from a 100-g sample of leaves of MDMV-inoculated, field-grown T8 X 07B. (A) Mosaic portion of leaves, and (B) dark green portion of the same leaves.

deflection equal to 0.0-0.25 absorbance units at 254 nm. The concentration of virus was determined by conversion of the virus peak area, including absorbance due to faster sedimenting aggregated virus, to total absorbance units. The absorbancy index at 260 nm of 2.4 cm^2/mg for tobacco etch virus (11) was used to convert total absorbance to μg of virus. A standard error is introduced by using this conversion factor, because the absorbance of purified MDMV at 254 nm is 4% less than at 260 nm (Jones & Tolin, *unpublished data*). MDMV has the same morphology and sedimentation coefficient as TEV, the only flexuous rod for which an extinction coefficient has been reported.

RESULTS.—*Virus concentration in susceptible hybrids.*—Three corn hybrids highly susceptible to MDMV under field conditions, Hy X C103, DeKalb 805A, and Golden Cross Bantam were used in all these tests. Symptoms appeared 4-5 days after inoculation when all hybrids were grown in the greenhouse. A slight flecking in the first leaf above the two inoculated leaves and a general mosaic in upper leaves were observed. The same hybrids grown under favorable conditions in the field showed mosaic symptoms 5-7 days after inoculation in all of the leaves above those inoculated. Stunting or dwarfing was not observed in any of the susceptible hybrids inoculated with MDMV in the field.

The concentration of virus in the third and fourth leaves of Hy X C103 and Golden Cross Bantam grown in the greenhouse was determined over a period of 28 days after inoculation, and the results of a single experiment are presented in Fig. 1. Virus was first detected in the third and fourth leaves of Hy X C103 plants 6 days after inoculation (Fig. 1-A). The concentration remained high (20-25 μg/g) in this hybrid from 9 through 18 days after inoculation, and declined gradually over the 4-week period. Virus was first detected on the 5th day after inoculation in the third leaf of Golden Cross Bantam (Fig. 1-B). The concentration was the highest (20-25 μg/g) in this hybrid 6-9 days after inoculation and decreased rapidly thereafter. Within 2 weeks after inoculation, no virus could be detected by this assay in Golden Cross Bantam.

The amount of virus extracted from the third and fourth leaves of Hy X C103 and Golden Cross Bantam at various times after inoculation from a number of greenhouse experiments in 1968, 1969, and 1970 is summarized in Table 1. Although variable, the pattern of virus concentration is consistent with that of the previous experiment. At no time was it possible to detect virus in the inoculated leaves by this assay.

The concentration of MDMV in three susceptible hybrids grown in the field is shown in Fig. 2. The concentration was highest (44 μg/g) in DeKalb 805A and Hy X C103, and lowest (22 μg/g) in Golden Cross Bantam. The results in 1969, though not so complete, were similar to those obtained in 1970. The concentration remained sufficiently high in all three hybrids to obtain a positive microprecipitin reaction with clarified sap and MDMV antiserum, and to be a source of the virus for purification until the leaves began to turn brown. The general pattern of virus concentration was the same as that observed in greenhouse studies, except that the concentration of MDMV in Hy X C103 and DeKalb 805A was increased 2-fold and declined more slowly. Even after 2-3 months, the concentration of MDMV in field-grown corn was nearly equivalent to the peak concentration of virus in greenhouse-grown corn, and corresponded to a dilution end point of 10^{-3} to 10^{-4}.

TABLE 1. Concentration of maize dwarf mosaic virus in clarified sap from corn hybrids grown in the greenhouse

Hybrid	Days after inoculation		
	7-13	14-20	21+
Hy × C103			
Range	3.0-47.5[a]	13.3-29.0	6.0-25.0
Average	20.1 (18)[b]	22.0 (6)	13.0 (4)
Golden Cross Bantam			
Range	1.0-39.0	0.0-19.8	0.0-4.5
Average	15.0 (16)	7.6 (12)	1.1 (4)

[a] μg of virus per g fresh weight of leaf tissue, determined by sucrose density-gradient analysis.
[b] Numbers in parentheses are the number of extractions.

Virus concentration in a resistant hybrid.—The resistant hybrid, T8 X 07B, showed a general mosaic pattern in the youngest leaf 9-10 days after inoculation in the greenhouse during the winter. The concentration of MDMV was as high as in comparable tissue of Hy X C103 and Golden Cross Bantam 11 days after inoculation (Fig. 4). In six different assays, sampled when T8 X 07B was showing general systemic mosaic symptoms in the winter, the amount of virus extracted from entire leaves showing symptoms ranged from 9.0 to 23.0 μg/g, with an average of 13.1 μg/g.

During the summer, some but not all T8 X 07B plants in the greenhouse and in the field expressed only a narrow, longitudinal band of chlorotic tissue through an otherwise normal, dark green leaf on some of the leaves of a particular plant. The narrow bands of mosaic tissue were usually limited to the tissue between two to six veins, and were as short as from 4 to 6 inches, but could extend the entire length of the leaf. Several leaves of T8 X 07B grown in the field showing the longitudinal banding symptoms were collected. The mosaic area of the leaf was separated from the dark green tissue of the same leaf, and virus concentration in each portion was assayed. The results (Fig. 3) show that MDMV occurred in high concentrations in the mosaic tissue (47 μg/g), whereas little or none was found in the green part of the same leaf.

DISCUSSION.—The concentration of MDMV in clarified sap of infected corn could be readily assayed by density-gradient centrifugation and photometric scanning. This technique for assaying virus has several advantages over the use of systemic infectivity assays, particularly when comparing the amount of virus in plants of different resistance levels, at different times

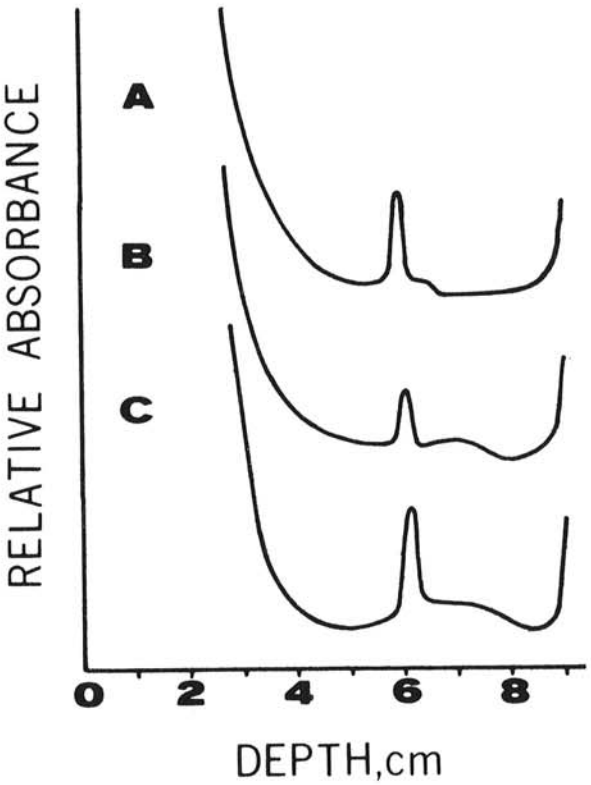

Fig. 4. Density-gradient scanning patterns of clarified sap, representing 1.5 g of leaf tissue, from three hybrids grown in the greenhouse in the winter, 11 days after inoculation. (A) T8 X 07B (17.0 μg virus/g of tissue). (B) Golden Cross Bantam (12.0 μg virus/g of tissue). (C) Hy X C103 (21.0 μg virus/g of tissue).

after inoculation, or grown under various environmental conditions. In a single day, 12-18 assays can be performed. Testing the same number of samples by infectivity assays would require a tremendous number of corn plants and several weeks to perform. In addition, this assay reproducibly detected differences of less than 5 μg/g.

Extensive comparisons of the density gradient assay, systemic infectivity assay, and microprecipitin assay were run in connection with purification studies. Details of these results will be published in a later paper. The density gradient assay described and used in these studies was found to be the most accurate, consistent, rapid, and simple of any tested. The increased absorbance in the gradient column upon which the virus concentration was based was due to virus. Numerous flexuous rods were observed in the electron microscope in samples collected from the gradient zone. Areas above and below the zone contained fragments and/or aggregated rods. Some virus sedimented through the gradient as samples from the pellets also contained aggregated rods. Density-gradient zone samples were infectious and reacted with MDMV antiserum. Samples collected above and below the zone showed little or no infectivity. Loss of virus due to aggregation cannot be determined with an infectivity assay, but can be observed in the density gradient except for that sedimenting into the pellet.

Recovery of virus at different times after inoculation and from different hybrids may vary. However, it must be assumed that the virus is the same and that losses of virus during the assay are similar. Density-gradient scanning patterns of clarified sap from three different hybrids shown in Fig. 4 indicate slight differences in aggregated virus sedimenting slightly faster than the main virus zone, but this area was included in the comparisons.

The rapid decrease in MDMV in Golden Cross Bantam grown in the greenhouse generally agrees with the results obtained by Tu & Ford (22). The low amounts of MDMV in Golden Cross Bantam 21-28 days after inoculation may explain the apparent low yield of purified MDMV in previous work (2, 17). Our results also show that field-grown plants can be used throughout the growing season as a source of large amounts of tissue containing a high concentration of virus. MDMV should be recoverable for diagnosis at any stage of development of the plant. The additional virus is undoubtedly due to more favorable growing conditions in the field than in the greenhouse. Greenhouse-grown plants are not so dark green nor so large, and the lower leaves turn yellow and die within 3 weeks after inoculation. Temperature and nutritional level are specific factors that have been shown to influence MDMV concentration in corn (19, 21).

MDMV can multiply and attain concentrations as high in resistant T8 X 07B as in susceptible hybrids in the greenhouse in the winter, even though symptom appearance is delayed. This suggests that the mechanism of resistance in this hybrid is not against the infection process, since it is capable of supporting virus multiplication under certain conditions. The delay of symptom appearance in the winter and the longitudinal banding or streaking symptoms in field-grown plants indicate that the resistance mechanism may either limit virus spread within the plant or decrease the rate of virus multiplication. The presence of high concentrations of MDMV in the mosaic bands with little or none in the green areas of the same leaf supports the former hypothesis. Delayed symptom appearance could be explained by both. Tu & Ford (23) found that MDMV did not move from the inoculated leaves of the resistant Illinois A and concluded the resistance mechanism was against virus movement. However, resistant plants occasionally showed systemic mosaic streaks under their conditions, but were not included in their study.

The resistance of certain cells of a plant to infection by a systemic virus is a well-known phenomenon in dicotyledonous plants, but has apparently not been described for monocotyledons. With tobacco mosaic virus (TMV) and turnip yellow mosaic virus (TYMV), for example, the first leaves showing mosaic symptoms often have areas that are a normal dark green in color, whereas the rest of the leaf is yellow green (1, 12, 18). The dark green areas contain less virus than the yellow green areas, and are

resistant to infection by the same virus or strain (1, 6, 12, 18).

The lack of infection of large areas of a corn leaf by MDMV does not appear to be an analogous situation. The mosaic bands comprise a very small part of the total leaf area of an otherwise resistant host, whereas the dark green areas occurring in leaves infected with TMV and TYMV make up a small portion of an otherwise susceptible host. The fact that the reaction is expressed differently in greenhouse-grown plants in the winter suggests that environmental factors influence the resistance mechanism. Similarly, temperature has been found to reverse the expression of resistance in *Pisum sativum* to bean mosaic virus 2 (14).

The ability of certain breeding lines of a plant to resist virus is common, and the expression of resistance by T8 X 07B to MDMV may be only one type of many found in corn. However, several different inbred lines and hybrids in Virginia nurseries exhibit the same type of streaking or banding symptoms following inoculation with MDMV, and are classed as resistant. Not all lines would be expected to react alike. McKinney (10) described a line of tobacco, 448A, which limited spread of tobacco mosaic virus into younger leaves but did not form the typical necrotic hypersensitive reaction. The mechanism of resistance of T8 X 07B to MDMV may involve a substance or substances found in resistant corn plants or produced upon infection under favorable environment for the growth of the host. This substance may act by limiting the spread of the virus or the rate of virus multiplication. Alternatively, resistance may simply be a mechanical barrier to virus movement such as fewer and smaller intercellular connections, thus limiting the lateral movement of virus beyond the vascular bundles adjacent to the cells that were originally infected.

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