Beet Western Yellows Virus in Phloem Tissue of *Thlaspi arvense*

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ABSTRACT

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An electron microscopic study was made of pennycress (*Thlaspi arvense*) infected with beet western yellows virus (BWYV) strain B-16. All leaves examined, whether symptomless or chlorotic, contained virus particles. However, the distribution of BWYV in leaves was uneven; many sections did not reveal virus particles. In contrast, large virus aggregations were readily found in stem tissue. In both leaves and stems, virions were seen in sieve elements, companion cells, and phloem parenchyma, but not in xylem or mesophyll cells. In sieve elements, virions were located peripherally, often between the stacked cisternae of the endoplasmic reticulum (ER). In companion and other parenchyma cells, the virus was most often visible in the cytoplasm near the plasmalemma. Occasionally, virions were seen in the nucleus, aggregated around the nucleolus. Virus particles were found in plasmodesmata connecting sieve and parenchyma cells and in those between adjacent parenchyma cells. The most common cytopathological change was proliferation of the ER. Some small vesicles were noted, most often within virus aggregates in sieve elements. Healthy pennycress contained no virus-like particles.

Additional key words: electron microscopy.

Beet western yellows virus (BWYV) is a member of the luteovirus group (4,10), which is composed of small (25–30 nm) icosahedral viruses which cause yellowing of plant hosts. BWYV has a wide host range, infecting more than 150 species in 23 plant families (6,7). Economically important hosts include sugar beet, table beet, lettuce, radish, and spinach. Many weed species of the Compositae and Cruciferae are hosts of BWYV and often play an important role in the epidemiology of the disease (5); pennycress (*Thlaspi arvense* L.) is one such cruciferous weed host of BWYV.

The symptoms of BWYV strain B-16 infection on pennycress are similar to those induced by the virus on many other hosts (4). Interveinal chlorosis is first found on the older leaves, beginning at the leaf tip and spreading towards the base. As the disease progresses, intermediate-aged leaves develop chlorosis, but the young leaves remain green. All leaves become thickened and brittle, and develop a distinct downward tip curl.

Esau and Hoefert (8) found BWYV to be largely phloem-limited in sugar beet leaf tissue. Sugar beet, however, cannot be used for virus purification due to a high concentration of phenolic compounds. In our laboratory, pennycress was being used as a source of purified BWYV, and we needed to know where the virus was located in that host. Special, often time-consuming, purification techniques are required to release virions from the cells of vascular bundles. Only if virions were indeed found to be concentrated in that tissue would the use of such purification techniques be justified.

MATERIALS AND METHODS

Tissue was harvested from two BWYV-infected pennycress plants 4 wk after inoculation by the green peach aphid (*Myzus persicae* Sulz.). Samples from each plant were taken from the stem, and from five leaves. On the oldest leaf sampled, chlorosis extended basipetally over more than half the leaf surface. The intermediate-aged leaf had tip chlorosis. Three young green leaves were examined, including the youngest leaf of each plant. Similar leaf and stem samples were taken from two healthy (noninoculated) pennycress plants. All plants were grown in an air conditioned greenhouse (22 C) equipped with supplemental fluorescent lights that were used to extend day length to 16 hr.

Tissue pieces approximately 1 cm square were fixed in 5% glutaraldehyde in 0.08 M cacodylate buffer, pH 7.4, under vacuum (3). After two 30-min rinses in the cacodylate buffer, the tissue was cut into 1-mm squares which were postfixed overnight in 2% Palade's osmium in veronal acetate buffer at 4 C. Dehydration was in a graded acetone series (30–100%), with uranyl acetate staining in the 70% acetone. Tissue was embedded in Spurr's low viscosity embedding medium (13), and sectioned on a Reichert OMU-3 ultramicrotome. The sections were stained with lead citrate for 5–10 min, and viewed in a JEM 7 electron microscope.

RESULTS

Examination of vascular bundles from the leaves or stems of healthy pennycress plants revealed ultrastructure typical of higher plants. Mature sieve elements contained degenerate plastids and peripheral endoplasmic reticulum (ER) and were often filled with fibers of p-protein. The cytoplasm of companion cells was very electron dense, compared with that of the phloem parenchyma cells. No viruslike particles were seen in healthy pennycress tissues.

In vascular tissue of BWYV-infected pennycress, virus particles were conspicuous, even at low magnification (Fig. 1), particularly between the stacked cisternae of the peripheral ER of sieve elements. Virus particles were differentiated from ribosomes by the

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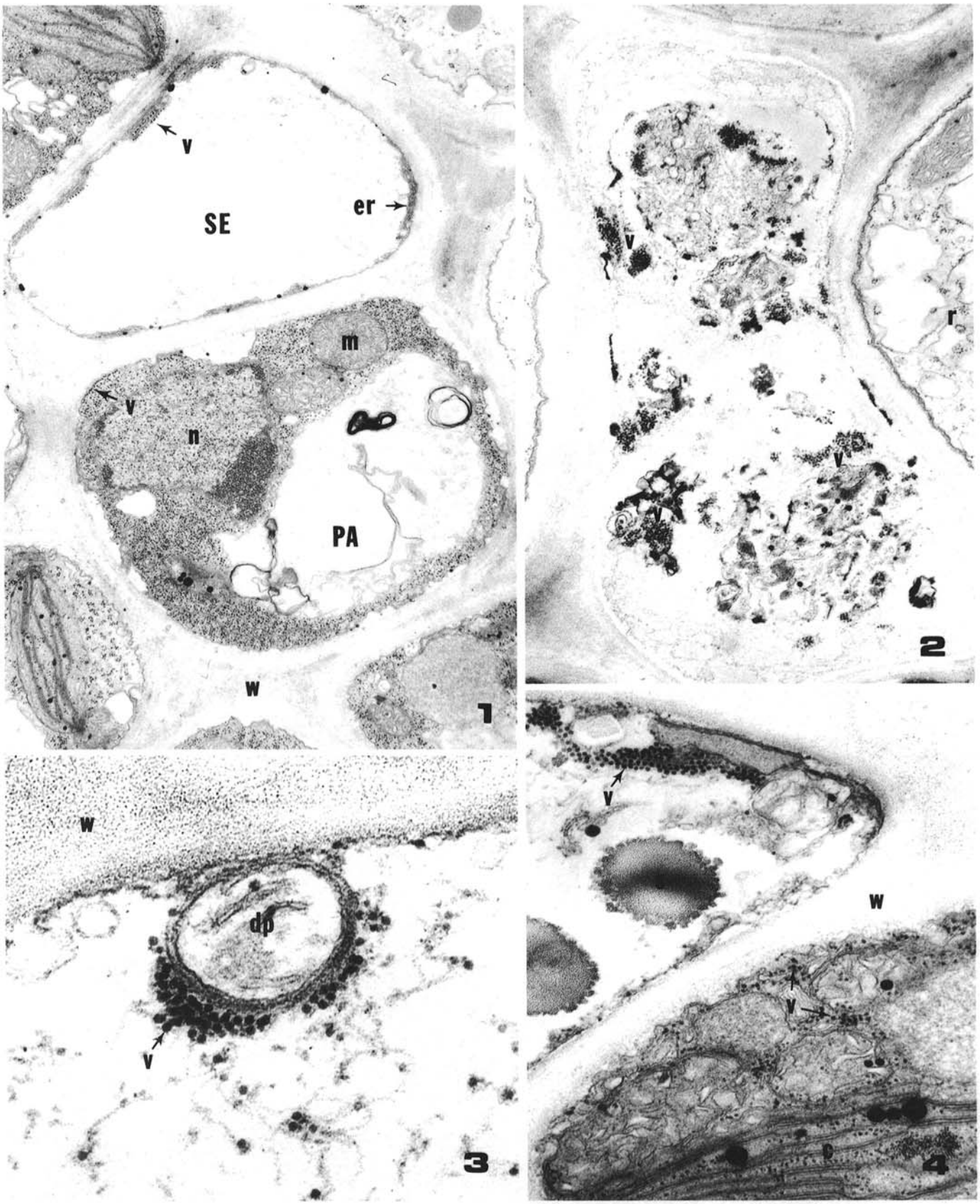
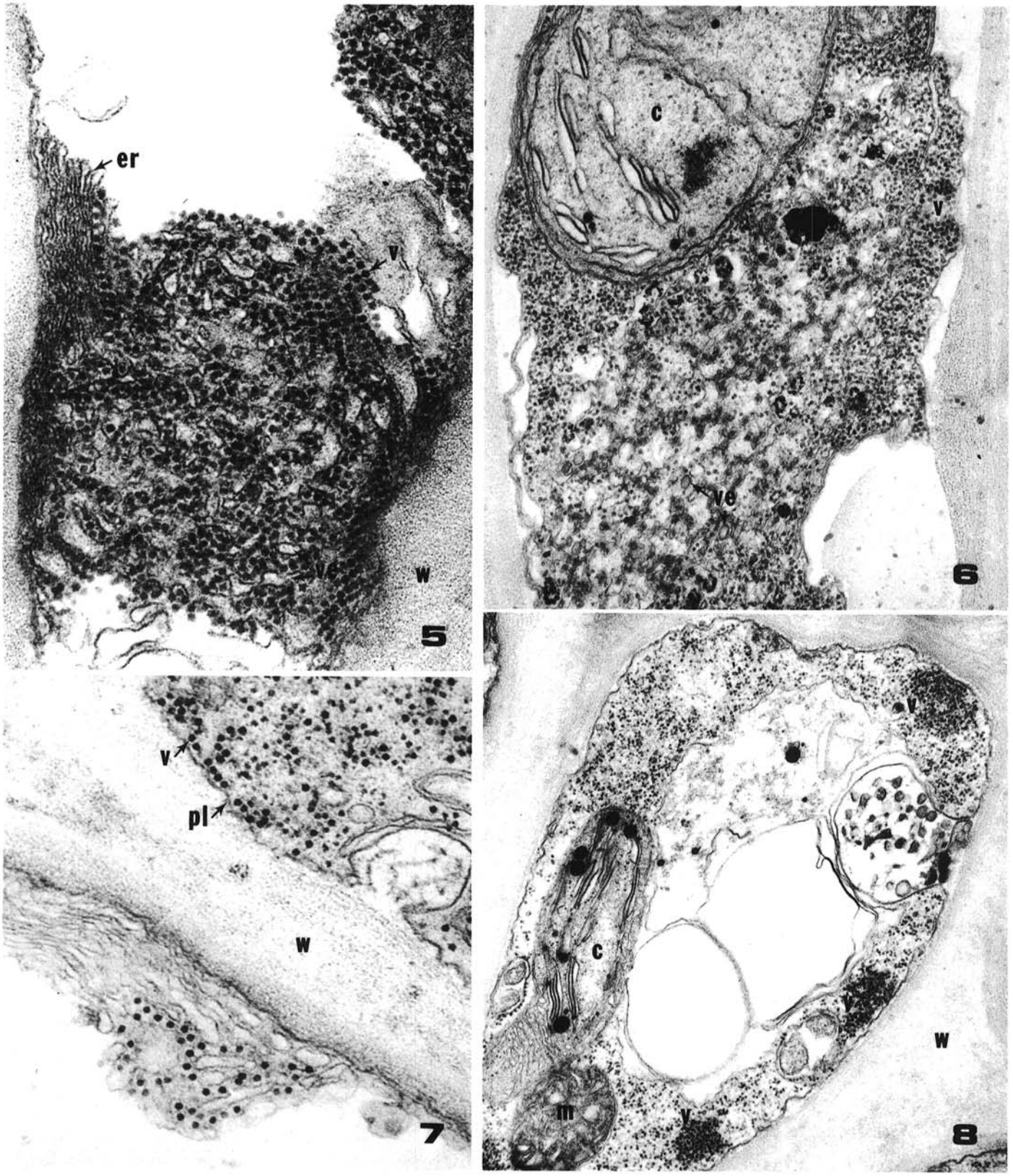
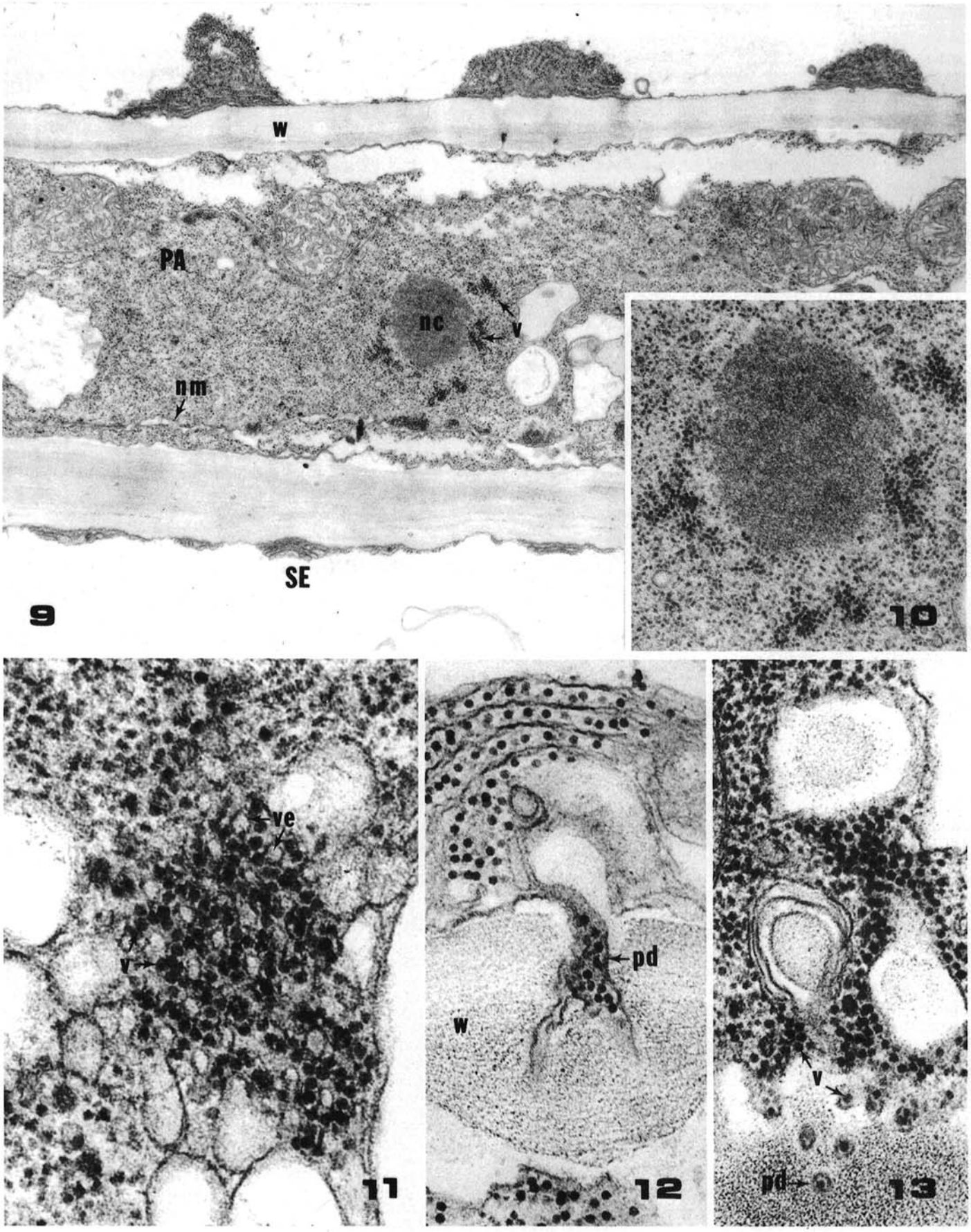


Figs. 1–4. Beet western yellows virus (BWYV) in phloem tissue of pennycress. **1,** Phloem tissue of a young leaf from a BWYV-infected plant. BWYV particles (**v**) between endoplasmic reticulum (**er**) cisternae in sieve element (**SE**) and in parenchyma cell (**PA**) cytoplasm. **m** = mitochondrion, **n** = nucleus, **w** = cell wall (×18,000). **2,** BWYV particles in maturing sieve element. **r** = ribosomes (×28,000). **3 and 4,** Phloem tissue of the youngest leaf from a BWYV-infected plant. **3,** BWYV particles associated with degenerate plastid (**dp**) (×92,000) and **4,** endoplasmic reticulum of sieve elements and scattered in parenchyma cell cytoplasm. **c** = chloroplast, **s** = starch, **w** = cell wall (×45,000).

criteria of Esau and Hoefert (8): virions were slightly larger, more regular in outline, and stained more densely than ribosomes. BWYV was located in the sieve elements of all types of BWYV-infected pennycress tissue: old leaves with pronounced interveinal chlorosis, middle-aged leaves with chlorosis near the tip, and young green leaves, as well as in the stem. Virions often were difficult to locate in old and middle-aged leaves. They were most readily visible in sieve elements, associated with the peripheral ER, or in degenerating sieve elements (Fig. 2), in which they were not peripherally located. Virions were also uncommon in the young leaves of the



Figs. 5–8. Beet western yellows virus (BWYV) in phloem tissue of pennycress. Vesicles (**ve**), virions (**v**), and proliferated endoplasmic reticulum (**er**) in **5,** sieve element (×72,000) and **6,** phloem parenchyma cell. **c** = chloroplast, **w** = cell wall (×43,000). **7 and 8,** BWYV (**v**) associated with the plasmalemma (**pl**) in **7,** single file (×78,000) and **8,** groups. **m** = mitochondrion, **w** = cell wall(×29,000).



Figs. 9–13. Beet western yellows virus (BWYV) in phloem tissue of pennycress. BWYV (**v**) associated with nucleolus (**nc**) in nuclei of phloem parenchyma cells in **9** (×17,000) and **10** (×45,000). **11,** Intranuclear array of virions and vesicles (**ve**). **nm** = nuclear membrane, **PA** = parenchyma cell, **SE** = sieve element, **w** = cell wall (×104,000). **12 and 13,** BWYV in plasmodesmata (**pd**). Virions in **12,** groups (×91,000) and **13,** single file (×83,000).

plants, but some accumulation could be found in every leaf examined, including the youngest leaves (Fig. 3,4). In sieve elements, virions were almost always membrane-associated, either with ER (Fig. 4), or with degenerate plastids (Fig. 3). Virions also were located in companion cells of the youngest leaf, most often near the plasmalemma (Fig. 4).

Virus particles were most readily seen in the sieve elements of the stem. Often the virus, located between stacked cisternae of the ER, formed large, various shaped aggregates (Fig. 5). Such aggregates of virus and ER sometimes did not appear to be attached to the cell membrane, but serial sections usually revealed that they were. Small vesicles often were associated with these large masses of virus particles (Fig. 5).

Pennycress cells infected with BWYV usually did not show conspicuous cytopathological changes; it was easier to locate cells which contained virus than cells with ultrastructural alterations. The most common change was a proliferation of ER in both sieve and parenchyma cells (Fig. 5,6). Vesiculation is such a common phenomenon in phloem that it was often difficult to attribute the presence of the vesicles that were seen here to infection by the virus. Small (~100 nm) vesicles were not uncommon in cells with ER proliferation (Fig. 5,6). Such vesicles were particularly obvious in virus aggregates in sieve elements (Fig. 5), but did not resemble those seen by Esau and Hoefert in BWYV-infected sugar beet, either in appearance or cellular location.

In phloem parenchyma cells, BWYV was found most often near the plasmalemma. When only a few virions were present, most of them were usually aligned single file along the cell membrane (Fig. 1,7). As more virions appeared, they were found in small, peripheral clusters (Fig. 8). When virions were not aggregated, but spread throughout the cytoplasm of parenchyma cells, it was difficult to positively distinguish them from ribosomes (Fig. 4,7). The most common intracellular location of BWYV in phloem parenchyma cells was thus in the cytoplasm. In a few parenchyma cells, virions were seen in the nucleus. Intranuclear aggregations were visible even at low magnification (Fig. 9). These aggregations always were located near the nucleoli (Fig. 10), but no paracrystalline arrays of BWYV were seen. In one cell, an area within the nucleus was seen which could possibly be an assembly site of BWYV (Fig. 11); virus particles were regularly arranged around small vesicles. No other possible site of virus replication or assembly was noted.

BWYV particles often were seen in plasmodesmata (Fig. 12,13). These plasmodesmata were devoid of their tubular core, and were not covered by ER cisternae, as they were in cells of healthy pennycress. Virions were seen in single file in narrow channels, and in groups in wider channels. Wherever virus particles were seen in plasmodesmata, they also were present in nearby cell cytoplasm.

DISCUSSION

Small icosahedral virions often are difficult to identify in electron micrographs because of their similarity to ribosomes. The presence of BWYV virions in mature sieve elements and in plasmodesmata clearly distinguished them from ribosomes, which are not found in either of these locations. Virus particles also could be seen in maturing sieve elements in which ribosomes were no longer present. By reference to virions in these areas as standards, it was possible to identify virus particles in other, less conspicuous, intracellular locations.

BWYV is phloem-limited in pennycress; sieve elements, companion cells, and phloem parenchyma were all invaded by the virus. No virus was seen in xylem, or in mesophyll cells. The distribution of BWYV was uneven; many sections did not reveal virus particles. In stem tissue, however, BWYV was visible in nearly every section examined. The concentration of virions in phloem tissue justifies the time and effort expended during purification of BWYV to release virions from leaf and stem vascular bundles. When tissue was frozen in liquid nitrogen and ground to a fine powder with a mortar and pestle, much higher concentrations of purified BWYV were obtained than when tissue was processed similarly but without grinding in liquid nitrogen (2). Brakke and Rochow (1) obtained similar results in the purification of another luteovirus, barley yellow dwarf virus, which also is localized in phloem tissue (11,12).

Esau and Hoefert (8,9) published an electron microscopic study of BWYV-infected sugar beet. In beet, virus particles only rarely were found between the cisternae of the endoplasmic reticulum in sieve elements. In pennycress, however, this was the most obvious virus location; the virus-infected areas were easy to distinguish even at low magnification. Esau and Hoefert reported that BWYV particles in sugar beet phloem parenchyma were "most conspicuous and most numerous in nuclei," associated with the nucleoli, and sometimes formed paracrystalline arrays. In pennycress, virions only occasionally were seen in parenchyma nuclei; more often, virions were located peripherally in the cell cytoplasm, often aligned along the plasmalemma. BWYV induced conspicuous vesicles in sugar beet. These vesicles contained fibrillar material resembling nucleic acid, and were hypothesized by Esau and Hoefert (9) to play a role in BWYV replication and translocation. Similar vesicles were not detected in pennycress, although smaller vesicles sometimes were associated with virus aggregates.

The differences noted between BWYV infection in pennycress and sugar beet could be due to at least two factors: the different hosts examined, or the different strains of BWYV used for infection. Different strains of alfalfa mosaic virus, for example, are known to induce varying degrees of vesiculation. The same could be true for BWYV strains, with B-16 producing only a few small vesicles which are not obvious in the electron microscope. The presumed function of the vesicles, that is the transport of viral nucleic acid, does not require a large structure. The overall pattern of location of BWYV in sugar beet and in pennycress tissues is quite similar. In both hosts virions are seen in sieve elements, in phloem parenchyma cell cytoplasm and nuclei, and in plasmodesmata connecting those cells. In sugar beet, BWYV was found in mesophyll outside the phloem; it was not seen outside the phloem in pennycress, but the search was not extensive.

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