J. gen. Virol. (1977), **34**, 107–114
Printed in Great Britain

Barley Yellow Striate Mosaic Virus in the Salivary Glands of its Planthopper Vector *Laodelphax striatellus* Fallén

By M. CONTI
Laboratorio di Fitovirologia applicata, CNR, Turin, Italy
AND R. T. PLUMB
Rothamsted Experimental Station, Harpenden, Herts., England

(*Accepted* 2 *August* 1976)

SUMMARY

The salivary glands of planthoppers, *Laodelphax striatellus*, transmitting barley yellow striate mosaic virus (BYSMV), cryptogram */* : */* : U/U : S,I/Au, were sectioned and examined in the electron microscope. BYSMV was detected in the cytoplasm but not in the nuclei or other organelles of infected cells which did not show structural changes. The BYSMV virions, 300 to 320 nm long and about 40 nm wide, were frequently arranged in parallel aggregates bound by a membrane. Long flexuous tubules of variable length, 18 to 20 nm wide were found in close association with BYSMV. The tubules were typically found in bundles surrounded by a membrane which also contained virus particles in different stages of organization. The virion of BYSMV is believed to consist of two coaxial helices, the inner derived from the tubules and the outer being formed between the inner helix and the outer envelope of the virus. A hypothesis is advanced for the morphogenesis of BYSMV in insect tissue which differs from that occurring in plants.

INTRODUCTION

Several viruses of Gramineae are transmitted in the circulative manner by the Delphacid planthopper *Laodelphax striatellus* Fallén (Kisimoto, 1973; Conti, 1974). One, a rhabdovirus, is barley yellow striate mosaic (BYSMV), cryptogram */*: */*: U/U: S,I/Au.

BYSMV has been reported only from Northern Italy (Conti, 1969, 1972) but it may be related or identical to two other rhabdoviruses having the same vector and host-range, northern cereal mosaic virus in Japan (Shikata & Lu, 1967) and a virus infecting durum wheat in Southern France (Signoret, Giannotti & Alliot, 1972; Leclant & Signoret, 1976).

In common with lettuce necrotic yellows virus (LNYV; Wolanski & Chambers, 1971) and American wheat striate mosaic virus (WSMV; Vela & Lee, 1974), BYSMV infection results in the formation of virus matrices or viroplasms in the cytoplasm of infected plant cells (Conti & Appiano, 1973). Viroplasms, however, are more frequently associated with rhabdoviruses which infect vertebrate animals (Hummeler, Koprowski & Wiktor, 1967; Shope *et al.* 1970; Zajac & Hummeler, 1970; Murphy *et al.* 1972).

BYSMV, LNYV and WSMV also multiply in the tissues of their insect vectors and may therefore be considered as 'bridging' viruses, having both invertebrate animals and plants as hosts. The appearance and intracellular localization of LNYV in aphid tissue has been studied by O'Loughlin & Chambers (1967) but attempts to detect WSMV in its leafhopper

vector have been unsuccessful (Sinha & Behki, 1972). This paper reports electron microscopic observations of BYSMV in the tissues of *L. striatellus*.

METHODS

The BYSMV isolate was obtained from planthoppers (Conti, 1969) and maintained in barley. The planthoppers used in this particular study were selected from *Laodelphax striatellus* collected near Turin, Italy, in a BYSMV-free area. The insects acquired BYSMV by feeding as young nymphs on infected barley. Planthoppers of the same colonies, not exposed to the virus, were used as controls.

One month after acquisition, the planthoppers were dissected in phosphate buffer, 0·1 M, pH 7·0, the salivary glands fixed in 3 % glutaraldehyde and 1 % OsO_4, both in 0·057 sodium cacodylate buffer, dehydrated in an acetone series including 70 % acetone saturated with uranyl acetate, and embedded in Epon 812 (Luft, 1961). During preparation the specimens were kept at 4 °C.

Sections, mounted on carbon-covered, collodion-coated grids, were stained with lead citrate for 5 min (Reynolds, 1963) and examined in a Siemens Elmiskop IA electron microscope.

RESULTS

The salivary glands of *L. striatellus* have a principal gland with eight kinds of follicles and an accessory gland with two main secretory cells and several non-secretory cells (Sogawa, 1965). Particles of BYSMV and related structures were found in the cytoplasm, but not the nuclei, mitochondria and other cell organelles of both the principal and the accessory salivary glands. Particles were not found in the tissues of planthoppers not exposed to BYSMV. The cells of infected insects were otherwise not very different structurally from those of the controls.

Large aggregates of BYSMV virions, identical with those observed in plant tissue, were frequently found in the infected cells. The aggregates were consistently located in dense, granular cytoplasm, interspersed between large vacuoles, and consisted of parallel alignments of bacilliform particles surrounded by a single-layer membrane (Fig. 1 *a*, *b*).

The virions of BYSMV were 300 to 320 nm by 40 nm and showed a thin outer layer (Fig. 1 *c*) which may consist of the ends of the knob-like projections typical of the rhabdovirus envelope (Appiano & Conti, 1974). A regular cross-striation representing the helical structure of the nucleocapsid was sometimes seen.

The cells of infected planthoppers also contained flexuous tubules of varying length, 18 to 20 nm wide, which were never seen in uninfected controls. Long bundles of such tubules were enclosed within membranous sacs derived from the endoplasmic reticulum (Fig. 2). Fig. 3(*a*) shows several bundles of tubules, in transverse and longitudinal section, which have been released into the cytoplasm by rupture of their enveloping sacs. Short fragments of tubules were occasionally found scattered in cytoplasmic areas (Fig. 3*b*); both these fragments, and the long tubules illustrated in Fig. 2, were in close association with BYSMV particles either 'complete' (virions) or in different stages of organization.

Two types of 'incomplete' particles were seen, one with an inner component separated from a loose envelope by an electron-transparent space, and one consisting of the inner component with an additional layer of electron-opaque material which partially fills the space between it and the envelope. The former were distinguishable in both cross and longitudinal section (Fig. 2, single arrows) while the latter were obvious only when cut longitudinally (Fig. 2, double arrows).

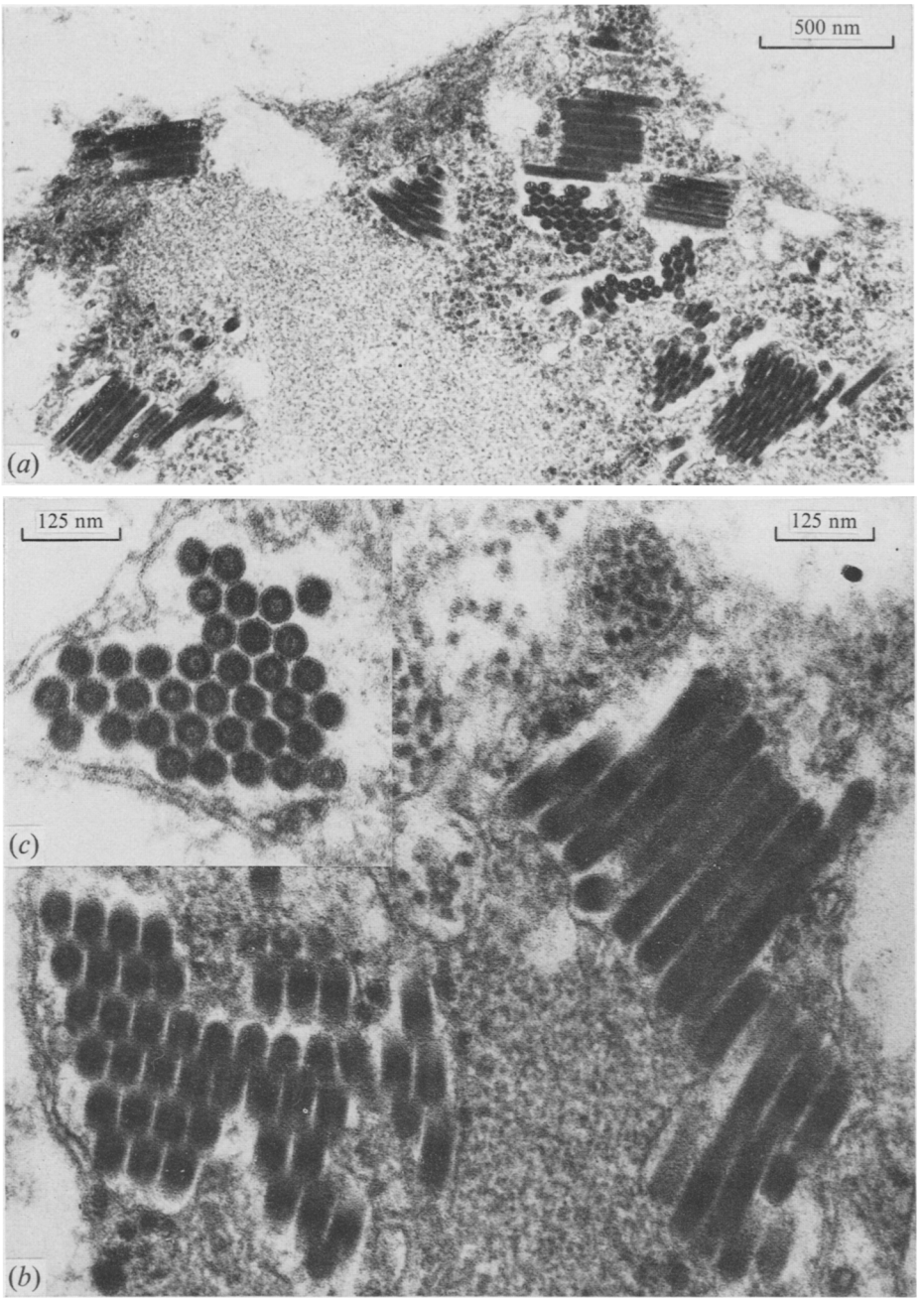

Fig. 1. (*a*) Aggregates of BYSMV virions in dense granular cytoplasm of the salivary gland cells of *Laodelphax striatellus*. (*b*) BYSMV virions at greater magnification. The single-layered membrane which envelopes the aggregates can be seen. The virions at bottom left are cut obliquely and appear bullet-shaped. (*c*) Transverse section of virions, showing outer diffuse layer, inner core and dense material between core and outer layer.

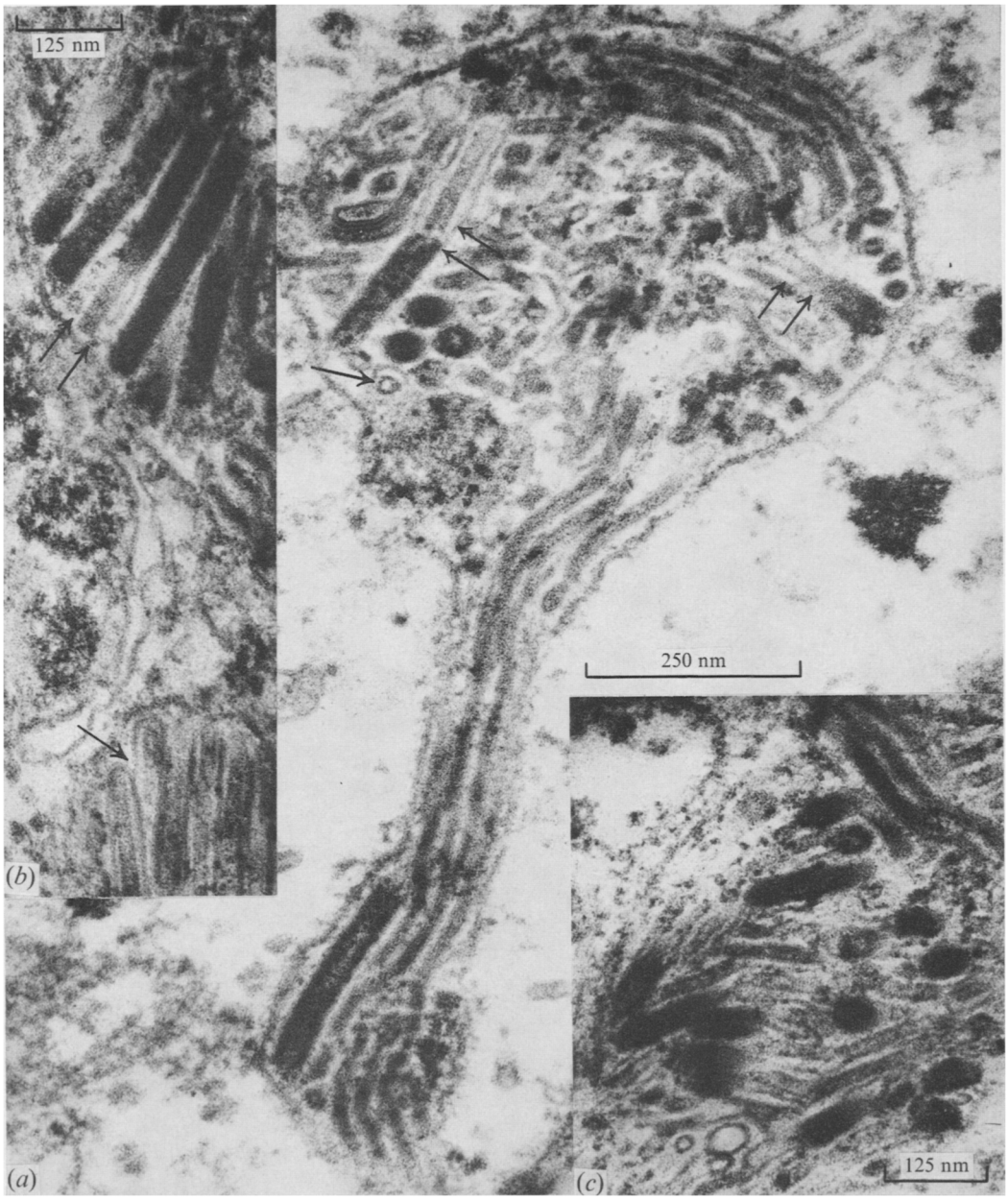

Fig. 2. Membrane-bound cytoplasmic enclaves containing BYSMV particles and the tubules which appear to be the source of the inner helical component of the virion. Different stages in particle organization can be seen where (i) the inner helix is surrounded by a loose envelope (single arrows), and (ii) where electron-dense material partially fills the space between the inner helix and the envelope (double arrows).

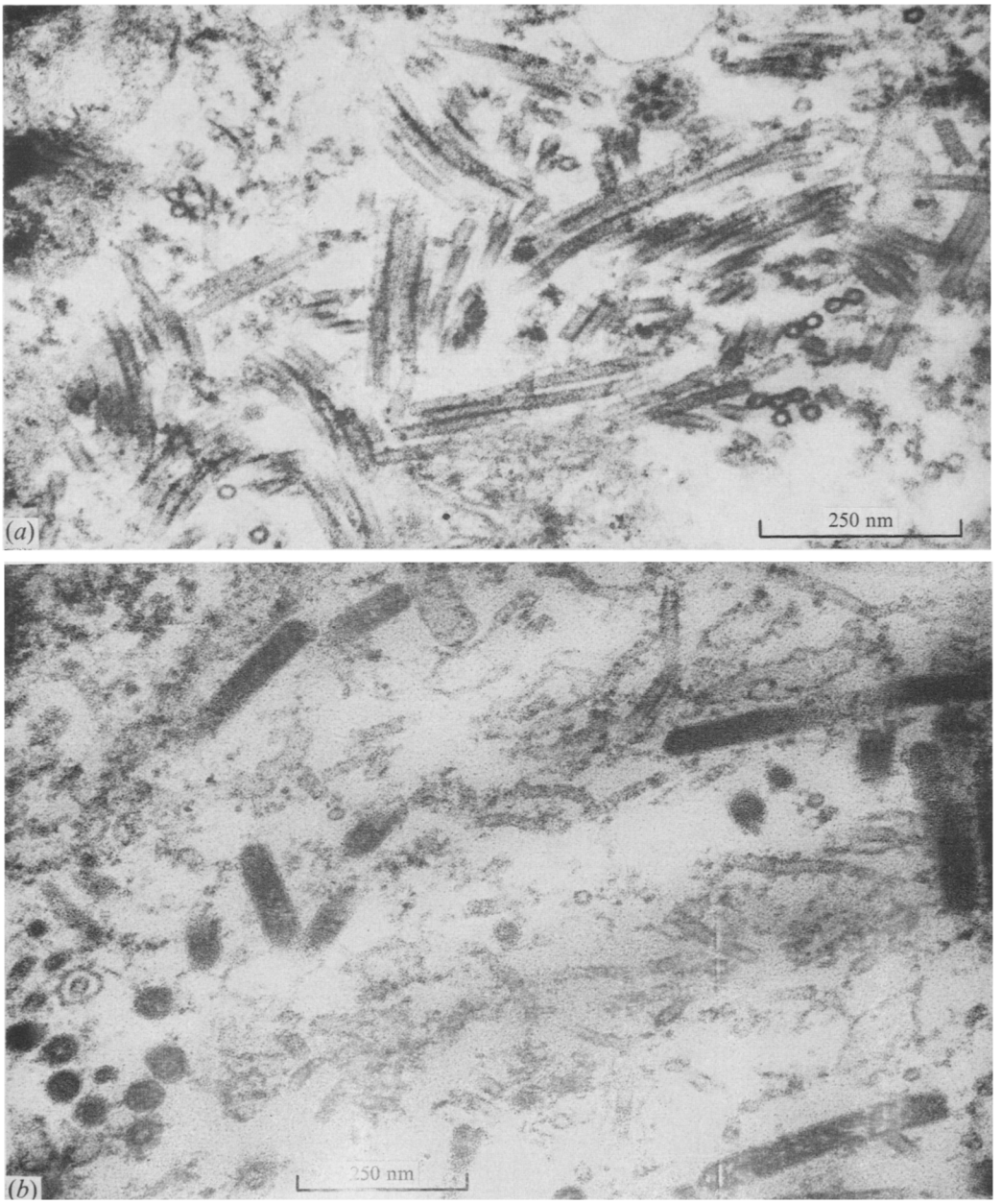

Fig. 3. (*a*) Bundles of tubules, sources for the inner helix of BYSMV. (*b*) Fragments of the inner helix and BYSMV particles.

DISCUSSION

BYSMV persists for more than 30 days in its vector and there is evidence that it is transmitted transovarially in *Laodelphax striatellus* (M. Conti, unpublished data). Our observations show that BYSMV virions occur and, apparently, also multiply in the salivary glands of viruliferous planthoppers, which further indicates that the virus–vector relationship is of the propagative type.

Although some controversy still exists, it is generally agreed that the morphology of plant-infecting rhabdoviruses is bacilliform while animal rhabdoviruses are bullet-shaped (Francki, 1973). In ultrathin sections of insect tissue, virions of BYSMV were bacilliform and were similar in size and appearance to those seen in plants (Conti & Appiano, 1973); bullet-shaped particles were seen only when virions were cut obliquely (Fig. 1*b*) or in particles apparently being assembled (Fig. 2).

The proposed structure of plant rhabdoviruses has been represented by slightly different models (Harrison & Crowley, 1965; Kitajima & Costa, 1966; Herold & Munz, 1967; Hills & Campbell, 1968; Wolanski & Chambers, 1971), which indicate that plant rhabdoviruses consist of a rod-shaped association of protein and RNA, the nucleocapsid, surrounded by a membranous envelope bearing 'bead-like' projections. The nucleocapsid has been generally considered as a unique morphological entity and variously described as an inner body (Harrison & Crowley, 1965), a tubular core (Kitajima & Costa, 1966; Kitajima, Lauritis & Swift, 1969*a*), a hollow cylinder (Herold & Munz, 1967) or a helical layer (Hills & Campbell, 1968).

However, Simpson & Hauser (1966) suggested that the nucleocapsid of vesicular stomatitis virus (VSV), a rhabdovirus that infects vertebrates, consists of two distinct helical structures. Nakai & Howatson (1968) thought the smaller of these helices might be an artefact due to disruption and rearrangement of material from the larger helix. Our observations on BYSMV now suggest that the nucleocapsid of this virus also consists of two coaxial helices which fit the model proposed for VSV by Simpson & Hauser (1966). As our results are based on thin sections rather than negatively stained preparations there should be less likelihood of interference by artefacts.

The inner helix of BYSMV nucleocapsid has been seen as long tubules organized in bundles (Fig. 2 and 3*a*), as short fragments scattered in the cytoplasm, either naked or surrounded by a loose envelope (Fig. 3*b*), or in partially assembled particles (Fig. 2, double arrows). In the latter the outer helix can also be seen partially surrounding the inner helix between it and the envelope. The outer helix is also obvious where it has completely encapsulated the inner helix and the virion is electron-dense.

In transverse sections of particles in plants, three electron-dense layers have been seen (Conti & Appiano, 1973) which may correspond to the virus envelope and the outer and inner helix. The 'central filamentous body' which bridges fragments of negatively stained particles (Conti, 1969) is probably the inner helix of the BYSMV nucleocapsid.

It could be argued that the structures in Fig. 2 were the results of virus disruption rather than formation but at least two facts suggest that this is not so: (i) the tubules are often much longer than the inner helix which would be released by the disruption of a virion, and (ii) the close association of tubules and incomplete particles is similar to that seen during the maturation of two rhabdo-like viruses which infect insects, the *Oryctes* virus (Huger, 1966) and the *Gyrinus natator* virus (Gouranton, 1972).

Peters & Schultz (1975) suggested that one general model for morphogenesis can probably be applied to all rhabdoviruses whether replicating in animal or plant cells while the process of BYSMV morphogenesis appears to be different in plant and insect tissue. It seems clear that BYSMV production in plants is associated with viroplasms (Conti & Appiano, 1973) as in the case of other rhabdoviruses (Wolanski & Chambers, 1971; Vela & Lee, 1974) as well as of viruses outside this group (Gerola & Bassi, 1966; Fujisawa *et al.* 1967; Kitajima, Lauritis & Swift, 1969*b*). With sowthistle yellow vein virus, virogenic matrices have also been detected in the aphid vector cells (Sylvester & Richardson, 1970) but this is not the case

with BYSMV, where, in infected planthoppers, tubular structures are found in association with virus particles.

Similar structures have been found in association with other rhabdoviruses in the tissue of their insect vectors (Chen & Shikata, 1972; Tinsley & Harrap, 1972) but their function is unclear. The long, cross-banded tubules found in *Rubus idaeus* infected with a rhabdovirus have been considered as possible cores of the virions (Jones, Roberts & Murant, 1974); other speculations on the possible functions of tubules associated with non-rhabdoviruses include their involvement in transport of virus (Dales & Chardonnet, 1973), virus assembly (Vidano, 1970) or possibly both (Mayhew & Carroll, 1974).

The multiplication of BYSMV in insect tissue may have the following characteristics. The inner helix of the nucleocapsid is assembled as a long tubule which becomes loosely surrounded by membrane of uncertain origin (possibly of *de novo* formation) which becomes the virus envelope. The outer helix is then assembled between the inner helix and the envelope and the virus is released by constriction of the tubule.

We thank Mr R. H. Turner for help with electron microscopy.

REFERENCES

APPIANO, A. & CONTI, M. (1974). Some observations on barley yellow striate mosaic virus (BYSMV) ultrastructure. *Journal of Submicroscopic Cytology* **6**, 103.

CHEN, M. J. & SHIKATA, E. (1972). Electron microscopy and recovery of rice transitory yellowing virus from its leafhopper vector, *Nephotettix cincticeps*. *Virology* **47**, 483–486.

CONTI, M. (1969). Investigations on a bullet-shaped virus of cereals isolated in Italy from planthoppers. *Phytopathologische Zeitschrift* **66**, 275–279.

CONTI, M. (1972). Barley yellow striate mosaic virus isolated from plants in the field. *Phytopathologische Zeitschrift* **73**, 39–45.

CONTI, M. (1974). *Laodelphax striatellus* as a vector of two cereal viruses in Italy. *Mikrobiologija* **11**, 49–54.

CONTI, M. & APPIANO, A. (1973). Barley yellow striate mosaic virus and associated viroplasms in barley cells. *Journal of General Virology* **21**, 315–322.

DALES, S. & CHARDONNET, Y. (1973). Early events in the interaction of adenoviruses with HeLa cells. IV. Association with microtubules and the nuclear pore complex during vectorial movement of the inoculum. *Virology* **56**, 465–483.

FRANCKI, R. I. B. (1973). Plant rhabdoviruses. *Advances in Virus Research* **18**, 257–345.

FUJISAWA, I., RUBIO-HUERTOS, M., MATSUI, C. & YAMAGUCHI, A. (1967). Intracellular appearance of cauliflower mosaic virus particles. *Phytopathology* **57**, 1130–1132.

GEROLA, F. M. & BASSI, M. (1966). An electron microscopy study of leaf vein tumors from maize plants experimentally infected with maize rough dwarf virus. *Caryologia* **19**, 13–40.

GOURANTON, J. (1972). Development of an intranuclear nonoccluded rod-shaped virus in some midgut cells of an adult insect, *Gyrinus natator* L. (Coleoptera). *Journal of Ultrastructure Research* **39**, 281–294.

HARRISON, B. D. & CROWLEY, N. C. (1965). Properties and structure of lettuce necrotic yellows virus. *Virology* **26**, 297–310.

HEROLD, F. & MUNZ, K. (1967). Morphological studies of maize mosaic virus. I. *Journal of General Virology* **1**, 227–234.

HILLS, G. J. & CAMPBELL, R. N. (1968). Morphology of broccoli necrotic yellows virus. *Journal of Ultrastructure Research* **24**, 131–144.

HUGER, A. M. (1966). A virus disease of the Indian rhinoceros beetle, *Oryctes rhinoceros* (L.) caused by a new type of insect virus, Rhabdion Virus *Oryctes* gen. n. sp. n. *Journal of Invertebrate Pathology* **8**, 38–51.

HUMMELER, K., KOPROWSKI, H. & WIKTOR, T. J. (1967). Structure and development of rabies virus in tissue culture. *Journal of Virology* **1**, 152–170.

JONES, A. T., ROBERTS, I. M. & MURANT, A. F. (1974). Association of different kinds of bacilliform particle with vein chlorosis and mosaic diseases of raspberry (*Rubus idaeus*). *Annals of Applied Biology* **77**, 283–288.

KISIMOTO, R. (1973). Leafhoppers and planthoppers. In *Viruses and Invertebrates*, pp. 137–156. Edited by A. J. Gibbs. Amsterdam: North Holland.

KITAJIMA, E. W. & COSTA, A. S. (1966). Morphology and developmental stages of *Gomphrena* virus. *Virology* **29**, 523–539.

KITAJIMA, E. W., LAURITIS, J. A. & SWIFT, H. (1969*a*). Morphology and intracellular localization of a bacilliform latent virus in sweet clover. *Journal of Ultrastructure Research* **29**, 141–150.

KITAJIMA, E. W., LAURITIS, J. A. & SWIFT, H. (1969*b*). Fine structure of Zinnia leaf tissues infected with dahlia mosaic virus. *Virology* **39**, 240–249.

LECLANT, F. & SIGNORET, P. A. (1976). Premières resultats concernant la transmission d'un rhabdovirus du blé par le delphacide *Laodelphax striatellus* Fall. *Compte Rendu IV^e Journées Phytiatrie et Phytopharmacie Circum Mediterranéenes*, Montpellier, Sept. 1974 (in the press).

LUFT, J. H. (1961). Improvements in epoxy resin embedding methods. *Journal of Biophysical and Biochemical Cytology* **9**, 409–414.

MAYHEW, D. E. & CARROLL, T. W. (1974). Barley stripe mosaic virions associated with spindle microtubules. *Science* **185**, 957–958.

MURPHY, F. A., JOHNSON, H. N., HARRISON, A. K. & SHOPE, R. E. (1972). Ultrastructural characterisation of a new member of the Rhabdovirus group – Klamath virus. *Archiv für die gesamte Virusforschung* **37**, 323–331.

NAKAI, T. & HOWATSON, A. F. (1968). The fine structure of vesicular stomatitis virus. *Virology* **35**, 268–281.

O'LOUGHLIN, G. T. & CHAMBERS, T. C. (1967). The systemic infection of an aphid by a plant virus. *Virology* **33**, 262–271.

PETERS, D. & SCHULTZ, M. G. (1975). A model for rhabdovirus morphogenesis. *Koninklijke Nederlandse Akademie van Wetenschappen – Amsterdam* **78**, 172–181.

REYNOLDS, E. S. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *Journal of Cell Biology* **17**, 208–212.

SHIKATA, E. & LU, Y. T. (1967). Electron microscopy of northern cereal mosaic virus in Japan. *Proceedings of the Japanese Academy* **43**, 918–923.

SHOPE, R. E., MURPHY, F. A., HARRISON, A. K., CAUSEY, O. R., KEMP, G. E., SIMPSON, D. T. H. & MOORE, D. L. (1970). Two African viruses serologically and morphologically related to rabies virus. *Journal of Virology* **6**, 690–692.

SIGNORET, P. A., GIANNOTTI, J. & ALLIOT, B. (1972). Particules de type viral chez *Triticum durum* Desf. presentant des symptômes de stries chlorotiques. *Annales de Phytopathologie* **4**, 45–53.

SIMPSON, R. W. & HAUSER, R. E. (1966). Structural components of vesicular stomatitis virus. *Virology* **29**, 654–667.

SINHA, R. C. & BEHKI, R. M. (1972). American wheat striate mosaic virus. *C.M.I./A.A.B. Descriptions of Plant Viruses*, No. 99.

SOGAWA, K. (1965). Studies on the salivary glands of rice plant leafhoppers. I. Morphology and histology. *Japanese Journal of Applied Entomology and Zoology* **9**, 275–290.

SYLVESTER, E. S. & RICHARDSON, J. (1970). Infection of *Hyperomyzus lactucae* by sowthistle yellow vein virus. *Virology* **42**, 1023–1042.

TINSLEY, T. W. & HARRAP, K. A. (1972). Viruses in vectors and other hosts. In *Moving Frontiers in Invertebrate Virology*, Monographs in Virology, vol. 6, pp. 27–59. Edited by J. L. Melnick.

VELA, A. & LEE, P. E. (1974). Morphogenesis of wheat striate mosaic virus – the internal nucleoprotein component. *Journal of Ultrastructure Research* **47**, 169–178.

VIDANO, C. (1970). Phases of maize rough dwarf virus multiplication in the vector *Laodelphax striatellus* (Fallen). *Virology* **41**, 218–232.

WOLANSKI, B. S. & CHAMBERS, T. C. (1971). The multiplication of lettuce necrotic yellows virus. *Virology* **44**, 582–591.

ZAJAC, B. A. & HUMMELER, K. (1970). Morphogenesis of the nucleoprotein of vesicular stomatitis virus. *Journal of Virology* **6**, 243–252.

(*Received* 3 *May* 1976)