Histological observations on maize leaf tissues infected with rayado fino virus\*——— E W KITAJIMA\*\*, RODRIGO GAMEZ\*\*\*

### COMPENDIO

Observaciones realizadas al microscopio electrónico mostraron que las células del parénquima de hojas de maiz infectadas con el virus del rayado fino (VRF) aparecian ligeramente contraídas en relación a las de hojas sanas. A hajas amplicaciones no se observó ningún cambio apreciable en los componentes celulares. A ampliaciones mayores se apreciaron masas deusas a los electrones en las vacuolas de células del parénquima de la epidermis y del parénquima vascular, formadas por agregados de particulas isométricas de 25 nm de diâmetro entremezcladas con material amorfo. Estas particulas no se observaron en células de plantas sanas, y son similares a las descritas para el VRF en preparaciones purificadas. Los efectos citopáticos de este virus y del virus del rayado del maiz de Brasil son idénticos, lo cual confirma observaciones anteriores sobre la similitud de estos virus.— Los autores.

#### Introduction

the Brazilian corn streak virus (CSV) (11) are small isometric viruses, 25-27 nm in diameter, transmitted in a persistent manner by the cicadellid leafhopper Dalbulus maidis DeLong & Wolcott. In a preliminary communication (10) we indicated that these viruses were serologically identical and induced similar cytopathic effects in leaf cells of infected maize plants. For these reasons RFV and BCSV are considered identical or closely related viruses. In this paper we describe some observations on intracelular location and cytopathic effects induced by RFV in cells of maize plants and compare these effects with those caused by the BCSV and other maize viruses.

## Materials and methods

Leaf samples from both uninoculated control and RFV-infected maize plants were fixed with 3% glutaraldehyde and post-fixed in 1% OsO<sub>4</sub>, buffered in phosphate, dehydrated and embedded in Epon at the University of Costa Rica The blocks were sectioned at

the University of Brasilia with a Porter-Blum MT-1 or with LKB ultratome III microtomes equipped with an IVIC diamond knife Thin sections were then stained with uranyl acetate and lead citrate and examined in a Zeiss EM9 electron microscope.

# Results

There was an accidental change in the labels of the blocks sent from Costa Rica; thus the material considered as healthy was indeed RFV-infected and viceversa. This, in part, was interesting because it eliminated possible bias in the examination of the sections. Preservation of the tissues was reasonable, with some few cells showing slight plasmolysis. Leaf parenchyma cells from RFV-infected plants were somewhat shrunken in relation to those from control plants. At low magnification, however, no remarkable changes could be noticed in the cell components. Vacuoles from cells of both RFV-infected and uninoculated control plants commonly exhibited electron dense specks (Fig. 1, 2,6). At higher magnifications it was possible to notice that these dense specks in the vacuoles from RFV-infected cells were made up of an aggregate of isometric particles, ca. 25 nm in diameter, commonly interspersed with an amorphous material (Fig. 3-5). In uninfected cells, these specks were composed of nonparticulated material (Fig. 6), and in no instance could the 25 nm particles be observed in these cells

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v\* Departamento de Biología Celular, Universidad de Brasilia, Braza-

<sup>\*\*\*</sup> Centro de Investigación en Virología y Fisiología Celular, Universidad de Costa Rica, Ciudad Universitaria. R. Gámez is Scientific Fellow of the Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICIT) of Costa Rica

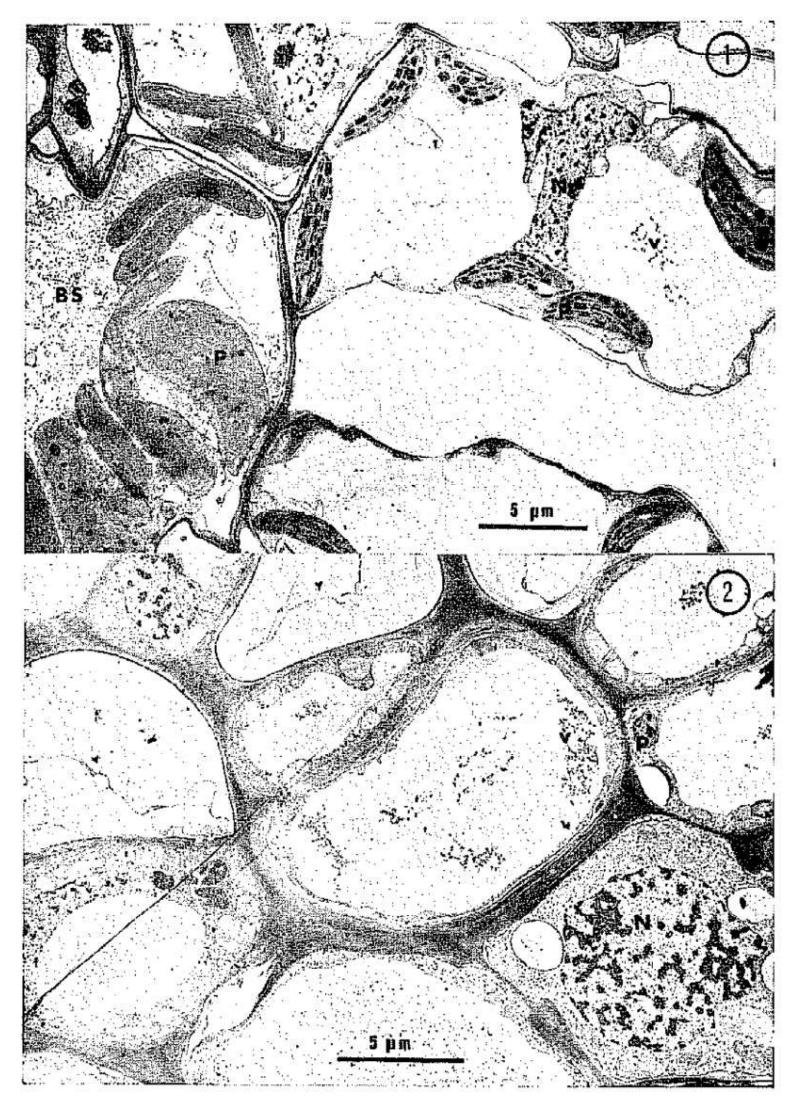


Fig. 1-2 -Virus-infected epidermal (Fig. 1) and phluem parenchyma (Fig. 2) cells. Electron dense specks containing virus particles appear in the vacuoles. BS bundle sheat. No uncleus; P. plastid, V. virus

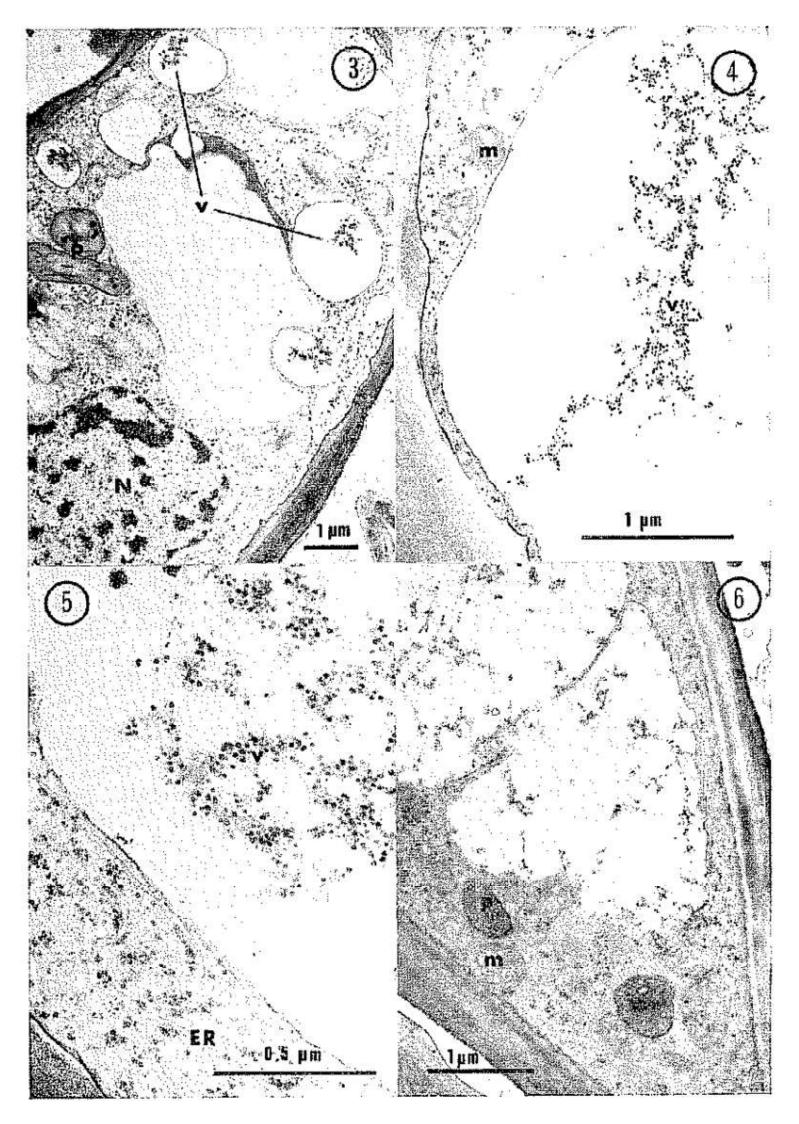


Fig 3-5 —Aggregates of isometric particles interspersed with amorphous material in caenoles of virus-injected opidermal parenchyma cells ER endoplasmic reticulum, m mitochondeia, N nucleus, P plantid, V virus

Fig 6 -Electron dense speeks of nonparticulated material in healthy phl-um parenchyma cell

## Discussion

The isometric particles observed in the vacuoles of RFV-infected cells from epidermis mesophyll and phloem parenchyma must represent the RFV in situ. Their presence only in RFV-infected tissues and their morphological similarity with the polyhedral particles found in purified and infective preparations (8, 11) are considered evidence in favor of this view The present results are also in agreement with previous studies that showed RFV and BCSV to be serologically similar or identical (10) Presumptive BCSV particles also occur in the vacuole of different types of leaf cells and were also observed in some few cases in the cytoplasm, associated with areas rich in vesicles (11). This was not observed in RFV-infected cells, but it could be due to the examination of a relatively small number of samples.

The similarity in the cytopathic effects of RFV and BCSV supports the view that both viruses are part of the same complex

The rayado Colombiano virus of maize is also serologically related to both RFV and BCSV (Gámez, R. and Martínez-López, G., unpublished data; Gámez, R. and Kitajima, E W, unpublished data) Originally it was thought to be 50 nm in diameter (13), but was recently shown to be identical to RFV and BCSV in size and morphology (14) The histological effects in maize of this virus are not known. Although there are other isometric viruses reported infecting maize such as the African corn streak (1,15), the maize stripe and maize line (12), the chlorotic dwarf (2,3), the chlorotic mottle (9), and the cucumber mosaic (4), most of them differ in several pathological and/or morphological properties, as well as in their insectvector and virus-vector relationships, and are thus probably unrelated to RFV and BCSV.

## Literature cited

- BOCK, K. R., GUTHRIE, E. J., and WOODS, R. D. Purification of maize streak virus and its relationship to viruses associated with streak diseases of sugar cane and Panicum maximum. Annals of Applied Biology, 77(3):289-296. 1974
- 2 BRADFUTE, O E Detection of maize stanting agent by electron microscopy of diseased tissues. Proceedings, Second International Congress of Plant Pathology Abstract Nº 70. 1973.

- BRADFUTE O E., LOUIE, R., and KNOKE, J. K. Isometric viruslike particles in maize with stunt symptoms Phytopathology, 52(8): 7-18. (Abstr.) 1972
- 4 COSTA A S and KITAJIMA, E W. Ocorrencia do mosaico em milho no Estado de Sao Paulo, causado pelo virus do mosaico de pepíno. Resumos 5º Congreso Sociedad Brasileira de Fitopatologia
- 5 GAMEZ, R. A new leafhopper-borne virus of corn in Central America Plant Disease Reporter, 53(12): 929-932, 1969
- 6 Iransmission of rayado fino virus of maize (Zea mays L) by Dalbulus maidis DeLong & Wolcott. Annals of Applied Biology, 73(3): 285-292 1973
- The leafhopper-transmitted maize rayado fino virus in Central America In Proceedings, International Maize Virus Disease Colloquium and Workshop. Ohio Agricultural Research and Development Center, Wooster, Ohio August 16-19, 1976 (In press)
- and RAMIREZ, C Purification, serology and electron microscopy of rayado fino virus of maize In Abstracts, Annual Meeting, Caribbean Division, American Phytopathological Soc, CIAT, Colombia December 1-6, 1975 p. 42
- HERBERT, T. T and CASTILLO, J A new virus of ma'ze in Perù. In Proceedings, Second International Congress of Plant Pathology, Abstract No. 70, 1973.
- to KTTAJIMA, E. W., GAMEZ, R., and LIN, M. T. A serological and histological comparison of the maize rayado fino virus from Costa Rica and the Brazilian corn streak virus In Abstracts, Annual Meeting, Caribean Division, American Phytopathological Society, CIAT, Cali, Colombia December 1-6, 1975 p. 50
  - YANO, T and COSTA, A. S. Purification and intracelular location of isometric particles associated with the Brazilian corn streak virus infection. Ciencia e Cultura (Brasil) 28(4): -127-430 1976
- 12 KULKARNI, H. Y Comparison and characterization of maize stripe and maize line viruses Annals of Applied Biology, 75(2): 205-216 1973
- 15 MARTINEZ-LOPEZ, G. and RICO DE CUJIA, LUZ M El virus del rayado colombiano del maiz Noticias Fitopatológicas -1(1): 27-32 1975.
- del rayado colombiano del maiz es un virus isométrico de alrededor de 30 mm de diámetro In Resúmenes, II Congreso, Asociación Colombiana de Fitopatología y Ciencias Afines. Bogotá Setiembre 1-3, 1976 p 52
- 15 PLASVIC-BAJAC, B. and MARAMOROSCH, K Electron microscopy of African maize streak Phytopathology, 62(6): 671 (Abstr.). 1972