## Rhabdoviruslike Particles Associated with Strawberry Crinkle Virus

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## ABSTRACT

Thin sections of petals from Fragaria vesca strawberries infected with strawberry crinkle virus (SCV), and of SCV-inoculative Chaetosiphon jacobi aphids, contained cytoplasmic areas with bacilliform particles ca. 190-380 nm long and  $69 \pm 6$  nm wide in the coated form. In the plant tissues, the particles frequently occurred in small aggregations, but those in the insect tissues tended to be more isolated. The particles were not associated with nuclei, and frequently were located at the periphery of cells.

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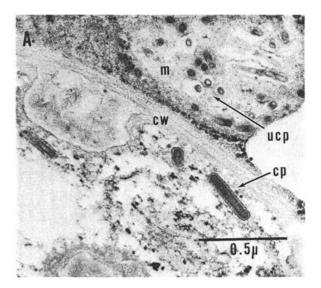
Additional key words: aphids, bacilliform particles.

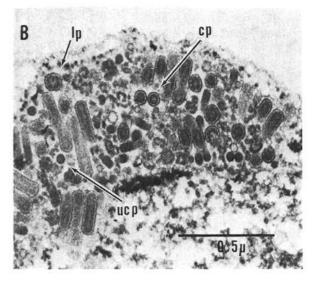
Ten aphid-borne virus diseases of strawberry have been described, and although the transmission and symptomatology have been reported with varying degrees of completeness (3), nothing is known of the nature of the virus particles involved. This is true even with the extensively studied strawberry viruses such as mottle, vein banding, mild yellow edge, and crinkle.

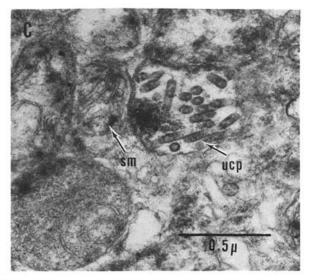
The strawberry crinkle virus (SCV) has a singularly unusual property that has served to focus attention to it; viz, a very prolonged latent period in the aphid vector (2, 4). Duffus (1) suggested that the sowthistle yellow vein virus (SYVV) might multiply in the aphid *Hyperomyzus lactucae* because of the long latent period, and he also cited SCV as the other aphid-borne plant virus with an unusually long latent period. Frazier (2) recently stressed the similarity in the vector-virus relationships of SCV and those of SYVV, and consequently electron-microscope examinations were made of SCV-infected material.

Initially, leaf dip preparations, negatively stained

Fig. 1. A) Thin section through two cells of a petal from a strawberry flower infected with strawberry crinkle virus (SCV) showing an isolated long coated particle (cp) and a number of uncoated particles (ucp) imbedded in a matrix at the periphery of a cell; cw = cell wall; m = matrix. B) A mass of encysted particles in a petal cell, with coated (cp) and uncoated (ucp) particles, and an occasional large particle (lp) containing multiple bodies. C) Thin section of a cell in the subesophageal ganglion from an inoculative apterous Chaetosiphon jacobi aphid vector of SCV, showing a small encysted area containing bacilliform particles; ucp = uncoated particles; sm = swollen mitochondrion.







with 1% sodium phosphotungstate, pH 6.5, were made, with negative results. G. M. Behncken, of our laboratory, suggested that petals might be a better source of material, and on one occasion he found a bullet-shaped particle suggestive of those found in leaf dip preparations of SYVV. However, only rarely have similar structures been found in additional preparations.

Petals with the petal streak symptom of SCV from Fragaria vesca L. strawberry plants and inoculative aphids, Chaetosiphon jacobi H.R.L., were prepared for thin-sectioning. Aphids were fed on infected plants, then tested on a series of healthy indicator seedlings. When the test plants showed symptoms of SCV, the aphids were fixed. Petals from infected test plants were also fixed after they developed the streak symptom.

Discs, 1-2 mm in diam, of plant material, as well as dissected organs from inoculative aphids, were fixed in cold 3% glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated through a graded series of ethanol and propylene oxide, and embedded in Spurr's low viscosity embedding medium (7). Thin sections were cut with a diamond knife on a Porter-Blum MT-I utramicrotome, stained with uranyl acetate and Reynolds' lead citrate (5), and examined with a Philips EM 200 electron microscope.

In the plant tissue, bacilliform particles were seen in epidermal cells, as well as in parenchyma cells near vascular bundles. The particles occurred singly and in groups in the cytoplasm (Fig. 1-A). In some cases, the particles had become completely encysted within a matrix in the cell. Under such conditions, many of the infected cells were misshapened and deeply stained. Both coated (Fig. 1-B, cp) and uncoated (Fig. 1-B, ucp) forms were seen. The uncoated particles consisted of a thickened electron-opaque ring with or without an electron-lucent core; the coated form had an additional thinner outer ring. At times, the inner ring seemed to fuse with the core material to form an electron-opaque circular mass. A larger particle was also found, one that appeared to consist of four subunits in a central core (Fig. 1-B, lp), but this may have been a fixation artifact. The particle structure was more variable than that found in plants infected with SYVV.

In the aphid tissues, bacilliform particles were seen in the salivary and accessory glands and in the subesophageal ganglion. These particles (Fig. 1-C), again in the cytoplasm, were uncoated, consisting of a thickened electron-opaque ring, with or without an electron-opaque central core. The structural differences in the particles seen in the plant in comparison to those found in the insect, in terms of coating, were similar to that initially found with SYVV (6).

The various types of particles found make measurement of doubtful significance. However, the mean diameter of the coated form in the plant was  $69 \pm 6$  nm (n = 25), while it was  $44 \pm 1$  nm (n = 10) for the uncoated form. The width of the coated form was somewhat less than the 80 nm reported for SYVV (6). The average diameter of the uncoated particles found in insect tissue was  $42 \pm 3$  nm (n = 22). Particle length ranged from ca. 190-380 nm in the few cases where entire longitudinal sections were found.

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