

A Field Survey for Serogroups and the Satellite RNA of Cucumber Mosaic Virus

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ABSTRACT

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Collections of tomato (*Lycopersicon esculentum*), pepper (*Capsicum annuum*), cucurbits, and other species were made in Bermuda and central and western New York State. All samples were assayed directly without passaging through greenhouse hosts. One hundred thirty-four samples tested positive for cucumber mosaic virus (CMV) by enzyme-linked immunosorbent assay (ELISA). Of these, only two harbored detectable satellite RNA as demonstrated by dot blot hybridization and confirmatory Northern hybridization. Some false-positive dot blot readings occurred with certain sample preparations, but these were easily detected by

Northern hybridization. CMV serotyping was performed by using ELISA and antisera specific to the two serogroups, WT and S. Both CMV serogroups occurred in the field, with the S group occurring mostly in peppers. Eighty-three percent of the CMV-infected samples could be assigned to these two groups with the remainder giving a mixed reaction. Infected pepper populations analyzed in two New York locales were found to have high serogroup homogeneity. This survey indicates that satellites are rare and both serogroups are common in the native CMV populations of this study.

Cucumber mosaic virus (CMV) is a cosmopolitan virus and a common pathogen of banana (*Musa* spp.) (23), cucurbits, tomato (*Lycopersicon esculentum* Mill.), and pepper (*Capsicum annuum* L.) (18). It can overwinter in weed hosts (32) and is easily transmitted by aphids (24,30). Symptoms are usually a mosaic, stunting, and often leaf deformation, though milder symptoms can occur (10).

The virion of CMV is icosahedral and has a genome composed of three RNAs (20,27) plus a subgenomic RNA which serves as a messenger RNA for coat protein production (34). A satellite RNA, usually 334–342 bases long (16), is often found associated with CMV cultures maintained in the greenhouse. This satellite has little homology to the genomic RNAs of CMV (31) yet is dependent on them for its replication (15) and is encapsidated in the CMV virion (19). The CMV satellite can ameliorate CMV symptoms or induce new severe symptoms, depending on the CMV strain, the satellite, and the host (10,17,26,38).

Studies on the serology of CMV have defined two major serogroups. Using immunodiffusion and ELISA, Devergne et al (2,29) defined the DTL and T₀RS groups, which are equivalent to the genomic RNA hybridization groups WT and S of Piazzolla et al (28) and to groups I and II of Owen and Palukaitis (25),

respectively. The two serogroups defined by Edwards and Gonsalves (4) are also equivalent to the WT and S groups of Piazzolla et al due to common strains shared by the studies. We will refer to these groups as the WT and S serogroups.

We sought to determine if satellite RNA is naturally present in field plants infected with CMV. Concurrently, we typed the CMV-infected samples according to the serogroups WT and S to investigate the natural distribution of these two groups. Collections were made in two distinct locations, central and western New York State, and the Bermuda Islands off the southeastern coast of the United States. Bermuda was chosen in part because of a recent finding of satellite in a CMV-infected banana sample (3). We found CMV satellite to be rare and both serogroups well represented in our samples.

MATERIALS AND METHODS

Collection and storage of field samples. Central and western New York State samples were collected on 21 sites in the following counties (Fig. 1): four sites in Onondaga County, four in Tioga County, one in Seneca County, one in Yates County, six in Ontario County, and five in Erie County. All samples from the Erie County sites were free of CMV; all other New York sites yielded samples testing positive for CMV. Two sites in Ontario County and one in Erie County were experimental farms; all other New York

sites were commercial farms. Bermuda samples were collected on 24 sites (not shown). Ten of these yielded samples positive for CMV. All Bermuda sites were commercial farms.

A wide range of symptom types was sampled to collect plants that may have contained a satellite altering the normal symptom syndrome caused by CMV. For example, tomato symptoms ranged from no symptoms or a very mild "mottle," to classical CMV leaf narrowing and mosaic, or to intense chlorosis and deformation.

At each New York site, 20–60 samples of a single crop species (two to four leaves from a single plant) representing a large portion of the field were taken, placed in separate plastic bags, and frozen for long-term storage at -20°C . For the Bermuda collection, leaf samples were sliced in strips and dried over calcium chloride and subsequently stored in screw top tubes with calcium chloride at 4°C .

Virus propagation, transfer, and purification. In one experiment, samples were taken through three passages of tobacco (*Nicotiana tabacum* L. 'Havana 423'), which stimulates satellite replication (12,14), in order to build up the concentration of any initially undetected satellite RNA. Frozen or dried field samples were ground in 10 mM potassium phosphate buffer (pH 7.0) at 1:20–1:50 (tissue/buffer) with a mortar and pestle, and the extract was rubbed onto Carborundum-dusted tobacco leaves with the pestle. Transfer from fresh leaves was accomplished in the same manner.

Virions were purified from infected squash or tobacco with the method of Lot et al (21). Virions were used to inoculate some of the control plants used in ELISA or hybridizations. Virion RNA was extracted with phenol, ethanol precipitated (11), and fractionated by sucrose density gradient centrifugation (5). Virion RNA from cultures harboring satellite was used as standards in hybridizations.

Serology. Control strains for serotyping had been maintained in zucchini squash (*Cucurbita pepo* L. 'Seneca') since the last serogroup analysis (4). Tomato control plants were inoculated with virions purified from zucchini squash. The WT group was represented by CMV strains C, B (4), and Chi (Kearney, Gonsalves, and Provvidenti, unpublished). The S group was represented by strains WL (10) L1, L2, and L3 (4). All field samples were used directly; none were passaged through a host except in the satellite amplification study. A direct, double-antibody sandwich ELISA was used to detect CMV in field and greenhouse samples and for serogrouping. Each sample was assayed using antiserum raised against CMV-C, and in a separate plate, antisera against CMV-WL and CMV-L2. Plates were first coated with either C antiserum or L2 antiserum. After binding of the sample

antigens, antiserum conjugated to alkaline phosphatase was bound to the antigen. For this purpose, C antiserum was used for the C antiserum-coated plates, while either WL or L2 antisera were used for the L2 antiserum-coated plates, each working equally well. All of these procedures followed those of Clark and Adams (1). Greenhouse control plates were generally read 60 min after the addition of substrate, while field sample plates were read 70–150 min after substrate addition. Greenhouse tomato, pepper, and zucchini squash, either uninoculated or inoculated with the CMV strains listed above, were used as controls for the field samples, with two to four infected controls and two to five healthy controls included in each plate. Duplicate wells were used for every sample.

Dot blot and Northern blot. Except in the satellite amplification study, field samples were used directly with no intermediate host passaging. One hundred fifty milligrams of frozen field or fresh control sample, or a somewhat smaller amount of dried field sample, was ground in 150 μl of buffer (0.1 M glycine, 0.01 M EDTA, 0.2 M NaCl; pH 9.5; plus SDS [sodium dodecyl sulfate] to 1% and 2-mercaptoethanol to 0.1%), and 300 μl of phenol/chloroform (22). A micropestle (Kontes Scientific, Vineland, NJ) was used to grind the samples in microcentrifuge tubes. After vortexing, the samples were centrifuged at 16,000 g for 5 min. The aqueous phase was transferred to a new microfuge tube and stored at -20°C until ready for use. Extraction procedures were carried out at room temperature. Ethanol precipitation did not decrease nonhomologous binding or increase sensitivity with the dot blot hybridization.

Samples were then treated with glyoxal and blotted onto a Gene Screen Plus nylon membrane according to company protocols (Dupont/NEN, Boston, MA). Briefly, the aqueous phase was brought to 1 M glyoxal (Sigma, deionized in BioRad AG 501-X8[D] resin), 12.5 mM sodium phosphate (pH 6.5), and 50% DMSO (Sigma). It was then heated to 50°C for 15 min, chilled on ice, and loaded onto the prewetted membrane with a commercial manifold. Northern blots were done in similar fashion, with electrophoresis of glyoxalated samples at 80 V through 1% agarose in TAE as listed in the company protocol, followed by blotting onto the Gene Screen Plus membrane by capillary action.

The hybridization probe was a ^{32}P -labeled in vitro transcript made from a clone of the white leaf variant satellite of CMV inserted in the transcription vector pT7T318U (Kearney and Gonsalves, unpublished). In vitro transcription in the presence of alpha- ^{32}P ATP was carried out according to Pharmacia Company protocols. Hybridization followed DuPont protocols, except that washing was done with $0.2\times$ SSC rather than $2\times$ SSC to increase

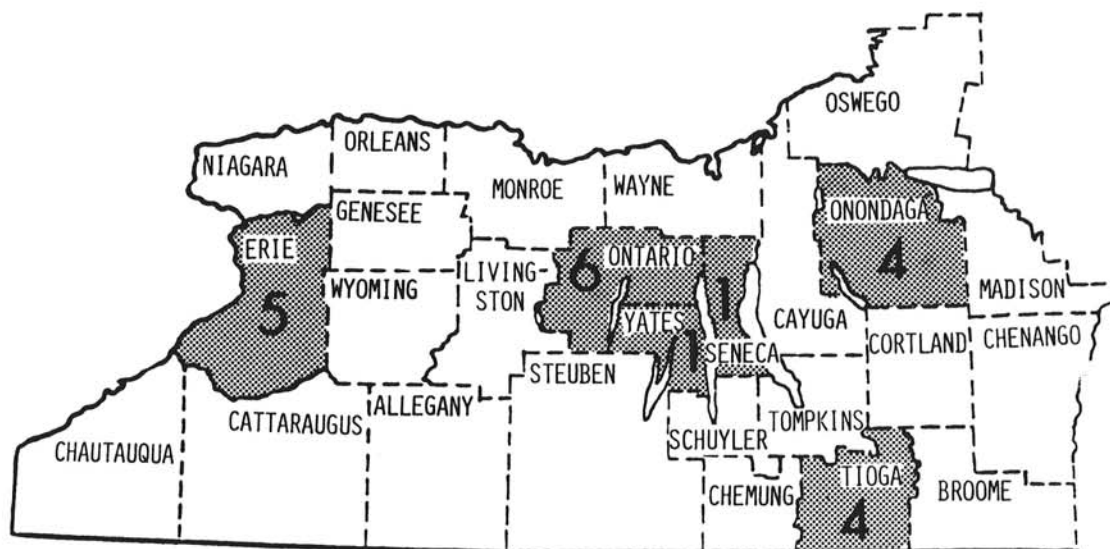


Fig. 1. Distribution of plant sample collection sites within central and western New York state. Collections were made in the shaded counties. The number of collection sites are indicated for each county. Cucumber mosaic virus was detected at all sites except the five Erie County sites.

stringency and 250 $\mu\text{g/ml}$ yeast tRNA replaced the 100 $\mu\text{g/ml}$ salmon sperm DNA in the hybridization buffer to decrease background.

RESULTS

Standardization of ELISA and dot blot tests. Some CMV isolates were poorly detected by an antiserum for the opposite serogroup. This is most easily seen by plotting the $A_{405\text{nm}}$ value obtained using WT serogroup antiserum in ELISA against the $A_{405\text{nm}}$ value obtained using the S antiserum for several greenhouse control plants infected with CMV strains representing the two serogroups (Fig. 2). For samples represented by points near the coordinates, CMV is easily detected by only one of the antisera.

In addition to more complete detection, we used two antisera for each sample to investigate the natural distribution of the two serogroups. From Figure 2 and other greenhouse data, a convenient rule for defining inclusion in a serogroup was derived. If the $A_{405\text{nm}}$ value using WT antiserum was greater than twice that of the $A_{405\text{nm}}$ using S antiserum, the sample was placed in the WT serogroup. For the reverse situation, the sample was placed in the S serogroup. Samples with WT and S values within a factor of two of each other were placed in a "mixed" group. In Figure 2 (and Fig. 6 below), lines are drawn with slopes of 2 and 0.5 to demonstrate the divisions graphically.

The mean $A_{405\text{nm}}$ for the healthy controls used in this study was, using WT antiserum, 0.027 for zucchini squash, 0.057 for tomato, and 0.023 for pepper; using S antiserum, 0.046 for squash, 0.042 for tomato, and 0.026 for pepper. With some sites, a sample $A_{405\text{nm}}$ value two to four times the mean of the healthy control value was considered a positive reading, but the positive threshold was usually set at a convenient $A_{405\text{nm}}$ value three to 10 times the healthy mean (e.g., 0.200).

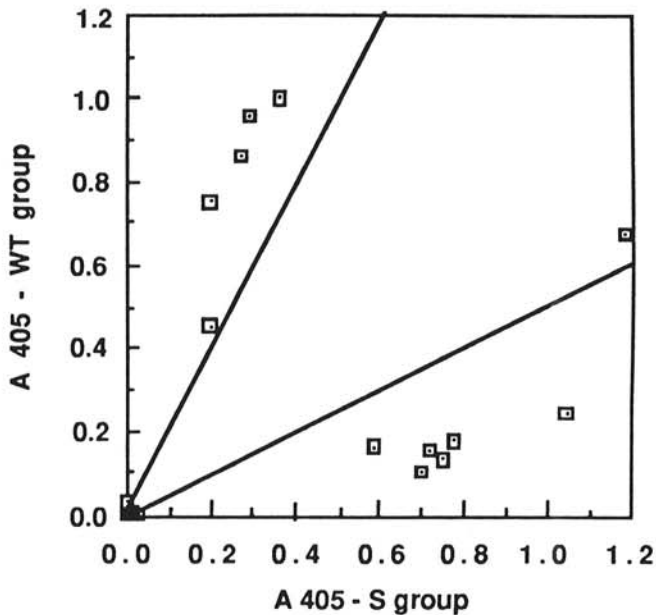


Fig. 2. A graphic means of serogrouping cucumber mosaic virus (CMV) samples, demonstrated with inoculated control plants. Greenhouse squash and tomatoes were inoculated (except for 10 healthy controls) with several CMV strains representing the two CMV serogroups. Enzyme-linked immunosorbent assay was done for each sample using antisera for the WT and for the S serogroups, generating two $A_{405\text{nm}}$ (absorbance at 405 nm) values. When these two values are plotted against each other for each sample, three clusters result: Healthy plants (origin), S group strains (bottom), and WT group strains (left). The two lines have slopes of 2 and 0.5, showing that, for almost all samples, the $A_{405\text{nm}}$ value obtained with antiserum to one serogroup is at least twice the value obtained with the antiserum to the other serogroup. For these inoculated control plants, the WT strains used were C and B in squash and C and Chi in tomato; the S strains were L1, L2, and L3 in squash and WL, L1, L2, and L3 in tomato.

Dot blot hybridization was used to detect satellite RNA in the field samples. The method used gave a very low response to healthy controls and a very high signal to CMV-positive satellite-containing controls (Figs. 3 and 4). Satellite can be detected in tobacco, tomato, pepper, and squash. Tobacco infected with CMV plus satellite required a dilution of 1/10,000 to lessen its signal to the range of healthy controls diluted 1/20 (Fig. 3); this represents a sensitivity range of 500-fold.

The detection of CMV and satellite in field samples. Samples were collected from 21 sites in central and western New York State (Fig. 1) and 24 sites in Bermuda. Frozen or dried field samples were first tested by ELISA to determine if they were infected with CMV. Antisera to both serogroups of CMV (WT and S) were used. Samples shown to be CMV-infected were then analyzed for the presence of satellite by dot blot hybridization.

CMV was common in the pepper (43%) and cucurbit (34%) collections but fairly rare in the tomato samples (9%) (Table 1). Sixteen of the 21 New York sites and 10 of the 24 Bermuda sites yielded CMV-infected samples (Table 1). No satellites were detected in the Bermuda samples. In the New York samples, a satellite was found in one pepper from Tioga County and in one tomato from Ontario County. The site of the tomato sample was an experimental farm owned by the New York State Agricultural Experiment Station and was the location of a previous outbreak of tomato white leaf disease which was shown to be due to a satellite associated with CMV (10).

Eight samples gave strong signals in the dot blot assay (Fig. 4). Two of these were the New York samples mentioned above. The remaining six comprised the only tomato and lettuce samples that tested positive for CMV by ELISA among the Bermuda

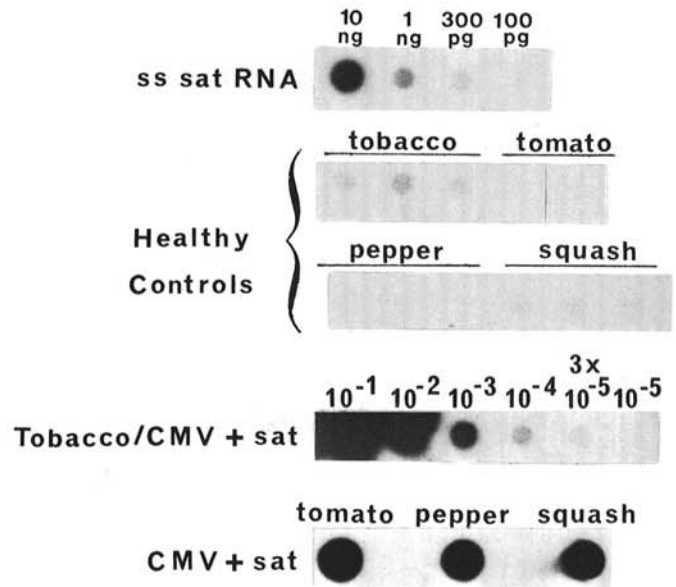


Fig. 3. Dot blot hybridization of greenhouse control plants for the detection of cucumber mosaic virus (CMV) satellite (sat) RNA. In the first (upper) row is a dilution series of single-stranded satellite RNA (ss sat RNA) purified from CMV virions. In the second and third rows are nucleic acid extracts of healthy controls, with each dot representing a separate plant and 1/20 of the total extract (150 mg of tissue). The fourth row is a dilution series of a nucleic acid extract of tobacco infected with CMV containing satellite. A 1/10 dilution implies that 1/10 of the entire nucleic acid extract, or 15 mg of tissue, was blotted. The bottom row contains three infected controls, inoculated with CMV containing satellite. The squash control was inoculated with a CMV strain containing a satellite that is able to multiply in squash, usually a poor supporter of satellite replication (sat-WL1, a gift of Peter Palukaitis, Cornell University). Samples were ground in buffer with phenol and chloroform, vortexed, and centrifuged. The aqueous phase was glyoxalated, loaded into a commercial blotting manifold, and blotted onto a nylon membrane. The membrane was probed with a ^{32}P -labeled in vitro transcript from a CMV satellite clone.

collection (five tomato and one lettuce). In Northern hybridization, the two New York samples yielded bands comigrating with satellite, but all six Bermuda samples were negative for satellite (Fig. 5) with no bands comigrating with satellite. To solve this discrepancy, a dot blot was done with samples of dried tomato, lettuce, and squash tissue from the Bermuda collection and frozen tomato samples from the New York collection, all of which tested CMV-negative by ELISA. All frozen tomato and dried squash samples gave no signal, but the dried tomato and lettuce samples gave strong signals.

CMV-infected New York tomato samples had symptoms ranging from virtually no symptoms or mild mottling or yellowing

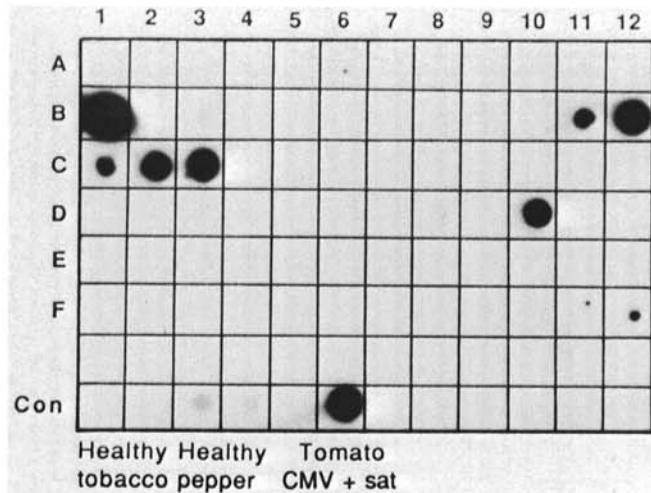


Fig. 4. Dot blot of field samples for the detection of cucumber mosaic virus (CMV) satellite (sat) RNA. The first six rows (A-F) were blotted with crude nucleic acid preparations of field samples while the bottom row contains the greenhouse control samples. The field samples giving signal were as follows: B1, tomato, Ontario County, New York; B11-12, C1-3, tomatoes, Bermuda; and D10, pepper, Tioga County, New York. A Bermuda lettuce sample also gave a signal, but was on another blot. The spot in position 12 F is an artifact. Nucleic acid preparations were made as for Figure 3. All samples were diluted fourfold.

TABLE 1. The presence of cucumber mosaic virus (CMV) and its satellite in field samples

Crop	Location	Number of plants collected	Number of plants with CMV ^a	Number of sites with CMV	Number of plants with satellite ^b
Pepper	Bermuda	50	13	5	0
	New York	131	64	6	1
Tomato	Bermuda	44	5	3	0
	New York	247	22	7	1
Cucurbit	Bermuda	16	1	1	0
	New York	31	15	5	0
Misc.	Bermuda ^c	60	9	6	0
	New York ^d	12	5	1	0
Totals	Bermuda	170	28	10	0
	New York	421	106	16	2

^aAs determined by direct double-antibody sandwich ELISA using antisera to both serogroups (WT and S) of CMV in separate tests for each sample. The coating antibodies were developed using CMV-C (WT group) or CMV-L2 (S group). Following binding with sample antigens, alkaline phosphatase-conjugated antibodies developed with CMV-L2 or -WL (S group) or CMV-C were bound to the sample antigens.

^bAs determined by dot blot and northern hybridizations (see Figs. 4 and 5).

^cMiscellaneous samples from Bermuda comprised six lettuce (*Lactuca saligna*), seven banana (*Musa* sp.), and various weed and ornamental species.

^dMiscellaneous samples from New York State comprised five bean (*Phaseolus vulgaris*) and seven weed samples. Enzyme-linked immunosorbent assay CMV positives were *Amaranthus* spp., *Solanum dulcamara*, and *Abutilon theophrasti*.

in most plants, to a moderate to severe mosaic and shoestringing in the remaining samples.

The single tomato with satellite had mild symptoms with no necrosis or chlorosis. CMV-infected New York pepper samples almost uniformly had a finely marked mosaic giving a dull appearance to the leaves. Stunting was also common and occasionally necrotic flecks, lines, or an oak leaf pattern occurred. The pepper sample containing satellite had the typical dull mosaic.

Amplification of satellite in field samples by tobacco passages. All frozen or dried field samples shown by ELISA to be infected with CMV were inoculated onto tobacco in order to encourage the replication of latent CMV satellite (12,14). Pepper samples were also transferred to pepper and then to tobacco. Only a low percentage of samples could be transferred to greenhouse hosts, probably due to a loss of infectivity with the 6 mo of storage at -20 C and with the unavoidable thawing and refreezing in the process of taking samples for ELISA and hybridization assays. Eleven samples were taken through three tobacco passages and were found to be ELISA positive for CMV. None of these samples contained satellite before passaging. Northern hybridization showed that satellite did not appear during tobacco passage in any of the samples. Symptoms were consistent throughout the three passages for each sample and were mainly vein banding and leaf tip blanching.

Occurrence and distribution of serogroups in the field. The field samples generally fell into one of the two serogroups (Table 2). Only 16.8% of the samples belonged in the mixed group. Along crop lines, pepper samples were predominantly infected with S group CMV, while the other crops were mostly infected with strains of the WT group for both Bermuda and New York.

Due to the large number of pepper samples, it was possible to examine the occurrence of serogroups within a site or locale. Peppers collected from two Tioga County sites located within two miles of each other were quite homogeneous in terms of serogroup membership. Except for one uninfected individual, all samples belonged in the mixed category (Fig. 6A). Similar

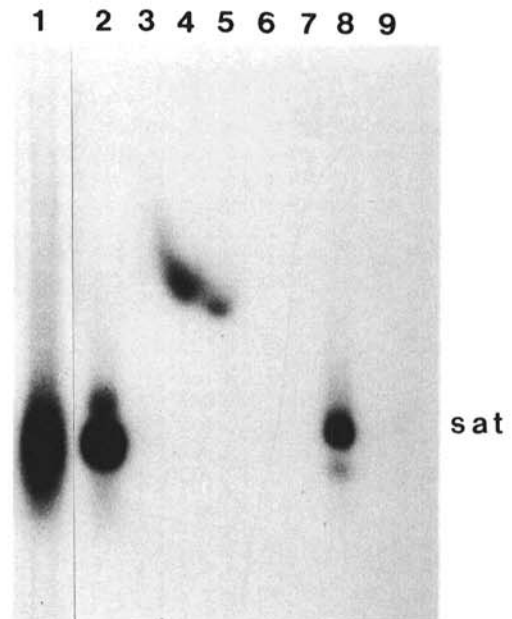


Fig. 5. Northern blot analysis of field samples testing positive for satellite in dot blot of Figure 4. Glyoxalated nucleic acid preparations of samples were electrophoresed through 1% agarose and transferred to a nylon membrane for hybridization with a ³²P-labeled satellite transcript. Lane 1, control tobacco inoculated with CMV-WL harboring satellite; lane 2, field tomato sample, Ontario County, NY; lanes 3-7, tomato samples, Bermuda; lane 8, pepper sample, Tioga County, NY; lane 9, lettuce sample, Bermuda. Only the New York samples (frozen tissue) yielded bands comigrating with satellite RNA; the Bermuda samples (dried tissue) gave either spurious or no hybridization, demonstrating that, among the dot blot positive samples, only the New York samples contained satellite.

homogeneity was found with two Ontario County sites located within two miles of each other. Thirty of the samples fell into the S group, with only one sample in the WT group and one in the mixed group (Fig. 6B).

DISCUSSION

Both serogroups of CMV were well represented in the field samples of this study. However, most of the S group samples were found in peppers, and of these a large number came from only two sites in Ontario County. Therefore, the most well-distributed serogroup in terms of crop type and location was the WT group. The association of the S group mainly with peppers in both Bermuda and New York needs the confirmation of a larger number of samples and sites. Of all samples assayed, 17% were placed in a "mixed" category. These presumably represented mixed infections of two or more strains or infections by a single strain possessing epitopes for both antisera. Serogroup homogeneity was high for two New York locales. Over the locales analyzed, aphids may have fed on a particular patch of overwintering infected weeds and then spread the homogeneous CMV population over several pepper fields.

The dot blot assay produced false positive readings in the detection of CMV satellites for none of the 106 frozen samples but for all six dried tomato or lettuce samples. Apparently, either a chemical change during drying or an excess of tissue used in the assay caused the false positive readings. However, by checking all dot blot positive samples by Northern hybridization, we easily detected the false positives and confirmed the presence of satellite in the remaining two frozen samples.

Avoiding false negative readings, on the other hand, depends on the sensitivity of an assay. It would be difficult to attain a level of sensitivity to confirm the absolute absence of satellite from our samples. Our dot blot assay was able to distinguish between healthy controls and a satellite-containing control sample which was diluted 500-fold more than the healthy controls. Even with this level of sensitivity, we cannot rule out that a residual, very poorly replicating amount of satellite RNA may be present in the samples tested, which could serve as a reservoir for future infections. Northern blotting using agarose or a polyacrylamide system (39) could have increased sensitivity.

However, it can be stated that in 132 of 134 field plants sampled, the level of satellite RNA was at least 500-fold less than found in greenhouse infections, if satellite was present at all. The absence of satellite in 11 samples after passaging in tobacco also argues for low levels or an absence of satellite in the original samples. Therefore, satellite RNA was either absent or probably played no role in disease development with the native CMV populations analyzed in this study.

TABLE 2. Typing of cucumber mosaic virus (CMV)-infected field samples by serogroup

Crop	Location	S group ^a	Mixed	WT group
Pepper	Bermuda	7	1	5
	New York	47	16	3
Tomato	Bermuda	0	2	3
	New York	5	2	15
Cucurbit	Bermuda	1	0	0
	New York	0	0	15
Misc.	Bermuda	0	2	8
	New York	0	0	5
Totals	Bermuda	8	5	16
	New York	52	18	38
	Combined	60	23	54

^aFurther analysis of enzyme-linked immunosorbent assay data set of Table 1. ELISA was performed on each field sample using antiserum against CMV from either the WT or S group, generating a pair of A_{405nm} (absorbance at 405 nm) values, A-WT and A-S. If $A-S > 2 (A-WT)$, then the sample was placed in the S group; if $A-WT > 2 (A-S)$, then in the WT group. If the two values were within a factor of 2 of each other, then the sample was designated "mixed."

The literature contains examples of both presence and absence of satellite in native CMV populations, though in many of these reports either a low sample size or a noncrop host were used, or samples were first propagated through greenhouse hosts. Propagation before satellite assay can lead to amplification (12,14) or changes (9,16,26) in the native satellite population, or possibly to contamination with another satellite (7). Dot blot analysis has been used in Israel (33) and double-stranded RNA analysis in California (37) to directly detect CMV satellite RNA from field samples, but only on the noncrop relative of cultivated tobacco, *Nicotiana glauca* Grah., which would be expected to encourage satellite replication. Hybridization has also been used to detect satellite in a single CMV-infected banana sample from Bermuda

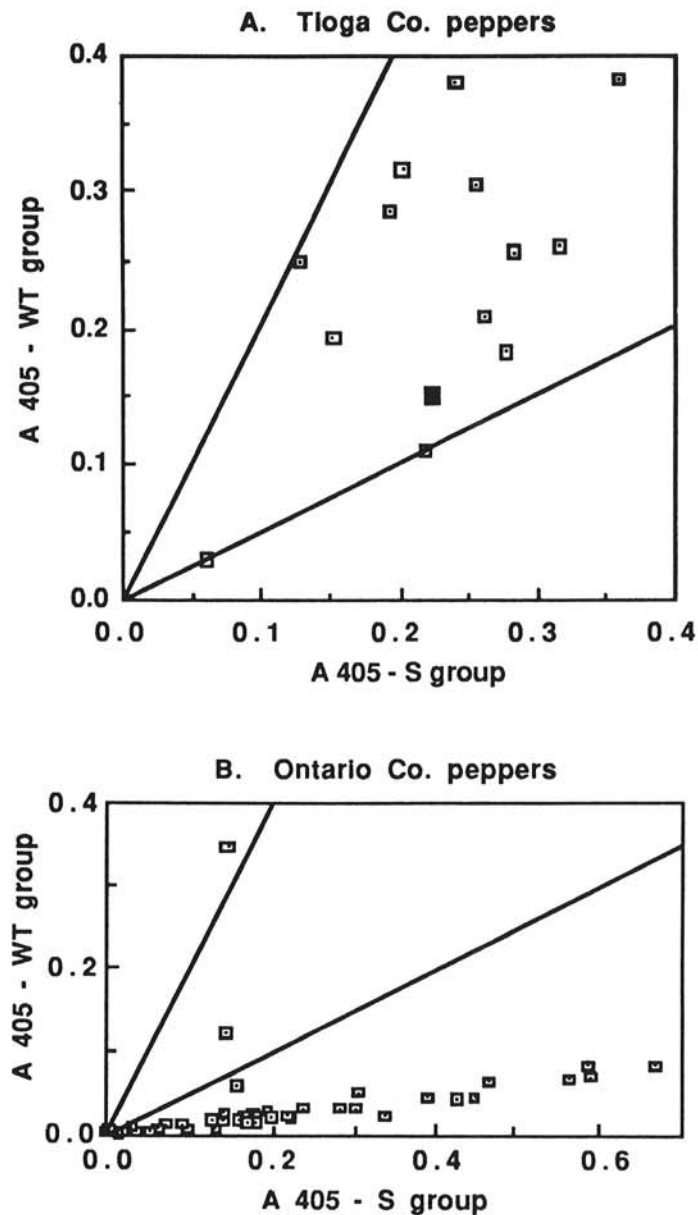


Fig. 6. Distribution of cucumber mosaic virus (CMV) serogroups (WT and S) among pepper samples collected from two different locales. **A**, Samples collected from two sites within two miles of each other in Tioga County, NY. All samples fall between the dividing lines described in Figure 2 and were placed in the "mixed" category. One sample had A_{405nm} readings below 0.1 and is designated uninfected. This sample was one of the two samples that did not have typical CMV symptoms (dull-leaf mosaic and/or necrotic oak leaf pattern). The pepper that contained CMV satellite (Figure 5) is indicated by a filled-in square. **B**, Samples from two sites within two miles of each other in Ontario County, NY. One of the samples is in the WT group, one is in the mixed group, 30 are in the S group, and 9 are designated uninfected (< 0.100). Roughly 80% of the samples had a dull mosaic and/or necrotic patterns on the leaves.

(3). However, an unpublished Australian survey, mentioned in a review article (7), uncovered no satellites. Among CMV isolates propagated in the greenhouse, some are free of satellite even while maintained on tobacco (6,13), though most harbor a satellite (9,16,26).

Perhaps the best evidence of the occurrence of satellite in the field is the occurrence of disease outbreaks that have been connected to a specific CMV satellite. A tomato necrosis outbreak in 1972 in the Alsace region of France was attributed to a satellite (17), as were outbreaks of tomato white leaf in New York (10) and tobacco chlorosis in Japan (35,36). Recently, an outbreak of tomato necrosis has occurred in Italy, and the involvement of a necrogenic CMV satellite has been demonstrated by dot blot analysis of tomato field samples (8). In this study, direct analysis of an extensive number of field samples detected satellite in necrotic tomato samples, but not in tomato plants exhibiting fernleaf, a symptom typically induced by CMV.

In combining these reports with our own findings, there is the suggestion that CMV satellite RNA is rare in CMV infections of some common crop species except during periodic satellite epiphytotics. More studies are needed to confirm that ameliorative or symptom-neutral satellites are generally absent from CMV crop hosts exhibiting typical or mild CMV symptoms. If these hosts did harbor such satellites, they could serve as reservoirs for severe satellite epiphytotics by mutation of the satellite to a virulent form or by transfer to a host susceptible to induction of severe symptoms.

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