Effects of Parsnip Yellow Fleck Virus on Plant Cells

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SUMMARY

Light and electron microscopy of spinach and Nicotiana clevelandii leaf tissue infected with parsnip yellow fleck virus (PYFV) and of chervil containing PYFV either alone or together with its ‘helper’ virus, anthriscus yellows (AYV), showed that cells of all species contained inclusion bodies not found in virus-free plants. Inclusions occurred in most types of leaf cell and consisted of vesicles probably derived from the endoplasmic reticulum, other smaller vesicular structures, and straight tubules about 30 nm in diam.; mitochondria and Golgi bodies often occurred around the periphery. Older inclusion bodies were composed almost entirely of the straight tubules.

Tubules about 45 nm in diam. and containing virus-like particles were often associated with the plasmodesmata and were sheathed by outgrowths of cell-wall material. Tubular structures occurred also in the sieve tubes and some of them contained virus-like particles. Most cell organelles appeared normal but many chloroplasts possessed peripheral vesicles bounded by a single membrane and some contained rectangular microcrystalline structures. No effects attributable to AYV were noted.

INTRODUCTION

Parsnip yellow fleck virus (PYFV; Murant & Goold, 1968) has a combination of properties unlike those of any other well-characterized virus. It is transmitted by the aphid Cavariella aegopodii in the semi-persistent manner but, unlike the few other viruses of this type whose particles have been seen, it has isometric particles about 30 nm in diam. Moreover, its transmission by aphids depends on the presence of a ‘helper’ virus, anthriscus yellows (AYV). Because of these distinctive properties it is of interest to know more about the distribution of the virus in infected plants and about its effects on plant cells. This paper reports the results of light and electron microscope studies on infected plants.

METHODS

Virus isolates. The PYFV-infected tissue examined was from plants manually inoculated with one or other of two local lesion isolates or infected with a field isolate by means of aphids (Cavariella aegopodii). The two local lesion isolates were those described by Murant & Goold (1968): A-421, from Anthriscus sylvestris (cow parsley), and P-121, from parsnip; they are distantly related to each other serologically and differ somewhat in host range (Murant & Goold, 1968). The field isolate was from A. sylvestris and was indistinguishable from A-421 serologically, and in host range and symptom production; umbelliferous plants infected with this isolate by means of aphids also became infected with AYV.

Preparation and examination of thin sections. The following kinds of plant material were

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examined: (i) *Nicotiana clevelandii* infected with isolate P-121. Samples were collected from systemically infected young leaves at several times between 15 and 25 days after the plants were inoculated. (ii) *Spinacia oleracea* (spinach) infected with isolate A-421 or isolate P-121. Samples were collected from systemically infected young leaves at several times between 7 and 21 days after the plants were inoculated. (iii) *Anthriscus cerefolium* (chervil) infected with isolate P-121 or with the field isolate of PYFV, together with AYV. Systemically infected leaves were collected 5 days after the plants were inoculated, immediately after the first appearance of systemic symptoms. (iv) As controls, comparable healthy material of each of the above species was also examined.

Leaf samples were fixed, dehydrated and embedded by the method of Jones, Kinninmonth & Roberts (1973) and sections cut for light and electron microscopy on an LKB Ultrotome I or a Cambridge Huxley Mark 2 ultramicrotome, using glass knives. For light microscopy, sections 0.5 μm thick were collected, stained with basic fuchsin and methylene blue, and mounted using the method of Roberts & Hutcheson (1974); they were examined by bright field, positive phase contrast and negative phase contrast techniques. For electron microscopy, sections were post-stained with uranyl acetate and lead citrate, using the grid-frame technique of Robertson & Roberts (1972), and examined in a Siemens Elmiskop I electron microscope operating at 80 kV. Examination of consecutive serial sections by either light or electron microscopy confirmed the identity of the structures seen by each method.

**RESULTS**

*Light microscope observations*

Only spinach and *Nicotiana clevelandii* leaf tissue was examined by light microscopy. Two kinds of abnormality were seen in PYFV-infected tissue: ‘inclusion bodies’, consisting of irregularly shaped clumps of material, usually adjacent to the nucleus; and ‘cell wall outgrowths’, similar to those described previously in *N. clevelandii* leaf cells infected with carrot mottle virus (Murant, Roberts & Goold, 1973).

In systemically infected leaf tissue of both species, inclusion bodies and cell wall outgrowths (Fig. 1) were first seen, about 11 days after the plants were inoculated, in cells of the vascular tissue except sieve tubes and xylem vessels. Soon afterwards, small neighbouring areas of palisade and upper epidermal cells became necrotic. Inclusion bodies and cell wall outgrowths later became visible in other parts of the leaf. They appeared first around the necrotic areas, then in cells of the palisade layer and later in cells of the spongy mesophyll; by about the 25th day after inoculation they were present throughout the leaf, including the upper and lower epidermis. About half the cells in any leaf section contained inclusions but in *Nicotiana clevelandii* there was a greater proportion in palisade tissue than in spongy mesophyll tissue. When first seen, the inclusions were about 2 to 5 μm in diam. and stained partly blue and partly red, but later they enlarged to 5 to 10 μm (*N. clevelandii*) or 7 to 23 μm (spinach) and stained only pale pink.

In *Nicotiana clevelandii*, cell-wall outgrowths occurred not only on walls shared with adjacent cells but also occasionally on walls bordering intercellular spaces, and were up to 10 to 15 μm long; in one instance an outgrowth was seen to traverse the whole width of the cell, a distance of 30 to 40 μm. Sometimes, outgrowths appeared to grow towards the nucleus or the inclusion body. This was not observed in spinach cells, in which the outgrowths were shorter (2 to 5 μm), broader and more irregular in shape, and occurred only on walls shared with adjacent cells.
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Fig. 1. Light micrographs of leaf cells infected with isolate P-121, showing cell-wall outgrowths (O), inclusion body (I) and nucleus (N). (a) Spinach, (b) and (c) Nicotiana clevelandii.

Fig. 2. Part of a young inclusion body adjacent to the nucleus (N) of a spinach leaf cell infected with isolate P-121. It is bordered by mitochondria (M) and contains ribosome-studded vesicles (V), clusters of smaller vesicles (SV), groups of randomly oriented tubules (T) and granular bodies (GB).

Electron microscope observations

Examination of sections

Ultrathin sections of PYFV-infected leaves of chervil, spinach and Nicotiana clevelandii were examined by electron microscopy. No differences were observed between chervil plants infected with PYFV alone and those infected with PYFV and AYV together, and only slight
differences were noted between spinach plants infected with the two isolates of PYFV. Several kinds of abnormality were found in infected leaves.

**Inclusion bodies**

The ultrastructure of the inclusion bodies was similar in all three species. In young inclusion bodies (Fig. 2), mitochondria and Golgi bodies were often arranged around the periphery
Fig. 4. (a) Plasmodesmatal outgrowths containing virus-like particles in *Nicotiana clevelandii* leaf cell infected with isolate P-121. One outgrowth is seen in longitudinal section, another (arrowed) in transverse section. Inset shows a plasmodesmatal outgrowth containing a double row of virus-like particles; (b) row of virus-like particles enclosed in two concentric tubules. Isolate P-121 in spinach leaf cell.

and the inner part consisted of ribosome-studded vesicles (presumably derived from endoplasmic reticulum), clusters of smaller, more lightly staining vesicles and groups of randomly oriented straight tubules about 30 nm in diam. and up to 1 μm long. In spinach cells infected with isolate A-421 the tubules occurred singly, whereas in cells infected with isolate P-121 they occurred in groups of five to twenty. Young inclusions also contained densely staining granular areas bounded by a single unit membrane.

Older inclusions (Fig. 3) were much larger, lacked the ribosome-studded vesicles and had few of the smaller vesicles. Instead they were composed almost entirely of the 30 nm diam. tubules, together with whorls of membranes (Fig. 3, inset). Mitochondria were still found around the periphery of older inclusions but Golgi bodies were uncommon. Very rarely, tubules containing a single row of virus-like particles were found in inclusion bodies. Although the boundary between the inclusion body and the surrounding cytoplasm was fairly sharply defined, there was no bounding membrane.

**Cell-wall outgrowths**

These were observed by electron microscopy in chervil as well as in spinach and *Nicotiana clevelandii*, and occurred in all kinds of cell, especially in the palisade and vascular tissue (except xylem vessels and sieve tubes). Like the cell-wall outgrowths in *N. clevelandii* infected with carrot mottle virus (Murant *et al.* 1973), they consisted of new cell-wall material sheathing plasmodesmatal tubules. In *N. clevelandii*, the plasmodesmatal outgrowths were
Fig. 5. Tubular structures in sieve tubes. (a) Section from spinach showing the structures in transverse section, with C-, S- and O-shaped configurations; (b) section from chervil showing the structures in longitudinal section, some containing virus-like particles.

up to 15 μm long and usually contained only one tubule (Fig. 4a), whereas in chervil and spinach the outgrowths were 2 to 3 μm long, broader and more irregular in outline and contained up to twenty tubules. The tubules were about 45 nm in diam. and usually contained a row of densely staining virus-like particles 25 to 30 nm in diam.; occasionally the particles formed a double row (Fig. 4a, inset). The plasmodesmatal tubules did not extend beyond the end of the sheathing plasmodesmatal outgrowth, but sometimes virus-like particles were seen in the cytoplasm around the end of the outgrowth. Tubules containing virus-like particles, but without a sheath of cell-wall material, were sometimes observed in the cytoplasm. They were not seen to be associated with plasmodesmata and it is therefore not certain whether they were similar in nature to the plasmodesmatal tubules or were distinct structures. On two occasions rows of particles were seen surrounded by two concentric tubules (Fig. 4b) similar to those found with strawberry latent ringspot virus (Roberts & Harrison, 1970).

**Tubular structures and virus-like particles in sieve tubes**

Tubular structures somewhat different from those seen in inclusion bodies and cell-wall outgrowths occurred in a small proportion of sieve tubes (Fig. 5a, b), and less frequently in *Nicotiana clevelandii* than in spinach and chervil. The structures were up to about 1 μm long and about 45 nm in diam. Most, however, were not complete tubes but were C-shaped or S-shaped in cross section. Virus-like particles occurred, apparently, only within the complete
tubules (Fig. 5a, b); in some sections the tubules seemed to be associated with the plasmodesmata connecting the sieve tubes to companion cells.

**Cell organelles**

Except for their association with inclusion bodies, the mitochondria and Golgi bodies seemed normal. Nuclei, however, were often invaginated by areas of cytoplasm or sometimes, in *Nicotiana clevelandii*, by the cell-wall outgrowths. As with carrot mottle virus (Murant et al. 1973), there was no evidence that the nuclear membrane was penetrated by the outgrowths.

Chloroplasts in spinach and *Nicotiana clevelandii* showed various abnormalities. They were often distorted, appearing spherical or pleomorphic, and contained many lipid droplets; frequently they possessed small vesicles (Fig. 6) arranged peripherally but not always immediately adjacent to the chloroplast membrane. The vesicles possessed only a single bounding membrane. In spinach, chloroplasts also sometimes contained rectangular microcrystalline structures of unknown composition (Fig. 6).

**DISCUSSION**

Many of the ultrastructural changes in cells infected with PYFV are similar to those reported for other viruses but, to our knowledge, no other single virus induces all of the features we have observed. Plasmodesmatal and cytoplasmic tubules containing virus particles are a common feature of cells infected with some kinds of isometric viruses, and plasmodesmatal outgrowths have been reported with several: maize rough dwarf (Gerola & Bassi, 1966), dahlia mosaic (Kitajima & Lauritis, 1969), cowpea mosaic (Van der Scheer & Groenewegen, 1971), bean pod mottle (Kim & Fulton, 1971), cauliflower mosaic (Conti et al. 1972), cherry leaf roll (Jones et al. 1973), carrot mottle (Murant et al. 1973) and tobacco ringspot (Halk & McGuire, 1973). Inclusion bodies containing membranous material are also common, but those induced by isometric viruses seldom contain tubules like those we have found with PYFV. Tubules are however reported as a component of inclusion bodies
induced by some rod-shaped viruses. The tubules in the X-bodies of tobacco plants infected with tobacco mosaic virus may be composed of virus protein (X-protein) (Kolehmainen, Zech & Von Wettstein, 1965; Esau, 1968). Large masses of loosely packed convoluted tubules about 40 nm in diam. occurred in cells infected with soil-borne wheat mosaic virus (Peterson, 1970), and inclusion bodies in cells infected with wheat spindle streak mosaic virus contained membranous tubes (Hooper & Wiese, 1972). However, none of these tubule-containing inclusions resembled the PYFV inclusions in the details of their fine structure. It seems probable that inclusion bodies induced by plant viruses are the sites of synthesis of virus products; those induced by cowpea mosaic virus are thought to be sites of virus RNA replication (de Zoeten, Assink & van Kammen, 1974).

Another unusual feature of leaves infected with PYFV is the presence of tubular structures with virus-like particles in the sieve tubes. Murant et al. (1973) observed tubules in sieve tubes of *Nicotiana clevelandii* infected with carrot mottle virus but they were smaller in diam. than those found with PYFV, did not contain virus-like particles and did not display C-shaped or S-shaped configurations in cross section.

The chloroplast vesicles in PYFV-infected cells differed from those that are characteristic of infection with tymoviruses (Hatta, Bullivant & Matthews, 1973; Matthews, 1973) in not being immediately adjacent to the chloroplast surface and in possessing only a single, not a double, bounding membrane. They also differed in appearance from those induced by barley stripe mosaic virus (Carroll, 1970).

The ultrastructural changes induced by PYFV were similar in three different hosts and with two serologically different isolates of the virus. The changes suggest that PYFV is present in all parts of the leaf including the epidermal layers. Therefore absence of virus from epidermal layers cannot be a reason for the relatively long times (more than 10 min; El Nagar & Murant, 1973) which are needed by aphids to acquire PYFV from source plants. Furthermore, throughout this work no ultrastructural differences were observed between chervil plants infected with PYFV alone and others infected with both PYFV and its 'helper' virus, AYV. Ultrastructural studies thus provide no information about the nature or appearance of AYV, nor do they give any clue to the possible mechanism of the dependent transmission of PYFV. However, it seems unlikely that this phenomenon is to be explained by an altered distribution of PYFV in plants that also contain AYV because other studies (El Nagar & Murant, 1973) show that aphids already carrying AYV can acquire PYFV from singly infected plants.

REFERENCES


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