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Mealybug transmission of grapevine virus A

by

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Übertragung des Rebenvirus A durch Schildläuse

Zusammenfassung. — Ein Closterovirus, das morphologisch und serologisch dem Rebenvirus A (GVA) sehr nahesteht oder mit diesem identisch ist, wurde durch Adulte und Larven der Schildlaus *Pseudococcus longispinus* TARGIONI TOZZETTI (Pseudococcidae) von an Blattrollkrankheit und Holzrunzeligkeit erkrankten Reben der Sorte Inzolia auf *Nicotiana clevelandii* GRAY übertragen. Durch Saftübertragung aus infizierten Reben konnte GVA nicht nachgewiesen werden; es wurde jedoch von *N. clevelandii* auf *N. bentamiana* DOMIN. mechanisch übertragen. Mittels ISEM konnte GVA nachgewiesen werden: 1. In den beiden oben genannten krautigen Wirtsarten; 2. in Reben der Sorte Inzolia, die als Spenderpflanzen für die Schildlaus-Übertragungsversuche verwendet worden waren; 3. in ursprünglich virusfreien Reben der Sorten Cabernet Franc und Procupac, die Blattrollsymptome gezeigt hatten, nachdem sie in einem Gewächshaus in unmittelbarer Nähe erkrankter Reben spontan von Schildläusen befallen worden waren; 4. in zerquetschten Schildläusen, die an infizierten Inzolia-Reben gesogen hatten. Die ISEM-Befunde wurden an Dünnschnitten bestätigt. In allen Fällen wurden im Phloemgewebe der infizierten krautigen Wirtspflanzen und Reben filamentöse Viruspartikeln gefunden. Zusätzlich enthielten die Spender- (Inzolia) und Empfängerreben (Cabernet Franc, Procupac und LN-33), nicht jedoch die krautigen Wirtspflanzen, isometrische virusartige Partikeln zusammen mit cytopathologischen Strukturen (vesiculäre Körper). Wegen des Vorliegens einer Mischinfektion in den Spenderreben und der offensichtlichen Übertragung beider Partikeltypen auf die Rebe ist es nicht möglich, Rückschlüsse auf die ätiologische Bedeutung von GVA im Verhältnis zur Blattrollkrankheit zu ziehen. Weitere Versuche unter Verwendung von Virusquellen, die nur mit GVA infiziert sind, wurden aufgenommen.

Introduction

In spring 1979, virus-free cuttings of LN-33 (Couderc 1613 × Thompson Seedless) and of *Vitis vinifera* L. cvs Procupac, Cabernet Franc, Mission, Alfonse Lavallée, Cardinal and Grillo, were imported to Sicily by the Institute of Plant Pathology of the University of Palermo from the University of California, Davis, through the courtesy of Dr. A. C. GOHEEN. These cuttings were rooted in plastic containers with sterile soil mix in a glasshouse. In the same house other pot-grown grapevine rootings from non selected mother plants of local varieties were present.

During summer a severe outbreak of the Pseudococcidae mealybug *Pseudococcus longispinus* TARGIONI TOZZETTI took place. All vines were heavily infested and remained so for several weeks until the infestation was eradicated. In the subsequent autumn, all rootings of virus-free red-berried varieties began to show downward rolling and reddish discolourations of the blades which were especially strong along the margins. Petioles were swollen and reddish. Localized swellings and crackings, reminiscent of those induced by corky bark, were present on the canes particularly in places where mealybug colonies had developed and fed.

In winter 1980, these rooted cuttings were transplanted outdoor and, by late summer of the same year, all were showing symptoms indistinguishable from those typical

of leafroll disease. The same symptomatology recurred in 1981, 1982 and 1983 (Fig. 3). Alterations of the canes, however, were no longer visible since 1980.

These observations were consistent with the notion that mealybugs could be vectors of grapevine leafroll as indicated, some 20 years ago, by the late Dr. H. F. DIAS and Dr. L. CHIARAPPA (HEWITT 1968). CHIARAPPA (personal communication), in trials with an unidentified species of *Pseudococcus* carried out in California in 1961, obtained the reproduction of the leafroll syndrome on virus-free Mission vines exposed to mealybugs which had previously fed onto grapevines with natural leafroll infection. Unfortunately these tests were not repeated and the results were not published. Specific transmission tests with *P. longispinus* were therefore carried out under controlled conditions and the preliminary results are reported in the present paper.

Materials and methods

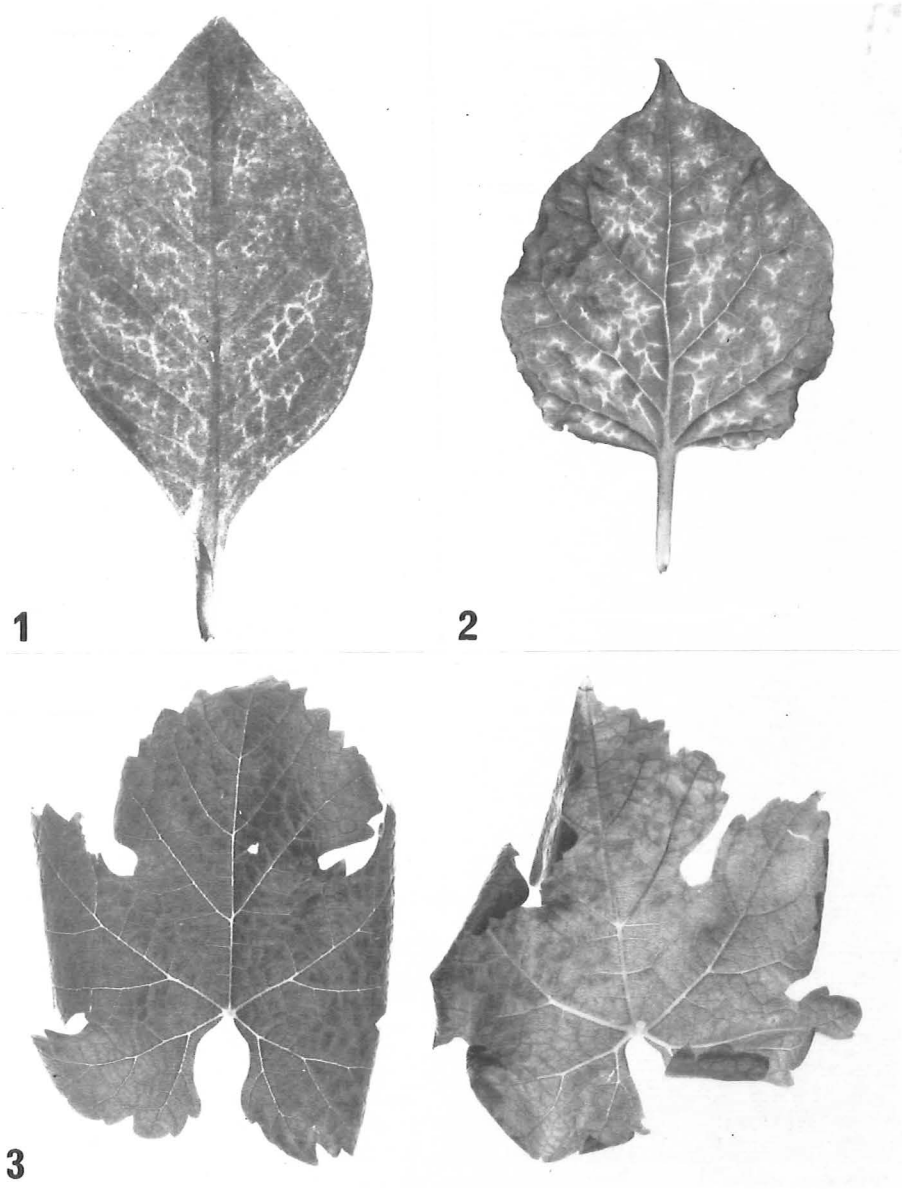
Transmission tests. — Non-viruliferous *P. longispinus* colonies were established in April 1982 by hatching eggs on sprouting virus-free potatoes grown in a growth chamber (18—20 °C and 12 h artificial illumination). Mealybug crawlers (about 20/plant) were transferred onto rooted cuttings of *V. vinifera* cv. Inzolia originating from vines known to be affected by leafroll and stem pitting (legno riccio) as ascertained by symptoms and indexing.

After an acquisition access time of 3 weeks, mealybugs in groups of no less than 15, were transferred onto 4 virus-free LN-33 rootings and 2 seedlings each of the following herbaceous hosts: *Phaseolus vulgaris* L., *Cucurbita pepo* L., *Cucumis sativus* L., *Pisum sativum* L., *Chenopodium quinoa* WILLD., *Chenopodium amaranticolor* COSTE et REYN, *Nicotiana tabacum* L. cvs White Burley and Samsun, *Nicotiana glutinosa* L., *Nicotiana rustica* L. and *Nicotiana clevelandii* GRAY. After 4 d of inoculation access time, all insects were killed. Disinfested plants were moved to an air-conditioned glasshouse at 22—24 °C and checked for appearance of symptoms.

Transmission tests were repeated in spring 1983. Mealybug instars were allowed to feed on the same Inzolia vines as above for 4 weeks, then transferred in groups of no less than 20 onto 7 seedlings each of healthy *N. glutinosa* and *N. clevelandii* and on 8 vegetating rooted cuttings of virus-free LN-33. In a parallel set of trials, mealybug adults were transferred in groups of 4 individuals onto 6 seedlings each of the above *Nicotiana* species. In both trials, inoculation access time was 4 d, after which all plants were disinfested and moved to a glasshouse.

Controls consisted of *N. glutinosa, N. clevelandii* seedlings and LN-33 rootings, grown under cage with a very fine mesh net in the same growth chamber as inoculated plants. Control plants were divided into two comparable groups one of which was artificially infested with non-viruliferous mealybug crawlers reared on virus-free potato sprouts.

Electron microscopy. — For immunosorbent electron microscopy (ISEM) and immunoelectron microscopy (IEM) for antibody coating (decoration) an antiserum to grapevine virus A (GVA) and the homologous antigen, kindly supplied by Dr. M. CONTI, were used. GVA is the same as grapevine stem pitting-associated virus described by CONTI *et al.* (1980) and recently re-named (MILNE *et al.* 1984). For ISEM the antiserum was diluted 1:500 in 0.06 M Soerensen's phosphate buffer at pH 6.5, whereas for antibody coating it was diluted 1:10 in the same buffer. ISEM was made according to ROBERTS and HARRISON (1979). Freshly prepared carbon-coated grids (400 mesh) were floated for 1 h at 37 °C on a drop of diluted GVA antiserum. After thorough washing in



Figs 1 and 2: Vein clearing in *N. clevelandii* and *N. benthamiana* leaves systemically infected with GVA.

Fig. 3: Incipient leafroll symptoms (late June 1983) in Cabernet Franc leaves from a vine that had contracted the disease following *P. longispinus* infestation in 1979.

Abb. 1 und 2: Adernaufhellung bei Blättern von *N. clevelandii* und *N. benthamiana* mit systemischer Infektion durch GVA.

Abb. 3: Beginnende Blattroll-Symptome (Ende Juni 1983) bei Blättern einer Cabernet-Franc-Rebe, die sich die Krankheit nach einem Befall durch *P. longispinus* im Jahre 1979 zugezogen hatte.

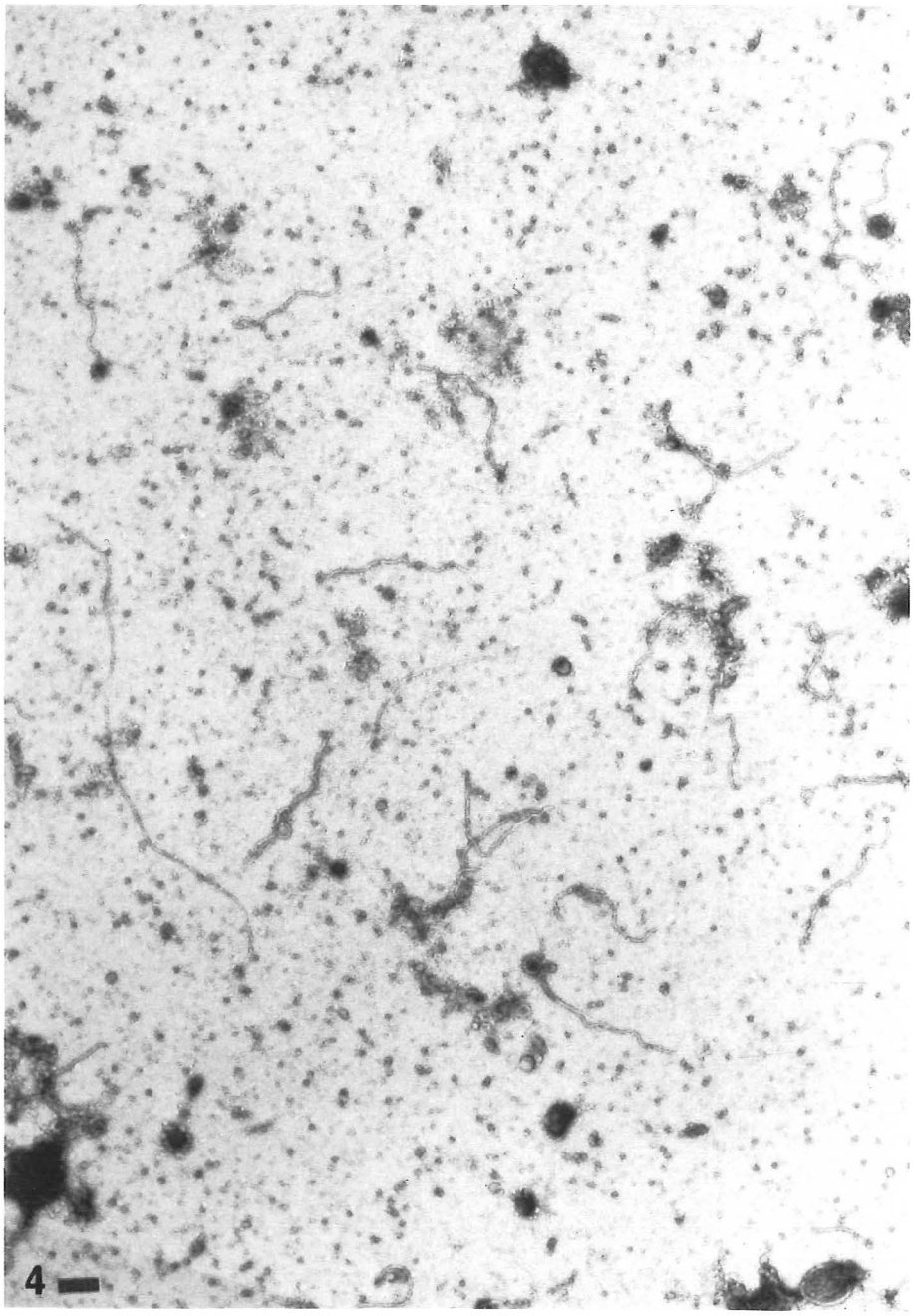
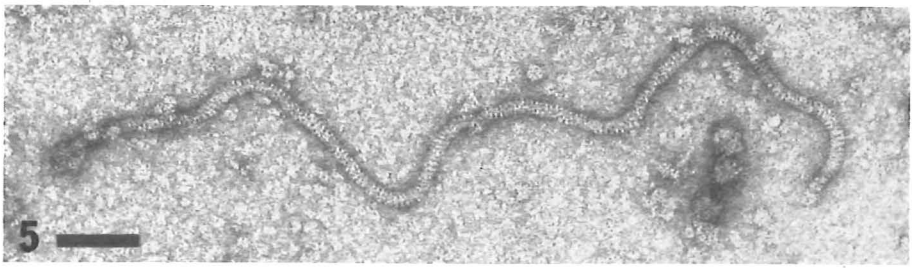
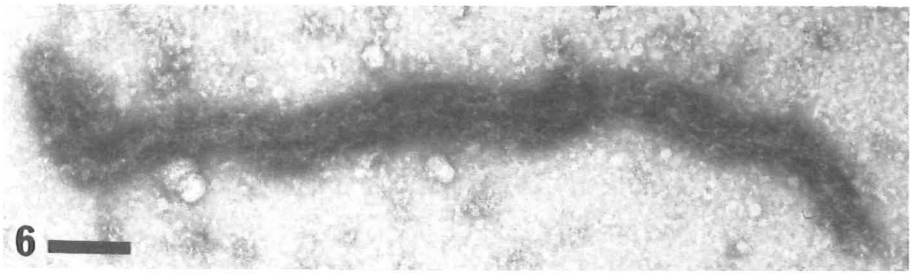


Fig. 4: ISEM preparation from a *N. clevelandii* leaf infected with GVA. Many flexuous virus particles are visible. Bar = 200 nm.

ISEM-Präparat eines Blattes von *N. clevelandii* bei Infektion mit GVA. Es sind zahlreiche gewundene Viruspartikeln zu sehen. Maßstab = 200 nm.





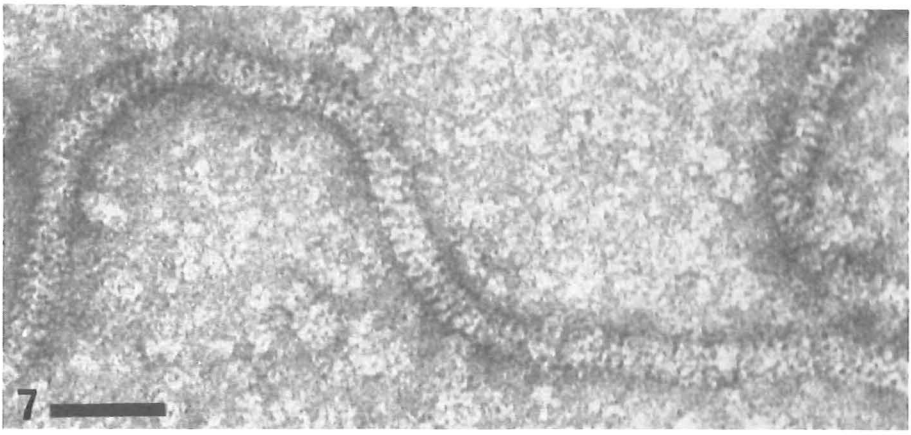


Fig. 5: A virus particle from a *N. clevelandii* leaf tip stained with uranyl acetate.

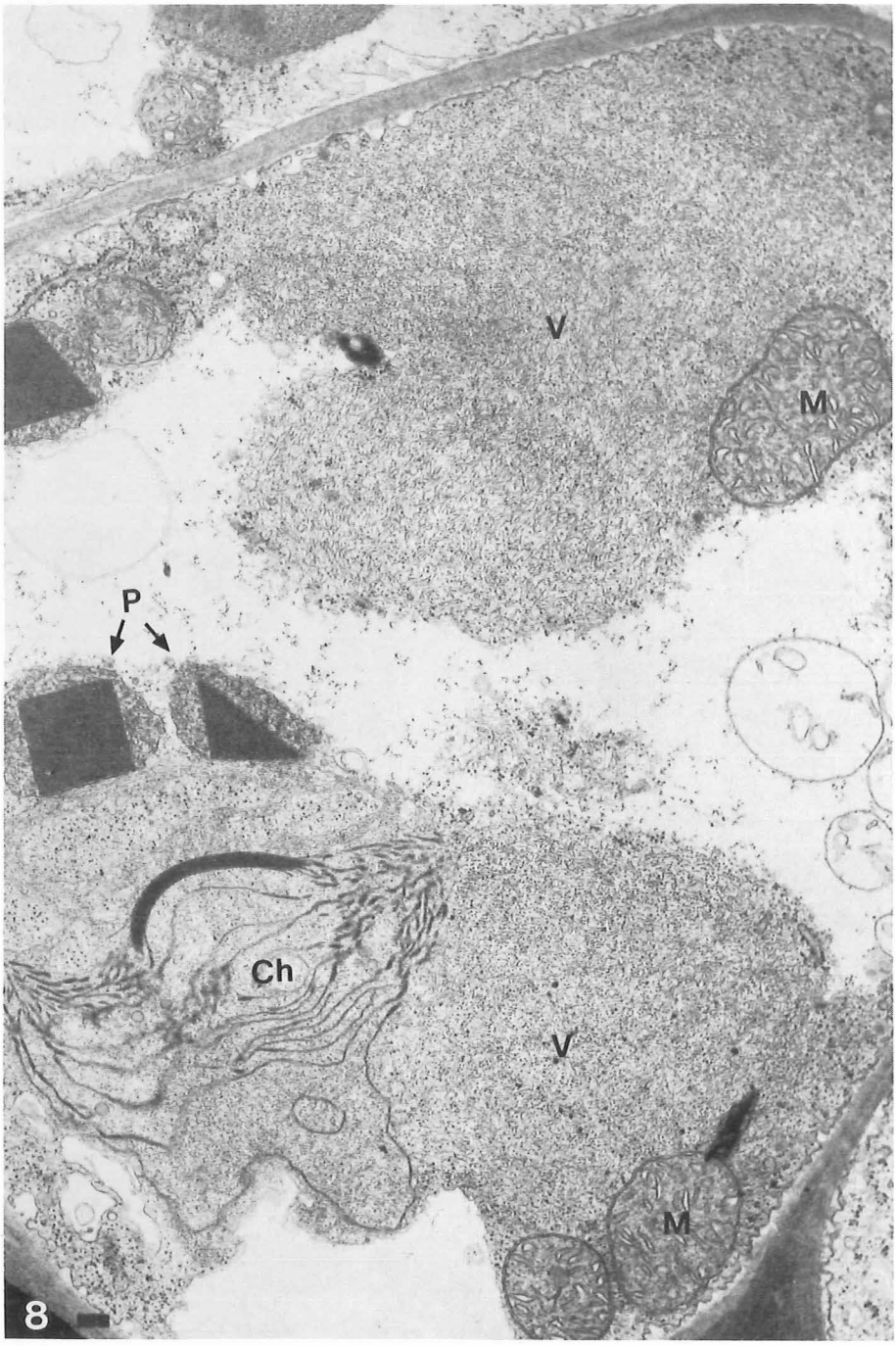
Fig. 6: Virus particle as above decorated with GVA antiserum.

Fig. 7: Substructural details of a GVA particle. Bars = 100 nm.

Abb. 5: Viruspartikel aus einer Blattspitze von *N. clevelandii* nach Färbung mit Uranylacetat.

Abb. 6: Viruspartikel wie oben mit angelagertem GVA-Antiserum.

Abb. 7: Feinstruktur eines GVA-Partikel. Maßstäbe = 100 nm.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buffer, the grids were transferred film-down onto drops of plant or mealybug extracts placed on a piece of dental wax in a moist chamber. The chamber was then transferred to a cold room (4 °C) and incubated for no less than 24 h.

Extracts were from: (i) mid-vein and petioles of Inzolia leaves from vines used as acquisition hosts for *P. longispinus;* (ii) leaves of *N. clevelandii* plants that had been exposed to viruliferous mealybugs and were showing symptoms; (iii) petioles and mid-vein of Cabernet Franc and Procupac vines that became infected after the natural infestation of 1979 and of LN-33 exposed to viruliferous mealybugs in spring 1983; (iv) groups of 3—4 mealybug adults from infected Inzolia vines, crushed with a glass rod in a drop of Soerensen's phosphate buffer and (v) symptomless *N. clevelandii* leaves from plants exposed to non-viruliferous mealybugs.

Extracts from leaf material were made by grinding tissues in a mortar in presence of carborundum powder and 0.5 ml of 5 % aqueous nicotine. The slurry was diluted with a few drops of Soerensen's phosphate buffer and centrifuged in conical glass tubes for 10 min at about 1,500 *g*. A drop of the supernatant was used for floating antiserum-sensitized grids.

For antibody coating of virus particles (MILNE and LUISONI 1977), the grids, before staining with 2 % aqueous uranyl acetate, were exposed to GVA antiserum. For thin sectioning, small pieces of the main vein with strips of adjacent parenchyma tissues were excised from leaves of: (i) *N. clevelandii* plants that had been exposed to viruliferous and non-viruliferous (control) mealybugs; (ii) *V. vinifera* cv. Inzolia, i.e. donor vines on which *P. longispinus* had fed; (iii) LN-33 exposed to presumably viruliferous mealybugs in spring 1983; (iv) Procupac and Cabernet Franc vines which had shown leafroll symptoms following mealybug infestation of 1979. Fixation in 4 % glutaraldehyde in 0.05 M neutral cacodylate buffer (2 h at room temperature) was followed by post-fixation in the cold (4 °C) for 2 h in 2 % osmium tetroxide, staining overnight in 0.5 % aqueous uranyl acetate, dehydration in graded ethanol dilutions and embedding in Spurr's resin. Thin sections were double stained with uranyl acetate and lead citrate before examination with a Philips 201C electron microscope.

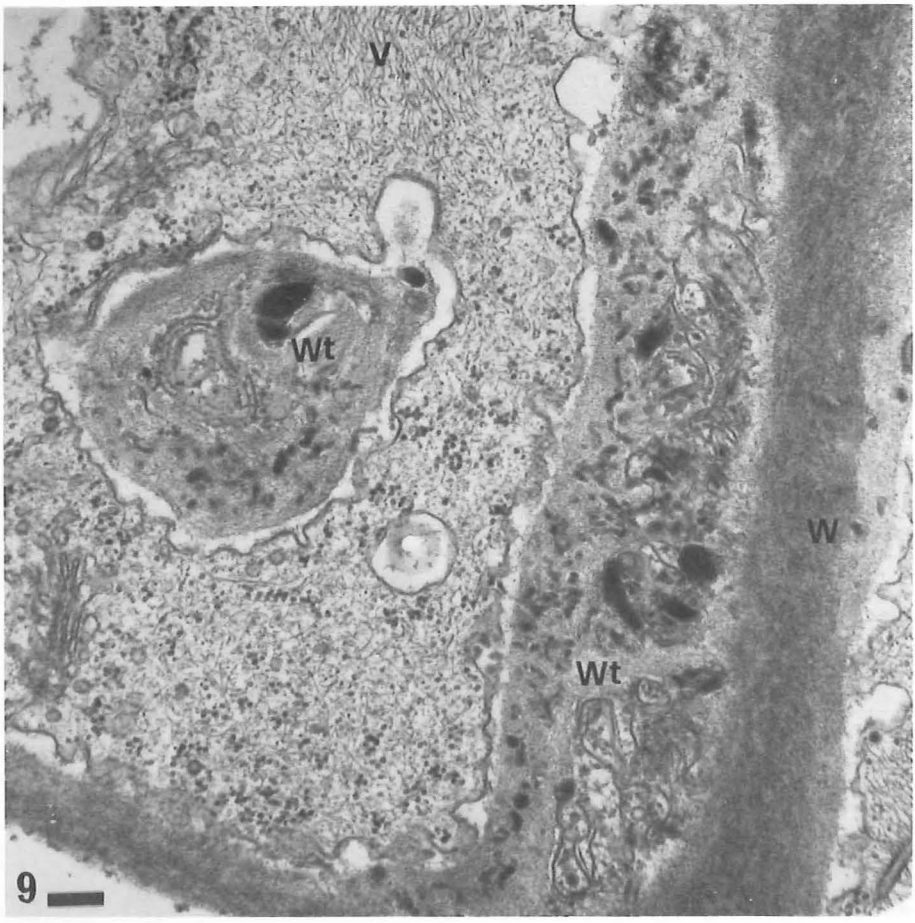
Results

Transmission tests

1. Mealybugs. — In 1982 tests, both *N. clevelandii* plants that had been exposed to *P. longispinus* crawlers previously reared onto infected Inzolia vines showed, 3 weeks after the end of the mealybug feeding period, a distinct clearing of the veins (Fig. 1). Newly formed leaves were smaller than usual, mottled and slightly puckered and the plants showed rosetting and bushy growth. All other herbaceous hosts used in the trial remained symptomless. In autumn, rolling and reddish discolourations appeared on the leaves of the 4 rooted cuttings of LN-33.

Fig. 8: *N. clevelandii* parenchyma cell infected with GVA. Massive aggregates of virus particles (V) are present, chloroplasts (Ch) are deeply disorganized whereas mitochondria (M) and peroxysomes (P) appear normal. Bar = 200 nm.

Mit GVA infizierte Parenchymzelle von *N. clevelandii*. Es sind dichte Ansammlungen von Viruspartikeln (V) vorhanden; die Struktur der Chloroplasten (Ch) ist tiefgreifend gestört, während die Mitochondrien (M) und Peroxysomen (P) normal erscheinen. Maßstab = 200 nm.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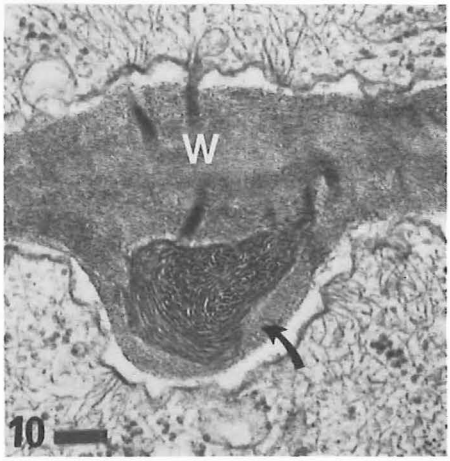
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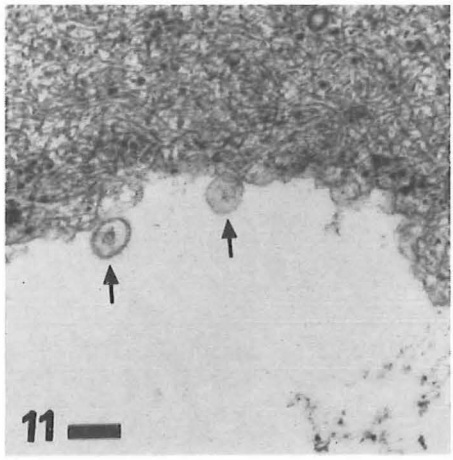
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N. clevelandii plants became infected in both sets of tests performed in 1983. In these tests, when mealybug crawlers were used, all plants (7 out of 7) showed symptoms as above, about 3 weeks after exposure to the insects. With adults, positive results were also obtained, but the transmission rate was lower, for only 3 plants out of 6 became infected. Thus a total of 10 *N. clevelandii* seedlings out of 13 exposed to viruliferous mealybugs exhibited a symptomatology that was remarkably similar to that induced in the same host by GVA (CONTI *et al.* 1980).

No symptoms were seen in control plants, including those on which non-viruliferous *P. longispinus* crawlers reared on virus-free potato sprouts had fed.

2. Sap inoculation. — All attempts to transmit a virus by inoculation of Inzolia (donor vines) or Cabernet Franc, LN-33, and Procupac (receptor vines) leaf sap expressed in presence of 2.5 % aqueous nicotine or 2 % polyvinylpyrrolidone in 0.1 M phosphate buffer at pH 7 failed.

Instead, a virus was successfully transmitted by manual inoculation from *N. clevelandii* to healthy *N. clevelandii* and *Nicotiana benthamiana* DOMIN. plants. Within 10—12 d from inoculation, *N. benthamiana* reacted with a pronounced vein clearing (Fig. 2), distortion and puckering of the leaves and generalized stunting.

Electron microscopy

1. ISEM and IEM decoration tests. — All preparations from *N. clevelandii* and *N. benthamiana* with symptoms contained a very high number of filamentous particles (Fig. 4) with the typical closterovirus structure (Figs 5 and 7). These particles were very flexuous, had a diameter of ca. 12 nm, a pitch of 3.8 nm (Fig. 7) and a length of ca. 800 nm (range 750—825 nm). All particles were very heavily decorated by the antiserum to GVA (Fig. 6).

Similar particles, equally well decorated by GVA antiserum were found in ISEM preparations from: (i) Inzolia donor vines (4 out of 9 different vines); (ii) crushed mealybugs taken from Inzolia donor vines (1 out of 3 samples); (iii) Procupac and Cabernet Franc vines that had become infected following natural mealybug infestation of 1979 (4 out of 6 different vines). No virus particles were seen in preparations from LN-33 exposed to viruliferous mealybugs in spring 1983.

In IEM tests, GVA antiserum decorated to the maximum reactive dilution (1:256) both the homologous virus (CONTI *et al.* 1980) and the virus transmitted by mealybugs from Inzolia vines.

Fig. 9: Extensive cell wall thickenings (Wt) in a GVA-infected *N. clevelandii* cell. The ground cytoplasm contains many scattered or loosely aggregated virus particles (V).

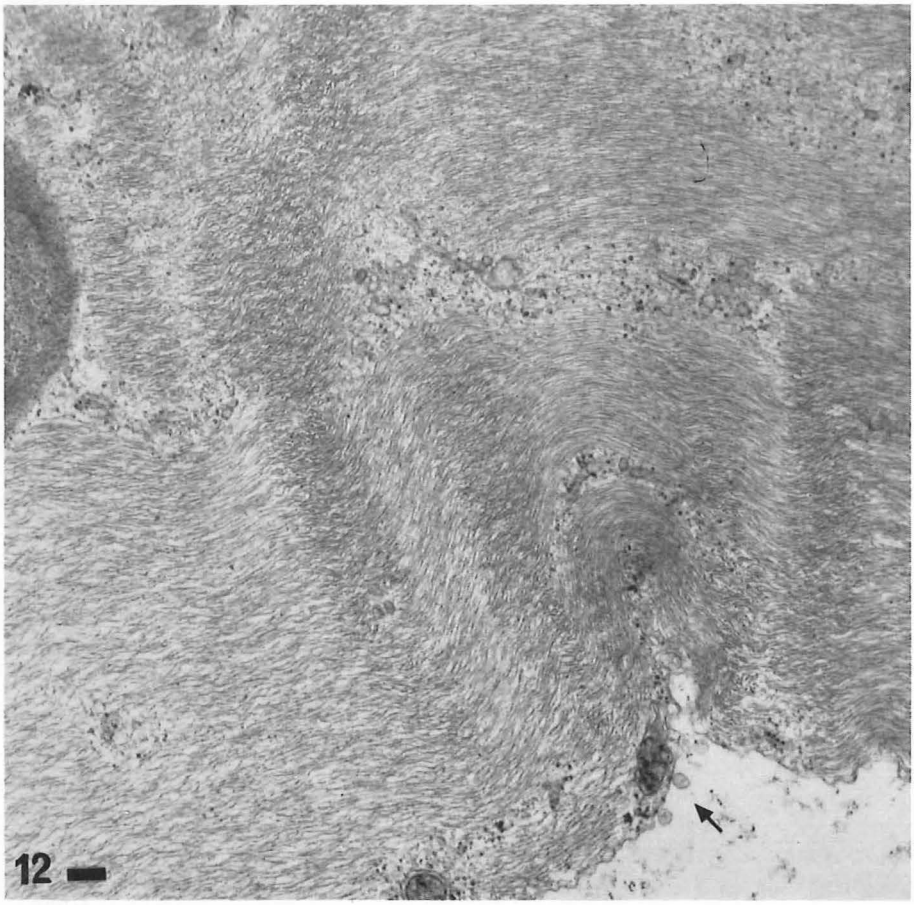
Fig. 10: A localized wall thickening engulfing an aggregate of virus particles (arrow). W = cell wall.

Fig. 11: Membranous vesicles containing fibrillar material on the tonoplast of an infected cell. Many virions are scattered in the cytoplasm. Bars = 200 nm.

Abb. 9: Ausgedehnte Wandverdickungen in einer GVA-infizierten Zelle von *N. clevelandii*. Das Cytoplasma enthält zahlreiche zerstreute oder locker zusammengeschlossene Viruspartikeln (V).

Abb. 10: Eine einzelne Wandverdickung, in ihrem Inneren eine Ansammlung von Viruspartikeln (Pfeil). W = Zellwand.

Abb. 11: Membranöse Bläschen, die fibrilläres Material enthalten, am Tonoplasten einer infizierten Zelle. Im Cytoplasma sind zahlreiche Virionen zerstreut. Maßstäbe = 200 nm.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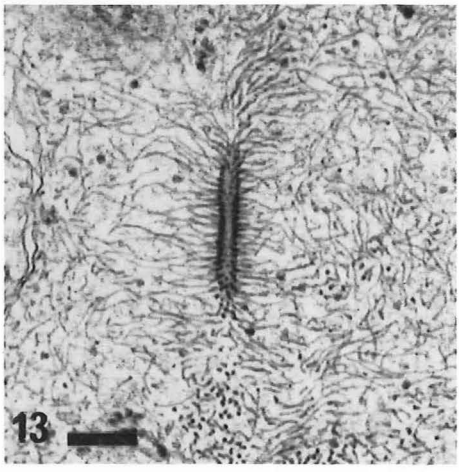
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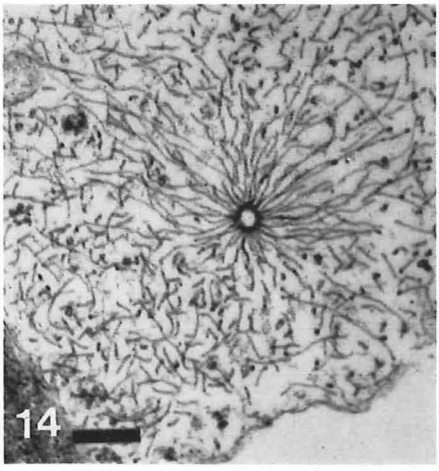
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2. Thin sectioning of *Nicotiana clevelandii*. — Regardless of the type of tissues examined (parenchymatous or conducting) all samples from plants with symptoms showed extensive ultrastructural modifications. No cytopathological changes were detected in control plants.

Infected cells had a deranged cytology. Although most of the major organelles such as nuclei, mitochondria and peroxysomes were apparently unaffected, chloroplasts were misshapen, had altered lamellar system or were totally disrupted (Fig. 8). Wall thickenings originating from deposits of callose-like substances were extensive in some cells (Fig. 9). The ground cytoplasm was loaded with intensely electron opaque dots about 10 nm across and flexuous filaments of variable length that were interpreted as profiles of virus particles in cross and longitudinal or tangential section, respectively. Virions often accumulated in huge masses that filled most of the cell lumen (Fig. 8). The particles occurred in bundles, whorls and banded bodies (Fig. 12) or were randomly spread throughout the cytoplasm. Occasionally, particles were within plasmodesmata or wall thickenings (Fig. 10), or radiated from tubular structures of unknown nature, onto which they were attached by one end (Figs 13 and 14).

Aggregates of virus particles were not intermingled with membranous vesicles containing viral RNA as with certain closteroviruses (ESAU 1968). However, groups of similar vesicles were seen protruding from the tonoplast of many infected cells (Figs 11 and 12).

3. Thin sectioning of grapevine. — The ultrastructure of leaf tissues of naturally infected Inzolia plants which had been used as donors for mealybug transmission trials, was comparable to that exhibited by other leafroll-affected vines (CASTELLANO *et al.* 1983).

Aggregates of filamentous particles were present in phloem parenchyma and companion cells (Figs 15 and 16) and in differentiating phloem elements. The outward appearance and aggregation forms of the particles was the same as observed in *N. clevelandii*.

Vesiculated bodies (Fig. 17) like those described by CASTELLANO *et al.* (1983) were also seen, together with profiles of rounded virus-like particles thus indicating that, as it often occurs with leafroll-affected plants (CASTELLANO *et al.* 1983), also the Inzolia vines used in the present work were doubly infected with a filamentous and an isometric virus.

The results of ultrastructural observations of leaf tissue samples of Cabernet Franc and Procupac which had contracted leafroll disease following *P. longispinus* infestation, were similar. Phloem tissues of all specimens contained vesiculated bodies and isometric virus-like particles as well as filamentous particles scattered at random in the ground cytoplasm or in small aggregates (Figs 18 and 19).

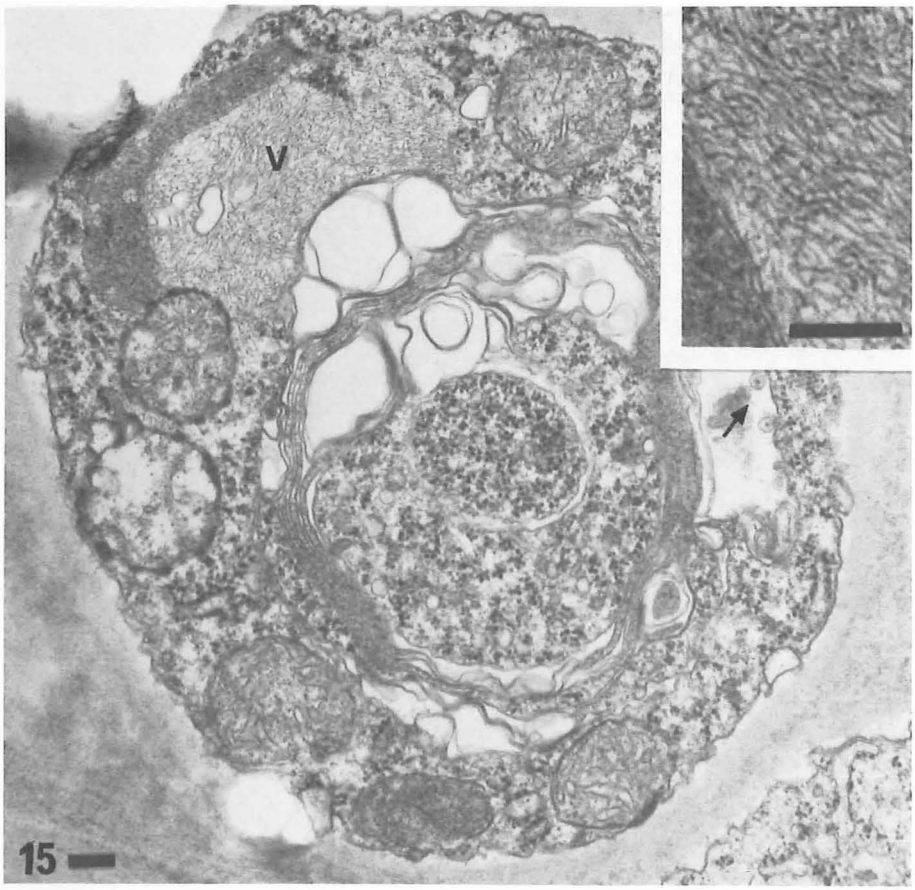
No such structures were seen in LN-33 leaf samples from vines exposed to mealybugs in spring 1983.

Fig. 12: A huge aggregate of GVA in a *N. clevelandii* parenchyma cell. Arrow points to membranous vesicles on the tonoplast.

Figs 13 and 14: Tubular structures with virus particles attached by one end viewed in longitudinal (Fig. 13) and cross (Fig. 14) section. Bars = 200 nm.

Abb. 12: Eine ausgedehnte Ansammlung von GVA in einer Parenchymzelle von *N. clevelandii*. Die Pfeile weisen auf membranöse Bläschen am Tonoplasten hin.

Abb. 13 und 14: Längs- und Querschnitt durch tubuläre Strukturen; an einem Ende befinden sich Viruspartikeln. Maßstäbe = 200 nm.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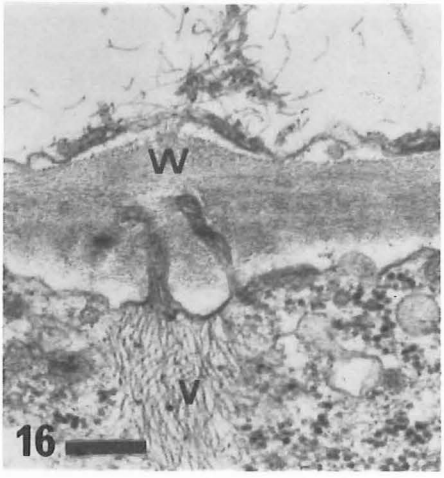
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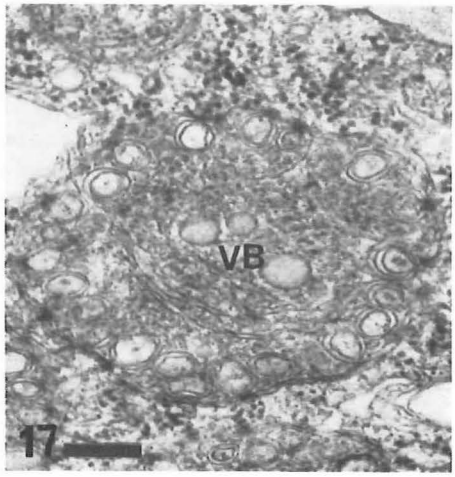
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Discussion

The results of the present study provide experimental evidence that a closterovirus morphologically, serologically and ultrastructurally very close or identical to GVA is: (i) acquired from grapevines by instars and adults of *P. longispinus;* (ii) transmitted by the same mealybug species to *N. clevelandii* and to grapevine; (iii) associated with leafroll symptoms in grapevine.

Although details of the transmission mechanism (i.e. acquisition and inoculation access time and retention time) have not yet been worked out, there is little doubt that adults and instars of *P. longispinus* were able to transmit GVA from naturally infected vines to both herbaceous plants and virus-free grapes. In fact, the same virus, whose identity was always ascertained serologically as GVA, was found in donor vines, crushed *P. longispinus* individuals, herbaceous hosts and grapevines that had been exposed to mealybugs. Moreover, the results of all transmission tests from grapevines to *N. clevelandii* carried out so far, though preliminary, were consistently positive, thus indicating that the transmission obtained was not occasional.

This finding is of no little consequence, for, up to now, *P. longispinus* has been reported as vector of tropical bacilliform viruses such as the agent of cocoa swollen shoot (POSNETTE 1950) and another morphologically similar virus involved in a complex disease of *Colocasia esculenta* (L.) SHOTT (GOLLIFER *et al.* 1977). Reports of mealybugs transmitting other types of viruses are not fully substantiated (ROIVAINEN 1980) and no record exists of a closterovirus being vectored by a mealybug. Closteroviruses are transmitted in a semipersistent manner by aphids (BAR-JOSEPH *et al.* 1979) with a few exceptions. GVA may be one such exception for it was not transmitted experimentally by *Myzus persicae* SULZ. and *Macrosiphon euphorbiae* THOM. (CONTI *et al.* 1980).

The transmission operated by *P. longispinus* may not be selective. Naturally infected donor vines, in addition to GVA, contained another virus with isometric particles whose inclusion bodies are readily identified in infected cells (CASTELLANO *et al.* 1983). This virus and related inclusions were not observed in thin-sectioned tissues of *N. clevelandii* but were present in vines on which viruliferous mealybugs had fed. This may indicate that *P. longispinus* is capable of transmitting the isometric virus from grapevine to grapevine but not from grapevine to *N. clevelandii* perhaps because the virus does not infect and multiply in this host. Evidently, this is just a working hypothesis requiring experimental proof through specific trials now under way. The occur-

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Fig. 15: Companion cell of grapevine cv. Inzolia used as donor for mealybug transmission tests. An aggregate of filamentous particles (close-up view in inset) is present in the cytoplasm. Arrow points to tonoplast-associated vesicles.

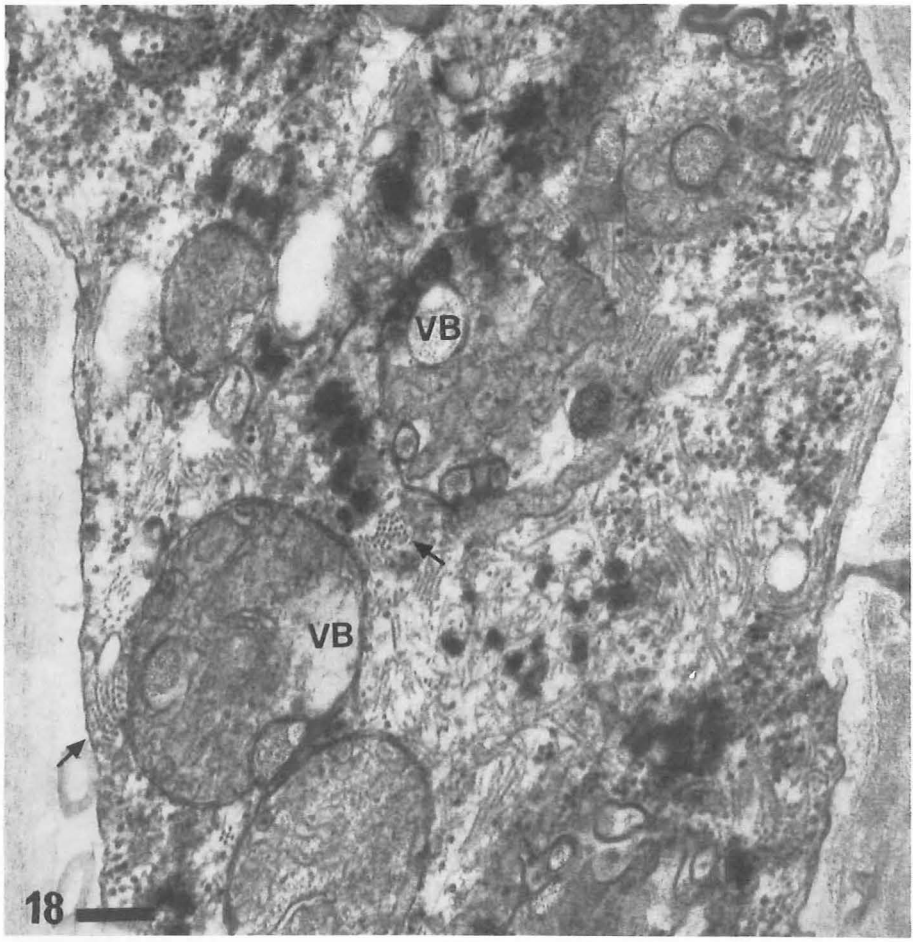
Fig. 16: Virus particles (V) next to and inside plamodesmata of an Inzolia grape cell. W = cell wall.

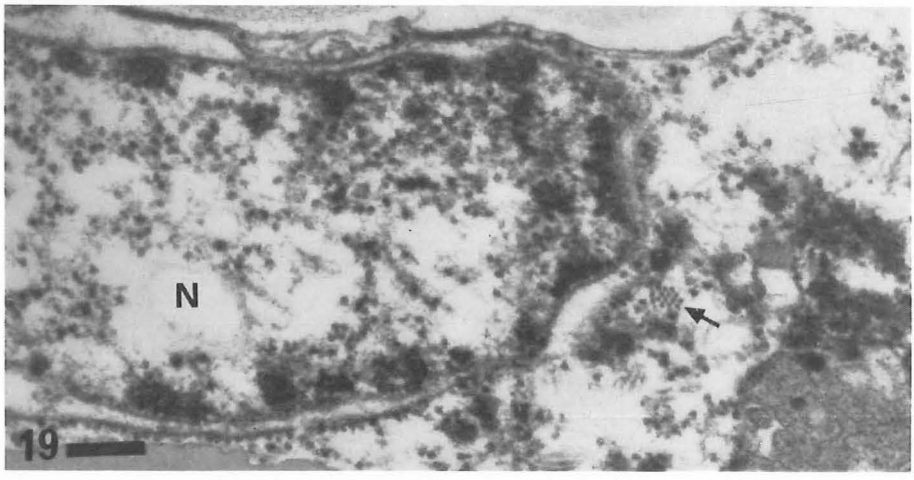
Fig. 17: A vesiculated body (VB) likely originating from a modified chloroplast in an Inzolia grape cell. Bars = 200 nm.

Abb. 15: Siebröhrengeleitzelle einer Rebe der Sorte Inzolia, die als Virusspender für Schildlaus-Übertragungsversuche verwendet wurde. Im Cytoplasma findet sich eine Ansammlung filamentöser Partikeln (im Einsatz stärker vergrößert). Der Pfeil zeigt auf die dem Tonoplasten angelagerten Vesikeln.

Abb. 16: Viruspartikeln (V) unmittelbar neben sowie innerhalb von Plasmodesmata. W = Zellwand.

Abb. 17: Vesiculärer Körper (VB) in einer Zelle der Rebsorte Inzola, der wahrscheinlich aus einem modifizierten Chloroplasten hervorging. Maßstäbe = 200 nm.


VB
VB
18


N
19

rence of mixed infections (GVA and the isometric virus) in vines that showed leafroll symptoms following exposure to mealybugs does not allow to draw definitive conclusions on the identity of the possible etiological agent of this disease. The present situation is complicated by the widespread occurrence of a second closterovirus (grapevine virus B, GVB) serologically distinct from GVA but often associated with it, in vines affected by leafroll alone or by leafroll and stem pitting (MILNE *et al.* 1984).

A further source of confusion is the report by VON DER BRELIE and NIENHAUS (1982) that leafroll-infected and healthy vines both contain filamentous particles "similar to closteroviruses". However, these particles are much longer than those of GVA and GVB (ca. 2000 nm viz. ca. 800 nm) and, in the published micrographs, they do not seem to show the flexuosity and substructure typical of closterovirus virions.

The role of mealybugs in the natural spread of leafroll is another point requiring elucidation.

Except for an occasional record (DIMITRIJEVIC 1973) leafroll is not reported as actively spreading in the field (BOVEY 1968, GOHEEN 1970). The most recent estimates of the rate of its natural spread in old Californian vineyards is of ca. 18 cm/year (GOHEEN 1982), whereas no apparent re-contamination has been detected in new Californian vineyards established with heat-treated stocks (GOHEEN, personal communication). On the other hand, the experimental transmission of leafroll by dodder from grapevine to grapevine, recently reported by WOODHAM and KRAKE (1983), does not have epidemiological significance.

Hence, leafroll is not at all a fast moving disease. Whether this is so because the vectors are not too efficient or because their natural attacks to grapevine are of negligeable importance, remains to be ascertained. The data available may be in favour of the second hypothesis. *P. longispinus* is reported to be an occasional parasite of grapevine (MCKENZIE 1967). Other Pseudococcidae such as *Planococcus ficus* SYN., *Pl. citri* RISSO and *Pseudococcus maritimus* EHRHORN are much more common grapevine pests and their outbreaks are becoming a serious problem in many viticultural areas of the world (TRANFAGLIA and VIGGIANI 1978). The vectoring ability of some of these mealybug species is now being investigated.

Fig. 18: Phloem parenchyma cell of a Procupac vine which had shown leafroll symptoms following mealybug infestation of 1979. Filamentous particles in longitudinal and cross (arrows) section are scattered throughout the cytoplasm. Vesiculated bodies (VB) and profiles of isometric virus-like particles are also present.

Fig. 19: Intranuclear occurrence of isometric virus-like particles in a Procupac phloem parenchyma cell. Arrow points to a bundle of cross-sectioned filamentous particles. N = nucleus. Bars = 200 nm.

Abb. 18: Phloemparenchymzelle einer Procupac-Rebe, die nach Schildlausbefall im Jahre 1979 Blattrollsymptome gezeigt hatte. Längs- und quergeschnittene (Pfeile) filamentöse Partikeln sind über das Cytoplasma zerstreut. Vesiculäre Körper (VB) und die Umrisse isometrischer virusartiger Partikeln sind gleichfalls zu sehen.

Abb. 19: Isometrische virusartige Partikeln im Zellkern einer Phloemparenchymzelle der Rebsorte Procupac. Der Pfeil zeigt auf ein Bündel quergeschnittener filamentöser Partikeln. N = Zellkern. Maßstäbe = 200 nm.

Summary

A closterovirus morphologially and serologically very close or identical to grapevine virus A (GVA) was experimentally transmitted by adults and instars of the Pseudococcidae mealybug *Pseudococcus longispinus* TARGIONI TOZZETTI from grapevine of cv. Inzolia affected by leafroll and stem pitting to *Nicotiana clevelandii* GRAY. GVA could not be recovered from infected vines by inoculation of sap but it was mechanically transmitted from *N. clevelandii* to *N. benthamiana* DOMIN. With immunosorbent electron microscopy (ISEM) tests, the presence of GVA was ascertained in: (i) both the above herbaceous hosts; (ii) Inzolia vines used as donor plants for mealybug transmission tests; (iii) formerly virus-free Cabernet Franc and Procupac vines that had shown leafroll symptoms following natural mealybug infestation in a greenhouse where they were grown next to diseased grapes; (iv) squashed mealybugs which had fed on infected Inzolia vines. ISEM findings were confirmed by thin sectioning. In all cases filamentous virus particles were found in phloem tissues of infected herbaceous hosts and vines. In addition, donor (Inzolia) and recipient (Cabernet Franc, Procupac and LN-33) grapevines, but not herbaceous hosts, contained isometric virus-like particles and associated cytopathic structures (vesiculated bodies). The occurrence of a mixed infection in donor vines and the apparent transmission of both types of particles to grapevine does not allow to draw conclusions on the etiological significance of GVA in relation to leafroll disease. Further trials with source plants infected with GVA alone are now under way.

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