MAIZE DWARF MOSAIC VIRUS: PURIFICATION, SOME PHYSICAL PROPERTIES, AND YIELD OF VIRUS FROM SEVERAL CORN HYBRIDS

by

Ronald Klair Jones

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APPROVED:

S. A. Tolin, Chairman

M. G. Hale

C. F. Genter

C. W. Roane

J. A. Fox

R. J. Stipes

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INTRODUCTION

Maize dwarf mosaic was first reported in the United States in 1964 (14, 38, 47) and was first observed in Virginia in 1964 (28). Since then, the virus has become very widespread and destructive in Virginia (29) and in the United States. The distribution of maize dwarf mosaic virus (MDMV) in the United States and Canada was summarized in 1968 (40).

From the data published to date, MDMV appears to belong to the potato virus Y (PVY) group of Brandes and Wetter (11) and to be a strain of sugarcane mosaic virus (SCMV). Viruses of this group are filamentous particles, 750 mμ long, consisting of 5% ribonucleic acid (RNA) and 95% protein and are aphid transmitted in a nonpersistent manner.

The viruses of the PVY group have been difficult to purify and characterize because of a low concentration of virus in host tissue (37), lack of stability of virus after extraction from the host (17, 37), and aggregation, both end-to-end and lateral, of virus particles during purification (12, 15, 16, 24, 32, 46). This is one of the largest groups of plant viruses and until methods are available to obtain highly purified virus in fairly large amounts, it will not be possible to study adequately relationships among the members of this group. Better purified preparations will be necessary before the relationship between MDMV and SCMV can be investigated critically. If the purification procedures can be improved, it may be possible to make detailed chemical, physical, and serological studies of the virions and of virus protein and RNA.

The most successful means of controlling MDMV is through the development of resistant hybrids. Several types of resistance against plant

viruses are known and have been bred into several crops. In most cases, particularly in monocotyledons, the type of resistance expressed by a resistant variety has not been determined. The terms hypersensitivity, tolerance, immunity, resistance to movement within the host, resistance to insect vectors, etc. have been used by various authors to describe the types of resistance. Corn hybrids resistant to MDMV have been developed in many states, but the type of resistance expressed by these hybrids has not been classified.

This work was undertaken to re-examine the purification and physical properties of MDMV and to develop a rapid, accurate assay to measure the amount of MDMV in clarified sap and at each step in purification. The assay was used to determine whether the reported low yield of purified MDMV was due either to a low initial concentration in corn tissue or to a loss of virus from aggregation and/or degradation during purification. Different hybrids and times after inoculation were evaluated as sources of virus for purification. The amount of virus lost at each purification step was measured and variations in the procedure were investigated to decrease the loss. The amount of extractable virus was measured in susceptible and resistant corn hybrids grown under greenhouse and field conditions to classify the type of resistance in corn to MDMV.

LITERATURE REVIEW

Soon after MDMV was first reported, several investigators attempted to purify the virus and determine its relationship to other viruses. MDMV was first purified by Shepherd in 1965 (36) using chloroform clarification followed by differential centrifugation. He found that the virus is a flexous rod and has a sedimentation coefficient of 168 S in sucrose density-gradient centrifugation. Up to 40% of the purified virus rods were within the size range 695-750 mμ. Purified virus, apparently concentrated, was infectious at dilutions as high as 10^{-3} to 10^{-4}. He concluded that MDMV was related to SCMV on the basis of particle morphology, physical properties, and serological reaction in microprecipitin tests. He also compared virus from naturally infected corn in the field with that from inoculated plants in the greenhouse and found them to be identical.

In 1966, Bancroft et al. used Shepherd's procedure and further characterized the virus (5). They reported that 43% of the virus particles after differential centrifugation and 47% of those from the light-scattering zone observed after density-gradient centrifugation were in the 725-775 mμ range. The virus had a sedimentation coefficient of 155 S in the analytical ultracentrifuge with ultraviolet optics, and 158 S in sucrose density-gradient centrifugation. The partially purified virus was contaminated with fraction 1 protein, observable as a slowly sedimenting component in analytical centrifugation and as light-scattering zones above and below the virus in sucrose density-gradients. Results showing nonpersistent transmission of MDMV by at least 11 species of aphids were also presented.

Sehgal purified MDMV by acid precipitation of host components, DEAE-cellulose filtration, and differential centrifugation (32, 33). The preparations contained virus particles 750-800 mμ long and some particles 1.5-2 times this length, indicating end-to-end aggregation. Such virus preparations were infectious at dilutions of 1:250, but the concentration in relation to crude sap was not given. For further purification, the virus was isolated from zones formed during equilibrium centrifugation in cesium chloride (35). Purified virus had a density of 1.3245 and an absorption spectrum typical for RNA viruses with 5% RNA and 95% protein but without the hump at 290 mμ typical for tobacco mosaic virus (TMV). They reported difficulty getting discrete zones in sucrose density-gradients (33, 35).

These reports indicate that the concentration of MDMV in corn is very low or the purification procedures are very inefficient. The dilution end point of crude sap was reported as 10^{-2} by Shepherd (36). He made a 50-fold concentration following the first high speed centrifugation while Bancroft et al. (5) suspended the final virus pellets from 200-400 g of tissue in 1 ml of buffer. The variability in particle length indicated either aggregation, degradation, or both.

Purification of fairly large quantities of non-aggregated virus has been difficult for viruses in the PVY group. Numerous workers have tried to improve the yield of virus by reducing aggregation during purification, since aggregated virus fails to resuspend after high speed centrifugation or is sedimented during low speed centrifugation (10, 15, 16, 33, 37, 46, 48). The selection of a buffering system is very important in the extraction and purification of filamentous viruses.

Increased purification of many of the filamentous viruses generally results in increased aggregation problems (9).

Borate buffer was used by Shepherd and Pound to prevent aggregation of turnip mosaic virus (37) and was used in the early purification studies with MDMV (5, 36). Ford, however, has since shown that borate buffer causes degradation of MDMV (17). Sodium citrate was used by van Regenmortal et al. to prevent aggregation of watermelon mosaic virus (WMV) after one cycle of high speed centrifugation, but it was not effective after several cycles of differential centrifugation (46). Reichmann had used sodium citrate previously in the preparation of nonaggregated potato virus X (26). The addition of sucrose to re-suspending buffers increased the recovery of tulip breaking virus after high speed centrifugation (48).

Pirone and Anzalone reported that more than 50% of the infectivity of SCMV was lost in one cycle of high speed centrifugation (24). It was not possible for these authors to obtain discrete zones in sucrose gradient centrifugation. The virus was completely lost by more than one cycle of either differential or sucrose gradient centrifugation, probably indicating aggregation. Delgado-Sanchez and Grogan studied many variables in an attempt to improve the purification procedures of PVY but still had problems with both aggregation and particles of a shorter than normal length (16). Damirdagh and Shepherd have recently shown that low concentrations of urea and 2-mercaptoethanol prevents lateral aggregation of tobacco etch virus (TEV) during purification. They stated that the same procedure would work for MDMV but no data were given as to the amount of virus obtained or to what degree aggregation was prevented (15).

A rapid and accurate assay technique is necessary to evaluate the source tissue and the various steps in the purification procedure. One of the deficiencies in the work reported to date on MDMV has been the lack of a good assay system. Sehgal et al. reported a sorghum hybrid that gave local lesions after inoculation with MDMV (34). However, this plant did not appear suitable for quantitative assay because lesions were few in number and were often confluent.

Tu and Ford have used systemic infectivity assays in their studies with MDMV and have reported the results in a variety of ways. In the study of the effect of host nutrition on virus multiplication, they presented actual numbers of plants infected when plants were inoculated with 7 dilutions of MDMV, calculated the total proportion infected with 6 of the virus dilutions, and applied the arcsin transformation and analysis of variance to determine significance between treatments (41). In later papers they used a dilution end point assay and reported the total proportion infected or the actual dilution end point (42, 43, 44, 45). However, no discussion was made of the statistical validity of their assay. Kuhn and Jellum used a systemic assay for MDMV and reported actual numbers of infected plants and total proportion infected for each dilution series from several corn hybrids having varying resistance levels (22).

Brakke has used a systemic assay for wheat streak mosaic virus (WSMV) in wheat and analyzed the data by the maximum likelihood calculation and the loglog transformation in order to compare virus concentrations (6). In 1968, Brakke and Ball determined the concentration of WSMV in clarified sap by analytical density-gradient centrifugation and

photometric scanning, which measures directly the particles of uniform size, shape, and density (10). This method was comparable to but more sensitive than the systemic infectivity assay used previously (6) and required less time.

Using systemic infectivity assays, workers in Iowa have compared the concentration of MDMV in susceptible corn hybrids, Golden Cross Bantam, Seneca Chief, and Ohio W49, in relation to environmental factors. Host nutrition, time after inoculation, temperature, and leaf position affected virus concentration. In nutrient solution culture, corn grown at N, P, and K levels optimum for growth of the plant contained the highest concentration of virus (41). The MDMV concentration reached a peak 4-8 days after inoculation, remained at this level for 1-2 days, and then decreased to a lower level (21, 42, 44). The rapid increase in virus concentration always coincided with symptom appearance. The virus peaked and declined most rapidly at 26°C, but the peak concentration was unaffected by temperature. Lower temperature delayed symptom appearance and increase in virus (43). The leaves emerging soon after inoculation contained more virus than successively emerging leaves (44).

Corn hybrids expressing resistance to MDMV in the field often show infection when inoculated in the greenhouse. A delay in symptom appearance after inoculation in the greenhouse has been observed with resistant hybrids (C. W. Roane, personal communication). Scott _et al_. in 1969 reported a delay in symptom appearance in resistant hybrids after inoculation in the greenhouse (31). Kuhn and Jellum evaluated 59 commercial corn hybrids in the greenhouse for their reaction to MDMV. They reported 5 reaction types, from highly susceptible to highly

resistant, based on time of symptom appearance after inoculation. They were further able to correlate the disease index in greenhouse tests with resistance in the field (22).

In a study to measure free amino acids, Tu and Ford compared the relative concentration of MDMV by dilution end point and symptom expression in the susceptible Seneca Chief and the resistant Illinois-A. Their data showed that the virus concentration in the inoculated leaves of both the susceptible and resistant corn was about the same, having dilution end points of 10^{-3} and 2×10^{-3}, respectively. A dilution end point of 5×10^{-4} was obtained from the systemically infected leaves of the susceptible, but virus could not be recovered from comparable leaves of the resistant. Only occasionally did resistant plants show systemic mosaic streaks; these plants were not included in the study. They concluded that Illinois-A has a genetic resistance that prevents virus movement within the plant (45).

METHODS AND MATERIALS

Cultural Conditions

The MDMV isolate used in these studies was isolated from naturally infected corn plants in Nelson County, Virginia. Its host range indicated it to be strain A (Johnsongrass strain), as reported by Roane and Tolin (29). A stock culture of the virus had been maintained in the greenhouse for 3-4 years by mechanical transfer in the corn hybrids, Golden Cross Bantam, Hy X C103, or DeKalb 805A, grown in 4 or 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 neutral 0.01M sodium phosphate buffer, or by spraying leaves with an artist's airbrush (Thayer and Chandler, Inc., Chicago, Ill., Model C) with inoculum containing 1% carborundum. Inoculum for the airbrush was prepared by grinding infected tissue in 0.1 M sodium citrate plus 0.1% mercaptoethanol (R. W. Toler, personal communication), straining through cheesecloth and diluting to a final concentration of 1:10 (w/v). Air pressure was maintained at 120 psi for inoculation with the airbrush in the greenhouse. Corn seedlings in the greenhouse were inoculated in the 1-2 leaf stage (second leaf not completely elongated), 7-10 days after planting. Field inoculations were made with the airbrush using a portable air compressor which maintained a pressure of 80-100 psi. Field studies were conducted with plants grown in the Blacksburg area, where MDMV does not occur naturally, and inoculated in the 2-3 leaf stage.

Purification and Analysis

MDMV was extracted from systemically infected corn leaves, with the midrib removed, by homogenizing the leaves in a cold Waring Blender in 2-3 stages with a volume of cold 0.1 M sodium citrate plus 0.5% mercaptoethanol equal to the weight of tissue. The homogenate was strained through cheesecloth and then centrifuged for 10 minutes at 10,000 rpm in the 9RA rotor of the Lourdes refrigerated centrifuge. The volume of the supernatant was measured, emulsified with one-half volume of chloroform, and centrifuged for 5 minutes at 5,000 rpm to separate the emulsion. The aqueous, upper phase was collected, held at 4°C for 3-4 hours, and then centrifuged for 15 minutes at 12,000 rpm. The supernatant was centrifuged at 30,000 rpm for 90 minutes in the Type 30 rotor of the Spinco Model L-2 65B ultracentrifuge. The resulting pellets were overlaid with 0.005 M sodium citrate, pH 7.0, stirred gently with a glass rod, and allowed to stand overnight at 4°C. The resuspended virus pellet was centrifuged at 10,000 rpm for 10 minutes; the supernatant was adjusted with 0.005 M sodium citrate to one-tenth the volume of the clarified sap. Two to 4 ml of the virus suspension were layered on sucrose density-gradient columns and centrifuged for 2.5 hours at 27,000 rpm in the SW 27 rotor. 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 3 1/2" 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) and was added to make a final concentration of 0.005 M citrate. Gradients were allowed to stand overnight at 4°C before centrifugation.

Gradients were scanned photometrically by the method of Brakke (8). A 5-inch strip recorder was attached to the ISCO Ultraviolet Analyzer and the densitometer was adjusted to give a full-scale deflection equal to 0-1.0 optical density units at 254 mμ. The virus zone was collected manually, diluted with an equal volume of 0.005 M citrate, pH 7.0, and centrifuged for 50 minutes at 50,000 rpm in the Type 65 rotor. The virus pellets were resuspended in 0.005 M citrate, pH 7.0, in a volume equal to one-tenth the volume of the clarified sap. Following an additional low speed centrifugation, this preparation is referred to as "purified virus."

The concentration of virus was determined by sucrose density-gradient centrifugation and by conversion of the virus peak area on the 5-inch strip recorder to μg of virus, according to Brakke (10). The peak areas were measured with a planimeter (Los Angeles Scientific Instrument Co., Los Angles, Calif., Model L 30 AB). The ISCO density-gradient fractionator was calibrated with a series of concentrations of $K_2Cr_2O_7$ in 0.05 N KOH. The optical density of each dilution was measured with the spectrophotometer. The optical density units in the virus peak were calculated by the following formula from the ISCO manual: (area of peak in $inch^2$) X (optical density/inch of meter deflection) X (ml/inch of chart travel). The absorbancy index at 260 mμ of 2.4 mg/cm for TEV (25) was used to convert optical density units to μg of virus. A sample calculation is given in Appendix Table I.

The ultraviolet absorption spectrum was determined with a Unicam Model Sp 800A spectrophotometer. The sedimentation coefficient for a single concentration of virus was determined in a Spinco Model E ultracentrifuge using Schlieren optics.

Samples to be viewed in the electron microscope were placed on carbon-backed, collodion-coated grids with hand-drawn micropipettes left on the grid 2-3 minutes, and drawn off. Samples containing sucrose were left on the grid 2-3 minutes, withdrawn, and then washed 10-12 times by placing a drop of double distilled water on the grid and drawing it off with a micropipette. Grids were shadowed with tungsten oxide and viewed in an RCA EMU-3H electron microscope.

Serology

Anti-MDMV serum was prepared by making 3 intramuscular injections into rabbits of purified virus emulsified with Freund's incomplete adjuvant at 10-day intervals. Each rabbit received a total of 21 mg of virus. Rabbits were bled by cardiac puncture 7 and 21 days after the final injections.

Specific reaction of the serum against the virus was tested by density-gradient serology according to Ball and Brakke (4). Either 0.2 ml of clarified sap or 0.1 ml of purified virus was mixed with an equal volume of anti-MDMV serum diluted 1/64 with phosphate buffered saline (neutral 0.01 M phosphate buffer plus 0.85% sodium chloride). After standing for 15 minutes at room temperature, 0.2 ml of the mixture was layered on sucrose density-gradients, made by pipetting 1.1 ml each of 100, 200, 300, and 400 g of sucrose/liter in 0.005 M citrate, pH 7.0, and centrifuged for 25 minutes at 55,000 rpm in the SW 65 rotor. Centrifuged gradient columns were analyzed on the ISCO fractionator.

For microprecipitin tests, performed according to Ball (3), all concentrated virus suspensions were diluted to 1:1 (w/v) in 0.005 M

citrate, pH 7.0, before making a 2-fold dilution series. Serial dilutions of serum, clarified sap, and purified virus were made in neutral 0.01 M phosphate buffered saline. Virus dilution end points were read after 24-36 hours at 4°C at a serum dilution of 1/8, which usually gave the best reaction.

Infectivity Assay

For systemic infectivity assays, 7-day old Hy X C103 corn seedlings were used. Each sample to be assayed was diluted to 1:10 (w/v) and 1:20, and by 10-fold steps thereafter. Crude sap and clarified sap were diluted with 0.1 M sodium citrate plus 0.5% mercaptoethanol. Approximately 1% carborundum (600-mesh) was mixed with dilution. Purified virus samples were diluted with 0.005 M citrate, pH 7.0. Inoculations were made by spraying 4.5 ml of each dilution on 10 seedlings with an artist's air-brush, which was washed thoroughly with distilled water between inoculations. Within each series, the highest dilution was inoculated first and the most concentrated was last. Infected plants were recorded 7 and 14 days after inoculation. The titer was calculated using a loglog transformation (See Appendix Table II). The results are reported as the logarithm and the standard deviation of the number of infectious particles per infection-initiating volume that infected 63% of the plants (6). The percentage loss of infectivity was calculated from the actual number of particles per infection-initiating volume for each assay (See Appendix Table II).

Concentration of Virus in Clarified Sap

To determine the concentration of MDMV in clarified sap, the clarification procedure was the same as that described previously except the clarified sap was given the final low speed centrifugation immediately after the chloroform emulsion was separated. Concentration determinations from greenhouse-grown plants were made from 2 g of fresh leaf tissue ground in a cold mortar and pestle, while those from field-grown tissue were made from 100 g of fresh tissue ground in a cold Waring Blender. A 2 ml aliquot of the clarified sap representing 1.50-1.75 g of tissue was layered on a sucrose density-gradient column and centrifuged for 3 hours at 27,000 rpm in the SW 27 rotor. Centrifuged gradient columns were scanned photometrically with the recorder set to give a full scale deflection equal to 0-0.25 optical density units at 254 mμ. The concentration of virus was calculated as described previously.

RESULTS

Properties of Purified MDMV

MDMV purified by chloroform clarification, differential and sucrose density-gradient centrifugation was colorless, opalescent, birefringent, and formed a discrete band in sucrose density-gradients at concentrations greater than 0.5 mg/ml. Density-gradient scanning patterns at 3 stages of the purification procedure for MDMV-infected and healthy corn tissue are shown in Fig. 1. MDMV from clarified sap formed a symmetrical peak indicating uniformity in particles (Fig. 1A). The concentration of virus decreased with purification. Absorbance on the leading and trailing edges of the peak indicates aggregation and degradation of virus from the resuspended first high speed pellet (Fig. 1B). Aggregation and degradation were also evident in the scanning pattern of purified virus, in which nearly all of the slowly sedimenting contaminating material was removed (Fig. 1C). Analysis of correspondingly treated healthy corn confirmed that no components sedimenting at the same rate as MDMV were present (Fig. 1D, E, F).

Purified preparations, as represented in Fig. 1C, showed an ultraviolet absorption spectrum typical (Fig. 2) for rod shaped viruses having 5% RNA and 95% protein. The $A_{260/280}$ of a typical purified preparation was 1.18. The spectrum also showed a slight shoulder at 290 mμ similar to that for TMV, but not as prominant, indicating the presence of tryptophan in the coat protein.

Purified virus at 0.42 mg/ml suspended in distilled H_2O or in 0.01 M NaCl had a sedimentation coefficient of 160 S or 162 S,

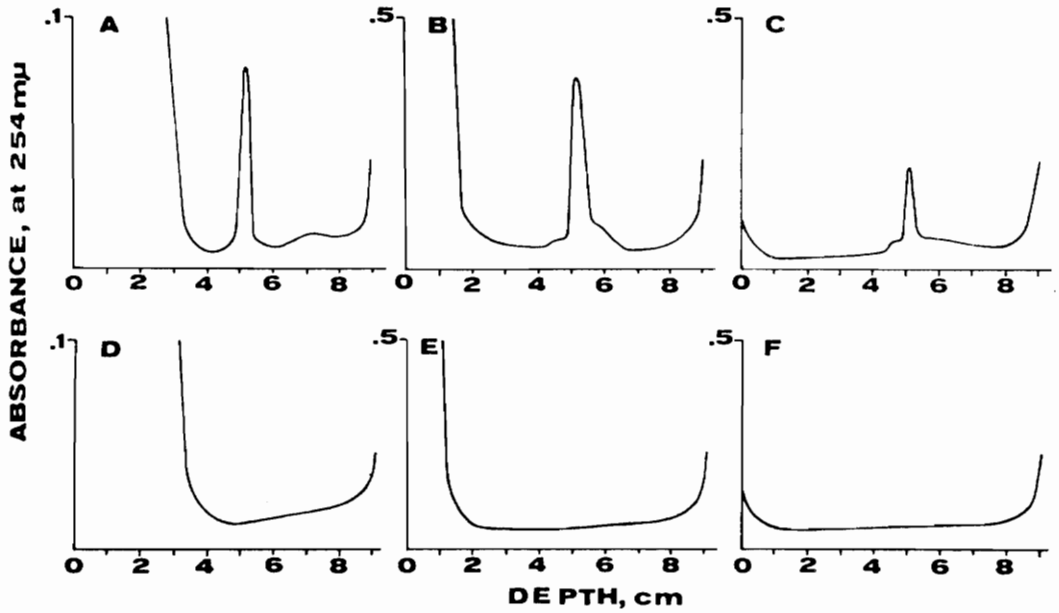

Fig. 1. Density-gradient scanning patterns of field grown, MDMV-infected Hy X C103 at 3 stages of the purification procedure. A) Clarified sap from 1.5 g of leaf tissue. B) Resuspended first high speed pellet from 15 g of tissue. C) Purified virus from 15 g of tissue. D), E), and F) Comparable treated healthy corn tissue.

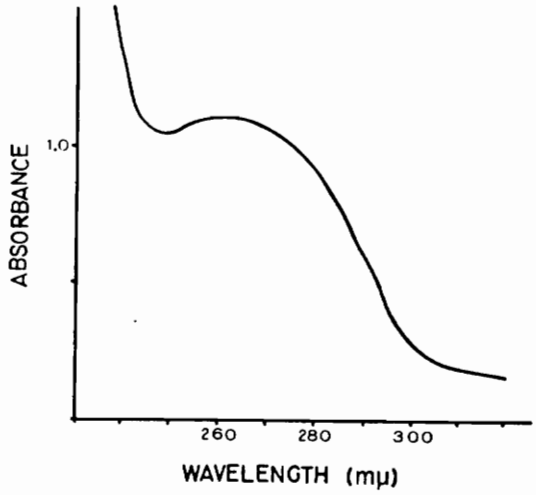

Fig. 2. Ultraviolet absorption spectrum of purified MDMV in 0.005 M citrate buffer, pH 7.0.

respectively, in the Spinco Model E analytical ultracentrifuge. Only one component was detected in the purified virus (Fig. 3).

Variation in Purification Procedure

All variations in the purification procedure were conducted using greenhouse-grown Hy X C103 or Golden Cross Bantam. The effect of a variation in one step of the procedure was determined while all other steps remained constant. Sucrose density-gradient analysis was used to evaluate the effects of each variation.

For homogenization and clarification, the tissue was ground in 5 molarities of sodium citrate, 0.5 M, 0.1 M, 0.05 M, 0.01 M, or 0.005 M. The highest yield of virus and the sharpest peak in sucrose density-gradients were obtained from tissue ground in 0.1 M sodium citrate. For clarification of crude sap, freezing leaf tissue or ground homogenate, charcoal adsorption, heat denaturation at 40°C for 15 minutes, and lowering the pH level of the ground homogenate to pH 5.5 with citric acid were compared to chloroform emulsification and evaluated both as clarified sap and after high speed centrifugation. No measurable virus was recovered after freezing either the leaves or the ground homogenate. Charcoal treatment did not give adequate clarification. Heat and acid treatment caused excessive aggregation after high speed centrifugation. Even though chloroform caused a loss of infectivity (Table I), it gave good clarification and did not cause excessive aggregation.

Several ratios of chloroform to homogenate (v/v) were evaluated. A 1:2 ratio gave adequate clarification, did not increase the total volume as much as the 1:1 ratio used earlier (5, 36), and the sap was a clear,

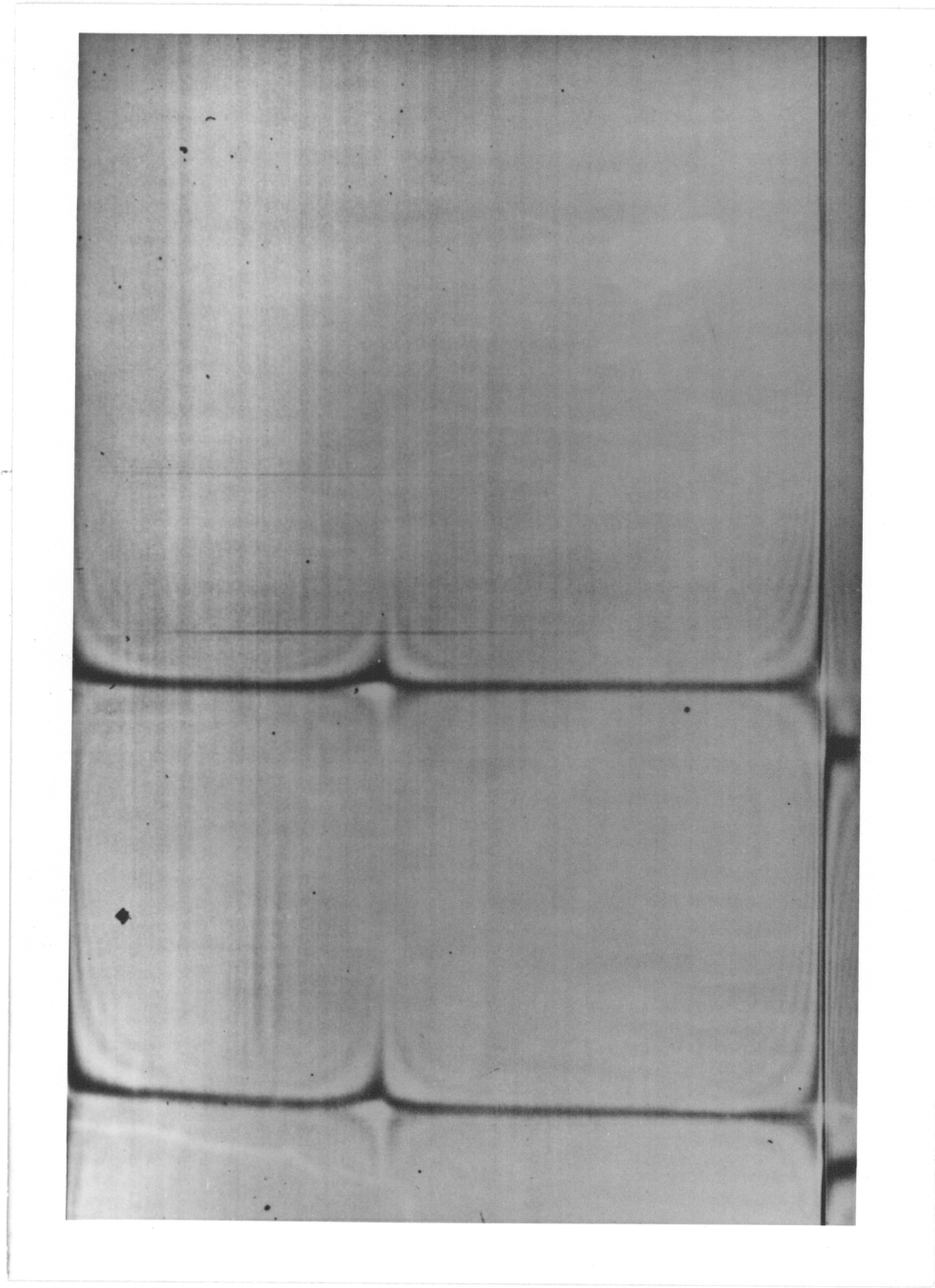

Fig. 3. Sedimentation pattern of purified MDMV (0.42 mg/ml), upper cell in distilled H_2O and lower cell in 0.01 M sodium chloride, 12 minutes after the An-D rotor reached 31,410 rpm in the Spinco Model E analytical ultracentrifuge.

Table I. Yield of MDMV from field-grown Hy X C103

Virus Preparation	Density-Gradient		Infectivity			
	Yield[a]	% loss[b]	Titer[c]	% loss		Dilution End Point
Crude sap						
Expt. 1[d]			3.43±0.11			10^{-5}
Expt. 2[e]			3.44±0.09			10^{-5}
Clarified sap						
Expt. 1	45.0		2.59±0.11	85.5[f]		10^{-4}
Expt. 2	41.2		3.13±0.12	51.0		$2x10^{-4}$
1st high speed						
Expt. 1	18.1	58	1.56±0.23	98.6	91.7[g]	$2x10^{-3}$
Expt. 2	17.8	57	1.35±0.11	99.2	98.4	10^{-3}
Purified virus						
Expt. 1	9.6	79	1.10±0.12	99.53	96.8	10^{-2}
Expt. 2	11.2	73	0.98±0.13	99.65	99.3	10^{-2}

[a] μg of virus/g of leaf tissue.
[b] % loss from clarified sap.
[c] Logarithm and standard deviation of the numbers of infectious particles per infection-initiating volume (See Appendix Table II).
[d] 6/14/70.
[e] 6/29/70.
[f] % loss from crude sap (See Appendix Table II).
[g] % loss from clarified sap (See Appendix Table II).

light yellow after separation of the emulsion. Allowing the clarified sap to stand at 4°C for 3-4 hours between the centrifugation to break the chloroform emulsion and the final low speed centrifugation resulted in the formation of a heavy precipitate. This precipitate, which contained no virus, was removed by centrifugation at 12,000 rpm for 15 minutes, leaving a clear, light yellow supernatant. The high speed pellets were smaller if the clarified sap was held for 3-4 hours before the final low speed centrifugation than if the clarified sap was centrifuged immediately.

Various dilutions from 0.5 to 0.001 M citrate, pH 7.0, were compared to determine the optimum molarity of citrate for resuspending high speed pellets; 0.005 M citrate buffer was the most satisfactory. The purified virus was stable in this buffer for at least one week, the longest time tested, while the virus in clarified sap was destroyed after 2 days. High speed pellets resuspended in 0.005 M citrate buffer equal to one-tenth the volume of the clarified sap and centrifuged 10 minutes at 10,000 rpm showed birefringence when swirled in a glass tube between crossed polarizing lenses.

To try to increase the recovery of virus after the high speed centrifugation, several different additives and buffers were evaluated. The detergent Igepon (7) at 0.1, 0.01, 0.005, and 0.001%, 5% sucrose (48), and 0.1% mercaptoethanol and urea (15) were tested as additives to 0.005 M sodium citrate buffer. Other buffers evaluated were 0.005 M sodium borate, pH 8.0, 0.01 M phosphate, pH 7.0, 0.005 M tris-HCl, pH 7.0, and distilled H_2O. None of the additives consistently increased the yield of MDMV after high speed centrifugation nor appeared to decrease the degree of aggregation.

Losses of MDMV During Purification

The loss of MDMV at each step of the purification procedure was determined by sucrose density-gradient analyses, by systemic infectivity assays, and by microprecipitin tests. The results of 2 typical experiments are shown in Table I.

The yield determined by sucrose density-gradients in a typical experiment was 45.0 μg of virus/g fresh weight of tissue in the clarified sap, 18.1 μg/g resuspended after the first high speed centrifugation, and 9.6 μg/g purified virus. The results in general show that 50% of the virus in the clarified sap was lost after the first high speed centrifugation and another 50% loss occurred during the final purification step. Total ultraviolet absorbance equivalent to the amount in the collected virus zones could be recovered following centrifugation to concentrate the purified virus and remove sucrose. Only half of the absorbance layered on the second density-gradient was recovered in the zone. The microprecipitin test for the 2 experiments gave virus dilution end points of 1/128 - 1/256, 1/64, and 1/32 for clarified sap, resuspended first high speed pellets, and purified virus, respectively. Thus, the overall yield of purified virus was only 25% of that in the clarified sap, based on sucrose density-gradient and serological dilution end point analyses.

Systemic infectivity assays of crude sap and the 3 purification steps showed that a tremendous loss of infectious virus occurred (Table I). An average of about 75% of the infectivity was lost during the chloroform clarification step. Another 20-30% was lost by high

speed centrifugation, but little further loss occurred in the purified virus stage. The total recovery of infectious purified MDMV was less than 0.5% of the infectivity in crude sap. Loss could also be expressed in terms of the infectivity dilution end point, which decreased 10-fold at each step.

Yield of purified MDMV varied greatly from one run to another but was probably influenced more by the concentration of virus in the tissue than by differences in the efficiency of the technique. For 3 different runs where the yield of purified virus was 9.6, 15.0, and 23.6 μg/g, the percentage loss from clarified sap to purified virus was 82, 79, and 73, respectively.

Samples collected from various depths in sucrose density-gradients of virus after one high speed centrifugation or of purified virus and viewed in the electron microscope (Fig. 4 and 5) showed that both aggregation and degradation of the virus particles occurred during purification. Particles shorter than the normal length for MDMV were observed in samples from the leading edge and below the major zone. Aggregated particles were observed in samples collected below the major zone. The main difference between the gradient of the first high speed pellet and of the purified virus was that the former contained more nonviral components than the latter. Numerous aggregated virus particles were observed in samples of the clear pellet in the bottom of density-gradient tubes after centrifugation.

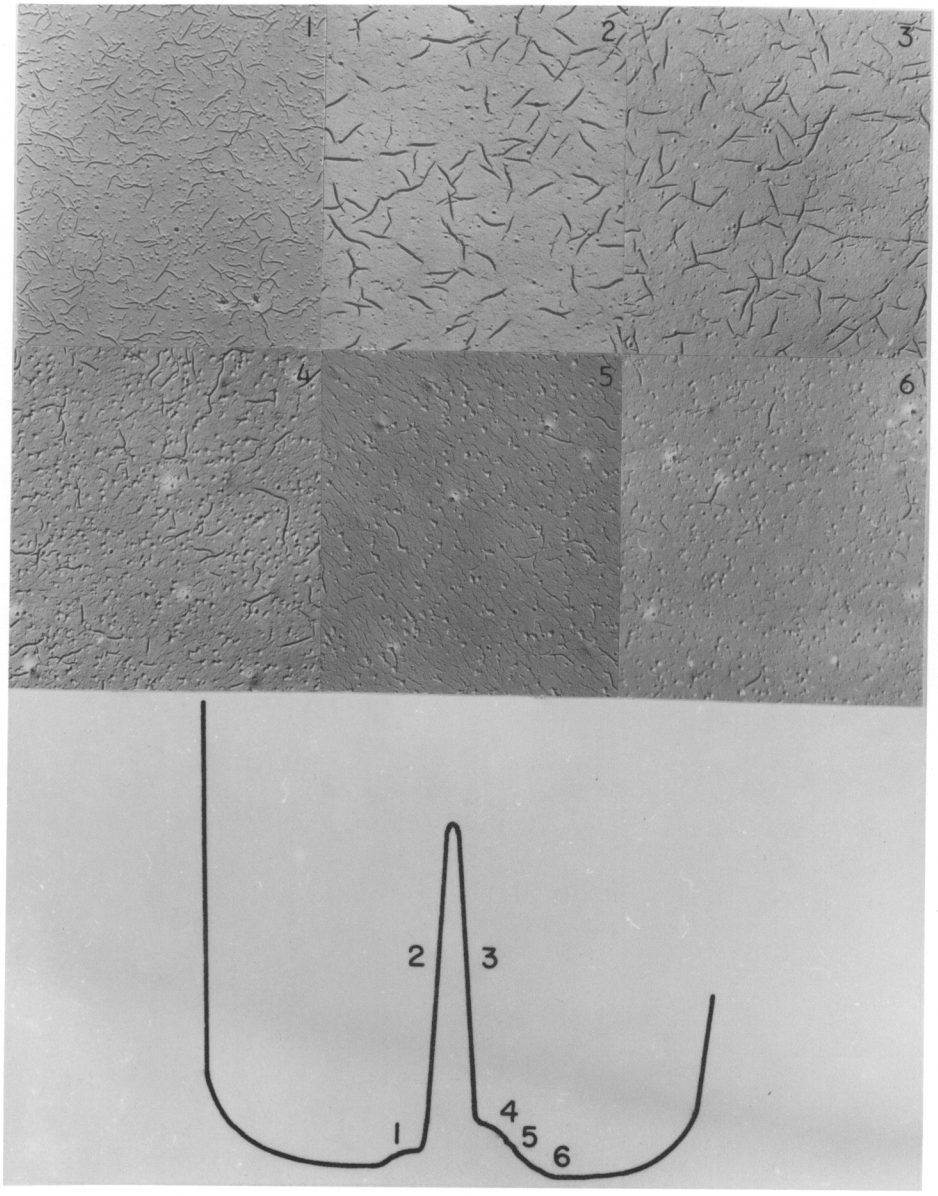

Fig. 4. Electron micrographs of MDMV in samples collected from a centrifuged density-gradient column. Two ml, representing 15 g of leaves, of the resuspended first high speed pellet from field-grown, MDMV-infected Hy X C103 was layered on the column. Numbers in upper right corner of each micrograph correspond to sample number. Samples 2 and 3 were diluted 1:10 with 0.005 M citrate buffer before placing on grid.

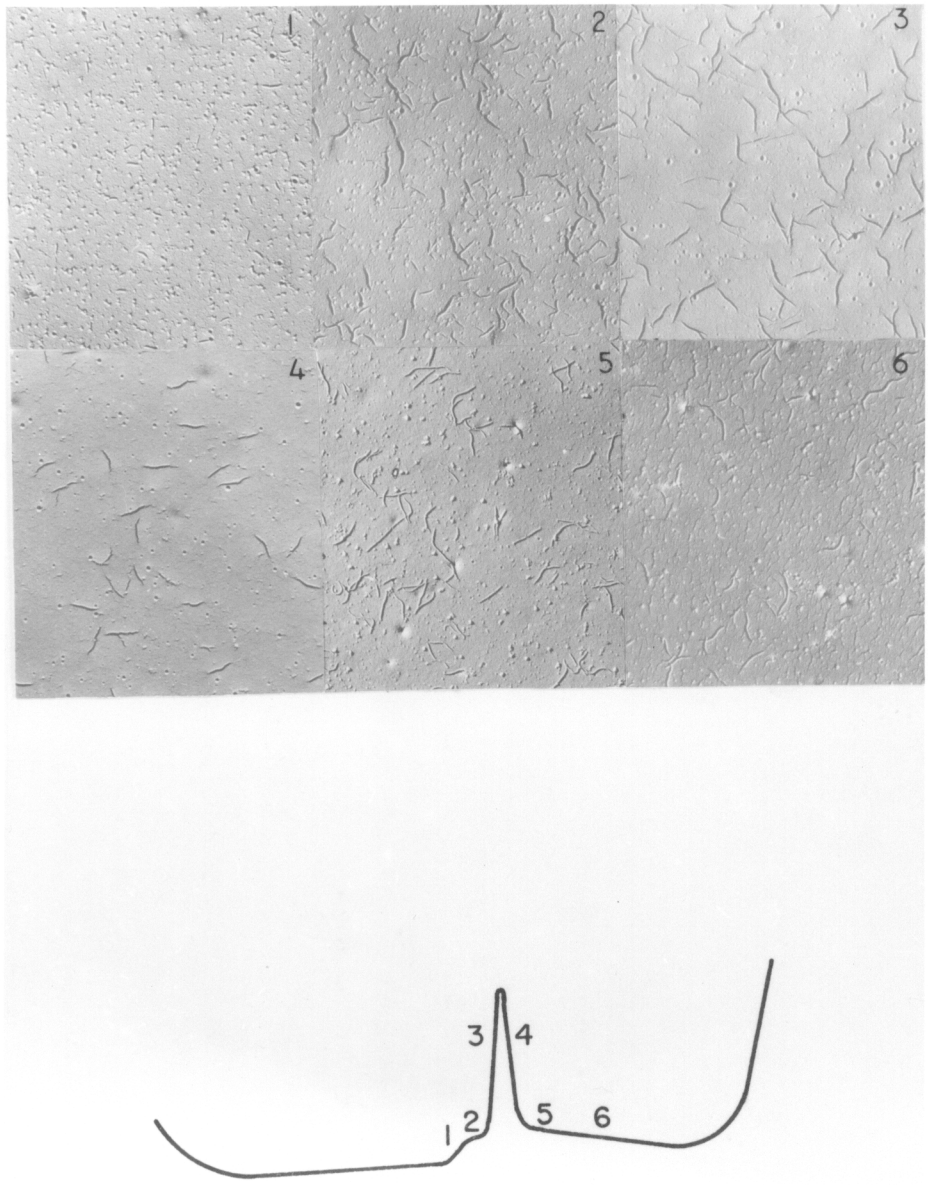

Fig. 5. Electron micrographs of MDMV samples collected from a density-gradient column after centrifugation. Two ml of purified virus from field-grown, MDMV-infected Hy X C103 was layered on the column. Number in upper right corner of each micrograph corresponds to sample number. Samples 3 and 4 were diluted 1:10 with 0.005 M citrate buffer before placing on grid.

Interaction of MDMV and Several Corn Hybrids

Susceptible

Three corn hybrids highly susceptible to MDMV under field conditions, Hy X C103, DeKalb 805A, and Golden Cross Bantam sweet corn, were used in these tests. Symptoms appeared 4-5 days after inoculation in all 3 hybrids grown in the greenhouse. A slight flecking in the first leaf above the 2 inoculated leaves and a general mosaic in leaves above this were observed in the 3 hybrids in the greenhouse. The same hybrids grown under favorable conditions in the field showed mosaic symptoms 5-7 days after inoculation. The mosaic symptoms appeared in all of the leaves above the inoculated leaves in susceptible plants. 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 leaf 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. 6. Plants had been inoculated in the 2-leaf stage. Virus was first detected in Hy X C103 6 days after inoculation in the third and fourth leaves. The concentration remained high (20-25 μg/g) in Hy X C103 from 9-18 days after inoculation and declined gradually over the 4-week period. Virus was first detected on the fifth day after inoculation in the third leaf of Golden Cross Bantam. The concentration was the highest (25 μg/g) in Golden Cross Bantam 6-9 days after inoculation and decreased rapidly thereafter. Within 2 weeks after inoculation, no virus could be detected.

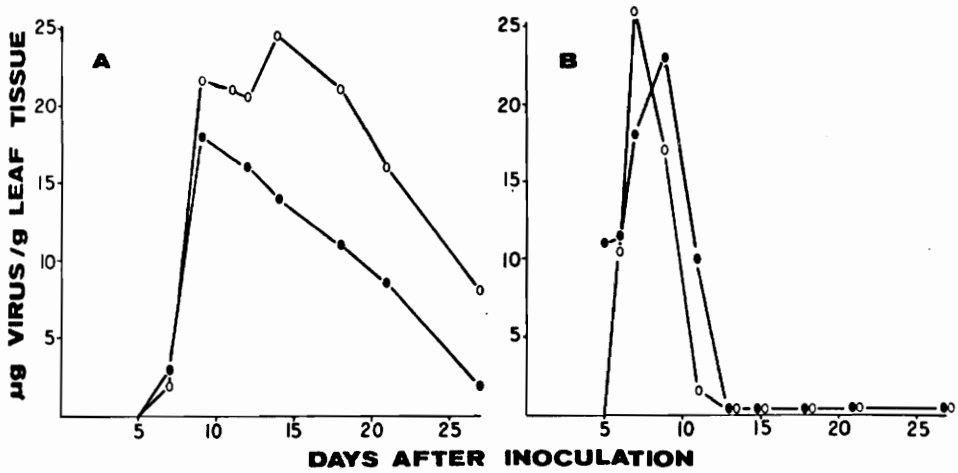

Fig. 6. Change in concentration of MDMV in the third (-●-) and fourth (-O-) leaves of A) Hy X C103 and B) Golden Cross Bantam for 28 days after inoculation in the greenhouse. Concentrations were determined by density-gradient analysis of clarified sap representing 1.5 g of leaf tissue.

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 II. (See Appendix Table III for complete data). Although variable, the pattern of virus concentration is consistent with that of the previous experiment. At no time was it possible to extract a measurable amount of virus from the inoculated first and second leaves.

The concentration of MDMV in the 3 susceptible hybrids, Hy X C103, DeKalb 805A, and Golden Cross Bantam, grown in the field is shown in Fig. 7. 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 incomplete, were similar to those in 1970. The concentration remained sufficiently high in all 3 hybrids to obtain a positive microprecipitin reaction with clarified sap, 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 in field-grown corn was nearly equivalent to the peak concentration of virus in greenhouse-grown corn.

Resistant

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 in T8 X 07B in comparison to

Table II. Concentration of MDMV in clarified sap from corn hybrids grown in the greenhouse

Hybrid	Days after inoculation 7-13	14-20	21+
Hy X 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/g fresh weight of leaf tissue, determined by sucrose density-gradient analysis.

[b] Number in parentheses is the number of extractions.

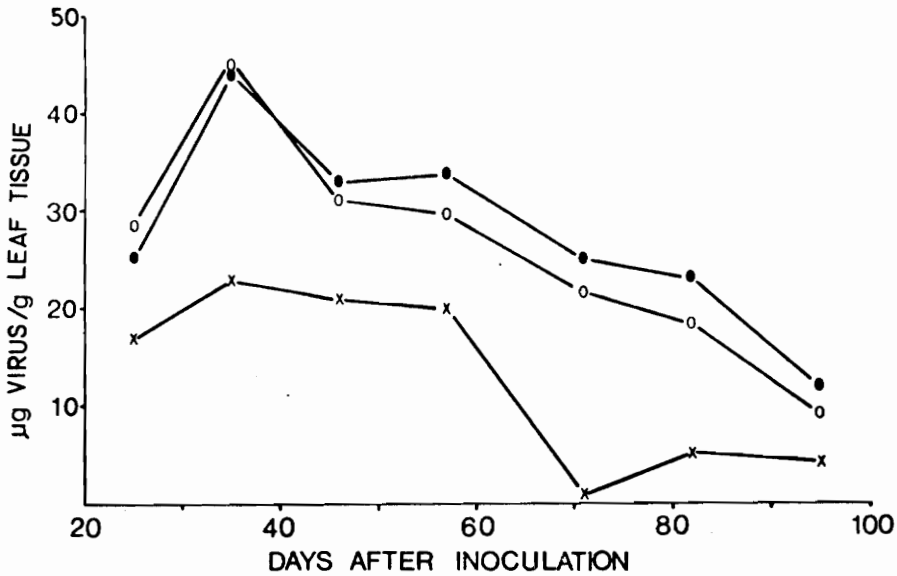

Fig. 7. Change in concentration of MDMV in Hy X C103 (-O-), DeKalb 805A (-●-) and Golden Cross Bantam (-X-) for 95 days after inoculation in the field in 1970. Concentrations were determined by density-gradient analysis of a 2 ml aliquot of clarified sap representing 1.5 g of tissue, from a 100 g sample of leaf tissue.

Hy X C103 and Golden Cross Bantam is shown in Fig. 8. In 6 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 9.0-23.0 μg virus/g of tissue with an average of 13.1 μg/g for 6 determinations.

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, but not all, of the leaves of a particular plant. The narrow bands of mosaic tissue were usually limited to the tissue between 2-6 veins and were as short as 4-6 inches but could extend the entire length of the leaf. Several leaves from T8 X 07B grown in the field showing the longitudinal banding symptoms were collected. The mosaic-band area of the leaf was separated from the remaining dark green tissue of the same leaf. The virus concentration in each was measured by density-gradient analysis of clarified sap. The results (Fig. 9) of several assays showed that the virus occurred in high concentration in the mosaic tissue (47.1 μg/g) while little or none was found in the green part of the same leaf.

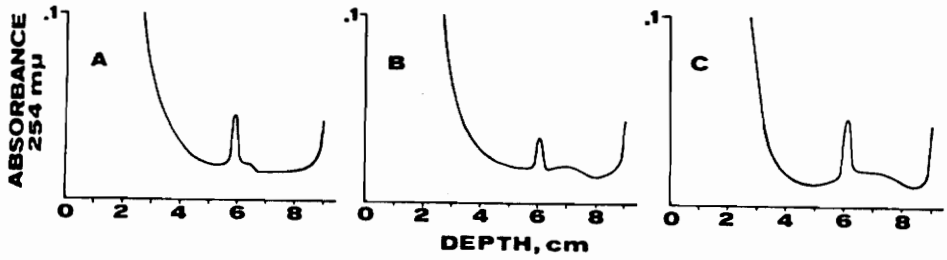

Fig. 8. Density-gradient scanning patterns of clarified sap, representing 1.5 g of leaf tissue, from 3 hybrids grown in the greenhouse in the winter, 11 days after inoculation. A) T8 X 07B (17.0 μg virus/g of tissue). B) Golden Cross Bantam (12.0 μg virus/g of tissue). C) Hy X C103 (21.0 μg virus/g of tissue).

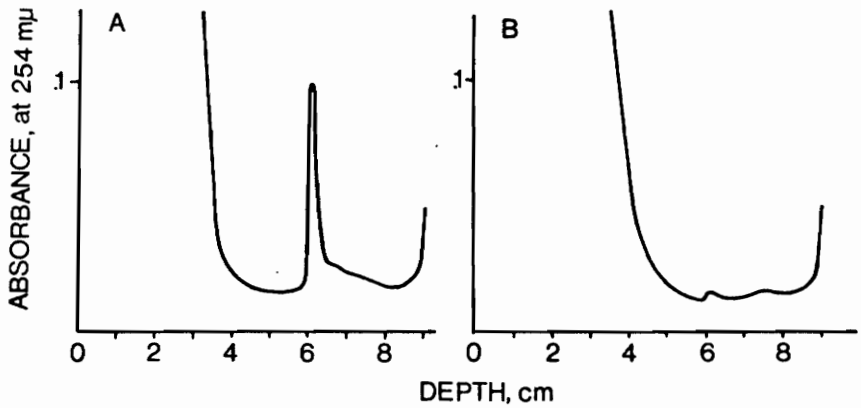

Fig. 9. Density-gradient scanning patterns of a 2 ml aliquot, representing 1.5 g leaf tissue, of clarified sap from a 100 g sample of leaves of MDMV-inoculated, field-grown T8 X 07B. A) Portion of leaves showing chlorotic bands, and B) Dark green portion of the same leaves.

DISCUSSION

The purification procedure used for this study differs from that used by others (5, 32, 33, 35, 36) in the use of citrate buffer instead of borate or phosphate buffers and in the use of sucrose density-gradient centrifugation. There appeared to be an optimum molarity of citrate for tissue extraction and for virus suspension after high speed centrifugation. Although clarification by chloroform emulsification caused considerable loss of virus, it was the most satisfactory and consistent treatment of any of those tested. The fact that several additives failed to increase the efficiency of resuspension indicates that MDMV differs from WSMV (10) and TEV (15) in chemical properties controlling aggregation. MDMV purified by this method appears to be stable. Fairly high yields of purified virus were obtained consistently. The sedimentation coefficient of 160-162 S is between those previously reported for MDMV (5, 36) and is similar to values reported for TEV (25) and WSMV (6). The slight shoulder at 290 mμ in the ultraviolet absorption spectrum varies from Sehgal's results (35). This may be an indication of a higher degree of purity of the virus obtained by this purification procedure. The $A_{260/280}$ agrees with the value of 1.18 obtained by Sehgal and Jean (35).

It is difficult to compare the infectivity of purified virus reported here to that of others (32, 36) because they presented too little data. Shepherd reported a dilution end point of 10^{-3} for purified virus concentrated 50-fold (36). Sehgal and Jean reported a dilution end point of 2.5×10^{-2} for purified MDMV, but the initial concentration of the purified virus was not given (32). The 10^{-2}

dilution end point reported here for purified virus apparently is higher than either of the previously reported values, since the inoculum was adjusted to 1 ml/g of tissue prior to dilution. These results indicate a higher percentage recovery of purified virus, a higher virus concentration in the starting tissue, or both. The dilution end point of 10^{-5} for crude sap of field-grown tissue is higher than that reported previously (32, 36, 41, 45).

The analysis of clarified sap and purified virus by density-gradient centrifugation and photometric scanning was a rapid, consistent method for measuring MDMV concentration. The results were comparable to, but more accurate than, infectivity or serological assays of the same preparations. The yield of purified MDMV from field-grown Hy X C103 of 9.6-23.6 μg/g fresh weight of leaf tissue is greater than the 4 μg/g obtained for WSMV by the same technique (10). Yields of MDMV reported here are less than the 40-60 μg/g obtained by Damirdagh and Shepherd using A_{260} with TEV from tobacco, but their values were apparently not based on purified virus recovered in zones (15).

The concentration of MDMV determined by serological methods is comparable to the other two methods, but is far less sensitive. In addition, the antigenic receptor sites of degraded or aggregated virus may not be changed sufficiently to be distinguished by the antibody molecules. Therefore, a higher value would be expected, if the test were run quantitatively.

The yield of purified virus represents only about 25% of that present in clarified sap. Of this, 50% was lost by failure to recover virus after the first high speed centrifugation. Approximately 50% of

the absorbance in the zones after concentration, as measured with the spectrophotometer, was recovered in the virus zone after subsequent density-gradient centrifugation. This loss of virus was probably due to aggregation. Working with WSMV, Brakke and Ball reported a 70-90% recovery of virus after the first density-gradient centrifugation, and a total recovery from clarified sap to purified virus of 50% (10). Infectivity assays of MDMV indicated a yield of less than 0.5% from crude sap to purified virus, compared with 1% for WSMV (10). Purified MDMV, like WSMV, apparently has a low specific infectivity.

As viewed in an electron microscope, samples collected from density-gradients showed that short pieces of virus particles occurred above and below the virus zone. The short pieces below the virus zone may indicate that some degradation takes place in the gradient because the short particles should have a lower sedimentation rate and remain above the virus zone. Aggregated virus particles observed below the monomer zone and in the pellet also account for part of the losses incurred. Previous reports on purification of MDMV have also shown that aggregation and degradation occur during purification. Shepherd reported that only 40% of the purified virus particles were between 695-750 mμ (36). Bancroft et al. reported 43-47% of the particles were 725-775 mμ (5), while Sehgal found some particles 1.5-2 times the normal length (32). Ford observed degraded MDMV particles in the electron microscope following purification and incubation in borate buffer, and pointed out that another buffering system was needed for MDMV (17).

For the purification of large amounts of MDMV, the importance of selecting a source plant which contains a high concentration of virus

cannot be overemphasized. The concentration of MDMV in Golden Cross Bantam grown in the greenhouse was highest during the period 7-12 days after inoculation and decreased after this time. This generally agrees with the results obtained by Tu and Ford with systemic infectivity assays for MDMV (41, 42, 43, 44). Since the concentration did not decrease as rapidly in Hy X C103, it is a more suitable hybrid as a source of virus. The very low concentration in Golden Cross Bantam 21-28 days after inoculation can partially explain the apparent low yield of purified virus in earlier work (5, 36). These 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 for purification.

More favorable growing conditions may explain why the concentration of MDMV is higher and remains high for a longer period of time in susceptible plants grown in the field than those grown in the greenhouse. The lower leaves of greenhouse-grown plants turn yellow and are dead 3 weeks after inoculation. Greenhouse-grown plants are not as dark green nor as large as field-grown plants. The nutritional level of greenhouse-grown corn plants has been shown to influence the concentration of MDMV in corn leaves (41).

The delayed symptom appearance with the resistant T8 X 07B grown in the greenhouse in the winter agrees with the data of Kuhn and Jellum (22) and Scott _et al_. (31). Kuhn and Jellum evaluated 59 varieties and inbred lines and showed that the higher the level of resistance to MDMV in the field the later symptoms appeared after inoculation in the greenhouse. The 3 susceptible hybrids used in this study correspond to

their Reaction A (highly susceptible); the resistant T8 X 07B fits their Reaction D (resistant) or Reaction E (highly resistant) (22).

The data presented here show that MDMV can multiply and attain concentrations as high in resistant T8 X 07B as in the susceptible hybrids in the greenhouse in the winter. This indicates that the mechanism of resistance in T8 X 07B is not against the infection process, since T8 X 07B can support the multiplication of MDMV. However, the delay in symptom appearance after inoculation in T8 X 07B in the winter and the longitudinal banding symptoms in field-grown plants indicate the resistance mechanism may be against either the spread of the virus within the plant or the rate of multiplication of the virus. The high concentration of virus in the mosaic bands with little or none in the green part of the same leaf supports the former hypothesis. The delay in symptom appearance in the greenhouse tends to support both hypotheses. Tu and Ford have shown that MDMV only rarely moves out of the inoculated leaves of the resistant Illinois-A. They concluded that the resistance mechanism in Illinois-A was against the movement of the virus in the plant (45). McKinney has reported that the tobacco line 448A limits the spread of TMV into the younger leaves without the necrotic hypersensitive reaction (23). This resistance mechanism in corn may be a form of tolerance in which the plant becomes infected and still produces high yields by limiting the movement of the virus from the site of infection. Further, the different reaction of T8 X 07B in the winter and summer indicates that some environmental factor appears to condition the expression of this resistance mechanism. Temperature has been found to reverse the expression of resistance in *Pisum sativum* to bean mosaic virus 2 (30).

The resistance of certain cells of a plant to infection by an unlimited virus is a well-documented phenomenon in dicotyledonous plants, but has not been described for monocotyledons. Following inoculation with TMV (1, 2, 13, 18, 19, 20) and turnip yellow mosaic virus (TYMV) (27), the first leaves showing mosaic symptoms often have areas of the leaf that are normal dark green in color while the rest of the leaf is yellow green. Goldstein (19) made a detailed cytological study of the dark green and yellow green areas of leaves showing TMV mosaic symptoms. She found the dark green areas appeared cytologically similar to healthy leaves whereas yellow green areas were abnormal. Since this report, several workers have noted that the dark green areas contain less infectious TMV than yellow green areas (13, 20, 39). Further, Fulton (18) and Atkinson and Matthews (1) have shown that the dark green areas are resistant to infection by the strain causing the mosaic but were not resistant to challenge inoculation with another strain. Atkinson and Matthews (2) used a density-gradient analysis to show that there was only 0-5.1% as much TMV in dark green tissue as in yellow green tissue. They also showed by electron microscopy that there was much less virus in the cells in the dark green areas compared to adjacent yellow green cells. It has been postulated that even though the cells in the dark green areas do contain a small amount of TMV, it may not be actively multiplying in these cells and that the virus spreads into these cells through plasmodesmata connections with infected cells. These cells in the dark green areas seem to be resistant to the multiplication of the virus (2). This situation differs from that in T8 X 07B and MDMV in that the mosaic bands make up a very small part of

the total leaf area of a resistant host whereas the dark green areas with TMV and TYMV make up only a small part of the leaf of a susceptible host.

SUMMARY

A rapid, accurate assay was developed to measure the concentration of MDMV either in clarified sap or in the purified form by analytical sucrose density-gradient centrifugation. The density-gradient assay was compared to a systemic infectivity assay on corn and to the microprecipitin test and was found to be more sensitive and more rapid to perform.

The purification of MDMV was re-evaluated to determine whether the low yield of virus was due to degradation during purification or to a low initial concentration in corn tissue. Several variations in each step of the purification were evaluated by sucrose density-gradient centrifugation and electron microscopy. The most consistent purification procedure consisted of extraction in sodium citrate and mercaptoethanol, chloroform clarification, and differential and density-gradient centrifugation in citrate buffer. Yields of 9.0 - 23.0 μg of purified virus/g fresh weight of field-grown, mechanically inoculated Hy X C103 were obtained by this method. The density-gradient assay and the microprecipitin test showed that approximately 25% of the virus in clarified sap was recovered in the purified form. Less than 0.5% of the infectivity in the crude sap was recovered in the purified form.

Purified MDMV showed birefringence, had an ultraviolet absorption spectrum typical for viruses having 5% RNA and 95% protein with an $A_{268/280}$ of 1.18, and had a sedimentation coefficient of 160-162 S.

In greenhouse tests, the concentration of MDMV in clarified sap reached a maximum in Golden Cross Bantam sweet corn 8-10 days after

inoculation and was very low 15-28 days after inoculation. Virus concentrations as high as 25 μg/g were attained after 8-15 days in Hy X C103 grown in the greenhouse and remained high for over 21 days. This corn hybrid, Hy X C103, is more suitable as a source of tissue for purification than is Golden Cross Bantam. Susceptible corn hybrids grown in the field and inoculated with an artist's airbrush contained approximately twice as much virus, and the concentration remained high for a longer period of time than in the same hybrids grown in the greenhouse. Virus concentrations as high as 49 μg/g leaf tissue were obtained in clarified sap from field-grown Hy X C103. Field-grown corn is an excellent source of large quantities of tissue with a high concentration of virus.

The resistant hybrid, T8 X 07B, showed general mosaic symptoms following inoculation in the greenhouse in the winter. However, the symptoms appeared 5-6 days later than in the susceptible hybrids grown under the same conditions. During the summer, T8 X 07B showed only an occasional systemic, chlorotic longitudinal band of tissue in an otherwise normal, dark green leaf of corn grown in the field or greenhouse. MDMV concentrations in the resistant hybrid showing general mosaic symptoms were as high as were those in susceptible hybrids grown under the same conditions in the greenhouse. In the summer when T8 X 07B showed chlorotic band symptoms, high concentrations of MDMV were extracted from the chlorotic band whereas little or no virus was extracted from the normal dark green tissue

of the same leaves. Apparently, the mechanism of resistance in T8 X 07B against MDMV is not against infection or viral multiplication but is against the movement of the virus within the plant.

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Appendix Table I. Sample flow sheet and calculation of μg virus/g leaf tissue

2 g leaf tissue, midrib removed

2 ml of 0.1 M sodium citrate + 0.5% mercaptoethanol

grind in cold mortar and pestle

centrifuge 10 minutes, 10,000 rpm 9RA rotor

2.75 ml homogenate recovered

1.4 ml chloroform (emulsify in test tube)

centrifuge 5 minutes, 5,000 rpm 9RA rotor

collect aqueous phase

centrifuge 10 minutes at 12,000 rpm 9RA rotor

2 ml layered on 10-40% sucrose gradient

centrifuge 3 hours at 27,000 rpm in SW 27 rotor

scan at 3 1/3 ml/minute, 0-0.25 O.D. range

0.12 $inch^2$ (area under virus peak measured with planimeter)

0.50 O.D. units per $inch^2$ (based on standardization of ISCO, as described in Methods and Materials)

1.56 g of tissue in 2 ml layered on column (2g/2.75 ml homogenate recovered)

2.4 absorbancy index (O.D./mg of MDMV)

$$\text{Yield} = \frac{0.12\ \text{inch}^2 \times 0.50\ \text{O.D./inch}^2}{1.56\ \text{g} \times 2.4\ \text{O.D./mg}} \times 10^3\ \mu\text{g/mg}$$

$$= \frac{0.12 \times 0.50}{1.56 \times 2.4}\ \text{mg/g} \times 10^3\ \mu\text{g/mg}$$

$$= 16.0\ \mu\text{g virus/g leaf tissue}$$

Appendix Table II. Sample calculation of infectivity assay

1	2	3	4	5	6	7	8	9	10	11
Z[a]	X/N[b]	q[c]	lnln[d]	App. Titer[e]	Y[f]	ω[g]	η[h]	Nω[i]	Nωη	$N\omega\eta^2$
-4.6	0/10	0		4.6	3.85					
-5.3	0/10	0		5.3	3.15					
-6.9	1/10	0.1	+0.83	7.73	1.55	.23	2.26	2.3	5.20	11.75
-7.6	2/10	0.2	+0.48	8.08	0.85	.58	.45	5.8	2.61	1.17
-9.2	5/10	0.5	-0.37	8.83	-0.85	.34	-.58	3.4	-1.98	1.15
-9.9	8/10	0.8	-1.50	8.40	-1.45	.20	.12	2.0	.24	.03
-11.5	9/10	0.9	-2.25	9.25	-3.05	.46	-1.2	4.6	-5.55	6.60
-12.2	10/10	1.0			-3.75	.23	1.0	2.3	2.30	2.30
-13.8	10/10	1.0			-4.35	.13	1.0	1.3	1.30	1.30
	Sums							21.7	4.12	24.20

Appendix Table II. (Con't)

a/ $\log_e$ or ln of the dilution inoculated. If the dilution is 1:1000, or 10^{-3}, Z = 2.3 log 1/1000 = 2.3(log 1 - log 1000) = 2.3(-log 1000) = 2.3(-3) = -6.9.

b/ The denominator, N, is the number of plants inoculated and the numerator, X, is the number remaining healthy.

c/ q is the fraction remaining healthy and equals X/N.

d/ lnln = loglog transformation of q, from Table XVI[1/].

e/ Apparent titer, calculated from the difference between column 4 (lnln) and column 1 (Z). For example at 10^{-4}, App. titer = -0.37 -(-9.2) = 8.83.

f/ An inspection of the values for lnln for the dilution series suggests that lnln = 0 (column 4) for an estimated titer of 8.45 (column 5). At this value, an estimated 37% of the plants would remain healthy. Expected lnln Y = Z = (est. $\ln_\chi$). For example at 10^{-4}, Y = -9.2 + 8.45 = -0.85.

g/ ω = weighting coefficient for corresponding values of Y, from Table XVII[1/].

h/ η = maximum working deviate for corresponding values of Y and ω, calculated from Table XVII[1/] and values of q.

i/ N, number of plants inoculated, multiplied by ω, the weighting coefficient.

$$\text{Corrected value of } \ln_\chi = \text{Est. } \ln_\chi - \frac{\Sigma N\omega\eta}{\Sigma N\omega}$$

$$= 8.45 - \frac{4.12}{21.7}$$

$$= 8.45 - 0.19$$

$$= 8.26$$

Appendix Table II. (Con't)

Conversion to $\log_{10}$: $\ln\chi = 2.3 \log_{10}\mu$

$$\log_{10}\mu = 8.26/2.3$$

$$= 3.59$$

Variance, based on sampling error $= \frac{1}{\Sigma N\omega}$

Standard deviation of $\log_{10}\mu$ $= \frac{1}{\sqrt{\frac{21.7}{2.3}}}$

$$= 0.09$$

$\therefore$ Virus titer $= 3.59 \pm 0.09$

The virus titer, or estimated mean value of virus concentration, times -1, gives the log of the dilution that would infect 63% of the plants. Thus, at a dilution of $10^{-3.59}$, 63% of the plants should have been infected, and according to the Poisson distribution, there was one particle of virus per "infection-initiating volume." Therefore, there must have been $10^{3.59}$ virus particles per infection volume in the undiluted preparation. The log of the number of particles is 3.59; the value of the antilog is 3891×10^3. This figure was used in calculations to determine percentage loss in infectivity with purification steps.

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VITA

Ronald Klair Jones was born January 31, 1939 in Kilmarnock, Virginia. He is the son of Mr. and Mrs. Carroll M. Jones of Sampson's Wharf, Virginia.

He is married to the former Cecilia Fay Satterfield of Hyattsville, Maryland. They have four children, Cheryl Linnett born August 1, 1962, Carolyn Annette born October 17, 1963, Micheal Lloyd born July 17, 1966, and Edna Fay born June 18, 1970.

He completed elementary school in Kilmarnock, Virginia and secondary school in Arlington, Virginia. He attended Emory and Henry College, Emory, Virginia from 1957 to 1958, entered Virginia Polytechnic Institute in 1958 and obtained a B.S. degree in Horticulture in 1961. In 1961, he entered the University of Delaware, Newark, Delaware and obtained the M.S. degree in Plant Pathology in 1963. He entered the University of Florida in 1963 to work toward the Ph.D. degree and resigned 1965. From 1965 to 1968, Mr. Jones was employed by Clemson University, Clemson, South Carolina as Extension Plant Pathologist. He returned to graduate studies in September 1968 under Dr. S. A. Tolin at the Virginia Polytechnic Institute, Blacksburg, Virginia to finish the Ph.D. degree in Plant Pathology.

Ronald Klair Jones

Ronald Klair Jones

MAIZE DWARF MOSAIC VIRUS: PURIFICATION, SOME PHYSICAL PROPERTIES, AND YIELD OF VIRUS FROM SEVERAL CORN HYBRIDS

by

Ronald K. Jones

Abstract

To better understand the nature of maize dwarf mosaic virus (MDMV) and its interaction with susceptible and resistant corn hybrids, the purification of MDMV and its physical properties were re-evaluated and a rapid assay procedure was developed.

The virus was purified by chloroform clarification and differential and sucrose density-gradient centrifugation. Corn tissue was homogenized in 0.1 M sodium citrate plus 0.5% mercaptoethanol. The virus was re-suspended after high speed centrifugation in 0.005 M sodium citrate, pH 7.0. Purified virus has an ultraviolet absorption spectrum typical for filamentous viruses having 5% RNA, shows birefringence, has an $A_{260/280}$ of 1.18, and a sedimentation coefficient of 160-162 S. The virus is filamentous and appears to be a member of the potato virus Y group. A yield of 9.0-23.0 μg of virus/g fresh weight of field-grown, mechanically inoculated Hy X C103 was determined by analytical sucrose density-gradient centrifugation. This represents a yield of purified virus of approximately 25% of that in the clarified sap. Results obtained from the density-gradient analysis were comparable to but more sensitive than those from the microprecipitin test and a systemic

infectivity assay. The systemic infectivity assay on corn, analyzed by maximum likelihood and loglog transformation, showed a yield of purified virus of 0.4% of that in the crude sap.

Sucrose density-gradient analysis of clarified sap was used to determine the concentration of MDMV in the susceptible Golden Cross Bantam, Hy X C103, and DeKalb 805A, and the resistant T8 X 07B. In the greenhouse, the virus concentration is at its peak in Golden Cross Bantam 8-10 days after inoculation and 8-15 days in Hy X C103. Yields in clarified sap as high as 25 μg of virus/g leaf tissue were obtained from Hy X C103 in the greenhouse. The concentration is higher in all 3 susceptible hybrids mechanically inoculated with an artist's airbrush in the field and remained relatively high throughout the growing season. Yields in clarified sap as high as 50 μg/g leaf tissue were obtained from field-grown Hy X C103.

Hy X C103 is a more suitable hybrid to use in the greenhouse as a source of virus than Golden Cross Bantam because the concentration remains higher for a longer period of time. Field-grown tissue is an excellent source of large quantities of tissue with a high concentration of virus.

The resistant corn hybrid, T8 X 07B, shows systemic mosaic symptoms in the greenhouse in the winter but the symptoms appear 5-6 days later than in the susceptible corn hybrids. Concentrations of MDMV as high as in the susceptible hybrids could be recovered from T8 X 07B in the winter. The resistant T8 X 07B shows only an occasional systemic chlorotic, longitudinal band of tissue in an otherwise normal dark green leaf in both the greenhouse and field in the summer. High concentrations

of MDMV could be extracted from the chlorotic bands but not from the dark green part of the same leaves. These data suggest that the mechanism of resistance in T8 X 07B is not against infection or multiplication but is against the movement of the virus in the plant.