Association of Squash Leaf Curl Virus with Nuclei of Squash Vascular Cells

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ABSTRACT

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Squash leaf curl is a whitefly-transmitted virus disease affecting members of the Cucurbitaceae and is caused by a geminivirus. It is associated with nuclei of vascular tissues in leaves of zucchini squash and with maturing phloem sieve elements. Whiteflies probe leaves mainly from the abaxial surface, and internal symptoms are expressed to a large extent in the vascular tissue of the abaxial phloem. Severe necrosis of sieve elements occurs after only 9 days of infection. The disease is discussed in comparison to other whitefly-transmitted geminivirus diseases of plants.

Whitefly-transmitted diseases have become increasingly important in California from an economic standpoint during the past few years, partially because of ideal environmental conditions for their development and spread (3–6) and partially because of the recognition and separation of various types of whitefly-transmitted viruses (5,13). The complex of whitefly-transmitted diseases has produced severe crop losses among the major crops in the desert southwest. One such disease affects members of the family Cucurbitaceae and has been named squash leaf curl (5,6). Clearly, a need for an understanding of the biological relationships of these viruses with their hosts exists, and it is toward this end that the present contribution addresses one aspect: the plant tissue-virus relationship at an ultrastructural level.

MATERIALS AND METHODS

Leaves of all ages were collected from seedlings of zucchini squash (*Curcurbita pepo* L.) 7, 8, and 9 days after inoculation with squash leaf curl virus (SLCV) via the whitefly vector (*Bemesia tabaci* (Genn.) (3). Symptoms of veinclearing and leaf curling were present on all leaves collected 9 days after inoculation. Symptoms were present only on the second leaf and older leaves collected 7 and 8 days after inoculation. Material for electron microscopy was fixed in paraformaldehyde-glutaraldehyde, dehydrated in acetone, and embedded in epoxy resin as previously described (8,9). Thin sections were stained with uranyl acetate and Millonig's lead stain (7).

RESULTS

Virus particles of SLCV have been isolated and characterized (3). The particles seen in the present study conformed to the size and shape of isolated dimer particles (3) and were found in many of the nuclei of vascular parenchyma cells (Figs. 1, 2, and 7). The minor differences encountered among the collections made at daily intervals were not intensively studied. The virus inclusions in the nuclei were closely associated with differentiation of the veins and appeared throughout the phloem and immature xylem parenchyma (Figs. 1, 4, and 5). Geminivirus particles were seen within immature and maturing sieve elements and were often not contained within nuclei because nuclei had degenerated (Figs. 6 and 7). Vascular tissues contained nuclei with virus inclusions even before the tissues were fully differentiated (Figs. 1 and 4). Fully mature sieve elements did not show gemini particles, nor was it possible to determine how the particles moved from the sieve elements into the adjacent phloem or xylem parenchyma cells. The nuclear inclusions in parenchyma cells were often very large and occupied more than half of the apparent volume of the nucleus (Figs. 4 and 5). Of course, the plane of sectioning of the nucleus must be considered when such a statement is made, because a cut at the top of a large more or less spherical inclusion body will appear like a small circle, but a cut through the center of such a body will be much larger (Fig. 5).

Lobing of nuclei appeared in areas of the leaf in which symptoms had progressed so that numerous cells of the bundle showed nuclear inclusions (Figs. 3 and 5). In longitudinal views, nuclei of phloem parenchyma cells contained several nuclear inclusions composed of dimer particles (Fig. 5). The occurrence of so-called fibrous rings (11,12) was rare, and more mature tissue did not show the rings with any greater frequency.

Nucleoli remained intact and relatively unaltered structures even while nuclear lobing and virus inclusion production proceeded to a striking extent (Figs. 1, 4, and 5). Sieve elements themselves contained dimer particles whether or not the nucleus remained intact (Figs. 6 and 7). In Figure 6, the sieve element marked SE contains virions and its associated companion cell shows a nuclear virus inclusion, as do the surrounding phloem parenchyma cells (Fig. 1). Degenerated sieve elements were seen in differentiating veins and showed virions in the degenerated cytoplasm (Fig. 4, D).

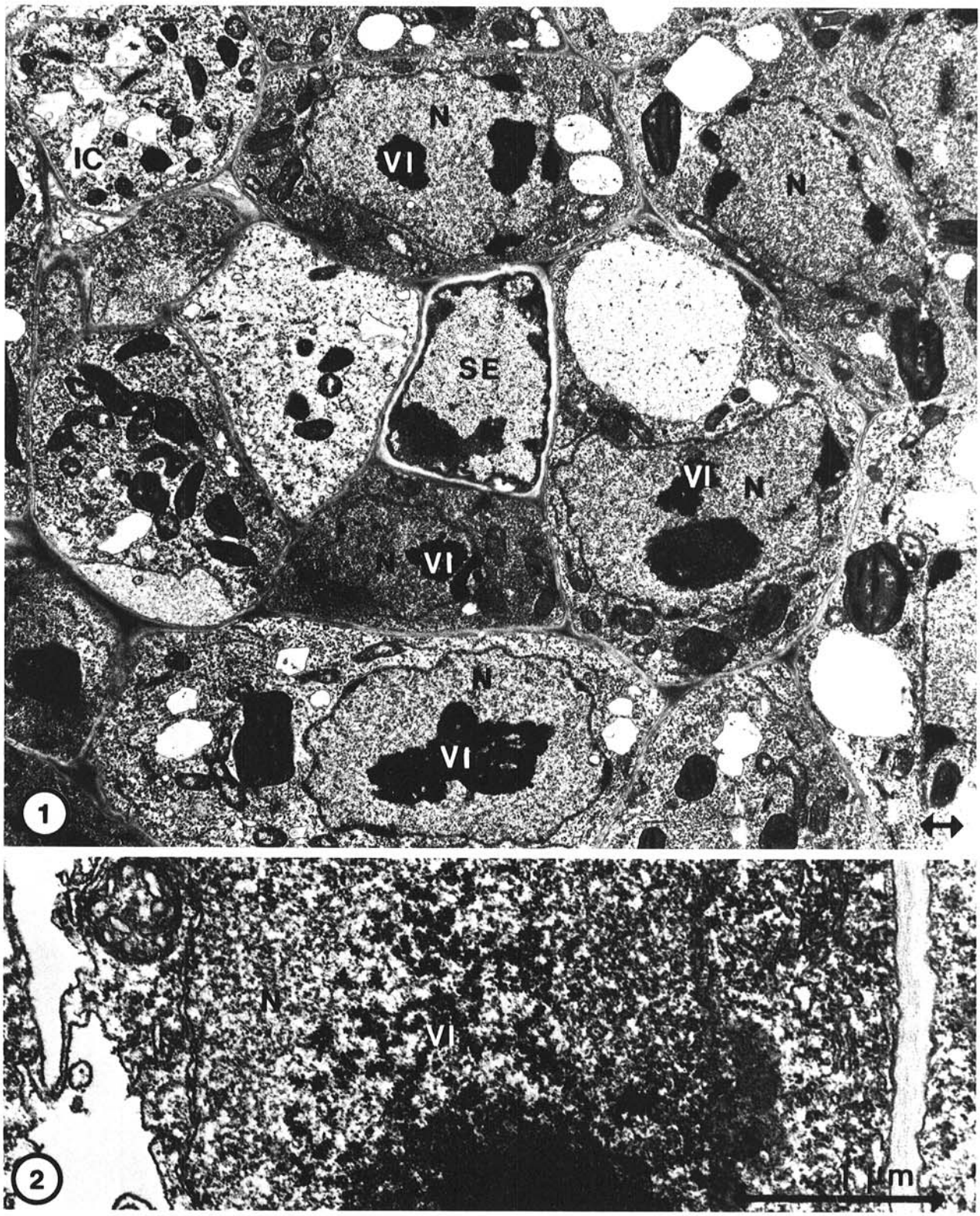
DISCUSSION

Whiteflies are recognized as phloem feeders. Evidence exists that they do far less structural damage to plants than either aphids or leafhoppers in their feeding activities (14). Stylets penetrate primarily from the abaxial leaf surface, where most of the whiteflies occur, and travel intercellularly between parenchyma cells to the phloem. Damage to intervening cells is rare, and stylet sheaths (often found in tissues probed by aphids) are not commonly found; indeed, whiteflies may probe little (14). Sieve-tube damage is slight, phloem is not blocked, and there is no wound response, according to Pollard (14).

The ultrastructure of vascular tissues in leaves of squash (*C. pepo*) has been described by Turgeon et al (15) from which the following account of the structure of minor veins is taken. Squash leaves possess bicollateral vascular bundles in which the xylem is centrally located. Phloem (including both sieve elements and parenchyma cells) is located on either side of the xylem. Beneath the adaxial epidermis is well-developed palisade parenchyma, below which the vascular bundles are embedded in spongy mesophyll that continues as a tissue to the abaxial epidermis. The abaxial phloem can be distinguished from the adaxial phloem by the large border parenchyma cells between the vascular tissue and the spongy mesophyll. These cells have been recognized as structurally distinctive and have been termed "intermediary cells." They contain numerous irregularly shaped vacuoles and so are easily recognized both in the light and electron microscopes (IC in

Figs. 1 and 4). In addition, the cells are distinguished from other vascular parenchyma cells by the unusually abundant plasmodesmatal connections in the cell walls between mesophyll and vascular bundle cells (15).

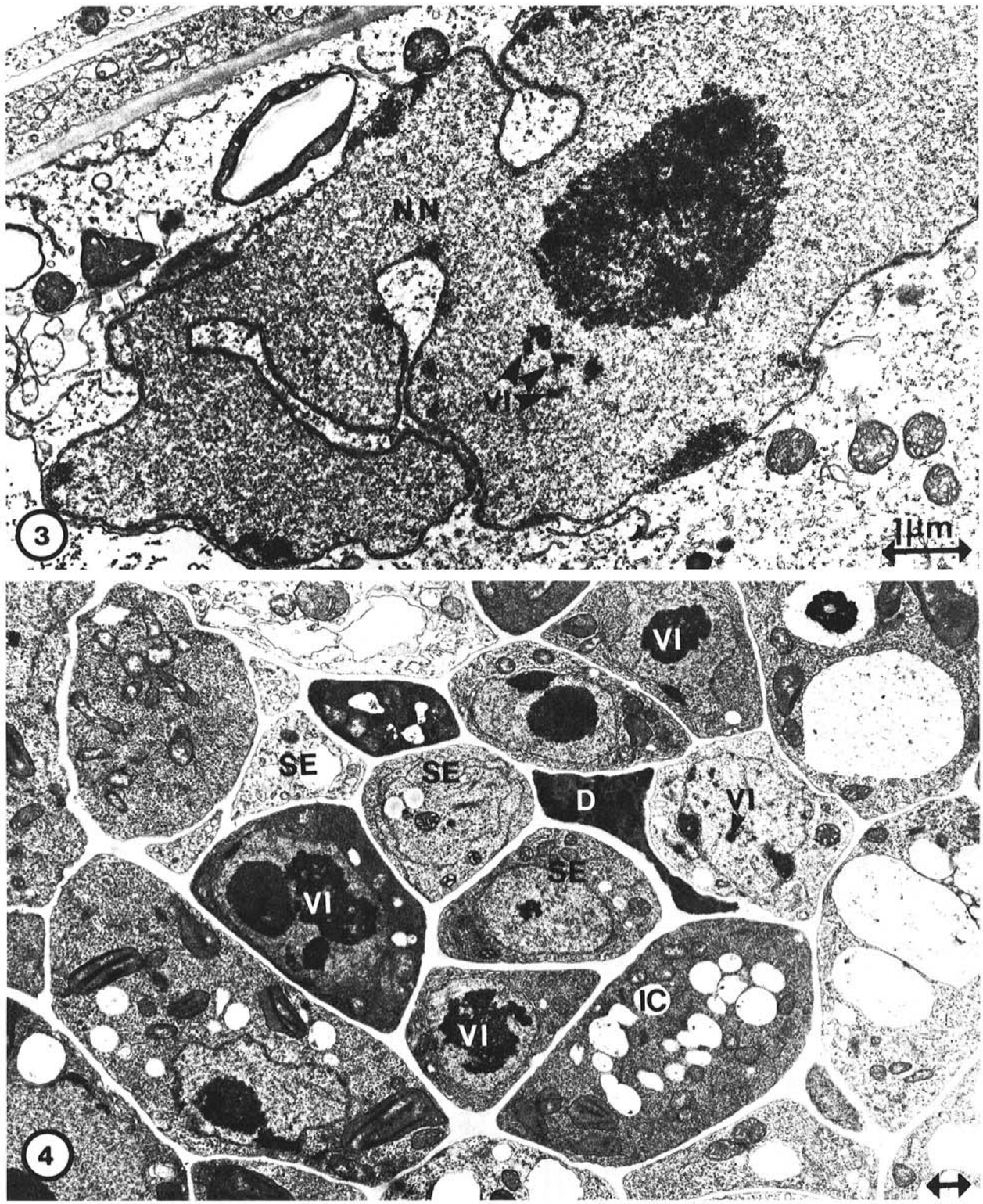
Fibrous rings and nuclear inclusions have been found to be associated with many of the plant-geminivirus infections. The fibrous rings occur in the nuclei that will or do contain nuclear gemini particles (10). Fibrous rings are uncommon in leaf cells infected with SLCV, and this observation may provide a diagnostic difference between this whitefly-transmitted geminivirus and others of similar vector affinity. Certainly, in SLCV-infected tissue, the fibrous rings cannot be a diagnostic feature at the light



Figs. 1 and 2. Portions of transverse sections of squash leaves inoculated with the squash leaf curl virus (SLCV) collected 9 days after inoculation. **1,** Immature sieve element (SE) and surrounding phloem parenchyma cells, many of which contain viral inclusions (VI). An intermediary cell (IC) can be distinguished from other vascular parenchyma cells by characteristic unusual and abundant vacuoles. Scale bar = 1 μm. **2,** Higher magnification of a viral inclusion showing typical dimer particles of SLCV, a geminivirus. Scale bar = 1 μm.

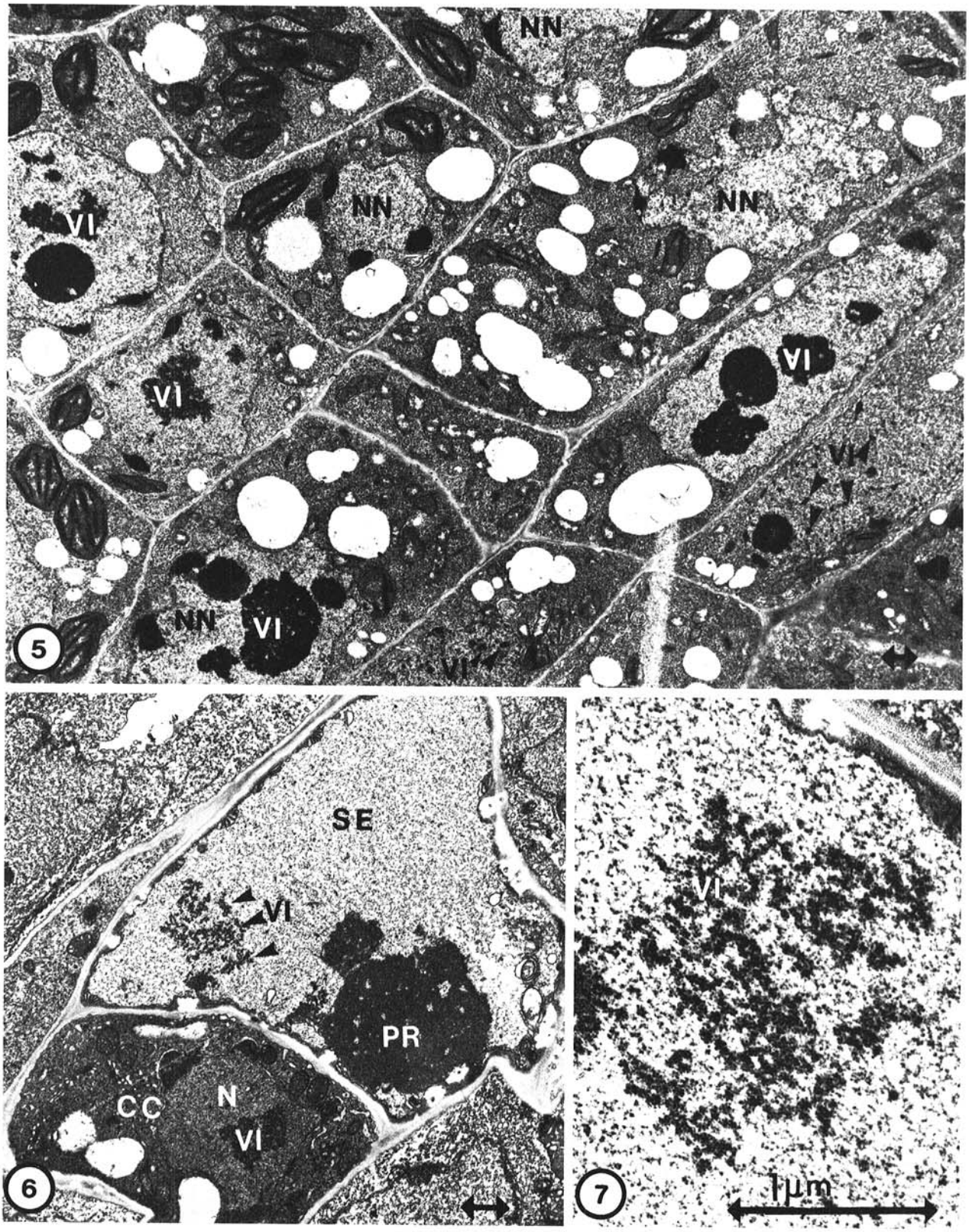
microscope level as they can in a number of geminivirus-induced diseases (2). It is not known at present if fibrous rings are associated with viral invasion of fully mature tissues or not. The present report is concerned with viral invasion of undifferentiated tissues and thus differs from most other studies that have been conducted on geminivirus cytopathology. Kim et al (12) reported fibrous rings in immature sieve element nuclei of bean leaves infected with the whitefly-transmitted geminivirus, bean golden mosaic.

Hypertrophy of the nucleolus was found in cells infected with bean golden mosaic virus (12). The extent to which the nucleoli were hypertrophied was striking—up to three-fourths of the nuclear volume was occupied by the nucleolus. These nucleoli became less prominent as the time after inoculation increased and they were rarely observed in cells 8 days or more after inoculation. In the present study, similar nuclear inclusions were found, but these are regarded as inclusions of virus particles and are not considered "hypertrophied nucleoli."



Figs. 3 and 4. Portions of transverse sections of squash leaves infected with squash leaf curl virus (SLCV) 9 days after whitefly inoculation. **3,** Nucleus that is highly lobed and contains a small viral inclusion (VI, arrows) near the lobed nucleus (NN). Scale bar = 1 μm. **4,** Portions of six sieve elements (SE), one of which has degenerated (D). The surrounding phloem parenchyma cells contain obvious virus inclusions (VI) within the nuclei. Scale bar = 1 μm.

It is reasonable to assume that nuclear inclusions are found often in the abaxial phloem parenchyma, because the abaxial phloem is the probable site of most feeding by the whiteflies. It may be significant that Botha and Evert (1) have shown that aphids feed preferentially on the abaxial phloem in *Cucurbita* leaves. Although several other diseases transmitted by whiteflies and associated with gemini particles have been investigated, no speculations as to the relationship between vector feeding and symptom expression at



Figs. 5–7. Longitudinal (Fig. 5) and oblique (Figs. 6 and 7) sections of squash leaves infected with squash leaf curl virus (SLCV) 9 days after inoculation. **5,** Vascular parenchyma cells that contain viral inclusions (VI) and lobed nuclei (NN). Otherwise the highly cytoplasmic young cells appear very normal. Scale bar = 1 µm. **6,** Immature sieve element (SE) and companion cell (CC) in the phloem are cut obliquely. The SE contains a large P-protein body (PR), degrading cytoplasm that is probably normal, and an area of dimer particles free in the cytoplasm. Scale bar = 1 µm. **7,** Higher magnification of particles from Fig. 6 showing the double or geminate aspect of these particles. The background cytoplasm of the immature sieve element contains the fibrillar type of P-protein and ribosomes. Scale bar = 1 µm.

the ultrastructural level have been advanced, nor have tissue relationships of whitefly feeding been looked at since the electron microscope has been available as a research tool. These areas and more careful investigations of timing of whitefly feeding relative to virion development in the same and adjacent cells would seem ripe for future research.

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