

Rothamsted Repository Download

A - Papers appearing in refereed journals

Plumb, R. T. and Vince, D. A. 1971. Fixation and electron microscopy of the Rothamsted culture of henbane mosaic virus. *Journal of General Virology*. 13 (Nov), pp. 357-359.

The publisher's version can be accessed at:

- <https://dx.doi.org/10.1099/0022-1317-13-2-357>

The output can be accessed at: <https://repository.rothamsted.ac.uk/item/8ww72>.

© 1 November 1971, Microbiology Society.

Fixation and Electron Microscopy of the Rothamsted Culture of Henbane Mosaic Virus

(Accepted 16 August 1971)

Thin sections of tissue infected by viruses of the potato virus Y (PVY) group often fail to show virus particles. Henbane mosaic virus (HMV) is more concentrated in infected tissue than most PVY group viruses and seemed a suitable subject for electron microscopy. We failed to detect any particles after tissues were subjected to a commonly used double fixation technique but found them in tissue fixed with osmic acid; parallel results were found when purified virus preparations were similarly treated.

This paper reports some details of the fine structure of infected tissue and the effect of fixation on virus within the host cell and in purified preparations.

The Rothamsted culture of HMV used has been maintained in *Nicotiana tabacum*, cv. White Burley, usually infected by mechanical inoculation but occasionally with aphids. The virus was purified from this host by a modification of Damirdagh & Shepherd's (1970) procedure using borate instead of phosphate buffer throughout, and infectivity was confirmed by inoculation on *Nicotiana sylvestris*.

Preliminary investigation of infected leaves involved fixation with both glutaraldehyde and osmic acid. Small strips of leaf were put in 2.5% (v/v) glutaraldehyde in 0.2 M-phosphate buffer (pH 7) and vacuum-infiltrated for 5 min. After 2 hr the specimens were washed in phosphate buffer containing 0.25 M-sucrose, and fixed for 2 hr in 1% (w/v) osmic acid in the buffer-sucrose mixture. After washing in buffer-sucrose and dehydrating in alcohol the specimens were embedded in epon. Subsequently the first fixation in glutaraldehyde was omitted.

Purified virus was suspended in 0.02 M-borate buffer (pH 8) containing 0.5 M-urea and 0.1% 2-mercaptoethanol, and fixed for 2 hr with 2.5% glutaraldehyde or 1% osmic acid. Virus suspensions fixed in glutaraldehyde were dialysed in sucrose-phosphate buffer before post-fixation in osmic acid. Dialysis was also necessary for particles fixed in glutaraldehyde before negative staining with phosphotungstic acid (PTA).

Sections were stained with 2% (w/v) uranyl acetate followed by Reynold's lead citrate, and dip and purified preparations stained in 1% PTA.

Thin sections of infected leaf fixed in glutaraldehyde and osmic acid gave very similar results to those reported by Harrison & Roberts (1971) for *Atropa* mild mosaic virus (AMMV) infection and support the serological relationship between HMV and AMMV found by Govier & Woods (1971).

Sections of HMV-infected tissue fixed in glutaraldehyde and osmic acid contained abundant pinwheels but no virus particles were seen; leaves fixed in osmium alone showed many filamentous particles in the cytoplasm associated with pinwheels, the filaments were 12 nm. wide and resembled HMV particles. The particle-like filaments were seldom seen free in the cytoplasm, and most were arranged in uniseriate rows closely appressed to the pinwheel lamellae and parallel to their long axes. This was confirmed by the association, in partially purified preparations, of particles with lamellae apparently derived from pinwheels. Particles cut transversely showed as dots, distinguishable from ribosomes by their regular spacing and small size. This arrangement of particles was also described by Edwardson, Purcifull &

Christie (1968) in tissue infected with water-melon mosaic virus, a member of the PVY group with particles 725 nm. long, and our results suggest that the much longer and stiffer particles of HMV (Watson, Plumb & Woods, 1971) behave in much the same way.

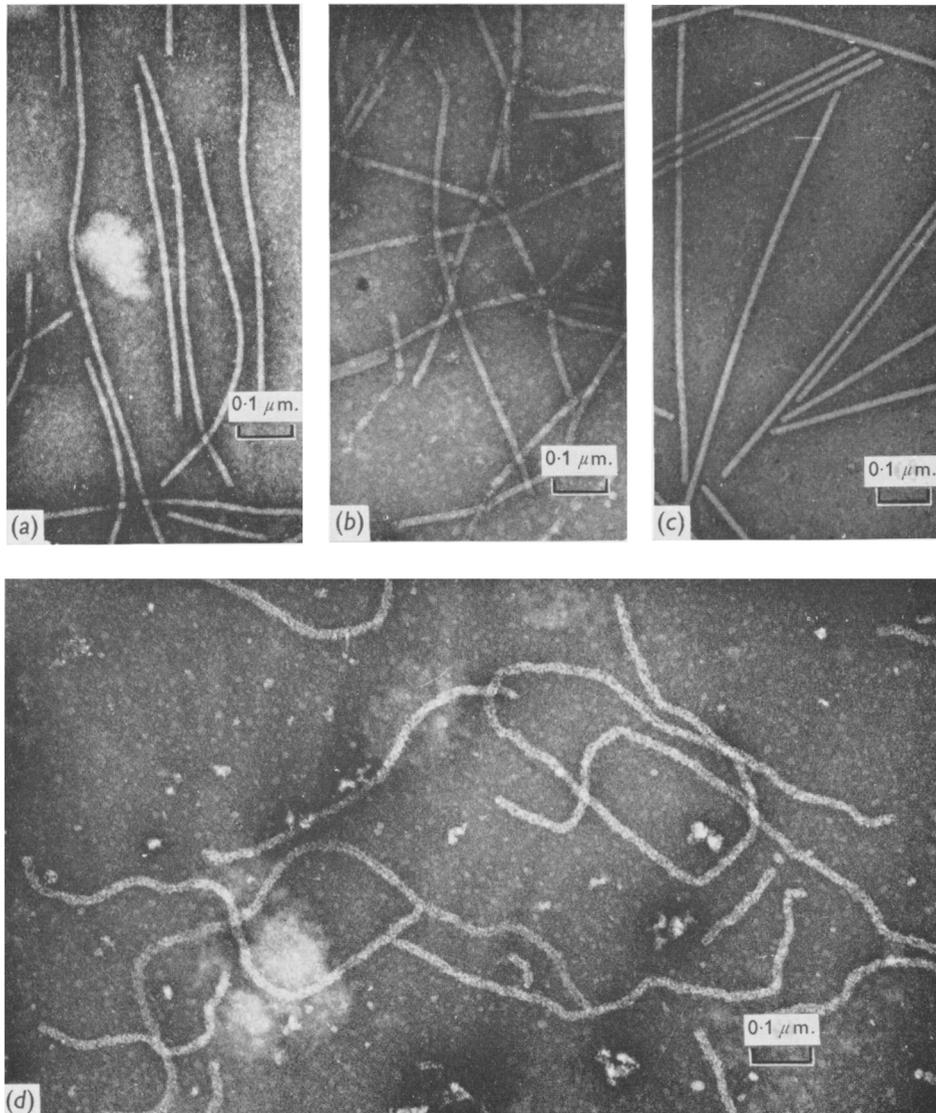


Fig. 1 *a*. Purified virus fixed in glutaraldehyde. *b*. Purified virus fixed in osmic acid. *c*. Purified virus untreated. *d*. Purified virus fixed in glutaraldehyde and then osmic acid.

When purified preparations of HMV were fixed in glutaraldehyde (Fig. 1 *a*) or osmic acid (Fig. 1 *b*) alone they differed little from unfixed particles (Fig. 1 *c*), but after double fixation they were swollen, deformed and flexuous and some showed an electron-translucent core (Fig. 1 *d*). If similar changes occurred in infected tissues during fixation it may explain why particles of HMV and other PVY group viruses are only rarely seen after double fixation.

Particles of sugar-beet mosaic virus were also found in infected tissue fixed with osmic acid alone but not after double fixation (Vince & Watson, 1971), although the effects of the fixatives on the purified virus has not been investigated.

Although this 'fixative effect' may explain why some PVY group viruses are not readily seen, it may be a very specific effect because virus-like filaments have been described for other PVY viruses in double-fixed tissue (Arnott & Smith, 1967; Herald & Weibel, 1963; Gardner, 1969; Russo & Martelli, 1969; Harrison & Roberts, 1971). However, our results do suggest that osmium alone may be a more useful fixative for PVY group viruses than the more commonly used double fixation procedures.

*Rothamsted Experimental Station
Harpenden, Herts.*

R. T. PLUMB
DOREEN A. VINCE

REFERENCES

- ARNOTT, J. H. & SMITH, K. M. (1967). Electron microscopy of virus-infected sunflower leaves. *Journal of Ultrastructure Research* **19**, 173.
- DAMIRDAGH, I. S. & SHEPHERD, R. J. (1970). Purification of the tobacco etch and other viruses of the potato Y group. *Phytopathology* **60**, 132.
- EDWARDSON, J. R., PURCIFULL, D. E. & CHRISTIE, R. G. (1968). Structure of cytoplasmic inclusions in plants infected with rod-shaped viruses. *Virology* **34**, 250.
- GARDNER, W. S. (1969). Ultrastructure of *Zea mays* leaf cells infected with Johnson-grass strain of sugarcane mosaic virus. *Phytopathology* **59**, 1903.
- GOVIER, D. A. & WOODS, R. D. (1971). (In the Press)
- HARRISON, B. D. & ROBERTS, I. M. (1971). Pinwheels and crystalline structures induced by *Atropa* mild mosaic virus, a plant virus with particles 925 nm. long. *Journal of General Virology* **10**, 71.
- HERALD, F. & WEIBEL, J. (1963). Electron microscopic demonstration of sugarcane mosaic virus particles in cells of *Saccharum officinarum* and *Zea mays*. *Phytopathology* **53**, 469.
- RUSO, M. & MARTELLI, G. P. (1969). Cytology of *Gomphrena globosa* L. plants infected by beet mosaic virus (BMV). *Phytopathologia Mediterranea* **8**, 65.
- VINCE, D. A. & WATSON, M. A. (1971). In preparation.
- WATSON, M. A., PLUMB, R. T., & WOODS, R. D. (1971). *Rep. Rothamsted exp. stn. for 1970, Pt. 1*, p. 124.

(Received 31 March 1971)