Acta Bot. Croat. 45, 1—6, 1986.

CODEN: ABCRA2
YU ISSN 0365—0588

UDC 576.858.8:582.823(497.1) = 20

INFECTIOUS VARIEGATION VIRUS OF *CAMELLIA JAPONICA* L. IN YUGOSLAVIA

DAVOR MILIČIĆ, IRMTRAUD THALER and MANFRED GAILHOFER*

(*Department of Botany, Faculty of Science, University of Zagreb, and Institute of Plant Physiology, University of Graz)

Received November 24, 1985

More than twenty years ago the senior author found *Camellia japonica* trees in Opatija (Croatia) with colour breaking on the petals and symptoms of virus infection on the leaves. At that time it was known that this viral disease was transmissible by grafting but not mechanically.

During recent investigation on *Camellia* from Opatija we found viral symptoms again on leaves in the form of variegation, ringspots and necrotic rings, and on red petals in the form white spots. We performed electron microscopic investigations of infected tissues and in the young petals of *Camellia* we found bacilliform particles with dimensions of 30×150 nm. This virus had the same form and similar dimensions as the virus described by Hiruki (1985). In the virus, a helicoidal structure was observed which stretched all along the elongated particle. This virus was named Camellia infectious variegation virus (CIVV) by Hiruki.

Introduction

Camellia as a decorative plant

Beautiful and valuable trees of *Camellia japonica* L., mostly with intensive red flowers, are cultivated in several places along the Adriatic coast. Especially the parks of Opatija (Abbazia) are well known as agreeable places where *Camellia* trees have been in culture for over a century. In this region *Camellia* is in flowers from January to April when other decorative plants are still dormant (Domac 1955). Camellia likes moist atmosphere and therefore prefers shady places in parks (Adamović 1915, 1929).

Linné denominated *Camellia* according to the name of the Jesuit Camelli, who brought the plant to Europe from Manila in the 18th century. In its native country (Japan) there are varieties of *Camellia* with pink and white flowers.

The history of *Camellia* cultivation in Europe is closely connected with the city of Dresden where it was brought in 1771 (Encke 1960). During the 19th century Dresden was the center of *Camellia* production. More than 50,000 flower-pots yearly were cultivated there, so that cca 800,000 specimens of camellias of different ages were enumerated in Dresden in 1893. Afterwards the cultivation of camellias came to a standstill because of competition with azaleas.

It seems that camellias were imported to Opatija by seamen from oversea countries (Japan?) about the year 1844. In the parks of Opatija there are red, pink and white varieties of *C. japonica*. This plant is the pride of the parks of Opatija and it is used as the symbol of tourism of this town (Mandić and Srdoč 1982).

The culture of camellias in England began about the year 1800. The plant were cultivated in the open and a large number of precious cultivars were cultivated.

In distinction to Europe, where the significance of camellias is slightly diminishing, in some parts of North America (Florida, California) the interest for this decorative plant is growing. There are cultivated hybrids with other *Camellia* species.

The various sorts of camellias in Japan were studied by Hiruki (1985), who estimated that about a thousand various cultivars exist in that country. The first data about the culture of camellias in Japan derive from the seventh century. This country and the region of South Asia are the homeland of *C. japonica*. This species has also some relatives (see Encke 1960) which served for elucidation of the nature of infectious variegation of *C. japonica*. The best known relative is *Camellia sasanqua*.

Virus symptoms on *Camellia*

The alteration of the flower is especially interesting because it can be caused by infection with *Camellia* infectious variegation virus (CIVV) or by genetical factors. By means of grafting experiments it has been established that CIVV causes the colour change in red petals so that white flecks appear on them. This phenomenon is known under the name of breaking. Plakidas (1962) distinguishes four types of petal spots. The commonest type (CV 1) consists of large and round white flecks on red petals. Partial disappearance of colour on petals (breaking) appears also on trees with pink petals.

In addition to the changes on the petals, various virus symptoms appear on the leaves. These symptoms are yellow mottle, yellow rings and necrotic rings (Plakidas 1954 and others) (Fig. 1).

Graft-transmission experiments performed in many laboratories (Milbrath and McWhorter 1946, Plakidas 1962, Inouye 1982) were always successful.

The attempts to transmit the virus mechanically with infectious sap were always negative.

Material and Methods

The *Camellia japonica* L. plant used in this investigation for the preparation of ultrathin sections (Fig. 1 and 2) originated from First of May Gardens in Opatija. The plant used for the dip preparation originated from a private collection in Sarajevo. For the latter plant material we are thankful to Mrs. Devleta Buturović.

Petals of buds were cut into small pieces and fixed at 4°C for 90 min. in 3% glutaraldehyde buffered in veronal acetate, dehydrated in ethanol and embedded in Polarbed 812. Sections were poststained in uranyl acetate and lead citrate, and observed in a Siemens Elmiskop IA.

Petals of well developed flowers with the symptoms of breaking were also used for electron microscopy. Especially the white spots were investigated by Prof. Biljana Plavšić in a dip preparation using 2% phosphotungstic acid.

Results

In the spring of 1962 the first author visited Opatija and examined the plantations of *Camellia*. A large number of trees there showed large white flecks on red flowers. Moreover many trees had symptoms of infectious variegation on leaves in the form of yellow spots, vein chlorosis and chlorotic rings. Sometimes chlorosis covered bigger part of the leaf blade. Necrotic rings were rarely observed. The chlorotic symptoms varied very much. The most prominent symptoms were rounded white flecks on intensively red flowers. It seems that they belong to the first type of breaking symptoms (CV 1) according to the classification by Plakidas (1962). The presence of these symptoms showed that camellias of Opatija were often infected with CIVV.

During recent investigation, made in autumn 1985 and in spring 1986, we found the same symptoms again on *Camellia* from Opatija (Fig. 1).

The ultrathin sections of a flower bud from virus infected Camellia originating from Opatija were made by the authors from Graz (Austria). Figs. 2 and 3 show large or small groups of virus particles, which are mostly laterally aggregated. The particles were very similar to those described and illustrated by Hiruki (1985).

A single virus particle was bacilliform (Fig. 1). It was about 30 nm wide, but the length varied because many particles were cut obliquely in preparing the sections. Along the particles, optically dense transverse lines alternated regularly with approximately equally thick lines which were optically more translucent. We tried to count the optically denser lines and we enumerated about 15 turns or groups of turns in every virus particle. It seems that in every particle there is a helicoidal structure which stretches in close turns from one end to the other of the virus particle. The particle was similar to a segment of a tracheid of *Cormophyta;* it seemed that in submicroscopic dimensions a microscopic structure of plant body is formed.

When the particles are cut across as is the case with two particles in Fig. 2 (arrows), the middle part of particles appears optically more translucent and the peripheral one optically more dense.

Four particles of CIVV are presented in Fig. 9 by Hiruki (1985). These particles are parallelly arranged; they originated from a dip preparation treated with 2% uranyl acetate. It is clearly visible that a cen-

tral cavity is present along the middle part of each particle. Similar cavities exist in TMV and many other elongated viruses. This »axial canal« in TMV has a diameter of 4 nm. It seems that the central cavity of CIVV has a diameter of about 10 nm. The cross section of this cavity is visible in our Fig. 2 (arrows) and the longitudinal one in Fig. 9 by Hiruki (1985).

Fig. 9 by Hiruki is similar to Fig. 11 of the nucleoprotein of Newcastle disease, which is presented in the book by Fraenkel-Conrat (1985). This nucleoprotein is thinner than that of CIVV and is only 18 nm thick, while that of CIVV is about 30 nm. Besides, both nucleoproteins have a helically symmetry.

In spring 1985 many flower-pots of *Camellia* were imported to Zagreb from abroad. All plants had large and round white spots on intensively red petals. In order to investigate these plants we took a small quantity of plant sap from white parts of petals for a dip preparation. After staining with 2% phosphotungstic acid we observed a large number of virus-like particles. They were longer than the ordinary CIVV particles so they could represent longitudinal aggregates of this virus. We are thankful to Professor Biljana Plavšić (Sarajevo) for this information.

Discussion

In order to investigate the significance and expansion of CIVV, Hiruki (1985) examined about 600 cultivars of *Camellia* in Japan. He examined camellias from botanical gardens, universities, temples and a large number of private collections. From 600 inspected cultivars, 400 were completely healthy, one hundred had the genetic variegation of flowers and the remnant (less than one hundred) had virus symptoms on the flowers (breaking). It is necessary to stress that colour breaking was the most prominent virus symptom.

During infection with CIVV, the symptoms of yellow leaf mottle preceded those of breaking of flowers. It is known that genetic variegation is very common on *Camellia*; it is caused by spotaneous change of a genetic factor. Investigations have also shown that virus infection is relatively rare in varieties with white flowers, in which the symptoms are expressed on the leaves only.

After our poststaining in uranyl acetate and lead citrate, the helicoidal structure of CIVV was well pronounced. In respect to the helicoidal structure, CIVV is very similar to a virus of the fungus *Agaricus bisporus* (Lange) Imbach (cultivated mushroom). This virus has bacilliform particles 19 × 50 nm large and was named mushroom virus 3. The virus was first found by Hollings (1962) by means of metal shadowing techniques and was then studied by Leistner (1980) in preparation of ultrathin sections of carpophores of mushroom. Fig. 3 by Leistner (1980) shows that light and dark lines, which probably represent parts of a helicoidal structure, alternate along the virus particles.

With regard to the relation of CIVV to other elongated viruses of *Cormophyta*, CIVV — according to its form, dimensions and axial canal — resembles tobraviruses, hordeiviruses and, to some extent, tobamoviruses, but it differs considerably from them by the presence of the helicoidal structure described and illustrated (Fig. 2, 3).

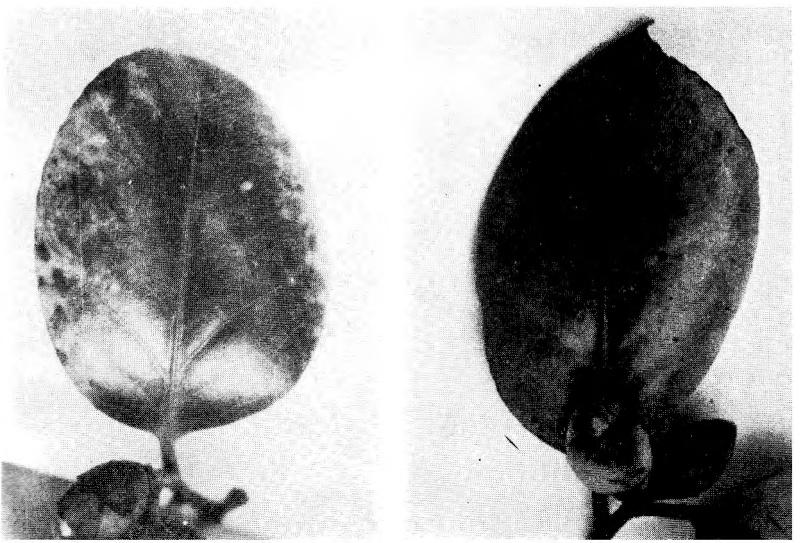

Fig. 1. Two variegated leaves of *Camellia* from Opatija.

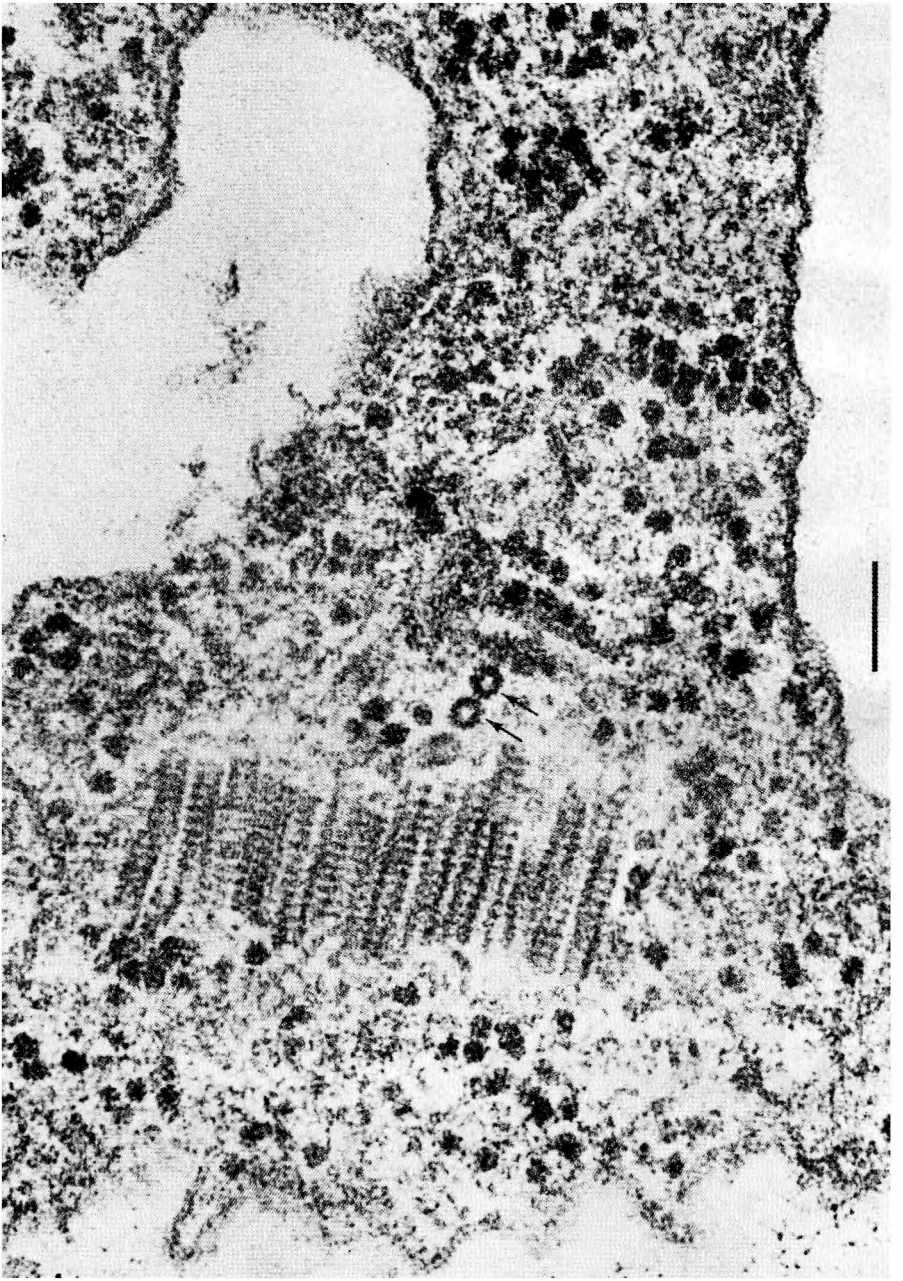

Fig. 2. Virus-like particles are arranged in parallel lines, two particles are sectioned transversely (arrows). Bar: 100 nm.

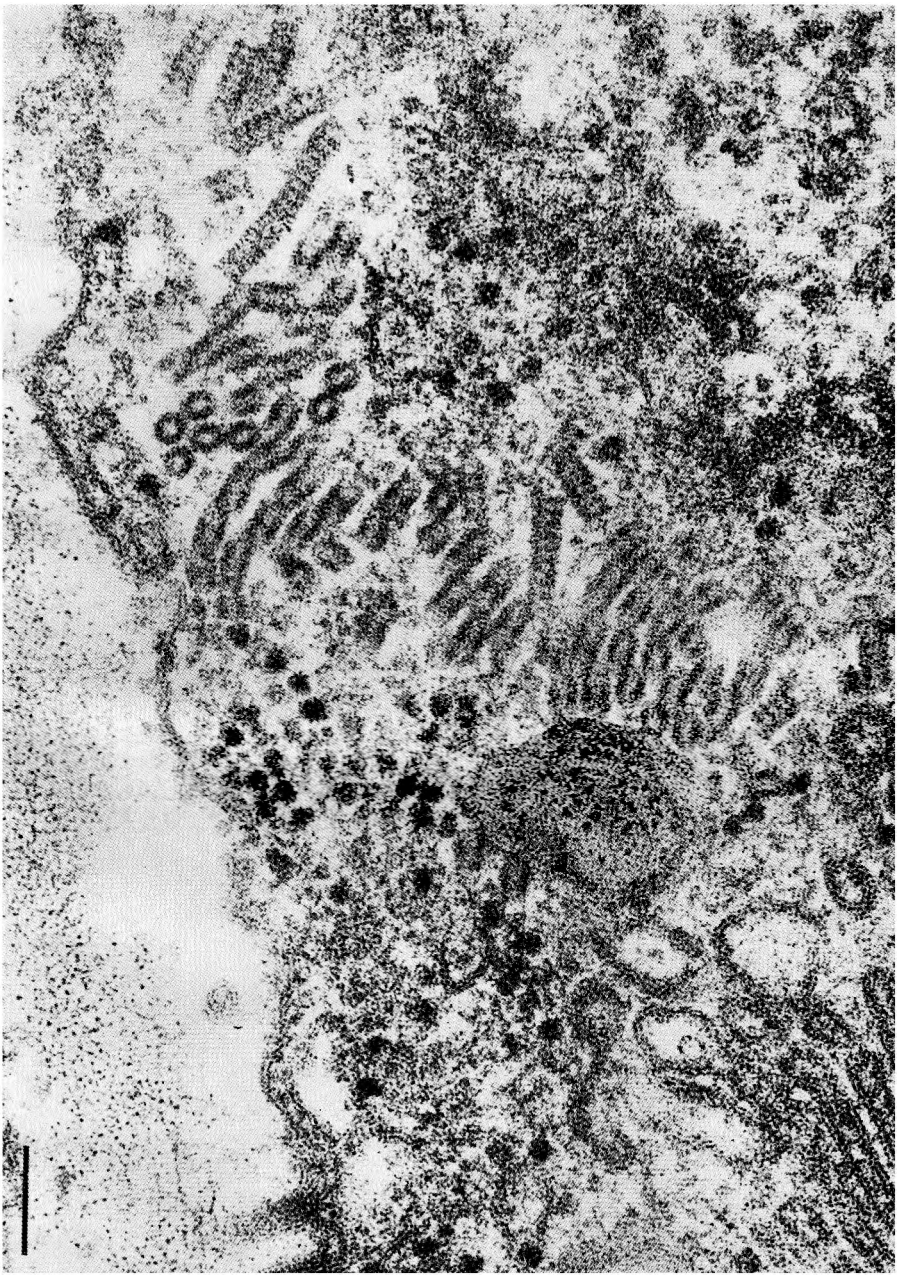

Fig. 3. Disarranged virus-like particles adjacent to the nucleus. Bar: 100 nm.

References

Adamović, L., 1915: Führer durch die Natur der nördlichen Adria mit besonderer Berücksichtigung von Abbazia. Hartlebens Verlag, Wien.

Adamović, L., 1929: Die Pflanzenwelt der Adrialänder. Gustav Fischer Verlag, Jena.

Domac, R., 1955: Opatija. Parkovi i šetališta. Hrvatska seljačka tiskara, Zagreb.

Encke, F. (Ed.), 1960: Pareys Blumengärtnerei. 2. Aufl., Paul Parey, Berlin und Hamburg.

Fraenkel-Conrat, H., 1985: The Viruses. Catalogue, characterization, and classification. Plenum Press, New York and London.

Hiruki, C., 1985: A preliminary study on infectious variegation of *Camellia*. Acta Horticulturae *164*, 55—62.

Hollings, M., 1962: Viruses associated with a die-back disease of cultivated mushroom. Nature *196*, 962—965.

Inouye, T., 1982: Graft transmission of virus-like symptoms of camellias. In Japanese. Ann. Phytopathol. Soc. Japan *48*, 117 (Abstract).

Inouye, T., and *N. Inouye*, 1975: Rod-shaped particles found in camellia leaves with necrotic ring spots. In Japanese. Ann. Phytopathol. Soc. Japan *40*, 133 (Abstract).

Leistner, H.-U., 1980: Elektronenmikroskopischer Nachweis von Viren in Fruchtkörper von *Agaricus bisporus* (Lange) Imbach in der DDR. Arch. Phytopathol. u. Pflanzenschutz, Berlin *16:* 153—158.

Mandić, O., i *V. Srdoč*, 1982: Opatija. Rivijera. 2. izdanje. Turist-komerc, Zagreb.

Milbrath, J. A., and *F. P. McWhorter*, 1946: Yellow mottle leaf, a virus disease of camellia. Amer. Camellia Yearbook, *1946:* 51—53.

Plakidas, A. G., 1948: Possibility of utilizing virus infection as means of producing new varieties of camellias. Amer. Camellia Yearbook *1948:* 107—109.

Plakidas, A. G., 1954: Transmission of leaf and flower variegation in camellias by grafting. Phytopathology *44*, 14—18.

Plakidas, A. G., 1962: Strains of the color breaking virus of *Camellia*. Phytopathology *52*, 77—79.

ZUSAMMENFASSUNG

INFEKTIÖSES SCHECKUNGSVIRUS AN *CAMELLIA JAPONICA* IN JUGOSLAWIEN

Davor Miličić, Irmtraud Thaler und Manfred Gailhofer

(Institut für Botanik, Naturwiss. Mathem. Fakultät der Universität Zagreb und Institut für Pflanzenphysiologie der Karl-Franzens-Universität Graz)

Von mehr als 20 Jahren fand der Senior-Autor in Opatija (Croatien) *Camellia japonica* Bäume mit Farbenbrechung an den Petalen und mit Symptomen der Virusinfektion an den Laubblättern. Damals war bekannt, daß diese Viruskrankheit durch Pfropfung, nicht aber mechanisch übertragbar ist.

Im Jahre 1985 fanden wir an *C. japonica* von Opatija wieder Virussymptome an den Laubblättern in Form von Gelbscheckung, ringförmigen Flecken und nekrotischen Ringen und weißen Flecken an den roten Petalen. Die elektronenmikroskopische Untersuchung ergab in jungen Petalen von *C. japonica* bazilliforme Partikeln mit Dimension 30 × 150 nm. Dieses Virus hatte dieselbe Form und ähnliche Größenverhältnisse wie das Virus von H i r u k i (1985). Das Virus zeigt über die ganze Länge eine schraubige Struktur. Es ist wahrscheinlich, daß dieses Virus ähnlich dem Virus von H i r u k i ist.

SAŽETAK

VIRUS INFEKCIJSKOG ŠARENILA KAMELIJE (*CAMELLIA JAPONICA* L.) U JUGOSLAVIJI

Davor Miličić, Irmtraud Thaler i Manfred Gailhofer

(Botanički zavod Prirodoslovno-matematičkog fakulteta Sveučilišta u Zagrebu i Institut za biljnu fiziologiju Sveučilišta u Grazu)

Prije više od dvadeset godina prvi autor ovog rada pronašao je u Opatiji kamelije *(Camellia japonica)* koje su imale simptome šarenila cvjetova (breaking) i simptome virusne bolesti na listovima. Već se tada znalo da se ta virusna bolest može prenositi cijepljenjem, ali ne može mehanički.

Prilikom nedavnih istraživanja na kamelijama iz Opatije pronašli smo ponovno simptome na listovima u obliku šarenila, prstenastih pjega i nekrotičnih prstenova te na crvenim cvjetovima u obliku bijelih pjega. Osim toga izvršili smo i elektronsko-mikroskopski pregled bolesnog tkiva, pa smo pronašli u mladim laticama virusne čestice baciliformnog oblika s dimenzijama 30 × 150 nm. Taj virus imao je jednak oblik i slične dimenzije kao virus koji je opisao H i r u k i (1985). U virusu je zapažena jasna helikoidalna struktura koja se pružala duž produženih čestica. Taj virus je H i r u k i (1985) nazvao virus infekcijskog šarenila kamelije.

Prof. dr. Davor Miličić
Botanički zavod
Prirodoslovno-matematički fakultet
Marulićev trg 20/II
YU-41000 Zagreb (Jugoslavija)

Prof. Dr. Irmtraud Thaler
Doz. Dr. Manfred Gailhofer
Institut für Pflanzenphysiologie
Schubert-Strasse 51
A-8010 Graz (Österreich)