

Virus Diseases of *Ginkgo biloba*?**Virová choroba jinanu dvojlaločného?**

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Abstract — On trees of *Ginkgo biloba* L. in Czechoslovakia and in Szeged (Hungary) different symptoms were found which suggested virus diseases: 1. Yellow stripe mosaic between the main veins. 2. Deformations of leaves. 3. A terminal leaf forms a cowl (ascidium) from one lobe, as the second lobe of the leaf is normal. The cowl has the shape more of an infundibulum and is 4.5 centimetres long.

After several years of study of the virosis of common spruce (*Picea excelsa*) (SMOLÁK 1947) I became interested in the virology of other trees of the group *Gymnospermae*, namely of *Ginkgo biloba* L. There are only a few of this remarkable species in our parks and gardens and therefore research on its virus diseases is rather difficult. The Reports of the Dendrological Section from 1958 state that there are only 63 known specimens of *Ginkgo biloba* in ČSSR (SVOBODA A. 1958). The list was made according to the questionnaires from 1927 and completed in 1957. I found, however, that a number of places where *Ginkgo* trees grow are still missing in the list (revision of the list is desirable). The subjects of my five years' study were mainly three *Ginkgo* trees in the park of the Hlávka foundation at Lužany near Plzeň, a tree about 15 metres tall in front of the Agricultural School at Louny, reported in "Zprávy dendrologické sekce", *Ginkgo* trees at Kutná Hora and the very remarkable avenue of *Ginkgo* plantation in Szeged in the South of Hungary. In addition I observed specimens at Potštejn, in a private garden in Praha-Vokovice and in the Praha Lobkowitz park.

Symptoms

The symptoms which first drew my attention to viroses were only observed by me in 1949 on the tree growing opposite the home of Prof. U. Jarník at Potštejn. But I found more marked symptoms on one of the two younger trees planted (by acad. Prof. B. Němec) in 1958 at Lužany at a distance of about 4 metres from each other.

1. Symptoms which may be called yellow stripe mosaic are as follows. They appear mainly in the summer months (VII.—IX.) in 1958 on the leaf blades in the form of very fine yellowish stripes, alternating paralelly with normal dark green stripes of the blade. There were many yellow striped leaves on these two specimens at the beginning of September 1958. Some of the blades had brown necrotic spots on their margins, and, according to the size of the dead parts, they were rolled up to renew the symmetry because of the morphostesy of leaves. The leaves of a much older tree, planted in the shade

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of a lime tree at about 5 metres distance from the two young *Ginkgo* trees, showed the yellow stripe mosaic only to a very small extent.

The yellow stripe mosaic of the *Ginkgo* tree often begins with relatively very small spots which do not strike the observer. Between two parallel veins a round yellow spot appears and below it a very fine yellow stripe passing along the vein. The stripe usually ends with disturbed mesophyll, which may give rise to a fine perforation. Deeper incisions with necrotic edges are often formed at the margin of the leaves near the stripes. Sometimes it is an intercostal perforation with a necrotic dying off of the tissues from which a yellowish stripe leads up to the margin of the leaves. These details were also observed on the Kutná Hora *Ginkgo* tree in a small garden of the Teachers Training College. The yellow stripes on the leaves become more or less brown, later on they tear.

Yellow stripe mosaic appeared most strikingly, as far as I could find out on the trees of the Szeged Avenue where one side has 25 trees and the other at a right angle 12 (Plate I, Fig. 1).

2. Other symptoms of disease in *Ginkgo* are deformation of leaves as shown in the picture (Plate I, Fig. 2). They were first found on one of the young trees in the park of Lužany. Some of the leaves are considerably dwarfed, and the blade is more or less abnormal in shape, being sometimes broadened, and with its tip shortened as if gnawed off. In some instances one lobe may partly cover the other as in the case of other viroses (e.g. on hop leaves). In other instances the distance between the lobes is considerable and the incisions deeper, so that the form of the leaf becomes almost "forma laciniata".

The number of deformations of the leaves in the Lužany Park differed from one tree to the other, even though they are only 4 metres apart. One of them shows symptoms of viroses (yellow stripe mosaic and deformations of leaves) to a greater extent than the other one which is healthier, though both grow under the same conditions. Therefore it is impossible to consider the deformation of the leaves as a result of spring frost. Differences of development of the two trees could already be observed during the spring shooting. The buds of the more infected tree were later in opening than those of the healthy one and continued to do so irregularly during the whole month of May. The shooting of the lateral buds was particularly slow and weak.

The Szeged trees in Hungary provided me with more important material for study. It is a double avenue, the only one of this kind that I have every met with, having 37 *Ginkgo* trees, each 4–5 metres tall. The majority of them are male, only a few are female and fertile. The yellow stripe mosaic shown in Fig. 1 was very striking here, more intensive than on the younger trees at Lužany.

3. I acquired 20 ripe seeds from the Szeged female trees, affected with yellow stripe mosaic, and had them planted each in one flower-pot in the greenhouse of the Institute of Plant Physiology of Caroline University in spring 1960. In spite of every care (by instructor J. Oppelt) only 6 stone fruits out of 20 germinated. The 14 which had not germinated finally rotted in the soil. The *Ginkgo biloba* seeds are known to be frequently subject to slimy decay with a terrible smell, similar to the smell of decayed teeth. This rot of *Ginkgo* seeds would be worth a more detailed study if there were more material at hand.

The six plants, getting on well, differ in size though they are grown in flower-pots under the same conditions. As the beginning of October 1960 five plants were about 10 centimetres high. Many of the leaves were already showing autumn colouring and were just beginning to fall off. The green leaves, not yet becoming regularly yellow, showed signs of yellow stripe mosaic. The sixth plant germinated last, and at above stated time, had attained only half the size (5 cm.) of the other five. It had two shoots (stems) growing out of the same seed. The leaves of this double specimen were and still are dark green, smaller than those of the other plants and slightly deformed. After two years of observation the plants from Szeged were as follows (16. 7. 62): The growth was irregular, the five stronger ones reached 45 cm., 40, 38, 31 and 21 cm. The specimen with two axes (vegetative shoots) had stems 12 and 8 cm. resp. high.

Observation on 7th September 1962 showed that the former plants had grown several centi-

meters each (in 7 weeks). The growth of the two-stem specimen was negligible. Each of the two stems grew only 1 cm and their leaves kept the striking dark green colouring and a tougher consistency.

All these plants show interesting symptoms, the most important of them on the specimens that are 40 and 45 cm. tall. On both of these the four top leaves growing crossways under the terminal bud are as if withered and limp, with the margins turned down like those of vine leaves affected by virus roll diseases. The other leaves, however, are more or less normal upright. One of the leaves of the four leaf top whorl is quite remarkable. One of the lobes has turned into a cowl, while the other lobe remains normal (Fig. 1). This ascidium, originating from half of the blade, is 4.5 centimetres long, in the shape of a funnel with an orifice of 10×12 millimetres diameter, so that it may be better called an infundibulum, rather than a cowl. The structure may be described as funnel-shaped even though it is classed as an ascidium in the symptomatology of viroses. According to my observation the *Ginkgo* cowl grows only slightly, its length and diameter after a month being only a few millimetres.

The rolling of the top leaves of the two largest plants increased as well and some of the leaves of these infected plants are remarkable. The incision between both lobes goes in some cases as far as the petiole, the lobes being bent far from each other so that the transformed leaf gives the impression of a compound leaf with two leaflets.

There is an extensive literature referring to cowl. In our country KLÁŠTERSKÝ I. (1949, 1951), has dealt with this problem. Besides, many observers have noted cowl (funnel)-shaped leaves. As early as 1938 BAYER A. considers a cowl-shaped leaf "a remarkable phenomenon" (Naučný slovník ovocnicko-vinařský a zahradnický 1938). PŘÍHODA A. (1949) reports on cowl on lime trees and on pelargonium, BLATTNÝ C. and OSVALD V. (1950) discuss cowl in spinach and cauliflower and VALENTA V. (1950) cowl in begonias. KLÁŠTERSKÝ (1951, pg. 104) points out that the leaf ascidium never originates from growing together of edges of the blade, but "congenitally" and that "A perfectly developed cowl is the most characteristic and surest symptom of the presence of this type of virus". I am convinced that in *Ginkgo biloba* L. cowl or infundibulum is a sure symptom of virosis especially when at the same time other common symptoms of virosis appear on the same plant.

Ascidium of *Ginkgo biloba* points to another fact. The seeds came from the trees in Szeged showing the above described yellow stripe mosaic. Thus it is almost certain that the virus of cowl in this case was carried by the seed, for it is impossible to assume that the cowl virus infected the young seedlings in the greenhouse in Praha. It would be technically very difficult to investigate

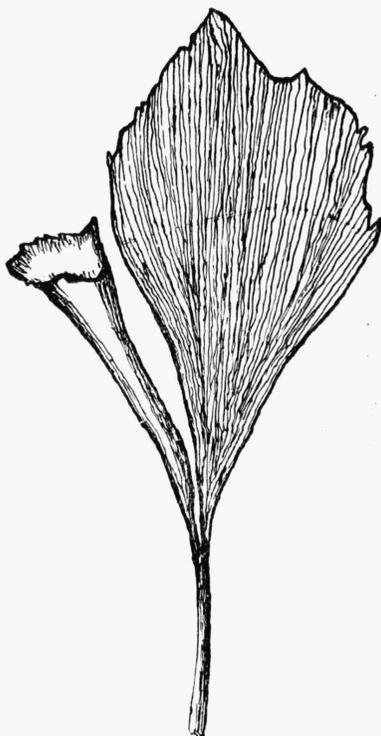


Fig. 1. — Ascidium (cowl) of *Ginkgo biloba* L. The lobe of the leaf is changed into a cowl-infundibulum. The second lobe is normal. On the *Ginkgo* seedling in the greenhouse of Caroline University. The seed of this seedling from Szeged. Orig. Sm.

the crowns of 37 comparatively tall trees for cowl formations nor would hardly anyone suppose that leaves with ascidium forms would occur on gymnosperm trees.

The high percentage of seeds of *Ginkgo* tree which did not germinate was also remarkable in our experiment (14 out of 20 seeds i.e. 70% did not germinate). Preliminary tests for germination are difficult with such rare material. Nor can it, naturally, be claimed that 14 out of 20 stone seeds did not germinate because their embryos were destroyed by a virus infection. It is not impossible but so far there is no proof for it. Nor are the causes of frequent rot in *Glinkgo* seeds known as yet.

4. Another serious symptom of *Ginkgo* virosis is the presence of leaf enations. I observed them in two places on a younger tree at Lužany and on an older one at Kutná Hora (garden in front of the former Teachers' Training College). This phenomenon as far as I could ascertain, does not occur on any healthy tree (Bílina, Praha 6, Kutná Hora Brauer garden). In the Brauer garden at Kutná Hora, which has only recently been opened to the public, there is a *Ginkgo* remarkable for its exceptionally healthy state. It is about

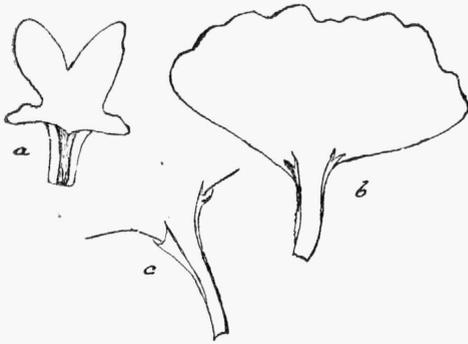


Fig. 2. *Ginkgo biloba* L.: a) Membrane wings of the petiole, b), c) Enations (excrecences) on the lower surface of leaves. From Kutná Hora. Orig. Sm.

18–20 metres high and divides at a height of 1.5 metres into two strong upward-growing branches with healthy dark green leaves throughout, most of which are of considerable size. The breadth of these leaves may be as much as 120 millimetres, the blade and petiole 100 mm and 90 mm long respectively. None of the ascertained symptoms of *Ginkgo* virosis has been observed on this tree. Another *Ginkgo* tree of about the same age in another part of the town (in front of the former Teachers' Training College) presents an entirely different appearance, that of a diseased tree. A certain kind of enation appears on the leaves which are greyish-brown in colour and smaller in size. The affected leaves have strikingly shorter petioles of about 10–28 millimetres with membranous wings on both sides that soon dry up and fringe. Where the petiole joins the blade, there grow on both sides green fork-shaped enations (Fig. 2), measuring 10–12 millimetres. They occur on both sides of one of the membrane wings of the petiole, they are green, fibrous and partly adnate to the edge of the blade. The fork-shaped points of these enations are free. As virus enations are comparatively rare symptoms in angiosperms, but are known from the leaves of sweet cherries and morello cherries, they are even more noteworthy in gymnosperms plants, where the study of virus infections is in its early beginnings.

Results and discussion

The symptoms of diseases described on *Ginkgo biloba* are suggestive of viroses. Even if the symptoms may be often diagnostic value we must admit

that in the case of these diseases a virus infection is not yet proved. To do that is the task of further investigation.

If the yellow stripe mosaic, the deformations of leaves, the cowl and the enations, all on the tree *Ginkgo biloba*, are in fact viroses, *Ginkgo biloba* would be included for the first time in the literature of virology, because, as far as I could find out, there is no mention of any virus disease of this gymnosperm plant in any Czechoslovak or foreign papers.

Generally it can be stated that this gymnosperm plant of great geological age, only very remotely related to angiosperm plants, reacts in its large fanlike leaves to virus infection almost in the same way as angiosperm plants do. Yellow stripe mosaic of *Ginkgo biloba* is similar to the virus stripe mosaic in monocotyledonous plants with parallel veined leaves, where the light coloured parts have a tendency to become elongated. We may consider the size and shape of ascidium, formed by one lobe of leaf, as an exception. The length and volume of this ascidium or infundibulum (4.5 centimetres and the ellipse-like mouth 12×10 millimetres) exceed those of any other ascidia known in our country.

Morphological malformations have attracted the attention of the botanists for a very long time. The cowl formations of *Ginkgo biloba* were described already in the Penzig's Pflanzen-Teratologie (PENZIG O. III, 2. ed. 1922, pg. 517) as follows: "Migliorato (Contribuzioni alla Teratologia vegetale, Ann. di Bot. II, 1905) beschreibt verschiedene Anomalien der Laubblätter, besonders die Bildung von Epascidien, welche entweder die ganze Blattspreite oder einzelne Lappen derselben in tutenförmige Gebilde verwandeln". Similarly PENZIG quotes E. Brown (An abnormal seedling, The Plant World IV, 1904, pg. 54). Nowadays teratology can hardly be a special branch of botany as many teratological phenomena, teratomata (BOS) are symptoms of diseases, some of which any viroses.

The above survey of symptoms observed in several *Ginkgo* localities tries to give a methodical outline, but does not include smaller details of one or the other kind of symptom, nor details showing that there need be a sharp borderline between the different kinds of symptoms. Thus, for example, the leaves of the top of the seedling, on which the described cowl was formed, were withered and with their margins turned down as if affected by leaf roll. The leaves of the lower part of the plant were normal. The rolling of these leaves discloses that especially those leaves in the top part of the plant, near the ascidium, are affected by the disease. The rolling of these leaves cannot be explained as a preparation for the formation of ascidium, as every ascidium, according to KLÁŠTERSKÝ, has its origin congenitally, and not by the margins of leaves growing together. The tendency of the leaves rolling up on the same plant (ascidium and leaf roll) is, however, characteristic. Also leaves having an enation on the lower side are usually deformed in different ways. A part of the blade is missing, the lobes may partially cover each other, many leaves on this tree are bent spoon-like or dishshaped. Deeper lobing of the leaf margins may be considered as the beginning of the so called "laciniata" form which is less of a taxonomic unit (variety), but more likely a plant infected by a certain virus.

The variability of our fruits trees is a well-known characteristic, not only in their growth but also in their response to one and the same virus, and this applies to *Ginkgo biloba* too. This tree is not only a decorative tree, but counts also as a fruit tree. Its variability is also pointed out by NĚMEC B. (1955, p. 59–60): "Similar to all cultivated fruit trees *Ginkgo biloba* is as well subject to a great variability". In the same paragraph he mentions also yellowish or striped *Ginkgo biloba* leaves.

As for the above described *Ginkgo biloba* diseases it is necessary to distinguish the viruses or rather to determine which of them affects this tree. Four groups of symptoms are not likely to represent four different viroses. According to findings in different localities, several kinds of symptoms, may be seen on the same tree at the same time, e.g. leaf deformations and enations may be symptoms of the same disease, even though there is a complex of viruses present. The same may be said for symptoms of ascidium plus leaf roll. Here, however, we deal probably with a systemic disease even if the leaves of the bottom half of the plant are not observed to have leaf roll. These problems are material for further study. I myself am going to follow up the *Ginkgo* viroses and try to transfer them by grafting, which I have not been able to do, so far, for technical reasons. I intend to continue in germination tests of *Ginkgo* seeds and in the transfer of these viroses by seed.

I have never observed on any *Ginkgo biloba* any insects which could be considered as vector. Except for virus diseases, *Ginkgo biloba* seems to be highly resistant to diseases and harmful insects.

Summary

On trees of *Ginkgo biloba* L. in Czechoslovakia and in Szeged (Hungary) different symptoms were found which suggested virus diseases:

1. Yellow stripe mosaic between the main veins.
2. Deformations of leaves.
3. A terminal leaf forms a cowl (ascidium) from one lobe, as the second lobe of the leaf is normal. The cowl has the shape more of an infundibulum and is 4.5 centimetres long.
4. Enations on the lower surface of the leaves.

Germination tests of *Ginkgo* fruits from the infected trees in Szeged showed the possibility of transferring the virus by seeds.

Shrnutí

Na jinanu dvojlaločném (*Ginkgo biloba* L.) bylo po několik let sledováno onemocnění, a to na různých lokalitách v Čechách (především na mladých stromech v parku Hlávčkovy nadace v Lužanech u Přeštice, na registrovaných stanovištích v Kutné Hoře aj.) a mimo ČSSR na památné ginkgové aleji o 37 starších stromech v Szegedu v již. Maďarsku. Na jinanových stromech zjištěny:

1. Žlutá pruhovitost listů, která v počátcích vyšetřována anatomicky.
2. Deformace listů, rozšířené a do výšky zkrácené čepule, částečné překrývání listových laloků, nesouměrnost, četnější zářezy čepule na způsob tzv. „forma laciniata“ apod.
3. Ascidiová forma (infundibulum) vzniklá z jednoho laloku listu vrcholového, ascidie rourovitěho tvaru, 4,5 cm dlouhá.
4. Na listech poněkud deformovaných, se zkrácenými, ale do plochy silně rozšířenými řapíky objevují se na spodní straně listů exkrescence (enations), 10–12 mm dlouhé nitkovitého tvaru, na konci vidlicovitě ukončené dvěma ostrými hroty. Tyto výrůstky vybíhají z blanitého křídla, kterými je plochý řapík po obou stranách olemován.

Pokusně provedeny klíčící zkoušky ginkgových peckovic odebraných z alejových stromů v Szegedu, které byly postiženy zřetelnou žlutou pruhovitostí. Z 20 semen vyklíčilo jich ve skleníku Ústavu pro fyziologii rostlin KU jen 6, na nichž se projevují různé patologické příznaky. Na jednom z těchto semenáčů vznikla zmíněná ascidie. Z pokusu možno soudit na přenos choroby semenem.

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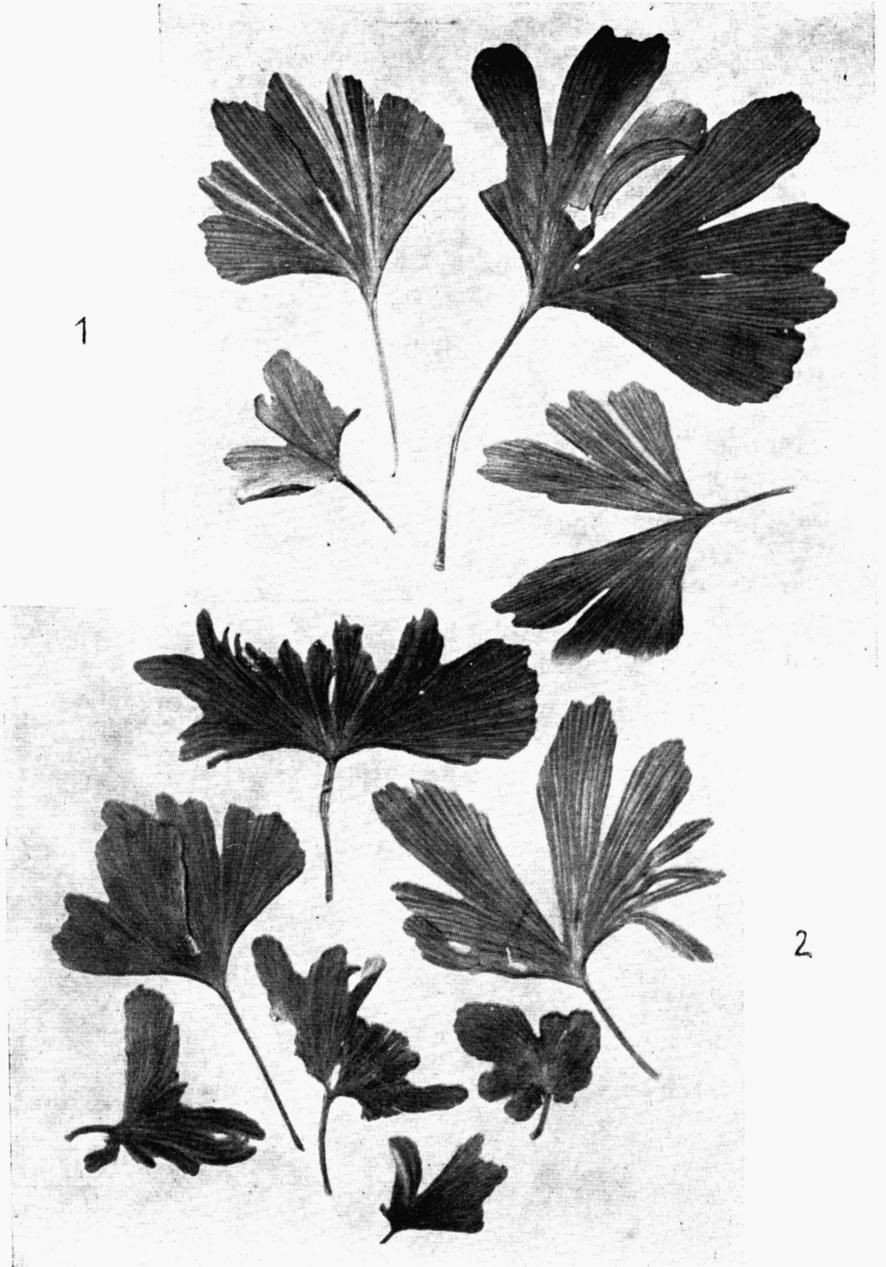
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Explanatio of the plate I:

Fig. 1. Yellow stripe mosaic of *Ginkgo biloba* L. From Szeged and Lužany. Photogr. by L. Kotrba.

Fig. 2. Different malformations of *Ginkgo biloba* L. leaves. From Lužany. Photogr. by L. Kotrba.



J. S m o l á k: Virus Diseases of *Ginkgo biloba*?