Pinwheels and Crystalline Structures Induced by *Atropa* Mild Mosaic Virus, a Plant Virus with Particles 925 nm. Long

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**SUMMARY**

*Atropa* mild mosaic virus is transmissible from tobacco to tobacco, both by green peach aphids and by inoculation of sap. It causes mosaic symptoms and has elongated slightly flexuous particles with a modal length of about 925 nm. It induces the formation of pinwheels resembling those elicited by viruses of the potato Y group (potyviruses), and infected cells also contain crystalline structures resembling hexagonal arrays of tubules.

**INTRODUCTION**

There is increasing evidence that viruses with close affinities can have similar ultrastructural effects on infected cells. Thus the potato Y group of viruses (Brandes & Bercks, 1965), here referred to as 'potyviruses', characteristically induce the formation of 'pinwheels', which have otherwise been found only in plants infected with the mite-transmitted wheat streak mosaic virus (Shepard & Carroll, 1967). These pinwheels were noted by Yamaguchi, Kikumoto & Matsui (1963), but not well understood and named until the work of Edwardson (1966), Purcifull & Edwardson (1967) and Edwardson, Purcifull & Christie (1968). They were found in cells infected with seven potyviruses and shown to consist of a series of plates radiating from a central axis and mostly curved in planes perpendicular to this axis. The plates can be digested by several proteolytic enzymes (Shepard, 1968; Weintraub & Ragetli, 1968) and therefore seem to contain protein which, moreover, is unrelated serologically to that in the virus particles (Shepard & Shalla, 1969).

In work started to study the cytological effects of viruses in other groups, we unexpectedly found pinwheels in cells infected with belladonna mottle virus, which has isometric particles and is allied to viruses of the turnip yellow mosaic group (Paul et al. 1968; Gibbs, 1969). Further work showed that our virus culture also contained a second and apparently new virus, with elongated particles. This has been named *Atropa* mild mosaic virus (Bode, Brandes & Paul, 1969; Dr O. Bode, personal communication). We now show that *Atropa* mild mosaic virus has particles considerably longer than those of potyviruses (730 to 800 nm.) but that, like them, it induces the formation of pinwheels.

**METHODS**

Infected tissue from young or well expanded leaves of tobacco plants (*Nicotiana tabacum* cv. Xanthi-nc) showing mosaic symptoms, was fixed, embedded, sectioned and stained by the 'modified method' of Harrison, Stefanac & Roberts (1970).
Fig. 1. (a) Particles of *Atropa* mild mosaic virus in phosphotungstate-treated sap from inoculated tobacco leaves. (b) Plate, presumably derived from a pinwheel, seen in phosphotungstate-treated tobacco sap. (c) Portion of plate at high magnification.
RESULTS AND DISCUSSION

In the first experiments, tissue was examined from leaves systemically infected with both belladonna mottle virus and *Atropa* mild mosaic virus. The cells contained abnormal chloroplasts, which had marginal vesicles of various sizes, similar to those found in cells infected with turnip yellow mosaic virus (Chalcroft & Matthews, 1966). Many of the cells also contained large masses of cytoplasm, in which pinwheels and crystalline structures could be seen in addition to the isometric particles of belladonna mottle virus. When sap from such plants was mixed with 2% sodium phosphotungstate and examined in the electron microscope, the isometric particles of belladonna mottle virus were found together with the slightly flexuous elongated particles of *Atropa* mild mosaic virus.

![Particle length distribution of *Atropa* mild mosaic virus in sap from inoculated tobacco leaves (142 particles). One unit = about 12.6 nm. Modal length = 92.5 nm.](image)

*Atropa* mild mosaic virus was freed from belladonna mottle virus by transmitting it to fresh Xanthi tobacco seedlings, using aphids (*Myzus persicae* Sulz.) fed for 5 min. on infected plants and then kept for 2 hr on healthy ones. When transmitted to further plants by inoculation of sap it produced mosaic diseases in Xanthi tobacco and *Datura stramonium*, but no symptoms in *Chenopodium amaranticolor*. The lengths of particles seen in phosphotungstate-treated leaf sap (Fig. 1a) showed a sharply defined modal value of about 925 nm. (Fig. 2), considerably longer than that of potyviruses but close to the value of about 900 nm, quoted by Bode et al. (1969). A similar value was obtained when the lengths of particles of *Atropa* mild mosaic and tobacco rattle viruses were compared by electron microscopy of mixed preparations. Preparations from plants infected with *Atropa* mild mosaic virus also
Fig. 3 to 5. Sections of tobacco leaves systemically infected with *Atropa* mild mosaic virus.

Fig. 3. Section of infected palisade cell showing pinwheels (P) in transverse and longitudinal section, and crystalline material (C).
Pinwheels and crystalline structures

contained plates (Fig. 1b, c) showing obvious longitudinal lines about 5 nm. apart and less obvious transverse markings at a spacing of 3 nm. These plates therefore appear similar to those derived from the pinwheels induced by watermelon mosaic (Edwardson et al. 1968) and other potyviruses.

Fig. 3 shows a large area of cytoplasm containing several pinwheels, some in cross-section and others in longitudinal section. They closely resemble the pinwheels induced by water-

Fig. 4. (a) Pinwheel, showing central axis and radiating curved plates. (b) Mass of virus-like particles. (c) Longitudinal section of parts of pinwheels showing association of the plates with the endoplasmic reticulum (arrows).
melon mosaic virus and, when examined in cross-section (Fig. 4a), dark spots of a similar diameter to that of particles of *Atropa* mild mosaic virus can be seen in contact with several of the plates. In longitudinal section, the ends of some of the pinwheel plates seem associated with the endoplasmic reticulum (Fig. 4c), a feature found also for two other potyviruses, pokeweed mosaic virus (Kim & Fulton, 1969) and maize dwarf mosaic virus (Krass & Ford, 1969). In both systemically infected and inoculated leaves some cells contained small areas of vesiculate material not seen in virus-free plants; virus-like particles were visible in some regions of the cytoplasm, occurring either individually or in masses (Fig. 4b). *Atropa* mild mosaic virus had no obvious effect on the chloroplasts.

Fig. 5. (a) Transverse section through crystalline structures seen in cytoplasm, showing honeycomb arrangement (arrow). (b) Longitudinal section through a similar crystalline structure.
Many of the infected cells contained crystalline structures (Fig. 3, 5a, b) apparently different from any previously reported in cells infected with plant viruses. They were often numerous in cells that contained them and were found only in the areas containing pinwheels. They mostly measured 150 to 300 nm. in cross-section, and many had a central unstained, irregular spot; their lengths ranged up to 2 μm. When the crystalline material was examined at higher magnification it appeared in cross-section as a honeycomb-like arrangement of hexagonal units with darkly staining walls (Fig. 5a). The centre-to-centre distance of these units was about 15 nm. In longitudinal section the crystalline material showed densely staining, parallel longitudinal bands at a spacing of about 12 nm. (Fig. 5b), depending on the angle of section. It seems unlikely that these patterns represent aggregates of virus filaments like those found in extracts of blue lupin infected with watermelon mosaic virus (Purcifull, Edwardson & Christie, 1968). They are interpreted more plausibly as hexagonally arrayed tubules of unknown composition.

Although a possible interpretation of our results might be that our culture of Atropa mild mosaic virus was contaminated with potato virus Y or another potyvirus, we think this unlikely because our culture caused no lesions in Chenopodium amaranticolor and there was no peak at about 750 nm. in the particle length distribution (Fig. 2). Also, pinwheels and crystalline structures were found in plants inoculated with preparations which had been diluted to 10⁻⁷ or heated to over 70°; both treatments which should have resulted in loss of infectivity by most potyviruses.

Like potyviruses, Atropa mild mosaic virus is transmissible by aphids in brief feeding periods, is transmissible by inoculation of sap, causes mosaic symptoms, has slightly flexuous elongated particles and induces pinwheel formation. It differs from potyviruses in reaching an apparently higher concentration in leaf sap and in the length of its particles. The description of the potyvirus group may need to be modified to include Atropa mild mosaic virus but more information is required about the properties and serological affinities of the new virus before a decision on this can be reached. At present it is not clear whether Atropa mild mosaic virus is best considered as a member of a previously undetected cluster of viruses distinct from the potyvirus group, or whether the group is larger than previously recognized, with Atropa mild mosaic virus at one extremity.

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REFERENCES


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