# Sugarcane Mosaic Virus and Maize Dwarf Mosaic Virus in Mixed Infections of Sugarcane and Other Grasses

A. G. Gillaspie, Jr. and H. Koike

Research Plant Pathologists, Southern Region, Agricultural Research Service. U.S. Department of Agriculture, Houma, Louisiana 70360. Current address of senior author: Applied Plant Pathology Laboratory, Agricultural Research Center-West, Beltsville, Maryland 20705.

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## ABSTRACT

Johnsongrass, itchgrass, and sugarcane were inoculated with one or more strains of sugarcane mosaic virus (SCMV) before, during, or after inoculation with maize dwarf mosaic virus strain A (MDMV). Inoculum from doubly inoculated itchgrass infected johnsongrass and 'Rio' sorghum, but the symptoms on Rio were milder than those of MDMV alone and distinct from those of SCMV alone. Inocula from simultaneously inoculated itchgrass were serially passaged through johnsongrass or Rio. When tested on Rio, symptoms from the johnsongrass series appeared to be MDMV, whereas those from the Rio series appeared to be variants of SCMV. The symptoms became stabilized after three serial passages through Rio, and further passages through other hosts, johnsongrass, itchgrass, or sugarcane ('Chunnee') did not change the symptom expression on Rio. These mixtures could be mistaken for new strains of MDMV. The mixtures could be separated biologically by passage through sugarcane ('Louisiana Striped') which yielded only SCMV, and physically, by purification methods detrimental to one component. When in a stabilized mixture, SCMV could multiply in johnsongrass, which is highly resistant to SCMV alone.

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The interaction of viruses or their strains has been studied since the discovery of cross-protection in plants in 1929 (20). These studies have been reviewed recently (14, 15). There are many examples of cross-protection between strains of a virus, of which one strain produces local lesions and the other does not. Strains that do not produce local lesions, usually appear not to cross-protect.

Certain mixtures of unrelated viruses cause symptoms on some hosts that cannot be explained by an additive effect of the symptoms of the viruses in the mixture. Some examples are double streak disease of tomato, caused by a combination of tobacco mosaic virus (TMV) and potato virus X (PVX); and the potato crinkle disease, caused by potato virus A and PVX together.

Mixed infections of virus strains have also been reported to produce a symptom pattern different from that of either strain separately. Mixed infections of TMV-common and TMV-yellow aucuba strains on *Nicotiana sylvestris* (4) and PVX-latent and PVX-B strains on tobacco (15) are examples of strain synergism. Such strain mixtures could easily be construed to be new strains.

There is evidence that sugarcane mosaic virus (SCMV) and maize dwarf mosaic virus strain A (MDMV) are strains of the same virus. They are similar in length (22) and are serologically related (5, 22). There has been some question whether all research has been done with the same isolate of SCMV-H, since SCMV-H has been reported not to cross-react serologically with MDMV (23). MDMV can infect sugarcane when inoculated mechanically (6, 8, 11, 13). There is a report that SCMV-H can partly cross-protect against MDMV in corn (27). The virus strains used in our experiments do not cause local lesions on the hosts used, except occasionally (12), and apparently two or more strains can persist in the same plant (3, 27).

In the experiments reported here, the mixed infections of SCMV strains with MDMV appear like new strains of MDMV. When these mixtures become

"stabilized", both components together will readily infect johnsongrass, a host which usually does not support SCMV alone (2, 26). Preliminary reports have been published (10, 17).

MATERIALS AND METHODS.—*Hosts and viruses.*—The host plants tested included sweet sorghum [*Sorghum bicolor* (L.) Moench 'Rio' and 'Atlas'], itchgrass or raoulgrass (*Rottboellia exaltata* L. f.), johnsongrass [*Sorghum halepense* (L.) Pers.], and sugarcane (*Saccharum sinense* Roxb. amend. Jeswiet 'Chunnee', *Saccharum officinarum* L. 'Louisiana Striped', and interspecific hybrid POJ 234). All plants were grown in a steam-sterilized soil in 7.5-cm peat pots or 10-cm clay pots in an aphid-free greenhouse. Sugarcane was grown from cuttings; other plants were grown from true seed.

SCMV strains A, B, D, H, and I (1, 3, 25, 26), and a serologically related isolate of MDMV from johnsongrass in Louisiana (16, 21), were used. SCMV-A was ATCC No. PV 181; SCMV-B, No. PV 186; SCMV-D, No. PV 52; SCMV-H, No. PV 51; and SCMV-I, No. PV 83. The SCMV strains were maintained on POJ 234, and the MDMV isolate was maintained on johnsongrass.

Young sugarcane plants were inoculated with an artist's airbrush (125-150 psi) (7). The inoculum contained infectious leaf extract, 0.01 N sodium sulfite (sulfite), and silicon carbide particles. The leaf extracts were prepared by grinding infected leaves in a meat grinder, mixing in sulfite (about 2 ml/g tissue), and filtering through cheesecloth.

Seedlings of sorghum, itchgrass, and johnsongrass at a two- to four-leaf stage were inoculated by rubbing leaves, dusted with silicon carbide, between thumb and forefinger. The inoculum of infectious leaf extract was prepared by grinding infected leaves, silicon carbide, and sulfite (about 2 ml/g tissue) in a mortar with a pestle. The leaves were rinsed with water after inoculation. This method of inoculation was also used for all of the assays in these experiments. Ten to 12 johnsongrass seedlings and 20 to 24 sorghum seedlings were used for each sample in each assay.

Symptoms on seedlings were read twice, at weekly intervals; symptoms on sugarcane were read over a 4-wk period. Data were recorded as a fraction—number infected per number inoculated—and a description of the symptoms was noted.

*Establishment, maintenance, and determination of mixed inoculations.*—For the superinoculation experiments, the seedlings of itchgrass and johnsongrass were inoculated with either MDMV or SCMV-H with an artist's airbrush. One week later, the itchgrass and johnsongrass were superinoculated with SCMV-H or MDMV. The inoculated johnsongrass and itchgrass seedlings were then assayed on Rio, on sugarcane POJ 234, and on johnsongrass, 2 wk after the second inoculation. Extracts from some of these infected test plants were subsequently inoculated to Rio, Atlas, and johnsongrass.

For the simultaneous inoculation of SCMV and MDMV to itchgrass, a mixed inoculum of SCMV strains A, B, D, H, or I from POJ 234 and of MDMV from johnsongrass was prepared by comminuting infected tissue (1 g/2 ml sulfite) of each separately, then combining equal volumes of the individual preparations. Combinations made were as follows: MDMV + SCMV-A (MDMV + A), MDMV + SCMV-B (MDMV + B), MDMV + SCMV-D (MDMV + D), MDMV + SCMV-H (MDMV + H), MDMV + SCMV-I (MDMV + I), and MDMV + SCMV-H + SCMV-I (MDMV + H + I). A seventh mixture was prepared by mixing 5 ml each of MDMV and SCMV-A, -B, -D, -H, and -I [MDMV + (A to I)] and centrifuging the mixture at 50,000 rpm for 30 min in a No. 50 rotor on a Spinco Model L ultracentrifuge (Beckman Instruments, Inc., Palo Alto, California). The resulting pellet was resuspended in sulfite of a volume equal to that of a sample with only two components. These seven mixed inocula and MDMV alone were inoculated onto itchgrass. After 3 wk, the itchgrass plants were assayed on johnsongrass, Rio, and Atlas and passaged serially on itchgrass.

*Methods of separation of MDMV from SCMV strains.*—The purification methods used were those reported by Gillaspie (9). Infected tissue was blended either in a solution of 0.5 M sodium citrate and 0.3% 2-mercaptoethanol (sodium citrate method) or in a solution of 0.3% ascorbic acid, 0.3% 2-mercaptoethanol, and 0.01 M sodium diethyldithiocarbamate (ascorbic acid method). The sample was filtered through cheesecloth and emulsified in one-third volume chloroform. The emulsion was broken by low-speed centrifugation. The aqueous layer received one cycle of high speed-low speed ultracentrifugation (30,000 rpm for 1.5 hr on a No. 30 rotor and 7,000 rpm for 10 min on a No. 40 rotor in a Spinco Model L ultracentrifuge). This was followed by a sucrose rate zonal density-gradient centrifugation and a sucrose "equilibrium" density-gradient centrifugation (23,000 rpm for 2 hr and 21,000 rpm for 17 hr, respectively, in a SW 25.1 rotor). Rate gradients contained 12.5 ml each of 100 and 400 g/liter of sucrose and equilibrium gradients 10 ml each of 200 and 600 g/liter sucrose. Both sets of gradients were prepared mechanically. Gradients were fractionated by puncturing the tube with a needle and withdrawing the sample with a hypodermic syringe or with a fractionator coupled to a 254-nm analyzer (ISCO, Lincoln, Nebraska, Model D and UA-2, respectively). Sucrose density-gradient electrophoresis was performed in a Steere-Davis electrophoresis apparatus (Bellco Glass, Inc., Vineland, New Jersey) (24).

For separation by serological cross-absorption, infected tissue was extracted by the ascorbic acid method. The virus pellet was resuspended in physiological saline after high-speed centrifugation. After a low-speed centrifugation, the supernatants were incubated (37 for 1 hr) with various dilutions of antiserum against the SCMV strain component, centrifuged in a clinical centrifuge to remove reaction materials, and assayed on Rio and johnsongrass seedlings. Normal serum controls were also included.

For separation by dilution, young plants of Rio or

johnsongrass were blended with 9 ml of sulfite to 1 g of tissue (a $10^{-1}$ dilution). This, and further dilutions were assayed on Rio and johnsongrass seedlings. After several weeks, the seedlings infected at the greatest dilutions were again assayed on Rio and johnsongrass seedlings, and the symptoms were noted.

RESULTS.—*Hosts and symptoms.*—SCMV-A, -B, -D, and -H produced a mild mosaic on Rio and a severe necrosis with reddening and stunting on Atlas. SCMV-A, -B, and -D do not infect johnsongrass and SCMV-H produced infection in 5% or less of inoculated johnsongrass plants. This infection was either symptomless or produced transient symptoms. This agrees with the work of others except that SCMV-H has been reported elsewhere to produce local lesions on the inoculated leaves of Atlas (2, 12, 26). These strains produced green or yellow-on-green mosaic symptoms on the sugarcane cultivars POJ 234, Chunnee, and Louisiana Striped.

SCMV-I produced reddening of the leaf sheath and midribs on Rio and a severe necrosis with reddening and stunting on Atlas. SCMV-I produced transient symptoms in 5% or less of inoculated johnsongrass plants; and after symptoms disappeared, virus could no longer be recovered. Our results on Rio and johnsongrass agree with those of Tippett & Abbott (26). This strain also produced green or yellow-on-green mosaic on POJ 234, Chunnee, and Louisiana Striped.

MDMV produced reddening and necrosis of leaf blades and spindles as well as stunting on Rio; mosaic symptoms on johnsongrass; and a mild mosaic on Atlas. This agrees with reported results except that MDMV has been reported to produce necrosis and stunting on Atlas (12, 13). MDMV produced either no symptoms or sparse streaking on POJ 234 and green-on-green mosaic on Chunnee. Louisiana Striped was not infected by MDMV (6, 11).

Mixed inocula produced a variety of symptoms on Rio. The symptoms were either a combination of the symptoms of each component alone but milder than either alone or the symptoms were decidedly in favor of one component. Thus, there were varying degrees of reddening, necrosis, stunting, and mosaic on Rio, but a given mixed inoculum produced uniform results. Rio was the only host on which mixed inocula could be differentiated from each other. Specific mixed symptoms on Rio are discussed later. The mixed inocula produced MDM-type mosaic on johnsongrass and SCM-type mosaic on POJ 234 and Chunnee. Atlas exhibited a mild SCM-type or MDM-type reacion, depending on the mixed inoculum.

The relation of symptoms to relative concentration of strains in the mixed inoculum was examined in a dilution experiment. Extracts from MDMV-infected johnsongrass at 1/3 and 1/30 dilutions were mixed with equal volumes of extracts from SCMV-B, -H, or -I-infected POJ 234 at dilutions ranging from 1/3 to 1/30,000. These mixtures were then inoculated onto Rio, Atlas, and johnsongrass. At lower levels of dilution of the SCMV component, SCM-symptoms were predominant on Rio and Atlas; at higher levels, the MDM-symptoms became dominant on Rio and Atlas, and the percentage of infection on johnsongrass was high.

*Superinoculation of SCMV-H and MDMV.*—Table 1 provides a summary of superinoculation results. Inocula prepared from superinoculated itchgrass produced, on Rio, symptoms less severe than those of MDMV alone, and produced mosaic symptoms on johnsongrass and POJ 234. The results suggested that both series of itchgrass plants contained mixed infections. Inocula prepared from superinoculated johnsongrass seedlings produced symptoms on Rio and johnsongrass like those expected from MDMV alone.

*Symptoms from simultaneous inoculation with SCMV strains and MDMV.*—Extracts of the itchgrass inoculated with one of the seven mixed inocula, or with MDMV alone, produced mosaic in at least 60% of inoculated johnsongrass plants. Passage of the inoculum from itchgrass inoculated with MDMV + A, B, or D to Rio, produced a yellow-on-green mosaic, red flecking of leaf blades, and less stunting than MDMV alone. MDMV + H produced reddening on leaf blades, some necrosis of the spindle, and stunting equal to MDMV alone. MDMV + I produced reddening of midribs and sheaths, with some necrosis of the spindle and less stunting than MDMV alone. MDMV + H + I produced the same symptoms as MDMV + I, except with stunting equal to that of MDMV. MDMV + (A to I) produced symptoms similar to those of MDMV + A, except that there was

TABLE 1. Symptoms produced by inocula from hosts inoculated with maize dwarf mosaic virus (MDMV) and sugarcane mosaic virus, strain H (SCMV-H) in succession

| Hosts | Inoculation | | Assay-host symptoms[a] | | | Interpretation of virus present | Number of host plants assayed |
|---|---|---|---|---|---|---|---|
| | 1st | 2nd | Rio | Johnsongrass | POJ 234 | | |
| Itchgrass | SCMV | MDMV | Atyp | + | +*[b] | mixed | 8 |
| | MDMV | SCMV | Atyp | + | +* | mixed | 8 |
| Johnsongrass | SCMV | MDMV | MDMs | + | +* | MDMV | 8 |
| | MDMV | SCMV | MDMs | + | – | MDMV | 8 |

[a] MDMs refers to symptoms associated with MDMV; Atyp refers to symptoms not typical of either above; and (+) refers to the presence of mosaic symptoms.

[b] (*) refers to the fact that these plants were assayed on Rio, johnsongrass, and Atlas. The results were used to obtain the interpretation of virus present.

red flecking on the sheath. Atlas plants gave erratic results with these inocula. Some plants showed SCM-type symptoms and others a mosaic only. A seed mixture was suspected, and this host was not used until after a new batch of seed could be obtained.

*Direct serial passage of simultaneously inoculated MDMV and SCMV strains.*—Mixed inocula from itchgrass were tested to see whether their symptom expression would change after serial passage through a host that might favor one strain over another. The pathogens present were serially passaged in johnsongrass and in Rio. After several passages, inocula prepared from johnsongrass and inoculated to Rio began to show symptoms indicative of MDMV alone. Those from Rio continued to infect johnsongrass, but on Rio produced a yellow-on-green mosaic with occasional red flecking on the leaves and a small amount of stunting (Fig. 1 and 2). The severity of the mosaic symptoms on Rio varied, depending on the original mixed inocula; for example, MDMV + B and MDMV + D produced a more pronounced mosaic than MDMV + A. The Rio symptoms remained more or less constant from transfer to transfer through 17 serial passages (Fig. 3).

The role of itchgrass in determining the characteristics of the mixtures was further examined in two experiments. In one experiment, after three passages through Rio or through johnsongrass, the inocula prepared from these hosts were passed to itchgrass as well as to Rio and johnsongrass (Fig. 3, step 6). After 4 wk, the infection on these hosts was assayed on Rio and johnsongrass. Plants inoculated from itchgrass showed symptoms similar to those inoculated from Rio and johnsongrass in each series. In a second experiment, mixed inocula initially inoculated to itchgrass were passaged serially in itchgrass eight times before serial passages in Rio or johnsongrass. The symptom changes that occurred in johnsongrass and in Rio were very similar to those that occurred after only one passage in itchgrass.


**Fig. 1.** Comparison of effects on 'Rio' sorghum due to maize dwarf mosaic virus (MDMV) and sugarcane mosaic virus, strain H (SCMV-H), singly and in combination. From left to right: healthy plant, and plants infected with SCMV-H, MDMV + H (Rio-to-Rio), MDMV + H (johnsongrass-to-johnsongrass), and MDMV.

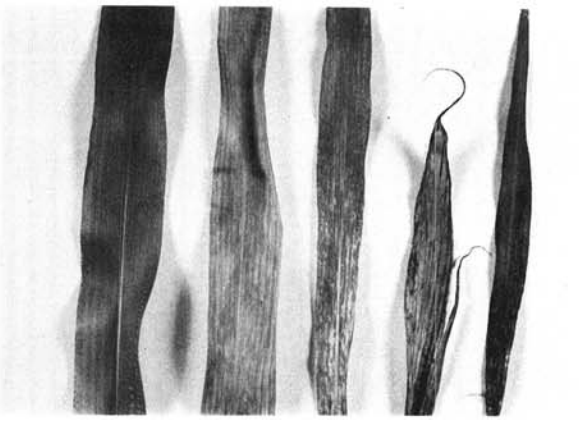

**Fig. 2.** Comparison of leaf symptoms on 'Rio' sorghum caused by maize dwarf mosaic virus (MDMV) and sugarcane mosaic virus, strain H (SCMV-H), singly and in combination. From left to right: healthy leaf and leaves infected with SCMV-H, MDMV + H (Rio-to-Rio), MDMV + H (johnsongrass-to-johnsongrass), and MDMV. Note severe necrosis of spindles of the two on the right.

When any of the seven mixed inocula originally inoculated to itchgrass and then passaged serially in johnsongrass were transferred to Rio, the symptoms elicited on Rio were those of MDMV, even after only three passages in johnsongrass. In sharp contrast was the behavior of an inoculum passaged in Rio before passing serially in johnsongrass. The inoculum, MDMV + A, was originally inoculated to itchgrass, passaged serially six times in Rio, then passaged (Fig. 3, step 8) serially five times in johnsongrass before returning to Rio. The symptom expression on Rio was the same as that of MDMV + A that had been passed through Rio only.

*Alternate serial passage of simultaneously inoculated MDMV and SCMV.*—Unlike the direct series, in which the mixture is passaged repeatedly in a single host, the alternate series involves more than one host. In the one experiment to be reported here, MDMV + I was initially inoculated to itchgrass where it was serially passaged twice, and then it was inoculated onto Chunnee (Fig. 4). From Chunnee, in one series, it was passed through johnsongrass back to Chunnee. In a second series, it was passed through itchgrass back to Chunnee in which it was passed serially two times at 6- and 10-wk intervals. Inoculations to Rio and johnsongrass showed the mixture to be present at each step of both series, though not in every sample of Chunnee tested.

Seed pieces from Chunnee stalks 4, 6, and 8 months after initial inoculation with MDMV + I were germinated in vermiculite. Symptoms on the shoots arising from the seed pieces were observed, and the shoots were assayed. The results showed that most of the canes still contained mixed infections. Some of those tested after 6 months contained only SCMV-I, however. In addition, top leaves of Chunnee were assayed after 6 months. Some plants contained mixed infections, and some only SCMV-I.

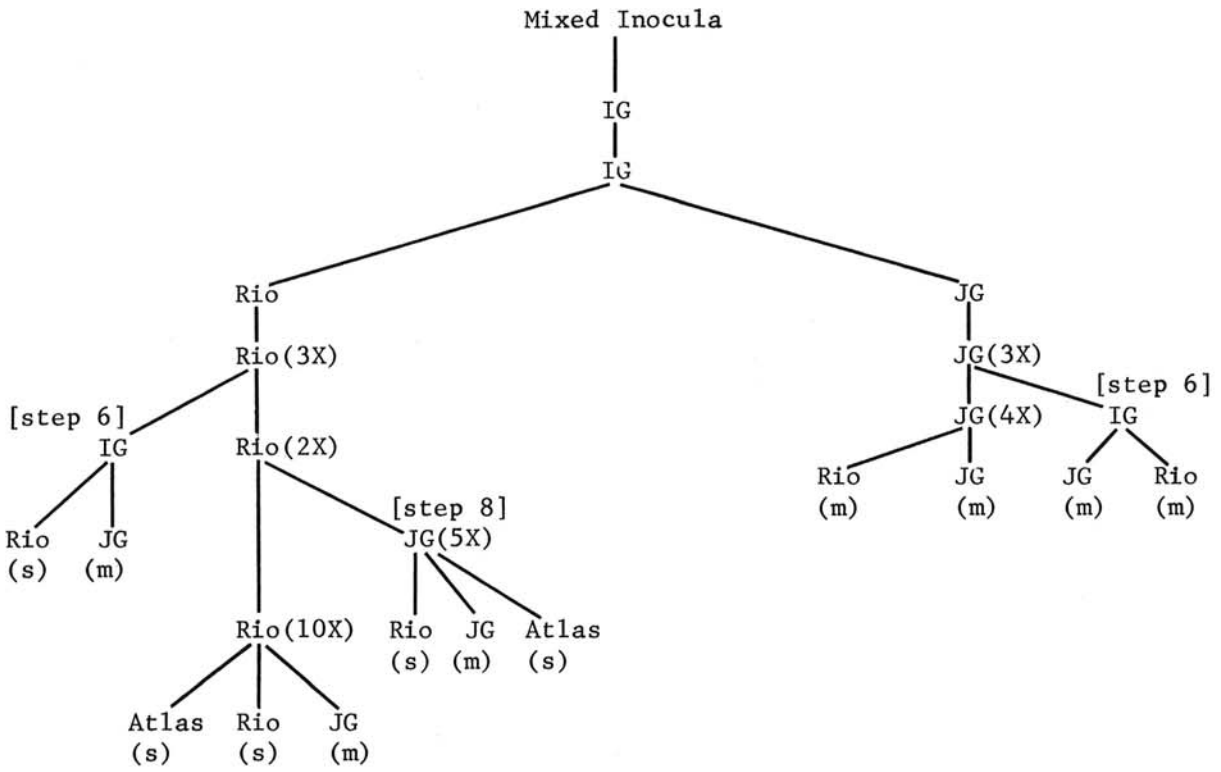


**Fig. 3.** Serial passage of maize dwarf mosaic virus (MDMV) and sugarcane mosaic virus (SCMV) strains and the production of symptoms on various hosts. Symbols: IG = itchgrass, JG = johnsongrass, (s) = symptoms associated with SCMV, (m) = symptoms associated with MDMV. This experiment was done between April 1971 and July 1972. Number of passages in a given host is indicated by 2X, 3X, 4X, or 5X.

*Component separation by purification.*—To examine whether the above results were caused by mixed infection or by new strains, a method of separation of the mixture components was sought. MDMV apparently can be extracted in much higher quantities by the sodium citrate method than by the ascorbic acid method (9). The latter method extracts SCMV in high amounts. The MDMV + A of the Rio-to-Rio series was purified from Rio tissue by the ascorbic acid method. The sample, after the equilibrium density-gradient centrifugation, was assayed on Rio, Atlas, and johnsongrass. The results indicated that only the SCMV component was present. MDMV apparently had been removed from the mixture. The sample migrated as a single band on the density-gradient electrophoresis column.

The MDMV + I from Rio assay plants of infected Chunnee was inoculated to *Zea mays* L. 'Sweetangold'. Both purification methods were tried on equal parts of the corn tissue after 3 wk. Both samples were subjected to density-gradient electrophoresis. The sample from the sodium citrate method contained many electrophorectic components, but the ascorbic acid method sample contained only two components. Samples before and after electrophoresis were assayed. The crude juice and samples taken before electrophoresis produced mixed symptoms on Rio and only mosaic on Atlas. Electrophoresis samples from the sodium citrate method were not infectious. The two bands from the electrophoresis of the ascorbic acid method sample inoculated to Rio produced mixed symptoms on some plants and MDM symptoms only on others. When the Rio plants were again assayed, the same symptoms appeared again. Thus, MDMV may have been separated from the mixture.

*Serological separation.*—The possibility of cross-absorption of one component in a mixture with antiserum against that component was tested. MDMV + A (Rio-to-Rio) was inoculated onto Rio and itchgrass. After 3 wk the tissue was harvested, purified, and incubated with various dilutions of SCMV-A antiserum. These samples were then assayed on Rio and johnsongrass. Some differences in symptom severity on Rio were observed. These Rio plants and some infected johnsongrass plants were assayed again on Rio and johnsongrass, but the test plants failed to show any significant differences in symptoms. It may be assumed that little or no separation occurred.

*Separation by dilution.*—Serial dilution is another technique which may separate components of virus mixtures, assuming that one strain may dilute out before the other. Extracts of plants thought to

contain a mixed infection were diluted and inoculated to Rio and johnsongrass. The results of these inoculations may be seen in Table 2. Different inocula varied widely from each other in dilution end points. The most dilute positives were assayed on Rio and johnsongrass to try to detect any symptom changes. The dilute positives on Rio produced by the MDMV + A (johnsongrass-to-johnsongrass) inocula showed stunting, severe mosaic, and flecking when passed to Rio, and those on johnsongrass showed severe stunting, reddening, and necrosis when passed to Rio. These differences in symptom expression may have been due to separation, but were probably caused by changes in component ratios during passage through Rio or johnsongrass. The other inocula failed to produce any differences when dilute positives were checked.

*Separation by passage through sugarcane.*—One successful method for separation of the components, was passage through a sugarcane cultivar that is resistant to one component. The cultivar Louisiana Striped was selected because it is resistant to MDMV. The direct johnsongrass-to-johnsongrass serially passaged mixed inocula did not produce any infection on Louisiana Striped, but all the Rio-to-Rio serially passaged mixed inocula, except MDMV + H + I, produced mosaic on this cultivar. Extracts from the Rio-to-Rio inoculated sugarcane plants produced only SCM-type symptoms on Rio and did not infect johnsongrass. Many *S. officinarum* canes are resistant to MDMV-infection (11) and might therefore serve the same purpose as Louisiana Striped.

DISCUSSION.—Our results indicate that mixed infections with MDMV and SCMV strains produce symptoms that could be mistaken for new strains of MDMV. These mixed infections simulate strains in other ways, also. The mixed infections could be maintained on certain hosts and serial passage through certain hosts (e.g., Rio) "stabilizes" symptom expression. The fact that johnsongrass, which is virtually immune to SCMV when inoculated alone, can support the multiplication of SCMV in a stabilized mixture is of particular interest.

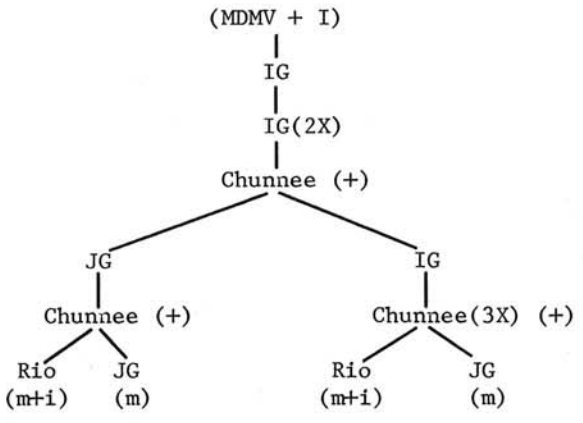


**Fig. 4.** Alternate passage of maize dwarf mosaic virus (MDMV) and sugarcane mosaic virus (SCMV-I) mixed inoculum. The experiment began in April 1971 and ended in June 1972. Symbols: (+) = mosaic symptoms present, (m) = symptoms associated with MDMV, (i) = symptoms associated with SCMV-I, IG = itchgrass, JG = johnsongrass. Number of passages in a given host is indicated by 2X or 3X.

TABLE 2. Dilution end point of mixed infections of maize dwarf mosaic virus (MDMV) and sugarcane mosaic virus (SCMV)

| Sample | Source | Dilution end points | |
|---|---|---|---|
| | | Rio | Johnsongrass (JG) |
| MDMV + A (Rio-to-Rio) | Rio | $10^{-4}$-$10^{-5}$ | $10^{-2}$-$10^{-3}$ |
| MDMV + A (Rio-to-Rio) | JG | $10^{-3}$-$10^{-4}$ | $10^{-4}$-$10^{-5}$ |
| MDMV + A (JG-to-JG) | Rio | $10^{-2}$-$10^{-3}$ | $10^{-1}$-$10^{-2}$ |
| MDMV + A (JG-to-JG) | JG | $10^{-3}$-$10^{-4}$ | $10^{-3}$-$10^{-4}$ |
| MDMV + B (Rio-to-Rio) | Rio | $10^{-3}$-$10^{-4}$ | $10^{-3}$-$10^{-4}$ |

Three groups of mixtures could be distinguished on the basis of symptoms after the Rio-to-Rio serial passages. The first group was MDMV with either A or H, the second group, MDMV with either B or D, and the third group, MDMV with I, with H + I, or with (A to I). The inocula used were of unknown titer, and these groupings may reflect the titer of the inocula used to establish mixed infections. The experiments with varied concentrations of inocula tend to support this interpretation.

There is much evidence that our results derive from mixed infections of SCMV and MDMV rather than from the isolation of a new strain. Changes in host range and in symptoms may be characteristic of new strains, but the separation into component strains by physical and biological methods can be explained only in terms of mixtures.

Some physical methods permitted separation of the component strains of the mixtures, and others did not. Separation by purification with a technique detrimental to one component was successful in some instances. This method seems to depend upon finding a mixture in which the component to be removed is present in small amounts. The viruses under study are closely related serologically and were not separated by serological cross-absorption, probably because of the common antigenic properties; neither were they separated by dilution. This suggests either that the concentration of the components is equal, or that some interference may be involved. Separation by density-gradient electrophoresis has not been successful because, under the conditions used, the electrophoretic mobilities of the components do not differ sufficiently.

The partial recovery from infection in Chunnee, leading to the loss of MDMV from MDMV + I-infected cane, was a second type of separation. A third type of separation was the host-induced

separation, leading to a selection favoring MDMV (itchgrass-to-johnsongrass) or favoring SCMV (Louisiana Striped). The different types of separation can lead to one or the other of the components, suggesting that it is unlikely that reversion of a new strain to an old one is involved.

It has not been possible to establish that the apparent separation of strains is complete. It has long been known that an excess of one strain may mask the presence of a second strain. The repeated assays from the itchgrass-johnsongrass direct series for example, showed only MDMV to be present. Methods for rigorous proof of strain purity do not exist in plant virology.

Infections of mixtures containing MDMV and SCMV may maintain themselves. The dilution experiments show that high titers of some mixtures are present in some hosts (Table 2). The alternate series experiments offer more evidence of the ability of the MDMV + I mixture to maintain itself through passage from itchgrass to Chunnee, then to itchgrass, and back to Chunnee three times, and then to Rio and johnsongrass. The mixtures are maintained for long periods in sugarcane; MDMV + I was recovered from a plant of Chunnee infected for 8 months.

The infections caused by mixtures of MDMV and SCMV can develop so that their symptoms become stabilized. Thus, serial passage through Rio has a stabilizing effect on symptom expression. In the direct-passage experiments, after only three passages through this host, the symptoms produced on Rio did not change after a single passage through itchgrass. Neither did passage through Chunnee usually change the characteristic symptoms of the mixtures on Rio. Also, MDMV + A, after a series of passages on Rio, was passed from johnsongrass to johnsongrass five times. When inoculum was returned to Rio, the symptoms remained the same as those from the MDMV + A that had been passed only from Rio to Rio. Some hosts, like itchgrass, do not cause this stabilization of symptoms. Thus, passage through johnsongrass, whether following after one or after eight passages through itchgrass, indicated MDMV alone to be present when assayed on Rio.

The mixtures were maintained through some 17 successive passages on Rio, and the symptoms remained essentially stable. Stable mixtures can be established from MDMV with any or all of the five strains of SCMV used. We think that the component strains of these mixtures must be able to achieve a proportion that guarantees infection by both, comparable invasiveness of new cells, comparable rates of multiplication, and comparable facility in spreading throughout the plant. In addition, we feel that the maintenance and stability of the mixtures could mean that the component strains multiply in the same cells of the host; there is no direct evidence to support this, however.

The results with itchgrass demonstrate that the establishment and stabilization of mixtures are different functions. Mixtures are readily established on this grass, but the mixtures, whether after one or eight serial passages, were not stabilized as shown by subsequent passage through johnsongrass. The host characteristics required for establishment and stabilization of mixtures are not understood.

There have been reports recently of many new strains and isolates of MDMV (18, 19, 28). The mixture experiments reported here suggest that different host ranges and symptomatology may result from mixtures as well as from new strains. Work is presently in progress to separate certain field isolates collected on sorghum in Louisiana to determine whether mixtures of MDMV and SCMV occur naturally.

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