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Light and electron microscopy of pea streak virus in crude sap and tissues of pea (Pisum sativum)

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Abstract

In epidermal strips of pea plants with the Wisconsin strain of the pea streak virus, studied from about three weeks after inoculation, extensive parts of the cytoplasm stained red when treated with phloxine and methylene blue. Usually the stained areas were not sharply delimited. Sometimes more or less well-defined granular and often vacuolated inclusion bodies were observed. They were mainly located near the nucleus and along the cell wall.

When sap of such plants was studied in the electron microscope, either negatively stained or shadow-cast, it contained very large amounts of elongate rigid particles typical of the virus. The particles were often found attached to cell organelles.

In ultrathin sections the virus particles could be easily detected occurring separately or in bundles of one particle long in the cytoplasm. Several of such bundles frequently occurred together and they were usually attached to membranes, e.g. around vacuoles, like other members of the carlavirus group (potato virus S group).

Introduction

The pea streak virus (PSV) ($*/_*$:*/5.4:E/E:S/Ap), first described by Zaumeyer (1938), is known to be an important virus in all pea growing areas of the USA, and is widespread in clover e.g. in Canada (Berkenkamp et al., 1966; Pratt, 1968). It is known to occur in Europe by the name of 'Steinkleevirus' (Wetter and Quantz, 1958). The virus may also be present in New Zealand (Chamberlain, 1939).

The virus is often confused with the red clover vein mosaic virus (RCVMV) (R/1:*/5:E/E:S/Ap), although it differs in particle length (619 and 654 nm, respectively) and is only distantly related serologically (Wetter et al., 1962). When identifying a highly deviating strain of RCVMV, Bos et al. (1972) also investigated the best-known or Wisconsin strain of PSV (described by Hagedorn and Walker, 1949) for comparison. They could further distinguish the two viruses, among others by absence of cross protection. Their publication presents some preliminary information on the intracellular accumulation of both viruses. The present paper reports in detail on light and electron microscopy of PSV in crude sap and in tissues of infected pea plants; a later paper will deal with RCVMV. We have recently published on the intracellular localization of the *Passiflora* latent virus also belonging to the potato virus S or carlavirus group (Bos and Rubio-Huertos, 1971).

Materials and methods

The virus used was the Wisconsin strain of PSV, usually designated 'Wisconsin pea streak virus' (WPSV), kindly provided by Dr D. J. Hagedorn, Madison, Wis., USA. It was maintained and propagated in pea, as a rule 'Koroza', but sometimes in other cultivars as well. *Vicia faba* 'Compacta' was often included as an indicator for its typical reaction to the virus (Bos et al., 1972).

For *light microscopy* epidermal strips of petioles, main veins and young stems of pea plants were stained with 1% phloxine and 1% methylene blue in Christie's solution (Bos, 1969).

Crude sap preparations for electron microscopy were made by finely chopping pieces of about 30 mm^2 in five to six drops of 2% PTA, pH 6.5, with a razor blade. One drop of liquid was then transferred with a glass capillary to a carbon-reinforced formvar-coated copper grid, and the excess liquid removed after half a minute with a piece of blotting paper. For shadow casting, the leaf pieces were chopped with distilled water or their cut-surface moved through a drop of distilled water on a formvar-coated grid for 10 to 20 seconds, and the excess water then removed. These water preparations were shadow-cast with palladium. For adding an internal size standard, the pea leaf pieces were often chopped together with a 5 mm^2 piece of a tobacco leaf with TMV. The sap preparations were studied in a Philips EM 300 electron microscope.

Ultrathin sections for electron microscopy were made of pieces of about 1 mm^2 of pea tip leaves 20 days after inoculation of lower leaves. The leaf pieces were treated for 2 h in 4% glutaraldehyde in 0.1 *M* Sörensen buffer pH 7 and washed for 3 h in the same buffer renewed each $\frac{1}{2}$ h. They were then fixed for 2 h at 4°C in a 2% osmium tetroxide solution in the Sörensen buffer, washed in distilled water for $\frac{1}{2}$ h and stained overnight in $\frac{1}{2}$% uranyl acetate. Thereafter the samples were dehydrated in a graded series of ethanol (25-100%) and propylene oxide and embedded in Epon araldite. They were cut with a glass knife and the sections examined in a Siemens Elmiskop 1 electron microscope.

Results

Light microscopy. At four different dates different groups of 'Koroza' peas, 18, 24, 31 and 36 days after inoculation, respectively, and showing characteristic symptoms of WPSV (Bos et al., 1972), were tested for inclusion bodies. In all instances infected epidermal strips at first glance, showed a diffuse, irregular red coloration. Upon closer examination the red colour was most intense near the nucleus and in elongated areas adjacent to the cell wall (Fig. 1 A and B). The 'bodies' mostly were diffusely bordered, but sometimes assumed the appearance of elongate, spindle-shaped or nearly globular true inclusion bodies. The more dense bodies usually were finely granular and rather frequently vacuolated.

Similarly staining condensations in the cytoplasm were detected in infected epidermal strips of the cultivars 'Dick Trom' (tested 24 and 37 days after inoculation), 'Perfected Freezer' (after 24 and 44 days), 'Victory Freezer' (after 24 and 44 days, Fig. 1 C and D), 'Perfected Wisconsin' (after 24 days) and 'Perfected Wales' (after 37 and 44 days).

Such deviations have never been observed in epidermal strips of healthy peas or in

Fig. 1. Light micrographs of epidermal strips of stems and petioles of pea with Wisconsin pea streak virus. A and B 'Koroza' 31 days, C and D 'Victory Freezer' 44 days after inoculation. Magnification × 750. *n* nucleus, *i* inclusion. (Photographs made by Dr R. E. Labruyère.)

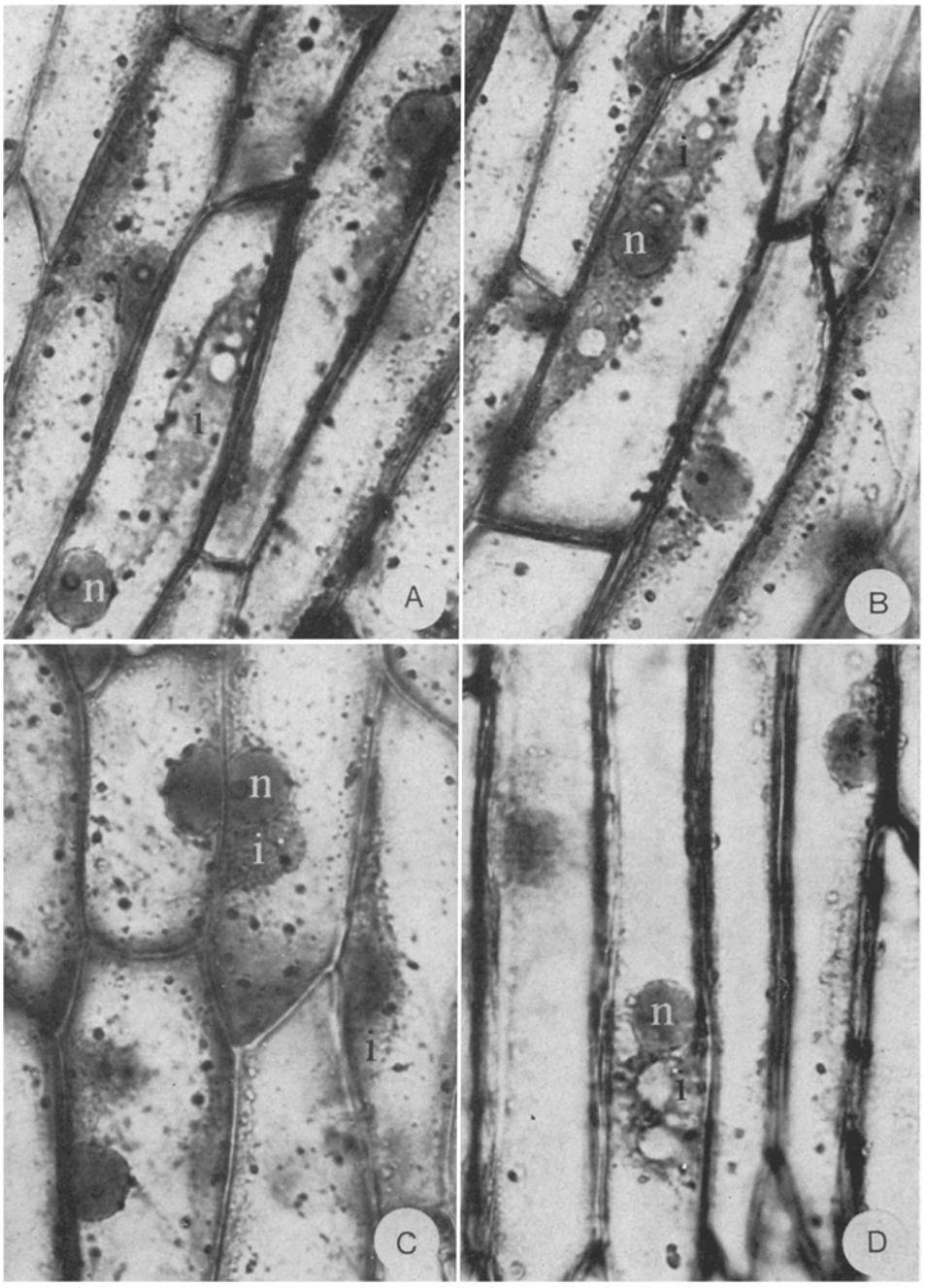

Fig. 1. Lichtmicroscopische foto's van epidermisstrookjes van erwtestengels en -bladstelen met Wisconsin-erwtestrepenvirus. A en B 'Koroza' 31 dagen, C en D 'Victory Freezer' 44 dagen na inoculatie. Vergroting 750 ×. n kern, i insluitsel. (Foto's gemaakt door dr. R. E. Labruyère.)

Fig. 2. Electron micrographs of negatively stained crude plant sap with WPSV and some added TMV particles; bar represents 500 nm.

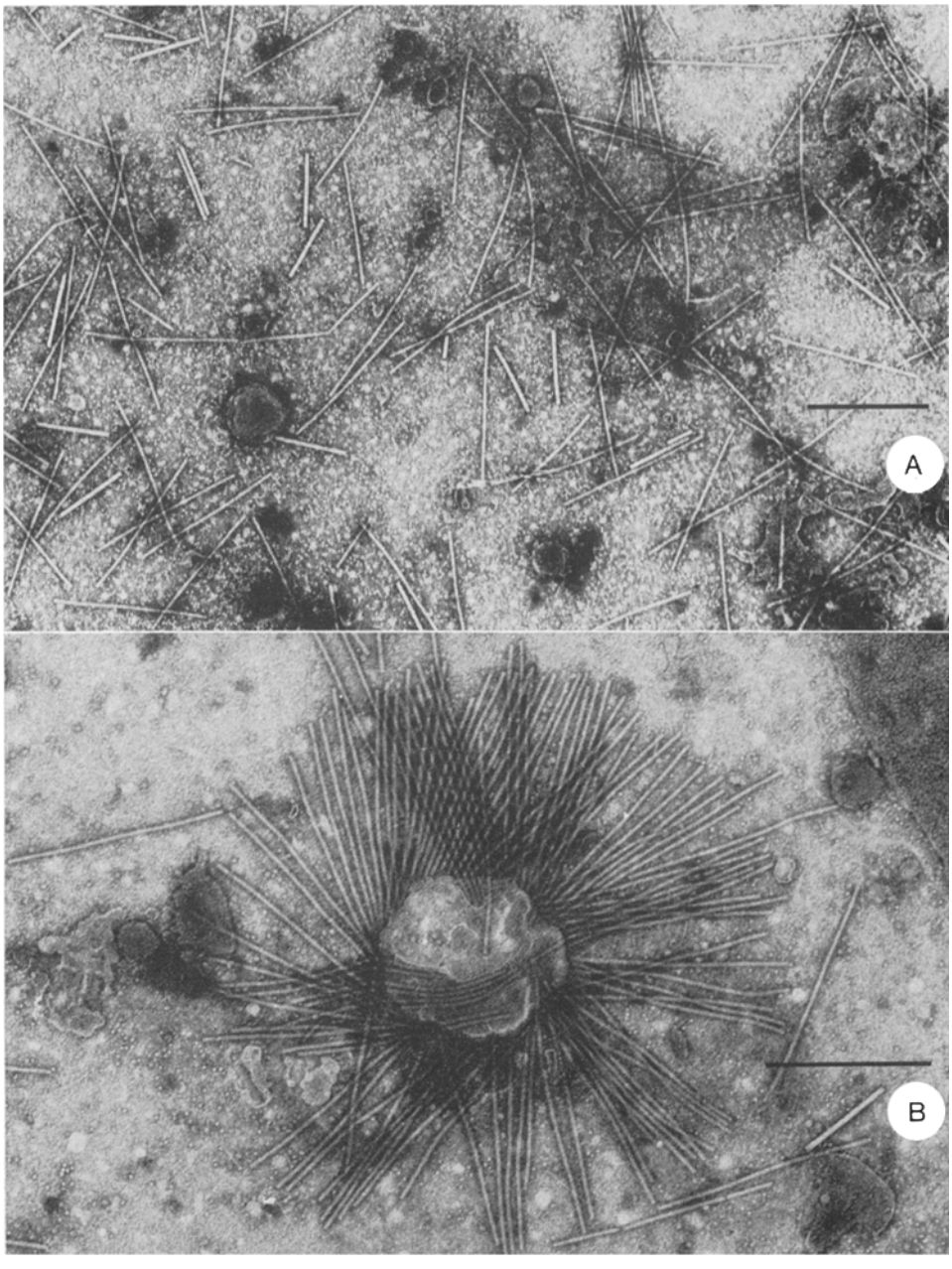

Fig. 2. Elektronenmicroscopische foto's van negatief gekleurd ruw plantesap met het WPSV en enige toegevoegde TMV-deeltjes; de vergrotingsstaaf geeft 500 nm weer.

Fig. 3. Electron micrograph of shadow-cast dip preparation of pea with WPSV; bar represents 500 nm.

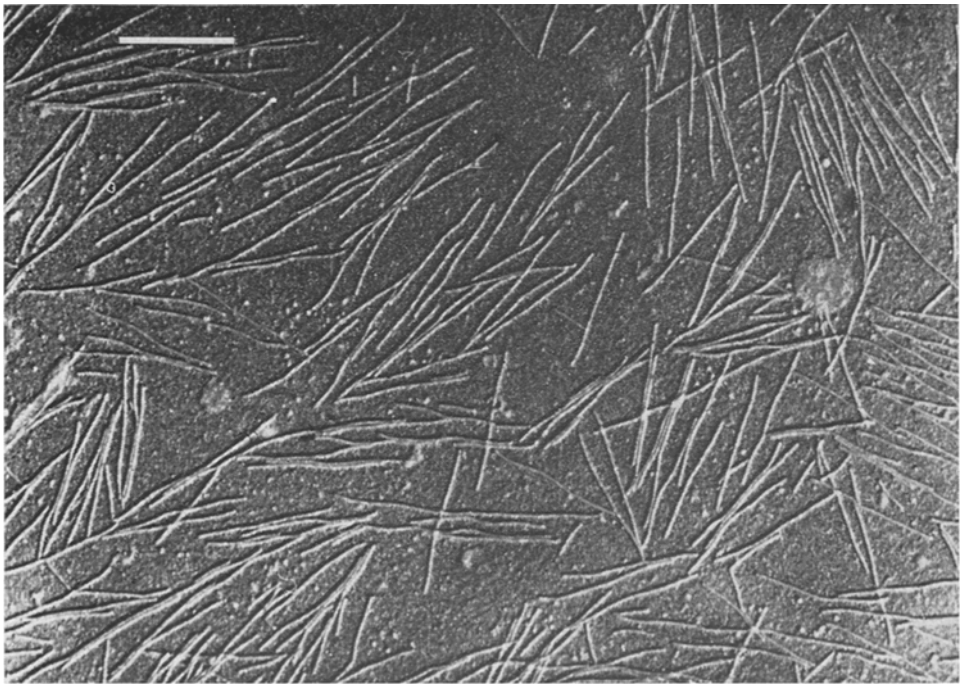

Fig. 3. Elektronenmicroscopische foto van geschaduwd indooppreparaat van erwt met WPSV; de vergrotingsstaaf geeft 500 nm weer.

peas infected with the related RCVMV. Crystals, typical of RCVMV, could never be detected.

Electron microscopy of chop preparations. As already mentioned by Bos et al. (1972), the WPSV could be very easily detected in virus-infected pieces of pea leaf finely chopped in PTA. The particles usually occurred in extremely high concentrations (Fig. 2A) like those of the E207 strain of RCVMV, although these appeared later. Such crude preparations as well as shadow-cast leaf dip preparations gave the impression that the virus constituted the major component of diseased cytoplasm. Often extensive mats or crowds of particles occurred as if dispersing from a dense accumulation. The particles were frequently found in groups with their ends attached to cell organelles (Fig. 2B). In shadow-cast preparations several spots had the appearance of a purified virus preparation (Fig. 3).

When occurring in complex with the E207 or the RK31 strain of RCVMV, the PSV particles could be easily distinguished from those of RCVMV by their particle lengths, (630 and 670 nm, respectively), the distribution curves of both viruses hardly overlapping each other (Fig. 11 of Bos et al., 1972).

Fig. 4. Ultrathin sections of 'Koroza' pea with WPSV 20 days after inoculation; *av* accumulation of virus particles, *iv* individual virus particles, *va* vacuole, *mi* mitochondrion, *w* cell wall, *c* chloroplast; bar represents 500 nm.

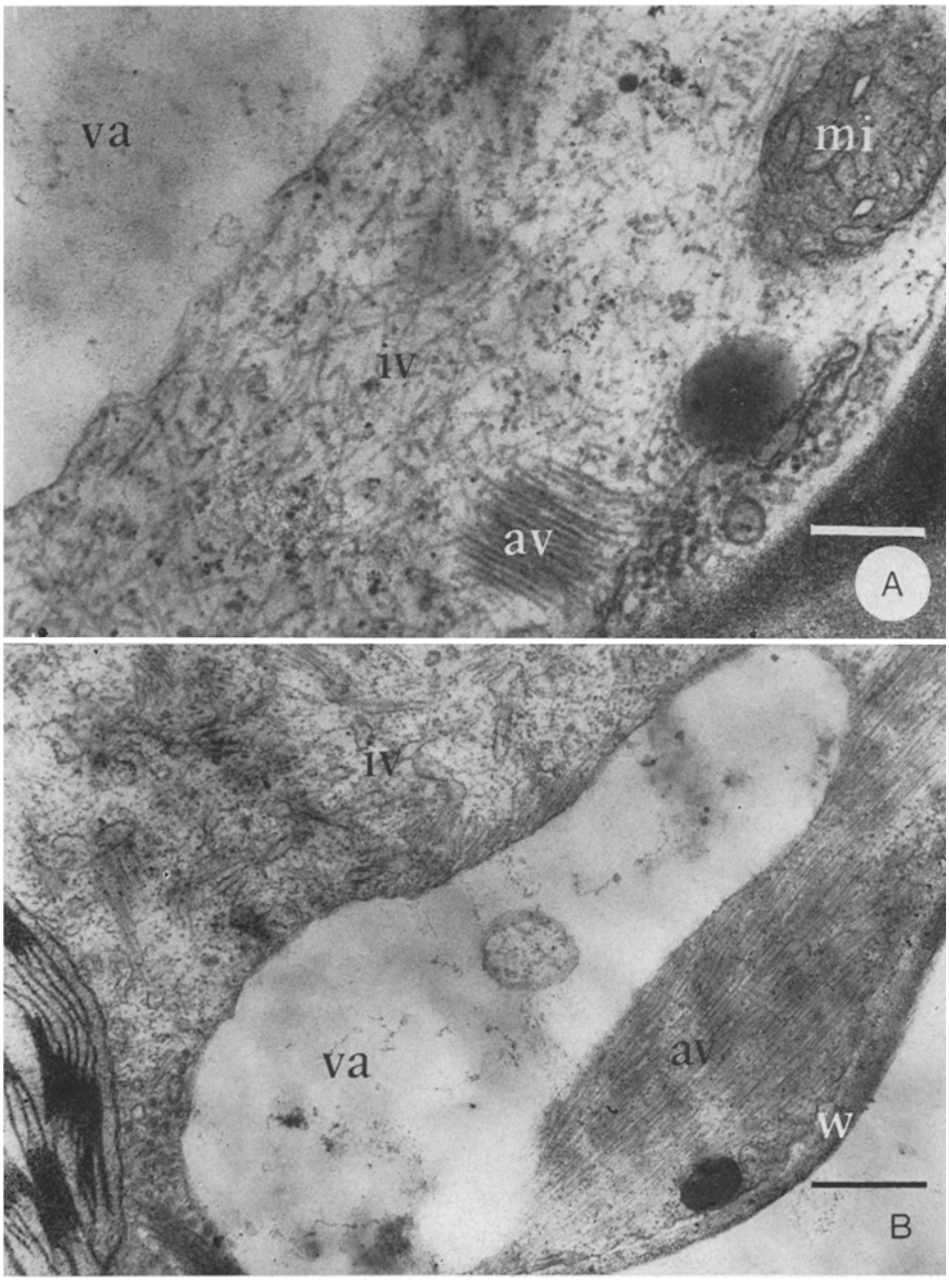

Fig. 4. Ultradunne coupes van 'Koroza' erwt met WPSV 20 dagen na inoculatie; av ophoping van virusdeeltjes, iv individuele virusdeeltjes, va vacuole, mi mitochondrion, w celwand, c chloroplast; vergrotingsstaaf geeft 500 nm aan.

Fig 5, Ultrathin sections of 'Koroza' pea with bundles of virus particles (*bv*) in cross section (A) or longitudinal section (B). Note attachment of bundles to tonoplast; *n* nucleus; bar represents 500 nm.

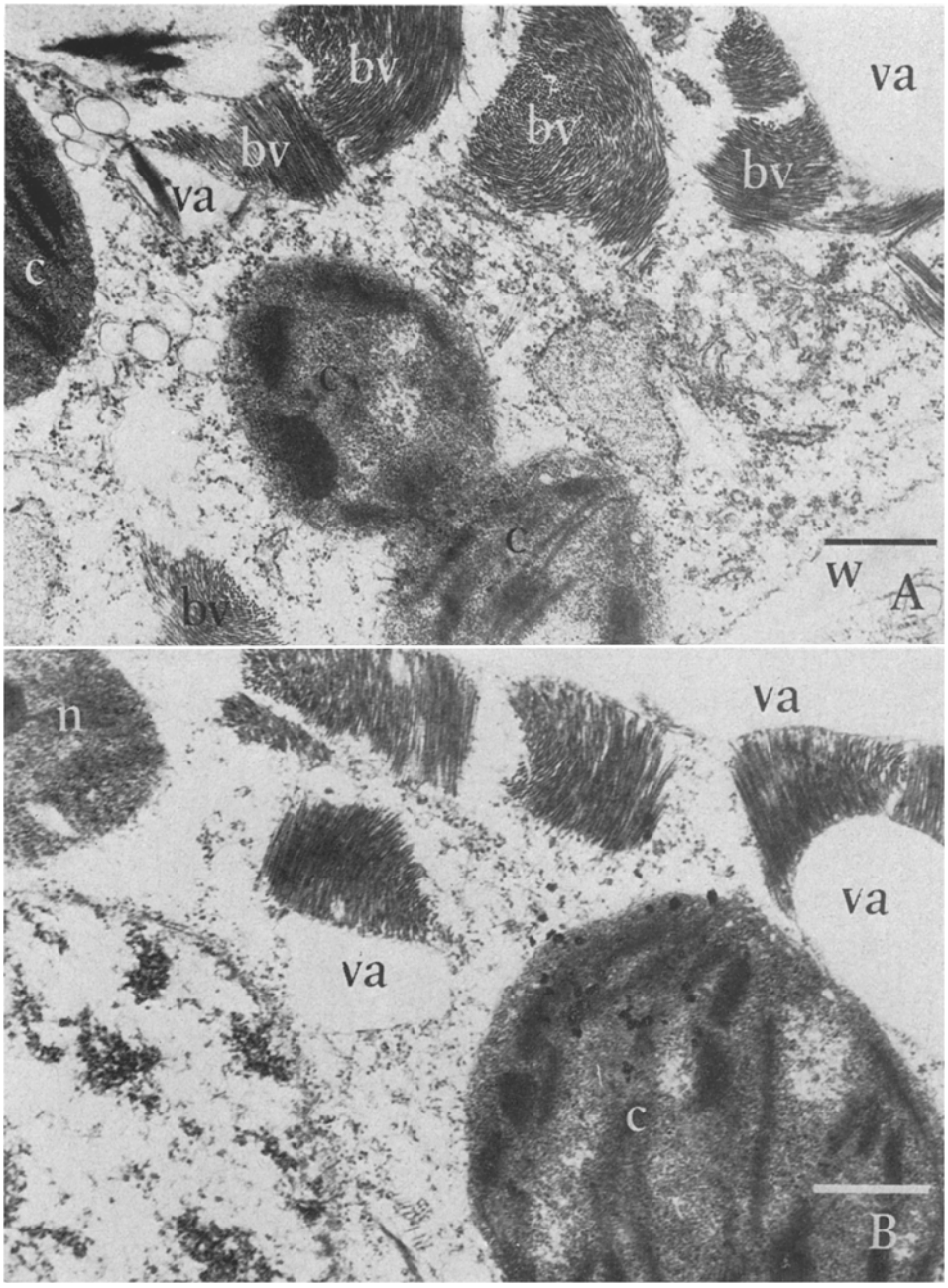

Fig. 5. Ultradunne coupes van 'Koroza' erwten met bundels virusdeeltjes (bv) in dwarsdoorsnede (A) of lengtedoorsnede (B). Let op hechting van bundels aan tonoplast; n kern; vergrotingsstaaf geeft 500 nm aan.

Fig. 6. Ultrathin sections at high magnification of bundles of virus particles in 'Koroza' peas in cross section (A) and longitudinal section (B); bar represents 500 nm.

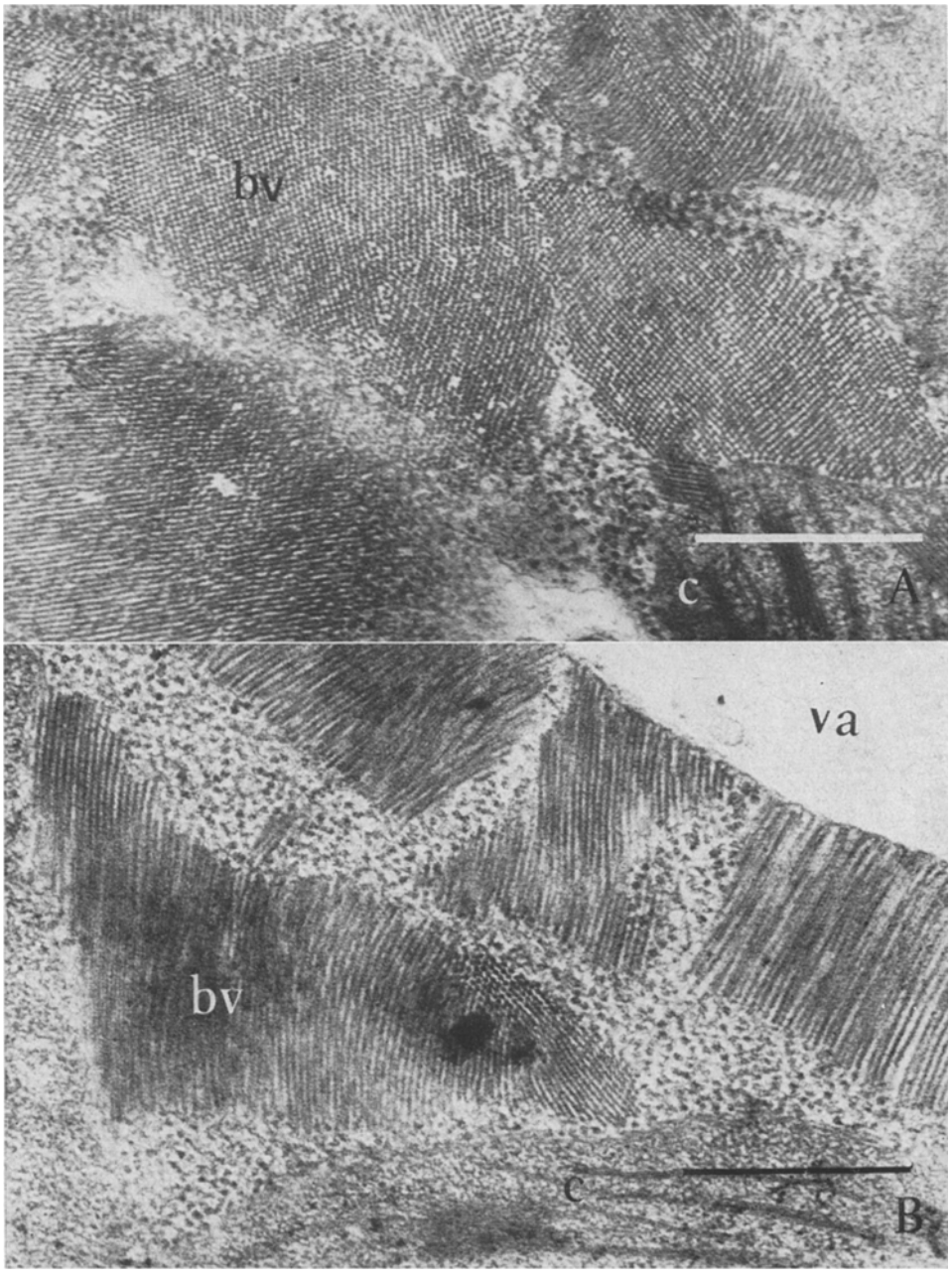

Fig. 6. Ultradunne coupes bij sterke vergroting van bundels virusdeeltjes in 'Koroza' erwt in dwarsdoorsnede (A) en lengtedoorsnede (B); vergrotingsstaaf geeft 500 nm aan.

Electron microscopy of ultrathin sections. In ultrathin sections the virus particles could be easily detected. They often occurred in masses randomly distributed in the cytoplasm (Fig. 4A). More frequently, however, they were observed in dense accumulations which were usually rather clearly bordered and of limited size (Fig. 5, 6). In some cases they were more extensive (Fig. 4B, lower right). In the dense accumulations particles were always present in more or less parallel array. In cross section such bundles often showed a whirlpool effect (Fig. 5A, top). Never was accumulation sufficiently regular to form true crystals. Often several 'bundles' occurred conglomerated (Fig. 6A). Very frequently the bundles were located so that particle extremities at one or both sides were attached to membranes, e.g. tonoplast surrounding vacuoles (Fig. 5A and B, 6B). Areas replete with individual or bundled virus particles often contained more or less extensive vacuoles (Fig. 4B, 5A and B, 6B).

In sections particle lengths were hard to determine because bundles were usually cut transversely or obliquely. When bundles were cut transversely, periodicity varied from about 11–15 nm, which may represent the particle diameter.

Discussion

The bundles in ultrathin sections undoubtedly are composed of virus particles, because their sizes are comparable to those of the particles in crude sap easily recognized as virus particles. Evidently, arrangement is not sufficiently regular to lead to the formation of large crystals visible with the light microscope. Their presence may explain the granulation visible with the light microscope.

The usually irregular delimitation of such accumulations could be traced back by electron microscopy of ultrathin sections. The same held for the vesicles seen with the light microscope and easily observed as vacuoles by electron microscopy of ultrathin sections. The tendency of virus particles to attach to membranes (Fig. 5, 6) also shows up in preparations of crude sap (Fig. 2B).

The immense accumulation of virus when studied in ultrathin sections also explains the enormous number of particles observed in dip and chop preparations. There are such large amounts of virus particles, either occurring separately or in bundles (these in turn often grouped together), that extensive parts of infected cells turn red when stained with phloxine and methylene blue.

Results obtained here when studying ultrathin sections of pea tissue infected with WPSV are basically comparable to those obtained with other members of the carlavirus group: *Passiflora* latent virus (Schnepf and Brandes, 1961; Bos and Rubio-Huertos, 1971), potato virus M (Tu and Hiruki, 1970), potato virus S (de Bokx and Waterreus, 1971) and carnation latent virus (Castro et al., 1971). With the first three viruses, however, virus concentrations usually are lower and the virus bundles, especially with potato viruses S and M, are less easy to find. However, the striking association of bundles with membranes is similar.

With the *Passiflora* virus parallel arrangement may be rather extensive and the association of several bundles showing whirlpool effects in cross section (Bos and Rubio-Huertos, 1971) resembles the situation with WPSV.

With the carnation latent virus, occurring alone or in complex with one or two other viruses, Castro et al. (1971) found large amounts of bundles of elongated particles

distributed at random in the cytoplasm of the host cell. In one case, bundles of particles were also observed within the nucleoplasm of some nuclei.

In contrast to RCVMV (strain RK 31), none of the mentioned viruses form hexagonal, triangular or irregular crystals (Bos et al., 1972; Fig. 48 of Bos, 1970).

Samenvatting

Licht- en elektronenmicroscopie van erwtestrepenvirus in ruw sap en weefsels van erwt (Pisum sativum)

Erwteplanten werden geïnoculeerd met de Wisconsin-stam van het erwtestrepenvirus ('pea streak virus'). Na behandeling van epidermisreepjes met floxine en methyleenblauw kleurden grote delen van het cytoplasma rood (Fig. 1). Gewoonlijk waren de gekleurde gebieden niet scherp begrensd. Soms echter hadden ze ook het karakter van korrelige, vaak van vacuoles voorziene insluitsels. Ze kwamen voornamelijk voor bij de celkern en tegen de celwand.

Wanneer sap van zulke planten met de elektronenmicroscoop werd bestudeerd, negatief gekleurd of na schaduwing van de preparaten, bleek dit zeer grote hoeveelheden stugge langgerekte deeltjes te bevatten die karakteristiek zijn voor het virus (Fig. 2 en 3). De deeltjes vertoonden een duidelijke neiging zich met hun uiteinden te hechten aan celorganellen en membranen (Fig. 2B).

In ultradunne coupes van bladweefsel kon het virus veelvuldig worden aangetroffen als afzonderlijke deeltjes in het celplasma of in bundels van één deeltje lang. Wel was de concentratie meestal hoog (Fig. 4). Veel van zulke bundels kwamen vaak samen voor en ze waren meestal gehecht aan membranen, bijv. rond vacuoles (Fig. 5 en 6).

De verkregen resultaten zijn vergelijkbaar met die van andere, tot dusver bestudeerde virussen van de aardappelvirus-S-groep. De hoeveelheden virus zijn bij het erwtestrepenvirus echter uitzonderlijk hoog.

Acknowledgments

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