TRISTEZA AND RELATED DISEASES

Comparison of Particle Characteristics and Cytopathology of Citrus Tristeza Virus With Other Morphologically Similar Viruses*

M. Bar-Joseph, G. Loebenstein, and J. Cohen

Threadlike particles (TLP) about 2000 x 10-12 nm in size were found by Katajima et al. (1974) to be associated with tristeza virus (CTV) infection. Although completion of Koch's postulates with TLP has not yet been achieved, several lines of evidence (Bar-Joseph, 1974; Kitajima et al., 1964), including confirmation of association of TLP with CTV infection in citrus (Bar-Joseph et al., 1970; Chen et al., 1971; Price, 1966, 1970; Primo et al., 1971; Schneider and Sasaki, 1972) and in the non-citrus host Passiflora gracilis (Kitajima et al., 1974) strongly implicate TLP as the particles of CTV. The morphological resemblance between TLP and the particles of beet yellows virus (BYV) (Russell, 1970) confirmed earlier propositions (Klotz, 1950), based on similarities in their mode of transmission by aphids, that these viruses be included in the same group. Most elongated plant viruses have been grouped according to particle length, but a special group has been suggested for viruses with threadlike morphology (Brandes and Bercks, 1965; Gibbs, 1969; Harrison et al., 1971). The name Klostervirus was recently coined for this group (Bar-Joseph and Hull, 1974). In addition to CTV and BYV, this group consists of beet yellow stunt (Duffus, 1972, 1973), apple stem-grooving virus (Lister, 1970b), apple chlorotic leafspot (CLSV) (Lister, 1970a), carnation necrotic fleck (CNFV) (Inouye and Mitsuhata, 1973), carnation yellow fleck (CYFV) (Smookler and Loebenstein, 1974), and wheat yellow leaf (WYLV) (Inouye et al., 1973). The modal lengths of these viruses range between 700 and 2000 nm (table 1). Gibbs (1969), noting the serial nature of these modal lengths said, “It is interesting that these lengths are approximately in the ratio of 1:2:3:4.” With the exception of BYV (Russell, 1970) and CLSV (Inouye and Mitsuhata, 1973) most threadlike viruses have a very restricted natural host range and infection associated with species within the same family. Viruses of this group, except the apple viruses, are transmitted by aphids in a semipersistent manner.

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TABLE 1
NORMAL LENGTH, DIAMETER, AND PITCH OF HELIX OF CITRUS TRISTEZA AND SIX OTHER VIRUSES

<table>
<thead>
<tr>
<th>Virus</th>
<th>Length (nm)</th>
<th>Diameter (nm)</th>
<th>Pitch of helix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple stem grooving*</td>
<td>600</td>
<td>12</td>
<td>3.7</td>
</tr>
<tr>
<td>Apple chlorotic leafspot*</td>
<td>720</td>
<td>12</td>
<td>3.8</td>
</tr>
<tr>
<td>Beet yellows</td>
<td>1250</td>
<td>12</td>
<td>3.7</td>
</tr>
<tr>
<td>Carnation yellow fleck†</td>
<td>1250</td>
<td>13</td>
<td>3.7</td>
</tr>
<tr>
<td>Carnation necrotic fleck</td>
<td>1400-1500</td>
<td>12-13</td>
<td>3.4</td>
</tr>
<tr>
<td>Wheat yellow leaf</td>
<td>1600-1850</td>
<td>10</td>
<td>3.4</td>
</tr>
<tr>
<td>Citrus tristeza (TLP)</td>
<td>2000</td>
<td>10-12</td>
<td>3.7</td>
</tr>
</tbody>
</table>

*Lister, R. M. (personal communication).
†Bar-Joseph, unpublished.

PURIFICATION

Past difficulties in obtaining preparations of CTV and CLSV were overcome by gentle grinding, since particles are sensitive to shearing during mechanical homogenization (Bar-Joseph and Hull, 1974; Bar-Joseph et al., 1970; Lister and Hadidi, 1971; Till and Shepherd, 1967), extraction with cold Tris buffer (not necessary for CYFV), and prevention of absorption of the particles to normal plant constituents by macerating the material in large volumes of buffer (up to 10 ml/gm fresh weight). These factors were also found critical for the purification of BYV and CYFV (Bar-Joseph and Hull, 1974; Bar-Joseph and Smookler, 1976). Bentonite clarification has been found effective for CLSV (Lister and Hadidi, 1971), BYV (Bar-Joseph and Hull, 1974) and CYFV (Bar-Joseph and Smookler, 1976). Bentonite clarification for CTV resulted in low yields, probably due to absorption to bentonite. Aggregation occurs when pelleting is done by ultracentrifugation, but this can be avoided by polyethylene glycol (PEG) precipitation. Resuspension of PEG precipitates is achieved by prolonged gentle stirring in large volumes of buffer. Centrifugation in sucrose gradients is valuable in the purification process of CLSV, BYV and CYFV. CTV appears to diffuse over a wide range of the gradients, probably as a result of the heterodispersity due to particle fragmentation, aggregation and absorbance to host membranes. A single sharp band containing concentrated CTV was obtained by density gradient centrifugation in cesium chloride (CsCl), the flocculate and the protein contaminants apparently banding separately (Bar-Joseph et al., 1972). CTV, like CLSV and BYV, was not stable in CsCl, unless it had previously been fixed by treatment with 2 per cent formaldehyde. CYFV is stable in CsCl. Following the work of Lot and Kaper (1972) on stability of cucumber mosaic virus in cesium sulphate (Cs₂SO₄), isopycnic gradient centrifugations were carried out with BYV (Bar-Joseph and Hull, 1974), CLSV (Bar-Joseph et al., 1974), and CTV (Flores et al., 1975) in this salt. Care has to be taken so as not to cause aggregation by overloading the gradients. Loading partially purified preparations of CTV directly on Cs₂SO₄ gradients failed to separate the virus from host contaminants (Bar-Joseph, unpublished). No efficient method of CTV purification without formaldehyde fixing is known. Virus yields (table 2) vary greatly among the viruses of this group so far purified. The extremely low CTV yields may result from CTV limitation to phloem tissue (Price, 1968).
PARTICLE CHARACTERIZATION

Some characteristics of these viruses are summarized in tables 1, 2, 3, 4. The single band formed by all these viruses in CsCl or Cs₂SO₄ gradients appeared to be very near to, or even co-banding with, tobacco mosaic virus (TMV). An RNA content of about 5 per cent may therefore be estimated for these viruses (Sehgal et al., 1970). The spectrophotometric analysis of purified preparations of CLSV, BYV and CYFV revealed an anomalous A₂₆₀/A₂₈₀ ratio of 1.72, 1.73 and 1.53, respectively. This is higher than expected for viruses with about 5 per cent RNA (Paul, 1959). Preliminary analysis indicated that BYV protein has no tryptophan, about 5 tyrosine residues and about 5½ cystine residues, and the estimated specific absorbance of BYV protein at 280 nm is about 0.356 OD per mg (Rees, Short, Bar-Joseph, and Hull, unpublished). The size of the coat protein of CTV (Bar-Joseph et al. 1972), CLSV (Chairez and Lister, 1973), BYV (Bar-Joseph and Hull, 1974) and CYFV (Bar-Joseph and Smookler, 1976) as determined by sodium dodecyl sulphate (SDS) acrylamide gel electrophoresis, is summarized in table 3. The differences in weights are small and perhaps reflect different experimental conditions.

The molecular weights of RNA prepared from BYV (Bar-Joseph and Hull, 1974), CLSV (Bar-Joseph et al., 1974) and CYFV (Salomon, Bar-Joseph, and Herzberg, unpublished) were estimated by a variety of methods (table 4). When

### TABLE 2

<table>
<thead>
<tr>
<th>Virus</th>
<th>Yield mg/100 gm tissue</th>
</tr>
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<tbody>
<tr>
<td>CTV (TLP)</td>
<td>40-320</td>
</tr>
<tr>
<td>CLSV</td>
<td>1000</td>
</tr>
<tr>
<td>BYV</td>
<td>4000-20000</td>
</tr>
<tr>
<td>CYFV</td>
<td>5200-21200</td>
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### TABLE 3

<table>
<thead>
<tr>
<th>Virus</th>
<th>Coat protein</th>
<th>Molecular wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLSV</td>
<td>23500</td>
<td></td>
</tr>
<tr>
<td>BYV</td>
<td>23000</td>
<td></td>
</tr>
<tr>
<td>CYFV</td>
<td>23500±500</td>
<td></td>
</tr>
<tr>
<td>CTV (TLP)</td>
<td>25000±1000</td>
<td></td>
</tr>
</tbody>
</table>

BYV and CLSV RNA were centrifuged through a 5-25 per cent sucrose gradient, a major UV absorbing band was detected, together with some slower sedimenting bands. Maximum infectivity coincided with the major band. Thus, these RNAs appear to correspond to the normal size in the intact virus. The ratio between RNA molecular weight (MW) and normal particle length in this group (table 5) is different from the ratios found for some other viruses. Using the ratios found in other viruses of this group for a normal length CTV, it can be estimated that the size of its RNA will be 6.3-6.9 x 10⁶ daltons.
**TABLE 5**

<table>
<thead>
<tr>
<th>Virus</th>
<th>RNA (M.W.) x10^6</th>
<th>Length (nm)</th>
<th>RNA/Length ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLSV</td>
<td>2.3</td>
<td>720</td>
<td>3194</td>
</tr>
<tr>
<td>BYV</td>
<td>4.0-4.3</td>
<td>1250</td>
<td>3200-3440</td>
</tr>
<tr>
<td>CYFV</td>
<td>3.95</td>
<td>1250</td>
<td>3166</td>
</tr>
<tr>
<td>CTV (TLP)</td>
<td>6.3-6.9*</td>
<td>2000</td>
<td></td>
</tr>
<tr>
<td>TMV</td>
<td>2.05</td>
<td>300</td>
<td>6833</td>
</tr>
<tr>
<td>Potato Virus X</td>
<td>2.1</td>
<td>520</td>
<td>4038</td>
</tr>
<tr>
<td>Turnip mosaic virus</td>
<td>3.5</td>
<td>730</td>
<td>4794</td>
</tr>
</tbody>
</table>

*Calculated for 3166 - 3440 RNA length ratios.

**CYTOPATHOLOGY**

The cytopathology of BYV infection in systemically infected hosts has been thoroughly characterized in a series of papers by Esau and associates (Cronshaw et al., 1966; Esau et al., 1966; Esau and Hoefert, 1971a, 1971b, 1971c). Ultrastructural changes, including banded inclusion bodies consisting of large masses of flexuous particles and characteristic vesicular structures containing fine fibrils, were observed. A similar intracellular organization was found in Chenopodium hybridum locally infected with BYV (Plaskitt and Bar-Joseph, 1973). Likewise, in Dianthus barbatus infected with CNFV, and in Dianthus caryophyllus infected with CYFV (Smookler and Cohen, unpublished; Josephs et al., unpublished), similar cytopathic symptoms have been observed. In Chenopodium quinoa infected with CLSV, cross-banded inclusions have been found, but limited examination has not yet revealed the vesicular structures (Plaskitt and Bar-Joseph, unpublished). Ultrastructural studies in leaves of various Citrus spp. (Chen et al., 1971; Hernandez-Yago and Forteza-Bover, 1973; Kitajima and Costa, 1968; Price, 1966, 1968; Schneider and Sasaki, 1972; Shikita and Sasaki, 1969; Tanaka et al., 1969) and Passiflora gratilis (Kitajima et al., 1974) infected with CTV revealed a large number of flexuous rods, apparently identical with TLP, accompanied by vesicular structures (Kitajima and Costa, 1968; Kitajima et al., 1974; Schneider and Sasaki, 1972) resembling those found in BYV (Esau and Hoefert, 1971a), CNFV (Inouye and Mitsuhata, 1973), and CYFV (Smookler and Cohen, unpublished). In addition, parallel tubes, presumably tubular P-protein—a normal host component, were found, sometimes in organized formations (fig. 2). The frequency with which such abnormalities occur in the phloem tissues was directly correlated with the severity of the virus strain and the susceptibility of the host plant (Kitajima and Costa, 1968). Cytopathological changes in sour orange leaves chronically infected with the ST, VT and CT strains of tristeza (Bar-Joseph and Loebenstein, 1973) were compared. No new types of particles or structures could be found in the seedling yellows types of tristeza (CT and VT strains) when compared with the ordinary ST strain. There was a quantitative difference. Phloem cells infected with CT contained large masses of TLP (fig. 1), while in cells infected with ST (fig. 2) only scattered regions of these particles were seen. In phloem cells infected with CT, the TLP content was lower than in carnation phloem and parenchyma cells infected with CYFV (fig. 3).

Sectioning purified concentrated CYFV pellets revealed aggregates of paracrystalline bundles resembling those found in vivo. The aggregates found in vivo are probably the consequence of the accumulation of virus particles in a limited space. It was also observed that the dense amorphic regions, presumed to
Fig. 1. Portion of a phloem cell in the main vein of a sour orange leaf infected with the seedling yellows strain (CT) of citrus tristeza virus (CTV). Tissue was fixed as described by Cohen and Loebenstein (1975) and embedded in Epon according to Luft (1961). Note the CTV particles (V) appearing in large masses.

Fig. 2. Portion of a phloem cell infected with the ordinary strain (ST) of CTV. Note the scattered regions of CTV particles (V) and organized formations (P) of “P protein.”

Fig. 3. A phloem cell of a carnation leaf infected with carnation yellow fleck virus (CYFV). Note the CYFV particles in the cytoplasm.
result from disorganization of virus in sieve elements (Esau and Hoefert, 1971c) contain normal particles. Tilting of specimens of both in vivo and in vitro packed particles (Josephs et al., unpublished), caused a change in the appearance of areas containing normal virus particles into areas of particles appearing deteriorated.

Available information justifies placing these viruses in one group. A better understanding of the relationships among members of this group will require additional biochemical information.

**LITERATURE CITED**

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