J. gen. Virol. (1971), 11, 193–195
Printed in Great Britain

An Unusual Inclusion in Plants Infected with a Tobacco Mosaic Virus Mutant

(*Accepted* 2 *March* 1971)

The mutant Ni 118 obtained from tobacco mosaic virus (TMV) treated with nitrous acid produces defective coat protein, especially when multiplying in plants kept at 35°. At this temperature, few complete virus particles are formed, and extracts of infected plants contain mostly free infective RNA and insoluble coat protein. At 20°, however, Ni 118 multiplies as well as the type strain of TMV (Jockusch, 1966; Kassanis & Bastow, 1971*a*). To gain more information about the conditions in infected cells we examined them by light and electron microscopy. Light microscopy was done by phase-contrast on living cells from epidermal strips of the undersides of Samsun tobacco leaves 1 week after inoculation. For electron microscopy the methods of Milne (1970) were used; pieces of leaf were fixed in glutaraldehyde and then osmium tetroxide, dehydrated in acetone, soaked in uranyl acetate in acetone and embedded in Epon. After sectioning, the material was stained in lead citrate.

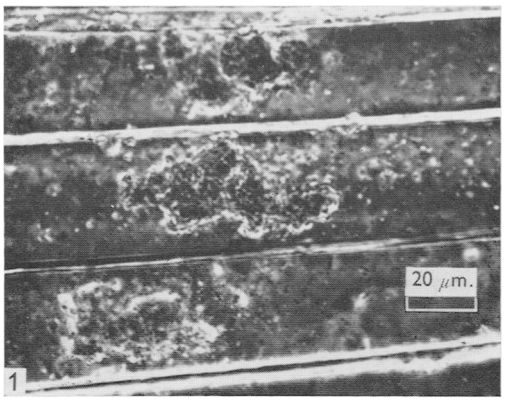

Fig. 1. Light microscopy of epidermal cells from a tobacco plant infected with Ni 118 at 35°.

The light microscope showed that most of the epidermal cells of infected plants at 35° contained 1 to 3 large, lobed, amorphous, dense inclusions or X-bodies (Fig. 1). Fig. 2 shows the appearance in the electron microscope of these inclusions, which are not crystalline and are of uniform density. Similar inclusions were seen in electron micrographs from Samsun tobacco infected with the flavum strain of TMV (Kolehmainen, Zech & von Wettstein, 1965), which reacts to elevated temperatures like Ni 118 (Jockusch, 1966). Virus particles were never seen in cells from plants infected with Ni 118 and kept at 35°.

Light microscopy of epidermal strips from infected plants grown at 20° showed fewer and smaller inclusions than at 35°, and also some small crystalline aggregates. There were occasional hexagonal crystals of virus particles of the type formed in cells infected with the type strain of TMV. In the electron microscope the amorphous inclusions resembled those produced at 35°. Complete virus particles were seen.

In plants infected with both Ni 118 and type strain and kept at 35° the amorphous inclusions were more broken up than in plants infected with Ni 118 alone. There is circum-

stantial evidence (Atabekov *et al.* 1970; Kassanis & Bastow, 1971*b*) that in dual infections at 35° some of the Ni 118 RNA is coated by protein of type TMV to produce stable particles containing Ni 118 RNA. Therefore, it is of interest that in the light microscope we have seen in the same cell both an Ni 118 inclusion and a crystalline hexagonal inclusion indistinguishable from that usually formed by the type strain when inoculated alone.

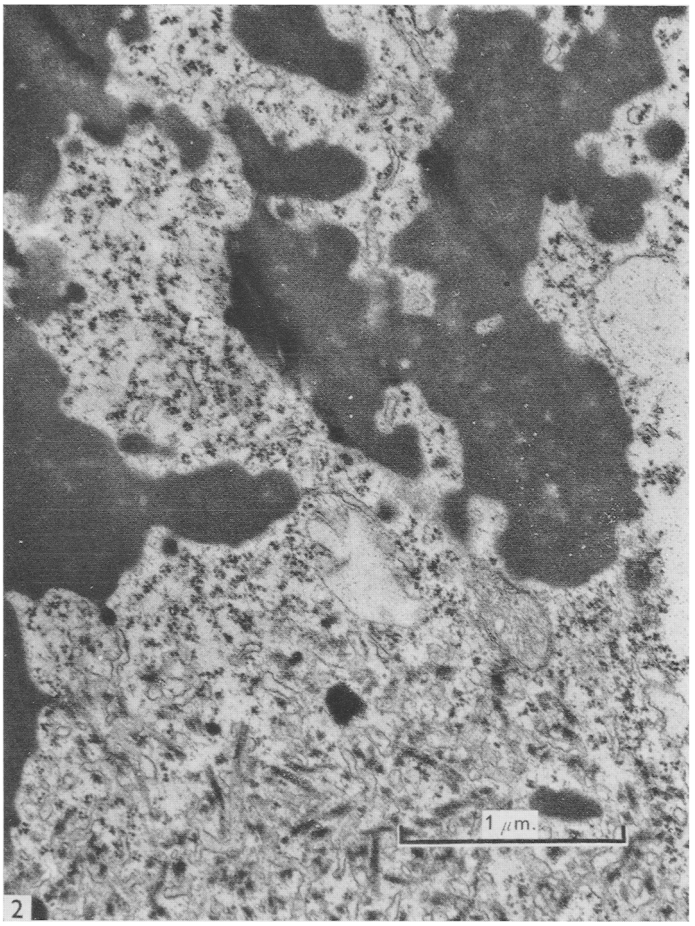

Fig. 2. Electron microscopy of a mesophyll cell from a tobacco plant infected with Ni 118 at 35°, showing part of an inclusion.

Tests were not made to discover the nature of the amorphous inclusions, but they probably consist of the insoluble defective Ni 118 coat protein. However, the inclusions stain very densely, whereas TMV particles, even in compact virus crystals, stain only lightly. They do not look like lipid or aggregated nucleic acid.

The lower half of Fig. 2 shows part of a region containing dense filaments such as described by Shalla (1964), Kolehmainen *et al.* (1965), Milne (1966) and others. These filamentous regions occurred as often in plants infected with Ni 118, whether kept at 35° or 20°, as they do in plants infected with type strain. There has been discussion (see Milne, 1966) as to whether the filamentous structures are precursors of virus particles; their occurrence in

conditions where virus particles are not formed (Ni 118 at 35°) does not clear this doubt because the multiplication of Ni 118 may have stopped at the precursor stage.

Rothamsted Experimental Station B. KASSANIS
Harpenden R. G. MILNE
Hertfordshire

REFERENCES

ATABEKOV, J. G., SCHASKOLSKAYA, N. D., ATABEKOVA, T. I. & SACHAROVSKAYA, G. A. (1970). Reproduction of temperature-sensitive strains of TMV under restrictive conditions in the presence of temperature-resistant helper strains. *Virology* **41**, 397.

JOCKUSCH, H. (1966). Temperatursensitive Mutanten des Tabakmosaikvirus. I. *In vivo* Verhalten. *Zeitschrift für Vererbungslehre* **98**, 320.

KASSANIS, B. & BASTOW, C. (1971 *a*). The relative concentration of infective intact virus and RNA of four strains of tobacco mosaic virus as influenced by temperature. *Journal of General Virology* **11**, 157.

KASSANIS, B. & BASTOW, C. (1971 *b*). Phenotypic mixing between strains of tobacco mosaic virus. *Journal of General Virology* **11**, 171.

KOLEHMAINEN, L., ZECH, H. & VON WETTSTEIN, D. (1965). The structure of cells during tobacco mosaic virus reproduction. Mesophyll cells containing virus crystals. *Journal of Cell Biology* **25** (2), 77.

MILNE, R. G. (1966). Multiplication of tobacco mosaic virus in tobacco leaf palisade cells. *Virology* **28**, 79.

MILNE, R. G. (1970). An electron microscope study of tomato spotted wilt virus in sections of infected cells and in negative stain preparations. *Journal of General Virology* **6**, 267.

SHALLA, T. (1964). Assembly and aggregation of tobacco mosaic virus in tomato leaflets. *Journal of Cell Biology* **21**, 253.

(*Received* 14 *December* 1970)