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Virus-like particles and ultrastructural modifications in the phloem of leafroll-affected grapevines

by

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Virusartige Partikeln und Veränderungen der Ultrastruktur im Phloem blattrollkranker Reben

Zusammenfassung. — Bei 42 Rebenstämmen mit Blattrollsymptomen wurde die Ultrastruktur des Blattphloems und Mesophylls untersucht. Die Reben stammten aus 12 verschiedenen Ländern (Italien, Portugal, Spanien, Frankreich, Jugoslawien, Ungarn, Sowjetunion, Bulgarien, Griechenland, Cypern, Malta und Nigeria). Insgesamt wurden 47 Exemplare elektronenmikroskopisch untersucht. Etwa die Hälfte der Proben (48 %) enthielt isometrische virusartige Partikeln in einem oder mehreren Phloemelementen (Siebröhren, Geleit- und Parenchymzellen); bei 15 % der Proben waren in denselben Zelltypen filamentöse virusartige Partikeln enthalten. Eine Saftübertragung der Viren war in keinem Falle möglich. Wichtige ultrastrukturelle Bildungen waren doppelwandige vesiculäre Körper, die möglicherweise stark abgewandelte Zellorganellen (Mitochondrien und/oder Plastiden) darstellen, sowie Bündel tubulärer Strukturen vom Durchmesser 40—45 nm oder 60—100 nm; die größeren Tubuli zeigten einen zentralen Kern, der anscheinend von einem oder mehreren Filamenten tubulären P-Proteins gebildet wurde. Die vorliegenden Ergebnisse werden im Zusammenhang mit den Literaturbefunden über blattrollinfiziertes Rebengewebe diskutiert.

Introduction

Leafroll is a long-known ubiquitous and economically important graft-transmissible disease of grapevine (GOHEEN *et al.* 1959) suspected to be of viral origin.

All attempts made so far for isolating its possible causal agent by mechanical means (i.e. inoculation of sap or of phenol-sodium dodecyl sulphate leaf extracts) have failed, except for a single case reported by TANNE *et al.* (1974) from Israel. TANNE *et al.* (1977) were able to isolate from vines with leafroll symptoms a virus with properties of members of the potyvirus group. This report stimulated new interest for this disease which, since then, is being reinvestigated in many laboratories using different approaches.

In our laboratory, electron microscope observations were carried out on thin-sectioned tissues mostly from naturally infected vines that showed leafroll symptoms. The results of this study are reported in the present paper.

Materials and methods

Source material. — 42 grapevine accessions of different geographic origin (Italy, Portugal, Spain, France, Yugoslavia, Hungary, Soviet Union, Bulgaria, Greece, Cyprus, Malta and Nigeria), most of which were growing in a collection plot near Bari (Italy) and showed variously intense leafroll symptoms, were selected. All mother plants were individually assayed by inoculation of sap to herbaceous hosts for presence of mechanically transmissible viruses. Tissue samples to be processed for electron

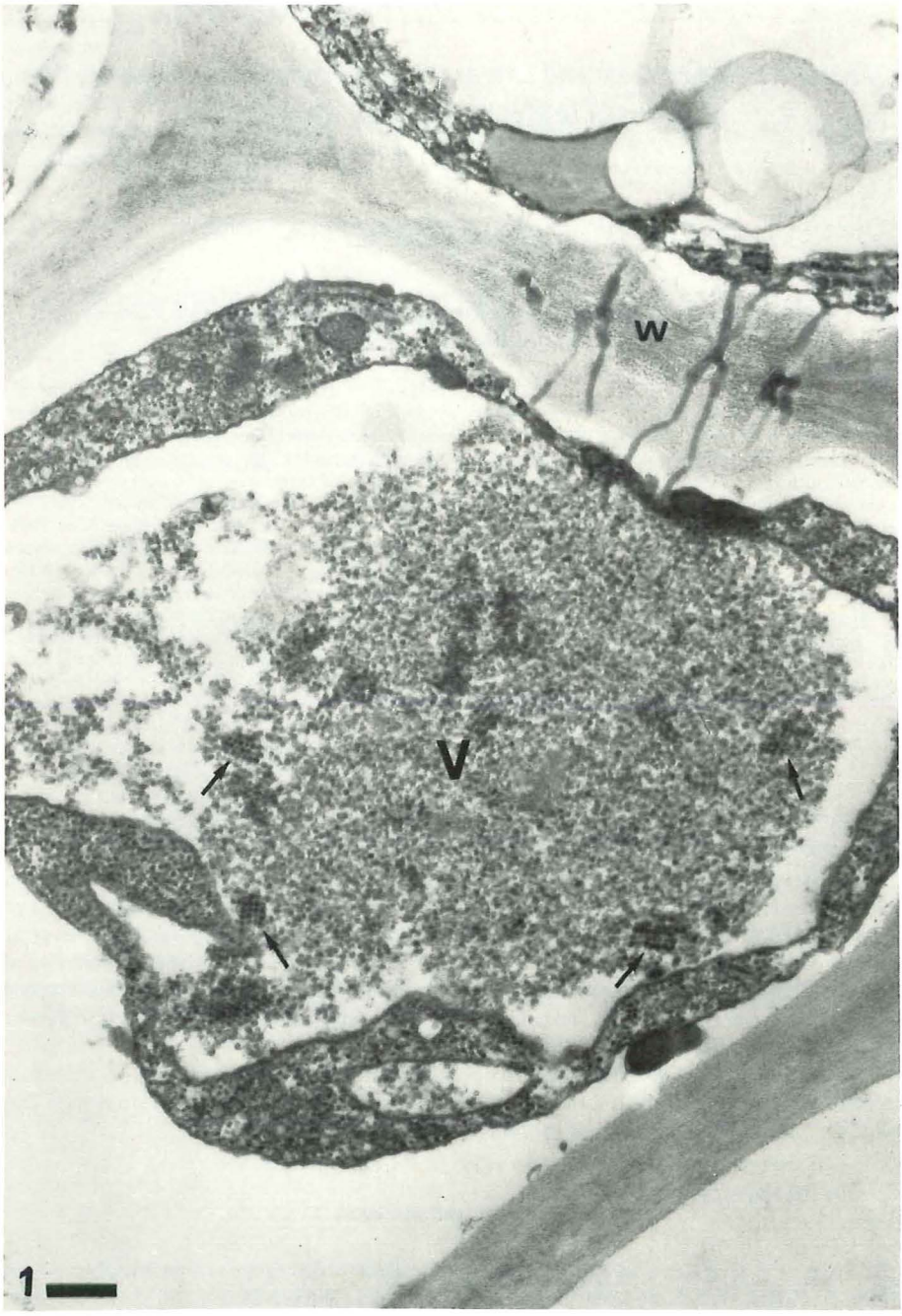

Fig. 1: Transverse section of a phloem companion cell showing a collapsed protoplast and a large aggregate of isometric virus-like particles (V), some of which are organized into microcrystals (arrows), in the central vacuole. W = cell wall. Bar = 250 nm.

microscopy were taken from the main vein of fully expanded leaves of field-grown vines. For five of the accessions under study, observations were made also on leaf tissues from glasshouse-forced cuttings. Tissue sampling was either in October or May-June for field-grown vines and in January-March for rooted cuttings.

Electron microscopy. — Small pieces of the main vein with strips of adjacent parenchyma tissues were excised in a drop of 4 % glutaraldehyde in 0.05 M neutral cacodylate buffer and fixed for 2 h at room temperature under slight vacuum in the same fixing solution. After thorough rinsing in buffer, the samples were postfixed in 1 % osmium tetroxide for 2 h at 4 °C, stained overnight in 0.5 % aqueous uranyl acetate in the cold (4 °C), dehydrated in graded ethanol dilutions and embedded in Spurr's medium. Thin sections were cut with a diamond knife and were double stained with uranyl acetate and lead citrate before examination with a Philips 201-C electron microscope. Comparable tissue pieces from a healthy vine were similarly processed to serve as controls.

Results

There was a difference between tissue samples depending on whether they came from glasshouse-grown or field-grown vines. The former were more readily sectioned and exhibited a rather well preserved ultrastructural organization of the phloem, in this resembling control samples. Collapsed or necrosing cells were few but inclusions and modifications similar to those of specimens from field-grown plants were present. The foliar phloem of field vines was much richer in tannins and more of its cells were necrotic or in an evident state of derangement. The ultrastructural modifications, however, were the same regardless of the origin of the material.

As summarized in the table, most of the samples examined (38 out of 47) contained intracellular inclusions and/or showed modifications of some sort. These changes were apparently limited to phloem tissues (sieve tubes, companion cells, phloem parenchyma cells) and were not observed in any of the mesophyll cells adjacent to the veins.

The most prominent ultrastructural changes were:

1. Presence of isometric virus-like particles

Two kinds of possible virus particles were seen: (i) rounded intensely electron opaque bodies, 22—24 nm in diameter with a smooth and regular contour (Figs. 2, 5 and 6); (ii) doughnut-shaped rounded bodies with an electron transparent centre and the same size of the above solid particles (Figs. 2, 3 and 5). By analogy with the reports of the literature (for review see MARTELLI and RUSSO 1977) these particulates were interpreted as profiles of isometric viral nucleoproteins (solid particles) and viral capsids deprived of nucleic acid (doughnut-shaped particles), respectively. In any case they differed and could be distinguished from ribosomes, though not always readily, because of the larger diameter, the higher electron opacity and the more regular contour.

Possible isometric virions were seen in all types of cells of phloem tissues including mature sieve elements. However, they were more abundant in differentiating sieve

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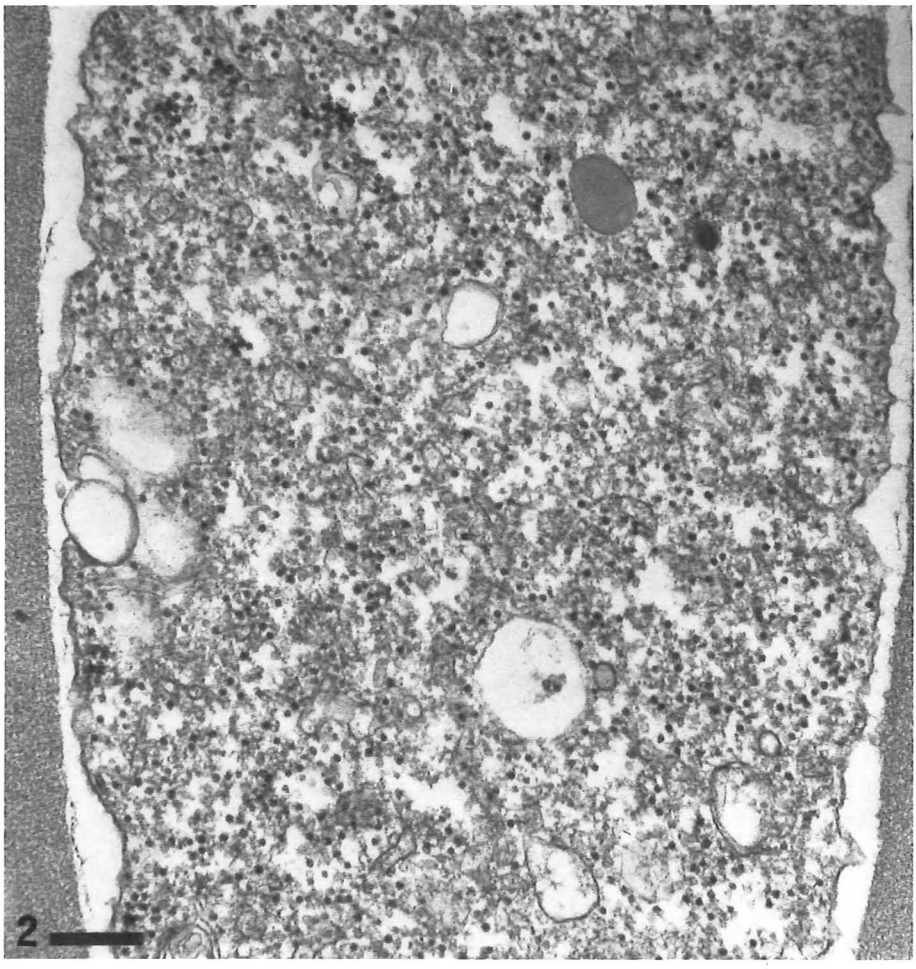
Querschnitt durch eine Siebröhrengeleitzelle mit kollabiertem Protoplasten und einem großen Aggregat isometrischer virusartiger Partikeln (V) in der Zentralvakuole; an etlichen Stellen sind diese in Form von Mikrokristallen angeordnet (Pfeile). W = Zellwand. Maßstab = 250 nm.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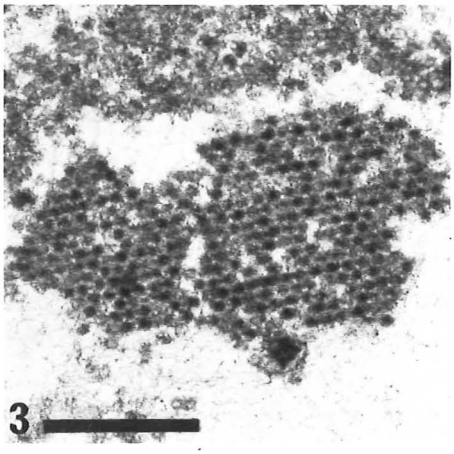
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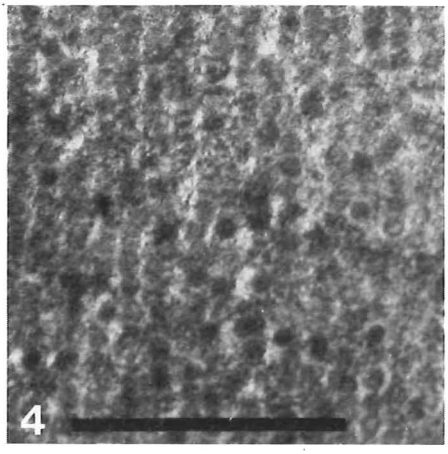
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tubes and, generally, in all cells containing an active and well developed protoplast. Virus-like particles were either scattered throughout the cytoplasm (Fig. 2) or were in massive accumulations in the central vacuole (Figs. 1 and 5). In the aggregates, individual particles were appressed in a disorderly fashion or, in two accessions (Table), were arranged in a crystalline lattice (Figs. 3 and 4). Possible virus crystals were variable in size, ranging from very small aggregates of a few elements (Fig. 1) to much larger structures (Fig. 3) which could attain a length of about 5 μm.

Virions were also seen, again in two accessions, in a single row within tubular structures reminiscent of the virus-containing tubules typical of nepo- and comoviruses and of a few ungrouped isometric plant viruses (reviewed by MARTELLI and RUSSO 1977, EDWARDSON and CHRISTIE 1978, MARTELLI 1980). The diameter of the tubular structures containing virus-like particles (42—45 nm) also conformed to the size of the virus-tubule associations elicited by the above virus groups.

With the exception of two dubious cases (Table), isometric virus-like particles were identified with a fair level of confidence in half (23 out of 47) the specimens examined. In particular, they occurred in a very high number of grape accessions from Italy (19 out of 27), in the two accessions from Soviet Union and one each from Spain, Yugoslavia and, possibly, Portugal and Greece.

Except for seven cases, no sap-transmissible viruses were recovered from mother vines, despite the repeated attempts made at different times of the year using diverse procedures and extraction media. Comparable particles were never seen in the mesophyll cells of the same tissue samples, including those from which grapevine fanleaf virus (GFLV) and an unidentified virus (Nigerian accessions) were isolated. Actually, in four of these samples (Table, accessions 14, 32, 41 and 42) no virus particles at all were detected either in phloem or mesophyll tissues. Hence, it seems unlikely that the isometric virus-like particles so frequently found in many of the grape accessions may be identified with GFLV or the Nigerian virus.

2. Presence of filamentous virus-like particles

The thread-like structures shown in Fig. 7 and 8 were interpreted as profiles of possible filamentous virus particles. These particles occurred in bundles of several to many loosely appressed elements each with a diameter of ca. 10 nm, and were preferentially located in the cytoplasm (Fig. 8). In one instance, comparable filaments were also seen within a nucleus of a companion cell (Fig. 7).

Owing to their outward aspect and size, the possible virions were in most cases distinguished from filaments of P-protein. In a few instances, however, (Table, accessions 8, 13 and 30) the scarcity of material did not allow for a clear-cut discrimination.

Thread-like structures were more commonly encountered in differentiating sieve tubes and companion cells. They were never seen in mesophyll cells. These filaments were identified as possible virions with a reasonable level of confidence only in seven accessions, all from Italy. Interestingly, the same plants contained also, often in the

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Figs. 2—4: Intracellular aspects of isometric virus-like particles. Possible virions scattered throughout the cytoplasm of an undifferentiated sieve element (Fig. 2) or in a crystalline array (Fig. 3). Detail of a virus crystal (Fig. 4) showing a few nucleoproteins (solid particles) intermingled with empty protein shells (doughnut-shaped particles). All bars = 250 nm.

Intrazelluläre Erscheinungsformen isometrischer virusartiger Partikeln. Mögliche Virionen sind im Cytoplasma eines undifferenzierten Siebröhrenelementes zerstreut (Abb. 2) oder in kristalliner Form angeordnet (Abb. 3). Der Ausschnitt eines Viruskristalles (Abb. 4) zeigt etliche Nucleoproteine (dichte Partikeln) gemischt mit leeren Proteinhüllen („pfannkuchenförmige" Partikeln). Alle Maßstäbe = 250 nm.

Table

Type of virus-like particles and major ultrastructural modifications found in grapevines with leaf-roll symptoms from different geographic origins

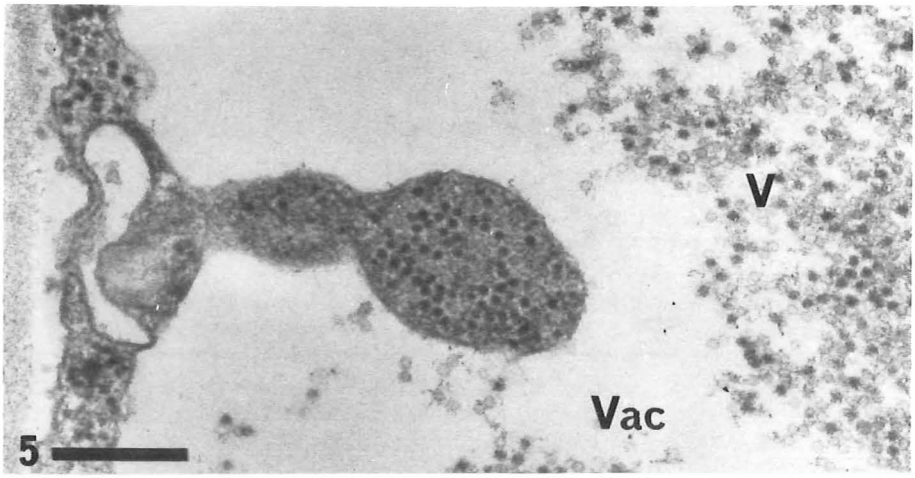
Der Typ der virusartigen Partikeln sowie die wichtigsten Veränderungen der Ultrastruktur bei blattrollkranken Reben unterschiedlicher geographischer Herkunft

Accession no.	Geographical origin and cultivar	Disease	Mechanical transmission	Isometric virus-like particles	Filamentous virus-like particles	Vesiculated bodies	Tubules (large)	Tubules (small)	Tubules containing virus-like particles	Crystals of virus-like particles
	ITALY									
1	LN-33	LR SP	–	+	+	+	–	+	–	–
1 (a)	LN-33	LR SP	–	+	+	+	–	–	–	+
2	Primitivo di Gioia	LR	–	+	–	+	–	–	–	–
2 (a)	Primitivo di Gioia	LR	–	+	+	+	–	–	–	–
3	Primitivo di Gioia	LR	–	+	+	+	–	–	–	–
3 (a)	Primitivo di Gioia	LR	–	+	–	+	–	–	–	–
4	Primitivo di Gioia	LR	–	+	–	+	–	–	–	–
5	Primitivo di Gioia	LR	–	+	+	+	–	–	–	+
5 (a)	Primitivo di Gioia	LR	–	+	–	+	–	–	–	–
6	Montepulciano	LR	–	–	–	–	+	–	–	–
7	Marchione	LR	–	+	+	+	–	–	–	–
8	Primitivo di Gioia	LR	–	+	+(?)	+	–	–	+	–
9	Nebbiolo	LR	–	+	–	+	+	–	–	–
9 (a)	Nebbiolo	LR	–	+	+	+	–	–	–	–
10	Primitivo di Gioia	LR	–	+	–	+	–	–	–	–
11	Primitivo di Gioia	LR	+(GFLV)	+	–	+	–	–	–	–
12	Rossese	LR SP	–	–	–	–	–	–	–	–
13	Vermentino	LR SP	–	+	+(?)	+	–	–	–	–
14	Sangiovese	LR SP	+(GFLV)	–	–	+	–	–	–	–
15	Montepulciano	LR SP	+(GFLV)	+	–	–	–	–	–	–
16	LN 33	LR SP	–	–	–	–	–	–	–	–
17	Montepulciano	LR	–	–	–	–	–	–	–	–
18	Montepulciano	LR	–	+	–	+	–	–	–	–
19	Montepulciano	LR	not made	+	–	+	–	–	–	–
20	Pascale di Cagliari	LR	–	–	–	+	–	–	–	–
21	Torbato	LR	–	–	–	–	–	–	–	–
22	Torbato	LR	–	–	–	–	–	–	–	–
	PORTUGAL									
23	Unknown	LR	–	+(?)	–	–	–	–	–	–

Table (continued)

Accession no.	Geographical origin and cultivar	Disease	Mechanical transmission	Isometric virus-like particles	Filamentous virus-like particles	Vesiculated bodies	Tubules (large)	Tubules (small)	Tubules containing virus-like particles	Crystals of virus-like particles
	SPAIN									
24	Unknown	LR	–	–	–	–	–	–	–	–
25	Pedro Jimenez	LR	–	+	–	+	–	–	–	–
	FRANCE									
26	Servant	LR	–	–	–	–	–	–	–	–
	YUGOSLAVIA									
27	Unknown	LR	–	+	–	+(?)	–	–	–	–
	BULGARIA									
28	Unknown	LR	not made	–	–	+(?)	–	+	–	–
	HUNGARY									
29	Unknown	LR	–	–	–	–	–	–	–	
	SOVIET UNION									
30	Unknown	LR	–	+	+(?)	+	–	–	+	–
31	Unknown hybrid	LR	–	+	–	–	–	–	–	–
	GREECE									
32	Corinthiaki	LR YM	+(GFLV)	–	–	–	+	–	–	–
33	Corinthiaki	LR YM	+(GFLV)	+(?)	–	+	–	–	–	–
	CYPRUS									
34	Sideritis	LR	–	–	–	–	–	–	–	–
35	Mavro	LR	–	–	–	–	+	–	–	–
36	Mavro	LR	–	–	–	–	+	–	–	–
37	Razaki	LR	–	–	–	–	+	–	–	–
	MALTA									
38	Unknown	LR VN	–	–	–	–	+	–	–	–
39	Unknown	LR	–	–	–	–	+	–	–	–
40	Unknown	LR	–	–	–	+	–	–	–	–
	NIGERIA									
41	Unknown	LR	+(b)	–	–	–	–	+	–	–
42	Unknown	LR	+(b)	–	–	–	+	+	–	–

Accessions marked with (a) are glasshouse-grown rooted cuttings; (b) a mechanically-transmissible still unidentified virus not related with grapevine fanleaf virus.
Mechanical transmission: + = positive; – = negative.
Following columns: + = present; – = not seen.
GFLV = grapevine fanleaf virus; LR = leaf roll; SP = stem pitting; VN = vein necrosis; YM = yellow mosaic.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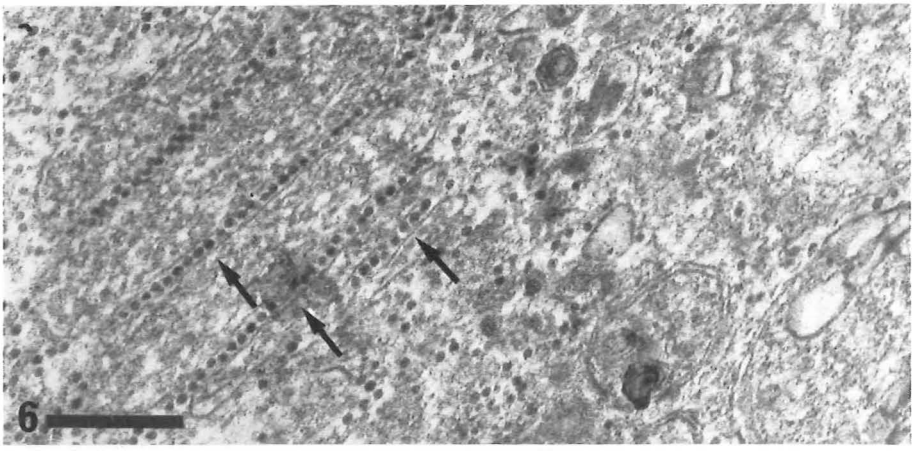
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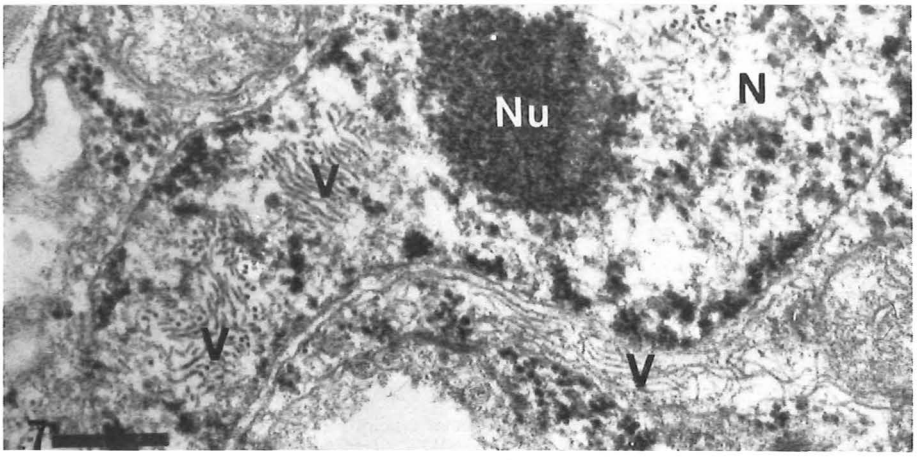
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same cells, isometric virus-like particles. Filamentous virions alone were not seen in any of the samples examined.

None of the repeated attempts to isolate a virus by inoculation of sap from vines containing possible filamentous virions was successful.

3. Vesiculated bodies

A most dinstinctive ultrastructural feature of phloem cells of many of the accessions under study was constituted by peculiarly structured ovoid to rounded membranous bodies, having the size of a mitochondrion or a small plastid (Fig. 9). These structures, which will be referred to as vesiculated bodies (VB), were bound by a double membrane which was lined internally by a row of roughly circular or pear-shaped vesicles measuring 80 to ca. 100 nm in diameter. These vesicles were also double-walled and were either electron clear and apparently empty or contained finely granular material of low electron opacity or a network of fine fibrils reminiscent of nucleic acid strands. In some VB the vesicles were scattered disorderly at a distance from the boundary membrane. However, in the majority of cases, the vesicles appeared to originate as invaginations of the membranous envelope to which they were obviously connected (Fig. 9, inset).

VB were present in companion and parenchyma cells but were also seen in differentiating sieve tubes. Their presence was almost invariably related to that of the isometric virus-like particles (Table) with which they usually coexisted in the same cells. Some of these cells, however, contained also filamentous virus-like particles. On the whole, VB were found in 24 out of the 47 specimens examined, 20 of which were from Italy and one each from Spain, Soviet Union, Greece and Malta (Table).

4. Tubular structures

Besides the tubules containing rows of isometric virus-like particles, two additional types of tubular structures were seen, which differed from one another both in size and outward aspect.

Small tubules (40—45 nm in diameter) were flexuous and apparently empty structures (Fig. 11) occurring in bundles of few elements in mature sieve tubes (Figs. 10 and 11). These tubules were found in one accession each from Italy and the Soviet Union (Table).

Fig. 5: Isometric virus-like particles (V) in a cytoplasmic protrusion and in the vacuole (Vac) of an immature sieve tube. Many of the particles look like empty protein shells. Bar = 250 nm.

Fig. 6: Tubules containing rows of virus-like particles (arrows) in the cytoplasm of a phloem parenchyma cell. Bar = 250 nm.

Fig. 7: Filamentous virus-like particles (V) in the cytoplasm and nucleus (N) of a companion cell. Nu = nucleolus. Bar = 250 nm.

Abb. 5: Isometrische virusartige Partikeln (V) in einem Cytoplasmafortsatz und in der Vakuole (Vac) einer unentwickelten Siebröhre. Viele Partikeln erscheinen wie leere Proteinhüllen. Maßstab = 250 nm.

Abb. 6: Tubuli, die Reihen virusartiger Partikeln enthalten (Pfeile), im Cytoplasma einer Phloemparenchymzelle. Maßstab = 250 nm.

Abb. 7: Filamentöse virusartige Partikeln (V) in Cytoplasma und Kern (N) einer Geleitzelle. Nu = Nucleolus. Maßstab = 250 nm.

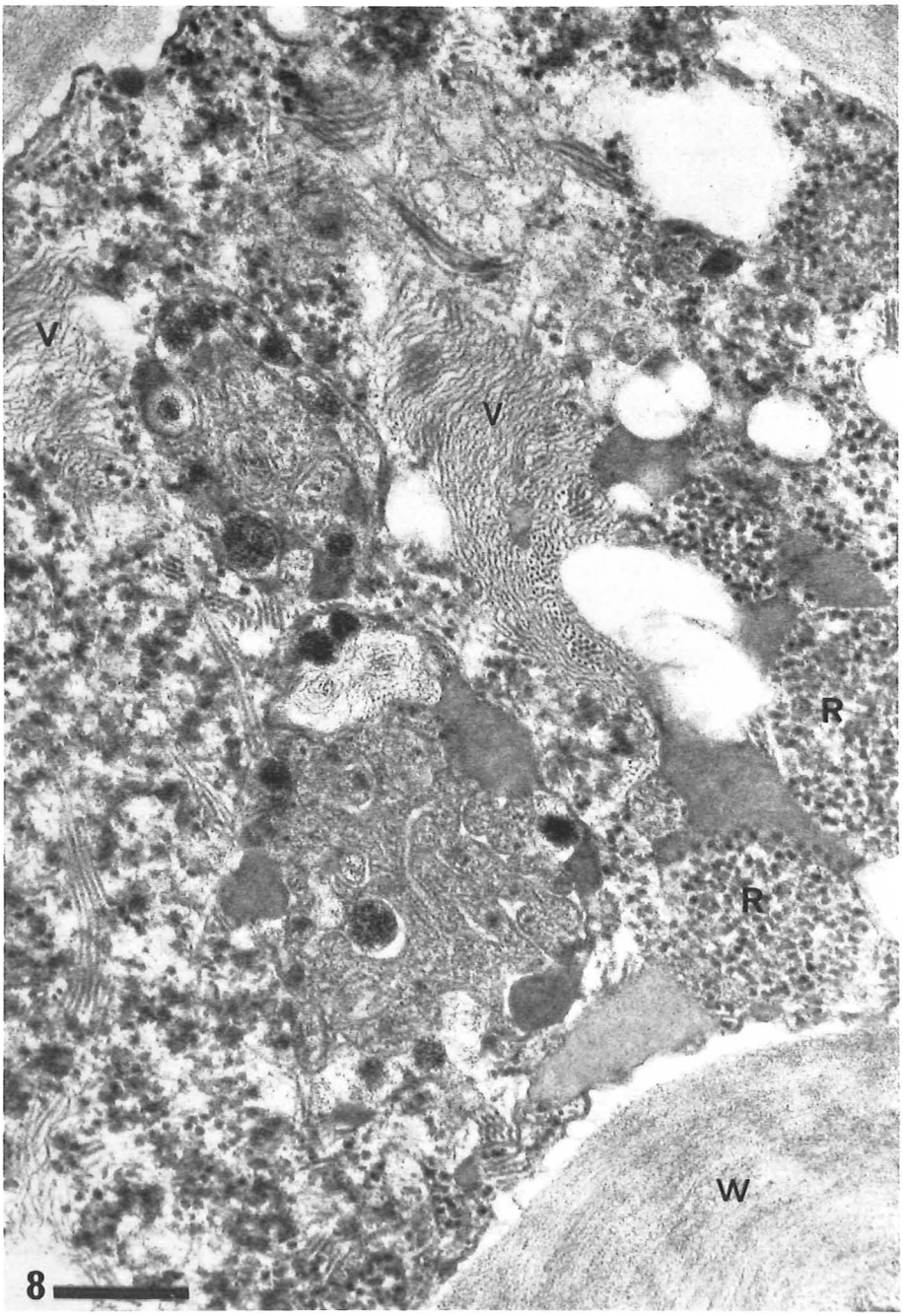


Fig. 8: Bundles of filamentous virus-like particles (V) in the cytoplasm of a companion cell. R = ribosomes; W = cell wall. Bar = 250 nm.

Bündel filamentöser virusartiger Partikeln (V) im Cytoplasma einer Geleitzelle. R = Ribosomen; W = Zellwand. Maßstab = 250 nm.

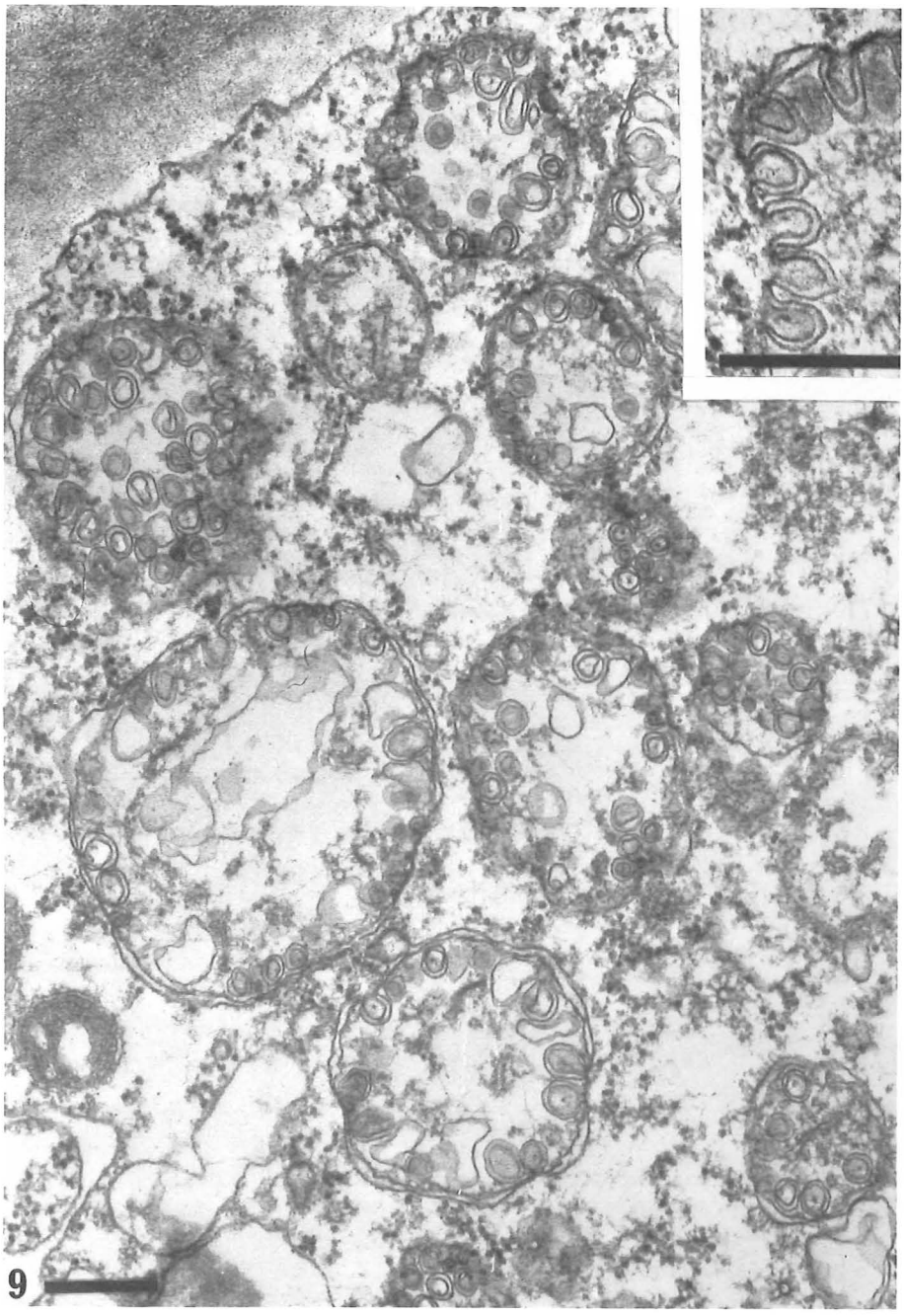
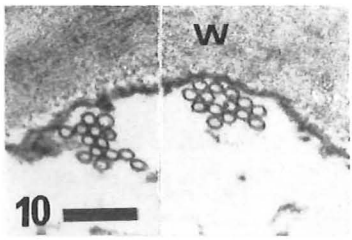


Fig. 9: Vesiculated bodies in the cytoplasm of a differentiating sieve element. Inset shows a detail of the double-membraned vesicular invaginations on the body surface. Bars = 250 nm.

Vesiculäre Körper im Cytoplasma eines sich differenzierenden Siebröhrenelementes. Das Nebenbild zeigt einen Ausschnitt der doppelwandigen blasenförmigen Einstülpung an der Körperoberfläche. Maßstäbe = 250 nm.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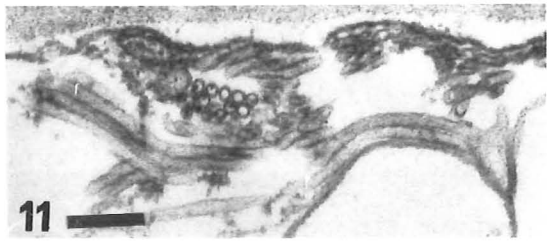
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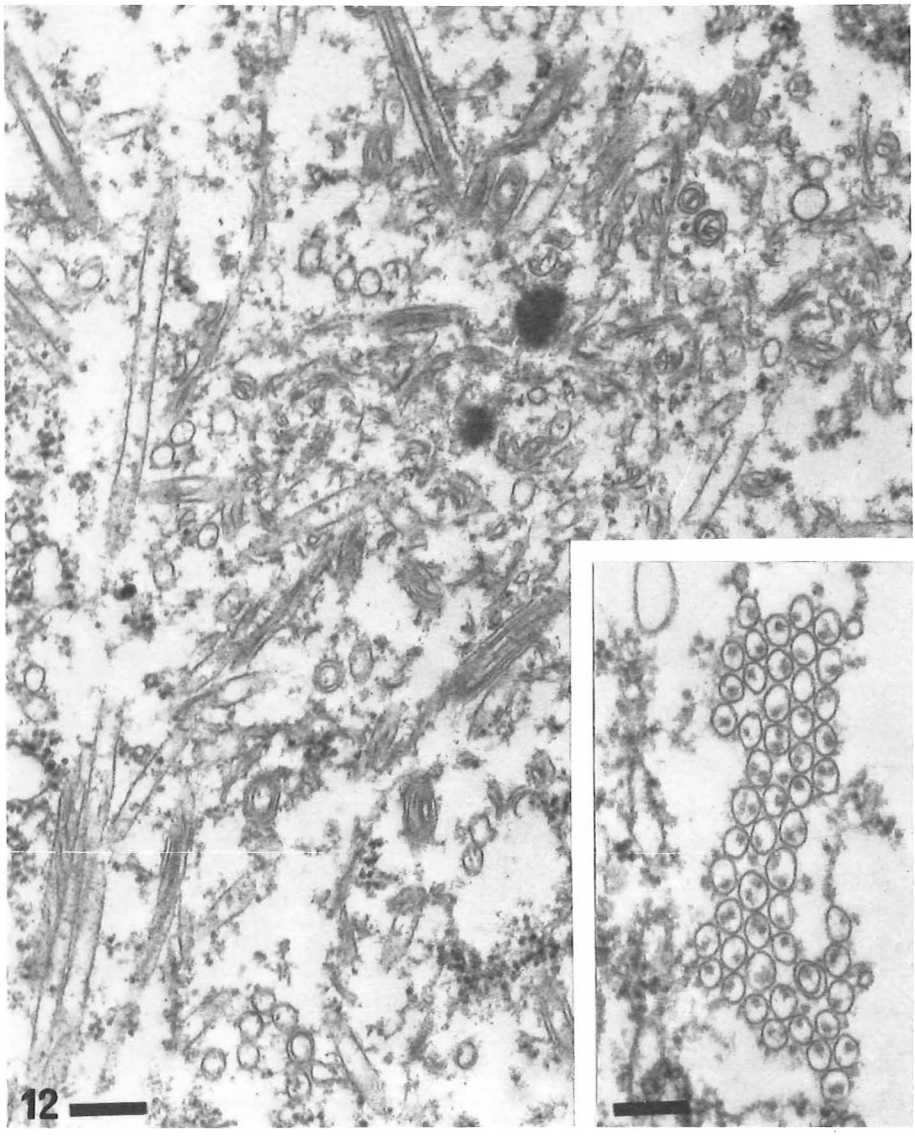
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Large tubules were apparently rigid structures of undetermined length (the longest one that was measured was about 850 nm long) and a diameter from 60 up to ca. 100 nm (Fig. 12 with inset). These tubules were single- or, sometimes, double-walled and were rarely empty. Ordinarily, they contained an electron dense core made up of one (less commonly two or three) rounded bodies 15 to 30 nm in diameter, sometimes exhibiting a ring-like aspect (Fig. 12, inset, and Fig. 13 with inset). At a closer examination, the doughnut-shaped cores appeared to be profiles of cross-sectioned tubular elements very similar to filaments of the tubular form of P-protein. A spatial relationship between large tubules and P-protein was detected in differentiating sieve elements (Fig. 13 with inset). When sieve tubes were differentiated, the tubules were still present, often in groups of many appressed elements (Fig. 12, inset) but P-protein was scanty or no longer visible.

Large tubules were more frequent than the smaller ones, being present in two accessions from Italy, two from Malta, three from Cyprus and one each from Greece and Nigeria to a total of 9 specimens out of the 47 examined (Table).

Discussion

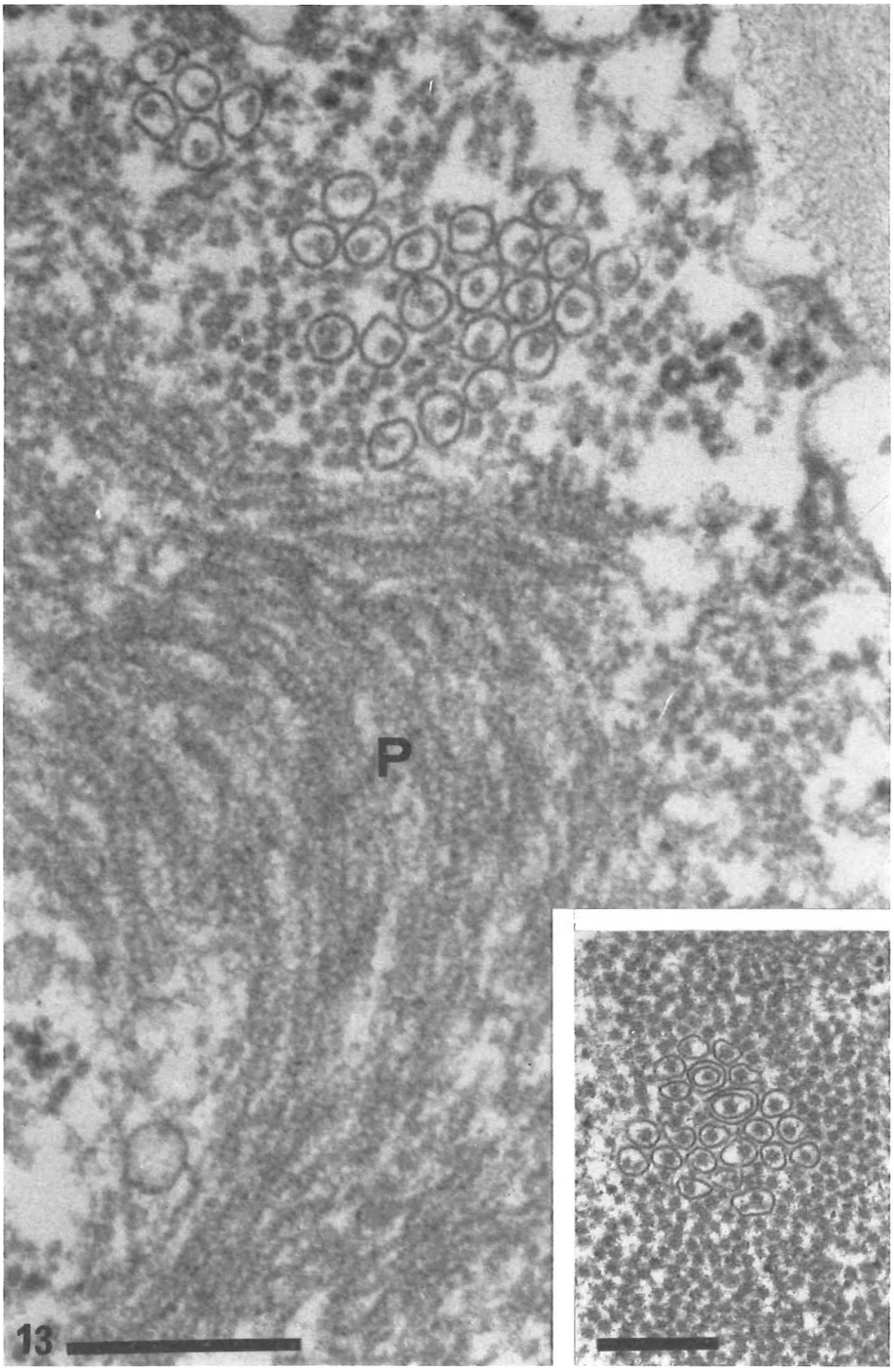
The results of the present study, however preliminary, have shown that specimens from field-grown vines and glasshouse-forced cuttings from these vines exhibited comparable intracellular inclusions and ultrastructural modifications, regardless of the environment in which the donor plants were grown and the time of the year when the sampling was made. This may be taken as a confirmation that electron microscopic observations of thin-sectioned infected grapevine tissues can be reliable and informative.

More than 80 % of the samples examined showed one or more features of possible pathological origin. Comparable changes were not seen in the control samples nor were recorded in any of the exhaustive electron microscopic studies on the anatomy and cytology of *Vitis* phloem reported in the literature (ESAU 1965, MENGDEN 1971).

Among the pathological features, vesiculated bodies were indeed the most striking and intriguing. The size of these structures and the presence of a double boundary membrane constitute strong indications that VB may be deeply transformed cell organelles such as mitochondria and/or plastids. Although transitional stages between normal mitochondria and plastids and VB were occasionally seen, this contention, to be proven, warrants additional specific investigations now under way. The point remains that, not only there is a striking similarity in the structure and form of the peripheral vesicles of VB and those elicited on the chloroplast surface by members of the tymovirus group (KOENIG and LESEMANN 1981, MATTHEWS 1981), but the overall outward aspect of VB recalls that of vesiculated mitochondria (HATTA and USHIYAMA 1973; GILL *et al.*

Fig. 10—12: Aspects of tubular inclusions. Small tubules in cross (Fig. 10) and longitudinal (Fig. 11) section in mature sieve elements. Longitudinally and transversely sectioned large tubules (Fig. 12) in a differentiating sieve tube. Inset shows a bundle of tubular structures in cross section. All bars = 250 nm.

Erscheinungsformen tubulärer Einschlüsse. Kleine Tubuli im Querschnitt (Abb. 10) und Längsschnitt (Abb. 11) in vollentwickelten Siebröhrenelementen. Längs und quer getroffene große Tubuli (Abb. 12) in einer sich differenzierenden Siebröhre. Das Nebenbild zeigt ein Bündel tubulärer Strukturen im Querschnitt. Alle Maßstäbe = 250 nm.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1981, RUSSO and MARTELLI 1982) and microbodies (MARTELLI and RUSSO 1982) found in cells infected with different viruses. Thus, one wonders whether VB may play a role similar to that of the above cytopathic structures, i.e. assistance in the replication of viral nucleic acid. If so, associating VB with phloem-related isometric virus-like particles becomes plausible for, so far, none of the filamentous plant viruses is known to induce comparable cytological modifications.

Another peculiar ultrastructural feature of leafroll-infected vines consisted of the bundles of tubular elements of unknown nature seen in the phloem of several accessions. Similar inclusions had already been observed in French grapevines affected either by red leaf, a recently described disease of the yellows type (LEGIN *et al.* 1979), and/or by ordinary leafroll (VUITTENEZ and STOCKY 1982). Hence, these tubules, rather than being fortuitous structures, are likely to have a pathological significance which, however, remains to be established.

Particles of a possible isometric virus ca. 28 nm in diameter were visualized *in situ* in Japanese grapevines naturally affected by "ajinashika disease" (NAMBA *et al.* 1979 a). Virions were only present in the phloem where they occurred in great abundance, often in crystalline arrays, and were not mechanically transmissible. These particles were identified as those of grapevine ajinashika virus, a name that has no reasons to stand since "ajinashika", rather than a disease of its own right, was shown to be a mixed infection of fleck and leafroll (TERAI and YANO 1982). Thus, the findings by NAMBA *et al.* (1979 a) come closer to the present ones.

Filamentous virus-like particles were first detected in the phloem of European grapes by MENGDEN (1971). The vines were affected by a yellows type disease ("Vergilbungskrankheit") but it is not known whether they also carried leafroll. Similar virus-like particles were more recently observed in Japanese (NAMBA *et al.* 1979 b) and Italian (FAORO *et al.* 1981) grapevines with leafroll symptoms. The authors tentatively identified the filamentous structures as particles belonging to a possible member of the closterovirus group and indicated it as the agent of the leafroll disease.

A feature common to all the above reports is the massive accumulation of possible virions in the sieve elements. This does not compare with the scantier presence of thread-like particles found in all samples examined in the present investigation. Despite this difference, the evidence gathered in our studies, suggests that at least two types of phloem-related viruses, none of which is mechanically transmissible, can be found with high frequency in naturally leafroll-infected grapevines. Hence, at the present status of knowledge, concluding that one or the other virus type is the causal agent of leafroll would be unsafe and unjustified.

The electron microscope investigations carried out so far on leafroll-infected vines, including the present one, have only begun to unravel some of the aspects of the complex nature of this disorder without providing any sound and concluding evidence that any specific type of virus can be regarded as its sole etiological agent.

Fig. 13: Cross-sectioned tubular inclusions next to an aggregate of P-protein (P) in an immature sieve element. Note the similarity of the doughnut-shaped core of the tubules and cross-sectioned P-protein filaments. Inset shows single- and double-walled tubules in the middle of a bundle of tubular P-protein filaments in cross section. Bars = 250 nm.

Quergeschnittene tubuläre Einschlüsse neben einem Aggregat von P-Protein (P) in einem unentwickelten Siebröhrenelement. Bemerkenswert ist die Ähnlichkeit zwischen dem „pfannkuchenförmigen" Kern der Tubuli und den quer getroffenen P-Protein-Filamenten. Das Nebenbild zeigt einfach- und doppelwandige Tubuli inmitten eines Bündels tubulärer P-Protein-Filamente im Querschnitt. Maßstäbe = 250 nm.

Summary

Ultrastructural studies were carried out on thin-sectioned phloem and mesophyll tissues of 42 grapevine accessions with leafroll symptoms, coming from 12 different countries (Italy, Portugal, Spain, France, Yugoslavia, Hungary, Soviet Union, Bulgaria, Greece, Cyprus, Malta and Nigeria). A total of 47 specimens were observed with the electron microscope. About half (48 %) the samples examined contained isometric virus-like particles in one or more phloem elements (sieve tubes, companion and parenchyma cells), whereas only 15 % of the samples contained filamentous virus-like particles in the same type of cells. None of these viruses could be transmitted by sap inoculation. Ultrastructural features of major importance were double-membraned vesiculated bodies possibly representing profoundly modified cell organelles (mitochondria and/or plastids) and bundles of tubular structures 40—45 nm or 60—100 nm in diameter, the larger of which exhibited a central core seemingly made up of one or more filaments of tubular P-protein. The present results are discussed in the light of the findings reported in the literature on leafroll-infected grapevine tissues.

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Literature cited

EDWARDSON, J. R. and CHRISTIE, R. G., 1978: Use of virus-induced inclusions in classification and diagnosis. Ann. Rev. Phytopathol. **16**, 31—55.

ESAU, K., 1965: Anatomy and cytology of *Vitis* phloem. Hilgardia **37**, 17—72.

FAORO, F., TORNAGHI, A., FORTUSINI, A. and BELLI, G., 1981: Association of a possible closterovirus with grapevine leafroll in northern Italy. Riv. Patol. Veg., Ser. IV, **17**, 183—189.

GILL, C. C., CHONG, J. and CAETANO, V. R., 1981: Cytopathological alterations associated with a virus-like disorder in *Panicum sabulorum*. Canad. J. Plant Pathol. **3**, 129—135.

GOHEEN, A. C., HEWITT, W. B. and ALLEY, C. J., 1959: Studies of grape leafroll in California. Amer. J. Enol. Viticult. **10**, 78—84.

HATTA, T. and USHIYAMA, R., 1973: Mitochondrial vesiculation associated with cucumber green mottle virus-infected plants. J. Gen. Virol. **6**, 127—140.

KOENIG, R. and LESEMANN, D. E., 1981: Tymoviruses. In: KURSTAK, E. (Ed.): Handbook of Plant Virus Infections and Comparative Diagnosis, 33—60. Elsevier, North-Holland, Amsterdam.

LEGIN, R., BASS, P. and VUITTENEZ, A., 1979: Une maladie de type chlorose accompagnée de rougissement foliaire autumnal et de chancres du bois, produite par un agent de type viral très résistant a la thermothérapie, transmissible par greffage à l'indicateur Pinot noir *V. vinifera*. Ann. Phytopathol. **11**, 136—137.

MARTELLI, G. P., 1980: Ultrastructural aspects of possible defence reactions in virus-infected plant cells. Microbiologica **3**, 369—391.

— — and RUSSO, M., 1977: Plant virus inclusion bodies. Adv. Virus Res. **21**, 175—266.

— — and — — , 1982: The fine structure od *Cymbidium* ringspot virus in host tissues. I. Electron microscopy of systemic infections. J. Ultrastruct. Res. **77**, 93—104.

MATTHEWS, R. E. F., 1981: Plant Virology. 2nd Ed. Academic Press, New York.

MENGDEN, K., 1971: Untersuchungen über eine Vergilbungkrankheit der Reben an Rhein, Mosel und Saar. Weinberg und Keller **18**, 345—431.

NAMBA, S., YAMASHITA, S., DOI, Y. and YORA, K., 1979 a: A small spherical virus associated with the ajinashika disease of Koshu grapevine. Ann. Phytopathol. Soc. Japan **45**, 70—73.

— — , — — , — — , — — , TERAI, Y. and YANO, R., 1979 b: Grapevine leafroll virus, a possible member of closteroviruses. Ann. Phytopathol. Soc. Japan **45**, 497—502.

RUSSO, M. and MARTELLI, G. P., 1982: Ultrastructure of turnip crinkle and saguaro cactus virus-infected tissues. Virology **118**, 109—116.

TANNE, E., SELA, I. and HARPAZ, I., 1974: Transmission of grapevine leafroll virus to herbaceous plants. Phytopathol. Z. **80**, 176—180.

— — , — — , KLEIN, M. and HARPAZ, I., 1977: Purification and characterization of a virus associated with the grapevine leafroll disease. Phytopathology **67**, 442—447.

TERAI, Y. and YANO, R., 1982: Ajinashika disease of the grapevine cultivar Koshu in Japan. Proc. 7th Meeting ICVG, Niagara Falls 1980, Agriculture Canada, 15—19.

VUITTENEZ, A. and STOCKY, G., 1982: Ultrastructure de vignes infectées par deux maladies de type viral: La mosaique des nervures, ou la "feuille rouge". Proc. 7th Meeting ICVG, Niagara Falls 1980, Agriculture Canada, 191—204.

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