Location of Grapevine Fanleaf and Yellow Mosaic Virus Particles in *Xiphinema index*

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Abstract: Particles of fanleaf and yellow mosaic viruses are reported in the lumen of the esophagus of *Xiphinema index*. Differences in cuticular morphology suggest differences in charged receptor sites which may offer an explanation for virus location and orderly arrangement. *Key Words:* NEPO virus, morphology, esophagus, odontophore.

A significant advance was made in understanding the relationship of nematode vectors and plant viruses when Taylor and Robertson (7) reported the location of raspberry ringspot and tomato black ring viruses in *Longidorus elongatus* (de Man). Similar reports have also been made on the site of viruses in *Xiphinema diversicaudatum* (Micol.) and *X. index* Thorne and Allen (8); *X. americanum* Cobb (4); and *Trichodorus pachydermus* Seinhorst (9).

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MATERIALS AND METHODS

Examination by electron microscopy of *X. index* from grapevines in California infected with fanleaf and yellow mosaic viruses has revealed virus particles in the esophagus. Soil samples were collected from commercial vineyards from *Vitis vinifera* L. 'Chardonnay' near St. Helena infected with fanleaf virus, and from 'Semillon' near Morgan Hill infected with yellow mosaic virus. In both cases, adult females of *X. index* were separated from the soil by wet-sieving, then selected ones transferred to tap water and chilled prior to infusion with 1.5% or 3% aqueous glutaraldehyde. The glutaraldehyde was added

two or three drops at 10-min intervals for 90 min. All solution was then drawn off and fresh glutaraldehyde added. The total fixation period was 17 hr. Four chilled-water rinses followed before a 1-hr postfixation in 2% osmic acid in phosphate buffer with 4.9% sucrose. After three rinses with the buffered solution, dehydration was carried out with a cold graded ethanol series of 35-, 50-, 70- and 95% for 15 min each. Final dehydration was done by immersion in 100% ethanol for 30 min. Subsequently, the specimens were passed twice through propylene oxide for 30 min, and embedded in an Epon-Araldite mixture. All preparations, up to and including propylene oxide, were carried out at 4 C. Diamond knives on a LKB Ultrotome were used to section the nematodes. The sections were picked up on Formvar-coated 100-mesh copper grids then stained with alcoholic magnesium uranyl acetate for 20 min followed by lead citrate for 10 min. Examination was done on an RCA-EMU-3G electron microscope having a 50-μ objective aperture and operating at 50 KV.

RESULTS

Fanleaf and yellow mosaic virus particles were found from the anterior end of the stylet extension [odontophore of Taylor and Robertson (7)] through the bulbar region of the esophagus (Fig. 1, A-C). At the junction of the odontostyle and the odontophore there was a very sharp demarcation with the virus particles present to the anterior limit of the odontophore but absent in the odontostyle. Oblique or longitudinal sections through the junction itself of odontostyle and odontophore were not found. However, many cross-sections through that region were definitely identified by surrounding tissues and consistently held virus in the odontophore but none in the odontostyle. This conforms with the results of Taylor and Robertson (8) working with *X. diversicaudatum* and Arabis mosaic virus. Their Fig. 2 illustrated an oblique section through the junction of the odontophore and odontostyle with a precise line separating virus in the former but absent in the latter. The particles were spherical, measured approximately 22-31 nm and lined the lumen in a monolayer. These physical characteristics conform with those described for fanleaf and yellow mosaic viruses. No visible distinction occurred between particles of the fanleaf and the yellow mosaic viruses.

DISCUSSION

Taylor and Robertson (7) suggest that a mucus-like layer accounts for the specificity of virus transmission by *Longidorus elongatus* and (9) that a layer of mucus accounts for the greater density of virus particles in the glandular part, than in the nonglandular part, of the esophagus of *Trichodorus pachydermus*. Their evidence for a mucus layer is inconclusive and has not been demonstrated in any other species of Nematoda. Furthermore, the presence of a mucus layer would only explain location, and would not account for the orderly, monolayered arrangement of the virus particles.

Electron micrographs of *Xiphinema index* (6, 10) and *Trichodorus* spp. (2, 5) have shown that there are distinct differences in the morphology of the cuticle of the odontostyle (which is similar to the external cuticle) and the odontophore (which is similar to the esophageal cuticle). Morphological differences in the cuticle of the odontostyle and odontophore suggest fundamental differences in tissue production and physiology which may be important in this phenomenon of virus particle location as well as to our understanding of phylogenetic relationships within Nematoda. Inglis (3) suggested that the two cuticular regions of the stoma, which are distinguishable on the basis of structure and differential staining, be recognized and named separately. Therefore, he proposed the term *cheilostome* for the anterior portion of the stoma with cuticle similar to external cuticle and *oesophastome* for that portion of the stoma surrounded by esophageal tissue with cuticle similar to that of the esophagus *per se*. These terms have physiological significance, as evidenced by structural and staining differences in stomatal cuticle. They have embryological significance, even though the cuticle of the body and entire stomodeum is ectodermal in origin because the *oesophastome* is part of the region of primary invagination and the *cheilostome* is from the terminal region of secondary elongation or overgrowth.

The location and arrangement of virus particles in different regions of the stomodeum of nematode vectors is additional evidence that the differences between *cheilostome* and *oesophastome* are physiologic as well as morphologic in nature. In *Xiphinema*, the virus is found in the odontophore and in the esophageal lumen. In these locations, the virus

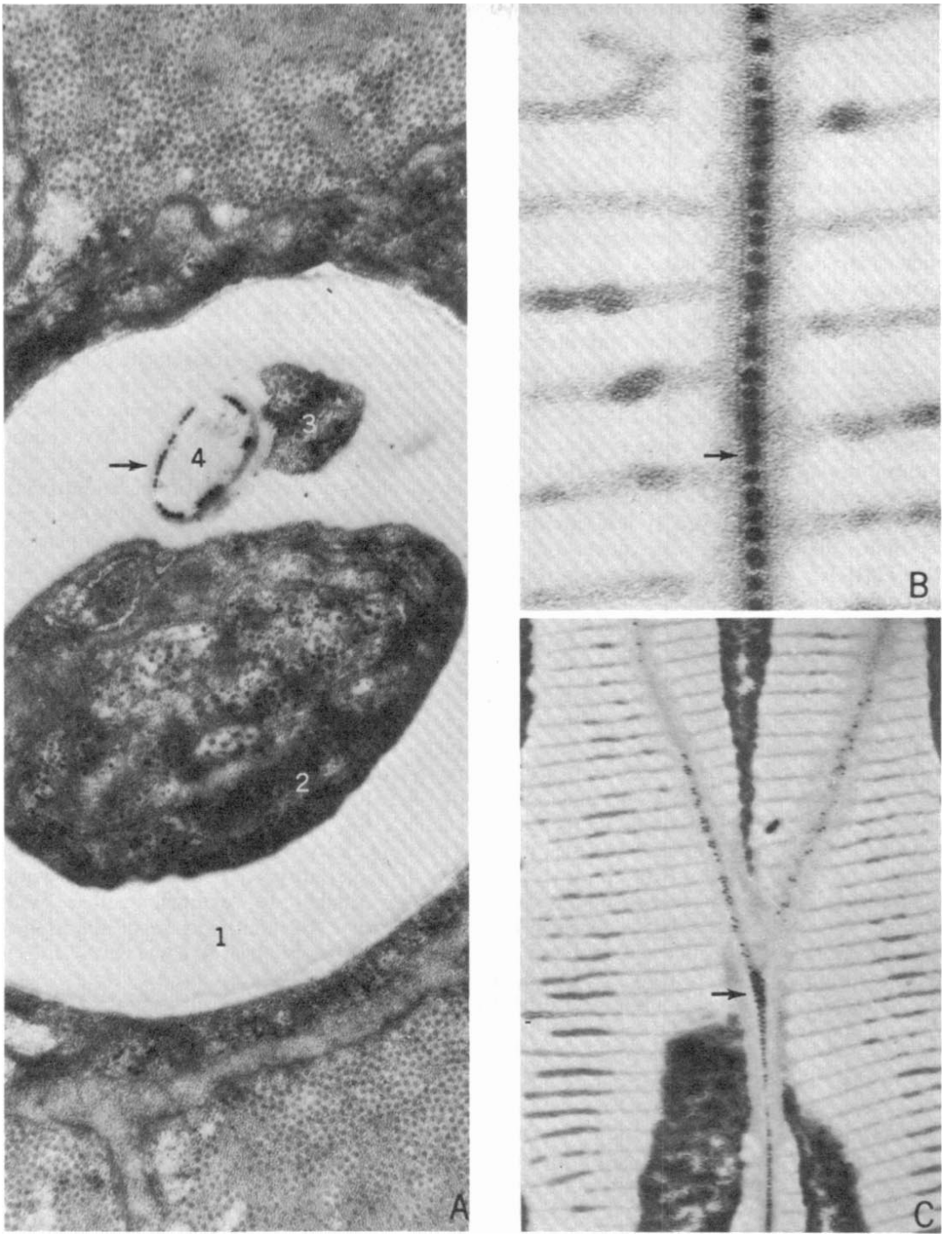

Fig. 1. **A)** Transverse section of *Xiphinema index* from yellow mosaic-infected vine. Odontophore appears as a white ring (1) containing the large dorsal cavity, (2) one of the two subventral cavities (3) and the lumen (4) lined with virus-like particles (arrow). (× 47,000). **B)** Longitudinal section through one of the rays of the triradiate lumen of the esophageal bulb of *X. index* from fanleaf-infected grapevine with virus-like particles (arrow). (× 135,000). **C)** Longitudinally-oblique section of the esophageal bulb of *X. index* from fanleaf-infected grapevine. Section is in the bulbar region with virus-like particles in all three rays of lumen. Banding effect is produced by the platelet thickenings of the lumen wall. (× 18,400).

particles are arranged in an orderly monolayer. The implication is that the forces or bonds which act on the particles in the odontophore and esophageal lumen are nonfunctional in the external cuticle and the cuticle of the odontostyle. This differential phenomenon is clearly demonstrated in *Trichodorus*. In the anterior stoma (cuticle similar to that of the external covering) disoriented virus particles are found; whereas, in the anterior esophageal lumen (cuticle similar to that of the esophagus *per se*) and adjacent to the stylet [= pharynx, Hirumi et al. (2); = *oesophastome*, Inglis (3)] the virus particles are in an orderly monolayer as they are in *Xiphinema*. The presence (*Trichodorus*) or absence (*Xiphinema*) of virus particles in the extreme anterior of the stomodeum is due not solely to cuticular differences but to morphological differences in stomatal structure and armature.

Taylor and Robertson (9) suggested that surface charges on virus particles as reported by Harrison and Roberts (1) may be a means by which virus particles are adsorbed on the cuticle of the digestive tract of nematodes. Inglis (3) reported that differential isoelectric points between internal and external cuticle are manifested through differential staining. Therefore, virus particle location and orderly arrangement in the lumen of the esophagus can be explained by availability of charged receptor sites on the esophageal tissue and surface charges on virus particles.

LITERATURE CITED

1. HARRISON, B. D. and I. M. ROBERTS. 1968. Association of tobacco rattle virus with mitochondria. J. Gen. Virol. 3:121-124.
2. HIRUMI, H., T. A. CHEN, K. J. LEE and K. MARAMOROSCH. 1968. Ultrastructure of the feeding apparatus of the nematode *Trichodorus christiei*. J. Ultrastruct. Res. 24:434-453.
3. INGLIS, W. G. 1966. The origin and function of the cheilostomal complex in the nematode *Falcaustra stewarti*. Proc. Linn. Soc. Lond. 177:55-62.
4. MC GUIRE, J. M., K. S. KIM and LINDA B. DOUTHIT. 1970. Tobacco ringspot virus in the nematode *Xiphinema americanum*. Virology 42:212-216.
5. RASKI, D. J., N. O. JONES and D. R. ROGGEN. 1969. On the morphology and ultrastructure of the esophageal region of *Trichodorus allius* Jensen. Proc. Helminthol. Soc. Wash. 36:106-118.
6. ROGGEN, D. R., D. J. RASKI and N. O. JONES. 1967. Further electron microscopic observations of *Xiphinema index*. Nematologica 13:1-16.
7. TAYLOR, C. E. and W. M. ROBERTSON. 1969. The location of raspberry ringspot and tomato black ring viruses in the nematode vector, *Longidorus elongatus* (de Man). Ann. Appl. Biol. 64:233-237.
8. TAYLOR, C. E. and W. M. ROBERTSON. 1970. Sites of virus retention in the alimentary tract of the nematode vectors, *Xiphinema diversicaudatum* (Micol.) and *X. index* (Thorne and Allen). Ann. Appl. Biol. 66:375-380.
9. TAYLOR, C. E. and W. M. ROBERTSON. 1970. Location of tobacco rattle virus in the nematode vector, *Trichodorus pachydermus* Seinhorst. J. Gen. Virol. 6:179-182.
10. WRIGHT, K. A. 1965. The histology of the oesophageal region of *Xiphinema index* Thorne and Allen, 1950, as seen with the electron microscope. Can. J. Zool. 43:689-700.