Characterization of a Defective Form of Tomato Spotted Wilt Virus

By F. N. VERKLEIJ AND D. PETERS*
Agricultural University, Department of Virology, Binnenhaven 11, 6709 PD Wageningen,
The Netherlands

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SUMMARY

Defective isolates of tomato spotted wilt virus (TSWV) which produce amorphous masses characteristic of TSWV in infected cells but do not produce enveloped virus particles have been studied. Nucleocapsid protein but no membrane proteins was detected by ELISA in plants infected with defective isolates. The protein of an infectious fraction purified from such plants consisted mainly of nucleocapsid protein. Electron microscope examination of this purified fraction revealed structures resembling the amorphous masses observed in thin sections of leaves infected with the defective isolates. This fraction from the three defective isolates studied contained three RNA segments, the mol. wt. of RNA 1 and RNA 3 being the same as those of normal TSWV particles. The mol. wt. of RNA 2 of the defective isolates was always smaller than that of the RNA 2 of normal TSWV particles. However, their mol. wt. were different for each isolate. Thus, the amorphous masses are aggregates of nucleocapsids of defective TSWV which do not produce normal particles since they have lost the ability to make at least one membrane protein species. This is possibly due to the deletion in RNA 2.

INTRODUCTION

Tomato spotted wilt virus (TSWV), a thrips-transmitted plant virus, has isometric membrane-bound particles of approximately 80 nm in diameter. Purified virus particles contain at least seven proteins; four of them occur in major quantities and three, with high mol. wt., in minor quantities. One of the major proteins (protein 4) is a glycoprotein with a mol. wt. of 78 000 and is located on the surface. The smallest major protein (protein 1) has a mol. wt. of 27 000 and is associated with the nucleocapsid (Mohamed et al., 1973; Tas et al., 1977a).

The single-stranded RNA genome, which occurs in three segments with mol. wt. of 2.7 x 10^6 (RNA 1), 1.7 x 10^6 (RNA 2) and 1.1 x 10^6 (RNA 3), is a positive strand (Verkleij et al., 1982).

In plant cells infected with TSWV, particles occur in clusters within dilated cisternae of the rough endoplasmic reticulum (Ie, 1964; Kitajima, 1965; Milne, 1970; Mohamed et al., 1973). In addition to the virus particles, dark, amorphous masses are observed in the cytoplasm. On repeated transfers by sap inoculation, these masses tend to increase in size and number whereas the spherical virus particles become less frequent. Eventually, isolates were obtained which produced only amorphous masses and no virus particles (Ie, 1982).

The absence of complete particles in infected cells suggests that the virus is defective. To elucidate the nature of the defect, the biochemical and serological properties of the defective isolates were analysed and the structure of the infectious agent was characterized.

METHODS

Isolates of TSWV used. Experiments were carried out mainly with two isolates of TSWV. TSWV-Y was kindly supplied by Dr Buzancić (Yugoslavia) in 1979. Electron microscopy of ultrathin sections of infected cells revealed that this isolate produced mainly the characteristic enveloped particles. The amorphous masses, typical of the defective form, were rarely present. Furthermore, we could readily purify complete TSWV particles from tobacco plants infected with this isolate (Verkleij et al., 1982). The other isolate, TSWV-P, which originated in Poland,
forms no complete particles, but only amorphous masses in infected cells. The origin and cytology of this isolate has been described by Le (1982). Some details of the other isolates are given in Table 1.

**Purification of the membrane proteins and nucleocapsid protein of TSWV.** TSWV was purified from TSWV-Y infected plants as described by Tas et al. (1977b). To dissociate the virus, Nonidet P40 (NP40) was added to a suspension of 0.25 ml containing approximately 3 mg TSWV (as estimated by its protein content) to a final concentration of 2%. The nucleocapsid fraction was separated from the membrane protein fraction by centrifugation of the dissociated virus suspension on a 10 to 50% linear sucrose gradient, using a Beckman SW41 rotor at 40000 rev/min for 5.5 h at 4 °C. The sucrose was dissolved in 0.01 M-sodium phosphate buffer pH 7.0, containing 0.01 M-Na2SO3, 0.5 M-NaCl and 0.5% NP40. Fractions were collected and their protein composition was analysed by SDS-polyacrylamide gel electrophoresis according to Laemmli (1970). The fractions containing either the nucleocapsid protein or the membrane proteins were pooled, dialysed against 0.01 M-sodium phosphate buffer pH 7.0, containing 0.01 M-Na2SO3 and 0.1% NP40, reduced in volume by placing the dialysis bags in solid sucrose and finally lyophilized.

**Preparation of antibodies.** The lyophilized proteins were dissolved in 0.5 ml demineralized water, mixed with an equal volume of Freund's incomplete adjuvant and injected subcutaneously into guinea-pigs. As estimated by gel electrophoresis, the amount of protein in each fraction was about 75 μg. A booster injection was given 2 weeks later and the animals were bled after a further 2 weeks. The immunoglobulin fraction was precipitated with ammonium sulphate. The immunoglobulins directed against the membrane protein fraction are designated as anti-G immunoglobulins and those against the nucleocapsid protein as anti-N immunoglobulins.

**ELISA.** The double antibody sandwich method of ELISA was carried out as described by Clark & Adams (1977). Sap was extracted by homogenizing leaf discs of 6 mm in diameter in 300 μl phosphate-buffered saline (140 mM-NaCl, 2.6 mM-KCl, 1.4 mM-KH2PO4 and 8 mM-Na2HPO4) containing 0.05% Tween-20 (PBS-Tween) in a small-sized Potter-Elvejhem tube. For each sample duplicate extracts were prepared, of which 200 μl was tested and, if necessary, diluted with PBS-Tween. The extracts were then incubated using p-nitrophosphate as substrate, and after incubation for 2 h at room temperature their absorbance was measured at 405 nm in a Titertek Multiskan.

**Purification of nucleocapsids and analysis of their RNA.** Leaves, systemically infected with the defective isolate were harvested 12 to 14 days after inoculation and midrids were removed. Batches of 25 g leaf material were homogenized in 100 ml 0.1 M-Tris-HCl pH 8.0 and 0.01 M-Na2SO3 and the homogenate was centrifuged for 10 min at 10000 g. The supernatant was centrifuged for 30 min at 110000 g and the pellet was dissolved in a small volume of 0.01 M-Tris-HCl, pH 8.0 and 0.01 M-Na2SO3 (resuspension buffer). The suspension was centrifuged for 30 min at 27000 rev/min on a 1 to 20% linear gradient of sucrose in resuspension buffer in a Beckman SW27 rotor. The absorbance of the gradient at 254 nm was recorded with a Uvicord spectrophotometer and the gradient was divided into 16 fractions. The fractions were assayed for infectivity on detached Petunia leaves (Tas et al., 1977a) and for the presence of nucleocapsid and membrane proteins by ELISA. The protein composition of each fraction was analysed by electrophoresis on 10% polyacrylamide gels according to the method of Laemmli (1970). The fractions containing nucleocapsids were also examined by electron microscopy. For this purpose, the nucleocapsid fractions were centrifuged for 2 h at 45000 rev/min in a Beckman SW50 rotor, after adding some egg-white. The procedures of fixation, embedding and ultra-sectioning of the pellets were as described by Le (1971).

To analyse the RNA, fractions containing nucleocapsids were centrifuged for 2 h at 40000 rev/min in a Beckman SW41 rotor and the pellets were resuspended in 1 ml resuspension buffer. The RNA was extracted from this suspension with phenol–SDS. The RNA was precipitated with ethanol, washed with 70% ethanol, dried and dissolved in a small volume of demineralized water. The RNA was analysed by electrophoresis on a 1% agarose gel containing 10 mM-methylmercury (Bailey & Davidson, 1976).

**Separation of the TSWV RNA segments and labelling with 32P.** A 24 μg amount of TSWV RNA, extracted with phenol–SDS from purified preparations of TSWV, was electrophoresed on a 1% agarose gel containing 10 mM-methylmercury as described by Bailey & Davidson (1976). The three bands containing RNA were excised under ultraviolet light illumination, wrapped in parafilm and stored at -20 °C. The RNA was recovered from the agarose slices by the freeze-squeeze method (Thuring et al., 1975). The extruded solution was dialysed against demineralized water and lyophilized.

The RNA segments were labelled *in vitro* at the 5' end with 32P, as described by Goldbach et al. (1978). The lyophilized RNA was dissolved in 50 μl buffer, after which the agarose residues were removed by centrifuging the solution through glass wool (Davies & Verduin, 1979). The reaction mixture consisting of 0.75 μl (7.5 units) T4 polynucleotide kinase (Boehringer) and 25 μl of the cleared solution was added to 40 μl[32P]ATP (Amersham International) and incubated for 30 min at 37 °C. The labelled RNA was recovered in 0.5 ml by passage of the reaction mixture through Sephadex G-50 with 0.15 M-NaCl and 0.015 M-sodium citrate (1 × SSC) as elution buffer. Each 10 μl contained approximately 10000 ct/min of which 75 to 90% could be precipitated with trichloroacetic acid (TCA). The RNA of these preparations, which was used for hybridization experiments, sedimented in a sucrose gradient at 45S. Apparently, the rather rough method of extraction of the RNA from the agarose degraded the RNA sufficiently to produce an RNA probe of small fragments.
Isolation of double-stranded RNA. Leaves of *Nicotiana rustiea*, systemically infected with TSWV-Y or TSWV-P were harvested 12 to 14 days after inoculation. Leaves of healthy plants of the same age were also harvested. The leaves were stored, after removal of the midribs, at -80 °C. Each batch of 100 g frozen leaf material was triturated using a pestle and mortar, mixed with 100 ml buffer containing 0.1 M- glycine, 0.1 M-NaCl, 0.01 M-EDTA and 0.01 M-Na2HPO4, pH 9.5, and put in a Waring blendor. After the addition of 200 ml of water-saturated phenol containing 10% (w/v) m-cresol and 0·1% (w/v) 8-hydroxyquinoline, 100 ml of a chloroform–isoamyl alcohol mixture (24:1), 10 ml 20% (w/v) SDS, 5 ml 20% (w/v) p-aminosalicylic acid, 3 ml diethlypyrocabonate and 3 ml 2-butanol, the homogenate was immediately blended at low speed for 1 min.

After a low speed centrifugation, the aqueous layer was collected and extracted with 1 vol. of 2·5 M-KH2PO4, pH 8·1, and 1 vol. of 2-methoxyethanol, to remove polysaccharides (Ralph & Bellamy, 1964). The nucleic acid in the aqueous layer was precipitated with 0·33% (w/v) cetyltrimethylammonium bromide. The precipitate was collected by centrifugation at 20000 g for 20 min and washed three times with 70% ethanol containing 0·1 M-sodium acetate. The precipitate was dried, dissolved in 5 ml 1 × SSC and dialysed against 1 × SSC.

To degrade DNA, magnesium acetate and DNase I (Sigma; DN-EP) were added to final concentrations of 5 mM and 10 μg per mg nucleic acid, respectively. This solution was incubated for 30 min at 25 °C. To degrade single-stranded RNA, the solution was incubated with a mixture of RNase A (10 μg/mg RNA) and RNase T1 (1 μg/mg) for 30 min at 37 °C. To remove the DNase and the RNase, the solution was treated with Pronase E. After extraction with a phenol–cresol–hydroxyquinoline mixture, 200 μg tRNA from *Escherichia coli* (Boehringer; RNase-free) was added as carrier to the aqueous phase and the nucleic acid (dsRNA) was precipitated with ethanol. The precipitated RNA was washed three times with 70% ethanol and finally dissolved in 300 μl 2 × SSC.

This preparation of dsRNA was used in the hybridization experiments. When analysed on 1% agarose gels containing 10 mM-methylmercury, only one band comigrating with tRNA was visible.

**Hybridization.** 32P-labelled TSWV RNA, dsRNA and 2 × SSC were mixed in a total volume of 110 μl in a 1·5 ml Eppendorf tube. Each tube contained 5000 to 7000 TCA-precipitable ct/min. The tubes were closed and heated in a pressure cooker for 5 min at 120 °C. Samples were annealed overnight at 68 °C. After incubation, each mixture was divided in two equal parts and 25 μl *E. coli* tRNA (10 mg/ml in 2 × SSC) was added. One sample was incubated with RNase A and RNase T1 (40 and 4 μg/ml, respectively, in 25 μl 2 × SSC) and the other with 25 μl 2 × SSC for 30 min at 37 °C. The undegraded RNA was precipitated after addition of 5 μl yeast RNA (10 mg/ml) by 300 μl 10% cold TCA. The collection of the RNase-resistant RNA on glass microfibre discs and the measurement of the radioactivity have been described by Toriyama & Peters (1980). Each hybridization was carried out in duplicate. All values were corrected for non-specific absorption on the filter and for self-annealing of the 32P-labelled TSWV RNAs; the values found averaged 50 and 30 ct/min, respectively.

**RESULTS**

**Purification of TSWV proteins and specificity of antisera**

Nucleocapsid protein was separated from membrane proteins by centrifuging dissociated virus particles on sucrose gradients. Fig. 1(a) illustrates the distribution of the proteins over the gradient. Fractions 1 to 4 (membrane protein fraction) and 8 to 14 (nucleocapsid protein fraction) were pooled and analysed by electrophoresis on polyacrylamide gels. No nucleocapsid proteins were found in the membrane protein fraction and no membrane proteins were found in the nucleocapsid protein fraction.

The immunoglobulins, prepared from the antisera against the membrane protein fraction (anti-G immunoglobulins) and against the nucleocapsid protein (anti-N immunoglobulins) did not cross-react in heterologous combinations. As can be seen in Fig. 1(b), the anti-G immunoglobulins reacted in ELISA only with the fractions containing membrane proteins, and the anti-N immunoglobulins only with those containing nucleocapsid protein.

**The detection of TSWV antigens in leaves infected with defective isolates**

Nucleocapsid protein was detected in sap from leaves infected with TSWV-Y (normal form) and TSWV-P (defective form) by ELISA. Sap from both sources gave ELISA readings of 1·0 and 0·95 at twofold dilutions and 0·25 and 0·35 at 32-fold dilutions, respectively (Fig. 2a). Clearly both isolates produce nucleocapsid protein in similar amounts during infection.

The anti-G immunoglobulins reacted positively in ELISA only with sap from leaves infected with TSWV-Y at a dilution of sap up to eightfold. The reaction with sap from leaves infected with the defective isolate, TSWV-P, was comparable with the background readings obtained
Fig. 1. (a) Analysis by SDS-polyacrylamide gel electrophoresis of the distribution of the viral proteins after centrifugation of NP40-dissociated TSWV in linear sucrose gradients. Lane V shows an electropherogram of TSWV proteins. Lanes 1 to 16 show the protein composition of the fractions 1 (top) to 16 (bottom of gradient). (b) Analysis of these fractions by ELISA. The black area shows the reaction with anti-G immunoglobulins which were used in a coating and conjugate concentration of 10 and 4 μg/ml; the fractions were undiluted. The white area shows the reaction with anti-N immunoglobulins which were used in a coating and conjugate concentration of 2 μg/ml; fractions were diluted 10-fold.

Fig. 2. ELISA tests with sap from leaves infected with TSWV-Y (○) and with TSWV-P (●) and from healthy leaves (□). (a) Anti-N immunoglobulins in a coating and conjugate concentration of 5 and 2 μg/ml. (b) Anti-G immunoglobulins in a coating and conjugate concentration of 10 and 4 μg/ml.

Fig. 3. ELISA tests with sap from leaves infected with TSWV-Y (□) and TSWV-P (●) at various periods after inoculation. Tests with sap from healthy leaves (□) of the same age as the infected leaves served as controls. (a) Anti-G immunoglobulins in a coating and conjugate concentration of 10 μg/ml; sap undiluted. (b) Anti-N immunoglobulins in a coating and conjugate concentration of 2 μg/ml; sap diluted 20-fold.
Table 1. \(A_{405}\) values from ELISA with sap from leaves infected with normal and defective isolates of TSWV*

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Form</th>
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<th>Anti-G</th>
</tr>
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<td>0.04</td>
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<td>0.04</td>
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<td>TSWV-B 21</td>
<td>Normal</td>
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<tr>
<td>TSWV-Thrips</td>
<td>Normal</td>
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<tr>
<td>TSWV-Amerine</td>
<td>Normal</td>
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<td>0.54</td>
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<tr>
<td>TSWV-T. majus</td>
<td>Normal</td>
<td>0.91</td>
<td>0.05</td>
</tr>
<tr>
<td>TSWV-Y</td>
<td>Normal</td>
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<td>0.66</td>
</tr>
<tr>
<td>N. rustica</td>
<td>(healthy)</td>
<td>0.01</td>
<td>0.04</td>
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</table>

* The isolates were propagated in \(N. rustica\). The isolate 'SNN' and 'T. majus' were propagated in \(N. tabacum\) cv. Samsun NN and in \(Tropaeolum majus\), respectively. Anti-G immunoglobulins were used in a coating and conjugate concentration of 10 and 4 \(\mu\)g/ml. Anti-N immunoglobulins were used in a coating and conjugate concentration of 2 \(\mu\)g/ml. Leaf sap was in all tests diluted twofold.

with sap from healthy leaves (Fig. 2b). TSWV-P-infected leaves therefore produced no detectable viral membrane proteins.

To examine the possibility that the defective form synthesized viral membrane proteins early in infection and that they subsequently disappeared, plants infected with TSWV-P were tested for viral membrane proteins 9 to 16 days after inoculation. Systemic symptoms first appeared about 9 days after inoculation and necrosis began 7 days later. Membrane proteins were undetectable in these plants (Fig. 3a), but nucleocapsid protein was found (Fig. 3b).

Several isolates of TSWV producing normal enveloped particles or exclusively amorphous masses were examined for the presence of viral membrane proteins and nucleocapsid protein. Leaves infected with normal isolates gave positive reactions with the anti-G immunoglobulins. In contrast, leaves infected with each of the three defective isolates did not react with these immunoglobulins (Table 1). Sap from all plants infected with the normal or defective isolates reacted with the anti-N immunoglobulins. These results support the hypothesis that no membrane proteins of TSWV are synthesized in plants infected with defective forms.

Purification of nucleocapsids from leaves infected with defective isolates and analysis of their RNA

The ELISA experiments indicate that defective isolates do not produce membrane proteins. Therefore, it may be assumed that the naked nucleocapsids of the defective isolates are infectious. It has been suggested that the electron-dense amorphous masses represent aggregates of nucleocapsids of a defective form of TSWV (Ie, 1982). To test this hypothesis, the aggregates were partially purified from leaves infected with defective isolates as described in the Methods section. The 110000 \(g\) pellet, which was infectious and contained structures similar to the electron-dense amorphous masses reported in infected cells, was centrifuged through a sucrose gradient. Fig. 4(b) shows that most of the ultraviolet-absorbing material, which was infectious, was found near the top of the gradient. When assayed by ELISA, the fractions of the gradient showed no significant reaction with the anti-G immunoglobulins. Although nucleocapsid protein was found throughout the gradient, most was found near the top. However, the ultraviolet-light absorbing profile did not completely coincide with the distribution of the infectivity over the gradient.

The fractions containing infectivity and showing ultraviolet light absorption contained appreciable nucleocapsid protein (Fig. 4a). Protein 4, the glycosylated membrane protein, was not detected. Polypeptides migrating in positions similar to those of proteins 2 and 3 were found in the top fractions but the identity of these polypeptides was not established. The position of the fractions with these polypeptides in the gradient differed slightly from those containing the nucleocapsid protein.
After pelleting, embedding and thin sectioning of this infectious fraction, structures resembling the intracellular amorphous masses were revealed by electron microscopy (Fig. 5).

The RNA extracted from the infectious fractions was analysed by electrophoresis on methylmercury–agarose gels. The RNA from nucleocapsids of TSWV-P appeared to consist of three RNA segments similar to those contained in normal TSWV particles. Two RNA bands had migration rates similar to RNA 1 and RNA 3 of the normal form of TSWV. The third band migrated faster than RNA 2 (Fig. 6, lanes 1 and 2). This new RNA segment had a mol. wt. of \(1.4 \times 10^6\), when the RNA segments of TSWV-Y \((2.7 \times 10^6, 1.7 \times 10^6 \text{ and } 1.1 \times 10^6)\); Verkleij et al., 1982) and of cowpea mosaic virus \((2.02 \times 10^6 \text{ and } 1.37 \times 10^6); \) Reijnders et al., 1974) were used as markers.

Like TSWV-P, two other defective isolates contained three RNA segments, two of which had the same migration rates as RNA 1 and RNA 3 of normal particles, while the migration rate of RNA 2 differed (Fig. 6, lanes 3 and 4). The RNA of isolate TSWV-SNN (lane 3), mol. wt. \(1.2 \times 10^6\).
Fig. 5. Electron micrograph of the material occurring in the pooled fractions 2 to 7 of a gradient as shown in Fig. 4.

Fig. 6. Gel electrophoresis of RNA species extracted from TSWV-Y particles (lane 1) and from nucleocapsids of defective isolate TSWV-P (lane 2), TSWV-SNN (lane 3) and TSWV-12 (lane 4).

Fig. 7. Hybridization of $^{32}P$-labelled TSWV RNA segments with dsRNA isolated from plants infected with TSWV-Y (●), TSWV-P (○), and with dsRNA from healthy plants (□). (a) RNA 1; (b) RNA 2; (c) RNA 3.
10^6, was not only smaller than RNA 2 of the normal form (lane 1), but also smaller than RNA 2 of isolate TSWV-P (lane 2). Moreover, this segment occurred in relatively large amounts whereas the segment migrating identically to RNA 3 of normal particles occurred only in small amounts. The RNA composition of the isolate TSWV-12 (lane 4) resembled that of the isolate TSWV-P, although RNA 2 had a somewhat lower mol. wt. (1.3 \times 10^6).

A smear of lower mol. wt. RNA was visible in all the RNA preparations of the purified nucleocapsids. This material is probably degraded viral RNA and contaminating ribosomal RNA.

**Comparison of the RNA 2 molecules of the normal and defective forms**

To determine whether the genome of the defective forms had lost nucleotide sequences present in the genome of the normal form, the three individual RNA segments of normal TSWV particles were degraded to fragments of 4S and thereafter labelled with 32P. These fragments were hybridized with increasing amounts of dsRNA isolated from plants infected with either the normal or the defective form.

As depicted in Fig. 7, the three labelled TSWV RNA segments hybridized with dsRNA from plants infected with the normal isolate TSWV-Y to a value of approximately 35%); they did not hybridize with dsRNA from healthy plants. 32P-labelled TSWV RNA 1 and 3 hybridized with dsRNA from plants infected with the defective isolate TSWV-P to levels which were comparable to those obtained in the hybridization with dsRNA from plants infected with TSWV-Y (Fig. 7 a and c). 32P-labelled TSWV RNA 2 hybridized to a lower value with dsRNA from plants infected with TSWV-P (Fig. 7 b). These results indicate that the genome of TSWV-P is composed of the same RNA segments 1, 2 and 3 as the genome of TSWV-Y, but that a part of RNA segment 2 has been lost.

**DISCUSSION**

The protein composition of three defective forms of TSWV was analysed serologically. Since proteins 3 and 4 predominate in the TSWV membrane fraction to which antibodies were raised, serum could contain antibodies to both proteins. Indeed, the serum contains antibodies to protein 4, the protein which is exposed to the outside (Tas et al., 1977a), as purified non-dissociated TSWV particles reacted positively in ELISA. Furthermore, protein 4 could not be detected in extracts from plants infected with defective isolates. These data indicate that the serum contains antibodies to protein 4 and that the defective form has lost the ability to produce at least protein 4 of TSWV. Our methods could not show whether or not the serum contained antibodies to protein 3.

We have also purified a fraction containing nucleocapsid protein and RNA from plants infected with the defective form. This fraction contained amorphous masses similar to those in the cytoplasm of the infected cells (Ie, 1982). Cells infected with the nucleocapsids of other membrane-bound viruses, such as measles virus (Robbins et al., 1980) and other paramyxoviruses, contain similar structures (Choppin & Compans, 1975). Our results indicate that the amorphous masses are aggregates of the TSWV nucleocapsid, as originally suggested by Ie (1971).

The RNA composition of the defective form has been studied by nucleic acid hybridization and by analysis of the RNA from purified amorphous masses. When hybridized with saturating amounts of dsRNA isolated from plants infected with TSWV-Y, one would expect that the three 32P-labelled TSWV-RNA segments, extracted from purified TSWV-Y particles, would reach a hybridization plateau of 100%. However, a maximum of 35% was obtained (Fig. 4c). The most likely explanation for this value is that the dsRNA preparations were contaminated with degraded viral ssRNA. In a comparable study, Bol et al. (1975) found a hybridization plateau of 45% in hybridization studies with 3H-labelled alfalfa mosaic virus RNA and virus-specific dsRNA. These authors have separated to a great extent the degraded viral ssRNA from the dsRNA by centrifugation of the dsRNA preparation over a sucrose gradient, a step we have omitted. Nonetheless, they ascribe the low hybridization value to residual viral ssRNA.

The hybridization experiments indicate that the defective form has conserved the nucleotide
sequences present in the RNA segments 1 and 3 of the normal form, but has lost a part of those present in RNA segment 2. This interpretation is reinforced by the analysis of the RNA from the nucleocapsids by gel electrophoresis. The nucleocapsids of the defective isolates studied possess RNA segments of the same size as RNA 1 and RNA 3 of normal particles, but have lost the RNA segment with the size of RNA 2. Instead a new segment of mol. wt. intermediate between RNA 2 and 3 of the normal form was observed.

The failure to detect protein 4 in sap from plants infected with the defective forms may indicate that this form of TSWV is no longer able to produce this membrane protein. The loss of this ability and the deletion in RNA 2 suggests that RNA 2 contains the genetic information for this protein. In a previous report, we have shown that RNA 3 contains information for the nucleocapsid protein (Verkleij et al., 1982). This is consistent with the occurrence of RNA 3 in the nucleocapsids of the three defective isolates.

Comparing defective TSWV with other defective viruses, it is clear that the defective form of TSWV is not a defective interfering (DI) particle. DI particles are not infectious by themselves, contain all the structural proteins and have deleted a part of the standard virus nucleic acid (Huang & Baltimore, 1977). However, the defective form of TSWV shares an important feature with DI particles, namely interference with the growth of standard virus. It is possible that the host plays an important role in this process, since after a few passages of a normal isolate of TSWV through cucumber plants, the amorphous masses predominate in infected cells and normal enveloped particles are difficult to detect (T. S. Ie, personal communication).

Certain isolates of wound tumour virus (WTV) and pea enation mosaic virus (PEMV) lose the ability to be transmitted by their vectors. These non-vector-transmissible counterparts differ in protein and nucleic acid composition. The non-vector-transmissible isolates of WTV have lost some of the twelve dsRNA segments and a protein which makes part of the outer protein shell of the virus (Reddy & Black, 1977). The non-vector-transmissible isolates of PEMV have lost a minor protein (Hull, 1976; Clarke & Bath, 1976) and a portion of the larger of the two RNA segments (Adam et al., 1979).

Ie (1982) suggested that it is unlikely that the defective form of TSWV will be transmitted by thrips, the natural vector of TSWV. One would expect that if the membrane protein were not essential for transmission, then it would have disappeared during evolution. However, final conclusions about the role of the membrane protein in transmission must await actual tests with thrips.

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F. N. VERKLEIJ AND D. PETERS


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