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Ultrastructural Study for the Characterization and Diagnosis of Pathogens in Tropical Fruits.

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The use of the electron microscopy permitted a greater insight into the study of the damage that fungi, bacteria and viruses produced to tropical fruits. Such ultrastructural characterization made also possible the design of appropriated methods for diagnoses of the plant diseases. Mango fruits (*Mangifera indica* L.) cv. Super Haden either healthy or with different levels of affectations for antracnosis, and papaya (*Carica papaya* L.) petioles with Rickettsia infection symptoms were sliced to sections of 5mm x 1mm. Then they were fixated with 4% glutharaldehyde in phosphate buffer pH 7.2-7.4, and subsequently post-fixated with 2% Osmium tetroxide in phosphate buffer pH 8 with dehydration further by a growing concentration gradient of acetone. Infiltration and polymerization was done as in Spurr (1969). Ultrathin sections (400 ºA) were sliced in a NOVA Ultramicrotome (LKB, Sweden) and placed on 400 mesh copper grids to be stained with uranyl acetate and lead citrate. Citrus Psorosis were studied by comparison of the partial purification of healthy plants and those having viral infection symptoms in Pinnapple orange (*Citrus sinensis* L. Osbec). A drop of each sample was applied to grids covered with Formvar-Carbon membrane, washed after two minutes and then stained with 2% uranyl acetate aqueous solution. All observations were carried out in a transmission electron microscope JEM2000 (JEOL). The ultrastructural analysis of healthy mango epidermal cells (fig. 1) showed mitochondria, chloroplast, nucleus, nucleoles and vacuoles as well as the rough endoplasmic reticulum within normal levels. However, the slices belonging to the proximity and center of the lesions (fig. 2) evidenced significant morphological alterations.
The papaya petioles healthy tissues showed densely contrasted zones in the center of the latex reservoir, some latex vesicles and membrane and cell debris, keeping the round shape and showing no trace of microorganisms (fig. 3). The infected tissue showed damaged latex tubes with loss of the round shape and collapsed walls in some portions. In the interior of the tubes the presence of bacillary bacteria with sizes of 0.3-0.5µm in length and 0.09-0.2 µm wide, surrounded by a non-dense area was observed. At a larger magnification (fig. 4) bacterias were perfectly defined and identified by the double cell wall and the periplasmic space. Besides, inside the bacteria several electron refringent bodies we observed. Similar results were outlined by Davis et.al (2). In samples from Pineapple orange plants showing similar Psorosis symptoms, viral particles having either closed, “O” or in some cases a spindle shape oscillating among 3.4 µm wide were detected, thus coinciding with previous reports for this virus (fig. 5 and 6). (1,3). In summary, in the present work we have characterized for the first time in Cuba the detection of both Rickettsia in papaya and Psorosis in the citrus.

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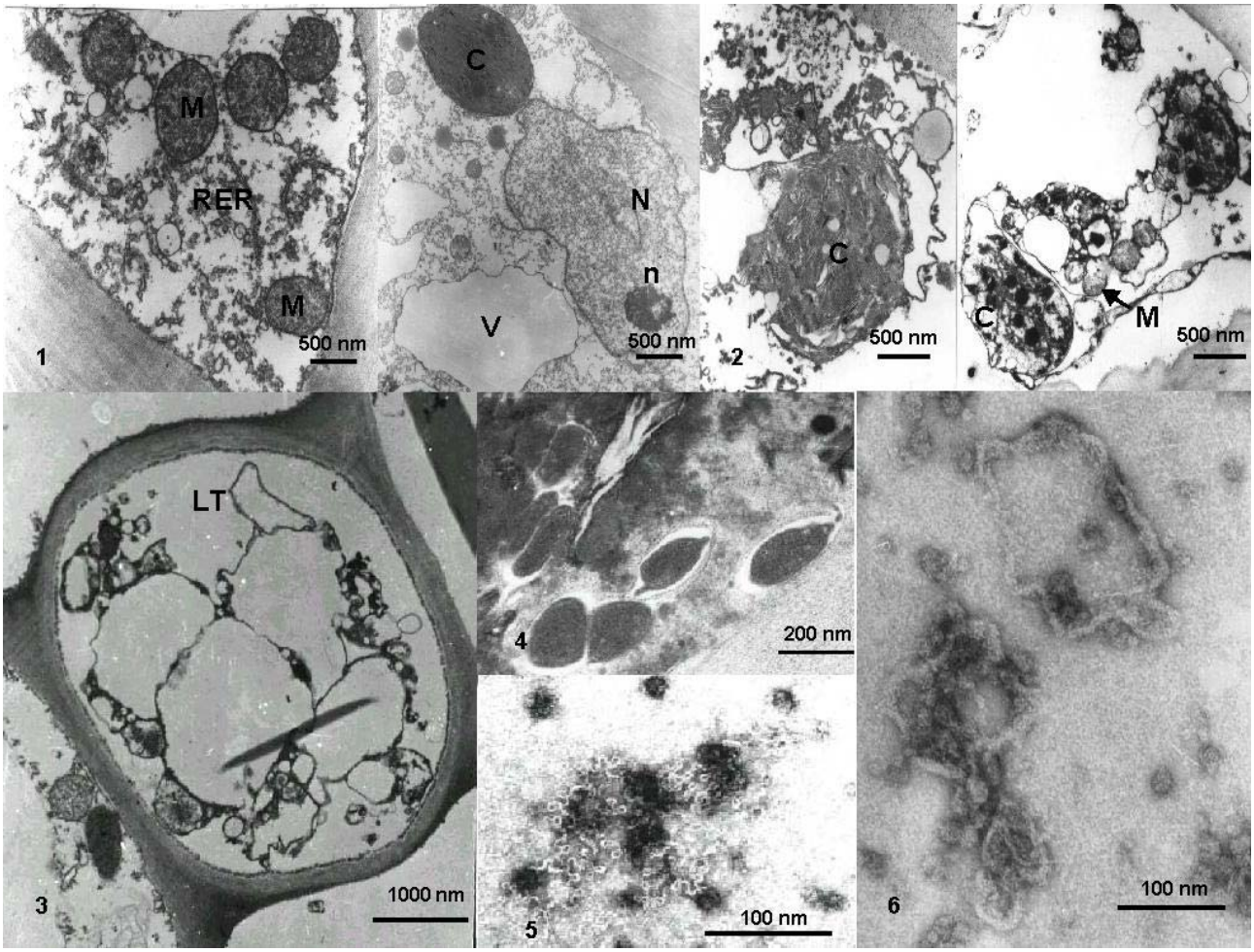

Figure 1: Fine section in health Mango fruit. The observed chloroplasts, mitochondria, endoplasmic reticulum, nucleus, nucleolus and vacuoles have the described characteristics.
Figure 2: Observation of Mango fruit damaged tissues with Collecotricum sp. Very affected chloroplasts and mitochondria with lost of the intern structure can be seen. The nucleus and nucleolus are not seen and the vacuoles lost their normal structure.
Figure 3: Section of health papaya petiole tissue. Observe the inside latex tube with cellular debris and latex drops
Figure 4: Bacteria inside the latex tube of a papaya petiole with disease symptoms can be seen.
Figures 5 and 6: Virus causing citrus Psorosis.

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