

## CYTOPATHIC EFFECTS OF OAT STERILE DWARF VIRUS IN ENATION CELLS OF OAT LEAVES

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*Summary.* — A Czechoslovak isolate of oat sterile dwarf virus caused thickening or distortion of cell walls and induced the formation of viroplasms containing incomplete virus particles in gall phloem. These effects were similar to those caused by other plant reoviruses but no tubular structures containing rows of virions were found. Dense bodies of a similar diameter to virions were occasionally seen inside mitochondria. Complete virions were distributed at random in the cytoplasm but clusters of virions were sometimes enclosed within a membrane. No crystalline arrays of virions were seen. Late in infection parenchyma cells contained membranous structures formed by membranes about 11 nm thick, close to viroplasms. As the cells degenerated further, there occurred scrolls of membranes, about 150 nm in diameter. Unbound masses of fine fibrous and tubular structures, strongly resembling P-protein, occurred in the cytoplasm. The fibrils were about 5 nm in diameter and the unbranched tubes 21 nm wide. Only complete virions were seen in these structures.

*Key words:* oat sterile dwarf virus; plant reoviruses; membranous structures; P-protein; viroplasm

### Introduction

Oat sterile dwarf virus (OSDV) is classified with the planthopper-transmitted plant reoviruses (subgroup 2, genus *Fijivirus*, fam. *Reoviridae*) that induce enations protruding from leaf veins. Virions of the members of this group are indistinguishable by electron microscopy, but by testing their subviral particles, three serological groups can be differentiated. OSDV, *Arrhenatherum* blue dwarf virus (ABDV) and *Lolium* enation virus (LEV) form a distinct serological group (Milne and Luisoni, 1977) and should be considered as forms of the same virus (Milne and Lesemann, 1978).

Particles of OSDV were first seen in the midgut cells of its vector *Javesella pellucida* (F.) (Brčák *et al.*, 1966) and later in enation cells of two host plants, *Avena sativa* L. and *Arrhenatherum elatius* (L.) Presl (Brčák *et al.*, 1972).

OSDV virions enclosed in tubes were observed in the cytoplasm of vector cells (Brčák *et al.*, 1966), but not in plant host cells, where they were distributed at random (Brčák *et al.*, 1972; Lindsten *et al.*, 1973). Distinct properties of OSDV encouraged us to continue our investigations on this virus.

### *Materials and Methods*

The OSDV isolate used was found by Ing. J. Vacke in field oats grown near the village Sá-zava in Českomoravská vrchovina. Its properties were described by Průša *et al.* (1959); it did not differ from other OSDV isolates obtained from this area. However, it was somewhat different from OSDV isolates collected by Vacke (1966) at Slapy near Tábor. The OSDV isolate was transmitted by *J. pellucida* to oats in a greenhouse, where the plants were kept for several months. Samples of enations were fixed at 0° C under partial vacuum in 6.25% glutaraldehyde in phosphate buffer, pH 7.4, for 1 hr. After washing, the samples were postfixed in 2% osmium tetroxide for 2 hr at room temperature and dehydrated in 30, 50, 70 and twice in 100% ethanol, transferred to propylene oxide and gradually embedded in Durcupan ACM medium which was polymerized at 60° C for 60 hr. Ultrathin sections were cut on a LKB Ultratome, stained for 10 min on 400 mesh copper grids with saturated water solution of uranyl acetate and lead citrate (Raynolds, 1963) and examined in Tesla BS 500 and JEM 100 B electron microscopes.

### *Results and Discussion*

Hatta and Francki (1976) suggested that particles of Fiji disease virus (FDV) were present in most gall phloem. This was also true for OSDV; most particles occurred in phloem parenchyma cells of the neoplastic tissue. Cell walls of degenerating phloem were thickened in places as they are in maize rough dwarf virus (MRDV) infections (Gerola and Bassi, 1966); sometimes the cell walls were distorted, as in the vein swellings caused by rice black streak dwarf virus (Shikata and Kitagawa, 1977). Degenerating cells which had completely lost their cytoplasm often contained masses of scattered virions (Fig. 1). Very occasionally the virions occurred in irregular clusters inside membranous structures (Fig. 3) similar to those observed in sieve tubes of plants infected with the pangola stunt virus (Schank *et al.*, 1972). Parenchyma cells forming the remnant of the bundle sheath and having a thin layer of cytoplasm at the cell wall, also contained scattered virions besides mitochondria, dictyosomes, endoplasmic reticulum, vesicles and lipid bodies; multimembrane whorls often occurred at cell walls. We failed to find virions inside sieve tubes.

Viroplasm (Figs. 4 and 6) were intensely electron-opaque bodies sometimes occupying most of the cytoplasm and similar to those described for MRDV (Gerola and Bassi, 1966; Gerola *et al.*, 1966). Incomplete virus particles (inner cores) were infrequent in the viroplasms, but complete virions occurred randomly and frequently outside the viroplasm (Figs. 4 and 6). At the edge of the viroplasm and also among the virions there occurred small electron-dense bodies (up to about 18 nm in diam.) that were probably ribosomes (Fig. 4).

Unusual membranous structures occurred near viroplasms, in portions containing virions. They resembled membranes of "empty tubes" observed with other reoviruses, especially with ABDV (Milne *et al.*, 1974). The mem-

branes were about 11 nm thick in cross section (Figs. 4 and 5). More degenerated cells contained clusters of membranes, sometimes adhering to the cell wall and shaped like scrolls (Figs. 1, 2, 6, 7 and 8). The scroll diameter was normally 130 to 170 nm and about 30 to 160 nm wide, when sectioned longitudinally. These structures showed mostly one, two or three dark stained layers and were similar to the tubes invested by an outer host membrane seen in ABDV-infected tissue (Milne *et al.*, 1974). Membranous structures never contained rows of complete virions as known with all other reoviruses except FDV (Milne and Lovisolo, 1977). They may originate from cell constituents, as in some cases they were associated with mitochondria (Fig. 8).

Parenchyma cells with large relatively unaffected nuclei also contained thin masses of fine fibrils and tubular structures (Fig. 9). The cytoplasm around them contained virions, but not in crystalline arrays, mitochondria, dictyosomes, remnants of endoplasmic reticulum, vesicles and lipid drops. Virions occurred in islands of thin fibrous masses or at tubular structures and exceptionally also within tubular masses (Fig. 9). Incomplete virions (inner cores) were not found inside fibrous and tubular masses. The fine fibrils had about 5 nm diameter, whereas the tubules were about 21 nm wide (Fig. 10). These fibrous and tubular masses were previously thought to be viroplasm-like (Brčák and Králík, 1973) or viroplasms, because in LEV-infected cells inner cores had been found inside the fibrous mass (Lesemann and Huth, 1975). In MRDV infections, viroplasms built up from a mass of highly electron-dense material included portions of thin fibrils containing few virus particles; the viroplasms contained both incomplete and complete particles (Bassi and Favali, 1972). Similar pale areas consisting predominantly of fibrils were also observed in viroplasms caused by pangola stunt virus infections (Giannotti and Milne, 1977). However, our electron micrographs suggested that the fibrous and tubular masses were not the site of OSDV assembly. In LEV viroplasms, the pale portions rarely contained incomplete virus particles, so that their origin and function are also obscure (Lesemann and Huth, 1975).

The fibrous and especially the tubular masses (Figs 9 and 10) strongly resembled the P-protein from phloem-parenchyma cells (Esau, 1968). The tubules are randomly oriented and their outside diameter and thickness of tubule walls are the same as in P-protein; they are not the same as microtubules whose walls are thinner (Pickett-Heaps and Northcote, 1966).

Membranous and tubular structures have often been described in connection with cytopathic effects of viruses. The fibrous and tubular masses may originate from disintegrating viroplasm at a late stage of infection, as viroplasms caused by related viruses are also formed from filamentous structures (Milne, 1977). But they may originate from degraded outer capsid material, or be a material for completing virions as suggested for a plant rhabdovirus (Fedotina, 1977). Cytochemical results (Hatta and Francki, 1978) may show these filamentous structures to be chemically diverse.

Kislev *et al.* (1968) reported MRDV particles inside mitochondria and chloroplasts. However, spherical particles occurring inside mitochondria and

having the same diameter as OSDV virions could not be identified in our electron micrographs (Figs 4 and 9).

We can conclude that an isolate of OSDV from Czechoslovakia causes cytopathic effects similar to those described for ABDV and LEV in Germany. This supports the conclusions of Milne and Lesemann (1978) that OSDV, ABDV and LEV are isolates of the same virus.

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*Explanation of Electron Micrographs (Plates XXVII–XXX):*

- Fig. 1.* OSDV particles scattered inside a degenerating cell containig two bundles of invested membranes (arrows) and several mitochondria (left). Two P-type plastids (pp) with cuneate crystalloids in a sieve tube (right).  $\times 14300$ .
- Fig. 2.* OSDV virions associated with fibrils and cross-section of a bundle of invested membranes  $\times 28600$ .
- Fig. 3.* OSDV particles and fibrils enclosed by a sac formation.  $\times 34300$ .
- Fig. 4.* A large viroplasm (Vp) composed of a fibrous mass. OSDV particles are scattered around the viroplasm and in the cytoplasm which contains thin membranous structures (arrows). The mitochondrion (M) contains a virion-like body.  $\times 25700$ .
- Fig. 5.* Edge of a viroplasm with complete OSDV particles and membranous structures.  $\times 61400$ .
- Fig. 6.* A portion of viroplasm (Vp) showing inner cores of OSDV particles. Complete virions are seen outside the viroplasm together with membranous scrolls (arrows).  $\times 61400$ .
- Fig. 7.* A portion of a degenerating cell containing individual virions and clumps of invested membranes.  $\times 42800$ .
- Fig. 8.* Randomly oriented invested membranes associated with two mitochondria.  $\times 42800$ .
- Fig. 9.* Parenchyma cell containing nucleus, mitochondria (rarely containing small dense bodies) and large fibrous and tubular P-protein-like masses.  $\times 20000$ .
- Fig. 10.* The tubes of a P-protein-like mass consisting of electron-lucent cores bounded by an electron-opaque cortex. Top left: one complete OSDV virion.  $\times 100000$ .