Rapid Diagnosis of the Citrus Tristeza Disease by Electron Microscopy of Partially Purified Preparations

M. Bar-Joseph and G. Loebenstein

Virus Laboratory, The Volcani Institute of Agricultural Research, Bet Dagan, Israel.

Contribution of The Volcani Institute of Agricultural Research, Bet Dagan, Israel, 1970 Series No. 1684-E. Supported in part by the Ministry of Agriculture and the Citrus Marketing Board of Israel.

Portion of a Ph.D. thesis to be presented by the senior author to the Hebrew University of Jerusalem.

The technical assistance of Ruth Naidik is gratefully acknowledged.

Accepted for publication 6 May 1970.

ABSTRACT

A modified procedure is described for the partial purification of the threadlike particles associated with the citrus tristeza disease. This method enables rapid identification of infected trees by electron microscopy. Diagnosis was accurate when 65 tristeza-infected and 62 control trees were examined by this method. Phytopathology 60:1510-1512.

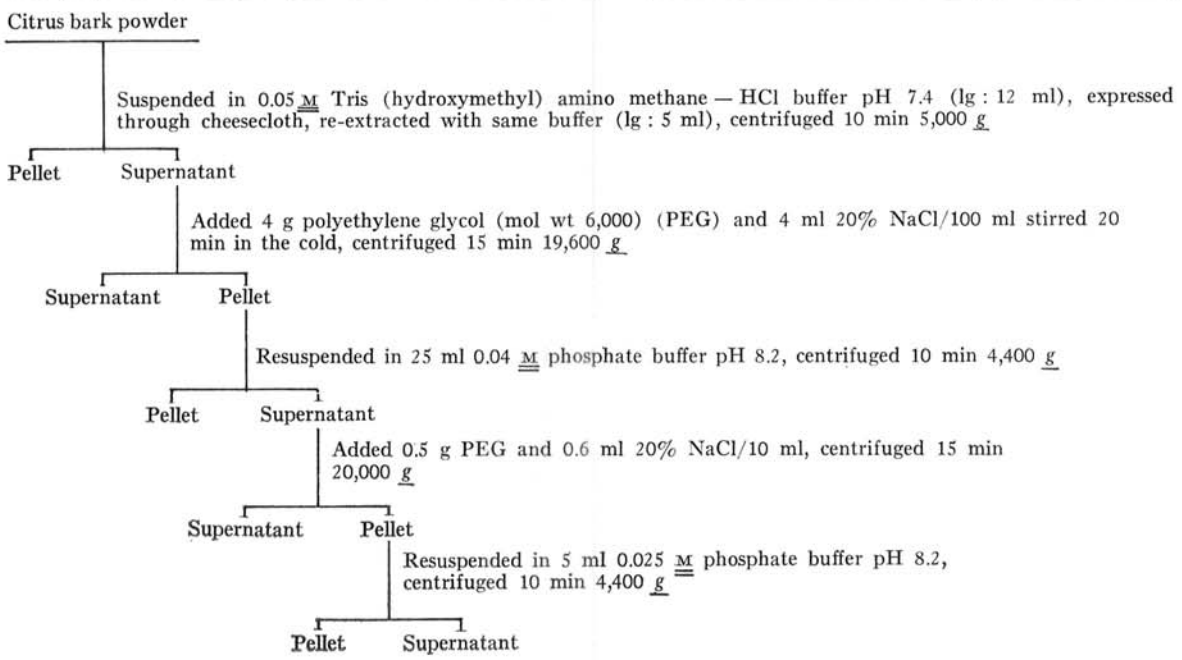
In a previous paper, a method was described for the partial purification of the threadlike particles associated with the tristeza disease of citrus (1). By employing a combination of gentle grinding, precipitation by polyethylene glycol (PEG), and differential centrifugation, and by using electron microscopy as an assay method, preparations with high concn of particles were obtained. In different tissues of four citrus cultivars, the highest concn of particles were always found in the stem bark.

Here we report a modification of the procedure enabling the rapid and regular detection of the threadlike particles associated with tristeza by electron microscopy and the adaptation of this method for a ready diagnosis (indexing) of tristeza-infected trees.

MATERIALS AND METHODS.—From the tree to be tested, 25 g bark (five to six branches, 50 cm long, 1 cm in diam) is peeled. The bark is pulverized, after freeze-drying, in a Wiley laboratory mill (intermediate model), and the powder extracted according to the procedure presented in the flow diagram (Table 1). A drop of the partially purified suspension is then placed on a Formvar carbon-coated grid. After 1 min, excess fluid is removed with filter paper and a drop of freshly prepared 1% uranyl formate (4) is placed on the grid for 15 to 20 sec. Excess fluid is then removed and the specimen dried in a desiccator for 15 min. Observations and electron micrographs were taken with a JEM-7A electron microscope at a magnification of 60,000 times.

RESULTS AND DISCUSSION.—Sixty-five trees of eight different cultivars, infected from 3 months to 5 years with a tristeza strain apparently identical to the T_3 strain (2), always showed threadlike particles with a

TABLE 1. Flow diagram for partial purification of threadlike particles associated with tristeza



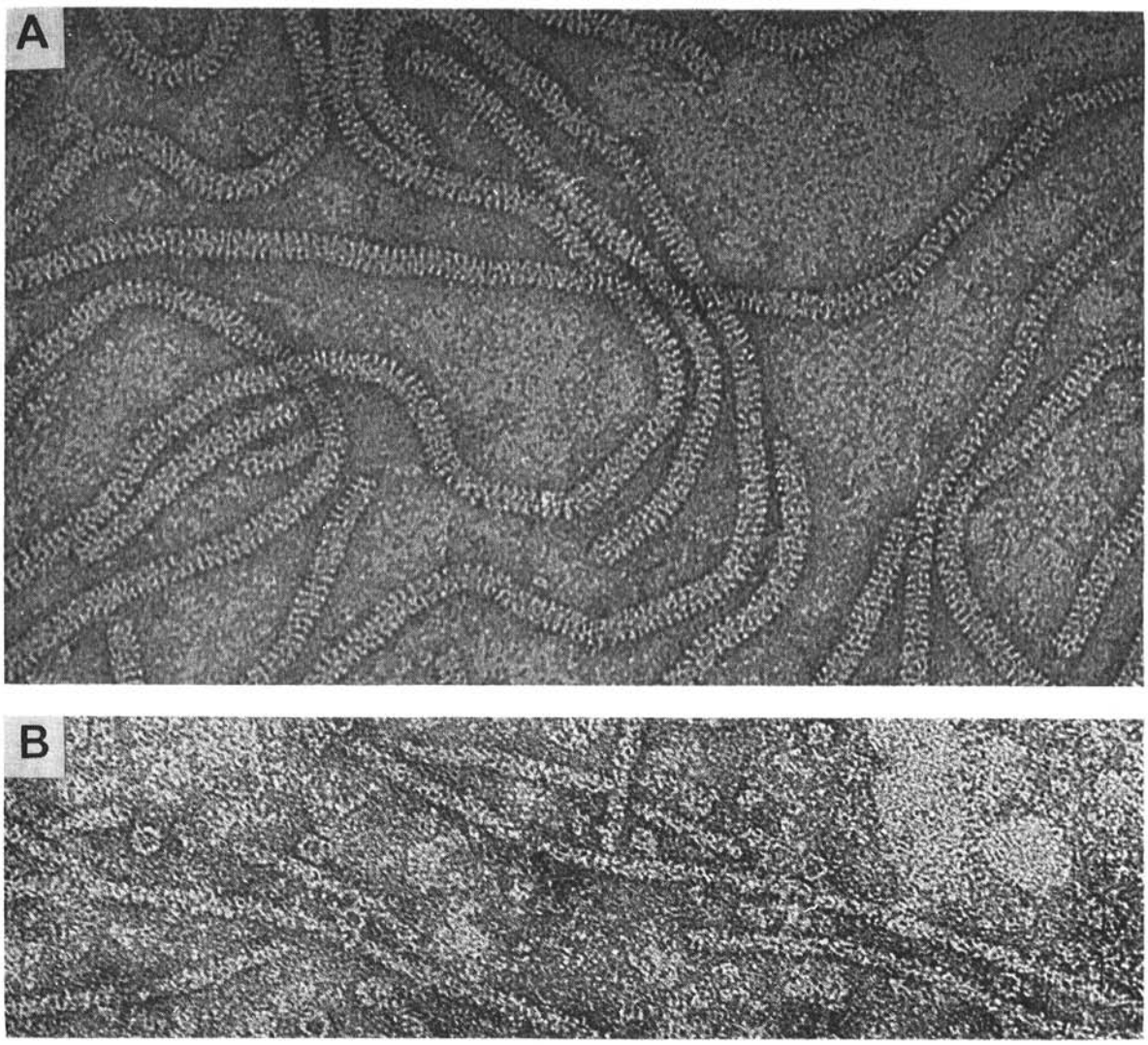


Fig. 1. Electron micrographs of **A)** threadlike particles associated with tristeza ×300,000; **B)** particles observed in control extracts ×300,000.

TABLE 2. List of trees examined for presence of threadlike particles

Variety	Rootstock	Tristeza-infected plants: No. trees examined and found to contain threadlike particles	Tristeza-infected plants: Time after inoculation	No. control trees examined
Citrus sinensis (L.)				
Osbeck, Shamouti sweet orange	Sweet lime	2	2 years	
Osbeck, Shamouti sweet orange	Sour orange	20	5 years	20
C. sinensis, Valencia	Sour orange	1	1 year	10
C. aurantium L. sour orange	Seedling	5	5 years	5
C. paradisi Macf. grapefruit	Sour orange	5	4 months	10
C. paradisi Macf. grapefruit	Madam Vinous	5	4 months	
C. paradisi Macf. grapefruit	K-lime	5	4 months	
C. paradisi Macf. grapefruit	Sweet lime	5	4 months	
C. limon (L.) Burm. f., Eureka	Seedling	5	12 months	5
C. limetta Risso, sweet lime	Seedling	5	5 months	5
C. macrophylla Wester	Seedling	5	3-8 months	5
C. volkameriana Pasq.	Seedling	2	2 years	2

typical helical substructure (Fig. 1-A, Table 2). At least 50 particles were found in each grid opening (200 mesh) in all the samples which were collected from August through March. From 62 other trees (Table 2) indexed negative for tristeza by the lime test (5), no similar particles were observed. In these preparations, however, particles of varying length, with a diam of 6-9 mμ, were often found (Fig. 1-B) (3). These particles could be easily distinguished from the tristeza-associated particles, as only the latter revealed a distinct helical substructure. Similarly, no substructure was observed in the particles when preparations from control trees were stained with neutralized 2% phosphotungstic acid or 1% uranyl acetate.

In two separate examinations made from air-dried bark taken from a tristeza-infected Marsh grapefruit tree (supplied by R. E. Schwarz, South Africa), typical threadlike particles were also easily detected.

Indexing for tristeza is currently done by the lime method (5), which requires considerable time and greenhouse space. The above-outlined procedure may have advantages for the rapid identification of infected trees, if the results are confirmed with additional citrus cultivars grown in other environments and infected by different strains of tristeza. The method may be particularly useful for screening large numbers of abnormal-appearing trees in areas where tristeza is not yet prevalent, thus preventing the establishment of infection centers.

LITERATURE CITED

1. Bar-Joseph, M., G. Loebenstein, & J. Cohen. 1970. Partial purification of viruslike particles associated with the citrus tristeza disease. Phytopathology 60:75-78.
2. Grant, T. J., & R. P. Higgins. 1957. Occurrence of mixtures of tristeza virus strains in citrus. Phytopathology 47:272-276.
3. Kitajima, E. W., D. M. Silva, A. R. Oliveira, G. W. Müller, & A. S. Costa. 1965. Electron microscopical investigations of tristeza, p. 1-9. *In* W. C. Price [ed.] 3rd Conf. Int. Organ. Citrus Virol. Proc. Univ. Fla. Press, Gainesville.
4. Varma, A., A. J. Gibbs, R. D. Woods, & J. T. Finch. 1968. Some observations on the structure of the filamentous particles of several plant viruses. J. Gen. Virol. 2:107-114.
5. Wallace, J. M. 1968. Tristeza and seedling yellows, p. 20-27. *In* Indexing procedures for 15 virus diseases of citrus trees. Agr. Handbook No. 333, Agr. Res. Serv. USDA, Washington, D.C.