

Electron Microscopy of Viruslike Particles Found in Mosaic-Diseased Raspberries in France

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ABSTRACT

Bacilliform viruslike particles have been observed in raspberry plants infected with raspberry mosaic virus. The particles were about 360-400 nm long and 60-80 nm in diam (measured in embedded sections).

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The study of the mosaic disease of red raspberries (*Rubus idaeus* L.) transmitted by the aphid *Amphorophora rubi* Kalt. (1) has been severely handicapped by the absence of information on the structure and properties of the causal agent concerned. We have tried to establish the morphology of the causative agent by means of electron microscopy.

Small pieces of leaves from healthy or mosaic-infected plants showing conspicuous symptoms (vein yellowing) were fixed in a phosphate-buffered 6.25% solution of glutaraldehyde for 1 hr at 0-4 C and post-fixed in a 1% solution of osmium tetroxide. Fixed tissues were dehydrated in a series of increasing concentrations of ethanol and propylene oxide and then embedded in a Epon 812-araldite mixture. The blocks were sectioned in a Reichert model OmU2 ultramicrotome, with glass knives made with an LKB knifemaker. Thin sections showing gold to silver interference colors were picked up on empty grids, colored with uranyl acetate saturated in 50% ethanol, and counterstained with lead citrate. Examinations were done in a Hitachi model HU 11 CS electron microscope.

Bacilliform particles were consistently found in preparations from infected plants, whereas no similar particles were ever recorded in those from control plants. These particles were relatively uniform in size, measuring 360-400 nm long and 60-80 nm in diam, with rounded ends. They have an outer membrane 8-10 nm thick, which envelops an inner tubular core, 320-360 nm long and 40-60 nm in diam. They appear uniformly dense, except for the membrane, and show no internal details as striations or helical arrangement. Nonpenetration of the stain into the particles could account for it. No bullet-shaped particles were seen. Transverse sections through particles showed a wide central canal in some particles and none in others, which suggests that material of some kind may fill the center of some particles. These sections of closely packed particles in cells also suggest that there is only one electron-dense layer of membrane (Fig. 1).

The particles seem to belong to the group of large rod-shaped plant and animal viruses (Rhabdovirales) which have complex structures, contain RNA, and possess an inner framework in the form of a helix (4). The particles observed resemble those of the potato yellow dwarf virus which have a similar common length, and those of the sowthistle yellow vein virus and lettuce necrotic yellows virus, which are both transmitted by the aphid *Hyperomyzus lactucae* L. No groups of particles were seen in close association with the outer edge of the nucleus or inside the nucleus as was observed in cells infected with *Gomphrena* virus (2) or with potato yellow dwarf virus (3), but many of the viruslike particles were found in bundles in the cytoplasm, enclosed in structures bounded by a single or a double membrane.

At this stage, we cannot prove that the particles observed in thin sections from mosaic-infected raspberry plants represent the causative agent of raspberry mosaic. However, the unusual morphology of these large particles, as well as the probable presence of lipids in their membranes, may explain our failure to purify this virus by classical extraction procedures (using inadequate centrifugation speeds and organic solvents).

Moreover, the morphological similarity of the bacilliform particles with those of other viruses that are transmitted by aphids closely related to *Amphorophora rubi* Kalt. (the vector of the raspberry mosaic virus), also suggests that the bacilliform particles are those of raspberry mosaic virus. As it is not possible to transmit raspberry mosaic virus

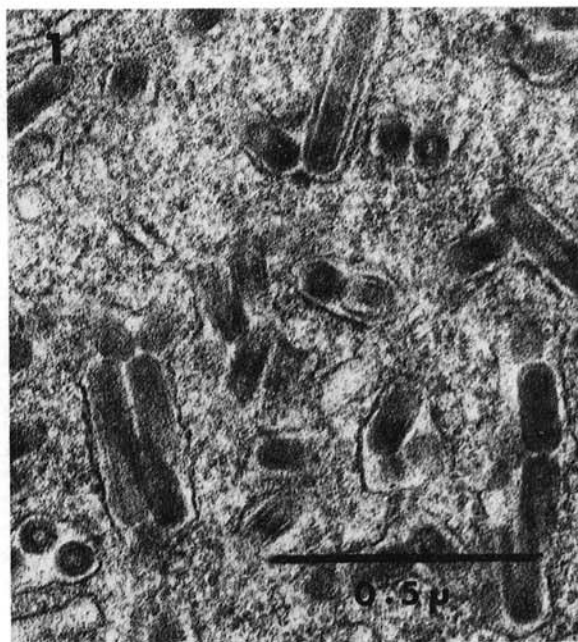


Fig. 1. Thin-sectioning in a mosaic-infected raspberry leaf cell showing rod-shaped viruslike particles. Bar indicates approximately 500 nm (X 67,000).

mechanically, further studies on aphid transmission of the virus are in progress.

LITERATURE CITED

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