Neth. J. Pl. Path. 91 (1985) 163-168 SHORT COMMUNICATION

Myelinic and granular inclusion bodies in non-inoculated tissues of both healthy and TMV-infected leaves of Xanthi n.c. tobacco

ANNA APPIANO and GIOVANNI D'AGOSTINO

Istituto di Fitovirologia applicata del C.N.R., Via Onorato Vigliani 104, 10135 Torino, Italy

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In several plant species the hypersensitive reaction to virus infection is accompanied by the appearance of new components in the soluble protein fraction of the leaf tissue (Redolfi, 1983). In tobacco the accumulation of these so-called pathogenesis-related proteins (PRs) is particularly evident in the inoculated leaf, around necrotic local lesions, but occurs, although in lower concentration (Gianinazzi et al., 1970), also in tissues far from the point of virus entry, for example in the half-leaf opposite the inoculated one, in which the severe cytological alterations correlated with necrogenesis do not occur.

In leaf tissues of *Nicotiana tabacum* L. cv. Xanthi n.c. (half-leaves opposite half-leaves inoculated with tobacco mosaic virus, TMV) the presence of peculiar 'myelinic bodies' has recently been reported (Fraser and Clay, 1983). We have made an electron microscopy study on the same virus-host combination, to see whether there is a correlation between the occurrence of myelinic bodies and the accumulation of PRs.

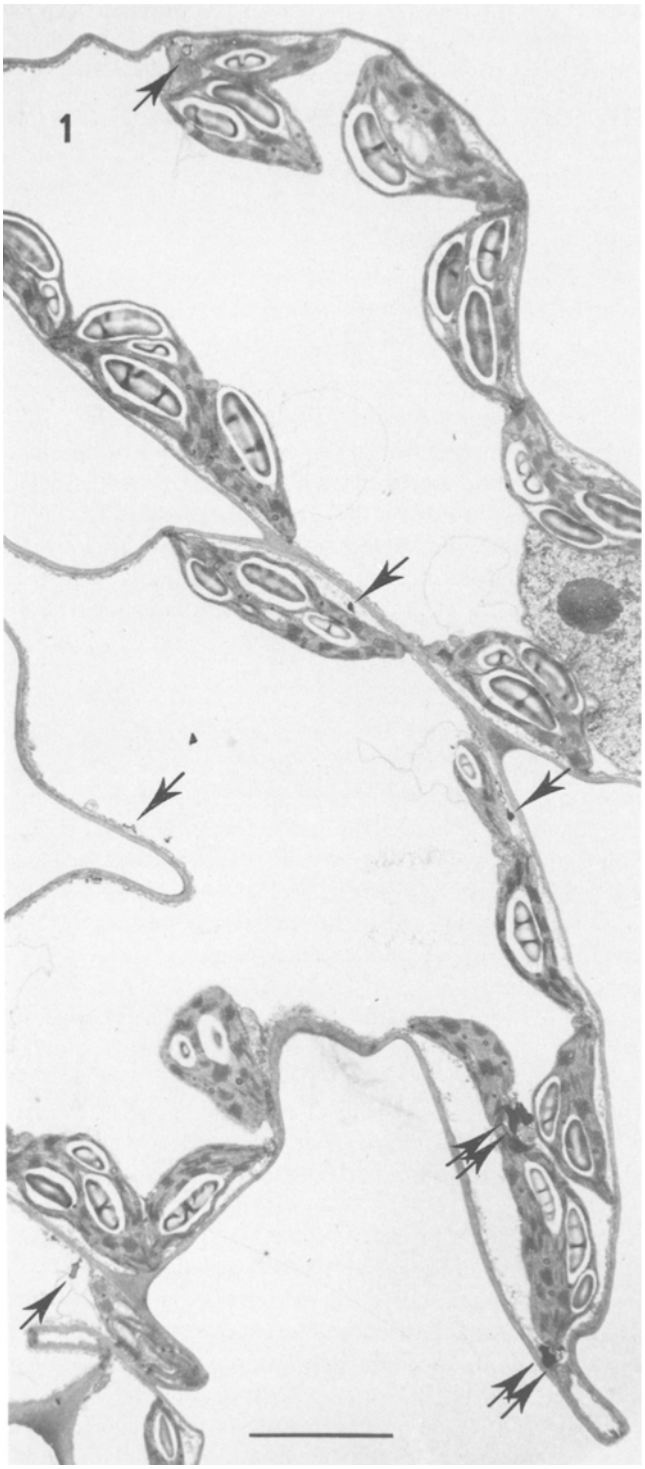
Plants of *N. tabacum* cv. Xanthi n.c. grown in the glasshouse were used at the stage in which they had 14 developed leaves, after having been preconditioned for 5-7 days in a growth chamber at 22 °C, 60% relative humidity and under 4 000 lx fluorescent light with a 12 h photoperiod. Only two fully expanded leaves were taken from each plant, to avoid variations due to leaf age.

Seven days after inoculation samples were taken from untreated half-leaves opposite half-leaves: *a)* inoculated with TMV, common strain; *b)* inoculated with buffer; *c)* not inoculated . Six different fixation schedules were used, changing the fixative (3% glutaraldehyde or 3% acrolein + 3% glutaraldehyde, always in phosphate buffer pH 7.2) and the molarity of the buffer (0.05, 0.1 or 0.15 M). After postfixation in osmium tetroxide, all the samples were dehydrated and embedded in the conventional way (Appiano and D'Agostino, 1983).

Some of the sections were treated with 3% hydrogen peroxide at room temperature for 20 min, to remove osmium (Marinozzi and Gautier, 1961). Serial sectioning was also made on some samples, the average thickness of the sections being about 80 nm.

The same half-leaves sampled for electron microscopy were checked for the presence of PRs: as expected (Gianinazzi et al., 1970), low but detectable amounts of PRs were present in the samples of the *a* series, not in the controls (*b* and *c* series).

The tissues of the three series of samples were cytologically similar. The features were those of mature but not senescent leaves. Mesophyll cells appeared well differen-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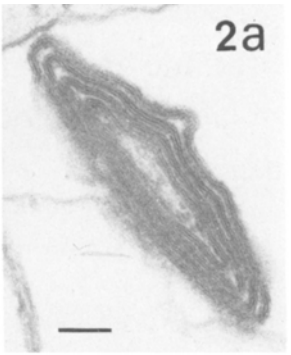
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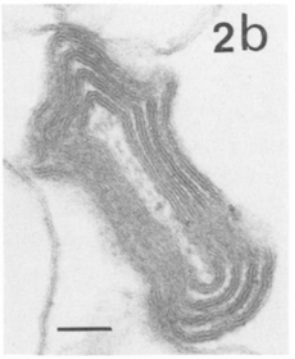
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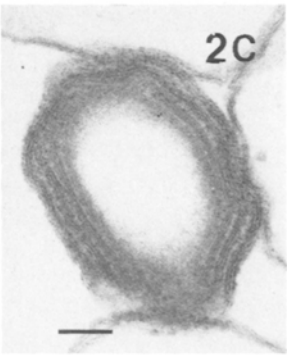
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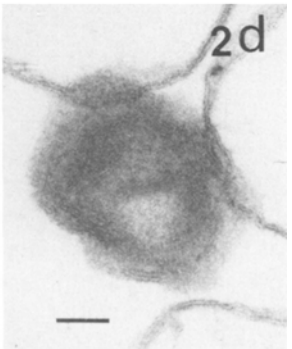
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Fig. 1. Part of three mesophyll cells of control tissue (*b* series). The chloroplasts show a well-developed lamellar system and a high starch content. Arrows point to myelinic bodies (MBs), double arrows to granular bodies (GBs). Bar represents 5 μm.
Fig. 2, a-d. One of the MBs of Fig. 1, bottom left corner: four serial sections, respectively No's 3, 4, 6 and 9, from a series of 14. In Fig. 2d the granular aggregate is a tangential view of one of the ends of the MB. In each figure bar represents 100 nm.

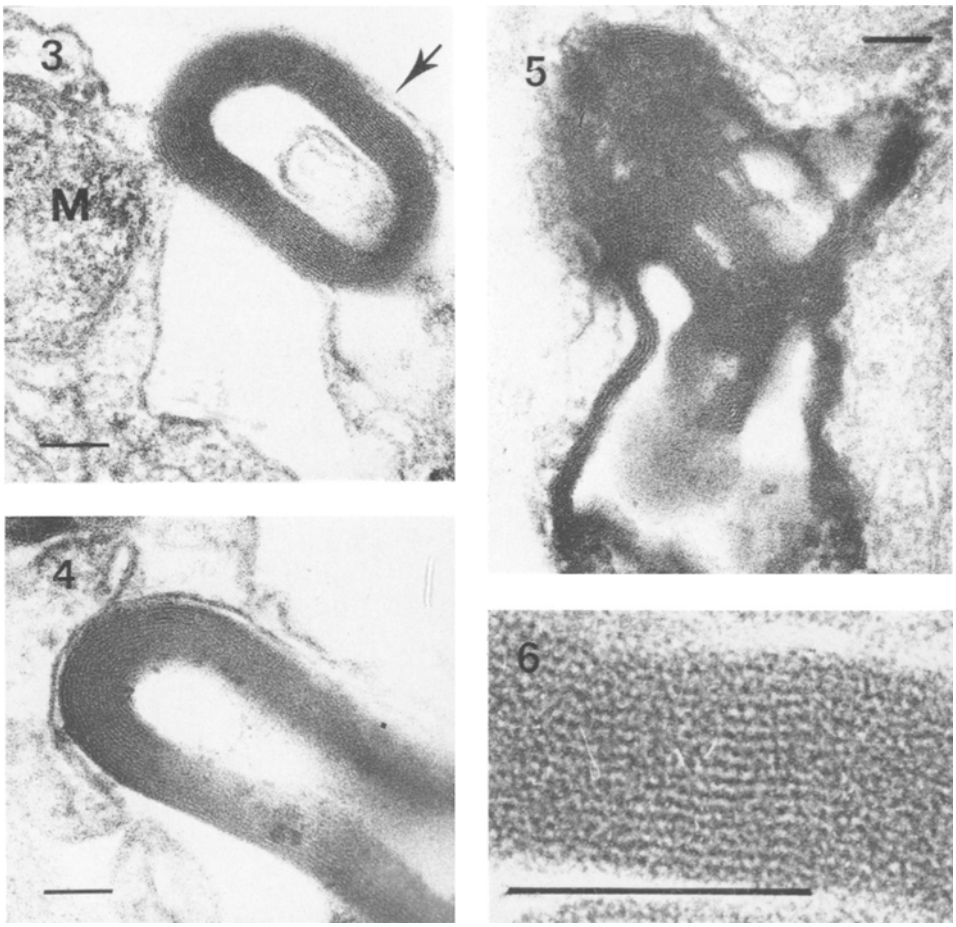
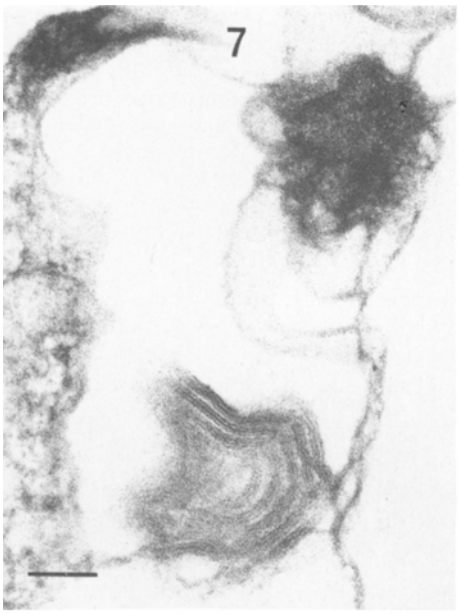
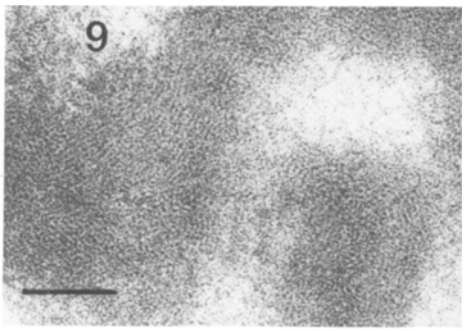
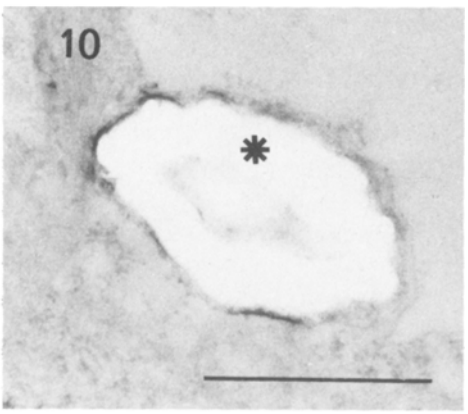
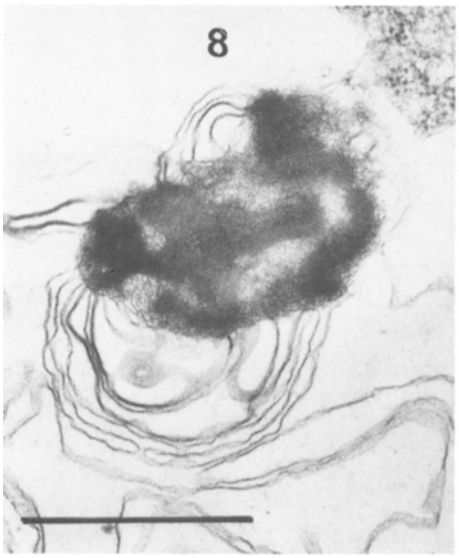


Fig. 3. An ovoidal MB in the cytoplasm (*a* series). Arrow points to the tonoplast. M, mitochondrion. Bar represents 100 nm.
Fig. 4. An ovoidal MB in the cytoplasm (*c* series). Bar represents 100 nm.
Fig. 5. A very convoluted MB in the cytoplasm (*a* series), showing both lamellar and granular structure. Bar represents 100 nm.
Fig. 6. A detail of Fig. 3 at higher magnification. The osmiophilic, parallel lamellae are well discernible. Bar represents 100 nm.









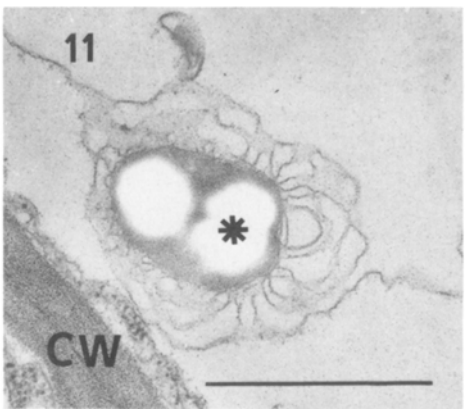


Fig. 7. An MB and a GB located side by side in the vacuole and joined by membranes (*a* series). Bar represents 100 nm.

Fig. 8. A GB surrounded by a tangle of membranes, located in the vacuole (*c* series). Bar represents 1 μm.

Fig. 9. A detail of Fig. 8 at higher magnification, showing a fingerprint structure in the GB. Bar represents 100 nm.

Figs 10 and 11. Sections analogous to those in Figs 3 and 8, respectively. After treatment with hydrogen peroxide, MBs (Fig. 10) and GBs (Fig. 11) disappear, leaving electron-lucent patches (*). CW, cell wall. In each figure bar represents 1 μm.

tiated, with large vacuoles and a cytoplasm rich in organelles; the chloroplasts had a well-developed lamellar system and prominent starch grains (Fig. 1).

In the mesophyll cells of all the series of samples two particular types of inclusion bodies were observed: myelinic bodies (MBs) (Figs 1, 2, 3, 4, 5, 6 and 7) and granular bodies (GBs) (Figs 1, 7, 8 and 9). The size and structure of both types of inclusion were similar, whether they occurred in half-leaves opposite those inoculated with TMV (Figs 3, 5, 6 and 7), with buffer (Fig. 2) or not inoculated (Figs 4 and 8). Also their frequency was comparable in the three series of samples: an average of 3-4 per cell profile, unevenly distributed among the cells.

The MBs had a diameter of 0.3 to 1.3 μm and a shape varying from ovoidal (Figs 3 and 4) to rather convoluted (Fig. 5), also depending on the plane of sectioning (Fig. 2, a-d). They consisted of strongly osmiophilic lamellae, concentric and parallel (Fig. 6), sometimes occurring in triplets (Figs 2 and 7). Close to these, fine granular aggregates, showing here and there a fingerprint substructure, were sometimes observed (Figs 2d and 5): serial sections reveal that they may be oblique or tangential views of the MBs (Fig. 2d). The MBs were located in the vacuole or, more often, in the cytoplasm, usually close to the plasmalemma. None was seen between plasmalemma and cell wall.

The GBs, about the same size as MBs but with a different structure, occurred only in the vacuole, and consisted of finely granular, osmiophilic material, sometimes with a fingerprint structure, surrounded by a tangle of membranes (Figs 7, 8 and 9). Sometimes MBs and GBs occurred side by side in the vacuole, and were joined by tangled membranes (Fig. 7).

The frequency and structure of MBs and GBs were not affected by the different fixation schedules, so we conclude that they were not a fixation artifact.

The two types of inclusion reacted in the same way to the hydrogen peroxide treatment, which selectively removes osmium: they disappeared leaving electron-transparent patches, while the plasmalemma, tonoplast and the tangled membranes around them remained nearly unaltered (Figs 10 and 11). This suggests that both inclusion types consisted mainly of lipid, and therefore that they were not plasmalemmasomes, even in the case of MBs located close to the plasmalemma. MBs and GBs may represent two different aspects or stages of the same phenomenon.

The MBs we observed resembled in size and structure the MBs noted by Fraser and Clay (1983) in samples analogous to those of our *a* series. However, the different location of MBs in our samples (in the vacuole or cytoplasm instead of the extraprotoplasmic space), as well as the presence of a second kind of inclusion, the GBs, suggest that the tissues in the two cases were possibly not in exactly the same condition. It cannot be excluded, for example, that growing conditions may influence the presence or structure of the inclusion bodies.

The presence of MBs and GBs in control tissues, as well as their lipidic nature, led us to consider rather unlikely an association of such bodies with the accumulation of PRs. The bodies are probably normal cell constituents, perhaps correlated with a particular leaf age or with the growing conditions used. This view is supported by the finding of MBs very similar in location and structure in cells of healthy fungi (Smith and Marchant, 1968) and higher plants (Robards and Kidwai, 1969). The authors suggested that MBs might have some role in the intracellular enzyme transport, and their presence was possibly bound to particular stages of cell development.

Acknowledgements

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Samenvatting

Myeline-achtige en granulaire insluitsels in niet-geïnoculeerd weefsel van gezonde en van met TMV geïnoculeerde bladeren van Xanthi n.c. tabak

Cytologisch onderzoek werd verricht aan helften van tabaksbladeren, waarin nieuwe eiwitcomponenten voorkwamen als gevolg van inoculatie van de tegenoverliggende bladhelften met tabaksmozaïekvirus. Er werden geen verschillen in ultrastructuur gevonden tussen dit materiaal en dat van niet met virus geïnoculeerde planten. In beide gevallen werden twee soorten insluitsels waargenomen, nl. myeline-achtige en granulaire lichaampjes. Aard en mogelijke functie van deze lichaampjes worden besproken.

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