Electron Microscopy of
Some Grasses and Cereals Infected with Cocksfoot Mottle, Phleum Mottle and Cocksfoot Mild Mosaic Viruses

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SUMMARY

Cells of five grass and cereal species infected with cocksfoot mottle virus, phleum mottle virus or cocksfoot mild mosaic virus were examined in ultra-thin sections. The intracellular distribution of the virus particles and their effects on cell ultrastructure appeared dependent more on the severity of the host/virus interaction than on the particular virus or host species involved. Particles of all three viruses occurred in the cytoplasm of mesophyll and phloem companion cells. Those of cocksfoot mottle virus were observed occasionally also in the nucleus. Relatively few particles, randomly distributed in the cytoplasm, were present in cells of plants with mild symptoms. In plants with severe symptoms the particles were numerous and often in near- or true-crystalline aggregates. In partially disrupted cells, virus particles occurred occasionally in membrane-bound, sometimes vesiculated, packets in the cell vacuole. These seemed to originate from the tonoplast. In more severely disrupted cells the tonoplast was lost and virus particles were scattered randomly throughout the vacuole.

Ultrastructural effects ranged from an increase in endoplasmic reticulum and vacuolation of the cytoplasm in the cells of plants with mild symptoms, to the total disintegration of all cell organelles in plants with the severest symptoms. Extrusions, which often encircled a volume of cytoplasm developed from the chloroplasts and mitochondria; membrane-bound vesicles, containing virus particles, appeared in the stroma of chloroplasts and in the mitochondria.

INTRODUCTION

Two similar, but apparently distinct, beetle-transmitted viruses infect cereals and grasses in Britain. Cocksfoot mottle virus (CFMV), cryptogram R/1:1/25:S/S:S/Cl, was isolated originally from Dactylis glomerata L. in south east England (Serjeant, 1967) and phleum mottle virus (PMV), cryptogram R/*:*/24:S/S:S/Cl, from Phleum pratense L. in Wales (Catherall, 1966). Both viruses have isometric particles about 28 nm in diam. and are transmitted by the same vector species, Oulema melanopa L. and Oulema lichenis Voet. (Coleoptera; Chrysomelidae: Catherall, 1970a, b; A’Brook & Benigno, 1972). However, the two viruses are serologically unrelated (Serjeant, 1967). Cocksfoot mild mosaic virus (CMMV), cryptogram R/*::*23:S/S:S/(Ap), was isolated originally from Dactylis glomerata in Germany (Huth, 1968; Huth & Paul, 1972) and later from the same species in Wales (Catherall & Chamberlain, 1975). CMMV is not serologically identical with PMV, but the
two are sufficiently closely related to be considered as strains of a single virus (Paul, Huth & Querfurth, 1974).

On the basis of their stability in vitro (thermal inactivation point, dilution end point and longevity in vitro), and because their particles sediment as a single component, Benigno & A'Brook (1972) concurred with a suggestion by Walters (1969) that CFMV and PMV might be similar to the beetle-transmitted southern bean mosaic virus. More recently, Bercks & Querfurth (1972) found a distant serological relationship between CMMV and two viruses in the beetle-transmitted turnip yellow mosaic group.

Though the particles of CFMV, PMV and CMMV have been studied in leaf-dip and purified preparations, nothing is known of their intracellular distribution nor of their effects on cell ultrastructure. The present work examines these viruses in ultra-thin sections and discusses the data obtained in relation to similar studies made on other plant viruses.

METHODS

PMV was isolated from, and maintained in, Phleum pratense L. CMMV and CFMV were isolated from Dactylis glomerata L.; CMMV was transmitted to, and maintained in Setaria italica (L.) Beauv., which is immune to CFMV; and CFMV was transmitted to, and maintained in, Triticum aestivum L., which is immune to CMMV. Leaves of P. pratense, S. italica and T. aestivum with conspicuous systemic symptoms were ground separately in a mortar with a little 0.1 M-phosphate buffer solution of pH 7 and rubbed on to the leaves of test seedlings of the five species listed in Table 1. Eight weeks later, when the test plants of the majority of susceptible host/virus combinations had developed obvious symptoms, leaf fragments were cut from green, yellow and necrotic areas of systemically mottled or symptomless leaves of similar age from plants of each host/virus combination and from comparable areas of leaves of uninoculated plants. Plants of the two perennial species (P. pratense and D. glomerata) were then cut back, overwintered in an unheated glasshouse and more leaf fragments were cut from the young regrowth the following spring. All leaf fragments were fixed immediately in 3% glutaraldehyde for 3 h at 4 °C and in 0.2% osmium tetroxide buffered with 0.2 M-sodium cacodylate buffer for 2 h at 4 °C. After dehydration in ethanol, the fragments were embedded in Spurr's low viscosity resin (Spurr, 1969). They were sectioned with an LKB Ultrotome I, stained first with uranyl acetate and then with lead citrate (Reynolds, 1963), and examined in an AEI EM 6M electron microscope at 80 kV.

RESULTS

Reaction to infection

The reactions of plants of the five species sectioned to infection with CFMV, PMV and CMMV are shown in Table 1. The susceptibility of barley and timothy to infection with CMMV is recorded for the first time. Such infection was, however, symptomless. CFMV differed from PMV and CMMV in its host range, but PMV and CMMV differed only in their symptomatologies. With the exception of PMV infection in Setaria italica, systemic symptoms appeared about two weeks after inoculation: in S. italica, symptoms of PMV developed after six weeks, but by the eighth week were similar in severity to those of CMMV.

Intracellular distribution of the virus particles

Distinctive spherical or near spherical particles, about 28 nm in diam., often with electron translucent centres (Fig. 1), were easily and consistently detected in the cells of plants from all host/virus combinations listed in Table 1 with the exception of the four recorded as
Having no infection. No similar particles were observed in the cells of uninoculated plants of any of these species; consequently, the particles were presumed to be those of the viruses.

In all hosts, the particles of each virus occurred mainly in the ground cytoplasm of mesophyll and phloem companion cells. The quantity present was closely related to symptom severity. They occurred in cells from both the green and yellow areas of infected leaves, but were more numerous in cells from the yellow areas. Cells from yellow and green areas of leaves from plants of each of the six mild or symptomless host/virus combinations (Table 1) contained only a few particles randomly scattered throughout the cytoplasm. Cells from similar areas from plants of each of the five severe or moderately severe host/virus combinations contained very large masses of particles which frequently filled the cytoplasm. Sometimes the particles were randomly distributed (Fig. 1), sometimes in near-crystalline aggregates and sometimes in crystalline arrays (Fig. 2). Crystalline arrangements were not specific to one virus or to one host, but occurred only in cells of plants with severe or moderately severe symptoms. The plant material was fixed immediately after removal from the plant so it is unlikely that these arrays were induced by wilting. Most frequently they occurred in mesophyll cells close to the vascular tissue, where the quantity of particles was usually greatest. In cocksfoot infected with CMMV, crystalline arrays were not found eight weeks after inoculation, but by the following spring the symptoms in the regrowth leaves were more severe than at eight weeks and the cells in the yellow areas of these leaves contained many near-crystalline arrays. By contrast, cells in the regrowth leaves of CFMV-infected cocksfoot, where the symptoms were even more severe, contained crystalline arrangements only in the green areas: cells in the yellow areas were almost devoid of tonoplast and the virus particles were randomly distributed throughout the cell vacuole. Rupture of the tonoplast with release of particles into the vacuole was a common feature in leaf cells of plants from all severe or moderately severe host/virus combinations. In the vacuole the particles were usually randomly distributed (Fig. 3), but in less disrupted cells they occurred in small membrane-bound packets which sometimes contained an inclusion vacuole (Fig. 4a). Possibly these packets originated from intrusions of the tonoplast into the vacuole (Fig. 4b). In more severely affected cells, the plasmalemma as well as the tonoplast was destroyed and the cytoplasm remained as remnants, sometimes in the form of long fingers extending into the vacuole.

Occasionally, particles of CFMV, but not those of PMV or CMMV, were seen in cell nuclei. Their distribution was usually random and they were therefore difficult to distinguish with certainty from normal nuclear material, but in one cocksfoot plant sampled in the spring when symptoms were at their most severe, small, near-crystalline aggregates were

Table 1. Reactions of five species of Gramineae to three viruses

<table>
<thead>
<tr>
<th>Host species</th>
<th>CFMV</th>
<th>PMV</th>
<th>CMMV</th>
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<tbody>
<tr>
<td><em>Triticum vulgare</em> cv. Hybrid 46 (wheat)</td>
<td>+++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Dactylis glomerata</em> cv. Sylvan (cocksfoot)</td>
<td>+</td>
<td>o</td>
<td>+</td>
</tr>
<tr>
<td><em>Hordeum vulgare</em> cv. Deba Abed (barley)</td>
<td>+</td>
<td>++N</td>
<td>o</td>
</tr>
<tr>
<td><em>Phleum pratense</em> cv. Sabre (timothy)</td>
<td>-</td>
<td>+</td>
<td>o</td>
</tr>
<tr>
<td><em>Setaria italica</em> (Italian millet)</td>
<td>-</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

* -, no infection; o, symptomless infection; +, mild mottling; + +, moderately severe mottling; + ++ , severe mottling; N, necrotic lesions.
Fig. 1. Section of cocksfoot cell infected with CMMV showing endoplasmic reticulum (ER) bounding many small cytoplasmic vesicles. Virus particles, some with electron translucent centres (arrowed) were distributed at random.

Fig. 2. Section of *Setaria italica* cell infected with CMMV showing virus particles in crystalline aggregates.
Fig. 3. Section of cocksfoot cell infected with CFMV showing absence of tonoplast and release of virus particles (Vp) into cell vacuole (Va).

Fig. 4. Section of cocksfoot cells infected with CMMV showing membrane-bound packets of virus particles in the vacuole; (a) with an included vacuole, (b) without an included vacuole and still attached to the tonoplast (arrowed).

Effects of the viruses on cell ultrastructure

The extent of change in cell ultrastructure was closely related to symptom severity and ranged from barely discernible effects to a complete destruction of all cell organelles. In cells of the plants from the three symptomless host/virus reactions (Table 1) the only changes in cell ultrastructure were the occurrence in the cytoplasm of a few small vesicles (Fig. 1) and a few amorphous inclusions (Fig. 7). In the cells of all plants from host/virus interactions leading to symptom development, many changes occurred, most of which were common to all three viruses. The number and size of membrane-bound vesicles were greater in plants with mild symptoms than in symptomless plants. This increase in vesiculation apparently resulted from an increase in smooth endoplasmic reticulum. Similarly,
amorphous inclusions were seen more frequently in cells of plants with mild or moderately severe symptoms than in cells of symptomless plants.

Infection with CFMV, PMV or CMMV was accompanied by marked changes in chloroplast structure. The chloroplasts in infected cells were usually elongated and often produced finger-like extrusions. Membrane-bound volumes of cytoplasm, often containing virus
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Fig. 7. Section of timothy cell infected with PMV showing an amorphous inclusion (A) and abnormal mitochondrion (M) partly encircling an opaque body (OB).

particles, were frequently observed within these extrusions. Either the extrusions encircled and engulfed a volume of cytoplasm, or hollows in the extrusions appeared totally enclosed when sectioned obliquely. In some cells, the chloroplast extrusions converged upon neighbouring chloroplasts or extrusions from them, and an electron-dense area then appeared between them (Fig. 6). In host/virus combinations with mild symptoms, these changes in chloroplast structure were observed only in cells from the yellow areas of infected leaves, but in those with moderately severe symptoms they occurred in cells from green as well as from yellow areas. Such changes were rarely seen in cells from the centres of severely yellowed areas and never in cells from necrotic areas where the chloroplasts were in an advanced state of degeneration or destroyed completely.

In plants infected with PMV and CMMV, the mitochondria were often elongate and in most instances the membranes on the inner curves of crescent shaped mitochondria were more swollen than those on the outer curves (Fig. 7). One or more membrane bound vesicles containing virus particles were sometimes observed within the mitochondria; these presumably originated in the same way as those in the chloroplasts. This vesiculation of the mitochondria was observed only in plants with mild symptoms. In plants with severe symptoms only mitochondrial remnants were observed. In plants infected with CFMV only remnants of mitochondria occurred.

Changes in the structure of the nucleus were observed only in cells with very high virus concentrations. In cells from the green areas of CFMV-, PMV- or CMMV-infected leaves with severe symptoms, the nuclear membranes disintegrated and in cells from the yellow and necrotic areas the nuclei were, like all other cell organelles, unrecognisable.
DISCUSSION

The intracellular distribution of CFMV, PMV and CMMV particles and their effects on host cell ultrastructure seemed dependent to a much greater extent on the severity of the host/virus interaction than on the particular virus or host species involved. Thus, many more virus particles were present, and ultrastructural damage was greater, in cells from yellow areas of infected leaves and in plants with severe symptoms than in cells from green areas or plants with mild symptoms. Only two differences were observed between cells infected with CFMV and those infected with the serologically unrelated PMV or CMMV: in CFMV infected cells, virus particles were occasionally found in the nucleus as well as in the cytoplasm and no virus-containing vesicles were observed in the mitochondria. However, because CFMV caused more severe symptoms than either PMV or CMMV and because particles were observed only in the nuclei of cells from plants with very severe symptoms whereas vesiculated mitochondria were observed only in plants with mild symptoms, it could not be concluded with certainty whether either feature was diagnostic.

Ultrastructural changes resulting from infection with CFMV, PMV or CMMV ranged from an increase in endoplasmic reticulum and vacuolation of the cytoplasm in cells from symptomless or near symptomless hosts to the loss of tonoplast, plasmalemma and organelle membranes in cells in the green areas of severely, or moderately severely mottled hosts and total disintegration of all cellular content in cells in the yellow and necrotic areas. Particles of all three viruses occurred in the cytoplasm where they were usually scattered, but sometimes in near-crystalline or crystalline aggregates. Particles were never observed in rows within membrane-bound tonoplastic channels in the cytoplasm nor in tubules embedded in the cell walls, as has been observed in radish cells infected with radish mosaic virus (Honda & Matsui, 1972) or in Cherokee Wax bean cells infected with bean pod mottle virus (Kim & Fulton, 1971; comoviruses – Harrison et al. 1971). Occasionally, membrane-bound packets of CFMV, PMV and CMMV particles which apparently originated from the tonoplast were seen in the vacuole. Packets of virus of similar origin have been observed in the vacuoles of artichoke cells infected with the artichoke mottled crinkle strain of tomato bushy stunt virus (Russo, Martelli & Quacquarelli, 1968), and in pelargonium cells infected with pelargonium leaf curl virus (Martelli & Russo, 1972).

In partially disrupted cells infected with CFMV, PMV and CMMV, the chloroplasts often developed extrusions in which membrane-bound volumes of cytoplasm were often observed. These inclusion vesicles usually contained virus particles, but none was observed to contain a mitochondrion or other cell organelle. Chloroplast extrusions and inclusion vesicles have been reported to occur in cells infected with several totally unrelated viruses and also in cells under physiological stress. Chloroplast inclusion vesicles in tomato cells infected with tobacco mosaic virus (tobamovirus group; Shalla, 1964) and in cowpea cells infected with southern bean mosaic virus (potyvirus group; Weintraub & Ragetli, 1970) sometimes contained a mitochondrion, but those in broad bean cells infected with bean yellow mosaic virus (potyvirus group; Weintraub & Ragetli, 1966) apparently did not. An additional feature in the present work, was that where extrusions from neighbouring chloroplasts came into close proximity, an electron-opaque zone appeared between them. By contrast, Hatta & Matthews (1974) reported that in chinese cabbage cells infected with turnip yellow mosaic virus, the chloroplasts became rounded and where they converged an electron translucent area appeared. Unlike cells infected with turnip yellow mosaic (Hatta & Matthews, 1974), wild cucumber mosaic (Allen, 1972), plantago mottle (Granett, 1973) and other tymoviruses,
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the chloroplasts in cells infected with CFMV, PMV and CMMV did not develop double-
membrane-bound, flask-shaped vesicles at, or near, their peripheries.

Inclusion vesicles similar to those in the chloroplasts were also observed in the mito-
chondria in cells of plants infected with PMV and CMMV. Weintraub & Ragetli (1970) re-
ported that mitochondria containing an electron opaque body within an inclusion
vacuole was a common feature of cowpea cells infected with southern bean mosaic virus,
but their observations provided little information as to how these inclusion vesicles may
have originated. No electron opaque bodies were seen within the mitochondria in cells
infected with PMV or with CMMV, but occasionally, crescent-shaped mitochondria
apparently about to encircle such a body were observed (Fig. 7). Weintraub & Ragetli
suggested that the disturbances which they observed in cells infected with southern bean
mosaic virus might be associated with viral synthesis. The changes in mitochondrial struc-
ture observed in the present study however, paralleled the changes which occurred in
chloroplast structure which, as Weintraub & Ragetli point out, can result from conditions
of physiological stress as well as from virus infections.

Though CFMV, PMV and CMMV resemble southern bean mosaic virus in many
respects, several notable differences seem to exist. For example, crystalline arrays of
CFMV, PMV and CMMV particles were a common feature of severely mottled plants
eight or more weeks after inoculation, whereas crystalline arrays of southern bean mosaic
virus in cowpea cells rarely persisted beyond the twentieth day after inoculation (Weintraub
& Ragetli, 1970). Further, we did not observe virus particles in the nuclei of cells infected
with PMV or with CMMV and only rarely in those of cells infected with CFMV, nor did we
observe crystalline aggregates of particles of any of the viruses in the cell vacuole. Amor-
phous inclusions similar to those found in cells infected with CFMV, PMV or CMMV
(Fig. 7) were not described in cowpea cells infected with southern bean mosaic virus. How-
ever, the material which composed these inclusions closely resembled that of the chloro-
plast extrusions. No evidence of fragmentation of the chloroplast extrusions was observed, but it
is possible that the amorphous inclusions are extrusions sectioned in the opposite plane.

Despite these differences however, there would appear to be no substantial evidence
against the grouping of CFMV, PMV and CMMV with southern bean mosaic virus as all
these viruses do have a number of other properties in common (e.g. type of vector, particle
morphology, stability in vitro) and although they have been placed by Gibbs (1969) in a
'salt stable 20% RNA group' few workers would consider this to represent an entirely
homologous group.

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