Papaya Apical Necrosis, a New Disease Associated with a Rhabdovirus

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ABSTRACT

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An apparently new disease affecting papaya (Carica papaya L.) was found in Zulia State, Venezuela. The initial symptom, a general yellowing of the plant, was followed by wilting of the younger leaves and necrosis of the plant apex. The disease was associated with a rhabdovirus measuring $210-230 \times 80-84$ nm and located in the nucleus of parenchyma cells usually in the phloem. The disease agent was transmitted by the leafhopper Empoasca papayae but could not be transmitted mechanically.

Papaya (Carica papaya L.), a fruit commonly grown in Venezuela, is severely affected by papaya ringspot virus (2), which brought about a decline in the planted area from 3,370 ha in 1976 to 2,335 ha in 1978. In 1979 in Zulia State, a new disease appeared and spread rapidly, killing a high percentage of the plants. Because neither fungi nor bacteria were associated with the disease, a study was undertaken to identify the causal agent. We report a rhabdovirus consistently associated with the diseased plants.

MATERIALS AND METHODS

Plant material showing early symptoms of the disease was triturated in 0.1 M

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Fig. 1. Apical necrosis in papaya (Carica papaya L.). Note wilting and chlorosis of leaves and apical necrosis.

phosphate buffer, pH 7.0, and inoculated with 600-mesh Carborundum into the following indicator plants for papaya viruses: C. papaya 'Criolla' and 'Cartagena', Cucurbita pepo L. 'Early Prolific Straightneck', Gomphrena globosa L., and Chenopodium amaranticolor L. After inoculation, the plants were placed in a greenhouse for observation.

Plant tissue was examined for virus particles with the dip technique and 2% buffered phosphotungstic acid as a stain.

For histologic studies, 1 mm² leaf segments were cut, fixed in 2% glutaral-dehyde in 0.1 M cacodylate buffer, pH 6.8, and postfiæed with 2% osmium tetroxide. After being dehydrated in an ethanol series, the samples were embedded in Epon 812 resin. The tissue was sectioned with a Sorval ultramicrotome equipped with a diamond knife and then stained with uranyl acetate and lead citrate. Copper grids were carbon-stabilized, coated with collodion, and observed with a JEOL 100 B electron microscope.

Insect transmission tests were carried out with leafhoppers, *Empoasca papayae* Oman, collected in the field from apparently healthy papaya plants. Insects were given an acquisition feeding of 2-3 days on infected papaya leaves and were then placed in groups of 10 onto 10 healthy papaya seedlings. The insects were killed after 20 days, and the plants were placed in a greenhouse for further

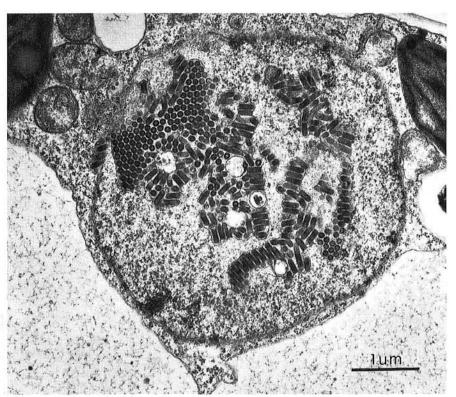


Fig. 2. Nucleus of phloem parenchyma cell of infected papaya with rhabdovirus particles in both longitudinal and cross sections. Particles inside the nucleus are surrounded by a membrane.



Fig. 3. Section through a vascular bundle (V) of a diseased papaya leaf. Parenchyma cells near the sieve tubes have rhabdovirus particles (R) in their nuclei.

observation. Two groups of 10 insects each that had no contact with infected tissue were placed on healthy plants as controls.

RESULTS

Initial symptoms of the disease were general yellowing of the affected plant followed by rapid decline of the younger leaves. Apical portions of the plant became necrotic, and later all the leaves died. Either the plant died or just its top died and new growth was produced on the lower part of the stem. Frequently, the new growth showed symptoms of the disease and the plant collapsed (Fig. 1). Several attempts to transmit the causal agent mechanically to indicator plants of papaya viruses failed.

Preparations from infected leaves consistently contained rhabdovirus particles. In tissue sections, rhabdovirus particles were found in membrane-bound pockets inside the nuclei of infected cells (Fig. 2) and occasionally in the perinuclear region. Only parenchyma tissue near the vascular bundles was affected, and infected cells were often found near the

sieve tubes (Fig. 3). One-hundred virus particles were measured in sections of infected cells. The particles were bullet-shaped with dimensions of $210-230 \times 80-84$ nm. Preparations from healthy plants were free of virus particles.

Eight of 10 papaya seedlings showed symptoms 20–30 days after being exposed to leafhoppers that had fed on diseased leaves. When insects from healthy plants were allowed to feed on healthy seedlings, no symptoms were observed. However, we were unable to find rhabdovirus particles in tissues that showed symptoms of the disease after being exposed to E. papayae.

DISCUSSION

The symptomatology we have described differs from that reported for other papaya viruses. Furthermore, no rhabdoviruses have been reported in association with papaya diseases (1,3-5). The rhabdovirus particles we observed were in the size range of other rhabdoviruses, and they were found mainly inside the nucleus and occasionally in the perinuclear space. Intranuclear

location has also been reported for other plant rhabdoviruses (3).

The main evidence that E. papayae is the vector of the disease agent is the fact that symptoms developed in papaya seedlings exposed to insects that had fed on diseased tissues. No comparable symptoms developed with unexposed insects. Furthermore, E. papayae was the only leafhopper consistently found on papaya plants in the affected area. The lack of electron microscopic evidence for transmission of the disease agent could be explained by the fact that the rhabdovirus is limited to the parenchyma cells of the phloem—in initial stages of the disease, few cells are infected, and these are difficult to locate.

E. papayae is also the vector of the causal agent of papaya bunchy top, a disease associated with a mycoplasmalike organism. We did not find mycoplasmalike bodies associated with phloem tissues of plants with apical necrosis. Moreover, the symptomatology of papaya apical necrosis is quite different from that of bunchy top.

Papaya ringspot virus is a limiting factor for papaya production in Venezuela. However, papaya ringspot virus incidence is very low in the coastal region of Zulia State, probably because the steady wind interferes with aphid transmission. The low incidence of papaya ringspot virus makes the zone potentially good for growing papayas, but papaya apical necrosis has nearly wiped out the papaya plants in this region in the last 2 yr.

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