TRISTEZA AND RELATED DISEASES

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

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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, 1970*b*), apple chlorotic leafspot (CLSV) (Lister, 1970*a*), 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.

*Contribution from the Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel. 1975 Series No. 290 E.

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

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

*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 (Cs_2SO_4), 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 Cs_2SO_4 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 Cs_2SO_4 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 A 260/280 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
VIRUS YIELDS OF CITRUS TRISTEZA AND THREE OTHER VIRUSES

Virus	Yield mg/100 gm tissue
CTV (TLP)	40-320
CLSV	1000
BYV	4000-20000
CYFV	5200-21200

TABLE 3
MOLECULAR WEIGHTS OF COAT PROTEINS OF CITRUS TRISTEZA AND THREE OTHER VIRUSES AS ESTIMATED BY GEL ELECTROPHORESIS

Virus Coat protein	Molecular wt.
CLSV	23500
BYV	23000
CYFV	23500±500
CTV (TLP)	25000±1000

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 10^6 daltons.

TABLE 4
MOLECULAR WEIGHTS (×10^6) OF THE RNA OF THREE VIRUSES AS ESTIMATED BY ANALYTICAL ULTRACENTRIFUGATION AND GEL ELECTROPHORESIS

	Analytical ultracentrifugation		Gel electrophoresis	
	Unmodified	Formaldehyde modified	Unmodified	Formaldehyde modified
CLSV	2.3 (infective)	2.35	2.4	2.2
BYV	4.3 (infective)	4.2	3.2-4.0	—
CYFV*	ND	ND	3.2; 3.95	ND

*Salomon, Bar-Joseph, and Herzberg, unpublished.

TABLE 5
RATIO OF RNA SIZE TO NORMAL LENGTH OF SOME ELONGATED PLANT VIRUSES

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

*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, 1971*a*, 1971*b*, 1971*c*). 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 (Plaskit 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 gracilis* (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, 1971*a*), 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

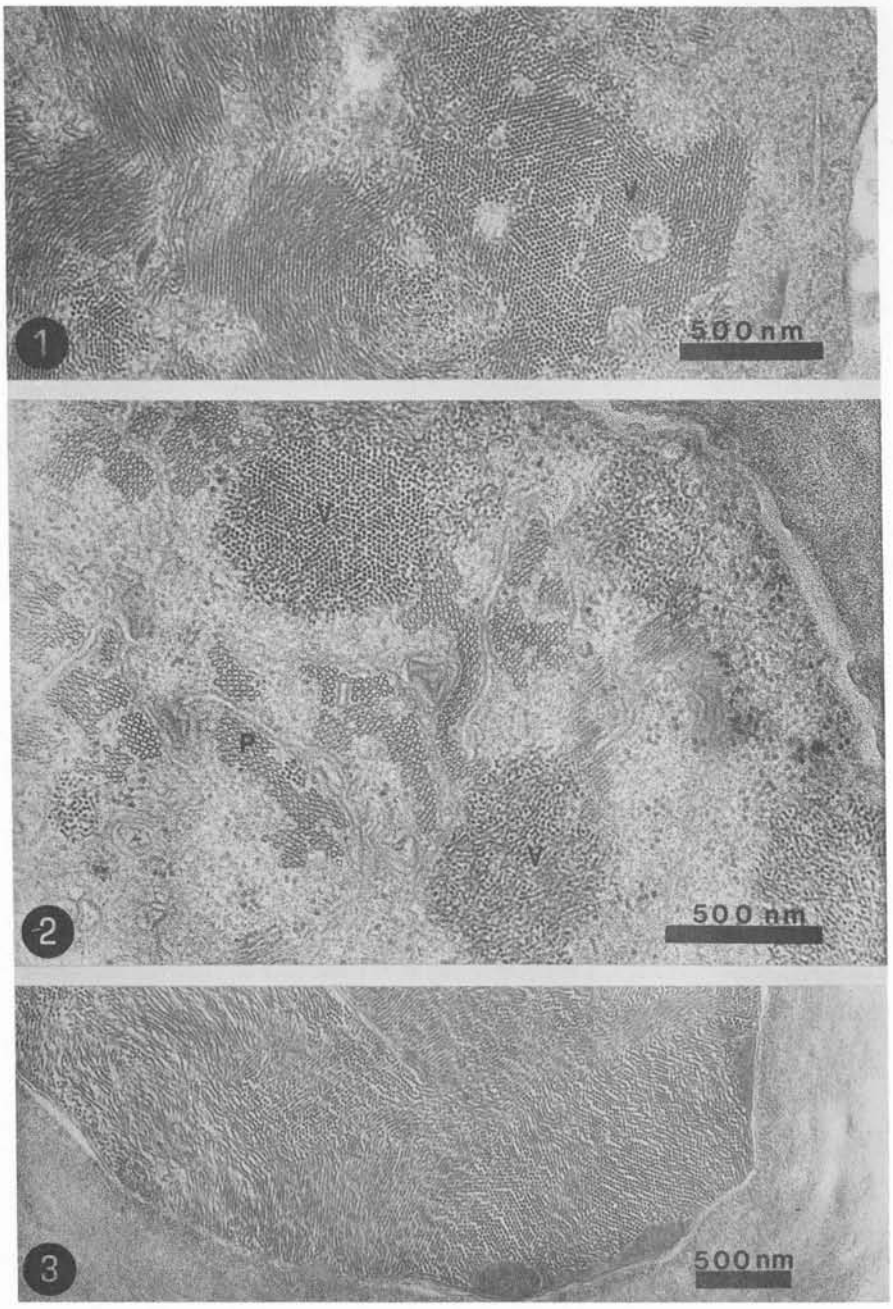

Fig. 1. Portion of a phloem cell in the main vein of a sour orange leaf infected with the seedling yellows strain (CT) of citrus tristeza virus (CTV). Tissue was fixed as described by Cohen and Loebenstein (1975) and embedded in Epon according to Luft (1961). Note the CTV particles (V) appearing in large 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, 1971*c*) 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.

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