Ultrastructure and Mitochondrial Vesiculation Associated with Closteroviruslike Particles in Leafroll-Diseased Grapevines

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Published with the approval of the director of the Arkansas Agricultural Experiment Station, Fayetteville. This research is supported in part by a grant from BARD (US-1036-85).

Accepted for publication 5 October 1988 (submitted for electronic processing).

ABSTRACT

Kim, K. S., Gonsalves, D., Teliz, D., and Lee, K. W. 1989. Ultrastructure and mitochondrial vesiculation associated with closteroviruslike particles in leafroll-diseased grapevines. Phytopathology 79:357-360.

Ultrastructural studies of Riesling and Chardonnay grapevines with typical leafroll-disease symptoms occurring in northwest Arkansas revealed flexuous, rod-shaped viruslike particles approximately 12 nm in diameter and of undetermined length occurring consistently in phloem cells. In phloem parenchyma cells, the particles were associated with clusters of membranous vesicles, 50–100 nm in diameter, containing fine fibrils characteristic of closterovirus infections. Unlike other known closteroviruses, however, the fibril-containing vesicles originated from modified mitochondria. Direct enzyme-linked immunosorbent assays indicated that the virus was serologically related to a New York isolate (NY-1) of a closterovirus associated with grapevine leafroll disease.

Additional keywords: cytopathology, grapevine leafroll virus, virus-induced vesicles.

Grapevine leafroll disease reportedly occurs in many grape-growing countries, but its etiology has not been fully determined. Because of its graft transmissibility and symptomatology and reactions on indicator hosts (2), the grapevine leafroll disease may be caused by a virus. Although viruslike particles have been observed in diseased plants, the types of these particles have been reported to be potyviruslike (21), small-isometric-viruslike (3,20) and closteroviruslike (6,17,18,19,23).

Stunting, chlorosis, mild mosaic and/or mottling, and cupping of leaves, suggestive of leafroll disease, were observed on Riesling and Chardonnay grapevines in northwest Arkansas. Because of the failure to mechanically transmit a viral agent to the herbaceous hosts tested, thin-section electron microscopy of affected leaves was carried out to investigate the presence of viruslike particles and associated cytopathic effects. This paper reports the presence of flexuous, rod-shaped viruslike particles and cytopathic effects characteristic of a closterovirus infection, a mitochondrial origin of the membranous vesicles, and a serological relatedness between the isolate and other closteroviruslike particles reported to be associated with grapevine leafroll disease.

MATERIALS AND METHODS

Leaf specimens, 1–2 mm^2, were taken from young leaves of three different Riesling grapevines with typical leafroll disease symptoms in late May 1983 through 1987. Similar leaf tissues also were taken from symptomless leaves of apparently healthy adjacent plants for a control. The tissues were prepared for thin-section electron microscopy as described previously (11).

The closteroviruslike agent found in this study was serologically compared with grapevine leafroll diseases occurring in other geographic locations. Enzyme-linked immunosorbent assays (ELISA) were performed with antiserum produced against the NY-1 isolate of leafroll from New York (23). Direct double antibody sandwich ELISA was done as described by Zee et al (23). Coating and alkaline phosphatase-conjugated NY-1 immunoglobulin were used at 1 μg/ml and 1/1,500 dilution, respectively. Leaf tissues of greenhouse-grown Riesling that originated from cuttings of symptomatic field grapevines were used at threefold dilutions from 1/15 to 1/405. Comparable Pinot noir grape leaves infected with the NY-1 isolates also were used as a positive control. Healthy Pinot noir leaves were used as negative controls.

RESULTS

Long, flexuous, rod-shaped virus particles and associated cytopathic changes were found consistently in leaves with symptoms but were absent in symptomless leaves. The particles were approximately 12 nm in diameter with undetermined length and occurred mainly in phloem parenchyma cells (Figs. 1–3), including companion cells and sieve elements. Only a limited number of phloem cells contained the particles, however. In a large vein only one or two cells contained particles, whereas the majority of other cells appeared normal.

In addition to the presence of viruslike particles, the most conspicuous cytopathic effects were structural changes in the mitochondria of phloem parenchyma cells. These changes included the occurrence of small membranous vesicles in the perimitochondrial space between the outer and inner mitochondrial membranes and the degradation of stromal structure of the mitochondria (Figs. 4–6).

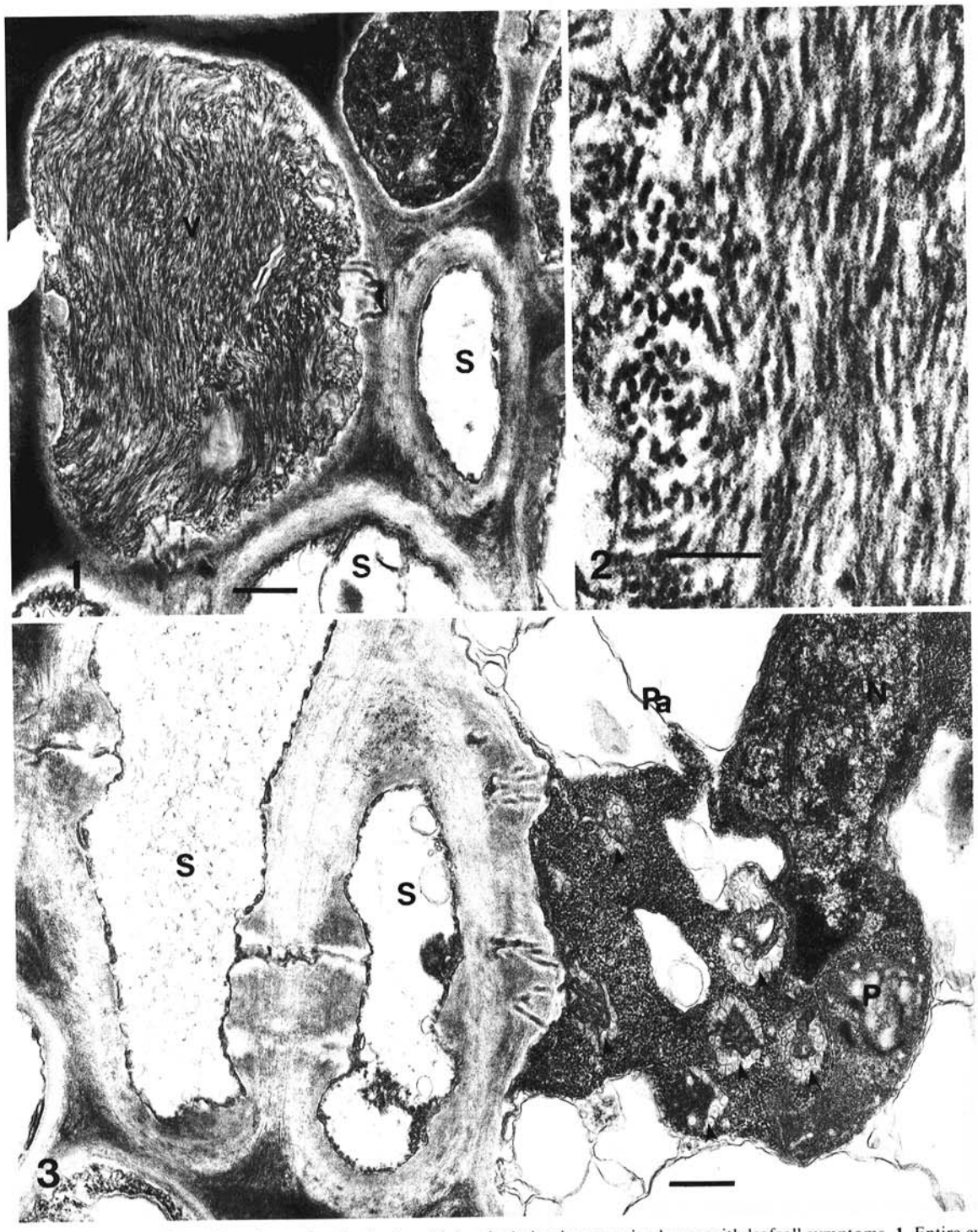
The vesicles, ranging from 50 to 100 nm in diameter, were bounded by a single membrane that often was continuous with the outer mitochondrial membrane (Fig. 4). Some mitochondria were surrounded by vesicles that filled their entire perimitochondrial space (Figs. 4–6). Most of these vesicles contained electron-dense fine fibrils that coalesced in the center (Figs. 7 and 8).

Some mitochondria with few vesicles retained their cristae and stroma (Figs. 4 and 5). In mitochondria with a large number of vesicles, however, the stroma became deeply undulated and contained only a few recognizable cristae (Figs. 5, 7, and 8). They also often contained a vacuolelike region in the stroma, indicative of degeneration (Figs. 6 and 8). These structurally altered mitochondria often were difficult to recognize unless they were closely associated with other mitochondria containing cristae (Fig. 6). The perimitochondrial space crowded with a large number of vesicles became greatly widened with little stroma (Fig. 8). Vesicle-filled perimitochondrial spaces appeared as single-membrane-bounded bodies containing aggregates of small vesicles when the stroma was not included in the section (Fig. 9). Clusters of vesicles

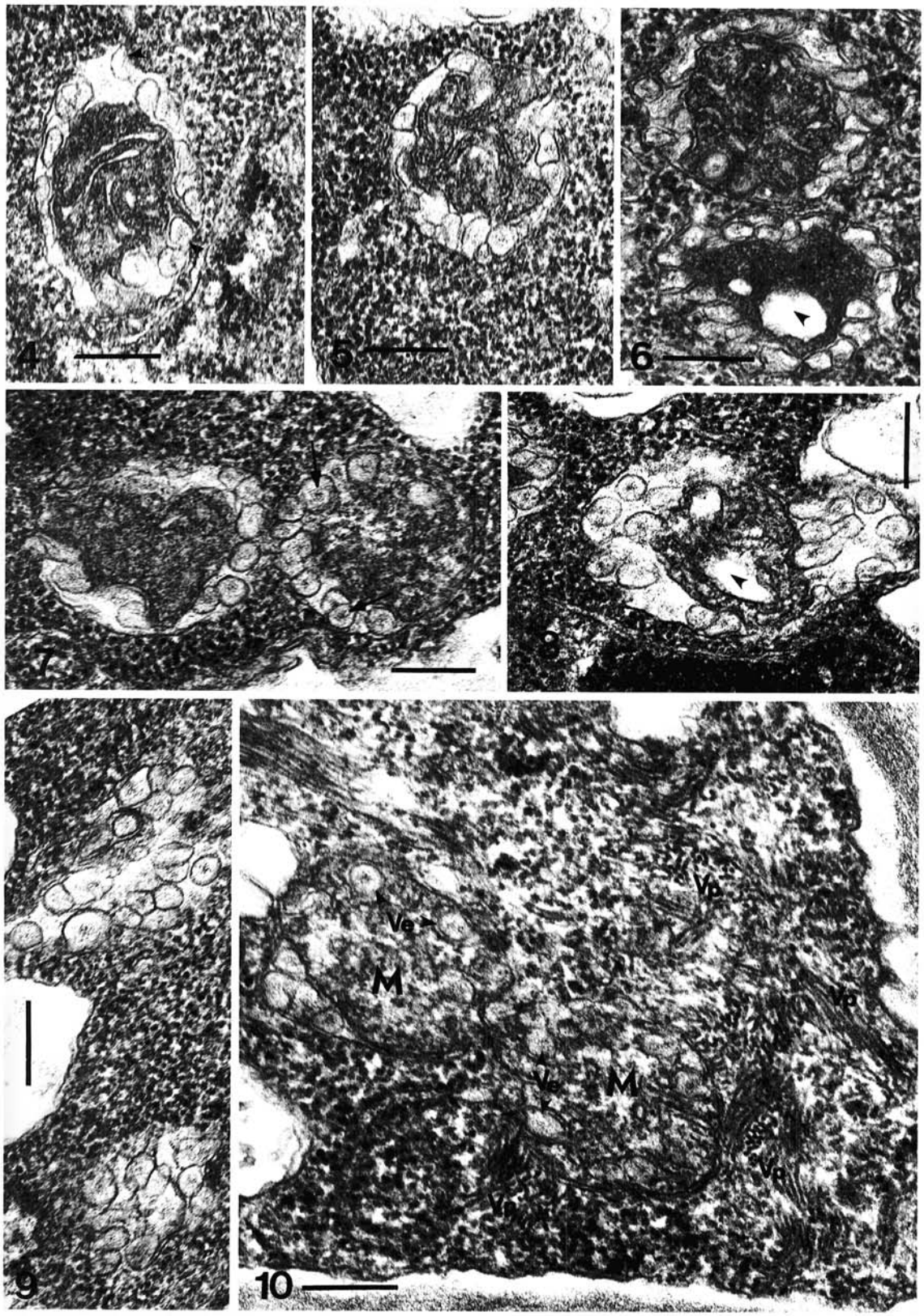
in the cytoplasm also were observed without a recognizable bounding membrane suggesting degeneration of the membrane (Fig. 9).

Viruslike particles also occurred in the cytoplasm of cells that contained vesiculated mitochondria (Fig. 10). The particles were not observed within the interior of such mitochondria, although they occurred adjacent to them. Not every cell containing vesiculated mitochondria also contained viruslike particles (Fig. 3). Phloem parenchyma cells containing vesiculated mitochondria without viruslike particles were frequent. Cells with virus particles did not always contain vesiculated mitochondria. The cytoplasm in such cells was mostly replaced by compact aggregates of virus particles (Fig. 1).

Leaf extracts from Riesling grapevines showing leafroll symptoms gave positive ELISA reactions with the NY-1 antiserum. Optical density readings of ELISA tests taken at 405 nm after 30 min were 0.633, 0.418, 0.217, and 0.077 for the Arkansas isolate as opposed to 1.460, 1.085, 0.549, and 0.226 for the NY-1 isolate of leafroll at the dilution of 1:15, 1:35, 1:45, and 1:405, respectively. Comparative healthy extracts gave readings of



Figs. 1–3. Closteroviruslike particles (V) and associated mitochondrial vesiculation in grapevine leaves with leafroll symptoms. **1,** Entire cytoplasm of a parenchyma cell in a small vein is occupied by virus particles which are arranged parallel to each other and have a wavy appearance. S = sieve element. Bar represents 500 nm. **2,** A higher magnification of a portion of Figure 1 showing transverse and longitudinal sections of the particles which measure approximately 12 nm in diameter. Bar represents 100 nm. **3,** A low magnification of a small vein showing two sieve elements (S) and a portion of a parenchyma cell (Pa) that contains several vesiculated mitochondria (arrowheads) in the cytoplasm. N = nucleus; P = plastid. Bar represents 500 nm.



Figs. 4–10. Mitochondria exhibiting different stages of vesiculation. Bar in each figure represents 250 nm. **4,** A mitochondrion containing single-membrane-bound vesicles in the space between the outer and inner mitochondrial membranes. Membranes of some vesicles are continuous with the outer mitochondrial membranes (arrowheads). The stroma containing several cristae is sufficiently intact to be recognized as a mitochondrion. **5,** The stroma of a vesiculated mitochondrion appears somewhat compressed with reduced number of cristae. **6,** Two vesiculated mitochondria. The upper one contains several cristae but the lower one has no cristae. An electron-lucent vacuole (arrowhead) is evident in the stroma of the lower mitochondrion. **7,** The stroma of the left mitochondrion is deeply undulated whereas the stroma of the right one appears to be depleted. Presence of coalesced fibrils (arrows) in the center of vesicles is clearly shown. **8,** Vesicle-containing perimitochondrial space is greatly widened and the stroma contains a vacuole (arrowhead). **9,** Two small clusters of vesicles in the cytoplasm without recognizable bounding membrane. **10,** A portion of phloem parenchyme cell containing two vesiculated mitochondria (M) and associated virus particles (Vp). Ve = vesicles.

0.01 or less. The test was repeated twice, and similar results were obtained.

DISCUSSION

Grapevine leafroll disease is apparently caused by a closterovirus. Particle morphology, phloem-limited cytopathic effects, and a consistent association of fibril-containing vesicles are ultrastructural characteristics similar to those occurring in other known closterovirus infections (8,15). The diseases include leafroll symptom-bearing grapevines found in Italy (6) and Japan (18). Because of difficulties in diagnosing closteroviruses by other means such as symptomatology and host range studies, a characteristic cytopathology has been a valuable aid in closterovirus diagnosis (8,15). In addition to the cytopathic effects, the serological relationship with the closteroviruslike particles associated with the New York isolate of the grapevine leafroll strengthen an etiology of the grapevine leafroll disease that involves a closterovirus. However, other evidence such as direct infectivity and/or insect vector involved, which would confirm that the closteroviruslike particles observed are the causal agent of the disease, are yet to be demonstrated.

Among the cytopathic effects, the fibril-containing vesicles are significant in establishing the viral etiology of grapevine leafroll disease. These structures have been found consistently in cells infected with many viruses belonging to a number of different taxonomic groups (7,8,16). In addition, virus-induced vesicles have been demonstrated in many cases to be the sites of viral RNA replication (1,12,22) and, therefore, are considered to be one of the most reliable cytopathic features of a virus infection (7).

Virus-induced vesicles are formed from various cell organelles such as endoplasmic reticulum, microbodies, chloroplasts, nuclei, tonoplasts, and mitochondria (8), and the origin of these vesicles has, in many cases, shown a high degree of specificity to a taxonomic group of related viruses or to a particular virus. For example, the vesicles induced by most tymoviruses are formed in the chloroplasts (14), whereas those induced by tombusviruses, with a few exceptions (7), develop from microbodies (20).

The vesicles induced by most closteroviruses have a characteristic morphology; however, the origin of vesicles has not been fully established. In studies on beet yellows virus (BYV), the type member of the closterovirus group, Esau and Hoefert (5) postulated that the vesicles originate "de novo" as receptacles of the fibrils that might be viral RNA. Vesicles induced by many other closteroviruses have since been referred to as the "BYV-type" if the source of these vesicles was uncertain (8).

Vesicles associated with the closteroviruslike particles in grapevine leafroll-diseased cells of this study are formed in modified mitochondria. Involvement of mitochondria in the genesis of virus-induced vesicles, unlike those of microbodies and chloroplasts, has been reported in viruses belonging to a number of different taxonomic groups such as the tobamovirus (10), tobravirus (9), and tombusvirus (4) groups and some unclassified viruses (8). In almost all cases, vesicles induced by typical closteroviruses have been reported to be "BYV-type" (8). However, mitochondrial vesicles similar to those observed in the grapevine leafroll are associated with dendrobium vein necrosis virus which has closteroviruslike particles (13). The relationships between these two viruses and the New York isolate of grapevine leafroll (23) should be investigated. Dendrobium vein necrosis virus has not been fully characterized.

Mitochondrial vesiculation has been reported previously in grapevine with leafroll symptoms, but the viruslike particles associated with the vesicles were isometric (3) rather than closteroviruslike particles. The mitochondrial vesicles associated with the isometric particles were, however, double membrane bound and originated as invaginations of both the outer and inner mitochondrial membranes, whereas those associated with the closteroviruslike particles of this study were single membrane bound and were apparently formed by invaginations of the outer membrane.

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