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## Investigation of Ring Spot Virus Disease of *Smilax aspera* L. (*Liliaceae*)

By

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With 11 Figures

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### Summary

THALER I., PLEŠE N., GAILHOFER M. & RUSAK G. 2000. Investigations of ring spot virus disease of *Smilax aspera* L. (*Liliaceae*). – *Phyton* (Horn, Austria) 40 (2): 303–313, with 11 figures. – English with German summary.

From Mediterranean (region of Croatia) climbing monocotyledonous plant *Smilax aspera* with prominent ring spot symptoms, an elongated virus, named *Smilax* virus, was isolated on test plants. It induced cylindrical virus inclusions and tubular structures within the cytoplasm of infected host cells. The finding of filamentous virus particles of about 730 nm and cylindrical virus inclusions showed that the isolated *Smilax* virus belongs to the family *Potyviridae*, perhaps to genus *Potyvirus*. The virus is associated with ring spot syndrome *S. aspera* but not proven cause.

### Zusammenfassung

THALER I., PLEŠE N., GAILHOFER M. & RUSAK G. 2000. Untersuchungen der virösen Ringfleckkrankheit von *Smilax aspera* L. (*Liliaceae*). – *Phyton* (Horn, Austria) 40 (2): 303–313, 11 Abbildungen. – Englisch mit deutscher Zusammenfassung.

In der Küstenregion von Kroatien wurden an *Smilax aspera* (*Liliaceae*) ausgeprägte Ringfleckensymptome beobachtet. Nach Übertragung des Extraktes auf Testpflanzen wurde ein längliches Viruspartikel etwa 730 nm isoliert und als *Smilax* Virus bezeichnet. Es induziert zylindrische Viruseinschlüsse und tubuläre Struktu-

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ren im Cytoplasma der infizierten Wirtspflanzenzellen. Die Länge des Viruspartikels und die zylindrischen Viruseinschlüsse zeigen, dass das *Smilax* Virus zu der Familie *Potyviridae*, vielleicht zur Gattung *Potyvirus* gehört. Das Virus scheint die Ringfleckenkrankheit von *Smilax aspera* zu verursachen, ist aber nicht als deren Erreger bewiesen.

## Introduction

The Mediterranean monocotyledon *Smilax aspera* L. (*Liliaceae*) is an evergreen semi-woody climbing plant with broad heart-shaped leathery leaves. On several parts of the Adriatic coastal region of Croatia we have observed the specimens of *S. aspera* with very prominent virus-like ring spot symptoms on the leaves. To the best of our knowledge, no virus infection in this monocotyledonous species has been recorded up to now. Therefore, we have decided to investigate this susceptible virus disorder in more detail.

The results of our investigations are presented in this paper.

## Material and Methods

Virus infected *S. aspera* plants were collected from several localities of Dalmatia: Trsteni, Lokrum and Mlini (near Dubrovnik), Split, Primošten, Zadar and islands Hvar and Lošinj. Predominant symptom on the leaves was chlorotic ring spot accompanied with "oak leaf" pattern and chlorotic spots (Fig. 1). During the hot summer the symptoms became weaker on the newly developed leaves and then gradually disappeared.

A virus was isolated repeatedly from infected *S. aspera* plants, which originated from different localities. For initial virus isolation the local lesion test plants *Chenopodium amaranticolor* and *C. quinoa* were mechanically inoculated with the extracts of symptoms bearing leaf lamina of *S. aspera*. The isolation of the virus succeeded only when the extracts were considerably diluted with 0.06 M neutral phosphate buffer containing 0.3% sodium ascorbate. Following the initial isolation of the virus the *Smilax* virus isolate was obtained after four successive passages through single lesion on *C. amaranticolor*. After the initial isolation the virus inocula were prepared without ascorbate additive. For further investigations the isolated *Smilax* virus was propagated and maintained in locally infected *Tetragonia expansa* test plant. Searching for experimental host range of the virus back inoculations were made on *C. amaranticolor* and *C. quinoa*. We also try to infect *Smilax* seedlings by mechanical inoculation of the virus.

Determination of properties of the virus in plant sap was done by common procedure using low speed centrifugated (2000g/20 min) crude sap from locally infected leaves of *T. expansa* and *C. quinoa* as test plant. Aphid transmission experiments were conducted with apterous adults of *Myzus persicae* in a non-persistent way. The aphids were starved for two hours, given an acquisition feeding period of two to three minutes on infected *T. expansa* leaves and then transferred to healthy *C. quinoa* and *T. expansa* using six specimens of each test plant and 15–20 aphids per plant.

Light microscopical analysis of infected tissue was done on leaf epidermal strips of *T. expansa* and on leaf hair cells of *Nicotiana clevelandii* from the local lesion area.

For ultrastructural observations samples of leaves of virus infected *S. aspera* and *T. expansa* and *C. amaranticolor*, together with comparable samples which lack symptoms, were fixed in 3 % glutaraldehyde with 0.1 M phosphate buffer at pH 7.2. After rinsing they were postfixed in 1 % osmium tetroxide, dehydrated in ethyl alcohol and embedded in Agar 100 resin. Ultrathin sections were stained with uranyl acetate and lead citrate. Micrographs were taken on a Philips electron microscope. For detection of virus particles and tubular structures in leaf extracts of *S. aspera* and *T. expansa* (Fig. 4, 5) negative staining in 2% uranyl acetate was performed. Cytochemical reaction with pepsin was done after THALER & GAILHOFER 1988.

## Results

After obtaining of *Smilax* virus through single local lesion (see Material and Methods) the virus was subjected to some other basic investigations described below.

### Host range

The virus isolate was mechanically transmitted to some other common herbaceous test plants. Besides on *C. amaranticolor* (Fig. 2) the *Smilax* virus provoked very distinct necrotic local lesions also on *T. expansa* (Fig. 3). *C. murale*, *C. quinoa*, and *N. clevelandii* reacted also with necrotic local lesions, whereas in *Cucumis sativus*, *N. tabacum* 'Samsun' and *Petunia hybrida* local infection was latent. Consequently, all the infected test plants have been infected only locally. No infection was established on *Celosia cristata*, *Datura stramonium*, *Gomphrena globosa*, *N. glutinosa*, *N. megalosiphon*, *Ocimum basilicum*, *Phaseolus vulgaris* 'Top Crop', *Pisum sativum*, *Vicia faba*, *Vigna sinensis*, and some other common test species. We have also not succeeded to transmit the isolated *Smilax* virus by back inoculation to *S. aspera* seedlings.

### Properties in vitro and aphid transmission

In crude leaf sap of *T. expansa* the *Smilax* virus retained infectivity up to 11 days at room temperature and its thermal inactivation point lied between 53° and 55° C. In our experiments the isolate was not transmissible by *M. persicae* in a non-persistent manner.

### Light and electron microscopy

Through light microscopic analysis of infected leaf tissue no amorphous virus inclusion bodies have been observed in living epidermal and hair cells from the local lesion area of infected *T. expansa* and *N. clevelandii*, respectively.

Ultrathin sections of living leaf mesophyll cells from the symptoms bearing areas of *S. aspera* and *T. expansa* contained in the cytoplasm conspicuous elements of cylindrical virus inclusions, recognised as one of the main characteristics of viruses of the family *Potyviridae* (e.g. EDWARDSON

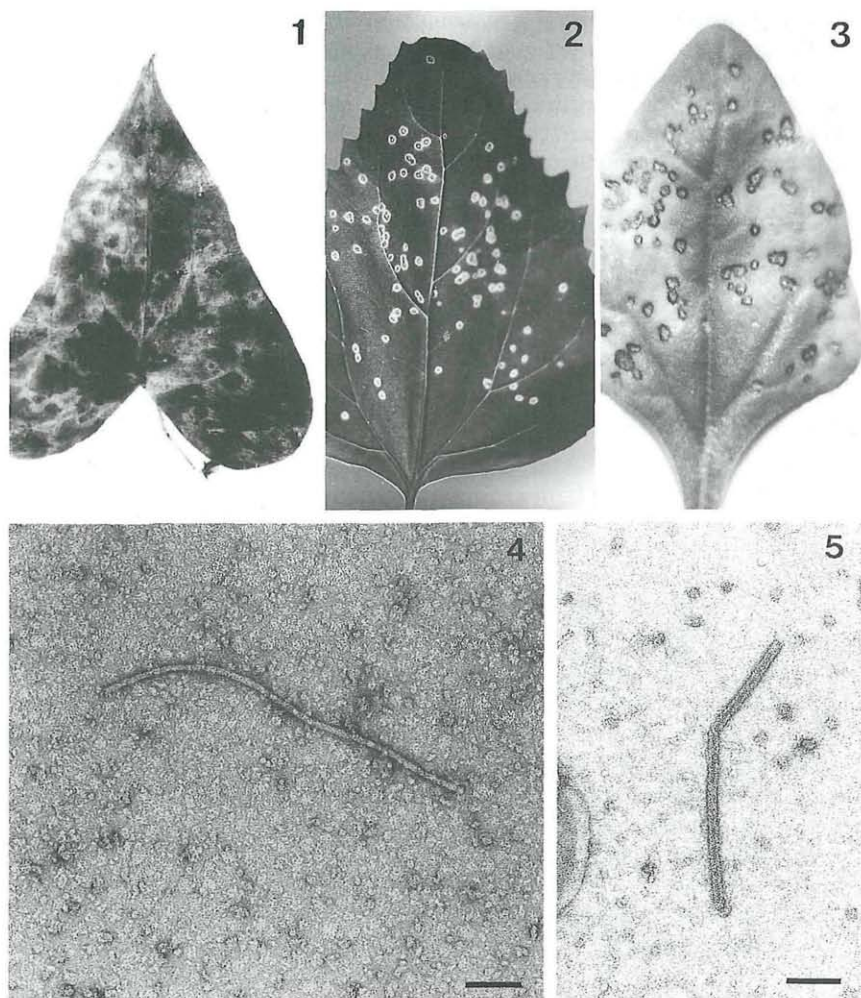


Fig. 1. *Smilax aspera* leaf with ring spot and "oak-leaf" pattern symptoms.  
 Figs. 2-3. Leaves with necrotic local lesions provoked by isolated *Smilax* virus. –  
 Fig. 2. *Chenopodium amaranticolor*. – Fig. 3. *Tetragonia expansa*.  
 Figs. 4-5. Flexuous virus particles of *Smilax* virus (Fig. 4) and tubular element (Fig. 5)  
 in crude leaf extracts of infected *Tetragonia expansa* after negative-staining. Bars =  
 100 nm

1974, 1992, LESEMAN 1988, SHUKLA & al. 1994, BARNETT & al. 1995, EDWARDSON & CHRISTIE 1996). Cylindrical inclusions showed pinwheel structures with radiating plates, and laminated inclusions out of which laminated aggregates were especially remarkable (Fig. 6, 7, 8). However, only early stages of infections contained fully developed cylindrical inclu-



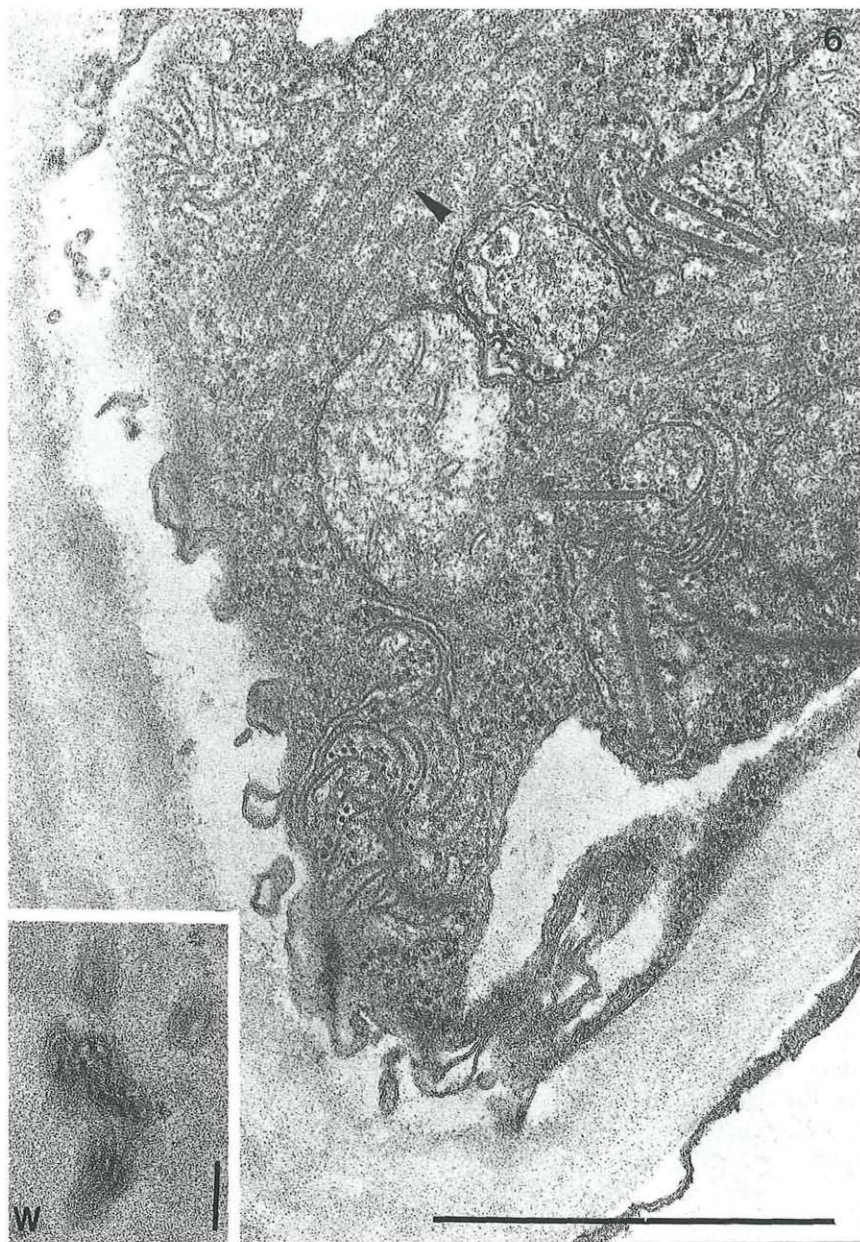


Fig. 6. Cylindrical inclusions – pinwheels and laminated aggregates, tubular elements (arrowhead) in the cytoplasm of *Tetragonia expansa* leaf cell infected with isolated *Smilax* virus. Bar = 1 µm. – Inset shows virus-like particles in plasmodesmata of cell wall (W). Bar = 100 nm.

sions, i.e. pinwheels and bundles (Fig. 6). At the later stages of infection large masses of disintegrated pinwheels, which occurred as laminated products (Fig. 7, 8), prevailed. The inclusions were never seen in the leaf parts without virus symptoms. Virus specific vesiculation originating from cytoplasmic endomembrane system often followed the inclusions.

Besides cylindrical inclusions, in virus infected cells of both sub-microscopically investigated plant species, conspicuous tubular structures could be frequently found in the cytoplasm (Fig. 9, 10). These tubules were predominantly brought together in small loose bundles or they appeared as rather large accumulations. Especially in *T. expansa* greater parts of cytoplasm were frequently filled with tubules (Fig. 10). Tubular structures, which could be easily isolated through leaf extracts (Fig. 5), measured about 23–26 nm in diameter and had a different length with a maximum at about 100 nm. Digestion of tubules with pepsin indicated their proteinaceous nature.

In addition to virus inclusions, filamentous virus particles were also observed in thin sections of infected leaf cells. In *S. aspera* they occurred aggregated in more or less dense bundles (Fig. 11) which could make larger accumulations or they were scattered through cytoplasm (Fig. 9). In *T. expansa* only scattered particles were detected. Sometimes virus particles have been also found in plasmodesmata of cell wall (Fig. 6, inset). About 100 elongated virus particles from leaf extracts of mother plant *S. aspera* were measured. Most particles were about 730 nm long. Their width was about 13 nm (Fig. 4).

## Discussion

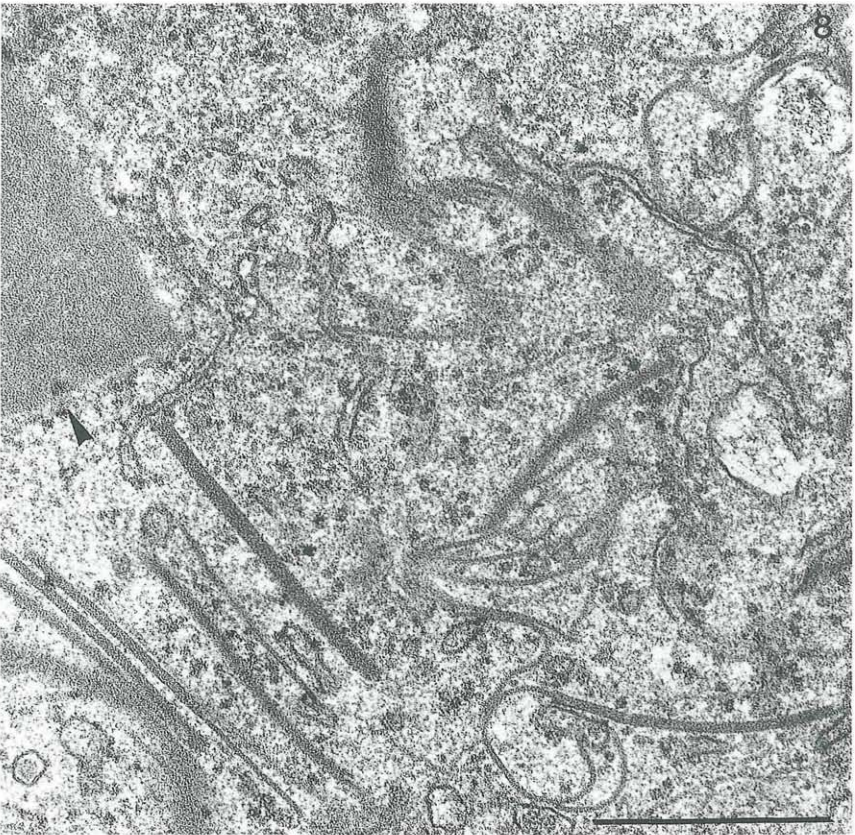
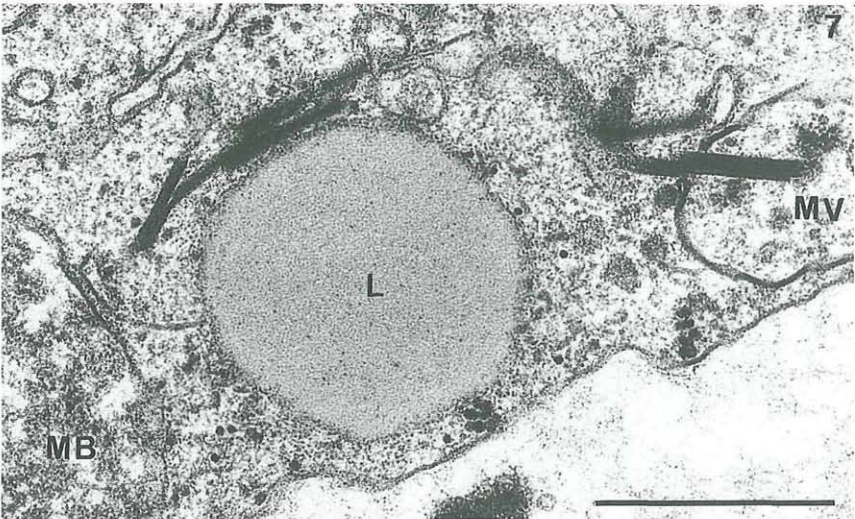
The described results of investigations showed that in the coastal region of Croatia the *S. aspera* plants with leaf ring spot symptom are virus infected. The presence of filamentous virus particles and cylindrical virus cell inclusions in the cytoplasm of infected mother plants *S. aspera* as well as in investigated experimental host (*T. expansa*) pointed out that the isolated *Smilax* virus belongs to the family *Potyviridae* (e.g. EDWARDSON 1974, 1992, EDWARDSON & CHRISTIE 1978, 1996, LESEMAN 1988, SHUKLA & al. 1994, BARNETT & al. 1995). *Smilax* virus induces subdivision II cylindrical inclusions (EDWARDSON & al. 1984), i.e. pinwheel and laminated inclusions. In origin host *S. aspera* as well as in analysed older infections of herba-

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Fig. 7. Laminated aggregates in the cytoplasm of leaf cell of *Smilax aspera* showing ring spot symptoms. L = lipid, MV = multivesicular body. Microbody (MB). Bar = 500 nm.

Fig. 8. Disintegrated cylindrical inclusions – pinwheels and laminated aggregates, in leaf cell of *Chenopodium amaranticolor* with ring spot symptoms; protein crystal (arrowhead). Bar = 500 nm.





ceous host *T. expansa* cylindrical inclusions were predominantly in fragments. Namely, it is known that in young infections cylindrical inclusions tend to pinwheel arrangement and later on their breakdown may occur (NOME & al. 1974, FRANCKI & al. 1985, LESEMAN 1988, EDWARDSON 1992, SHUKLA & al. 1994 and others).

The size of measured filamentous virus particles from leaf extracts of *Smilax* fell within the bounds reported for particle sizes of genus *Potyvirus* (FRANCKI & al. 1985, LESEMAN 1988, SHUKLA & al. 1994, BARNETT & al. 1995). Their presence in plasmodesmata is also noted for potyviruses (e.g. RUSSO & MARTELLI 1969, WEINTRAUB & al. 1974, NOME & al. 1974, LESEMAN 1988, SHUKLA & al. 1994). In *Smilax* and *Tetragonia* plants virus particles were scattered in the cytoplasm, but in origin plant their more or less dense bundles in considerable accumulations have been also found. Such remarkable amassing of virions is not peculiarity of potyviruses, although virus particles in bundle-like aggregates of various size may occur by potyviruses and *Potyviridae* at all (e.g. KIM & FULTON 1969, KAMEI & al. 1969, PLUMB & JAMES 1973, LANGENBERG & SCHROEDER 1973, EDWARDSON & CHRISTIE 1978, FRANCKI & al. 1985, LESEMAN 1988, SHUKLA & al. 1994); in young infections these aggregates are often intimately attached to cylindrical inclusions and endomembrane system. It is also possible that our *Smilax* virus in origin plants could be accompanied with one other elongated virus, which we failed to isolate by mechanical inoculation. However, the size of a greater part of virus particles from infected *S. aspera* corresponded to that characteristic for potyviruses.

Cytoplasmic proteinaceous tubular structures, observed in thin sections of both submicroscopically investigated infected hosts are noted earlier in host cells infected with some potyviruses (MARTELLI & RUSSO 1969, HARRISON & ROBERTS 1971, WEINTRAUB & al. 1973, BEGTRUP 1976, CHRISTIE & EDWARDSON 1977, KITAJIMA & COSTA 1978, MIGLIORI & GOURRET 1987, LESEMAN 1988). Various tubular structures could be also found in cells of some apparently healthy plant species, especially in gland cells (see THALER & GAILHOFFER 1988).

Light microscopic amorphous inclusions provoked by some potyviruses are commonly induced in chronically infected plants (HOLLINGS & BRUNT 1981). However, we have analysed only leaf cells of herbaceous hosts in young infections, but not the tissue of infected mother plant.

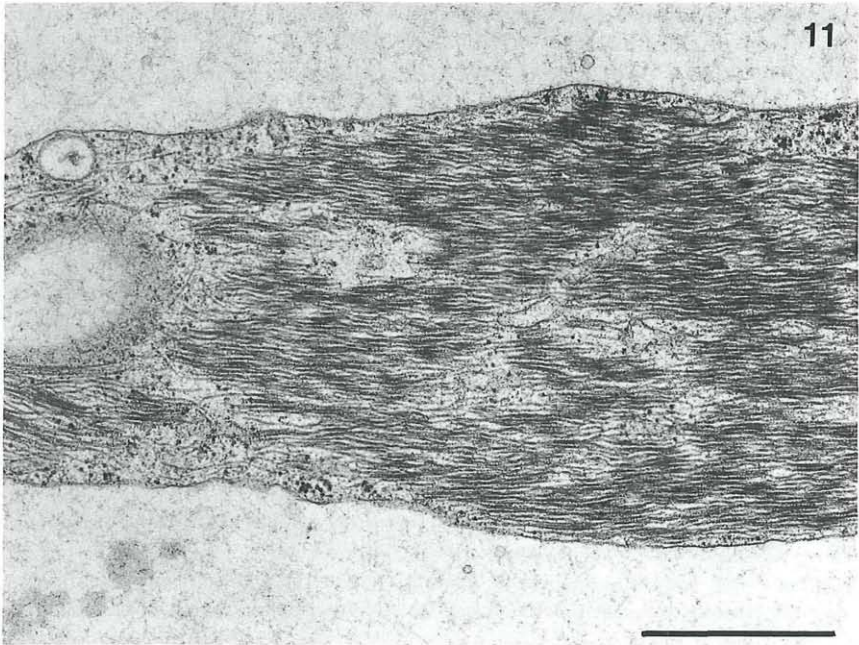
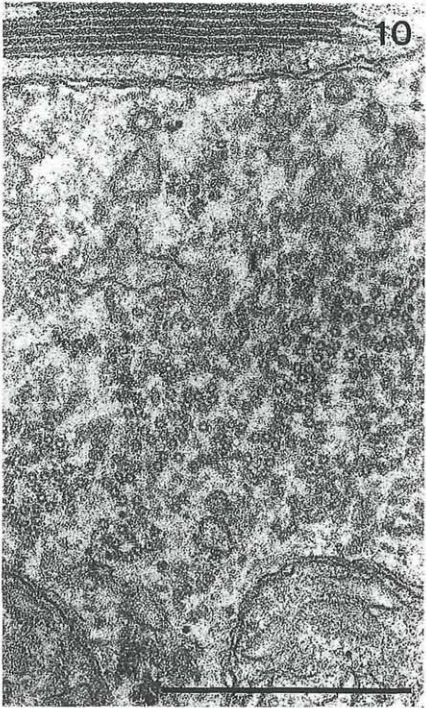
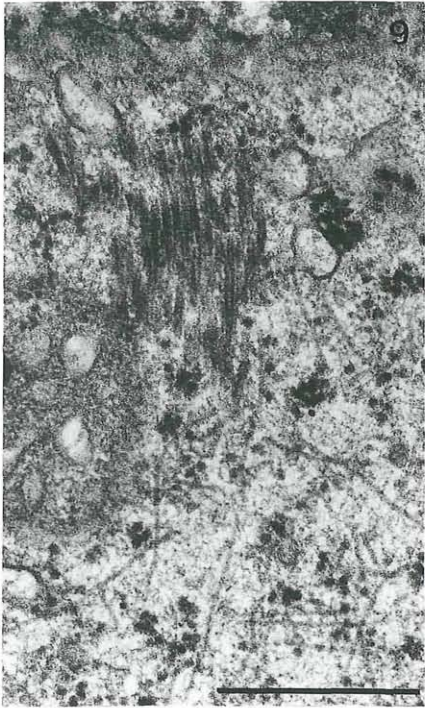
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Fig. 9. Tubular structures and scattered filamentous virus-like particles in the cytoplasm of leaf cells of *Smilax aspera* with ring spot symptoms. Bar = 500 nm.

Fig. 10. Infected *Tetragonia expansa*; large accumulation of tubules in cross-section. Bar = 500 nm.

Fig. 11. *Smilax aspera* showing ring spot symptoms; bundles of virus particles in the cytoplasm. Bar = 1 µm.





Otherwise, *Smilax* virus is a virus with rather narrow experimental host range, fairly stable in crude plant sap but sensitive to heating. It is a virus with uncertain transmissibility, apparently not vectored by aphid *M. perisicae*.

On the basis of our investigations suggested that isolated *Smilax* virus belongs to family *Potyviridae*, perhaps to genus *Potyvirus*. It is associated with the ring spot syndrome of climbing plant *S. aspera* but not proven cause.

#### Acknowledgement

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## Recensiones

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