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Gentiana asclepiadea L. Infected with a Rhabdovirus

By

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With 16 Figures

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Summary

THALER I. & GAILHOFER M. 1996. *Gentiana asclepiadea* L. infected with a rhabdovirus. – Phyton (Horn, Austria) 36 (2): 209–220, 16 figures. – English with German summary.

Gentiana asclepiadea found in Styria/Austria often shows mottling symptoms on adult leaves. The rhabdovirus is named provisionally Gentiana rhabdovirus Graz (GRVG) and it is the first report of a rhabdovirus in *Gentiana asclepiadea*. GRVG-particles were stabilized in the crude extract of leaves and negatively stained with ammonium molybdate. They have a length of 210 - 620 nm and a diameter of about 70 nm.

The virus particles are localized in cisternae of the endoplasmic reticulum, which often surround chloroplasts, mitochondria or microbodies. In some mesophyll cells a cytoplasmic area resembling a "viroplasm" occurs . By this area cisternae of the endoplasmic reticulum with GRVG-particles occur. Rarely GRVG-particles or incomplete particles occur in the perinuclear space in a granular matrix. Budding of nucleocapsids from the inner nuclear membrane or from the "viroplasm" was not observed.

GRVG-particles are not detected in plants grown from seeds of virus diseased individuals. This rhabdovirus particles cannot be transmitted mechanically to *Nicotiana glutinosa.*

Zusammenfassung

THALER I. & GAILHOFER M. 1996. *Gentiana asclepiadea* L. infiziert mit einem Rhabdovirus. – Phyton (Horn, Austria) 36 (2): 209–220, 16 Abbildungen. – Englisch mit deuscher Zusammenfassung.

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In der Steiermark (Österreich) findet man häufig *Gentiana asclepiadea* mit gefleckten Blättern. Es ist der erste Bericht über ein Rhabdovirus in *Gentiana asclepiadea* und es wird vorläufig Gentiana Rhabdovirus Graz (GRVG) genannt. Die im Rohextrakt stabilisierten und mit Ammoniummolybdat negativ kontrastierten GRVG-Partikel sind 210–620 nm lang mit einem Durchmesser von ungefähr 70 nm.

Die GRVG-Partikel liegen meist in Zisternen des ER, die Chloroplasten, Mitochondrien und Microbodies umgeben. Ein auffallender Cytoplasma-Bereich, vermutlich ein Viroplasma, kommt in manchen Mesophyllzellen vor. Am Rande dieses Bereiches sind in ER-Zisternen GRVG-Partikel vorhanden. In wenigen Pflanzen liegen unvollständige Virus-Partikel und GRVG-Partikel in einer granulären Matrix im perinuclearen Raum. Ein „budding" der Nucleocapside an der inneren Kernmembran oder an Membranen des ER wurde nie beobachtet.

Keimpflanzen, die aus Samen viruskranker *Gentiana asclepiadea* gezogen wurden, enthalten keine GRVG-Partikel.

Eine mechanische Übertragung der GRVG-Partikel auf *Nicotiana glutinosa* ist nicht gelungen.

Introduction

Plants of *Gentiana asclepiadea* with mottled leaves are often found in forests of Koralpe, Schöckel, and Gleinalm (Styria, Austria) at an altitude of about 1000 m. Host reaction starts with vein clearing, which later disappears totally. On adult leaves mottling was observed (Fig. 1). It is suggested that the symptoms are caused by a virus infection. Infected plants show a reduction of growth as well as retarded flowering. Finally the leaves turn brownish and wither prematurely.

The occurence of virus-particles in the cells and their morphology are investigated.

Material and Methods

Samples of leaves with vein clearing and mottled leaves, 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 tetraoxide, 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 CM 10 electron microscope.

Leaf tissue samples were crushed in 0.1 M Tris-HCl, pH 8.4 , that contained 0.1 M magnesium acetate, 0.04 M sodium sulfite, and 0.001 M mangane chloride (JACKSON & CHRISTIE 1977, CHRISTIE & al. 1987) and negatively stained with ammonium molybdate adjusted with ammoniac to pH 7.1. Furthermore it was tried to fix the GRVG-particles in the crude extract with glutaraldehyde followed by negativ staining with uranyl acetate.

Results

In mottled leaves of *G. asclepiadea* rhabdovirus particles can be observed in xylem parenchyma and mesophyll cells of the chlorotic area,

and also in leaves with vein clearing. The rhabdovirus is named provisionally Gentiana rhabdovirus Graz (GRVG). The GRVG-particles seem to be absent in cells of the stem, root and flower of plants with mottled leaves.

Particles in sections

The particles occur single or in small groups in the cisternae of the endoplasmic reticulum. The small groups form hexagonal patterns. Most particles are found in cisternae close to chloroplasts, mitochondria and microbodies (Fig. 2). In sections the diameter of GRVG-particles is about 70 nm, the length is variable and ranges from 210 to 1300 nm. In the transverse section the GRVG-particle shows a structure of two concentric rings. The outer of these rings appears to form an envelope, the inner forms the nucleocapsid with a channel, about 38 nm wide. The center of the channel may contain an electron-dense axial element (Fig. 3). In the longitudinal section the inner part of the enveloped particles is cross striated. The variability of the lenghts of the particles suggests that two or more nucleocapsids form an end-to-end aggregation (Fig. 4).

In addition to complete GRVG-particles unenveloped but striated particles occur. These particles have a diameter of about 47 nm and a length of 580–620 nm. Both enveloped and incomplete particles may be found in the same endoplasmic reticulum cisterna (Fig. 5). In some mesophyll cells an area, probably a viroplasm can be found. It is localized near the nucleus and contains endoplasmic reticulum many large vesicles of 280–475 nm with fibrous content and numerous small „vesicles" of 28–40 nm diameter (Fig. 6). By this area endoplasmic reticulum cisternae containing GRVG-particles occur (Fig. 7).

In the nucleus of mottled leaves and leaves with vein clearing particles with a helicoidal organization occur. In cross sections these particles form a ring (Fig. 8). Similar particles were also observed in the cytoplasm (Fig. 9). The budding of these particles from the inner membrane of the nuclear envelope or from the cytoplasma into the endoplasmic reticulum has not been observed so far. Plants grown from seeds of mottled *G. asclepiadea* also contain particles with a helicoidal pattern in the nuclei of cotyledons. However, GRVG-particles were never observed in such plants.

Plants with vein clearing and two individuals of mottled plants collected in autumn show a changed nucleus. The nucleus has an area of thread-like elements in cross (Fig. 10) or longitudinal sections. The envelope of the nucleus shows numerous invaginations of the inner membrane into the nucleoplasm and evaginations of the outer nuclear membrane into the cytoplasm. The perinuclear space contains either circular granula (Fig. 11), or threadlike structures, GRVG-particles, and incomplete particles (Fig. 12, 13).

Chloroplasts of cells infected by GRVG appear degenerated. The thylakoids are dilated and the number and the diameter of the plastoglobuli is increased. Phytoferritin crystals were observed in the chloroplasts of infected and uninfected plants.

Negative staining

GRVG-particles from the crude extract of mottled leaves were stabilized with sodium sulfite und negatively stained with ammonium molybdate. They are bacilliform, the channel is not positively stained (Fig. 14). After glutaraldehyde fixation particles appear with both ends

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Erklärung der Abbildungen

Fig.1. *Gentiana asclepiadea* with mottled leaves infected with a rhabdovirus (GRVG).

Figs. 2–13. Parts of cells of the mesophyll ; bars represent 0.5 μm.

Fig. 2. Cross sections of GRVG-particles in cisternae of the endoplasmic reticulum surrounding a part of a chloroplast.

Fig. 3. Cross section of GRVG-particle surrounded of a cisternae of the endoplasmic reticulum (arrow).

Fig. 4. Longitudinal section of GRVG-particle in a cisternae of the endoplasmic reticulum.

Fig. 5. Parallely arranged incomplete virus particles (asterisk) and GRVG-particles (arrows) in cisternae of the endoplasmic reticulum . Inset : cross section of a cluster of incomplete particles and of a single GRVG-particle.

Fig. 6, 7. "Viroplasm", contains endoplasmic reticulum, large vesicles with fibrous content among many small "vesicles", next to it occur single GRVG-particles in cisternae of endoplasmic reticulum.

Fig. 8. Part of a nucleus. Nucleocapsids with a helicoidal pattern in longitudinal (arrows) and cross sections (arrowheads).

Fig. 9. Nucleocapsids with a helicoidal pattern occur in the cytoplasm.

Fig. 10. Part of a nucleus contains a thread-like inclusion (asterisk), in the perinuclear space occur GRVG-particles (arrow).

Fig. 11. Part of a nucleus. Folds of the inner membran of the nuclear envelope, the perinuclear space contains numerous granula (asterisks).

Fig.12. Part of a nucleus. GRVG-particles, incomplete particles, and thread-like structures occur in the dilated perinuclear space (arrow).

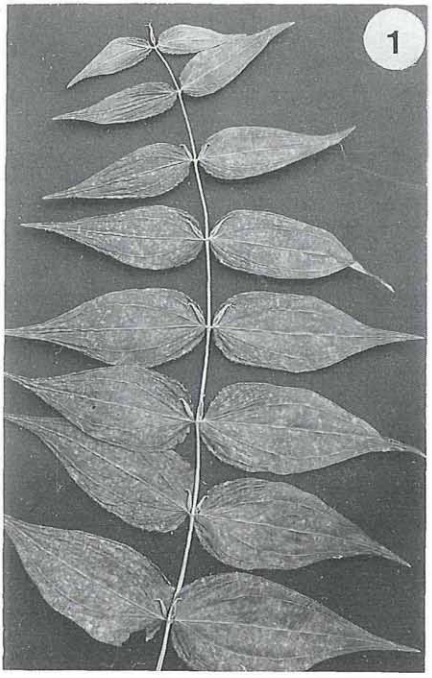
Fig.13. Part of a nucleus. The dilated perinuclear space contains granula (arrowhead), numerous GRVG-particles (asterisk) and incomplete particles (arrow).

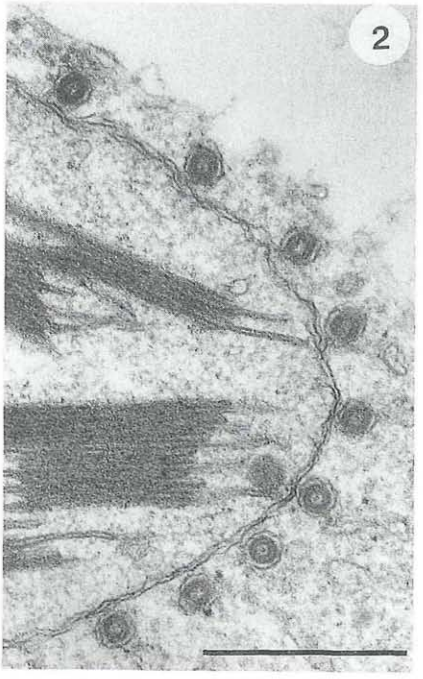
Figs.14–16. Negatively stained particles from the crude extract of mottled leaves. Bars represent 100 nm.

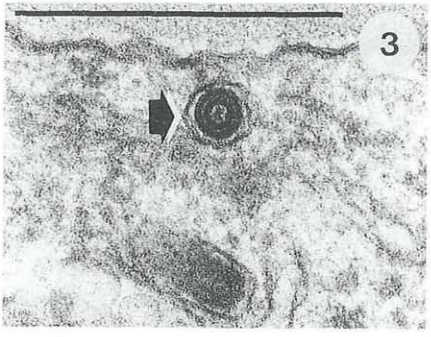
Fig. 14. GRVG-particles stabilised with sodium sulfite and negatively stained with ammonium molybdate.

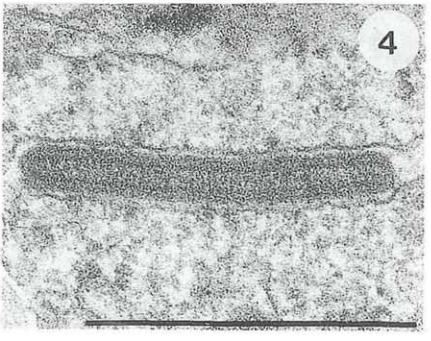
Fig. 15. GRVG-particles fixed with glutaraldehyde and negatively stained with uranyl acetate.

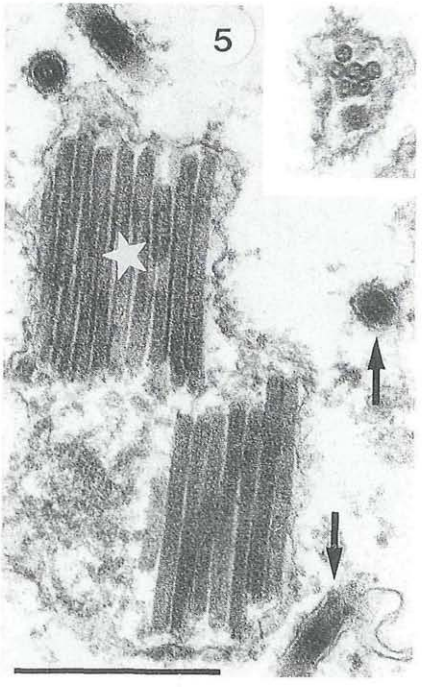
Fig. 16. Nucleocapsids after treatment of the crude extract with glutaraldehyde and negative staining with uranyl acetate.

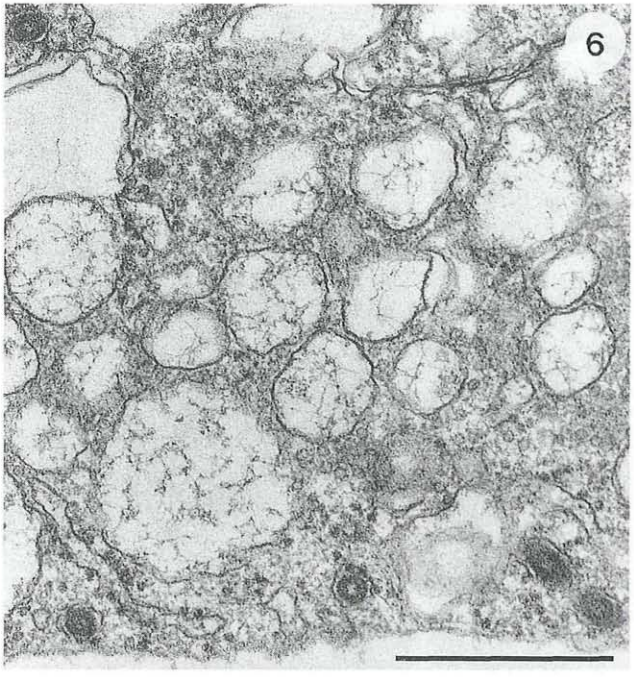


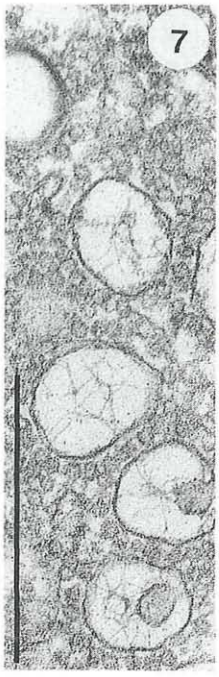


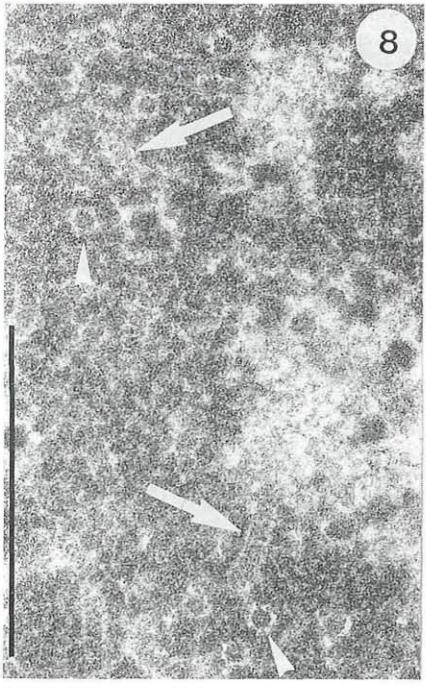


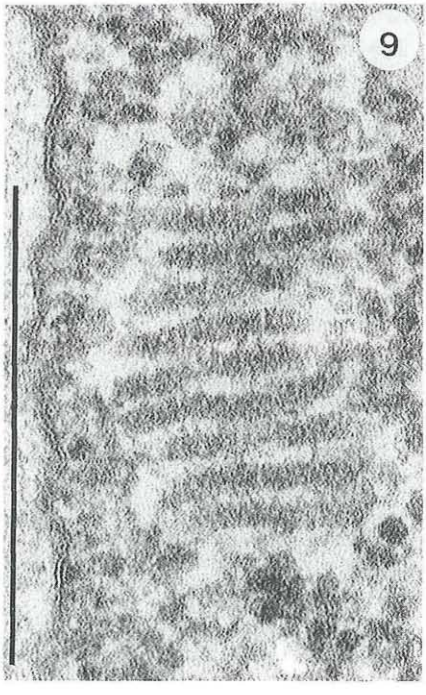


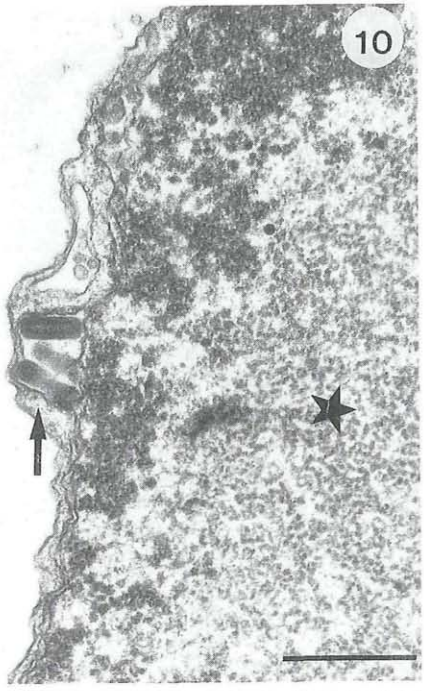


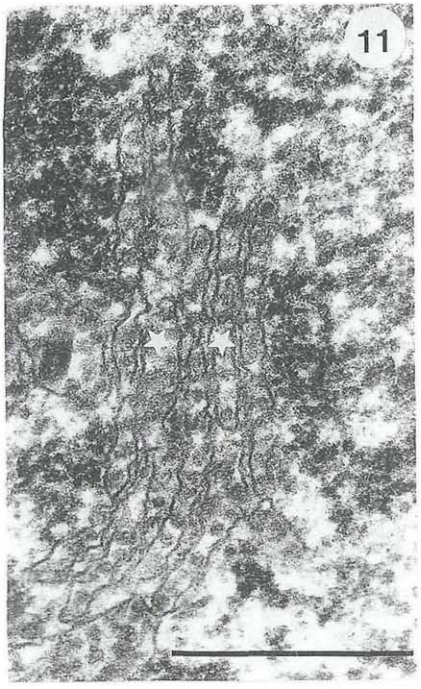


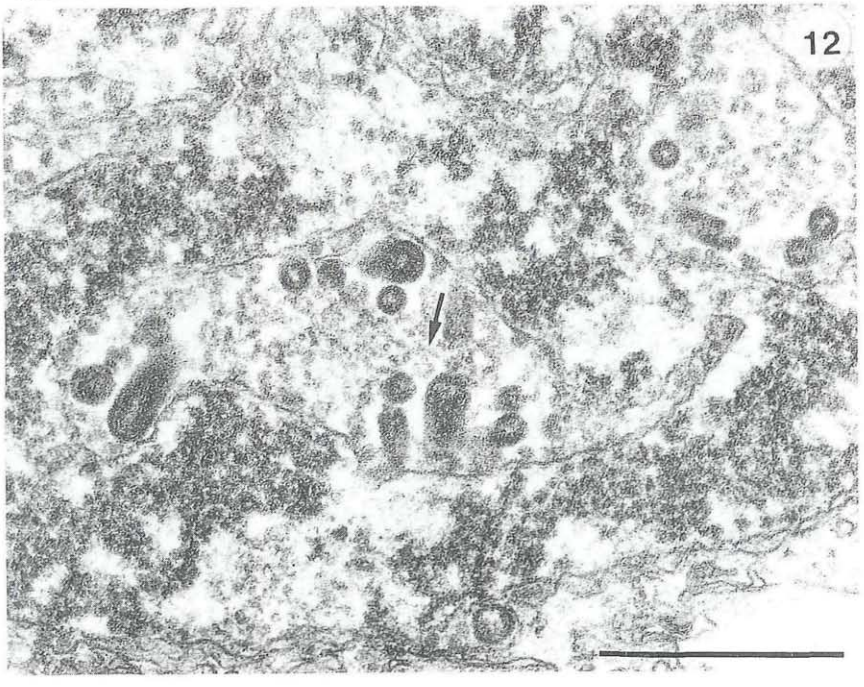


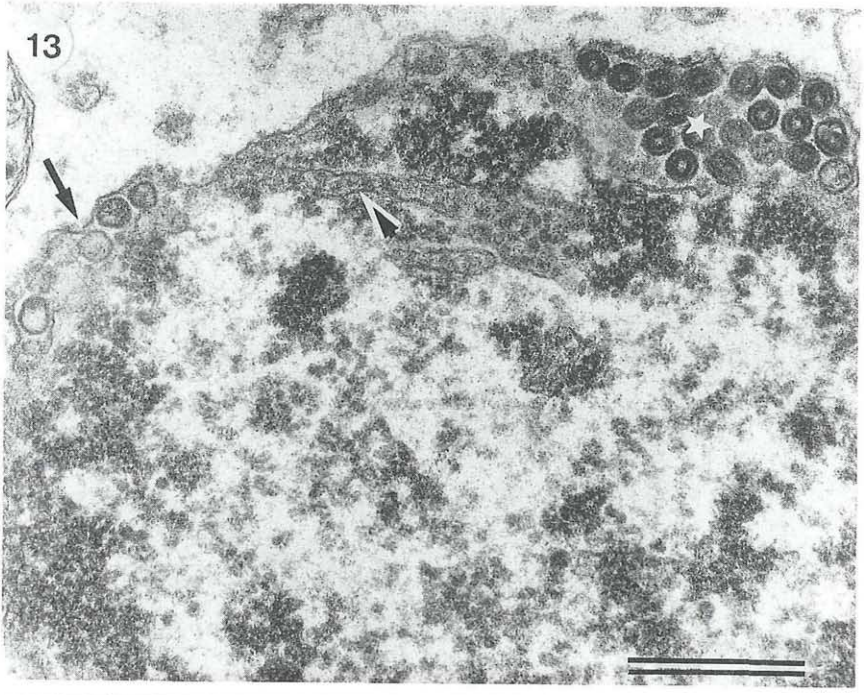


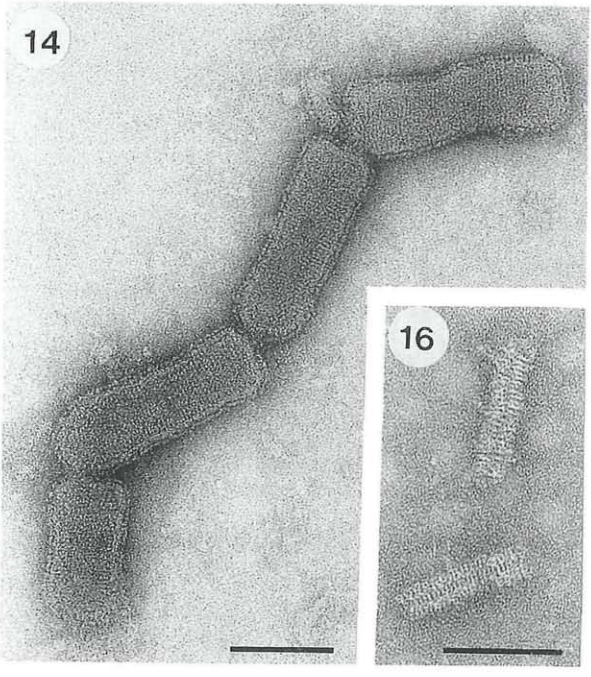


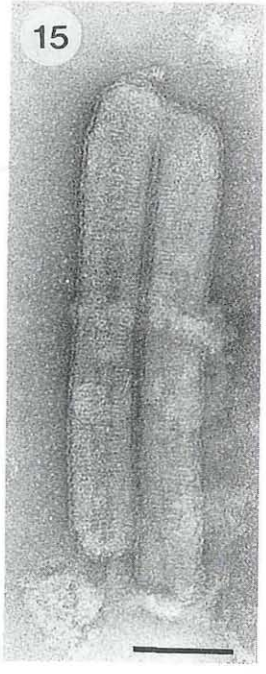












rounded, the channel is positively stained (Fig. 15). Many particles appear broken, and numerous nucleocapsids were seen (Fig. 16). The low number of GRVG-particles allows only a rough estimation of the dimensions. They are 68-70 nm in diameter and 210, 400 and 620 nm in length (Fig. 14, 15).

Attempts to transmit GRVG by sap inoculation to *Nicotiana glutinosa* were unsuccessful.

Discussion

With respect to the morphology and cytopathology we propose to assign the bacilliform particles in *G. asclepiadea* to the rhabdoviruses. The particles are named provisionally Gentiana rhabdovirus Graz (GRVG).

Negative staining of rhabdoviruses may produce artefacts (FRANCKI 1973), therefore particles are stabilized by sodium sulfite and negatively stained with ammonium molybdate (JACKSON & CHRISTIE 1977, CHRISTIE & al. 1987). GRVG-particles were isolated from the crude sap of leaves of *G. asclepiadea*. The diameter of the particles was 68–70 nm , the length of about 210, 400, or 620 nm. It is supposed that they represent double length particles and tandem formations. After stabilization with sodium sulfite and negative staining with ammonium molybdate the central channel of the particle is often not stained. This may indicate an intact envelope of such particles. In addition cross striated particles without envelope occur. After fixing the crude sap with glutaraldehyde followed by negative staining with uranyl acetate nearly exclusively unenveloped particles are observed. The central channel of the few enveloped particles is always stained. A similar result is received by D'AGOSTINO & al. 1992 when negative staining rhabdovirus particles of *Apium graveolens* var. rapaceum. In crude extracts they found many broken particles but also particles with nucleocapsids extruded.

The meagre knowledge of the GRVG leads to a classification "only as rhabdovirus virus-like particles" (FRANCKI & al. 1991: 260). In general plant rhabdoviruses are divided in two subgroups (A and B), which differ in replication and morphogenesis of virus particles in the cells. The subgroup A viruses replicate in the cytoplasm in association with a viroplasm. Morphogenesis occurs in association with vesicles of the endoplasmic reticulum. A nuclear phase appears to be involved in replication of some members; type species: lettuce necrotic yellows virus. The subgroup B viruses multiply in the nuclei. Large granular inclusions are thought to be sites of replication. Virus morphogenesis occurs at the inner nuclear envelope. Complete virus particles accumulate in perinuclear space; type species: potato yellow dwarf virus (FRANCKI & al. 1991).

Therefore GRVG seems to belong to the subgroup A. If GRVG-particles occur in vesicles of the endoplasmic reticulum attached to different organelles, then nuclei are unaltered.

In some mesophyll cells a conspicuous plasma region is noticeable, probably a viroplasm. It is surrounded by cisternae of the endoplasmic reticulum with GRVG-particles and contains large vesicles with fibrous content, endoplasmic reticulum and numerous small „vesicles". Similar vesicles can be observed in the dilated perinuclear space of lettuce necrotic yellows virus infected *Nicotiana clevelandii* (WOLANSKI & CHAMBERS 1971). The viroplasms described in literature consist of filamentous or granulated substance and nucleocapsids. They are sometimes budding into the endoplasmic reticulum, which surrounds the viroplasm (FRANCKI & al. 1985, JACKSON & al. 1987). In *G. asclepiadea* nucleocapsids are also found in a granulated area of the cytoplasm, however budding into endoplasmic reticulum cisternae is not observed.

In GRVG infected *G. asclepiadea* noticeable alterations of the nuclei are found only in two specimens. In a dilated perinuclear space nucleocapsids and complete GRVG-particles are embedded in a granulated matrix and thread-like structures. Similar alterations are described in *Dendrobium,* infected by rhabdovirus (LAWSON & ALI 1975). Budding of the nucleocapsids from the inner nuclear membrane has neither been observed in *Dendrobium* nor in *G. asclepiadea.* The rhabdoviruses of *Evonymus* found in some countries of Europe are similar in size, but differ in symptomatology and localisation in the cell. *Evonymus* collected in France and in Yugoslavia contains particles in the perinuclear space and in cisternae of the endoplasmic reticulum (JONSSON 1974, PLEŠE & ERIĆ 1980). *Evonymus* plants in Italy show the rhabdovirus particles only in the endoplasmic reticulum cisternae closely attached to chloroplasts and mitochondria (PISI & BELLARDI 1991).

Nucleocapsids are observed in the nucleoplasm of various plants after infection with rhabdoviruses (cf. FRANCKI 1973). In the nuclei of mottled leaves, leaves with vein clearing and cotyledons grown of infected plants nucleocapsids occur. In healthy *G. asclepiadea* no nucleocapsids are found.

In protoplasts inoculated with SYNV of FLSV nucleocapsids were observed in the cytoplasm (VAN BEEK & al. 1985 a,b). It is supposed that the nucleocapsids serve for the distribution of the rhabdovirus in the plant. In *Oncidium flexuosum* the penetration of nucleocapsids from the nucleus through the nuclear pores is described by LESEMANN & DORAISWAMY 1975. Also in *G. asclepiadea* the nucleocapsids seem to migrate through nuclear pores into the cytoplasm. Particles similar to nucleocapsids were found in *Camellia* showing symptoms of *Camellia* leaf yellow mottle disease (INOUVE & INOUVE 1975, HIRUKI 1985, MILIČIĆ & al. 1986). MILIČIĆ & al. 1986 suppose that CLYM disease is caused by viruses. These particles have a helical symmetry and an axial channel, and occur in the cytoplasm and rarely in the nucleus (GAILHOFER & al. 1988). The particles are absent in plants which lack symptoms. However, rhabdovirus-like particles are not known in *Camellia.*

The etiology of the disease remains yet unknown, because mechanical transmission of GRVG to *Nicotiana glutinosa* was unsuccesful. Several rhabdoviruses are not mechanically transmissible (JACKSON & al. 1987, PISI & BELLARDI 1991, D'AGOSTINO & al. 1992).

The development of GRVG could be elucidated by transferring of particles in protoplasts of suitable plants, which will be done in our next experiments. The development of festuca leaf streak virus (FLSV) and sonchus yellow net virus (SYNV) in protoplasts of *Vigna unguiculata* was studied by VAN BEEK & al. 1985 a, b.

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