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Virus Aggregates and Pinwheels in Plants Infected with Mite-transmitted Ryegrass Mosaic Virus

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Mulligan (1960) reported that ryegrass mosaic virus (RMV) had long rod-shaped particles that were often broken in preparation. Brandes (1964) confirmed the particle shape and determined a modal length of 703 nm and Brandes & Bercks (1965) placed RMV in the potato virus S group together with another mite-transmitted virus, wheat streak mosaic (WSMV), because their particles were slightly shorter and stiffer than those classified with potato virus Y. Shepard & Carroll (1967) found pinwheel inclusions, thought typical of the PVY group (Edwardson, 1966), in wheat infected by WSMV. Gibbs (1969) suggested that WSMV and RMV should be placed in a subgroup of the PVY group.

We maintained RMV in the glasshouse by mechanical inoculation to Italian ryegrass (*Lolium multiflorum*) cv. S. 22 and oats (*Avena sativa*) cv. Blenda. It was distinguished from other grass viruses by particle size and morphology, host range and vector transmission, as described by Catherall (1970).

Pieces of infected leaf were fixed in 2·5 % glutaraldehyde in 0·05 M-phosphate-buffered 1 % osmium tetroxide, dehydrated in acetone and embedded in Epon. Tissue was stained with uranyl acetate during dehydration and, after sectioning, with lead citrate (Reynolds, 1963).

The virus usually occurs in aggregates seen in transverse and longitudinal section in Fig. 1 (*a*, *b*). The aggregation of virus in some tissues suggests a reason for the large variations in particle number seen in different leaf-dip preparations from the same leaf. However, a few unaggregated particles occur in some cells. Most virus particles were restricted to mesophyll tissue and were never seen in vascular tissue or adjacent cells and infected cells became vacuolate and degenerated as described by Shepard & Carroll (1967). Sections also showed frequent pinwheels and laminar cell inclusions (Fig. 1 *c*, *d*). The length and diameter of the filamentous particles agreed with those of RMV seen in negatively stained leaf-dip preparations. No particles were seen associated with cell inclusions or pinwheels. This agrees with Shepard & Carroll's (1967) observations on WSMV. Infected tissue appeared the same in ryegrass or oats.

The presence of pinwheels and cytoplasmic inclusions support Gibbs's (1969) suggestion that, at least in their ultrastructural effects on infected cells, some mite-transmitted viruses have affinities with the PVY group. Harrison & Roberts (1971) found pinwheels induced by *Atropa* mild mosaic virus which is now known to be a strain of henbane mosaic virus (Govier & Woods, 1971) speculated that the PVY group should be considered as having wider limits of particle length than previously supposed, although retaining other characteristics such as non-persistent aphid transmission, sap transmissibility, mosaic symptoms and having flexuous, elongated particles. The presence of pinwheels in wheat infected by wheat spindle streak mosaic virus (WSSMV) (Hooper & Wiese, 1972) is further evidence for a possible modification of the limits of the PVY group. Slykhuis & Polak (1971) and Hooper & Wiese (1972) reported a range of particle lengths in section from 600 to 3000 nm but observations of leaf-dip preparations and purified particles suggested an infectious particle length of more than 1000 nm. Although WSSMV shares with PVY group viruses other characteristics as well as particle morphology, it is soil-borne and is not

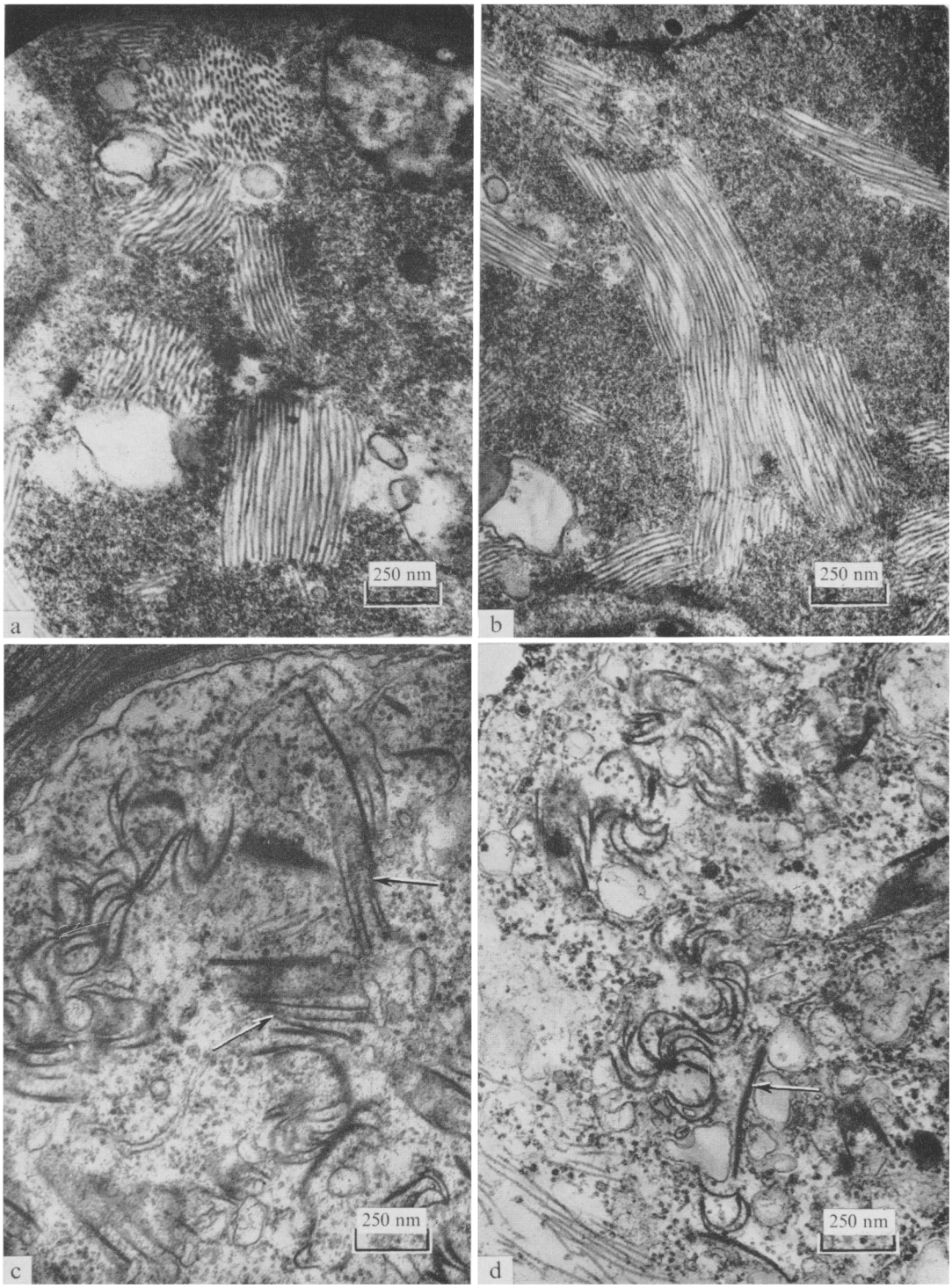

Fig. 1. Ryegrass mosaic virus infected leaves. (*a*) Virus aggregates in transverse and longitudinal section. (*b*) Virus aggregates in longitudinal section. (*c*) Pinwheels and inclusions (arrowed). (*d*) Pinwheels, virus particles + inclusions (arrowed).

transmitted by aphids. These results, together with the work of Shepard & Carroll (1967) and that reported here, suggest that, although pinwheels remain diagnostic for infection by viruses with elongated particles, other characters of the virus, especially the vector, may not correspond to those associated with the PVY group. Specifically, pinwheel inclusions need not necessarily be associated with an aphid vector.

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