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Research Note

A non-mechanically transmissible isometric virus associated with asteroid mosaic of the grapevine

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K e y w o r d s : asteroid mosaic, virus-like disease, isometric virus, electron microscopy.

Introduction: Asteroid mosaic, a long-known virus-like disease of the grapevine (*Vitis vinifera*), was originally described from Napa Valley in California (Hewitt 1954), where it remained localized without apparent spreading and is now disappearing (A. C. Goheen, personal communication). Naturally infected vines exhibit star-shaped chlorotic spots, sometimes with necrotic centre, irregularly distributed over the leaf blades which may be asymmetric, twisted and puckered along the veins. Foliar symptoms are often accompanied by stunting and unfruitfulness. The disease is caused by an unknown graft-transmissible agent which induces highly diagnostic localized clearing of the main and secondary veins in *V. rupestris* (Hewitt *et al.* 1962).

Asteroid mosaic-like symptoms have been repeatedly observed in *V. vinifera* in Europe and South Africa (Refatti 1970) but no published records exist of their graft transmissibility to *V. rupestris*. Recent successful graft-transmission trials carried out in Greece, constitute, therefore, the first experimental evidence of the occurrence of asteroid mosaic outside USA (Kyriakopoulou 1991).

In the assumption that the agent of asteroid mosaic could be a virus, investigations were initiated using an authentic diseased Californian source which was maintained and propagated in isolation in *V. rupestris* under glasshouse conditions. This paper reports the preliminary results of these investigations.

Materials and methods: Asteroid mosaic-infected *V. rupestris* cuttings which showed prominent leaf symptoms (Figure, a) were rooted in sand and forced to grow under glasshouse conditions. Donor vines did not contain grapevine fanleaf (GFLV) or grapevine fleck (GFkV) viruses, as ascertained by repeated mechanical inoculations to herbaceous hosts and/or ELISA tests (Clark and Adams 1977) using locally produced antisera. However, all attempts to transmit a virus from these cutting by inoculation of sap expressed from leaves or roots were unsuccessful. Thus, virus recovery was attempted directly from grapevine tissues.

Virus sources were leaf (whole blades or separated main veins or petioles), root and cortical tissues from symptomatic rootlings, which were processed with either of two slightly modified versions of the technique used for the purification of GFkV (Boulila *et al.* 1990): (i) tissues were homogenized in 5 vol. of extraction buffer (0.05 M phosphate pH 6.2, containing 5 mM mercaptoethanol, 5 mM DIECA and 0.05 % polyethylene glycol mol. wt 6000) and the homogenate was stored overnight in the dark at room temperature after the addition of 1 % pectinase and 2 % cellulase, or (ii) tissues were ground in liquid nitrogen, then homogenized in 5 vol. of extraction buffer without enzyme treatment. With both procedures, the extracts were squeezed through cheesecloth, centrifuged at 6,000 *g* for 20 min and treated with 10 % PEG 6,000 plus 1 % NaCl at 4 °C for 1 h prior to alternate cycles of low- (10,000 *g* for 10 min) and high-speed (120,000 *g* for 1 h). Pellets were resuspended in 0.05 M phosphate buffer pH 6.8, containing 5 mM mercaptoethanol, 5 mM EDTA and 0.2 M NaCl or, before density gradient centrifugation (25 % sucrose in citrate buffer at 24,000 rpm for 2 h 45 min), in 0.02 M citrate pH 6.1. Purified preparations were observed with an electron microscope (Philips 201C) after staining with 2 % aqueous uranyl acetate and/or exposure to antisera to GFLV, GFkV and grapevine ajinashika disease-associated virus (GAaV) (Namba *et al.* 1991). Tissues from healthy *V. rupestris* rootlings were collected and similarly processed to serve as controls.

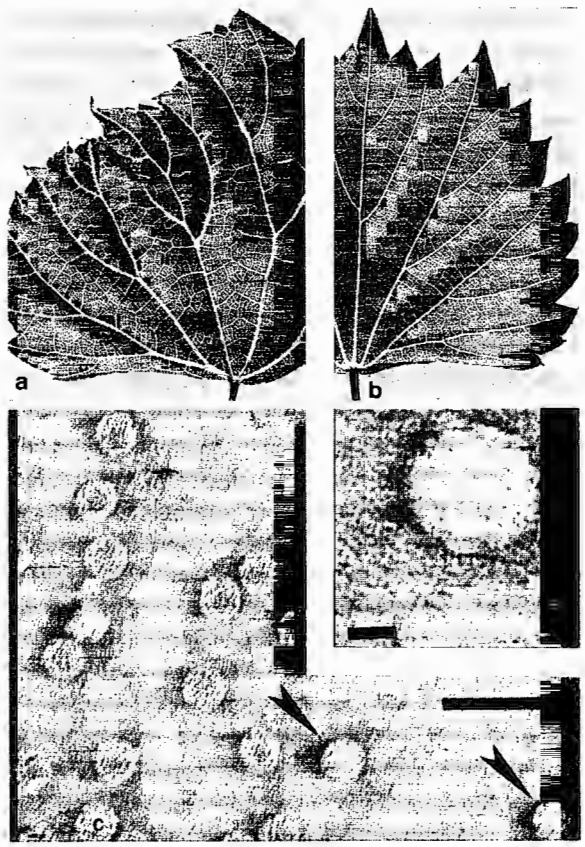

Figure: (a) Typical vein clearing included in *V. rupestis* by asteroid mosaic following graft inoculation. (b) A healthy leaf on the left. (c) Negatively stained virus-like particles in a purified preparation from cortical scrapings of asteroid mosaic-infected *V. rupestris* rootlings. Arrow heads point to apparently intact particles. Bar = 100 nm. Inset shows the surface structure of a particle at higher magnification (bar = 10 nm).

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Tissue fragments excised from symptomatic leaves or young roots were processed for thin sectioning according to standard procedures, i.e. double fixation in aldehydes and osmic tetroxide, dehydration through graded ethanol dilutions, embedding in Spurr's resin and staining with uranyl acetate and lead citrate (Martelli and Russo 1984) proir to observation under the electron microscope.

Results and discussion: Whereas the presumed agent of grapevine asteroid mosaic was readily transmitted by grafting to *V. rupestris*, in which it induced outstanding and specific symptoms (Figure, a, b), as already mentioned, it could not be recovered by inoculation of sap from symptom-showing vines despite of repeated attempts. Likewise, no virus particles nor prominent cytopathological structures could be detected in thin-sectioned tissues from symptomatic leaves or roots.

The results of purification trials directly from grapevine tissues were more encouraging. In density gradient centrifugation, concentrated, partially purified preparations showed a sedimentation diagram suggestive of the presence of two centrifugal components (i.e., two distinct, though very low peaks when monitored with a ISCO density gradient fractionator). Preparations before or after density gradient centrifugation contained isometric virus-like particles of two types: particles penetrated by the stain to varying extents, possibly representing empty shells, and apparently intact particles (Figure, c). Empty shells were significantly more numerous when tissues had been frozen in liquid nitrogen. However, liquid nitrogen treatment yielded the highest particle recovery, both from cortical scrapings and young roots. No particles were seen in preparations from whole leaves or separated main veins or petioles, regardless of the extraction procedure used (Table), nor in extracts from healthy controls.

Table

Efficiency of virus purification in relation to host tissue and extraction procedure

Tissue processed	Treatment	
	Enzyme	Liquid nitrogen
Whole leaves	-	-
Main veins	NT	-
Petioles	-	-
Cortical scrapings	NT	+++
Young roots	++	+++

+ = presence of particles; - = absence of particles;
NT = not presented

Virus-like particles had a rounded outline and a diameter of about 30 nm. Those particles that were apparently intact, showed a substructure suggestive of clustering of coat protein subunits in pentameric and hexameric capsomeres (Figure, c, inset), and resembled very much GFkV virions (Boulila *et al.* 1990). However, the source material used for purification was not infected by fleck, and purified preparations did not react in ELISA with monoclonal antibodies (Boscia *et al.* 1994) and a polyclonal antiserum to GFkV, nor were they decorated by the same antiserum. Negative results were also obtained in ELISA, immunodiffusion or decoration tests with antisera to GFLV, GAaV and antisera to several nepoviruses known to infect grapevines (i.e. arabis mosaic, grapevine chrome mosaic, grapevine Bulgarian latent, raspberry ringspot, tomato black ring, grapevine Tunisian ringspot, tobacco ringspot and tomato ringspot viruses). Repeated attempts to infect herbaceous hosts by mechanical inoculation with purified virus preparations failed.

The results on the present study, however preliminary as they are, can be taken as an indication that a novel non-mechanically transmissible isometric virus is associated with asteroid mosaic. It seems to be located primarily in phloem tissues, as suggested by its successful recovery from cortical scrapings where it occurs in low concentration.

The virus-like particles associated with the authentic Californian source of asteroid mosaic are quite different from those of all viruses recorded from grapevines, except for GFkV with which they bear a strong resemblance. Points of similarity are: (i) lack of transmissibility by inoculation of sap; (ii) localization in the phloem; (iii) size and shape of the particles; (iv) structural arrangement of coat protein subunits into pentamers and hexamers; (v) presence of two centrifugal components made up of empty protein shells and complete virions. This similarity is even more intriguing if one takes into account that fleck and asteroid mosaic are the only two diseases of *Vitis* known to have *V. rupestris* as the sole indicator, which reacts to both with somewhat similar symptoms. However, asteroid mosaic-infected vines were ELISA negative when tested with antisera to GFkV, and the virus-like particles associated with them were equally unreactive in ELISA and immunoelectron microscopy tests.

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