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Necrosis of *Aesculus hippocastanum* L.

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Nekrosa jírovce maďalu

V práci trvající tři léta byla sledována choroba jírovce maďalu (*Aesculus hippocastanum* L.), u nás i jinde v střední Evropě všeobecně rozšířená. Hlavním pracovištěm byl zámecký park v Lužanech u Přeštic (Hlávkova nadace). Příznaky a jejich vývoj byly pozorovány také na mnohých jiných místech. Podle třídění L. Bose (Wageningen) patří tyto symptomy do skupin Barevné změny (II), Nekrosa (IV), Deformace (VII) a částečně i Redukce růstu (plodů). Jde o chorobu, kterou před 60 lety popisoval SORAUER a THOMAS jako abiosu. Poněvadž některé zjevy poukazovaly na chorobu virového původu, byly podniknuty diagnostické zkoušky. Serologická byla negativní pro chemismus maďalových listů, které nejsou vhodným materiálem pro tuto metodu. Zkoušky roubovací a očkovací na zdravých semenáčích byly positivní. Virová nekrosa maďalů je choroba systémová, která není přenášena mechanicky dotekem. Snadno se však přenáší roubováním a očkováním. Některé známky nasvědčují též pravděpodobnosti, že do jisté míry může být přenášena i semenem.

Summary

The work described here was carried out during the last three years mainly on the castle premises at Lužany near Přeštice (Hlávka's foundation). It dealt with the disease of the horse chestnut-tree (*Aesculus hippocastanum* L.). The symptoms and their development have also been observed at a number of other places. According to the classification of BOSE (Wageningen) the symptoms belong to the groups of Colour changes (II), Necrosis (IV), Deformation (VII) and partly also Growth reduction (of fruits). We are dealing here with a disease that was described by SORAUER and THOMAS 60 years ago as abiosis. Since some of the symptoms suggested a virus origin of the disease some diagnostic tests were carried out. The serological test was nega-

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tive on account of the chemical composition of chestnut leaves which are not amenable to such tests. Grafting and inoculating tests on healthy seedlings were positive. Viral necrosis of the horse chestnut is a disease of the system which is not transferred by contact. It is readily transferred by grafting and inoculating. Some symptoms suggest that it can also partly be transferred by seeds.

The pathology of the horse chestnut is only little known. Three papers on the diseases (mycoses) of this decorative tree can be found in Phytopathology VI 1916. Nursery horse chestnut material has been reported to contain malignant blotch disease caused by the ascomycete *Guignardia aesculi* (PECK.) STEW.; adult trees are known to be affected by fungi of the following genera: *Nectria, Calonectria, Cryptospora, Gloeosporium, Botrytis* and others. Perthophytic *Nectria cinnabarina* (TODE) FR. which is a common pathogenic fungus of various deciduous trees is by no means infrequent. A recent paper on chestnut mycosis was published by SCHNEIDER (1961). For the rest, however, no mention can be found about a dangerous distribution of any of the above parasitic fungi in our horse chestnut trees.

On the other hand, the horse chestnut is affected at all places where it has been planted by a disease which usually escapes attention and which does not belong to the above-described mycoses or to abioses. It is by no means a new disease; on the contrary, it has been known to occur for several decades but the explanations found in the literature vary considerably. I shall call this disease a necrosis.

I have studied it mostly in the park and the gardens of the Hlávka Foundation at Lužany near Přeštice, but I have found it at a number of other localities. In Prague itself the disease can be found in the tree avenues of the street Pod kaštany and at many other places. The Lužany park reserve represents a very suitable working place, both in view of the presence of numerous old planted chestnut trees and of abundant horse chestnut seedlings which grow under suitable conditions at various places from seeds fallen to the ground and can be used as suitable experimental material. In addition to the chestnut trees growing in the main part of the park there are two such trees flanking a side gate to the castle. Both were planted a number of years ago at the same time. Both enjoy the same soil and atmospheric conditions but one of them has exhibited pathological symptoms for a considerable length of time while the other can be considered as healthy or relatively healthier.

Disease symptoms have been examined in the Lužany trees quite regularly since 1957. The two trees in question displayed the following differences during the first days of May 1959.

Healthy tree: more powerful growth, long branches form a more or less extensive pyramidal canopy, leaves are intensely dark-green, inflorescences found only in about 20% of the canopy (during that year horse chestnut trees were generally relatively poorly flowering in that region), under the tree a considerable amount of wind-blown seeds fallen in 1958.

Diseased tree: weaker growth, about 20% lower, branches clustered to form a dense canopy, leaves generally light-green with light spots in passing light, no inflorescences (in 1958 there were but a few), no seedlings under the tree, seeds collected in 1958 markedly wrinkled and smaller than seeds

of equal age from horse chestnuts elsewhere in the park. Seeds of 1958 did not germinate (tested also in green-houses).

Situation after 3 weeks (May 20th, 1959): The entire diseased horse chestnut-tree had more leaves with yellowish spots. Some necrotic brown spots were present at the blade edges and particularly at leaf tips. Between the principal nerves there were indications of perforations with a pink selvage (Fig. 1). The same symptoms were also observed in a number of trees in Prague.

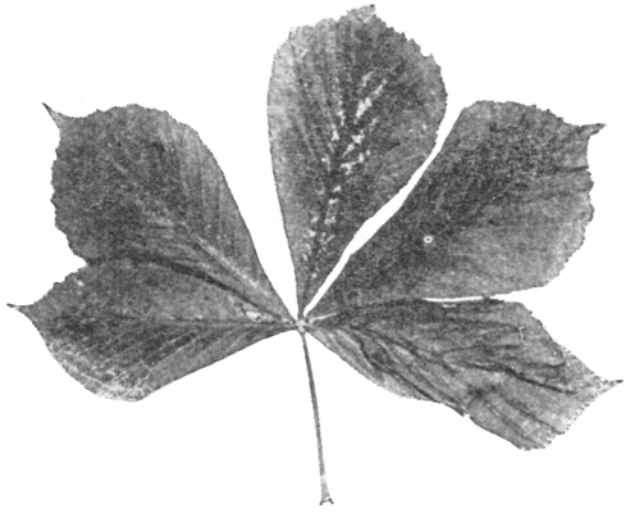

Fig. 1. Leaf with yellowish spots and beginning perforation. Author's photograph.

After 9 more days (May 29th, 1962) there were still more yellowish spots on the leaves and they were more marked. There were also more necrotic spots near the leaf tips and occasional similar spots on the leaves near the insertion of leaflets on petiolules. More marked intercostal perforations were also present, some were only rudimentary, the tissue between the principal nerves being paler and thinner. Occasionally, however, so far in small numbers only, the leaflets showed trellised broken-up patches (Fig. 2) between ribs. These perforations appeared only on the diseased tree. Not even now did the entire horse chestnut exhibit any inflorescence.

In addition to the above symptoms the diseased tree had some leaves with curled leaf-blades. These blades were thicker than those of the healthy ones, were darker green and usually deformed to a patelliform or spoon-like shape.

The described symptoms of horse chestnut necrosis were found not only in the Lužany trees. The same symptoms were observed at all places where chestnut trees had been planted. The disease appears to be a common one. Even the curliness with leaflets either patelliform or spoon-shaped (a less frequent symptom) can be found in many different localities. I observed it, for example, in chestnut trees at Luhačovice and especially in a dying chestnut tree in a cemetery at Ovesné Kladruby near Mariánské Lázně.

Perforation and its development in leaves is a rather remarkable symptom. Some leaves of practically normal appearance and visually healthy appear to be "preparing" for the disease. Their tissue between the principal nerves becomes somewhat yellow, weakens and thins out until cracks occur, minute at first but widening more and more. The origin of perforation starting with a weakening or an internal disturbance of the mesophyll (the epidermis remaining anatomically intact for a long time) would rather point to an internal causative agent. It is obvious that the disturbance is not caused by a me-

chanical factor, such as wind, rubbing of leaves against one another or against a branch and also not by frost as supposed earlier. Trellised cracks are not a regular phenomenon. Occasionally cracks are formed at irregular places, such as at the edge of a leaflet or in an intercostal part. At other times, cracks are formed apparrently at the central nerve rather than intercostally. Regular intercostal perforations along the entire length of the leaf-blade represent the most malignant destruction of the assimilatory apparatus (Fig. 3). The affected leaflets with residues of mesophyll are curled and fall off. As contrasted with the long blades of healthy leaflets, many of which are over 30 cm. long and as much as 15 cm. wide, the destroyed leaf is considerably reduced in size, pointing to a dangerous pathogenic agent such as could not be caused by abiotic factors.

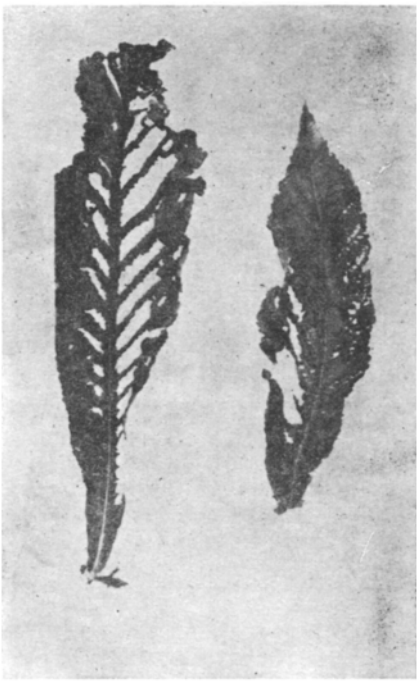

Fig. 2. Leaves with advanced perforation. Author's photograph.

The "trellised" horse chestnut leaves were described in 1903 by SORAUER as a consequence of spring frost. Low temperature (frost) was often blamed for causing many a pathological symptom. There is no necrotic tissue or necrotic part of an organ, there are no colour changes in plant organs or morphogenetic changes in leaves (culriness, rolling, cracks of nictitropic position) that have not at one time or another been explained as due to the effect of low temperature. SORAUER even attributes some other grotesque forms of leaves such as those resembling the *foliae laciniatae* to spring frost and cites various transitions between different types of perforation of horse chestnut leaves and describes light and translucent lines, spots, swollen parts of the leaf-blade etc. as being due to spring frost. This author was an outstanding observer but he could hardly cope with the mixture of variegated symptoms. A year later THOMAS (1904) attributed the perforation of chestnut leaves to the action of wind which causes the leaves to rub against one another and scratch their surface. He also mentions some leaf deformations which can hardly be explained as due to the action of wind. He was not satisfied with the explanation presented by SORAUER and was looking for a new one.

It is of interest that SORAUER compares the trellised leaves with the form *Aesculus hippocastanum* f. *laciniata*. At present this form is readily explained as a virosis (leaf atrophy). The amount of leaf tissue decreases until even the laciniate mesophyll edge along the principal nerve disappears and the leaf-blade is reduced practically to the main nerves which roll up as simple flexible

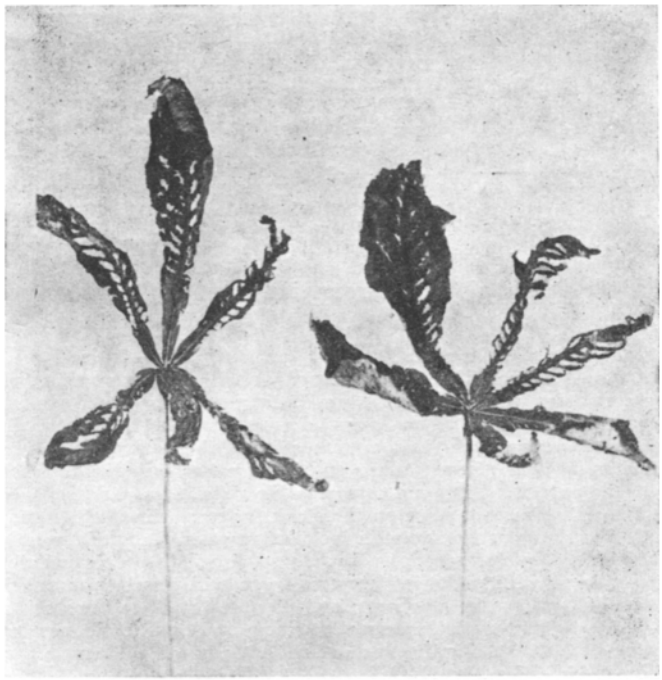

Fig. 3. Leaves dammaged by perforation and partly by atrophy. Author's photograph.

filaments (Fig. 4). HEGI (1912) mentions 10 taxonomic forms of *Aesculus hippocastanum* and it would certainly be correct to verify some of the uncertain ones from the point of view of virology, in particular: *Aesculus hippocastanum* f. *memmingeri* (white spotted leaflets), f. *variegata* (yellow spotted leaflets), f. *pumila* (stunted type), f. *baumanni* (full-blossomed type), f. *tortuosa* (twisted branches), f. *incisa* (leaves with deeper notches).

In explaining horse chestnut necrosis aetiologically the idea presented itself that we might be dealing here with a virus disease. This was suggested by the various symptoms and their development even if symptoms themselves cannot be taken as diagnosis. The serological test was negative. It was

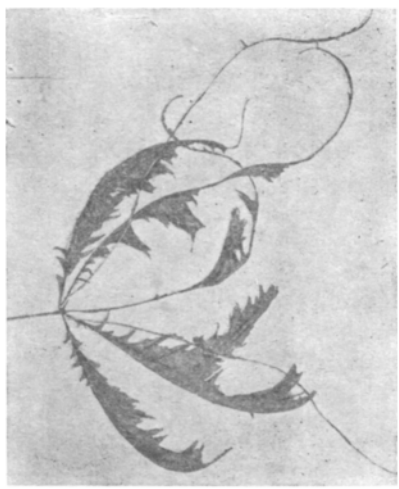

Fig. 4. Atrophy of leaf of *Aesculus hippocastanum* L. Photograph J. Dirlbek.

carried out by JERMOLJEV of the plant pathology department of the Research Institute for Plant Production at Ruzyně. Beginning on May 22nd, 1959, diseased material was sent from Lužany to the institute mentioned where injection material was prepared from the leaves and applied to rabbits. According to JERMOLJEV the negative result was to be expected since the horse chestnut is rather unsuitable for serological diagnostic tests. This is caused by the relatively large amount of tannic acids and of pectin bodies. The leaves also contain quercetin, quercetrin or another related glycoside (queraestrin), further there is carotene and in addition to tannins some resins which are a well-known obstacle to serological diagnosis.

Even before the serological tests experiments with grafting and inoculation were begun.

In the spring of 1958 and 1959 as well as 1960, grafts from the diseased tree at the southern edge of the park were transferred to visually healthy seedlings in a northern part of the park. The seedlings grew there under several mature healthy trees in which the above symptoms were not observed. The distance of the diseased tree from the healthy seedlings was about 150 m. Both places are separated by a natural section of the park with a growth of generally old big trees such as *Juglans nigra*, *Quercus* sp., *Fraxinus diversifolia*, *Liriodendron tulipifera*, *Ginkgo biloba* and many others, but not *Aesculus hippocastanum*. Grafts placed on numerous seedlings under the rind remained alive on the stock for some time but did not grow together and finally dried. The case was similar with inoculation when the grafts were placed onto the lower part of the trunk. The stock used, i.e. healthy seedlings, then occasionally revealed leaves beginning to be affected with yellowish necrotic spots with marked morphological changes. It can thus be concluded that the infection passed from the graft to the stock without it being necessary for the two to unite. Similar cases are known today. I made the experience myself while collaborating with J. B. Novák in transferring the virus willowy disease of pear trees (SMOLÁK and NOVÁK 1957, SMOLÁK 1958).

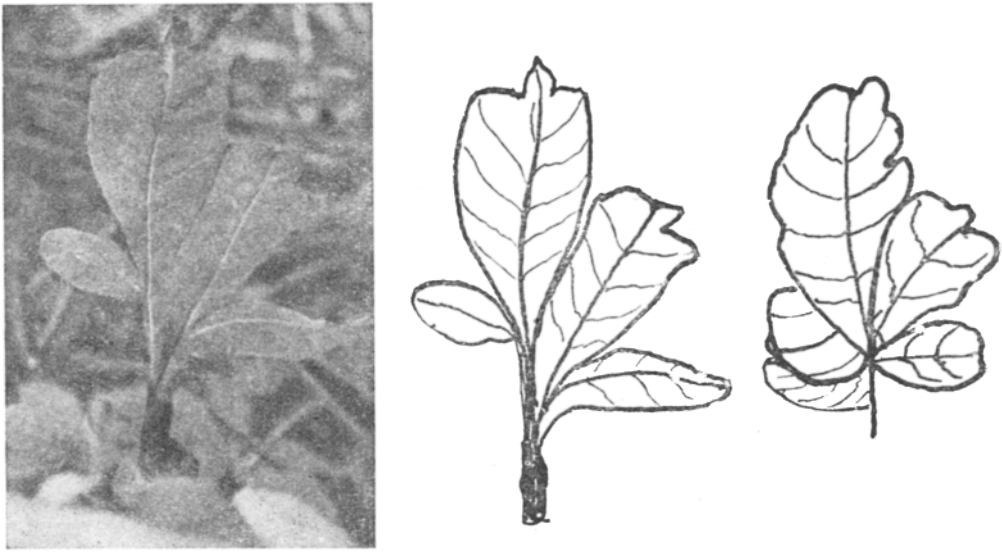

Fig. 5. Two leaves (photo and drawing) of *Aesculus hippocastanum* L., grown from a graft-infected seedling. Author's photograph.

Changes in leaves growing from grafted horse chestnut stock were generally of the following types:

1. Many leaf-blades began to display yellowish necrotic spots, the same as appearing in trees from which they were removed. The leaflets of grafted seedlings were slightly deformed at the tip or at the sides. Some of the leaves had only four leaflets.
2. The central leaflets were markedly elongated and formed a long filamentous structure proceeding from the principal nerve. Some leaves had somewhat curly blades.
3. Some leaves are composed of two very unlike leaflets or of several irregularly composed leaflets of various sizes (Fig. 5).
4. In a number of grafted seedlings very dark-green and leathery leaves appeared. They were of altered shape, in addition. Their leaflets were much narrower and coarsely indented.

Changes in inoculated seedlings resembled changes in grafted ones. The yellow and later brown necrotic spots are a frequent phenomenon in grafted or inoculated seedlings. They appear particularly in the uppermost leaves. The variously deformed leaves and in the beginning also a perforation of leaves are striking here as contrasted with normal leaves on untreated seedlings. One inoculated seedling produced three quinquefoliate leaves at the same level just below the growing point, the leaves remaining spread until the end of summer but being of dwarf size, only about 10 mm. in length.

Discussion

The disease of horse chestnut trees described here presents many interesting points. It is a common disease just as its host is one of the most common decorative trees. It is very likely an old disease (SORAUER 1903) but rather neglected, perhaps in view of the fact that we are dealing here with „only" a decorative tree.

The symptoms of the necrosis are rather variegated and little distinguishable. It is a known fact that there are no clear boundaries between visible and invisible reactions in virus diseases. It is equally difficult if not impossible to distinguish between normal and abnormal, between normal and pathological growth. The identification of symptoms in horse chestnut necrosis is also complicated by the fact that they appear at the very beginning of sprouting and persist till autumn. Toward the end of the vegetation season there is a tendency to confuse the symptoms with the normal autumn necrosis. Necroses can be very characteristic in virology, possessing a high diagnostic value and being possibly due to a system reaction.

Necrosis of horse chestnut trees appears to be a system virosis. The same trees display it year after year even if not with the same intensity (different virus strains ?) Transfer is feasible through grafts removed from various places in the canopy, deformed leaves growing from dormant buds in the lower and central part of the tree trunk. Even if no experimental evidence were available there are symptoms suggestive of a virosis, such as disappearance of

flower buds or initiation of stunted fruit which soon fall off, as well as curly leaf-blades and in some dark-green leaves a striking stiffness (unremoved starch). In order to study the symptoms of chestnut necrosis great numbers in various regions have to be investigated. Dwarfiness and premature falling off of fruits cannot be explained away by draught since neighbouring chestnut trees in the same locality can bear the normal amount of fruit which are healthy and do not fall off.

Frequent examinations convince us that the chestnut necrosis is not transferred by mechanical contact. Infected horse chestnut trees affected by a weak or strong necrosis often grow in avenues right next to healthy trees without the latter contracting the infection even if the branches of the trees are frequently in contact. The healthy specimens remain so throughout the vegetation season and even during the subsequent year without any necrosis symptoms (e.g. in the tree avenue of the Pod kaštany street in Prague).Cases are known when a necrotic horse chestnut was flanked by two completely healthy trees. The soil and atmospheric conditions here were identical and all the trees in the avenue are of the same age. We thus can rule out all abiotic factors (soil, light, temperature, climate). It is also very unlikely that we should be dealing here with a prolonged (several years) incubation. No minute insect species could be detected on the trees under examination.

On the other hand, it seems likely that the necrosis can be transferred to a certain degree through the seeds. In addition to previous observations this can be inferred from the appearance of seedlings growing from apparently healthy seeds in plots of S. Prát. In the clean environment of the green-house of the Plant Physiology Institute they grow in pots and possess a high percentage of deformed or curled leaves, some of them being dark-green and tough, thus indicating the presence of symptoms caused by grafting or inoculation. The seeds of necrotic chestnut trees can be affected by the virus infection to a greater or lesser degree. Then the possibility presents itself that a weak infection of seeds can result in various pathological symptoms which can also be obtained artificially, by grafting or inoculation. A stronger infection of different virus strains can explain the dying away of flower buds, dwarfiness and lack of germination of fruits and their premature falling off. Large horse chestnut trees even if exhibiting stronger infection can resist and vegetate for a number of years. In the end, however, the totally infected trees die under a variety of extreme symptoms when practically no leaf on the tree remains healthy and when branches begin to dry and die. This is the case with the trees in the cemetery at Ovesné Kladruby and in the horse-chestnut avenue of the Red Riding Hood Hotel in Mariánské Lázně.

The work described here is not terminated. It will be continued and attention focussed on the virus or complex of viruses as the causative agents of horse-chestnut necrosis.

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Некроз конского каштана

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В продолжении трех лет изучалось заболевание конского каштана (*Aesculus hippocastanum* L.) общераспространенное у нас и в средней Европе. Главным местом работы был парк замка Лужаны у Пржештиц. Симптомы заболевания и их развитие наблюдались тоже на других местах. По классификации L. Bose (Wageningen) симптомы принадлежат в группу Цветные изменения (II), Некроз (IV), Деформации (VII) и немного Редукция роста (плоды). Речь идет о заболевании, которое как анабиоз описал Sorauer и Thomas. Так как некоторые явления вызывали подозрение что заболевание является вирусным, были сделаны диагностические измерения. Измерения серологические были отрицательные для химизма листьев конского каштана, так как они не являются подходящим материалом для этого метода. Прививка на здоровых растениях была положительной. Вирусное заболевание каштана является заболеванием системным, которое не переносится механическим прикосновением. Однако очень легко его перенести прививкой. Некоторые признаки свидетельствуют до некоторой степени о возможности перенесения заболевания семенем.