BIOLOGIA PLANTARUM (PRAHA)

1 (3) : 223—234, 1959

Oat Sterile-Dwarf Virus Disease

VLADIMÍR PRŮŠA, EVŽEN JERMOLJEV and JOSEF VACKE

Crop Production Research Institute of the Czechoslovak Academy of Agricultural Science, Ruzyně near Praha

Received January 29, 1959

Souhrn

V práci jsou shrnuty nové důkazy o virovém charakteru sterilnosti a zakrslosti ovsa (VSZO), jakož i stručné zmínky o zjištění dalšího viru v ČSR — virové proužkovitosti pšenice (VPP). Oba viry mají stejného přenášeče — křísa *Calligypona pellucida* F. Virus proužkovitosti pšenice může být na rozdíl od VSZO předáván nakaženými samicemi vektora na potomstva transovariálně. Tato vlastnost, jež umožnila vzájemné oddělení obou virů, byla tím prokázána u fytopathogenních virů na evropské pevnině prvně. VSZO může být přenášen larvami i imagy křísa. Značně variabilní cirkulační doba VSZO v těle vektora proběhne většinou za 3 až 4 týdny. Inkubační doba VSZO činila u ovsa 3 až 4 týdny, u pšenice a ječmene 4 až 7 týdnů; žito projevovalo za 4 týdny pouze zaostávání v růstu. K přenesení infekce byl nutný minimálně půlhodinový pobyt vektora na testační rostlině; po třídenním pobytu došlo již ke 100% infekci. Destruktivita VSZO na hostiteli se nezvětšuje úměrně ani s délkou sání křísa, ani s přibývajícím počtem vektorů na rostlinu. Koncentrované extrakty z rozdrcených těl nakažlivých křísů aplikované mechanickým vtíráním i injekcemi do ovesných rostlin nezpůsobily u nich chorobné symptomy. VSZO ani VPP se nepodařilo přenést půdou nebo kokoticí. VSZO byl však přenesen roubem. Oba viry vyvolaly charakteristické symptomy u *Avena fatua* L. a *Poa annua* L. Zjištěné skutečnosti diskutovány z etiologického hlediska.

Summary

This paper presents a summary of new evidence for regarding sterility and dwarfing of oats (OSDV) as a virus disease. Brief references are also made to the identification of a further virus in Czechoslovakia—the wheat striate virus (WSV). Both viruses are transmitted by the leafhopper *Calligypona pellucida* F. The wheat striate virus differs from the oat sterile-dwarf virus

in that it can be passed transovarially by the infected females of the vector to their progeny. This characteristic, which made it possible for the two viruses to be separated from each other, was demonstrated for the first time for pathogenic viruses on the European continent in this case. OSDV can be transmitted by leafhopper larvae and adults. The time during which OSDV circulates in the vector's body is very variable; it is usually three to four weeks. The incubation period of OSDV was three to four weeks for oats, four to seven weeks for wheat and barley; rye only showed retarded growth after four weeks. For infection to take place it was necessary for the vector to remain for a minimum of half-an-hour on the test plant; 100% infection was obtained when the vector remained on the plant for three days. The injury inflicted by OSDV on the host does not increase proportionately either with the duration of the feeding period or with an increase in the number of vectors on the plant. Concentrated extracts of the crushed bodies of infectious leafhoppers produced no symptoms of disease, when rubbed or injected into oat plants. Nor were attempts to transfer OSDV and WSV through soil or dodder successful. OSDV was, however, transferred by grafting. Both viruses gave rise to characteristic symptoms in *Avena fatua* L. and *Poa annua* L. These findings are discussed from the etiological point of view.

Introduction

The significance of virus diseases in European cereals and fodder grasses has increased considerably in recent years, and numerous reports have appeared on the subject (KLINKOWSKI and KREUZBERG 1958). The economic losses caused by this category of disease are of such magnitude that they can no longer be underestimated. Czechoslovakia may serve as an example of one of the countries in which virus on cereals had not been definitely identified up to 1957.

For several decades, however, the periodic occurrence of very harmful diseases on oats had been observed in south-east Bohemia. The disease appeared in some years and there seemed also to be some correlation with climatic factors. Until recently the causes of the disease were sought among various agents both of a parasitic and non-parasitic nature. In 1943 BLATTNÝ, after examination of oat crops near Pelhřimov, expressed the view (v. also BOJŇANSKÝ and BLATTNÝ 1956) that the symptoms he observed might be due to a virus. He repeated this at the conference at Liblice in the winter of 1951/2.

The systematic study of the etiology of oat disease did not begin until the epidemic was spreading with unprecedented rapidity in 1954 to 1956, when losses reached catastrophic proportions. The only measure for resisting the disease, of course a provisional one, was a ban on the cultivation of oats in the threatened districts of the Jihlava region.

The authors of the present paper first met with the disease in July 1956. On the basis of an analysis of the symptoms, type of distribution and gradology of the disease, and from data on the occurrence of probable vectors,

the most likely cause was considered at that time to be a virus of the yellows type (PRŮŠA 1957). During the 1957 vegetative period it was so far possible to support the correctness of this conception that the actual originator of the calamity could be described as a new virus (PRŮŠA 1958a, 1958b). This virus was found to have characters distinguishing it from those viruses which could be considered on the basis of certain features to be related (PRŮŠA 1958b).

Oat sterile-dwarf virus disease (OSDV), as the new disease has been named (PRŮŠA 1958b), is not, however, confined only to Czechoslovakia. In the authors' opinion the same virus has been causing increasing damage in the Scandinavian countries, particularly since 1949, especially in Finland, where it caused an even greater catastrophe than in Czechoslovakia (KANERVO et al. 1957). It was in Finland, also, that the connection between the distribution of the disease and the leafhopper *Calligypona pellucida* F. was first established (HEIKINHEIMO 1956, 1957; KANERVO et al. 1957). The specific relation between this leafhopper and the spreading of this dangerous disease was experimentally confirmed shortly after, and independently of the Finnish workers, in Czechoslovakia (DLABOLA 1957, 1958; PRŮŠA 1958a, 1958b). According to some as yet unconfirmed reports, a similar disease has also occurred in Slovakia in the Orava basin. BLATTNÝ (1958) discovered a symptomatologically similar type of disease in Rumanian Transylvania.

Although the nature of the pathogenic agents of sterility and dwarfing in oats has been studied in its main outlines, it was still necessary to provide further and still more convincing evidence in order to dispel any doubt as to its virus origin. This has been the main aim of the work here presented.

At the same time the presence of another virus was determined. A detailed report on this will be given separately. This virus produces, in addition to dwarfing and other less obvious symptoms, a marked striation in cereals (PRŮŠA 1958b), which is not produced by OSDV. It has been named, according to the most characteristic symptom on the host where it was first found, wheat striate virus.

These two viruses often occur together, which is connected with the fact that they have the same vector (PRŮŠA 1958b). WSV is not of great economic importance so far; not perhaps because it is less injurious to the host cereals than OSDV, but because it has not yet occurred on a mass scale. It is, however, theoretically noteworthy for its behaviour in the vector, the infected females of the leafhopper *Calligypona pellucida* having the ability of transmitting it to their progeny transovarially, which is the first case of this kind to be demonstrated for a phytopathogenic virus on the European continent. This virus is probably identical with that described under the name "European cereal (wheat) striate mosaic" in recent times in England, and diseases observed in Germany and Denmark (SLYKHUIS 1958) probably belong to it too.

Material and Methods

In order to elucidate the question of the existence or non-existence of toxic and non-toxic populations of the leafhopper *Calligypona pellucida* it was necessary to substitute the simpler practice of mass transfer of leafhoppers to whole groups of test plants by a method of caging them individually on the plants, one insect on one plant. In this way it is possible to follow the pathogenic power of each leafhopper independently throughout the postembryonal development up to its death in a series of passages on individual test plants (v. fig. 1 and 2). The leafhoppers which were shown by sufficiently prolonged preliminary testing on healthy plants to be virus free could then be selected and used in later phases of work for experiments with their artificial infection. The uninfected insects were divided into two groups, experimental and control. Individuals from the experimental group were caged on diseased plants for a certain time for acquisition feeding. They were then transferred at definite time intervals on to a series of test plants in order to establish whether they had acquired infective power and, if so, how long after the period of feeding from the source of infection. The insects from the control group were put through a similar process with the difference that they were never brought into contact with infected plants. During the time when the experimental insects were feeding on infected plants the controls were isolated on healthy plants.

Using the method described it was possible to determine whether leafhoppers are able to become infected by feeding on diseased plants. This was a matter of primary importance in this investigation, since if the leafhoppers became infected under these circumstances it could not be explained as being due to toxins, because toxins differ from viruses in not being infectious. The experimental procedure described also ensured that in the event of results being positive it would be possible to obtain information on the length of the circulation period of OSDV in the body of the vector.

Results

Determination of the degree of natural infection of leafhoppers and artificial infection of virus-free adults of *Calligypona pellucida*

Experiments were started at the beginning of April 1958 in the glasshouse and were continued from May to about the middle of July in a frame made of hotbed windows in which the glass had been replaced by light silon material, allowing the insects and plants to develop under natural conditions. One hundred and sixty larvae in the hibernating stage of the leafhopper *Calligypona pellucida* were placed individually on 160 plants (fig. 3). The larvae were caught shortly before on the boundary of a field on which the oat crop had been totally infested by OSDV (experimental locality Sázava, Pelhřimov district).

The insects were divided into 16 groups of ten each, so that it was possible to carry out several variants of the experiment simultaneously. The individual variants differed as to the species of cereal on which the percentage of naturally infected larvae was determined, further as to the order in which larvae were caged on the cereals, the developmental stage of the cereal at the time of infection and the length of the test feeding on individual plants, which ranged from one to three weeks. The great majority of the leafhoppers in these experiments fed on five healthy cereals (of these at least three times on oats), some however survived for nine passages.

In the first phase of the experiments it was already shown that not all leafhoppers in populations from localities most strongly infested with OSDV were infected. Only about 86% of the individuals possessed the power of producing symptoms of disease. The remnant of about 14% of leafhoppers, although coming from a population of the same origin, never caused the changes characteristic of disease in oats, wheat or other cereals. Only after very thorough observation was it possible to note an extremely slight retardation of growth in plants on which they had fed. This became somewhat more marked when the period of sojourn of the leafhopper on the host plant was prolonged. This phenomenon, which is atypical for the disease, was usually noticable immediately after the application of the longest experimental period of test feeding

(three weeks) in plants at the earliest developmental stage (first leaf about 3 cm. long). However, after removal of the leafhoppers the majority of the plants concerned almost caught up with the unaffected control plants. This phenomenon might be due to the relatively great loss of assimilates caused by the intensive feeding by the leafhopper dependent on the young plant for its entire nutrition.

A certain proportion, always composed of the same insects (about 12% of the total), sometimes transmitted the striate virus as well as the sterile-dwarf. There were also some individuals among the experimental leafhoppers which exclusively transmitted the striate virus to the test plants.

In the majority of cases it was possible to distinguish the oat sterile-dwarf virus disease in oats with certainty only after four weeks from the time when the leafhoppers began to feed. Retardation of growth appeared a week earlier. In wheat, barley and rye dwarfing became evident approximately four weeks after infection; the final decision whether OSDV had really been successfully transmitted to wheat and barley was usually possible only after a further two to three weeks, when other symptoms of disease also appeared (stimulation of sprouting, retardation of earing, yellowing of the tips of the youngest leaves). In rye, which is clearly the most tolerant to OSDV, no symptoms were developed other than dwarfing. Differences in the damage caused by OSDV to individual cereals are to be seen in figs. 4, 5, 6 and 7.

According to DLABOLA (1957, 1958) sterility and dwarfing in oats was caused only by larvae and females of the leafhopper *Calligypona pellucida*. The present experiments have shown that males transmitted OSDV to the same extent as larvae and females.

As a supplement to the experiments described the question was simultaneously investigated why in DLABOLA'S work (1957, 1958) using mass transfer of leafhoppers from areas not affected by the catastrophic occurrence of OSDV (the locality of Radotín near Prague) a similar picture of the disease was obtained on test plants as when insects taken from infested crops were used. In this investigation the authors were also concerned with the question of possible differences in the power of transmitting OSDV between populations of *Calligypona pellucida* from the Radotín area, where the disease was not known up to that time, and from Sázava, an epidemic focal point.

For this purpose 64 leafhoppers of the hibernating generation, caught on a meadow in the Berounka basin at Radotín, were individually caged on oat plants. It was found that something more than 6% of individuals in this locality were infected with OSDV. This finding points, above all, to a more general distribution of the virus. Further it makes it possible to explain Dlabola's observations as being due to a slight admixture of infected leafhoppers, which could easily distort the results when using his technique of mass caging of the insects on the test plants.

In a further phase of research an attempt was made to induce artificial infection of leafhoppers which had been shown to be uninfected over a month und half test period. In the Sázava population there were altogether 23 insects (cca 14%) which never caused infection with the sterile-dwarf or the striate viruses. By the end of May seven of them had died, however. The remainder, already as adults, were divided into two parts, one (control) was passaged on healthy plants, while an attempt to infect the other (experimental) was made as follows: three leafhoppers were isolated individually on sterile-dwarf oat plants, two on sterile-dwarf wheat plants and three on striate wheat for a period of one week.

The leafhoppers were then individually caged for test feeding on healthy plants in order to find out whether they had been infected. Eight transfers of such infected insects were carried out in all. The intervals of test feeding lasted three to ten days. At the end of the eighth passage (from July 14 to 18, 1958) no insect was still alive. In spite of this high mortality among the leafhoppers it was recorded that from the fourth test feed, when only four insects remained, two of them began to transmit OSDV regularly. Infectivity was acquired by one male, which had been infected on virus-infested sterile-dwarf wheat and one female, which had been infected on diseased oats. The circulation period of the virus in the bodies of these leafhoppers corresponded to an interval of 25 to 32 days. The remaining leafhoppers caused no infection to the end of their lives. The parallel testing of control leafhoppers did not infect healthy plants at all.

Similar experiments were carried out with a part of the leafhoppers from Radotín. In this case 23 adults, which in preliminary tests had shown themselves to be incapable of infection, were caged on virus-infected sterile-dwarf oats for acquisition feeding for nine days. They were then passaged as usual on to healthy plants until they died. In the first passage, which corresponded to a circulation period of 9 to 17 days in the body of the leafhopper, three out of

23 insects caused infection (cca 17%). The proportion of infectious leafhoppers then increased in spite of a sharply rising natural mortality, until in the last passage corresponding to a circulation period of 36 to 42 days it amounted to two-thirds (2 insects out of three survivors were infectious). The control insects, which were tested in parallel, did not infect the host oats at all.

In this way the first proof was provided that leafhoppers which were originally incapable of infection can acquire it if they are allowed to remain in contact with a source of sterile-dwarf virus. It was also possible to demonstrate that uninfected members of leafhopper populations both from epidemic areas and from other areas can act as vectors for OSDV. Probably viruliferous and non-viruliferous lines, such as those observed in the leafhopper *Cicadulina mbila* NAUDE, vector of *Zea virus* 2 (Storey) SMITH (v. e. g. KÖHLER and KLINKOWSKI 1954), do not exist in *Calligypona pellucida*.

Artificial infection of *Calligypona pellucida* larvae bred from eggs

In order to provide final proof of the infectiousness of the disease it was necessary to try to introduce a greater number of uninfected leafhoppers into the infection experiments. The easiest way of obtaining sterile leafhoppers was to breed them from eggs. This procedure could, however, only be successful on the assumption that it was a transovarially non-transmittable virus.

To this end gravid females from a highly infected population (80 to 90%) from Sázava were left to lay eggs (fig. 8) in stalks of healthy barley which was at the flowering stage and therefore practically immune from infection by OSDV. After three weeks the ripe straw with the eggs was cut off below the lowest nodes and placed in a flower-pot with young, just-sprouted oats in a silon isolator. The interval between the beginning of laying to the hatching of the first larvae was about 3 to 4 weeks. The whitish, very mobile larvae, which are difficult to see with the naked eye, were collected as soon as possible after hatching so that the theoretical possibility of their contamination by the dry barley straw should be reduced to a minimum.

The larvae so obtained were divided into several groups and simultaneous experiments were carried out with them in order to demonstrate the infective character of both diseases under investigation. More detailed mention will be made here only of the groups in which the infective character of OSDV was followed. The first group (control) was composed of 74 larvae, which were caged on healthy oats immediately on the first day after hatching. They were then transferred again to other cereals (mainly oats) at definite time intervals. The second group (experimental) contained a somewhat greater number of larvae, which were first placed together in a silon isolator for acquisition feeding on sterile-dwarf oat plants. The majority were left there for 13 days, some for a longer period. A total of 92 larvae was individually transferred to healthy oats after infection with the sterile-dwarf virus. After seven to twelve days of test feeding they were then in all cases transferred to new cereals.

The control larvae, which were never able to feed on diseased plants, were individually transferred during a period from the middle of July to the end of September to new plants (usually oats) ten times in all. No case of transmission of the sterile-dwarf virus occurred, although these larvae came from females infected to a high degree by the disease. The originator of sterility and dwarfing in oats is, therefore, not transferable transovarially.

On the other hand, however, from the third passage, i. e. from the time when the larvae were two to three weeks old, the typical signs of wheat striate virus disease began to appear in a varying percentage of plants. Throughout the series of passages it was observed that wheat striation was transmitted by about 12% of the leafhoppers and these were always the same individuals. This virus is, therefore, transovarially transmittable and it was possible to separate it in this way from the OSDV.

Quite different pathogenic characters are exhibited by larvae (experimental) which were left on sterile-dwarf oats. They infected 17% of the oats plants on which they fed with the sterile-dwarf virus immediately in the first passage after their transfer from the source of infection. In the majority of the remaining plants there was also a slight retardation of earing as compared with those fed on by larvae of the same population which had not, however, come into contact with plants suffering from the sterile-dwarf virus. In addition, about 10% of the plants in this experiment became diseased with the striate virus.

During the second passage of these larvae, which took place 12 days later and lasted 10 days, there was a 58·5% infection with the sterile-dwarf virus and in the further passage 81% of the

plants were infected. Although these experiments have not yet been completed, because they will be continued in 1959 following the period of vegetative passivity, there can be no doubt that the great majority of the larvae became infected and convincing proof has thus been provided that the originator of sterility and dwarfing in oats is infectious and therefore a virus. At the same time it was observed that the circulation period of the sterile-dwarf virus in the body of the vector is very variable and that in the majority of individuals it lasts for three to four weeks.

Determination of the time essential for leafhoppers to remain on host plants in order to infect them

Investigation of the time essential for leafhoppers to remain on healthy plants in order to infect them was carried out as follows: infected leafhoppers were individually caged on plants (10 to 15) for test feeding for 5 min., 30 min., 1 hour, $1^1/_2$ hours, 2 hours, 3 hours, 6 hours, 9 hours, 24 hours, 30 hours, 2 days, 3 days, 4 days and 6 days. The experiment was carried out twice.

Following five to ten minute sojourn of leafhoppers on oats no case of transfer of the sterile-dwarf virus was obtained. Isolated cases of infection were recorded following 30 minute isolation of the insects on healthy plants. The percentage of infection then rose sharply with increase in the length of sojourn on the plants, particularly in the intervals up to 30 hours, when 57% of the leafhoppers caused infection. After 3-day and longer periods all the insects transmitted infection.

Thus, in order for infection by one or both viruses to be achieved it was sufficient in principle for one leafhopper to stay for a relatively very short time on the test plant—the lowest limit being 30 minutes. It was found that the infected plant in general undergoes an equally severe form of the disease when it is infected by an insect feeding e. g. for one hour or for three weeks.

Investigation of the toxicity of extracts from homogenates of infected leafhoppers

The experimental procedure was as follows: 342 *Callipygona pellucida* adults (weight 0·77 g.) of a highly infected population from an epidemic area were cooled at 0° C to immobility. They were then quickly transferred into a cold mortar, 3 ml. of cold physiological solution for cold-blooded organisms (0·64% solution NaCl in water) was poured over them, some coarse-grained carborundum was added and they were thoroughly homogenized. The leaves of 121 oat plants in a silon frame were then mechanically inoculated with the liquid. The plants used were at the stage of one to two leaves.

After a week, when the experimental plants had reached a height of 12 to 15 cm., had three leaves and tiny, two to five cm. long shoots (still lacking nodes—first internodium), they were inoculated again; this time inoculation was carried out by injection. For this purpose 300 adults from a highly infected population were homogenized in a similar way in a porcelain mortar with 3 ml. of physiological solution for cold-blooded organisms. The homogenate was poured into small test-tubes for centrifuging, the mortar being washed out with a further ml. of physiological solution, which was added to the test-tubes. Following 3 mins. of centrifuging, at 4000 r. p. m. (g = 1608) the supernatant was further purified through a paper filter. The filtrate was immediately injected with a fine one-cubic syringe into oat stalks, the leaves of which had been mechanically inoculated the week before. Injections were made at regular intervals right through the stalk so that the excess liquid appeared in drops at both sides. Out of the 121 plants so treated 30 were treated in addition by the sediment formed during centrifuging being rubbed into the stalks by the carborundum technique.

All these operations were carried out rapidly and as far as possible in the cold (cooled vessels and physiological solution), so that the denaturation processes should be impeded to the maximum. It can therefore be assumed that relatively large quantities of the most various substances that can be extracted from leafhopper organisms were successfully introduced into the young plants in concentrated form. Oat plants of the same age and cultivated under the

same conditions as the experimental plants were used as controls and were inoculated only with pure physiological solution.

None of the 121 plants inoculated as described above developed symptoms of the viruses under investigation. They all grew normally in the same way as the control plants, came to ear and produced seeds.

This result is evidence of the fact that OSDV is not mechanically transferable. As regards the possibility that a toxin may be involved, it must be remembered that toxins are usually fairly stable substances and it is therefore unlikely that, using the quite careful method described, denaturation of such a substance could take place, if such a substance were indeed present.

Infection experiments using other methods than the agency of insect vectors

In 1958 new attempts were made, with negative results, to transfer cereal viruses through soil to which a considerable quantity of chopped straw and roots of sterile-dwarf oats and striate wheat had been added in the autumn of the previous year. Oats and wheat sown in this soil grew quite normally.

The attempt to transmit the viruses by means of the dodder (*Cuscuta campestris* YUNCK.) was not carried out on a sufficiently large scale for the results to be fully significant. Another reason for the lack of a positive result so far was evidently the fact that dodder fixes itself on to oats and wheat with great difficulty.

A successful experiment on the transfer of OSDV by an agent other than the leafhopper *Calligypona pellucida* may be considered to be that of grafting carried out by BLATTNÝ (1958), both by contact grafting in 1957 (v. PRŮŠA 1958b) and bottle grafting in the following year. Grafts from diseased plants as distinct from those from healthy plants produced a marked stimulation of sprouting and other symptoms of OSDV. This is a further proof of the virus character of this disease.

Investigation of the range of host plants of OSDV

Examination of the range of hosts of OSDV has so far been carried out only in pilot experiments. In the case of 14 grass species (*Lolium perenne* L., *L. italicum* A. BR., *Bromus inermis* LEYSS., *Phleum pratense* L., *Dactylis glomerata* L., *Avena fatua* L., *Agropyrum repens* P. B., *Setaria viridis* P. B., *Hordeum murinum* L., *Anthoxanthum odoratum* L., *Briza media* L., *Arrhenatherum avenaceum* P. B., *Poa annua* L., *Sieglingia decumbens* BERNH.) and two other plants (*Stellaria media* (L.) VILL., *Trifolium pratense* L.) observations were made whether they reacted to artificial infection and with what symptoms. Attempts at reverse transfer were not made. Infection was carried out by the caging of groups of 10 to 20 leafhoppers on about the same number of young plants. Although it was later ascertained that only a small percentage of the leafhoppers used (from Čejkov, Pelhřimov district) was infected with the oat sterile-dwarf virus, the characteristic symptoms were caused in two grasses, *Avena fatua* L. and *Poa annua* L. (figs 9 and 10). *Avena fatua* seemed to be more strongly affected by the oat sterile-dwarf virus than was the very susceptible cultivated variety of oats, Valečovský bílý, on which all experiments had been made. Both these grasses were also successfully infected with the wheat striate virus.

This result indicates that in studying the epidemiology of both viruses we should not underestimate the part which may be played by some grasses, and perhaps other plant species, as sources and particularly as reservoirs of infection in nature.

Discussion

It was ascertained in Finland that much greater damage is caused to oat crops by the mass transfer of leafhoppers *Calligypona pellucida* from areas of epidemic oat disease than by those caught in districts where the disease

was not widespread (HEIKINHEIMO 1957; KANERVO et al. 1957). The Finnish authors evolved two hypotheses from their results as to the actual cause of the disease: either a new virus is involved, or two population strains must exist among leafhoppers differing in the toxicity of their salivary excretions (KANERVO et al. 1957). DLABOLA (1957, 1958), who worked parallel with the present authors, also assumed that the cause of the disease might be either of the above factors, i. e. toxin or virus. NUORTEVA (1958) rejected—without proof— possibility of a virus being the cause and maintained that it is a toxin. This author tried to investigate the effect of extracts of the crushed salivary glands of *Calligypona pellucida* on the growth of cuttings from etiolated bean shoots, using the method which is otherwise employed for evaluation of the phytotoxicity or phytostimulation of insecticides (CASIDA and ALLEN 1951a, 1951b).

So far, however, no one, either in Finland or in Czechoslovakia, has succeeded in obtaining convincing proof to justify the toxin hypothesis. The mass transfer of leafhoppers to test crops, which has so far been the only method employed in infection experiments with OSDV, was incapable of providing a solution regarding the existence of toxic and non-toxic strains because it is not possible in this way to determine whether the disease has been caused by all or only some of the leafhoppers concerned. On the other hand, the individual study of the pathogenic powers of single insects on the plants enabled the authors of the present paper to separate individuals which caused disease in the plants on which they fed from those which did not influence the health of the plants on which they fed. Non-infectious individuals, both from OSDV epidemic areas and from areas where the occurrence of the disease was unknown, were then successfully infected by artificial means.

The results of this work, which have been sufficient to exclude the assumption that the cause of the symptoms under investigation is a non-infectious toxic substance, can be supplemented by the following findings.

It was observed that about four weeks elapse from the time when infectious leafhoppers are caged on oat plants to the time when it is possible to record reliable symptoms of OSDV. Neither local lesions nor necroses develop. The incubation period in other plants is still longer. Only after a considerable incubation period does a clear picture of systemic disease appear. For example, oats takes on a darker green colouring, the youngest internodes do not lengthen, the bases of the stalks swell, sprouting increases, etc. The systemic character of the disease is also illustrated by the following experiment: after oats had come to ear the main stalk was removed above the first node in 60 plants suffering from OSDV and in 60 healthy plants. The plants were then richly manured with nitrogenous and phosphatic manures and adequately watered. While shoots of the healthy plants developed normally and in most cases formed ears, those of the diseased plants remained dwarfed to the end of the vegetative period, although their number increased several fold as compared with the healthy plants.

If the originator of the disease is a toxic substance injected by the leafhoppers it must exert a systemic effect, which is extremely rare. If, however, the existence of systemic action by a toxin were nevertheless to be assumed

we should come into conflict with the pathogenesis of the disease. The disease would necessarily grow worse proportionately to the length of the feeding time of the insect on the plant. This does not, however, happen. Further, injury by a toxin should be most evident in the host just at the time when the insect is feeding, or shortly after its removal from the plant. Then the plants should recover more or less quickly. Nothing of the sort, however, occurs. The disease acts quite to the contrary, for its symptoms appear and then show a sharp progressive intensification quite a long time after the infected leafhoppers have fed on the plants.

The course of the disease clearly indicates that its originator reproduces itself in the host plant, because as long as it does not attain a certain accumulation the plant does not react to its presence with the characteristic symptoms. It is significant that the length of the incubation period during which this reproduction takes place is practically independent of the length of the feeding time (hours or weeks) of the infected insects on the plants. A plant can develop the most severe form of the disease quite independently of the number of insects caged on it. Feeding by a single infected leafhopper, which may last in some cases for a very short time (30 min.) is quite sufficient to cause infection. These facts are certainly in direct contradiction to the toxin hypothesis.

The fact that two different diseases have been found to be spread by the same species of leafhopper is also in itself a serious argument against the theory that symptoms of disease could be caused by toxins. If this were to be the case it would be necessary to assume that one insect species, homogeneous both in origin and morphology, must possess four physiologically differing populations, i. e.: non-toxic, with toxin producing the sterile-dwarf disease, with toxin producing the striate disease and finally with both toxins. Anything of the kind is most improbable.

References

BLATTNÝ, C.: Verbal communication, 1958.

BOJŇANSKÝ, V., BLATTNÝ, C.: Vírusové choroby obilovin v SSSR. [Virus diseases of cereals in the U.S.S.R.] — Za vysokou úrodu (Praha) **4** : 299—300, 1956.

CASIDA, J. E., ALLEN, T. C.: A laboratory method for evaluating the phytotoxicity or phytostimulation of insecticides. — Science **113** : 553—555, 1951a.

CASIDA, J. E., ALLEN, T. C.: Criteria for evaluating insecticidal phytotoxicity—root growth. — J. econ. Entomol. **44** : 741—746, 1951b.

DLABOLA, J.: Nové výsledky výzkumu příčiny nevymetání ovsa. [New results of research on the causes of failure to come to ear in oats.] — Za socialistické zemědělství (Praha) **7** : 1310 — 1314, 1957.

DLABOLA, J.: Calligypona pellucida Fabr. — ein Haferschädling und eventueller Vektor einer Getreidevirose. — Nachrichtenblatt dtsch. Pflanzenschutzdienst (Berlin) n. F. **12** : 36—38, 1958.

HEIKINHEIMO, O.: Delphacodes pellucida F. als Verheer von Hafer. — Ann. entomol. fennici, **22** : 184—187, 1956.

HEIKINHEIMO, O.: Über die Wiesenzirpe Delphacodes pellucida F. (Hom., Auchenorh.) als Haferschädling in Finnland. — IV. Int. Pflanzenschutz-Kongress, Hamburg—1957, Kurzfassungen der Vorträge VIII. **30** : 116—117, 1957.

KANERVO, V., HEIKINHEIMO, O., RAATIKAINEN, M., TINNILÄ, A.: The leafhopper Delphacodes pellucida F. (Hom., Auchenorh.) as the cause and distributor of the damage to oats in Finland. — Publications of the Finnish state agricultural research board, No. 160, Helsinki, 1957.

KLINKOWSKI, M., KREUZBERG, G.: Vorkommen und Verbreitung von Gramineenvirosen in Europa. — Phytopath. Ztschr. **32** : 1—24, 1958.

KÖHLER, E., KLINKOWSKI, M.: Viruskrankheiten. — Sorauers Hdb. d. Pflanzenkrankheiten, Bd. II., 6. Aufl., 1. Lief., Berlin-Hamburg, 1954.

NUORTEVA, P.: On the nature of the injury to plants caused by Calligypona pellucida (F.) (Hom., Areopidae). — Ann. entomol. fennici, **24** : 49—59, 1958.

PRŮŠA, V.: Mohl být virus příčinou kalamity na ovsech v Jihlavském kraji v roce 1956? [Could a virus be the cause of the catastrophic oats disease in the Jihlava region in 1956?] — Za socialistické zemědělství (Praha) **7** : 1054—1060, 1957.

PRŮŠA, V.: Virová choroba na ovsu v ČSR. Nové nálezy z fytopathologie, přednesené na schůzi Fytopathologické sekce Čs. společnosti botanické v Praze 13. I. 1958. [A virus disease of oats in Czechoslovakia. New findings in phytopathology, delivered at a meeting of the Phytopathological Section of the Czechoslovak Botanical Society in Prague on Jan. 13, 1958.] — Preslia, **30** : 363, 1958a.

PRŮŠA, V.: Die sterile Verzwergung des Hafers in der Tschechoslowakischen Republik. — Phytopath. Ztschr. **33** : 99—107, 1958b.

SLYKHUIS, J. T.: A survey of virus diseases of grasses in northern Europe. — FAO Plant Protection Bull. **6** : 129—134, 1958.

Addresses: Ing. V. Průša, Hop Research Institute of the Czechoslovak Academy of Agricultural Science, Žatec, Leninova 1070, Czechoslovakia.
Ing. Dr. E. Jermoljev and Ing. J. Vacke, Crop Production Research Institute of the Czechoslovak Academy of Agricultural Science, Praha-Ruzyně.

Вирусная стерильность и карликовость овса

ВЛАДИМИР ПРУША, ЕВГЕНИЙ ЕРМОЛЬЕВ, ИОЗЕФ ВАЦКЕ

Резюме

В статье даются новые доказательства, окончательно подтверждающие вирусный характер стерильности и карликовости овса (ВСКО), эпидемия которой была в 1956 г. в Йиглавском крае.

Приводятся замечания об обнаружении другого вируса желтухи, который в отличие от ВСКО вызывает заметную полосатость у хлебных злаков и, повидимому, идентичен с вирусом, описанным недавно (*Slykhuis* 1958) в Англии, и, может быть, также с вирусом, наблюдаемым в Германии и Дании под названием European cereal striate mosaic.

Вирусная полосатость пшеницы (ВПП), как мы называем эту болезнь, интересна в теоретическом отношении, так как зараженные самки *Calligypona pellucida* F. могут передавать ее потомству трансовариально; это свойство было впервые доказано у фитопатогенных вирусов на европейском континенте. На этом основании стало возможным разделить оба вируса, так как ВСКО трансовариально не переносится.

В статье даны доказательства того, что у цикадки *Calligypona pellucida* F. не существуют две генетически и физиологически отличающиеся популяции, существенно отличные токсичностью своих слюнных выделений. Гипотеза о существовании таких популяций возникла в Финляндии и ее сторонники имелись также в ЧСР.

Оба вируса являются первыми обнаруженными на хлебных злаках в ЧСР вообще. У обоих заболеваний переносчиками являются цикадки *Calligypona pellucida* F. ВСКО может переносится как личинками так и имаго. Продолжительность циркуляции ВСКО в теле переносчика довольно варьирует (от 9 до 42 дней), но в большинстве случаев не превышает 3—4 недель.

Инкубационный период ВСКО длится у овса 3—4 недели, у пшеницы и ячменя 4—7 недель; рожь отстает в развитии примерно 4 недели после инфекции, однако другие болезненные признаки у нее не проявляются. ВСКО более разрушительно действует на овес, чем на пшеницу или ячмень, на рожь действует меньше всего.

Для переноса ВСКО достаточно и получасовое пребывание одной зараженной цикадки на восприимчивом растении. После 3-дневного нахождения зараженной цикадки на овсе всегда имело место заражение вирусом.

Распространение ВСКО не ограничивается одной только областью эпидемии, так как наличие вируса удалось экспериментально доказать в гораздо меньшем масштабе в одном из местонахождений вблизи Праги.

Степень разрушительности ВСКО не увеличивается пропорционально продолжительности сосания цикадки на растении. Поэтому увеличение количества цикадок на растении не может иметь значение для степени повреждения растения, так-как уже одна цикадка способна перенести заболевание с самыми тяжелыми последствиями.

Концентрированные экстракты из гомогенатов тел зараженных цикадок, применявшиеся в избытке, как путем механического втирания в растения, так и путем инъекции в стебли, никогда не вызывали заметного повреждения у овса. Опыт с переносом ВСКО и ВПП почвой дал отрицательный результат. Так же опыт с переносом обоих вирусов через повилику (*Cuscuta campestris* Yunsk.) был с отрицательным результатом, хотя в этом случае это могло быть связано с тем, что повилика не склонна к паразитизму на хлебных злаках.

Единственным успешным опытом по переносу ВСКО иным способом, чем через насекомое, мы считаем результат опыта с прививкой (Blattný 1958).

При предварительном исследовании ареала растений-хозяев в природе было обнаружено, что оба вируса хлебных злаков могут вызывать характерные симптомы у *Avena fatua* L. и *Poa annua* L.

В дискуссии анализируются некоторые дальнейшие факты с точки зрения этиологии.

BIOLOGIA PLANTARUM — Издается Биологическим институтом Чехословацкой Академии Наук в Издательстве ЧСАН, Прага II., Водичкова 40. — Выходит 4 раза в год. Подписная цена на 1 год Кчс 30,—, цена одного номера Кчс 7,50. — Адрес редакции: Biologický ústav ČSAV, Na cvičišti 2, Praha 6. — Заказы: Artia, Smečky 30, Praha 2, Československo. — Типография: Knihtisk, n. p. závod 4, Praha 13, Sámova 12.

Issued by Biologický ústav Československé akademie věd. Yearly subscription (4 numbers) Kčs 30,—. Single number Kčs 7,50. — Address: Biologický ústav ČSAV, Na cvičišti 2, Praha 6. Orders: Artia, Smečky 30, Praha 2, Czechoslovakia.

Zájemcům v ČSR dodá Poštovní novinový úřad, Jindřišská 14, Praha 3.