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ELECTRON MICROSCOPY OF WHEAT PLANTS INFECTED WITH EUROPEAN WHEAT STRIATE MOSAIC DISEASE (*)

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INTRODUCTION

European weath striate mosaic (EWSM) is a disease of cereals first described by SLYKHUIS and WATSON (1958). It causes fine clorotic striae in weath leaves followed by general chlorosis, stunting and finally death of the infected plants. This disease is known to occur in England, Denmark, Germany and Spain (SLYKHUIS and WATSON, 1958) as well as in Sweden, Finland and Czechoslovakia (NORTEVA, 1965). It is transmitted only by two species of planthoppers: *Javesella pellucida* (Fab.) and *J. dubia* (Kirsch.) (KISIMOTO and WATSON, 1965).

Previous attempts to find a virus particle associated with EWSM-disease by normal virological means, such as leaf dip

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preparation or virus purification, have so far been unsuccessful (WATSON, personal communication). SERJEANT (1967) found some spherical particles in clarified extracts of infected individuals of the vector *J. pellucida,* but similar particles were also found in extracts from uninfected individuals. In the present investigation, the etiological agent of EWSM-disease was looked for by electron microscopic examination of ultrathin sections of infected wheat plants.

METHODS

The culture of EWSM-disease used was obtained from Rothamsted Experimental Station (courtesy of Dr. Marion Watson). Weat plants (*Triticum aestivum.* L. var. Capelle Desprez), were infected with EWSM-disease by feeding infective planthoppers (*Javesella pellucida* Fab.) on healthy seedlings, for a 7-day period. Control seedlings were fed upon by non-infective hoppers for a similar period as above. Phosdrin was sprayed weekly on both infected and control plants until symptoms developed on the former. None of the control plants showed any disease symptoms.

Pieces about 1 mm wide and 6-7 mm long were excised from the leaf-blades and leaf-sheaths of wheat plants showing symptoms of EWSM-disease, ranging from early chlorotic streaks to complete chlorosis. Pieces of leaves of comparable position on the plant, were also taken from control (healthy) plants of the same age as the diseased ones. The excised leaf material was fixed for 17-24 h in 2% glutaraldehyde in 0.02 M phosphate buffer (pH 7.0). It was washed once in equal parts of 10% sucrose and 0.1 M buffer and then twice in 0.1 M buffer. It was then post-fixed in 1 or 2% osmium tetroxide in 0.1 M buffer for 3-4 h, and then dehydrated in graded ethanol or acetone. Fixation and washing was carried out at 4°C, but during dehydration the processed material was transferred gradually to room temperature. Staining in uranyl acetate (1% in absolute ethanol or 5% in 70% acetone) was followed by embedding either in butyl methacrylate-Styrene, according to the schedule of MOHR and COCKING (1968), or in Epon 812. Ultrathin sections, cut with glass or diamond knives, were stained with Rynolds lead citrate and examined in JEM 6, AEI EM6B or in Siemens Elmiscop 1A electron microscopes.

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RESULTS

Morphological changes in cell organelles

In the parenchymatous cells from healthy wheat leaves the cytoplasm forms a very thin layer lining the cell wall and enclosing the hloroplasts, mitochondria and the nucleus (fig. 1). In cells from leaves showing moderate chlorosis, i. e. clorotic streaks, the cytoplasm often bulges into the central vacuole forming large pockets (fig. 2). The chloroplasts of such cells appeared normal except for a tendency to contain starch grains larger in size than those from cells of healthy plants. However, in cells from leaves showing almost complete chlorosis the chloroplasts did not contain much starch and appeared to be higly invaginated. The nuclei did not seem to suffer any morphological change in leaves showing moderate chlorosis. No morphological differences were detectable between the mitochondria of the parenchymatous cells from healthy and diseased plants. However, as will be elucidated below, crystalline inclusins were found in structures that had some resemblance to the mitochondria (fig. 7), in the phloem cells of diseased plants.

Cell inclusions

Three types of inclusion bodies were found in the parenchymatous and phloem cells of leaves from EWSM-diseased plants, that were absent in similar cells from healthy plants. These inclusions, however, were found only in leaves showing moderate chlorosis but not in leaves showing near complete chlorosis. The inclusions found are as follows:

A) Long thin bands of filamentous units, nearly 10 nm in diameter, closely packed longitudinally (fig. 2, 3). These bands differed in length and thickness, but they reached up to 20 μ in length and 700 nm in diameter. Some of these bands showed apparent crystalline structure (fiig. 3), but some did not show such structure and were fluffy in appearance (fig. 7). In most cases, but not in all, a membrane was seen surrounding a group of these bands (fig. 3, 7). The long-thin-band inclusions were the most abundant type of inclusion; it was found in the cytoplasm of the mesophyl and phloem

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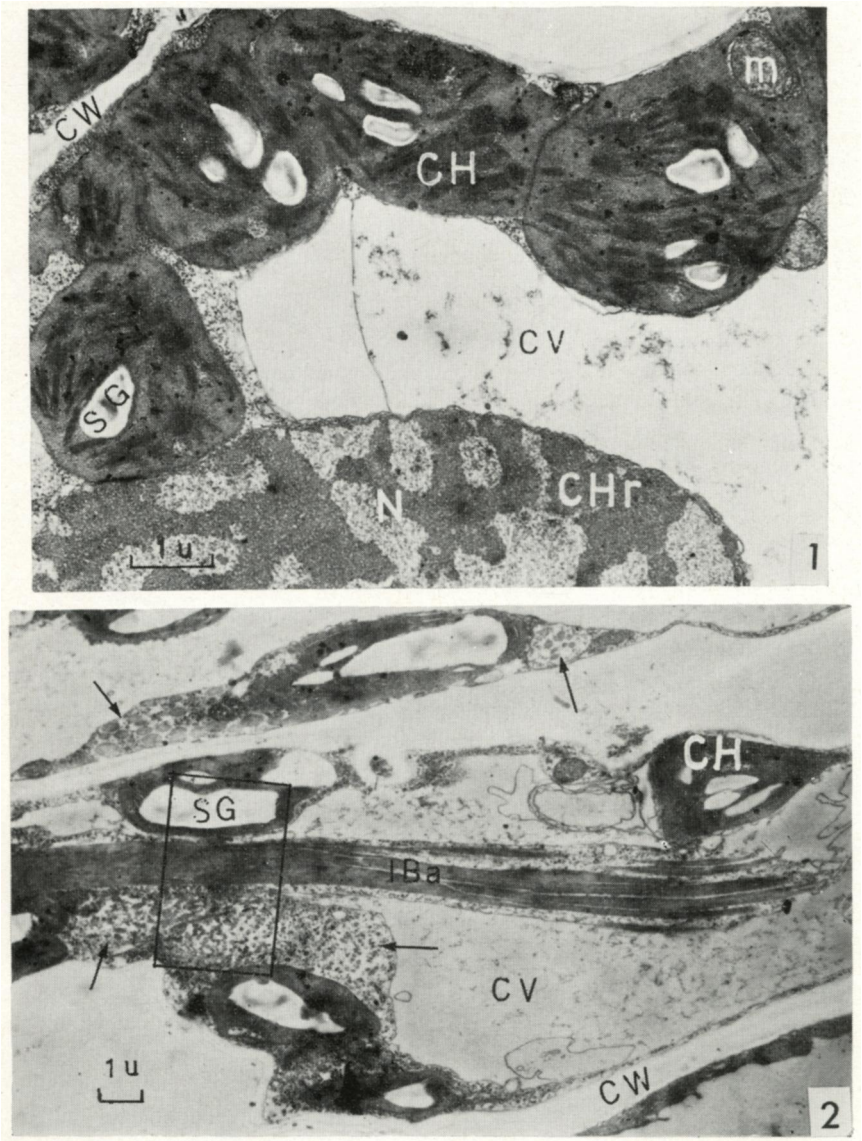

FIG. 1 and 2 - Parenchymatous cells from leaves of healthy (Fig. 1) and diseased (Fig. 2) plants; the latter plants showed moderate chlorosis. In Fig. 2, arrows indicate pockets of inclusion bodies « type a »; inset area is enlarged in Fig. 3, CH = chloroplast, CHr = nuclear chromatin, CV = cell vacuole, CW = cell wall, IBa = inclusion bodies « type a » in the form of long thin bands, m = mitochondrion, N = nucleus, SG = starch grain.

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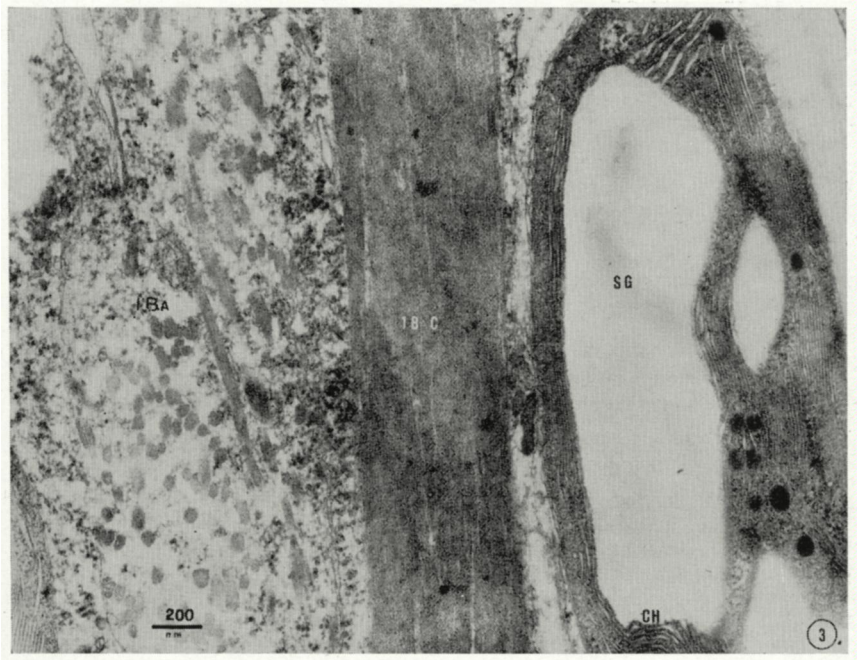

FIG. 3 - An enlarged area from Fig. 2, showing inclusion bodies « type a » in the form of long thin bands (IBa), some of which are large and show chrystalline structure (IBaC). Arrows indicate a membrane surronding a group of these inclusions. CH = chloroplast, SG = starch grain.

cells of diseased plants. It was also found, but less frequently, in the nuclei of such cells, free in the nucleoplasm, i. e. unbound by membranes (fig. 4).

B) Crystalline inclusions in the form of polygons (fig. 7, 8) were found in the cytoplasm of phloem cells from diseased plants. These crystals, like those of the former type of inclusions, consisted of units repeated every 10 nm approximately, but the packing and periodicity were more regular in type (B) inclusions than in type (A) ones. The polygonal crystals were either free in the cytoplasm, i.e. unbound by membranes (fig. 8) or were surrounded by a membrane (fig. 7). Some of these membranes resembled the mitochondrial membranes, and in some cases contained structures that resembled the mitochondrial cristae. This might suggest a mitochondrial

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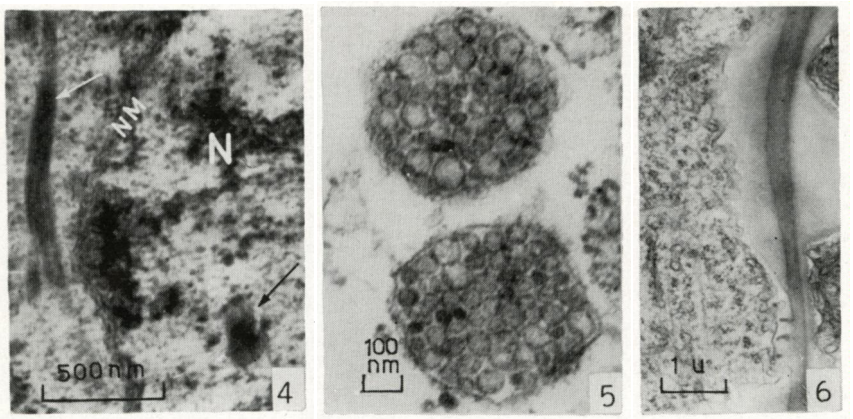

FIG. 4 - Intranuclear inclusions (black arrow), inside the nucleus (N), unsurrounded by membranes except the nuclear membrane (NM). Note similar inclusions in the adjacent cytoplasm (white arrow).

FIG. 5 - Membrane-bound clusters of bubble-like inclusion bodies « type c », found in the phloem of diseased plants.

FIG. 6 - Part of a sieve tube, of a diseased plant, that was devoid on inclusions.

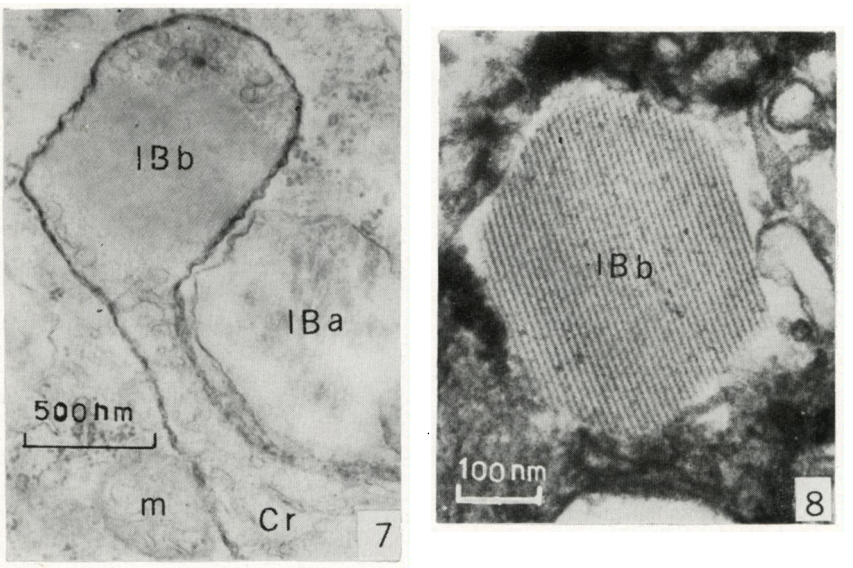

FIG. 7 and 8 - Crystalline inclusions « type b », in the form of polygons (IBb), found in the phloem cells of diseased plants. In Fig. 7 the crystal is enclosed in a membrane-bound structure that contained cristae (Cr), like those of the adjacent mitochondrion (m). In this figure, a membrane-bound pocket of « type a » inclusion bodies (IBa) is also present.

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origin for some of these crystal containing membrane-bound structures, but these structures were much larger in size than the normal looking mitochondria that were present in the same cells (fig. 7).

C) Round, bubble-like bodies were encountered occasionly in the cytoplasm of phloem cells from diseased plants. These bodies, which ranged from 30-85 nm in diameter, were devoid of any clear internal structure and were aggregated in membrane-bound clusters (fig. 5).

No other types of inclusions, and no virus- or *Mycoplasma*-like structures were found in the mesophyl or phloem cells (fig. 6) of leaves from diseased plants.

DISCUSSION

Inclusion bodies in diseased plants have been described in mosaic diseases more frequently than in yellows diseases (ESAU, 1967). Crystalline inclusions were either crystallized virus particles, as in tobacco mosaic virus (WARMKE and EDWARDSON, 1966), or crystals of a proteinaceous nature — probably a side product of the infection — as in bean yellow mosaic virus (WEINTRAUB and RAGETLI, 1968). The inclusion bodies reported in the present work, associated with the EWSM-disease in weath plants, do not resemble any known form of virus aggregation. The long-thin-band inclusions (type a), particularly the large ones (fig. 2, 3) may appear in the light microscope like the needlee-shaped crystals found in the phloem of carrot plants infected with aster yellows (STRUCKMEYER, 1963). The latter disease, wich is transmitted only by leafhoppers like the EWSM-disease, is now thought to be caused by *Mycoplasma*-like organisms (SHIKATA, MARAMOROCH and LING, 1969) that are found consistently in the phloem of infected plants. But the failure to find any virus- or *Mycoplasma*-like structures in weath plants infected with EWSM-disease makes the etiology of this disease still uncertain. There are, however, several possible explanations for this failure:

1) The causative agent might have been present, but it occurred at too low concentration to be detected in the ultrathin sections examined.

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2) If the causative agent was a virus of small isometric particles as suggested by its sedimentation coefficient (S20 = probably 120, as estimated by SERJEANT, 1967), it might have been comfused with the cell ribosomes, particularly in the lack of conspicuous virus aggregates. Such a situations is sometimes found with sowbane mosaic, turnip yellow mosaic and cowpea mosaic viruses (MILNE, 1967).

3) The EWSM-disease, or at least the strain used in the present investigation, may arise from infectious nucleic acid which is not detectable by the electron microscopical methods adopted. Certain variants of tobacco necrosis virus are believed to exist in the host plant as free ribonucleic acid (KASSANIS and WELKIE, 1963). But such cases are rarely found in nature, since in the absence of a protein shell the infectious units are liablee to be destroyed by the host ribonuclease enzymes. The etiology of EWSM-disease cleary needs further investigation. Further electron microscopy to locate the agent *in situ* aught to involve hosts other than wheat in addition to various tissues of the insect vectors.

I am indebted to Dr. Cocking, Botany Dept., Nottingham Univ. for the use of the electron microscope in the above Department, and to Dr. Marion Watson, Dr. R. G. Milne and Dr. B. Kassanis (Rothamsted Experimental Station, Horpenden, England) for their interest, helpful discussions and suggestions.

SUMMARY

Ultrathin sections of healthy and diseased wheat leaves were examined by the electron microscope, in the hope of identifying *in situ* a possible etiological agent of the European wheat striate mosaic disease. No virus - or *Mycoplasma*-like bodies were detected in the parenchymatous or phloem cells examined. However, various forms of inclusion bodies were consistently found in the cytoplasm of cells from diseased plants. Cytoplasmic inclusions included: a. crystalline inclusions in the form of elongate bands consisting of filamentous units, each about 10 nm in diameter, b. crystalline inclusions in the form of polygons, and c. bubble-like inclusions, 30-85 nm in diameter

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each, that occurred in membrane-bound clusters in the phloem. Intranuclear inclusions were apparently of the type (a) described above.

Morphological changes in cell organelles associated with different stages of the disease are described.

RIASSUNTO

Sezioni ultrasottili di foglie di grano sane ed ammalate sono state esaminate mediante microscopia elettronica allo scopo di esaminare *in situ* il possibile agente eziologico dell'« European wheat striate mosaic disease ».

Non sono state identificati nelle cellule parenchimatiche e floematiche né virus né micoplasmi. Sono stati rinvenuti tuttavia frequentemente diversi tipi di inclusioni citoplasmatiche nelle cellule di piante malate. Tali inclusioni sono di vario tipo: a) inclusioni sotto forma di bande allungate consistenti di unità filamentose ciascuna di 10 nm di diametro — b) inclusioni cristalline di forma poligonale — c) inclusioni tipo bolle (30-85 nm di diametro) riunite a grappolo e avvolte da una membrana presenti nel floema. Le inclusioni intranucleari erano apparentemente del tipo a, sopra descritto.

Vengono infine discusse variazioni morfologiche a carico degli organi cellulari associate ai diversi stadi della malattia.

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