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**CAMELLIA JAPONICA L. AND C. SASANQUA  
THUNB. — TWO HOSTS OF CAMELLIA LEAF  
YELLOW MOTTLE VIRUS**

**A REVIEW**

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Camellia leaf yellow mottle virus, CLYMV (Hiruki 1987) is the name of the agent which causes frequent symptoms of yellow mottle on the leaves of two camellia species, *Camellia japonica* and *C. sasanqua*. There are some difficulties in investigating this virus because up to now it has not been possible to transmit it mechanically but only by grafting.

A very important breakthrough was when T. and N. Inouye (1975) found the infective agent of CLYMV in *C. japonica*. The particles of this virus in *C. sasanqua* were found by Miličić et al. (1988). The particles were bacilli-form and about  $140 \times 30$  nm large. They had a helical structure.

During our present experiments we found that CLYMV particles had mostly 14 to 15 turns which run along a clearly visible central canal 10 nm wide. Subsequently we studied the aggregates which sometimes contained more than 200 virus particles being only laterally aggregated (Fig. 1). It seems that the impossibility to form true crystals is due to the fact that particle ends are not flat but somewhat irregularly rounded. In studying virus particles we have found that their size ranges from 120 to 170 nm accompanied by a different number of turns. During the research the presence of nucleic acid in virus particles was established. However, it remains unknown as yet whether CLYMV contains RNA or DNA.

Gailhofer et al. (1988) found virus particles in various parts of leaves of *C. japonica* and *C. sasanqua* and of the stamen of *C. sasanqua*. They described the anomalies and the collapse of the stamen, deformities of mitochondria and an electron lucent area in *C. japonica*.

## Structure and Properties of CLYMV

*Name of the virus*

*Camellia japonica* L. often has yellow spots on the leaves. Sometimes they can cover the whole surface of the leaf blade. This leaf yellowing is contagious and is of a virus character so that it can be easily transmitted by grafting from an infected plant to a healthy one. This virus was named camellia yellow mottle leaf virus by Milbrath and McWhorter (1946) and Inouye (1982), but the name was later modified to camellia leaf yellow mottle virus (CLYMV) by Hiruki (in schedis 1987). Some papers use the name infectious variegation virus for the same virus.

*Symptomatology*

According to Hiruki (1985) about 10% of *C. japonica* cultivars suffer from an infectious disease which is caused by CLYMV. Under the influence of this virus chlorotic flecks appear on the leaves in the form of a yellow variegation. The morphology of these flecks was excellently presented by Plakidas (1954, Fig. 1A-H). The same disease produces of white flecks (breaking) of a characteristic form on the red or pink petals (Plakidas 1954, Fig. 2B; 1962, Fig. 1—4). The symptoms of the disease are quite conspicuous so that it is easy to find infected plants.

As the symptoms of yellow mottle and breaking can disappear, it is useful to know that according to Inouye (1982) the cultivars Bonibotan and Hagoromo are reliable as the test plants because they often show yellow mottle symptoms on leaves and breaking on petals.

According to Inouye (1982) it is not sure yet whether the fairly frequent symptoms of necrotic rings are connected with CLYMV.

Hiruki (1985) gave a valuable review of the symptoms and spread of this disease in the world.

*Hosts and strains*

Plakidas (1954) has established, by means of grafting experiments, that CLYMV is transmissible to *C. sasanqua*, a very near relative of *C. japonica*. Consequently, CLYMV has two hosts: *C. japonica* and *C. sasanqua*.

It is worth mentioning that Encke (1960) cites ten species of the genus *Camellia*, among which are also *C. japonica* and *C. sasanqua*. It would be interesting to know whether some of the eight remaining camellia species can be hosts to CLYMV. Among these species the best known is *Camellia (Thea) sinensis* from which tea is obtained. This last plant is often cultivated in botanical gardens. As *Thea sinensis* has white flowers, obvious yellow symptoms can appear only on the green leaves.

As the form of white flecks on petals is inheritable, it is possible to characterize the strains of CLYMV by means of flower breaking symptoms. The first common strain of CLYMV forms round or oblong white flecks on the petals (Plakidas 1954, Fig. 2B; 1962, Fig. 1C). Virus infected exemplars of the variety of Ville de Nantes exhibited two distinct types of virus infection suggesting the presence of two different strains of virus. One of those strains showed large irregular white splotches and was named severe (Plakidas 1962, Fig. 1A). The other caused a very mild pattern forming only some white points on the petals

(Plakidas 1962, Fig. 1B). The fourth strain was found in the variety Adolph Audisson and was named special (Plakidas 1962, Fig. 1D). This strain is rare, therefore the plants are more prized and characterized by a wavy or feathery pattern.

#### *Transfer of virus by grafting*

As CLYMV is not mechanically transferrable to other *Camellia* plants, it was necessary to develop methods for transmission by grafting. Using these methods several authors transmitted the disease from infected to healthy plants (Milbrath and McWhorter 1946, Plakidas 1954, Inouye 1982). Very illustrative results were obtained by Plakidas (1954). After unsuccessful mechanical transmission trials with CLYM, Plakidas (1954, Fig. 2A) used as rootstock a healthy *C. sasanqua* which had two graftable branches. Healthy scions of *C. japonica* were grafted on one branch and infected scions on the other one. The scions easily coalesced with the rootstock. After a period which lasted 3.5 to 16 months, symptoms of disease appeared on the scions which had been healthy until then. This was the confirmation that the yellow mottle and variegation are of infectious nature and that the same agent could infect *C. sasanqua* and *C. japonica*.

Plakidas (1954) used many cultivars of *C. sasanqua*, for instance Hinode-gomo, Hiodo-shi, Shishi Gashira and Super Rosea, which were infected with CLYMV. Those isolates of the virus were all transferred to healthy scions of *C. japonica*. In horticultural practice *C. sasanqua* is very often used as rootstock for the cultivation of *C. japonica*.

#### *Some data about the particles of CLYMV in Camellia japonica*

In 1975 T. and N. Inouye found particles of CLYMV by electron microscope for the first time. The virus particles had the form of straight rods which were 140 nm long and 30 nm thick. However, a lot of particles was longer or shorter, but the thickness of particles was always stable and measured 30 nm.

The particles can occur singly, as shown by Miličić et al. (1986) in Fig. 3, or they can form aggregates of a large number of virus particles (Fig. 1; s. Miličić et al. 1986, Fig. 2). This form of aggregates is called »lateral aggregates« and it appears in the mesophyll of infected camellias. As noted by T. and N. Inouye (1975), there is also an additional form of virus inclusions in which the virus particles are not parallelly but irregularly arranged and are similar to a whirlpool. These virus inclusions are very rare. We noted this form of inclusion only once.

The virus particles are well visible after staining with phospho-tungstic acid which was applied by T. and N. Inouye (1975). During our investigation we used uranyl acetate and lead citrate (s. Miličić et al. 1986, p. 3) for staining the particles of CLYMV.

It must be pointed out that along the middle part of virus particles a central canal stretches which is 10 nm wide and it is usually quite transparent. It is specially well visible when the virus aggregates are sectioned transversally (Fig. 1 right).

The CLYMV particles were studied in *Camellia* materials from many localities which are very distant one from another: in Japan, Canada and Central Europe. Therefore, it seems that some of the main properties of this virus are already established.

Hiruki (1985) examined about 600 cultivars of *C. japonica* from various parts of the world. Of these, less than a hundred cultivars had virus symptoms. Thus, about 10% of cultivars are infected with CLYMV. The disease is spread on all continents where the conditions are favourable for *Camellia* cultivation.

#### *Finding of particles of CLYMV in Camellia sasanqua* Thunb.

*C. sasanqua* is not only used as rootstock for the cultivation of *C. japonica* but also as a very precious decorative plant (s. Encke 1960). Therefore this species is cultivated independently in the Botanical Gardens of the island of Lokrum near Dubrovnik. This plant flowers from November to March, i.e. a little earlier than *C. japonica* which flowers from January to April. *C. sasanqua* originates from the south Japanese island of Kyushu.

Early in the year 1987 Thaler and Gailhofer had an opportunity to micrograph the material from *C. sasanqua* gathered in the Botanical Gardens of the island of Lokrum. In the tissue of *C. sasanqua* infected with CLYMV a lot of rod-like particles were found (s. Miličić et al. 1988, Fig. A). The particles were singly and irregularly dispersed in the cells. The material originated from the region of stamen. The virus particles showed very clear spiral structure and contours of the central canal. In the cells some round vesicles about 60 nm large were also seen. This finding shows that not only *C. japonica* but also *C. sasanqua* contains rod-like particles characteristic of CLYMV in the cells of infected plants.

Even before after Plakidas (1954) it was known that CLYMV could infect *C. sasanqua*. However, at that time electron microscopy was not yet enough developed so that only in 1975 virus particles in *C. japonica* were found. The particles of the other host of CLYMV, i. e. *C. sasanqua*, were observed in the electron microscope only (more) recently.

In the cells of *C. sasanqua* we found helical structures (Fig. 2 b) near the cell walls. These helical bodies were thinner than the virus particles and a little smaller (cf. also Miličić et al. 1988, Fig. C). Moreover, the spiral character was more remarkable than in virus particles. About the significance and meaning of these helical structures we can say nothing as yet.

#### *Lateral aggregates of virus particles*

Like many other viruses, CLYMV also builds aggregates of virus particles but they are scarce. Already Hiruki (1985, Fig. 10) observed an aggregate of virus particles in the leaves of *C. japonica*. Afterwards Miličić et al. (1986, Fig. 2) found an aggregate of virus particles in a flower bud. In both preparations the particles were longitudinally sectioned. Those micrographs showed that the particles were laterally aggregated (Fig. 1).

As in both preparations of Hiruki (1985) and Miličić et al. (1986) the particles were longitudinally sectioned we bring now a preparation in which the aggregates are cross sectioned. On Fig. 1 right, a large aggregate is presented in which the particles are cross sectioned. This figure is specially interesting because it contains in the left part of Fig. 1 also an aggregate which is visible in longitudinal section.

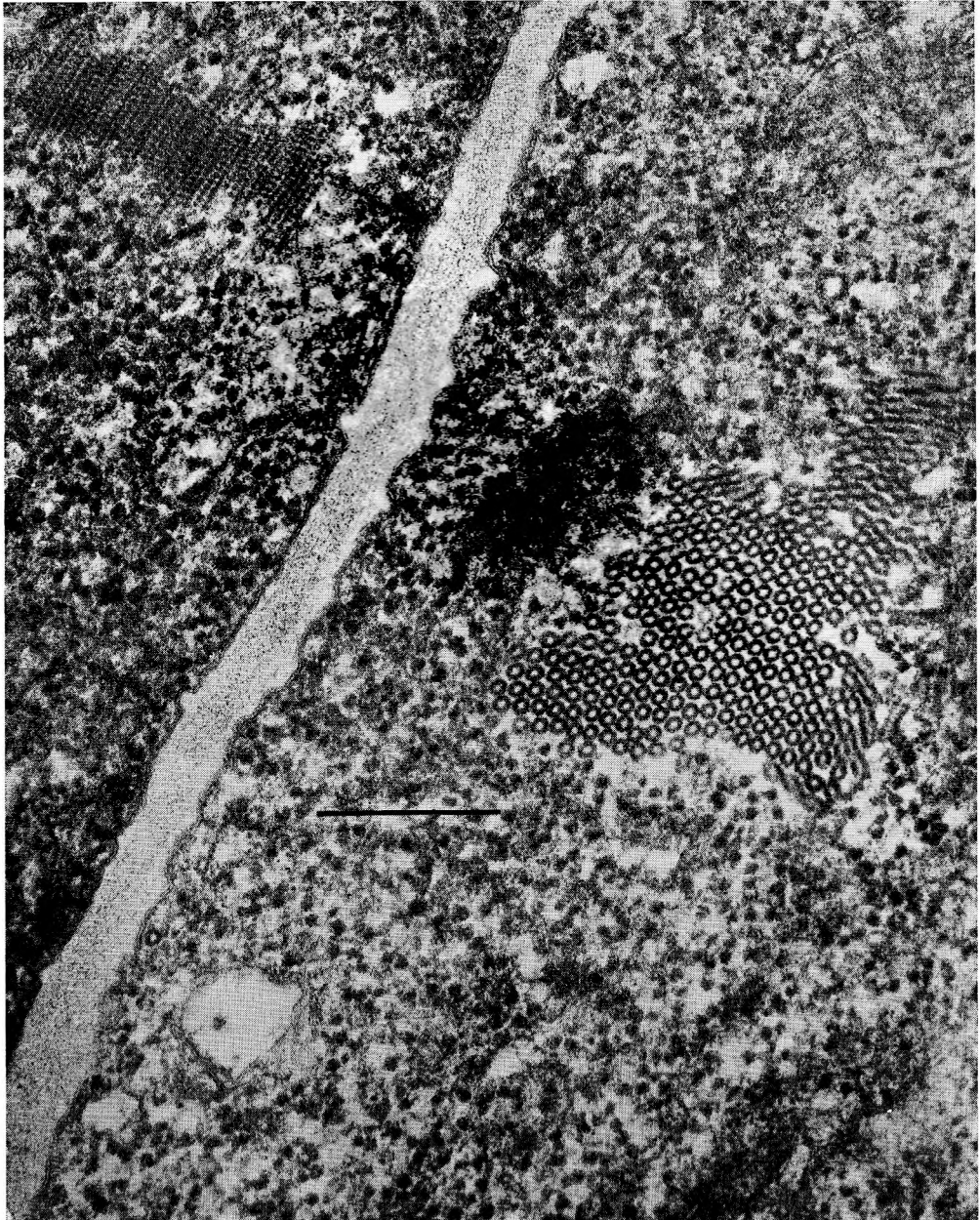


Fig. 1. Lateral aggregates of CLYMV particles. In the left cell a large longitudinally sectioned aggregate. The ends of particles are not aligned. In the right cell, the aggregate is cross sectioned. Bar = 300 nm.

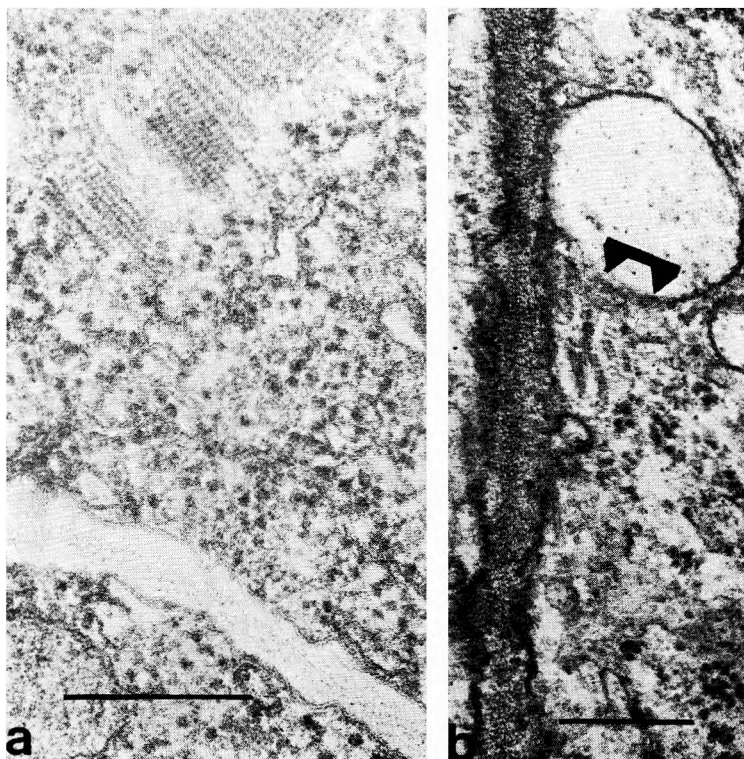


Fig. 2. a. Lateral aggregate in side view. Stained with uranyl acetate and lead citrate. It is visible that dark and light lines alternate in the turns. Bar = 300 nm. b. *C. sasanqua*. Small helical structures can be seen in some places near the wall (arrowheads). Bar = 200 nm.

The cross sectioned aggregate presented in Fig. 1 contains about two hundred virus particles which show a partially regular, paracrystalline arrangement of particles. The white dot in the middle of every particle represents the central canal of the particles, but the peripheral parts of the particles are black.

It is interesting that the ends of particles of the longitudinally sectioned aggregate (Fig. 1, upper left) are not aligned but are very irregularly distributed. It is probable that the surface of particles is not smooth but uneven in some places. It seems, that the central part of turns of virus particles protrudes outside forming a protuberance which hinders the movement of the virus particles.

If we observe the lateral aggregates of Fig. 1, upper left, attentively, we shall see that in every elongated virus particle there is about 15 turns. The number of turns is relatively easy to see. However, it is more difficult to see that inside of the turn one part of the turn is light and the other is a little darker.

The relations are clearer on Fig. 2a, where is also presented one lateral aggregate. However, in distinction from the first lateral aggregate this second one is very thin sectioned so that the relations are more easier to understand. On Fig. 2a the particles of this second form of virus aggregate are presented.

#### *Recent investigations*

Gailhofer et al. (1988) performed cytochemical investigations in order to establish whether RNA or DNA was the genetic substance of CLYMV. During these experiments they used various enzymes and chemicals which dissolved the objects partially but the results were not reliable.

Recently Martin and Kim (1987) have found by means of Bernhard's regressive staining technique that an interesting virus of mimosa (*Albizia julibrissin* Duruazzini) belongs to the group of DNA viruses. Therefore we think that it would be useful to apply colour reactions also in the analysis of CLYMV.

Gailhofer et al. (1988) continued investigations of the spread of CLYMV particles in camellia tissues. During this research the virus particles were found in the upper and lower leaf epidermis and in the mesophyll. Once the virus particles were observed also in the nucleus. In the mesophyll of *C. japonica* lateral aggregates are sometimes present, which occasionally contain over two hundred virus particles (Fig. 1 right).

Gailhofer et al. (1988, Fig. 1) have micrographed a large part of the stamen of *C. sasanqua*. The figure shows that the stamen contains many cells filled with virus particles. However, the turns in the virus particles are densely packed. Only in some places are the turns pushed aside leaving small interspaces.

This richness of virus particles gave us the idea that the region of stamens could serve for isolation of CLYMV to suitable test plants. A successful transfer to the test plants and analysis of the test plants could teach us very much about many important properties of this interesting virus.

During electron microscopic investigations Gailhofer et al. 1988, Fig. 2, studied an interesting anomaly in the region of stamen. In this place numerous of virus particles were laterally aggregated and more or

less loosely arranged. It was specially interesting that some virus particles were partially or completely unfolded (s. figure).

The virus particles of CLYMV consist of turns which form the living part of the virus. These turns are 10 nm thick and wrap the round central canal. In order to unwind the turns, it is necessary to interrupt the lateral connections between the neighbouring turns. It seems that these connections in some cases become dissolved and then the turns can be separated. The detached turns have the form of an elongated structure which is about 10 nm thick.

Gailhofer et al. (1988, Fig. 5) found interesting anomalies on mitochondria which were provoked by CLYMV. Some mitochondria were giant and measured nearly 1000 nm in length. As the end of this mitochondrion had the form of a cup, it was named »cup-like mitochondrion«.

That mitochondria can be rather deformed under the influence of viruses, was pointed out especially by Hatt a et al. (1971). Under the influence of various viruses deformations of mitochondria can appear and their volume can be enlarged. The deformations described by Hatt a et al. (1971) appeared under the influence of cucumber green mottle mosaic virus.

An electron lucent area was found by Gailhofer et al. (1988, Fig. 4) in young petals of *C. japonica*. In the vicinity of electron lucent area, loosely arranged and uncoiled virus particles were found. This anomaly was accompanied also by a small number of ribosomes in the vicinity of this area. There was also an area of very fine fibrils. The appearance of electron-lucent places is characteristic of some groups of viruses, e. g. orchid fleck virus (D o i et al. 1977).

## Discussion

Although CLYMV is a very interesting virus and *C. japonica* and *C. sasanqua* valuable decorative plants, nevertheless the virus has not been sufficiently investigated. It seems that the reason for this situation is that this virus occurs in host plants in a low concentration so that sometimes it is necessary to spend much time in order to find virus particles. Quite recently Gailhofer et al. (1988) have found many virus particles in the stamina of *C. sasanqua*. They are probably also present in *C. japonica*. However, it is easy to find diseased plants because the symptoms are frequent on the leaves and flowers of camellias.

It would be very important to transmit the camellia virus to test plants. Firstly Plakidas (1954) tried to transmit it to herbaceous test plants by means of carborundum, but the trial was unsuccessful. Afterwards Hiruki (1985, p. 57) attempted to infect *Chenopodium amaranticolor* and *Nicotiana clevelandii* with CLYMV but the results were negative. The possibility of using the test plants could considerably help to gain an insight into the properties of CLYMV.

In order to point out some similarities or relations among the viruses related to CLYMV we bring here Table 1. In this table CLYMV is compared with Orchid fleck virus (OFV) and with Cacao Shoot Virus Cluster. The common properties of these two viruses and virus cluster are that they are about 30 nm thick but the length is variable and ranges between 80 and 180 nm. Consequently they are moderately elongated viruses.



As it is visible from Tab. 1, the turn of nucleic acid of CLYMV is about 10 nm long. It seems that this turn is relatively very long. The pitch of OFV is 4,5 nm long and is also large (Doi et al. 1977, Lesemann and Bergtrup 1971). The same pitch of 4,5 nm have the largest elongated rhabdoviruses which dimensions are 160—380 x 50—95 nm (data from Bos 1983).

Table 1. Properties of virus particles

<i>Camellia Leaf Yellow Mottle Virus</i>	<i>Orchis Fleck Virus</i> (Thin sections)
30 x 120—170 nm	32—35 x 100—140
Electron lucent?	Electron lucent
Turn 10 nm	Pitch 4,5 nm
<i>Cacao Swollen Shoot Virus Cluster</i>	
23—31 x 80—180 nm	
Without visible substructures	

The third agglomerate of viruses named Cacao Swollen Shoot Virus Cluster with the above mentioned dimensions was established by Franki et al. (1987, II, p. 237). This group has about 7 elongated viruses which have similar morphology. However, there is no visible substructure in the particles. Neither are there any data available on their nucleic acid and their proteins. This virus cluster is not completely characterized. The members of this cluster differ very much from the structure of CLYMV.

It is certain that these three virus units, which are compared in Tab. 1, have few common properties except the dimensions.

Note: A. A. Brunt (1988: 506) considers that the morphology and the size of particles of CLYMV suggest that this virus might have affinities with tobacco stunt and lettuce big vein viruses, both of which are spread by *Olpidium brassicae*.

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## ZUSAMMENFASSUNG

CAMELLIA JAPONICA L. UND C. SASANQUA THUNB., ZWEI WIRTE VOM VIRUS  
DER GELBEN BLATTFLECKIGLEIT DER KAMELIE

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Vor drei Jahren hat der Autor mit I. Thaler und M. Gailhofer in dieser Zeitschrift die Arbeit »Infektiöses Scheckungsvirus in *Camellia japonica* L. in Jugoslawien« betitelt war, veröffentlicht. Jetzt veröffentlicht der Autor eine zweite Arbeit über dasselbe Virus, dessen Name in der Zwischenzeit verändert wurde: heute hat den Namen »Virus der gelben Blattpfleckigkeit der Kamelie«. Dieses Virus hat zwei Wirte, *Camellia japonica* und *C. sasanqua*, und ist weit verbreitet. Das Virus konnte bis jetzt nicht mechanisch, sondern nur durch Pfropfung übertragen werden.

Während die Dicke der Virusteilchen beständig ist und 30 nm beträgt, ist die Länge der Partikeln veränderlich und schwankt von 120 bis 170 nm, die häufigsten Längen 140 und 150 nm betragen.

Die Viruspartikeln wurden in beiden Wirtspflanzen (*C. japonica* und *C. sasanqua*) gefunden. Bei *C. sasanqua* befinden sich die Partikeln in den Staubblättern und in beiden Epidermen und im Mesophyll des Blattes. Sehr interessant sind die Staubblätteranomalien, die Deformationen von Mitochondrien und die Anwesenheit von einer »electron lucent« Zone.

## SAŽETAK

CAMELLIA JAPONICA L. I CAMELLIA SASANQUA THUNB. — DVA DOMAČINA  
VIRUSA ŽUTOG ŠARENILA LISTA KAMELIJE

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Prije tri godine objavili smo u ovom časopisu rad pod naslovom »Virus infekcijskog šarenila kamelije u Jugoslaviji«. Sada objavljujemo drugi rad o istom virusu. Taj virus još nije uspjelo prenijeti mehaničkim putem nego samo cijepljenjem. U međuvremenu je virus dobio drugi naziv. Danas ga nazivamo »virus žute pjegavosti lista kamelije«. On ima dva domaćina *C. japonica* L. i *C. sasanqua* Thunb.

Dok je debljina čestica stalna i iznosi 30 nm, dotle je dužina čestice najčešće 140 do 150 nm, ali ima ih i od 120 do 170 nm.

Virusne čestice su pronađene u oba domaćina. U listovima *C. sasanqua* nalaze se u epidermi i mezofilu. Zanimljive su anomalije prašnika, mitohondrija i pojava »electron lucent« područja u plazmi.

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