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RHABDOVIRUS IN *LABURNUM ANAGYROIDES* SUFFERING FROM VEIN YELLOWING DISEASE

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Introduction

The earliest literature on the symptoms on *Laburnum* species believed to be of viral origin, is about hundred years old and is cited by Schmelzer (1962). Somewhat later Baur (1906, 1907) demonstrated that the yellowing symptoms on *Laburnum anagyroides* Medic. were associated with an infectious disease. From *L. alpinum* with yellows symptoms Schmelzer (1962) isolated arabis mosaic virus.

Another type of virus symptoms named mosaic was described on several *Laburnum* species (van Katwijk 1952, Hollings 1959, Schmelzer 1962). The symptoms consisted of mosaic mottling and spotting on the leaves and were often associated with vein yellowing; the yellow colour extended along the major veins. Laburnum mosaic was transmissible by grafting but not by sap inoculation.

Another type of virus symptoms named mosaic was described on several *Laburnum* species (van Katwijk 1953, Hollings 1959, trees showing both mosaic and vein yellowing symptoms. The presence of the bacilliform particles was correlated only with the leaf areas showing vein yellowing symptoms but not with the mosaic ones. Therefore, in the authors' opinion the rhabdovirus is a causal agent of vein yellowing and the name laburnum yellow vein virus was proposed.

This report supports the finding of the above mentioned authors, namely that the appearance of bacilliform virions is associated with vein yellowing of *L. anagyroides*. This paper describes the morphology and distribution of rhabdovirus-like particles in the leaf cells of *L. anagyroides* with symptoms of vein yellowing.

Material and Method

In one locality in Zagreb specimens of *L. anagyroides* with symptoms of vein yellowing were noticed. The leaflets along the mid-rib and lateral veins were chlorotic or coloured yellow (Fig. 1. A, B). Very frequently the symptoms appeared as yellow lines which made an oak-leaf pattern spreading along the main veins (Fig. 1. B). Sometimes these changes extended in small patches between the lateral veins. The symptoms described appear to be very similar or identical to some of those illustrated by van Katwijk (1953, Fig. 1. left) and Schmelzer (1962, Fig. 7. B) and to those described by Schultz and Harrap (1975). However, the mosaic symptoms noticed by the cited authors were not visible on the material from Zagreb.

For electron microscopy small leaf pieces were fixed in 1% glutaraldehyde buffered with 0.1 M sodium cacodylate pH 7.2 and after washing in buffer were postfixed in 1% buffered OsO_4. Fixed samples were dehydrated in a graded ethanol series and embeded in Araldite resin. The ultrathin sections were stained with uranyl acetate and lead citrate. Stained sections were examined in a Siemens Elmiskop I electron microscope.

Results and Discussion

L. anagyroides from Zagreb bearing vein yellowing symptoms was subjected to electron microscope studies. The sections of infected leaves from the regions of yellow veins showed the presence of bacilliform virus particles in the parenchyma cells (Fig. 2., 3.), which corresponds to the finding of Schultz and Harrap (1975).

The virus particles were mostly situated in the perinuclear spaces which, because of the presence of virions, evaginated toward the cytoplasm in the form of larger or smaller pockets (Fig. 2.). It was found that the virus partices were also amassed in the inside of the nucleus (Fig. 3. A). In this case they represent intranuclear virus enclaves which apparently originate from invaginated perinuclear pockets (Plavšić et al. 1978). Thus, the virus enclaves are surrounded with the inner nuclear membrane. Vela and Rubio-Huertos (1974) also found virus partices within the nuclei, but their particles were immature (nucleocapsids) and lay free in the nucleoplasm. Single virus particles were also seen in the cytoplasm surrounded by a membrane (Fig. 3. B, C). In general, the particles have usually an irregular arrangement, but some of them make small aggregates with a regular hexagonal packing visible in cross section (Fig. 2. A).

The structure of bacilliform particles also corresponds to that of laburnum yellow vein virus described by Schultz and Harrap (1975). The particles consist of an electron dense core with a rather wide axial channel and an envelope on their surface. An osmiophilic ring visible in transversal section of particles corresponds in diameter to the core.

The diameter of complete particles is about 90 nm. Measurable virus particles are very long, i. e. about 500 nm. A clear transversal gap in the middle of the core, separating a long particle in two parts, is clearly visible (Fig. 2. A). Such long particles some of which have a gap, can also be seen in *Laburnum* infected with yellow vein virus of Schultz

Fig. 1. The leaves of *Laburnum anagyroides* with vein yellowing symptoms: A, B — yellowing along the main veins of the leaflets; B right — yellow oak-leaf pattern along the mid-rib of middle leaflet.

Sl. 1. Listovi vrste *Laburnum anagyroides* sa simptomima žućenja nerava: A, B — žućenje duž većih nerava liski; B, desno — žute linijske šare u obliku hrastova lista duž glavnog nerva srednje liske.

Fig. 2. Ultrathin sections of infected leaf parenchyma cells of *Laburnum anagyroides:* A, B — bacilliform virus particles, some of which sectioned transversally, in the perinuclear space; at the top of figure A one particle in the cytoplasm; a transversal gap in the middle of a long virus particles is marked with arrow; n nucleus, om outer nuclear membrane, im inner nuclear membrane, c cytoplasm, v vacuole, pl plastid, m mitochondrion, vs vesicles, pg plastoglobules. Bar represents 150 nm.

Sl. 2. Ultratanki presjeci kroz zaražene parenhimske stanice lista vrste *Laburnum anagyroides:* A, B — baciliformne virusne čestice, od kojih su neke poprečno presječene, u perinuklearnom prostoru; gore na slici A jedna čestica u citoplazmi; poprečna pukotina u sredini dugih virusnih čestica označena je strijelicom; n jezgra, om vanjska membrana jezgre, im unutarnja membrana jezgre, c citoplazma, v vakuola, pl plastid, m mitohondrij, vs vezikuli, pg plastoglobuli. Skala iznosi 150 nm.

Fig. 3. Ultrathin sections of infected leaf parenchyma cells of *Laburnum anagyroides:* A — two virus enclaves invaginated into the nucleus; bacilliform virus particles sectioned mostly transversally; between virus particles many empty vesicles of various size are present; B, C — single virus particles in the cytoplasm surrounded by unit membrane; n nucleus, om outer nuclear membrane, im inner nuclear membrane, c cytoplasm, v vacuole, w cell wall, vs vesicle. Bar represents 150 nm.

Sl. 3. Ultratanki presjeci kroz zaražene parenhimske stanice lista vrste *Laburnum anagyroides:* A — dvije nakupine virusnih čestica okružene unutarnjom jezgrinom membranom; baciliformne virusne čestice presječene uglavnom poprečno; između virusnih čestica nalaze se vezikuli različite veličine; B, C — pojedinačne virusne čestice unutar elementarne membrane u citoplazmi; n jezgra, om vanjska membrana jezgre, im unutarnja membrana jezgre, c citoplazma, v vakuola, w stanična stijenka, vs vezikul. Skala iznosi 150 nm.

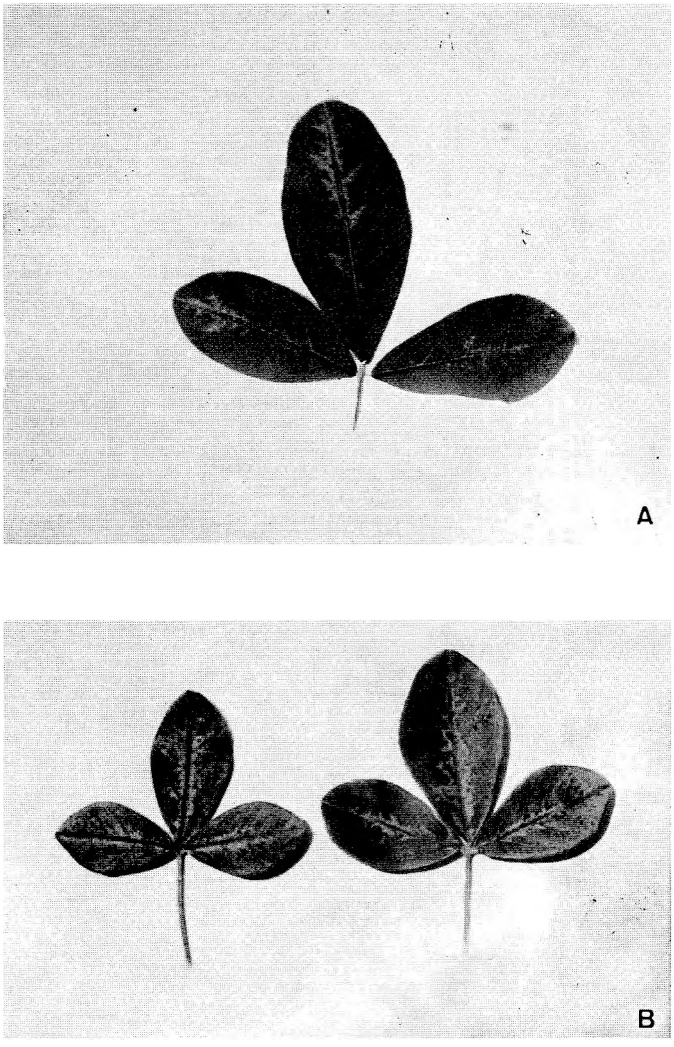

Fig. 1. — Sl. 1.

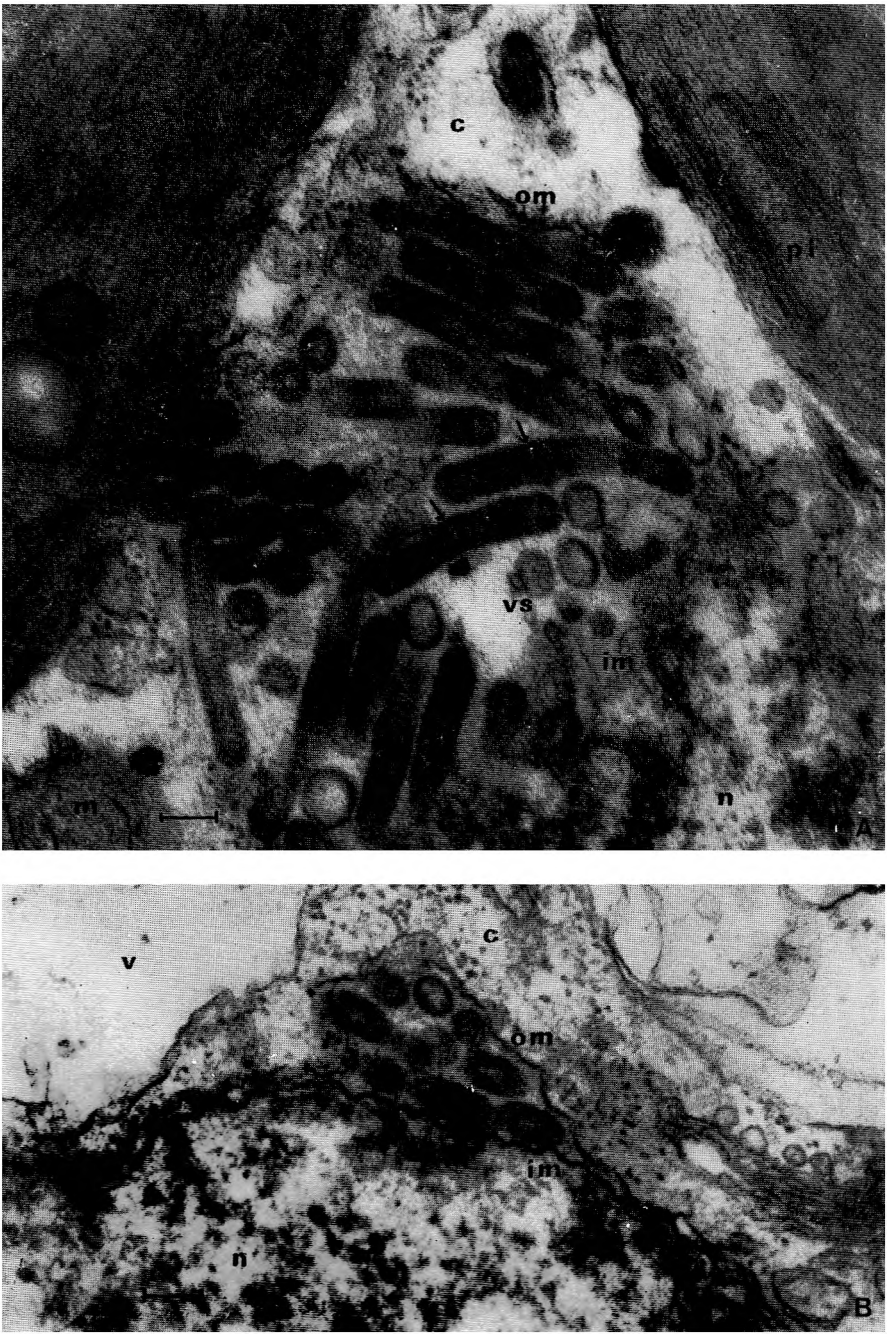

Fig. 2. — Sl. 2.

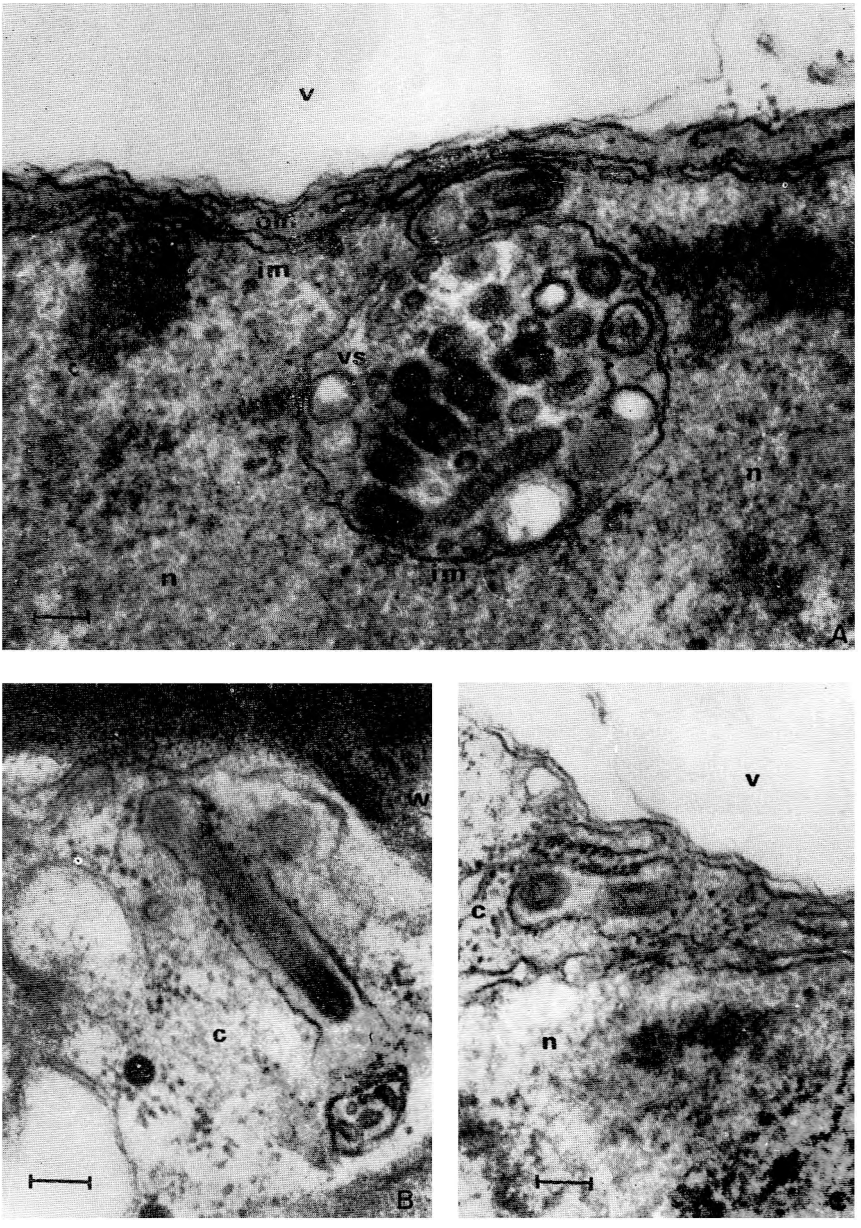

Fig. 3. — Sl 3.

and Harrap (1975), and were noticed in other plant rhabdoviruses as well (Vela and Rubio-Huertos 1974, Rubio-Huertos 1978, etc.). The length of our measurable particles was twice of that quoted by Schultz and Harrap (1975). We think that our particles are also about 250 nm long, but the measurable particles were very long because two nucleocapsids were incorporated into one envelope (Francki 1973).

Between virus particles in the perinuclear spaces and in the virus enclaves inside the nucleus a number of empty vesicles of various size was observed (Fig. 2. A, 3. A). They were also recorded by Schultz and Harrap (1975). One should not exclude the possibility that such vesicles derive from the electron transparent ends of virus particles which are seen in some plant rhabdoviruses (Knudson 1973, Vela and Rubio-Huertos 1974, Plavšić et al. 1976).

The results of our investigation are in agreement with those of Schultz and Harrap (1975) and they contribute to the statement of the authors quoted that their rhabdovirus, i. e. laburnum yellow vein virus, provokes vein yellowing symptoms on *L. anagyroides*. Moreover, the specimens of our *L. anagyroides* in which a bacilliform virus is present, expressed only vein yellowing and no mosaic symptoms. It seems, that rhabdoviruses of woody plants are often associated with vein yellowing or vein clearing symptoms on the leaves (Stace-Smith and Lo 1973, Jones et. al. 1974, Plavšić et al. 1976, Plavšić-Banjac et al. 1976).

Summary

Bacilliform particles characteristic of plant rhaboviruses were found in ultrathin sections of *Laburnum anagyroides* growing in a park in Zagreb (Yugoslavia). Specimens of *Laburnum* in which bacilliform virions were present showed only yellowing along the main veins but not mosaic symptoms. Virus particles were found in yellow areas of the leaflets and were situated in the nucleus as well as in the cytoplasm. Results of this investigation support the opinion of Schultz and Harrap (1975) that the vein yellowing symptoms on *L. anagyroides* are provoked by a rhabdovirus named by them laburnum yellow vein virus.

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SAŽETAK

RABDOVIRUS U VRSTI *LABURNUM ANAGYROIDES* SA SIMPTOMIMA ŽUĆENJA NERAVA

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U ultratankim presjecima kroz lisno tkivo grmova vrste *Laburnum anagyroides* Medic. (zanovijet) koji pokazuju simptome žućenja duž glavnih nerava utvrđena je prisutnost baciliformnih čestica karakterističnih za biljne rabdoviruse. Čestice su nađene u parenhimskim stanicama iz područja žutih promjena uz nerve, a lokalizirane su i u perinuklearnom prostoru i u citoplazmi. Prisutne su kratke i duge čestice. U srži dugih čestica zapažena je poprečna pukotina koja upozorava na mogućnost da je u takvim česticama uklopljen dvostruki genom. Rezultati istraživanja izneseni u ovom radu podupiru nalaz i mišljenje Schultza i Harrapa (1975) da simptome žućenja nerava u vrste *L. anagyroides* uzrokuje jedan rabdovirus veličine 245 × 89 nm, za koji su predložili naziv virus žutih nerava zanovijeti (laburnum yellow vein virus).

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