Etiology

Symptom Modification by Satellite Tobacco Mosaic Virus in Pepper Types and Cultivars Infected with Helper Tobamoviruses

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ABSTRACT


The presence of satellite tobacco mosaic virus (STMV) in coinfection with eight helper viruses in 23 hosts has previously been reported to cause no change in symptoms induced by the helper viruses alone. This is not the case for systemic infection of certain types and cultivars of pepper, Capsicum annum. In Jalapeño and Pimiento pepper plants, the presence of STMV in coinfections with TMV-U2 caused increased chlorosis in the form of abundant, bright-yellow patches relative to the mosaic caused by TMV-U2 alone. Increased chlorosis also was observed when TMV-U5 and pepper mild mottle virus (PMMV) were used as helper viruses. In Jalapeño the presence of STMV ameliorated severe blistering (leaf distortion) caused transiently by TMV-U2 at approximately 6 wk after inoculation. The concentration of TMV-U2 in Jalapeño plants was reduced by 87% in coinfection with STMV compared to infection by TMV-U2 alone.

Additional keywords: pepper symptoms, satellite virus symptoms, symptom-modulating effect.

Satellite tobacco mosaic virus (STMV) is a subviral pathogen that requires the presence of strains of tobacco mosaic virus (TMV) or other tobamoviruses for replication (20,21). It is called a satellite virus rather than a satellite RNA because its genome encodes its own virion capsid protein. The STMV virion is formed by 60 copies of the 17.5-kDa capsid protein subunit (13). STMV has a ssRNA genome of 1,059 bases (17), with a molecular weight of 0.3 X 10^6.

STMV was originally found in coinfection with TMV-U5 (also known as tobacco mild green mosaic virus [TMGMV]) in tree tobacco, Nicotiana glauca, in several areas of southern California (22). In previous studies (20,22), the presence of STMV did not induce modifications in the symptoms caused by several helper tobamoviruses. Ten of 23 plant species showed the ability to support systemic coinfection after mechanical inoculation. The other plant species responded with local lesions or were not susceptible to infection with the helper virus and STMV. Capsicum annum L. ‘Yolo Wonder’, which was included in one of those studies (22), showed local lesions but not systemic infection.

Plant disease modulation by viral satellites has been reviewed in great detail elsewhere (1,4,7). There are examples in which satellites cause disease attenuation, disease exacerbation, and situations in which disease-modulating effects do not occur. Because of previous reports (20,22), STMV was thought to belong to the non-disease-modulating group. We report here that STMV does cause alterations in symptomatology in coinfection with several helper tobamoviruses in various pepper hosts.

MATERIALS AND METHODS

Virus isolates. The U1 strain of TMV (TMV-U1) was progeny from the full-length infectious TMV-U1 clone, pTMV024 (2), obtained from W. O. Dawson (University of Florida, Citrus Research and Education Center, Lake Alfred, FL). The U2 strain of TMV (TMV-U2) was supplied by A. Siegel (Wayne State University, Detroit). The TMV-U5 strain was a California isolate described in previous studies (20). The older terminology of TMV-U2 and -U5, rather than isolates of TMGMV, will be used in this report because these two virus isolates could be distinguished by their reaction on peppers in mixed infection with STMV. Pepper mild mottle virus (PMMV) was obtained from C. Wetter (University of Saarland, Germany) (25). STMV was a natural isolate previously described (11,20). Plus-sense STMV-RNA transcripts were obtained from the clones pSTMV5 and pSTMV6 previously described by Kurath et al (11), both of which represent the two major sequence variants within the STMV type strain population. These clones differ by single bases at nucleotide positions 3, 494, 682, 751, and 805, and biological variation has not been detected among them.

Pepper hosts. Seeds of the pepper types Jalapeño, Anaheim, and Pimiento, were obtained from Greenfield (Shenandoah, IA), and cultivars Yolo Wonder B and Keystone Resistant Giant No. 3 from Petoseed (Saticoy, CA). These peppers were selected because they are commonly grown in California.

Plant growth and inoculation conditions. Seeds were planted in sterile soil. When seedlings were 2-3 wk old, they were dusted with Carbromundum and inoculated on the cotyledon leaves and/or on the first two true leaves with: 1) 3 µl of helper-virus buffer (50 mM sodium phosphate, pH 7.0) plus 3 µl of satellite buffer (50 mM Tris-phosphate, pH 8.6) (mock), 2) 3 µl helper virus in helper-virus buffer plus 3 µl of satellite buffer, 3) 3 µl STMV RNA in satellite buffer plus 3 µl of helper virus buffer, or 4) 3 µl helper virus in helper-virus buffer plus 3 µl STMV RNA or transcript RNA in satellite buffer. Each inoculated leaf received two 3-µl drops that were mixed and rubbed on the leaves by glove-covered fingers. Helper viruses were inoculated at a concentration of 200 ng/µl, and STMV RNA or transcript RNA was inoculated at 20 ng/µl. Inoculated plants were kept in a greenhouse and observed regularly for 3-4 mo. The experiments were replicated two or three times, with experiments conducted during Spring through Summer (34 C day and 20 C night) and Fall through Winter (26.8 C day and 17.5 C night).

Satellite RNA Detection. Satellite RNA was detected in infected plants by a dot blot hybridization method with radioactive probes prepared as described by Feinberg and Vogelstein (3) with some
modifications (11). The template for probe synthesis was a complete cDNA-STMV insert released from clone pSTMV6 with XbaI (Pharmacia, Uppsala, Sweden) and HindIII (Bethesda Research Laboratories, Inc., Gaithersburg, MD) and gel purified. Sample preparation and blot hybridization were described previously (11). In some cases the presence of STMV in infected plants was detected by dsRNA analysis using 2 g of leaf tissue. Purification was carried out by one cycle of chromatography on columns of CF-11 cellulose powder as previously described by Valverde et al. (22).

Concentration of virus in single- and double-infected plants.
At the end of each experiment, leaves were harvested, divided into two samples (sample 1 consisted of plants numbered 1–5, and sample 2 consisted of plants numbered 6–10), and kept frozen at −20°C. The methods used for virus purification and determination of virus concentration were based on a previous study (22). A series of fourfold dilutions were made beginning with 200 μl of virus suspension diluted in 800 μl of double-distilled water. A 200-μl sample of each dilution was loaded onto 10–40% sucrose gradients in distilled water, which were centrifuged in a rotor at 32,000 rpm for 2 h at 4°C. Centrifuged gradients were fractionated and analyzed using an absorbance monitor with a type 6 optical unit (254-nm filter) and a density gradient fractionator. Absorbance profiles of optimum dilutions were used to compare areas under the peak with comparable profiles of standard virus preparations of STMV and TMV at known concentrations. The data obtained were used to estimate virus concentration and virus yield.

RESULTS

Preliminary survey of peppers. A previous study by Valverde et al. (22) that included several solanaceous hosts and various STMV helper tobamoviruses showed no changes in the helper-induced symptomatology when STMV was present in local or systemic infection. Peppers that are commonly grown in California were tested for susceptibility to infection by the helper tobamoviruses and STMV. Plants inoculated with buffer (mock) or with STMV alone showed no symptoms, and dot blot hybridization with a STMV-specific probe gave negative results. When TMV-U1, -U2, or -U5 was inoculated alone or together with STMV, local lesions developed on all the peppers 3-5 days post-inoculation (Table 1). A severe local reaction on Anaheim, Keystone, and Yolo Wonder B caused the inoculated leaves to collapse and die. There were no additional symptoms, and sap of leaves of Yolo Wonder B plants above those inoculated was not infectious when mechanically inoculated onto N. tabacum L. ‘Xanthi nc’, indicating that no systemic infection with the tobamoviruses occurred. A STMV-specific probe also gave negative results. Jalapeño and Pimiento plants were infected both locally and systemically by the three helper tobamoviruses when inoculated with and without STMV (Table 1). STMV was detected in Jalapeño and Pimiento in systemically-infected tissues with a STMV-specific probe and dsRNA analysis when STMV was co-inoculated with TMV-U2 or -U5. In plants co-inoculated with STMV and TMV-U1, STMV was not detected in systemic tissues of Pimiento and was found in only one of the two Jalapeño plants. This is not surprising, because adaptation of STMV to replication with TMV-U1 as the helper virus appears to require a specific sequence modification (12).

Modifications of TMV-U2 and -U5 symptoms due to co-infection with STMV were clearly observed in Pimiento plants. The mosaic that developed in the doubly infected Pimiento plants consisted of green areas and bright-yellow patches and was most fully expressed in the mature older leaves. Pimiento plants infected with the helper viruses alone developed a mild mosaic of dark- and light-green areas. Jalapeño plants also developed yellow patches when coinfected with TMV-U2 and STMV but not when infected with TMV-U2 alone.

Jalapeño plants infected with TMV-U2 alone developed a severe blistering on some systemically infected leaves approximately 4-7 wk postinoculation. This blistering appeared to be transient in that it was less evident at later dates. This severe blistering phase was not observed in Jalapeño plants infected with TMV-U2 and STMV. In these plants, a gradual deformation in the leaf lamina that became a mild blistering was observed. Symptoms induced by TMV-U5 alone in Jalapeño plants were so severe that it was not possible to conclusively assess the effects of STMV and TMV-U5 co-infection, although the presence of STMV did not appear to have an ameliorative effect.

PMV-U did not induce a local reaction in any of the pepper types and cultivars tested when inoculated alone or with TMV. All five peppers were susceptible to systemic infection. Anaheim, Jalapeño, and Pimiento showed a mild mosaic when infected with PMV-U and a more striking mosaic when the helper and the satellite viruses were present together in infected plants. In Keystone and Yolo Wonder B, PMV-U alone induced mosaic, and PMV-U with STMV induced mosaic with yellow patches.

The symptom effects of STMV infections observed in the preliminary experiment in a small number of plants were unexpected and were investigated further with larger numbers of plants for those combinations exhibiting the most clear symptom modi-

TABLE 1. Reaction of five pepper hosts after mechanical inoculation with tobacco mosaic virus (TMV) strains TMV-U1, -U2 or -U5, pepper mild mosaic virus (PMV), and type strain satellite tobacco mosaic virus (STMV)

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>No. of plants</th>
<th>Anaheim</th>
<th>Pimiento</th>
<th>Jalapeño</th>
<th>Keystone</th>
<th>Yolo Wonder B</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMV-U1</td>
<td>2</td>
<td>LL</td>
<td>LL</td>
<td>LL</td>
<td>LL</td>
<td>LL</td>
</tr>
<tr>
<td>TMV-U2</td>
<td>2</td>
<td>LL</td>
<td>LL</td>
<td>LL</td>
<td>LL</td>
<td>LL</td>
</tr>
<tr>
<td>TMV-U2 + STMV</td>
<td>2</td>
<td>LL</td>
<td>LL</td>
<td>LL</td>
<td>LL</td>
<td>LL</td>
</tr>
<tr>
<td>TMV-U5</td>
<td>2</td>
<td>LL</td>
<td>LL</td>
<td>LL</td>
<td>LL</td>
<td>LL</td>
</tr>
<tr>
<td>TMV-U5 + STMV</td>
<td>2</td>
<td>LL</td>
<td>LL</td>
<td>LL</td>
<td>LL</td>
<td>LL</td>
</tr>
<tr>
<td>PMV-U</td>
<td>5</td>
<td>MM</td>
<td>MM</td>
<td>MM</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>PMV-U + STMV</td>
<td>6</td>
<td>MM</td>
<td>MM</td>
<td>M</td>
<td>M, YP</td>
<td>M, YP</td>
</tr>
<tr>
<td>STMV</td>
<td>2</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>MOCK</td>
<td>4</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

*LL = local lesions; SN = severe systemic necrosis; M = mosaic; ST = stunting; SST = severe stunting; MM = mild mosaic; SB = severe blistering; MB = mild blistering; YP = yellow patches; NS = necrotic spots; INR = initial necrotic reaction; SINR = severe initial necrotic reaction; and N = no symptoms. Underlining indicates differences ± STMV.

*Number of plants shown is the total from two independent experiments.
Fig. 1. Effect of tobacco mosaic virus (TMV) strain TMV-U2 and TMV-U2 and STMV on Pimiento leaves from 6-wk infected plants. Light (yellow) patches are seen on doubly infected leaves.

TABLE 2. Reaction of Pimiento plants to mechanical inoculation with tobacco mosaic virus (TMV) strain TMV-U2 or -U5 and type strain satellite tobacco mosaic virus (STMV) or transcript RNA of two STMV cDNA clones

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>No. of plants*</th>
<th>Symptomatology&lt;sup&gt;b&lt;/sup&gt;</th>
<th>First week</th>
<th>1-3 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMV-U2</td>
<td>20</td>
<td>LL, INR</td>
<td>M, ST</td>
<td></td>
</tr>
<tr>
<td>TMV-U2 + STMV</td>
<td>20</td>
<td>LL, INR</td>
<td>M, ST, YP</td>
<td></td>
</tr>
<tr>
<td>TMV-U2 + Tr6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9</td>
<td>LL, INR</td>
<td>M, ST, YP</td>
<td></td>
</tr>
<tr>
<td>TMV-U2 + Tr5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9</td>
<td>LL, INR</td>
<td>M, ST, YP</td>
<td></td>
</tr>
<tr>
<td>TMV-U5</td>
<td>5</td>
<td>LL, SINR</td>
<td>M, SST</td>
<td></td>
</tr>
<tr>
<td>TMV-U5 + STMV</td>
<td>5</td>
<td>LL, SINR</td>
<td>M, SST, YP</td>
<td></td>
</tr>
<tr>
<td>STMV</td>
<td>5</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>MOCK</td>
<td>5</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Number of plants shown is the total from two independent experiments.

<sup>b</sup> LL = local lesions; INR = initial necrotic reaction; SINR = severe initial necrotic reaction; ST = stunting; SST = severe stunting; M = mosaic; YP = yellow patches; S = severe necrosis; N = no symptoms. Underlining indicates differences ± STMV.

<sup>c</sup> Tr6 is transcript of RNA of clone pSTMV6, and Tr5 is transcript RNA of clone pSTMV5 (10).

TABLE 3. Time course of the symptom reactions of Jalapeño plants to mechanical inoculation with tobacco mosaic virus (TMV) strain TMV-U2 and natural satellite tobacco mosaic virus (STMV) or transcript RNA of two STMV cDNA clones

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>No. of plants*</th>
<th>Symptomatology&lt;sup&gt;b&lt;/sup&gt;</th>
<th>(weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMV-U2</td>
<td>30</td>
<td>LL, INR</td>
<td>1</td>
</tr>
<tr>
<td>TMV-U2 + STMV</td>
<td>30</td>
<td>LL, INR</td>
<td>4</td>
</tr>
<tr>
<td>TMV-U2 + Tr6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12</td>
<td>LL, INR</td>
<td>5</td>
</tr>
<tr>
<td>TMV-U2 + Tr5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12</td>
<td>LL, INR</td>
<td>6</td>
</tr>
<tr>
<td>STMV</td>
<td>12</td>
<td>N</td>
<td>12</td>
</tr>
<tr>
<td>MOCK</td>
<td>15</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

<sup>a</sup> Number of plants shown is the total from three independent experiments.

<sup>b</sup> LL = local lesions; INR = initial necrotic reaction; M = mosaic; ST = stunting; IYP = initial yellow patches; YP = yellow patches; S = severe necrosis; N = no symptoms. Underlining indicates differences ± STMV.

<sup>c</sup> Tr6 is transcript of RNA of clone pSTMV6, and Tr5 is transcript RNA of clone pSTMV5 (10).
TMV-U2 developed a severe blistering on the surface of some leaves, formed by depressions and sometimes bumps of dark-green tissue islands. This severe phase lasted for approximately 2-4 wk, and at the end of that period, the plants began to develop branches with milder blistering on their new leaves. The plants inoculated with TMV-U2 and STMV-RRNA or TMV-U2 and transcript RNA never had the severe phase of blistering, but they did have a mild blistering stage beginning approximately 8-wk post-inoculation. The mild blistering along with mosaic, necrotic spots, and stunting lasted throughout the experiment (Table 3). An average reduction of 30% in height was measured in plants infected with TMV-U2, TMV-U2 and STMV, or TMV-U2 and transcript RNA when compared to mock-inoculated plants in three experiments.

Yields of viruses. Analysis of sucrose-density gradient profiles of partially purified virus from the TMV-U2 and STMV experiments is shown in Figure 2. Virus particles were purified from Jalapeño plants from two experiments. A severe reduction in TMV-U2 virus yield was found in TMV-U2 and STMV coinfected plants. The range of TMV-U2 yield per 10 g of leaf tissue in singly infected plants was 1.32-1.75 mg and in doubly infected plants was 0.19-0.21 mg. The range of yield for STMV in doubly infected plants was 0.77-1.25 mg. Using the average of both purifications, the yield of TMV-U2 was reduced by approximately 87% in doubly infected plants compared to the TMV-U2 concentration in singly infected plants.

DISCUSSION

The pepper types Jalapeño and Pimiento supported replication and systemic infection of STMV in coinfection with the tobamoviruses TMV-U2, TMV-U5, and PMMV. TMV-U1 infection did not replicate STMV in Pimiento plants, but it did replicate in one of two infected Jalapeño plants. The presence of STMV in Pimiento plants induced changes in the symptoms caused by three of these helper viruses (TMV-U2, TMV-U5, and PMMV) in single infection. In Jalapeño plants, changes in symptoms also were induced by STMV in coinfection with TMV-U2 and PMMV. This is the first report describing STMV as a symptom-modulating agent. This report should be considered preliminary, in that few pepper types and cultivars were tested, and it should be noted that Jalapeño and Pimiento cultivars not tested here may exhibit different symptom reductions. However, this work clearly demonstrates that STMV can modify tobamovirus symptoms. The potential for finding additional combinations of pepper cultivars and tobamoviruses that support STMV replication and have symptom-modulating effects. STMV, satellite tobacco necrosis virus (STNV), satellite maize white line mosaic virus (26), and satellite panicum mosaic virus (SPMV) (14) are definitive satellite plant viruses. Of these four viral agents, only SPMV has been mentioned as having symptom-modulating properties, in the form of exacerbation (7). STNV, which is a well-characterized satellite virus, has no disease-modulating effects other than a reduction in the number and size of local lesions in Phaseolus vulgaris plants (8). However, this effect in local lesions could be related to the suppression of the tobacco necrosis virus (TNV) coat-protein synthesis during coinfection with STNV (6). The reported effect of STNV on TMV replication and local lesions is similar to the effect of STMV on TMV-U2 yield and amelioration in Jalapeño described here.

Two types of reactions were found in Jalapeño plants coinfected with TMV-U2 and STMV. One was a STMV-associated amelioration of the transient deformation of the leaf lamina (blistering) caused by TMV-U2 alone. Leaf blistering or distortion is a macroscopic symptom related to a reduction of cell size in those cells bordering dark-green islands (15). The other type of reaction was observed in Pimiento plants—a bright yellowing of the normally light-green patches forming the mosaic (found in helper alone infections). These changes in chlorosis were more pronounced when TMV-U2 was the helper virus for STMV than when the other tobamoviruses mentioned above were used as helpers. These findings have some similarities to what has been found for cucumber mosaic virus (CMV)-satellite RNA (satRNA) interactions, in which coinfection of strains from subgroup II of CMV but not those from subgroup I induced chlorosis in tobacco with some satRNAs (18).

The symptom modulation in STMV/TMV-U2 coinfactions in Jalapeño appears to be an example in which exacerbation (yellowing) and amelioration (blistering) of symptoms occur in the same plant during satellite-helper interaction. At this time, the relative distribution and concentration of TMV and STMV in the bright-yellow tissue patches or in the nonblistered tissues compared to other tissues and compared to single infections has not been investigated. The knowledge that STMV can induce specific subcellular cytotoxic effects (9) and the observation from the present study that the TMV accumulation is severely depressed in these doubly infected pepper plants may have some bearing on the symptom reactions. Infection of two CMV satRNAs (CMV-Y and CMV-WL) with their helper virus causes a phenomenon similar to that observed here with TMV-U2 and STMV, because in some hosts amelioration occurs and in other hosts exacerbation occurs (5,19). In both cases, a reduction of virus-helper yields in coinfactions was observed.

The concentration of TMV-U2 in Jalapeño plants was reduced by 87% when it was coinfected with STMV. In a previous study, TMV-U1 yield was reduced by 36% in N. glauca, and by 32% in N. glauca (22). When TMV-U5 was used as helper virus, the reduction in tobamovirus accumulation in N. tabacum was 37.5% and in N. glauca was 48% (22). The greater reduction of TMV-U2 in Jalapeño plants compared to the reductions observed in the tobacco hosts with TMV-U1 and -U5 could be related to the fact that Jalapeño is not a common natural host for TMV-U2, whereas this is the case for TMV-U5 in N. glauca. Furthermore, TMV-U2 in single and double infection with STMV induced severe symptoms, and virus yields were low compared to TMV-U5 in single and double infection with STMV in N. glauca, which caused symptomless infection and virus yields were 10-40 times higher for the helper virus in single and double infection, respectively. N. tabacum is taxonomically close to N. glauca, and the helper viruses, TMV-U1 and -U5, had similar yields in these hosts. TMV-U2 is usually found in widespread infections of to-

![Fig. 2. Absorbance profiles of virus purified from 0.4 g of Jalapeño leaf tissue from two experiments (EXP 1, EXP 2). Upper profiles show tobacco mosaic virus (TMV) strain TMV-U2 accumulation from singly infected plants. Lower profiles show TMV-U2 and satellite tobacco mosaic virus (STMV) accumulation from doubly infected plants. Sat = STMV.](image-url)
bacco fields (16,23), but it also has been detected once in pepper crops in Italy (24). The availability of natural variants of STMV (10,20,22) and the ability to use site-directed mutagenesis techniques to generate mutant, full-length RNA transcripts may allow the mapping of the area(s) of the STMV genome involved in the symptom-modulating effect. An initial attempt to analyze variants compared STMV RNA transcribed from clones pSTMV5 and pSTMV6, which represent the major sequence variations within the STMV type strain population, and showed differences at four nucleotide positions (11). These differences were not sufficient to distinguish these two variants by their reaction in pepper. The observation that transcript RNA from either pSTMV5 or pSTMV6 gave the same results as STMV viral inoculum suggests that a minor variant in the strain virus population is not responsible for the STMV-specific symptom modulations.

None of the pepper cultivars mentioned in this work have been reported to harbor STMV in commercial plots. However, a strong effort has not been made to detect STMV in pepper crops in California. The finding of a visible symptom-modulating effect by STMV in the form of mosaic with yellow patches should provide a visual aid for the selection of infected pepper plants in the field.

LITERATURE CITED