

Cytological Alterations in Cells Infected with Corn Leaf Aphid-Specific Isolates of Barley Yellow Dwarf Virus

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ABSTRACT

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Electron microscopic examination of tissue from oat (*Avena sativa*) leaves infected with *Rhopalosiphum maidis* (corn leaf aphid)-specific isolates of barley yellow dwarf virus (BYDV) revealed that alterations in infected phloem cells were basically similar to those described earlier for *R. padi* (cherry oat aphid)-specific isolates but differed from those in cells infected with *Macrosiphum avenae* (English grain aphid)-specific and aphid-nonspecific isolates of BYDV. Alterations that were the same for *R. maidis*-specific and *R. padi*-specific isolates were the presence in the cytoplasm of vesicular membranous inclusion bodies that contained fibrillar material and that were enclosed by a second membrane; alterations

in the nucleus involved the association of viruslike particles with distorted nucleoli. These particles also became distributed through the nucleoplasm and the cytoplasm. The nuclear heterochromatin showed progressive dissolution. Masses of proliferated tubular membranes in the cytoplasm were much more extensively developed than those with *R. padi*-specific isolates. Also, there were no densely staining filaments associated with virus particles in mature sieve elements and no pathological alterations in mitochondria; cell wall deposits were extensive in most infected cells. Massive clumps of virus particles in the cytoplasm late in the infection also were unique.

Isolates of barley yellow dwarf virus (BYDV) in North America have been grouped in five categories according to the relative transmissibility by five species of cereal-infesting aphids (5,17). These categories, referred to as variants or strains of the virus, are termed aphid nonspecific, *Macrosiphum avenae*-specific, *Rhopalosiphum padi*-specific, *R. maidis*-specific, and *Schizaphis graminum*-specific.

In the past, queries have arisen about whether these variants all belong to BYDV or whether some may be different viruses (1,2,21). Studies on properties of the virus variants have been hindered by very small amounts of virus in the tissue and by the disadvantage of having to use aphids as vectors when transmitting the virus, as in infectivity studies. Serologic studies have shown that some of these variants are closely related and that others may be only distantly related (1,15,18). Cross-protection studies have generally supported the serologic findings (1,10-12,16,19-21), but further serologic and cross-protection studies between some variants are required to provide a more complete picture.

Our recent studies on the sequence and type of alterations in cells infected with BYDV (8,9) indicate that electron microscopy may provide useful, supplementary evidence for determining relationships between the virus variants. The two serologically related *M. avenae*-specific and aphid nonspecific variants induced similar cytopathological alterations in oat cells, but the *R. padi*-specific variant, serologically unrelated to the other two variants, induced basically different cytopathological changes. The importance of such detailed developmental studies in helping to derive a meaningful classification of plant viruses was pointed out by Esau and Hoefert (3). The remarkable specificity found for the cellular inclusion bodies induced by some viruses or virus groups was also discussed in a recent review by Martelli and Russo (13). A further benefit accruing from these studies is that electron microscopy can now be used as an aid in identifying the BYDV variants according to the nature of the cytopathological alterations. In a recent study in Italy (4), for example, cytopathological alterations in rice tissues infected with rice giallume virus were similar to those found in our study with the *R. padi*-specific variant of BYDV in oats (9), thereby providing

support for the etiology of the rice giallume disease.

In this investigation we examined alterations in oat cells induced by the *R. maidis*-specific variant, which has been difficult to purify. Consequently, little is known of its serologic and other properties. Our electron microscopic observations with this variant showed that cellular alterations were basically similar to those induced by the *R. padi*-specific variant, but minor differences in certain alterations distinguish the two.

MATERIALS AND METHODS

The two *R. maidis* (corn leaf aphid)-specific isolates from Manitoba used were 6417 (6) and 7413 (7). The transmission characteristics of the isolates were reexamined on several occasions, with five species of aphids. *R. maidis* (Fitch) was the most efficient vector, and *S. graminum* (Rondani) also occasionally transmitted these isolates, but *R. padi* (Linnaeus), *M. avenae* (Fabricius), and *M. dirhodum* (Walker) did not transmit, thus confirming the isolates' original specificity. Individuals of *R. maidis* were used as vectors when transmitting the viruses maintained in oat plants (*Avena byzantina* C. Koch 'Coast Black') to *A. sativa* L. 'Rodney' test seedlings. Methods for growing and inoculating the oat seedlings at the one-leaf stage with the virus, for producing virusfree oats as controls for electron microscopy, and for sampling the plants have been described (9). Samples of plant tissue were collected from the first and second leaves 5-13 days after the beginning of the inoculation feeding period. Tissue samples were prepared for electron microscopy as already described (8).

Because preliminary work on these isolates and previous work on other BYDV isolates (8,9) showed that infection occurred exclusively in phloem cells, only longitudinal sections of the vascular bundle and, where possible, serial sections were examined. This enabled detection of any changes along the entire length of the elongated phloem cells. The developmental sequence of the infection was reconstructed by observing more than 600 infected phloem cells in about 40 samples taken mainly from the second leaves 8, 9, and 10 days after inoculation. At least three and usually many more similar observations were made at each developmental stage.

RESULTS

Cells infected with the virus were sieve elements, phloem parenchyma, and companion cells. Infected cells were identified by the presence of one or more inclusion bodies in the protoplast. Early, intermediate, and late phases in the infection of the cells refer, respectively, to cytoplasmic alterations occurring before alterations in the nucleus, alterations occurring thereafter in the nucleus, and further alterations in the cytoplasm and nucleus.

With both isolates, the earliest sign of infection was the occlusion of plasmodesmatal channels by very densely staining material, some of which often intruded into the cytoplasm. Virus particles usually were visible in the mature sieve elements adjacent to the infected cell (Fig. 1) at or near the connecting pitfield. Occasionally, one or a few virus particles also were visible in the plasmodesmatal channels. The dark staining material in plasmodesmata during the earliest infection stage was described earlier (8,9). Subsequent developments involved the appearance in the cytoplasm of membranous vesicles containing fibrils, surrounded by a second membrane. In some sections the second membrane was continuous with rough endoplasmic reticulum. These inclusions were scattered sparsely through the cytoplasm. Limited amounts of another type of inclusion with large-diameter membranous tubules containing amorphous material that stained with moderate density also were visible, usually nearer the walls than the center of the cell lumen (Fig. 2). An important feature was the development of extensive inclusion bodies consisting of highly proliferated and convoluted membranous tubules, narrow in diameter and associated with moderately staining amorphous material (Fig. 3). In some sections the tubules appeared to be continuous with rough endoplasmic reticulum (inset, Fig. 3). Aggregates of very densely staining material were observed frequently in particular areas of both types of tubular membranous inclusions (Fig. 4).

A fourth type of inclusion body often occurred in the cytoplasm adjacent to the highly proliferated tubular inclusions. These bodies were circular in section and had a maximum diameter of about 90 nm and short arms extending radially from the periphery (Fig. 5). They were similar in morphology and size to the spiny vesicles described by Newcomb (14) in differentiating procambial cells of bean root tips and in differentiating cells of phloem strands of *Dianthus* roots and *Coleus* petioles. In our work, however, spiny vesicles were not detected in differentiating phloem cells of uninfected, control tissue from oats, but they were common in most cells infected with the *R. maidis*-specific isolates. These vesicles were, on rare occasions, also associated with short lengths of tubular membrane in longitudinal section, from which arms protruded at right angles (Fig. 6). Also observed were areas in the cytoplasm without structure, but containing densely staining filaments, similar to those observed with *R. padi*-specific isolates (9).

Virus particles in moderate numbers were first observed in cells at the beginning of the intermediate phase. With isolate 7413, virus particles were first observed scattered thinly through the cytoplasm. Slightly later, particles were observed around the nucleolus, which was distorted, and within the outer granular layer of this organelle. Other cells also had virus particles scattered through the nucleoplasm (Fig. 7). The nucleus appeared normal otherwise; Fig. 8 shows a nucleolus from healthy control tissue for comparison. With isolate 6417, however, virus particles were first seen in association with the nucleolus, similar to that with isolate 7413, and subsequently particles also were scattered through the nucleoplasm but not the cytoplasm.

With both isolates, the late phase of the infection began with small clumps of virus particles in the cytoplasm against the plasmalemma (Fig. 9, 10). This was the first record of virus particles in the cytoplasm for isolate 6417 but represented a small increment in the number of particles in the cytoplasm for isolate 7413. Subsequently, the number of virus particles increased markedly, in both the cytoplasm and nucleoplasm. In the cytoplasm, the increase was mainly in the form of large clumped masses of particles throughout the cell lumen. One of these large masses is shown in Fig. 11 and details are shown in Fig. 12. Also, the

heterochromatin in nuclei became less densely staining and showed dissolution (Fig. 13), when compared with a cell nucleus from healthy control tissue (Fig. 14). At this stage, deposits were apparent over the entire walls and often protruded considerably into the lumen of most infected cells (Fig. 15). Incipient deposits of this type and localized deposits at pitfields first were noticed at the intermediate stage. The proliferated, membranous tubules of narrow diameter and the small, circular bodies with radial arms, formed during the early phase, persisted into the late phase after ribosomes disappeared.

DISCUSSION

The cytopathological alterations induced by the two *R. maidis*-specific isolates were remarkably similar, in both the sequence of events and the nature of alterations. These cellular alterations also were similar in major respects to those of cells infected with *R. padi*-specific isolates (9).

With all these isolates, there was always a close association of virus particles with the nucleolus. After virus particles appeared in the nucleus, the heterochromatin always gradually dissolved. A second constant feature in the cytoplasm, was membrane-bound vesicles containing densely staining fibrils. These vesicles were surrounded by a second membrane that was continuous with the endoplasmic reticulum (9). Such vesicles, however, appeared to be much more numerous with the *R. padi*-specific isolates than with the *R. maidis*-specific isolates. Proliferated, tubular, membranous structures also were a feature of cells infected with these variants. With *R. padi*-specific isolates, however, these inclusion bodies were very small, whereas those with the *R. maidis*-specific isolates were considerably larger.

Despite the similarity of alterations in cells infected with *R. maidis*-specific isolates and those infected with *R. padi*-specific isolates, some differences could be used to distinguish between the two variants. The spiny vesicles in the cytoplasm were not observed with other isolates of BYDV. Mitochondria did not exhibit the pathological alterations with *R. maidis*-specific isolates that were so typical in cells infected with *R. padi*-specific isolates (9). Deposits that covered the entire cell wall were found only in infected parenchymal cells with *R. padi*-specific isolates, whereas with *R. maidis*-specific isolates, extensive cell wall deposits were a feature of most infected cells in the phloem. Unlike the observations with other BYDV variants, densely staining filaments commonly associated with virus particles in mature sieve elements (9) were not found with *R. maidis*-specific isolates, and the arrangement of virus particles as small clumps against the plasmalemma or as large clumps in the cytoplasm with *R. maidis*-specific isolates also was unique.

Cytopathological alterations with *R. padi*-specific isolates differed from those with either *M. avenae*-specific (8) or aphid-nonspecific isolates of BYDV (9). These results and results from the present study, which show basically similar alterations with *R. padi*-specific and *R. maidis*-specific isolates, support the concept that BYDV may consist of at least two groups of viruses. A recent serologic finding by Paliwal (15) indicated only a distant relationship between an *R. maidis*-specific and an *M. avenae*-specific isolate. Also, synergistic reactions were found in cross-protection studies with an *R. maidis*-specific isolate and an aphid-nonspecific isolate (10). These facts provide further evidence that the *R. maidis*-specific variant may be unrelated, or only distantly related, to the two cytopathologically and serologically related *M. avenae*-specific and aphid-nonspecific variants.

R. maidis-specific isolates have been difficult to purify (18). It became obvious from our studies that low virus content is an important factor in this problem. Infected cells in tissue samples were much less common, and concentrations of virus particles in cells were much lower than with the other variants of BYDV. Systemic infection with *R. maidis*-specific isolates also occurred 2-4 days later. Wall deposits with the *R. maidis*-specific isolates were more extensive than with any of the other variants. Callose deposits normally form on the walls of aging sieve elements. In infected cells, however, wall deposits even on areas away from

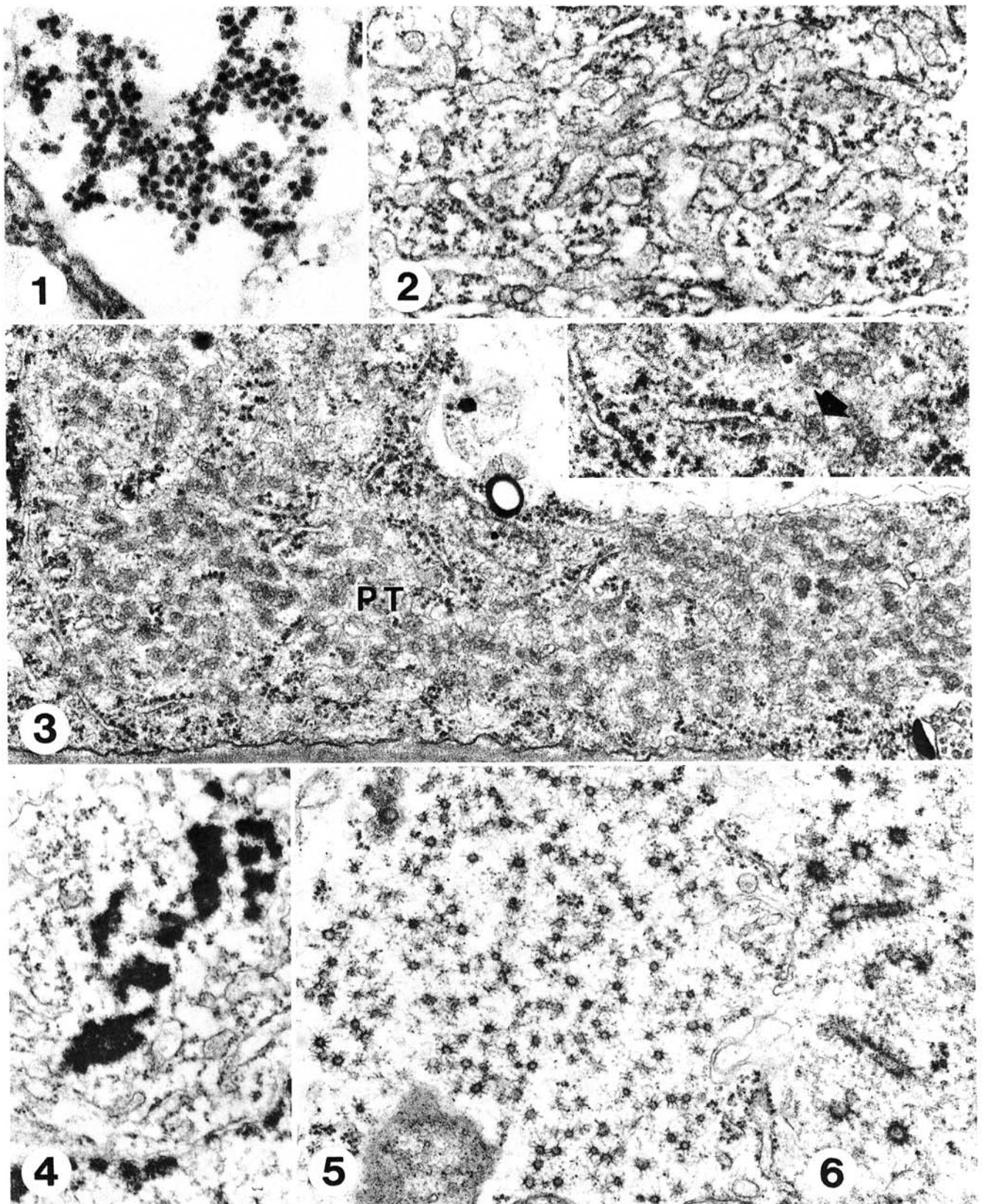


Fig. 1-6. Longitudinal sections through phloem cells of oats, *Avena sativa* L. 'Rodney'. Cytopathological alterations in cells were identical for both virus isolates of BYDV. Thus, electron micrographs are of cells infected with isolate 7413. **1.** Virus particles with some amorphous material in a mature sieve element. $\times 66,400$. **2-6.** Cytoplasmic inclusions in immature sieve elements or companion cells in the early phase of infection. **2.** Part of an inclusion body consisting of wide-diameter tubular membranes. Tubules contain amorphous material, and ribosomes are visible, attached to the outside of some membranes $\times 30,000$. **3.** Part of another type of inclusion body consisting of highly proliferated, convoluted membranous tubules (PT). The tubules are narrower in diameter than those in Fig. 2 and also are associated with amorphous material. $\times 34,900$. Inset shows the continuity (arrow) of one tubule with rough endoplasmic reticulum. $\times 69,900$. **4.** Aggregates of densely staining amorphous material associated with a membranous inclusion of wide-diameter tubules, close to the area shown in Fig. 2. $\times 25,700$. **5.** Cytoplasm largely devoid of ribosomes containing small, circular bodies with short arms extending radially from the periphery. $\times 32,900$. **6.** Cytoplasm in which circular bodies with radial arms occur together with short membranous tubules with similar arm projections. $\times 64,300$.

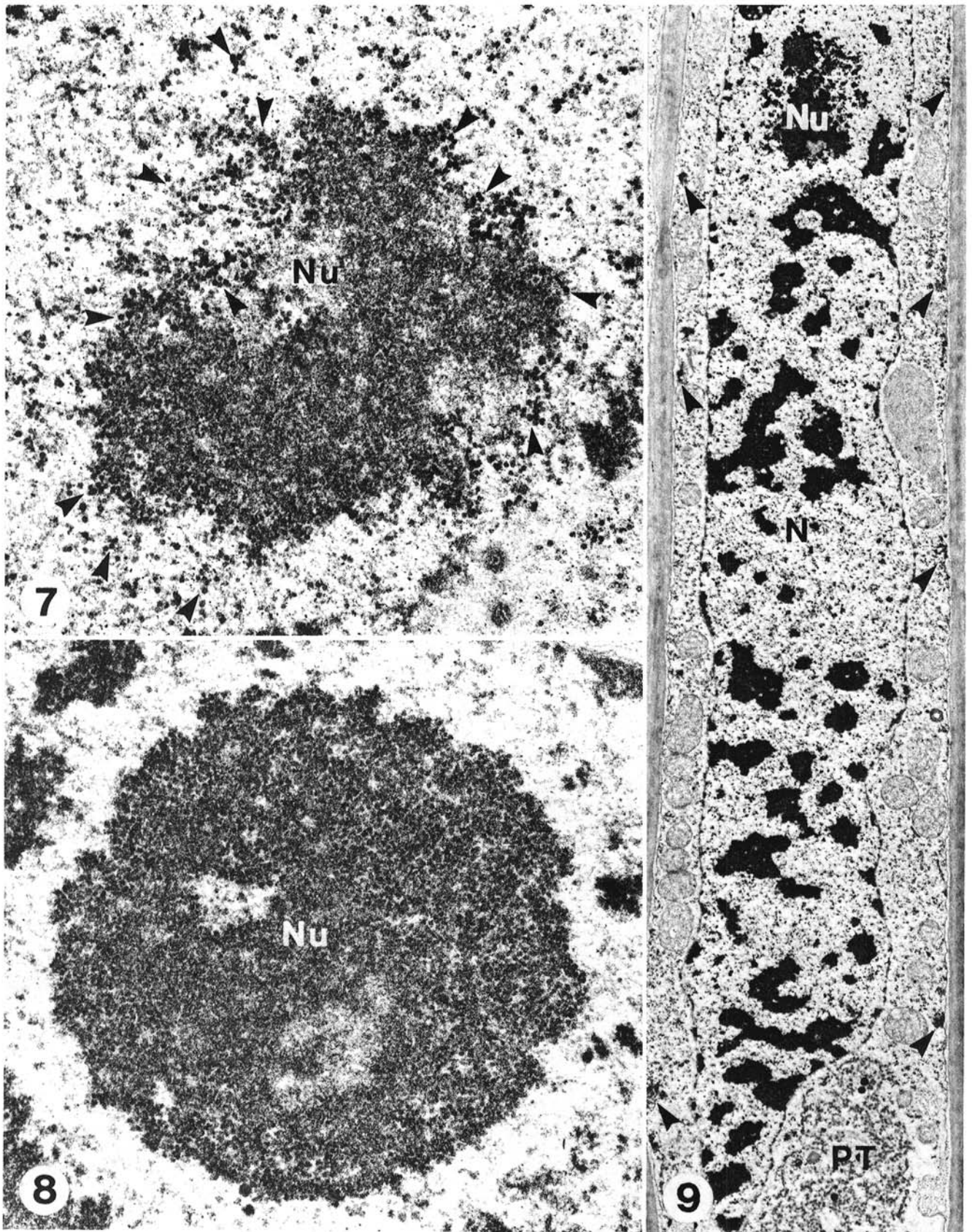


Fig. 7-9. Longitudinal sections through phloem cells of oats infected or not infected with BYDV. **7.** The nucleolus (Nu) of a cell at an intermediate stage of infection. Virus particles occur in small clusters (arrowheads) at and just within the periphery of the nucleolus and are thinly scattered also in the nucleoplasm. The nucleolar outline is distorted. $\times 48,200$. **8.** Nucleolus (Nu) of a cell from uninfected control tissue for comparison. $\times 48,200$. **9.** Low magnification of a cell at the beginning of the late phase of infection with isolate 6417, showing the distribution of virus particles as densely staining masses around the nucleolus (Nu) and as small clumps along the walls (arrows). (Details of the small clumps of virus particles are shown in Fig. 10.) Part of a membranous inclusion of highly proliferated, convoluted tubules (PT) is visible. (See details in Fig. 3.) At this stage the staining intensity of the heterochromatin was still normal. N = nucleus. $\times 8,000$.

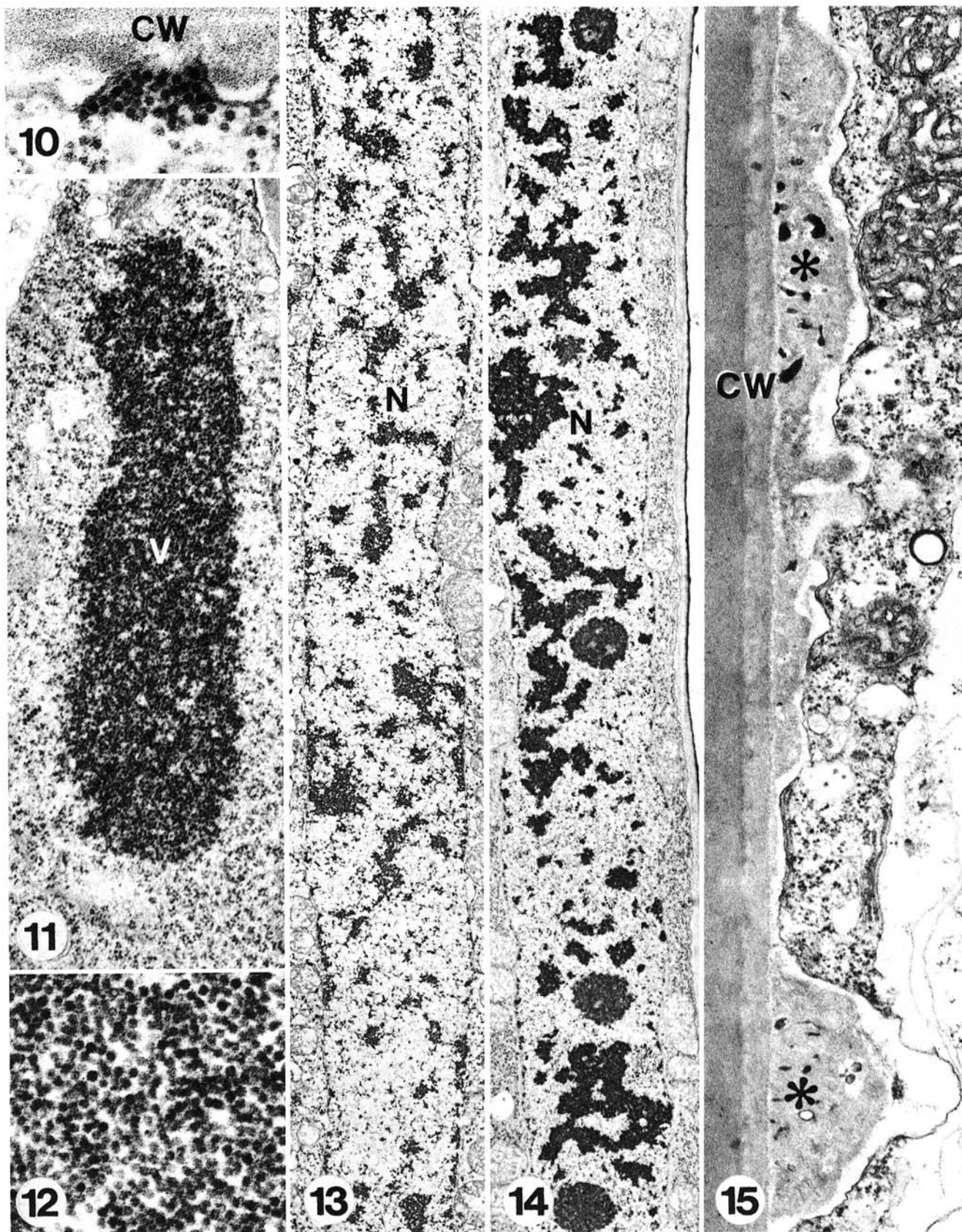


Fig. 10-15. Longitudinal sections through phloem cells of oats in the late phase of infection with BYDV or through infected cells. **10**, A small clump of virus particles in the cytoplasm against the cell wall (CW). $\times 75,000$. **11**, Large mass of densely staining virus particles (V) in the cytoplasm. $\times 24,900$. **12**, Part of the large mass of virus particles in Fig. 11, showing individual particles. $\times 75,000$. **13**, Part of a nucleus (N) showing the lighter staining and partially eroded heterochromatin. $\times 8,300$. **14**, Part of a nucleus (N) in a cell from uninfected control tissue. $\times 6,900$. **15**, Part of an extensive cell wall (CW) deposit (asterisks). $\times 26,000$.

pitfields were obvious well before these cells became devoid of cytoplasm. Therefore, the infection induces the host to produce calloselike deposits much earlier than normal. These deposits may serve to reduce or prevent egress of progeny virus particles from the infected cells, thereby increasing host resistance to the virus.

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