A NOTE ON THE OBSERVATION OF VIRUSES IN THE CELLS OF INFECTED PLANTS

by

KENNETH M. SMITH

Plant Virus Research Unit, Molteno Institute, Cambridge and
Laboratory of Physical Biology, National Institute of Arthritis and Metabolic Diseases,
National Institutes of Health, U.S. Public Health Service, Federal Security Agency,
Bethesda 14, Maryland (U.S.A.)

By the use of the electron microscope much information has been obtained on the size and shape of virus *in vitro*. Little, however, is known of the appearance and distribution of viruses as they occur in the living cells of the host. This is due to the fact that it is not possible to examine by means of the electron microscope the virus in the living cell. The next best thing, therefore, is the observation of the virus in the cell after fixation. This is best achieved by the examination of thin sections.

So far as the plant viruses are concerned, not very much work has been carried out on these lines apart from a study by Black, Morgan and Wyckoff¹. They cut sections of tobacco leaves infected with tobacco mosaic virus and observed the virus sometimes in fibrous masses and sometimes as single filaments in the cytoplasm. In the present study, the viruses selected were those of tomato bushy stunt and turnip yellow mosaic, and sections were cut of *Datura Stramonium* and Chinese cabbage plants infected with these two viruses respectively. On *Datura* the bushy stunt virus causes a marked green and yellow mottle, together with some necrosis, and portions of young leaves showing the yellow mottle were used for the work. Infected Chinese cabbage plants which show a still brighter mosaic mottle, were treated similarly. Necrotic areas were avoided.

Small pieces of leaf were cut out and fixed in osmic acid, using a two per cent solution with a citrate-phosphate buffer added. They were embedded in a mixture of 50 parts butyl, and 10 parts methyl, methacrylate. The sections were cut on a heavy-type rotary microtome, using the method of thermal expansion. They were shadowed with palladium and photographed on an R.C.A. electron microscope. Careful examination was made at the same time of comparable sections cut from healthy *Datura* and Chinese cabbage plants.

In examining sections of virus-diseased tissues of plants with the electron microscope, one of the difficulties is to recognise the virus particles among the varied constituents of the plant cell. In the case of a virus with a characteristic shape such as the tobacco mosaic virus this is less difficult. Where small spherical viruses like those of tomato bushy stunt and turnip yellow mosaic are concerned, the only method is to make careful comparison with exactly similar virus-free tissues. Even so it does not do to be too dogmatic and the accompanying electron micrographs are published on the assump-

Reference p. 214.

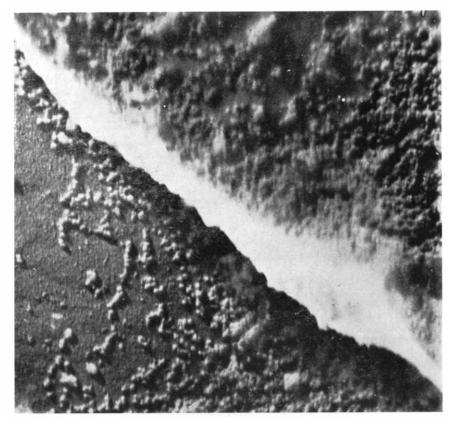


Fig. 1. Section through part of two adjoining cells in a leaf of *Datura Stramonium*, infected with tomato bushy stunt virus.

tion that the spherical particles seen in the cells are those of the two viruses concerned. This assumption is supported by the facts that the spherical particles are about the right size for these viruses and that similar particles were not observed in healthy cells of the same age.

At first sight it is rather astonishing, if these particles are indeed the viruses, that there should be such a great quantity in the cells. The section shown in Fig. 3 actually resembles a purified preparation of the bushy stunt virus *in vitro*. In Fig. 1 the section is cut through two adjoining cells and the cell wall can be seen in the middle of the photograph. In Fig. 2 may be seen further apparent masses of bushy stunt virus particles.

Fig. 4. is a section through an infected Chinese cabbage leaf. The cell wall can be seen at the top of the photograph with the apparent turnip yellow mosaic virus particles beneath. The particles appear smaller than those in the bushy stunt preparations and this is in accord with what is known of the sizes of these two viruses.

Grateful acknowledgements are due to Dr R. W. G. WYCKOFF in whose laboratory this work was carried out, to Dr H. Noda for much helpful advice, and to the National Institutes of Health, Bethesda, Maryland for an appointment as Visiting Scientist which made these studies possible.

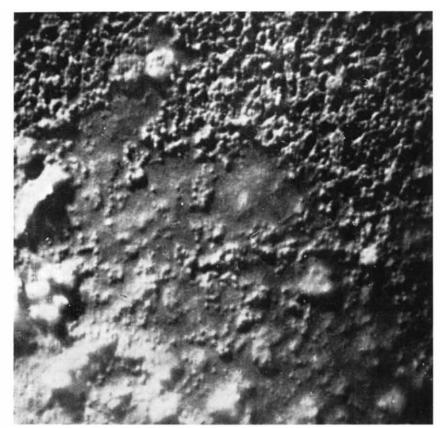


Fig. 2

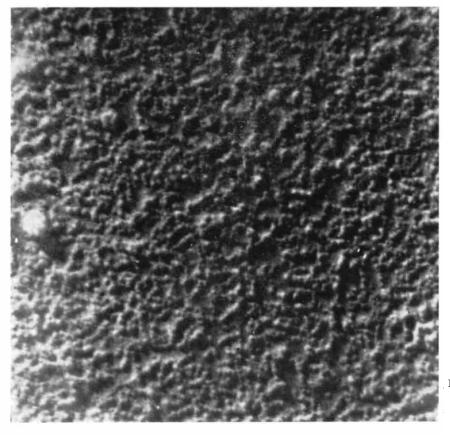


Fig. 3

Opposite page:

Figs. 2 and 3. Sections through portions of cells of Datura Stramonium infected with tomato bushy stunt virus. Note the large masses of what are apparently virus particles.



Fig. 4. Section through part of a cell in a leaf of Chinese cabbage infected with the turnip yellow mosaic virus. Note the similar but slightly smaller particles.

All magnifications approximately × 50,000

SUMMARY

Thin sections of plant tissue infected with two viruses have been examined on the electron microscope. The plants were *Datura Stramonium* and *Brassica Chinensis* (Chinese cabbage), infected respectively with tomato bushy stunt and turnip yellow mosaic viruses.

Spherical particles of approximately the same size as the two viruses can be observed in the cells. If these particles are the actual virus, then the cells contain extremely large quantities, especially in the case of tomato bushy stunt.

RÉSUMÉ

Nous avons examiné au microscope électronique des sections minces de tissus végétaux infectés par deux virus. Il s'agissait des plantes *Datura Stramonium* et *Brassica Chinensis* et des virus "bushy stunt" de la tomate et mosaíque jaune du navet.

L'on peut observer dans les cellules des particules sphériques qui ont approximativement la taille des deux virus. Si ces particules sont les vrais virus, alors les cellules contiennent des quantités de virus extrêmement grandes, particulièrement dans le cas de "bushy stunt" de la tomate.

ZUSAMMENFASSUNG

Dünne Schnitte von mit zwei Viren infizierten Pflanzengeweben wurden unter dem Elektronenmikroskop untersucht. Die Pflanzen waren *Datura Stramonium* und *Brassica Chinensis* (chinesischer Kohl), die mit Tomaten-"bushy stunt"-Virus, bzw. dem Gelben Mosaic Virus der Rübe infiziert waren.

Sphärische Teilchen von ungefähr der gleichen Grösse wie die beiden Viren konnten in den Zellen beobachtet werden. Wenn diese Teilchen die tatsächlichen Viren sind, dann enthält die Zelle davon äusserst grosse Mengen, bes. im Falle des Tomaten-"bushy stunt"-Virus.

REFERENCE

¹ L. M. Black, C. Morgan and R. W. G. Wyckoff, Proc. Soc. Exptl. Biol. Med., 73 (1950) 119.

Received Sept. 2nd, 1952