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A FILAMENTOUS VIRUS ASSOCIATED WITH MOSAIC OF *EUONYMUS JAPONICA*

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Introduction

More than seventy years ago Baur (1908) established by grafting experiments that the spotting and vein yellowing on the leaves of the evergreen ornamental shrub *Euonymus japonica* Thunb. (Japanese spindle--tree) is not a genetic characteristic of this species but »eine infektiöse Chlorose« induced probably by a virus. Later on this disease, spread on *E. japonica* in many countries of Europe, was named Japanese spindle-tree mosaic (das Mosaik des japonischen Spindelstrauchs, Schmelzer 1977). However, the true cause of disorder was not known.

Codaccioni (1972) and Jonsson (1974) ascertained the presence of a rhabdovirus in *E. japonica* 'Microphylla' with stem fasciation. The virus was transmissible by grafting to healthy plants, but it was not proved that the fasciation of the stem was caused by a rhabdovirus (Codaccioni and Cossard 1975, 1977).

Recently we have found bacilliform particles of a rhabdovirus as well as filamentous particles of another virus in the same leaf parenchyma cells of *E. japonica* (type form) and *E. japonica* 'Microphylla' suffering from mosaic disease (Pleše and Erić 1980). The infected plants displayed very prominent spotting and vein yellowing on the leaves but they did not show any fasciation of the stem.

In this paper we present the results of our further investigation of Japanese spindle-tree mosaic. Repeated finding of the filamentous virus is an important fact because it can solve the problem of the agent of this virus disease.

Material and Methods

As all attempts to transmit the virus to herbaceous plants remained without any success, the investigation of mosaic bearing *E. japonica* was continued by analysis of leaf tissues by electron microscopy. The leaves of specimens examined were collected from seven *Euonymus* shrubs from different localities in the area of Zagreb. Four specimens, three of which belonged to *E. japonica* (type form) and one to *E. japonica* 'Aureovariegata', displayed prominent spotting and vein yellowing or vein clearing symptoms (Fig. 1 A) which are characteristic of the Japanese spindle-tree mosaic (Schmelzer 1977). Two specimens of *E. japonica* (type form) and one of *E. japonica* 'Aureovariegata' were without any sumptoms and were used as control.

For electron microscopic analysis ultrathin sections of leaf tissue were processed in the same manner as described earliner (Pleše and Erić 1980). Leaf dip preparations were negatively stained with Na-phosphotungstate. The examinations were performed in a Siemens Elmiskop I electron microscope.

Results and Discussion

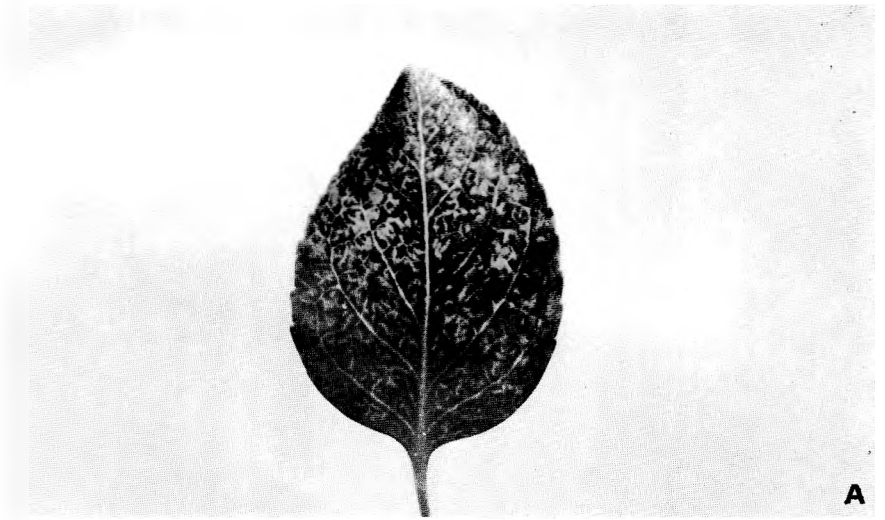
During the examination of ultrathin sections through leaf tissue only elongated particles of the filamentous virus were detected in all specimens which displayed mosaic. No virus particles could be found in control symptomless specimens of *Euonymus*.

The virus particles were situated in the cytoplasm of the parenchyma cells and were usually distributed randomly. In some regions the particles were amassed in considerable amount (Fig. 2. A). In such places they were frequently laterally aggregated forming small bundles scattered over the cytoplasm (Fig. 2. B). The accumulation of virus particles in bundles is especially characteristic of carlaviruses. However, the bundles of carlaviruses are usually very large, rather clearly bordered from the cytoplasm and composed of dense packed virus particles having the tendency to attach to the cytoplasmic membranes or to form banded inclusions (Bos and Rubio-Huertos 1971, 1972; Christie and Edwardson 1977).

In order to measure the virus particles, leaf dip preparations were also made from *Euonymus* with mosaic symptoms (Fig. 1. B, C). The measurable particles were about 650 to 680 nm long and 18 nm wide. The particle length corresponded to those of carlaviruses or some closteroviruses but the thickeness was unusual for these viruses. According to data cited by Matthews (1979) carlaviruses are about 13 nm thick and closteroviruses about 10 nm. Otherwise, the particles of our filamentous virus were rather straight like those in carlaviruses.

The filamentous virus is apparently wide-spread in *E. japonica* in the region of Zagreb. No doubt the virus particles detected during this investigation and filamentous particles found in the same species earlier in mixed infection with rhabdovirus (Pleše and Erić 1980) belong to the same virus.

On the basis of data presented we consider that only the filamentous virus is the agent which causes Japanese spindle-tree mosaic. In our opinion the rhabdovirus, which we have found in only two specimens of *E. japonica* (Pleše and Erić 1980), is present in this ornamental shrub



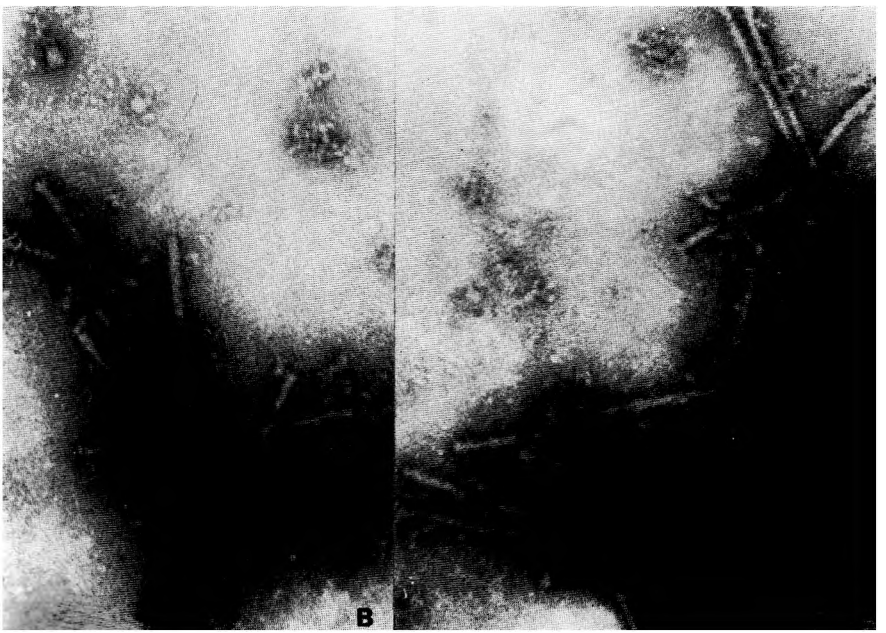


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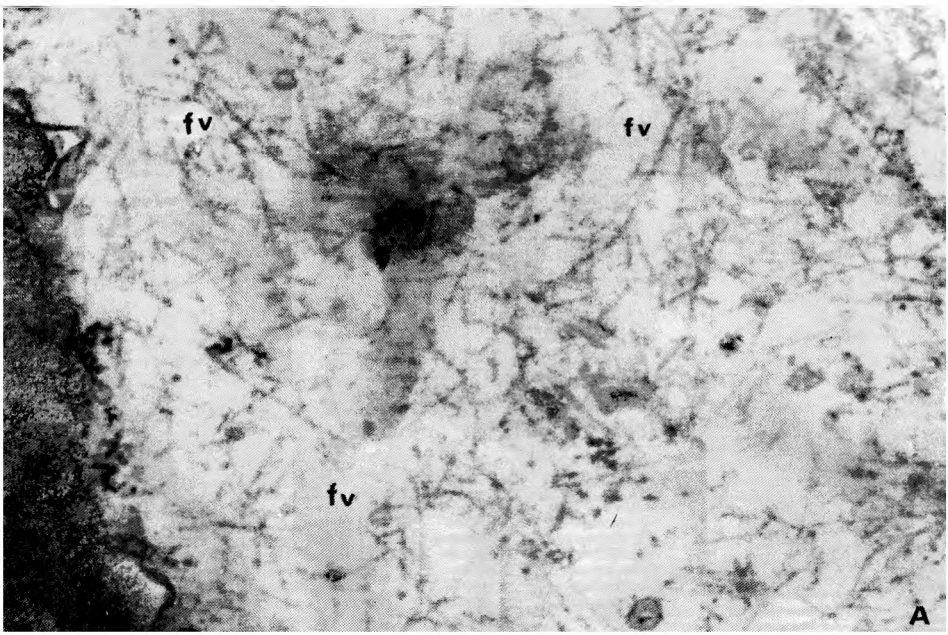
Fig. 1. A — the leaf of *Euonymus japonica* (type form) with mosaic symptoms. B, C — leaf dip preparations of *E. japonica* infected with mosaic; particles of a filamentous virus are present.

▶

Fig. 2. Ultrathin sections through leaf parenchyma cells of *Euonymus japonica* suffering from mosaic: A — particles of filamentous virus randomly distributed in the cytoplasm (fv); B — bundles of virus particles (b) scattered in the cytoplasm.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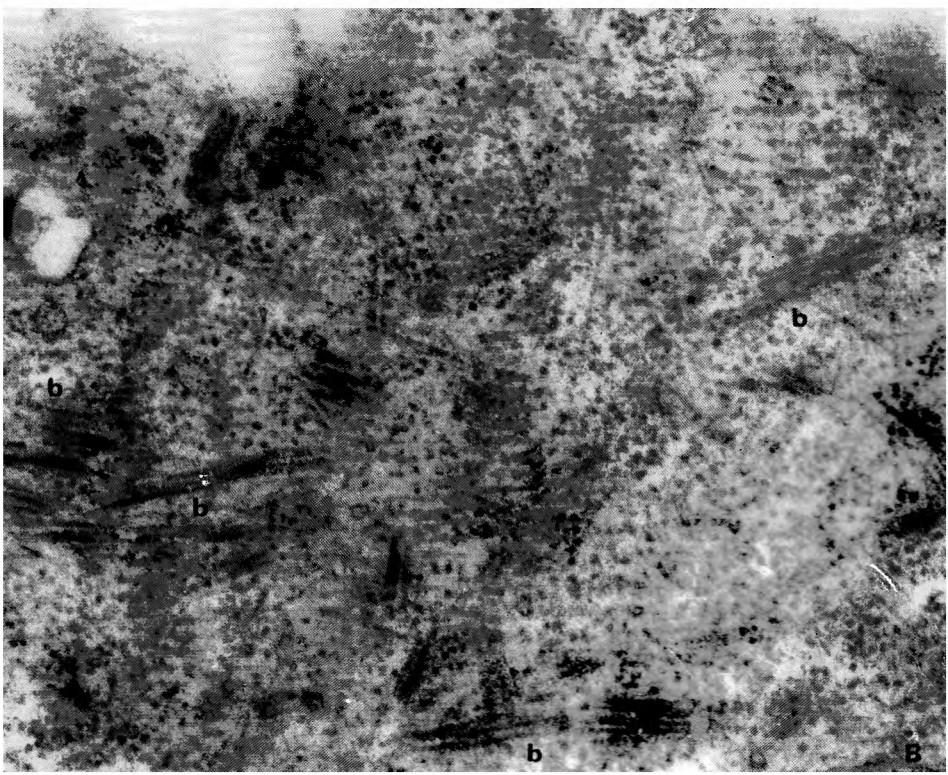
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only sporadically and has not a remarkable influence on the expression of the leaf symptoms. As the rhabdovirus was not usually present in all ultrathin sections of infected *Euonymus* (Pleše and Erić 1980), our submicroscopic analysis was repeated several times, but the rhabdovirus was never detected.

While the mosaic disease is wide-spread in *E. japonica* in the inland part of Croatia (Zagreb), the specimens in the coastal region (Zadar, Split, Primošten) are mostly symptomless and without virus particles in the cells. It is possible that the vector of the virus is absent or rare in the coastal region.

Summary

Ultrathin sections through the leaf tissue of ornamental shrubs *Euonymus japonica* Thunb. suffering from mosaic disease (Japanese spindle-tree mosaic) and of symptomless ones were analysed by electron microscopy. Elongated particles of a filamentous virus were present in the cytoplasm of the parenchyma cells of all shrubs displaying mosaic. The particles about 650 to 680 nm long and 18 nm thick were randomly distributed or laterally aggregated forming small bundles scattered in the cytoplasm.

We consider that this filamentous virus, apparently wide-spread in *E. japonica* in the inland part of Croatia (Zagreb), is the agent which causes Japanese spidle-tree mosaic.

References

Baur, E., 1908: Über ene infektiöse Chlorose von *Evonymus japonicus*. Ber. dtsch. bot. Ges. *26a*, 711—713.

Bos, L., M. Rubio-Huertos, 1971: Intracellular accumulation of *Passiflora* latent virus in *Chenopodium quinoa*. Neth. J. Pl. Path. *77*, 145—153.

Bos, L., M. Rubio-Huertos, 1972: Light and electron microscopy of pea streak virus in crude sap and tissues of pea (*Pisum sativum*). Neth. J. Pl. Path. *78*, 247—257.

Christie, R. G., J. R. Edwardson, 1977: Light and electron microscopy of plant virus inclusions. Fla. Agric. Exp. Stn. Monogr. Ser. *9*, 47—55.

Codaccioni, M., 1972: Description de particules intracytoplasmiques dans de jeunes tiges fasciées de *l'Evonymus japonica* Th. Bull. Soc. bot. Fr. *119*, 51—60.

Codaccioni, M., C. Cossard, 1975: Etude de la transmission expérimentale de diverses inclusions intracellulaires observées dans les tiges fasciées du *Forsythia* sp. et d'une variété de Fusain du Japan. C. R. Acad. Sc. Paris, *280*, série D, 1497—1501.

Codaccioni, M., C. Cossard, 1977: Transmission expérimentale de particules du type Rhabdovirus, naturellement présentes dans les tiges fasciées d'une variété de Fusain. C. R. Acad. Sc. Paris, *284*, série D, 701—704.

Jonsson, G., 1974: Localisation nucleaire et cytoplasmique de particules d'allure virale chez des sujets fasciées de Fusains (*Euonymus japonica* Thunb. et var. *microphylla* Jaeger). Revue gen. Bot. *81*, 135—150.

Matthews, R. E. F., 1979: Classification and nomenclature of viruses. Intervirology *12*, 131—296.

Pleše, N., Ž. Erić, 1980: Rhabdovirus in *Euonymus japonica*. Acta Phytopath. Acad. Sci. Hung. *15*, 291—295.

Schmelzer, K., 1977: Zier-, Forst- und Wildgehölze. In: M. Klinkowski, Pflanzliche Virologie. Band 4, pp. 335—336. Akademie-Verlag, Berlin.

SAŽETAK

FILAMENTOZNI VIRUS, MOGUĆI UZROČNIK MOZAIČNE BOLESTI VRSTE *EUONYMUS JAPONICA*

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Ultratanki presjeci kroz lisno tkivo ukrasnih grmova vrste *Euonymus japonica* Thunb. koji su bili zaraženi virusnim mozaikom (Japanese spindle-tree mosaic) i presjeci kroz lisno tkivo grmova bez mozaičnih simptoma analizirani su elektronskim mikroskopom. U citoplazmi parenhimskih stanica svih istraživanih grmova s mozaičnim simptomima nađene su filamentozne virusne čestice. Čestice dužine oko 650 do 680 nm i širine oko 18 nm bile su u citoplazmi ili raspršene ili agregirane u male svežnjiće koji su se također nalazili razbacani u citoplazmi.

Smatramo da je taj filamentozni virus, koji je, čini se, vrlo rasprostranjen u vrsti *E. japonica* u unutrašnjosti Hrvatske (Zagreb), uzročnik mozaične bolesti navedene ukrasne vrste.

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