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Diagnosis of sharka (plum pox) and host range of its inciting virus

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Abstract

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Diagnosis of sharka could be improved by distinguishing specific and unspecific symptoms. Two types of discoloration (broad red bands and thin red rings and lines) were specific, whereas the grooves and pits were not. Light and electron microscopy revealed the presence of nuclear and cytoplasmic inclusions in infected tissues. The cytoplasmic inclusions (bundles of needles) in plum fruits were reliable for diagnosis.

From the known herbaceous test plants *Chenopodium foetidum* and *Nicotiana clevelandii*, only *C. foetidum* was useful for diagnosis. *Ranunculus arvensis* and *Nicandra physaloides* were presented as possible new test plants.

Sixty herbaceous plant species were described as host of sharka virus. Some common weeds became systemically infected upon inoculation and may play a role in epidemiology of the virus.

Purified sharka virus was used to prepare antisera. Some properties of the virus were described.

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Introduction

Sharka is considered as one of the most serious threats to plum culture in Europe mainly because of the symptoms in the important varieties: grooving and pitting of the fruits, necrosis of the fruit flesh and a premature dropping of affected fruits. Considerable yield losses have been reported (Christoff, 1968; Darke, 1968). The causal agent of the disease, sharka (plum pox) virus, is transmissible by aphids. A rapid spread of the disease from tree to tree has been reported from several areas (Jordović, 1965). Sharka was first observed in 1915 - 1916 in Macedonia (Christoff, 1958) and the disease described in Rumania in 1922 as 'degeneration of the Agen-plum' was obviously the same (Savulescu & Pop, 1961). Later sharka was reported from Yugoslavia (Jossifowits, 1936), Hungary (Húsz & Klement, 1950) and Czechoslowakia (Smolak, 1954). During the last twenty years the disease has been reported from many other countries in Europe, including The Netherlands (Darke, 1968; Sutić, 1971).

In contrast with some countries in South-East Europe plum culture is not very important in The Netherlands. About 20 years ago plum orchards covered 5000 ha, about 8% of the total area fruit trees, but this has been reduced now to 1000 ha which is about 3.5% of the total fruit area (Anon., 1972). The cause of this diminished importance of plum growing is mainly a shift in cultivation. The shift to smaller trees in apple and pear growing, made possible by weak rootstocks, could not be achieved in plum culture. Another factor was the presence of serious diseases like bacterial canker and silverleaf, that shortened life of many plum trees. Sharka could become another threat to Dutch plum culture. It was therefore necessary to combat the disease immediately and to obtain precise data about the disease under Dutch circumstances.

The investigation comprised:

1. developing reliable and quick methods of diagnosis and
2. testing herbaceous wild plant species for their ability to act as hosts of the virus.

Review of literature

The first to study this serious disease of plum was Atanasoff (1932). He described symptoms on leaves and on fruits. Infected plum trees may show diffuse, chlorotic or light green flecks and rings on their leaves and a grooving and pitting on their fruits. He demonstrated, that the causal agent was obviously a virus since the disease was transmissible by grafting and also by the plum aphid *Brachycaudus helichrysi*.

Atanasoff proposed the name sharka which is Bulgarian for plum pox. The disease is now still known under this name although alternatives have been suggested. At the EPPO conference on sharka in 1968 it was decided to call the disease preferably: sharka (plum pox), (Darke, 1968).

After Atanasoff's study, sharka was reported from several European countries. This resulted in numerous investigations mainly dealing with symptoms of sharka on leaves and fruits of many local plum varieties, but also with the spread of the disease in plum orchards (Jossifowits, 1936; Ostojić, 1952; Christoff, 1958; Jordović, 1961; Jordović & Janda, 1963). In addition, several scientists did remarkable work on symptom expression: symptoms identical with those of sharka did occur in fruits of trees free from sharka virus (Christoff, 1958; Posnette & Ellenberger, 1963; Schuch, 1963; Kegler et al., 1964). Posnette and Ellenberger suggested the name pseudo-pox for this disease. This phenomenon and the now known varietal differences in symptom expression complicated diagnosis of sharka by symptoms.

For this reason various authors have investigated other methods for diagnosis, such as the use of indicator plants. The very susceptible plum variety Pocegaca was first introduced as an indicator but it does not react for some months or a season after infection at the earliest. A great improvement was the introduction of peach seedlings as indicator plants; in the glasshouse specific symptoms (vein yellowing and leaf deformation) could be expected 3 - 6 weeks after infection (Sutić, 1963).

At about the same time Németh (1963) succeeded in transmitting the virus to a herbaceous plant: *Chenopodium foetidum*. The inoculated leaves

reacted with characteristic yellow-ochre lesions, 7 - 10 days after inoculation with sap of leaves of infected plum trees. Thus, a very quick diagnosis with herbaceous indicator plants seemed possible. However, Kegler et al. (1964) were able to transmit the virus to *C. foetidum* in spring but not in summer.

In 1965, Kassanis & Sutić reported a second herbaceous test plant species: *Nicotiana clevelandii*. After inoculation with the virus, leaves showed necrotic rings within a week and a systemic mottle within 2 weeks. *N. clevelandii* was reported to show symptoms after inoculation throughout the whole season.

The transmission of sharka virus to herbaceous plants greatly facilitated the study of the virus itself. Some properties of the virus in sap of *C. foetidum* and *N. clevelandii* were reported and the first data about the morphology of the virus also became available. Flexuous particles with a length of about 764 nm were observed in extracts of infected plum leaves (Kegler et al., 1964), whereas particles with an estimated length of about 725 nm were seen in sap of infected leaves of *N. clevelandii* (Kassanis & Sutić, 1965). Their preliminary purification experiments failed, however.

The transmission of sharka virus to herbaceous plants could also have implications for the understanding of the epidemiology of sharka virus. However, apart from the two herbaceous test plants no other hosts of sharka virus were known than some *Prunus* sp. Up to now spread of sharka virus has been only observed from plum to plum in orchards (Jordović, 1963). The spread of sharka virus is mainly thought to be caused by man (grafting and budding of infected material) and in a natural way by the aphid species *Brachycaudus helichrysi, Myzus persicae* and *Phorodon humuli* (Atanasoff, 1932; Vaclav, 1960; Jordović, 1963; Kassanis & Sutić, 1965). Kassanis & Sutić (1965) proved the non-persistent transmission of the virus by *M. persicae*. They were able to transmit sharka virus from *N. clevelandii* to plums by this aphid species. They concluded that epidemiology of sharka may be far more complicated if plants of the natural vegetation served as hosts for the sharka virus. Baumann (1968) proved that *Prunus spinosa*, the hawthorn, which is widespread in nature throughout Europe should be considered as a host of the virus. But the investigations of Németh (1963), Kegler et al. (1964), Savalescu & Macovei (1965) and Kassanis & Sutić (1965) on a possible range of herbaceous plants as hosts for sharka virus revealed only *C. foetidum* and *N. clevelandii* as such. Therefore, the number of herbaceous hosts of sharka virus seemed rather limited.

Thus, in the past 40 years many investigations on sharka have been done but several aspects are still not clear. This study aims at contributing to the knowledge of sharka and its inciting virus.

Discussion

During the first observations of sharka disease in The Netherlands, the symptoms seemed to be fairly similar to the descriptions in the literature. This was especially so with leaf symptoms (diffuse chlorotic rings and flecks). Although Savalescu & Macovei (1965, 1968) encountered problems in distinguishing plum pox and line pattern symptoms, for Dutch varieties this was seldom a point of confusion. Most difficulties were caused by seasonal influences on symptoms expression and by the difference in distinctness of symptoms between the varieties. For example, symptoms on the leaves of 'Mirabelle de Nancy' were usually absent, whereas on the leaves of 'Czar' the symptoms were distinct between May and October. In general, symptoms on the leaves were easiest to detect in June and early July whereas later the symptoms were less prone. It seemed therefore better to use fruit symptoms for diagnosis during summer.

Fruit symptoms became visible 3 - 4 weeks before picking and thus, depending on the variety, they could be expected between the end of July and the beginning of September. The symptom 'grooving and pitting' was usually distinct and much easier to detect than leaf symptoms. However, similar symptoms on fruits of trees free from sharka virus ('pseudo-pox') have been reported (Christoff, 1958; Kegler et al., 1964; Posnette & Ellenberger, 1963; Schmid, 1968; Schuch, 1961). On fruits of Dutch varieties free from sharka virus the symptom could be established and, unfortunately, no differences were seen with the grooving and pitting on fruits of infected trees.

It has been suggested that other viruses in plum were causal agents of 'pseudo-pox'. Line pattern virus (Kegler et al., 1963) and dark green sunken mottle virus (Anon., 1967; Cropley, 1968; Marenaud, 1971) were reported in relation to the symptom. Van Oosten (1971, 1972) postulated a cumulative or synergistic effect of viruses on symptom expression. This suggestion was based on the observation that grooving and pitting in the presence of sharka virus was more severe than without this virus.

In the literature on sharka disease nearly all attention has been given to the grooving and pitting of the fruits: 'sharka' and 'grooving and pits' seemed to be interchangeable. Even the later detected comparable symptoms on fruits of trees free from sharka virus were named : 'pseudo-pox' or 'sharka-like'. It was therefore quite surprising to distinguish two more types of symptom on the fruits of several varieties: 1) broad red coloured bands, diffuse on one side and sharply defined on the other and 2) thin red coloured rings and lines, mainly on the lower side of the fruit. These discolorations also appeared 3 - 4 weeks before picking and were completely independent of the grooves and pits. On varieties with red fruits the discolorations remained visible during that period but on varieties with purple fruits the discolorations were only seen 5 - 10 days before colouring of the fruit and later vanished.

Discolorations were apparently also observed by Christoff (1958) and Jordović (1961) but they obtained no further attention. The discolorations were probably neglected in the past because these symptoms were hardly of economic importance on the varieties in the areas were sharka originated. The discolorations are present on the fruits of infected trees of Pocegaca (without doubt the most widespread variety in Yugoslavia), but are not visible on ripe fruits which are dark purple. The variety Pocegaca is similar or probably identical to 'Kjustendilska sliva' and 'Bistrita' (the main varieties of Bulgaria and Rumania, respectively) and Hauszwetsche (DDR, DBR, Belgium, The Netherlands). Varieties with red fruits are rare: only 'Victoria' has some distribution in north-west Europe. In The Netherlands 'Victoria' is the main variety: 40% of all plum trees belong to this variety.

Thus, the diagnosis of sharka virus by fruit symptoms was greatly improved by distinguishing between specific and unspecific symptoms. Surprisingly, the most important symptom economically was not reliable for diagnosis of the disease.

If external symptoms are not conclusive or not present at all some test methods might be used for diagnosis. The simplest method seemed to be the use of herbaceous indicator plants *Chenopodium foetidum* and *Nicotiana clevelandii*. The reliability of these species for diagnosis was tested throughout the year by using sap of leaves and fruits of plums infected with sharka virus. No reactions were obtained on *N. clevelandii* (in some cases a local latent infection could be traced). Németh (pers. commun.) encountered the same difficulties with *N. clevelandii* and suggested that the different results obtained with this test plant were caused by strains

of the virus. In contrast, Kassanis & Sutić (1965) obtained positive reactions on this test plant throughout the year.

According to data of Kegler et al. (1964) *C. foetidum* proved to be useful in the spring with buds and young leaves as source of inoculum but not in summer and autumn with expanded leaves as source of inoculum. This is most likely due to an increasing amount of inhibitors (polyphenols, tannins) in the leaves and their negative influence on virus transmission (Fulton, 1966). However, during summer, ripe fruits were excellent sources of inocula and sap transmissions to *C. foetidum* were quite reliable.

Thus, with this test method grooves and pits of sharka disease can be distinguished from those of 'pseudo-pox'. Usually a second virus was transmitted to *C. foetidum*. This virus was latent but gave distinct symptoms on *C. quinoa* and *C. amaranticolor* (van Oosten, 1970). The virus was transmitted to peach seedlings by grafting and it was shown to be dark green sunken mottle virus (van Oosten, unpublished).

As a result of the study on a herbaceous host range for sharka virus, two plant species were found to be potential test plants for sharka virus: *Ranunculus arvensis* (yellow local lesions within 8 - 10 days on the primary leaves) and *Nicandra physaloïdes* (pin point black necrotic local lesions within 5 - 6 days). If leaves of both plant species were inoculated with sap from leaves of plum infected with sharka virus, lesions were easily obtained on *R. arvensis* but often inconspicious on *N. physaloïdes*. However, the practical value of these species has not yet been established in large scale tests.

A still quicker and more reliable method for diagnosis of sharka virus became available after the detection of inclusion bodies in the cytoplasm and nucleus of herbaceous plants infected with sharka virus (Plese et al., 1969; van Oosten & van Bakel, 1970). These inclusions were also present in the cytoplasm of cells of petals and fruits of plum. The parenchyma cells of plum fruits are very large and inclusion bodies were easy to detect. Parenchyma cells of fruits of all examined varieties with a sharka virus infection contained these inclusions, whereas fruits of trees free from sharka virus did not. Thus this method is also useful for distinguishing between grooves and pits on fruits of trees with and without sharka virus.

It was hoped to improve diagnosis of sharka by serological means. However, the purification of the virus was difficult and this complicated the preparation of antisera. Therefore most attention was given to the improvement of the purification procedure. Partial purified virus suspensions were

obtained by several investigators (György & Németh, 1968; Schade, 1969; Ranković & Jordović, 1970; Babović et al., 1971). However, the considerable loss of virus during the first stages of purification was very unsatisfactory. This loss of virus was apparently due to aggregation of the virus with plant constituents and the subsequent removal by low-speed centrifugation. An attempt was made to avoid such losses by dissolution of membranes with the nonionic detergent Triton X-100. This worked quite well. A comparison of purification procedures based on clarification by ether/tetra (the mildest conventional procedure) and Triton X-100 showed large differences in virus yields and purity. At about the same time Noru & Yamaura (1971) published the successful purification of tobacco mosaic virus from Zinnia with the use of Triton X-100. My experiments showed that Triton X-100 might have a much wider application in purification as several other elongated viruses were readily purified in this way.

Sharka virus could thus be obtained in fairly large quantities and the preparation of antisera was quite easy. Specific titers of sera of 4096 were obtained. When such sera were used in spring some positive reactions were obtained with sap of leaves of infected peaches, plums and apricots. After the beginning of June, however, not a single positive reaction in microprecipitation tests was obtained. This is therefore comparable with the situation of testing of sap of leaves to *Chenopodium foetidum*.

There are so far only a few reliable indications about the possibilities of serological detection of sharka virus. György & Németh (1968) obtained promising results with sera-diagnosis of sharka in apricots, but not in plum. Schade (1969) reported positive serological reactions with leaves and fruits of infected plum trees but considered later that transmissions of sap to test plants were more reliable (Schade, in press).

Thus, several methods for purification of sharka virus are now available and antisera can easily be prepared. A routine method for serological detection of sharka virus is not yet available, however.

Sharka virus was well-known for its natural spread in orchards. Several data indicated a very quick spread in some areas of Yugoslavia, but elsewhere the spread was slow or apparently absent (Jordović, 1963, 1965). Jordović pointed to the presence of aphid vectors, their effectiveness of transmission and the difference in susceptibility between the varieties: The most important variety, viz. Pocegaca was the most susceptible one. These factors together could not entirely explain the difference in spread between the several parts of this country. After the detection of two

herbaceous plant species as hosts for sharka virus, Kassanis & Sutić (1965) suggested a possible role of weeds as hosts being undetected factor in the epidemiology of the virus. Their attempts, as well as these of several colleagues (Kegler et al., 1964; Savalescu & Macovei, 1965; Cropley, 1968) to enlarge the herbaceous host range failed, however. Later on, Schmid (1968) and Posnette (1968) also speculated about the role of weeds in the epidemiology of the virus but no new data were available to support this idea. It was therefore somewhat surprising to find that many herbaceous plant species could be infected experimentally. Apparently two factors have influenced the results: The use of sap of leaves of *N. clevelandii* instead of sap of leaves of *C. foetidum* or plum and the routine back inoculations to *C. foetidum*. It was easy to show that the use of sap of *C. foetidum* was very ineffective probably due to inhibitors in the sap (Schmelzer, 1959). Back inoculations were a necessity since many infected plant species were symptomless.

Thus, the virus has an impressive experimental herbaceous host range. This host range includes several common weeds, some of which became systemically infected. In the glasshouse sharka virus could easy be transmitted by *Myzus persicae* from peaches and plums to *Lamium amplexicaule* and vice versa. This demonstrated clearly the possibility that herbaceous plants may act as hosts under natural conditions.

Recently Kröll (in press) detected 10 different weed species naturally infected with sharka virus in an orchard containing sharka-diseased plum trees, and this may be the final proof of the actual situation. Baumann (1968) already observed some hedges of *Prunus spinosa* naturally infected with sharka virus and in addition, Jordović et al. (1971) found that *Prunus spinosa*, which is widespread in the natural vegetation, is infected with sharka virus to an important degree in some areas of Yugoslavia. The fact that weeds and shrubs act as sources for sharka virus contributes to the explanation of the difference in the speed of the spread between several areas: the degree of infection in the natural vegetation may be different. If the virus has escaped from infected orchards into the natural vegetation, this vegetation might be an important virus source after some time. This may be the case in areas where sharka virus has been known already for a long time. In areas with recent introductions of infected trees the spread may be initially slow as only the infected trees are sources of infection. Thus, the difference in infection potential may certainly contribute to the differences in the speed of the spread between several areas.

In short, the host range of sharka virus is less restricted as supposed before: *Prunus* species as well as weeds of several plant families may act as sources of infection and may be important factors in epidemiology of the virus.

Apart from the data on diagnosis and host range, more information was obtained about the inclusion bodies and the virus itself.

The observations with the light microscope on inclusion bodies revealed some interesting phenomena. The most remarkable one is probably the transiency of the nuclear needles: they first appeared about 2 weeks after infection in *N. clevelandii* leaves, remained for 5 - 7 days and then disappeared. Shortly before or after their disappearance similar needles became apparent in the cytoplasm. These cytoplasmic needles were seen for months. A comparable transiency of nuclear inclusions was observed by Robb (1963) for dahlia mosaic virus. She explained the disappearing of nuclear inclusions by extrusion from the nucleus into the cytoplasm. This is apparently not the case for inclusions evoked by sharka virus; not a single situation was observed suggesting this phenomenon. It seemed more likely that nuclear inclusions were broken down and transported to the cytoplasm.

It was interesting to examine the appearance of the needle-like inclusions (seen with the light microscope) in ultrathin sections (observed with an electron microscope). The observations in ultrathin sections showed an abundance of 'pinwheels' and 'lamellar aggregates' in the cytoplasm of the cells. Less frequent were elongated 'crystalline structures'. Pinwheels could also be observed without trouble with the light microscope in cells of epidermal strips of *N. clevelandii*; they were not identical with the needle-like inclusions. Edwardson et al. (1968) suggested that the bundles of needles observed with the light microscope in cells of plants infected with watermelon mosaic virus were, in fact, concentrations of pinwheels. For sharka virus this seemed to be incorrect. The needle-like inclusions in the nucleus and the cytoplasm were of similar appearance. However, in ultrathin sections no pinwheels were observed in the nucleus; the nuclear inclusions seemed to be much longer than thick with a crystalline structure and had a same appearance as the crystalline structures seen in the cytoplasm. It is now suggested that the needle-like inclusions observed with the light microscope are identical with the 'crystalline structures' observed in ultrathin sections with the electron microscope and not with 'concentrations of pinwheels' (Edwardson et al., 1968). This is in agreement with the opinion of Bovey (1971).

Nuclear inclusions have been reported from ultrathin sections of leaves of apparently healthy *Dianthus* species (Weintraub et al., 1968; Rubio-Huertos et al., 1968). These inclusions showed a clear striation in their length. It is, however, difficult to say whether these nuclear inclusions in apparently healthy *Dianthus* sp. are comparable with those in *N. clevelandii* infected with sharka virus. In healthy *N. clevelandii* nuclear inclusions did not occur at all.

The presence of 'pinwheels' and 'lamellar aggregates' in tissues infected with sharka virus has now been shown by Plese et al. (1969), Bovey (1971), Macovei (1971), van Bakel & van Oosten (1972). Edwardson (1966) disclosed that only viruses of the potato virus Y group evoke this kind of inclusion and therefore their presence is another argument for classifying sharka virus into this group.

The reported particle length of 764 nm and particle width of 16 nm are in good agreement with the values given by several investigators. From dip preparations and preparations of partially purified suspensions, Kegler et al. (1964), Kassanis & Sutić (1965) and Babović et al. (1971) reported particle length between 725 en 764 nm. Bovey (1971) and Macovei (1971) determined particle length in ultrathin sections of infected tissues. Bovey (1971) found values between 750 and 800 nm, but Macovei reported remarkably different length between 1000 and 2000 nm.

For particle width, values of 20 nm and 17 nm were reported from shadow-cast dip preparations by Kegler et al. (1964) and Bovey (1971), respectively. The latter author found a width of 15 nm for particles in ultrathin sections of infected tissues.

The procedure used for the purification of sharka virus resulted in virus suspensions of high purity. This virus proved to be comparable with other viruses of the potato virus Y group, like tobacco etch virus (Purcifull, 1966) and maize dwarf mosaic virus (Seghal & Jean Jong-ho, 1970). The somewhat differing data published by Ranković & Jordović (1970) and Babović et al. (1971) might be caused by the use of less pure virus suspensions.

Samenvatting

De virusziekte 'sharka' wordt algemeen beschouwd als één van de ernstigste ziekten die bij pruim kunnen voorkomen. Dit is voornamelijk gebaseerd op het effect, dat de ziekte op de vruchten heeft èn op de wijze waarop de ziekte wordt verspreid.

De symptomen zijn beschreven als putten en groeven in de vrucht, gepaard gaande met gomvorming en necrose in het vruchtvlees en een vroegtijdige vruchtval. Enorme opbrengstdervingen zijn gemeld.

In gebieden waar sharka reeds vele jaren bekend is, heeft men soms een zeer snelle verspreiding van de ziekte in boomgaarden waargenomen. Als vector kunnen enige bladluissoorten fungeren. De ziekte heeft zich vooral de laatste 20 jaar vanuit Midden- en Zuidoost-Europa naar West-Europa uitgebreid. In 1966 werd de ziekte voor het eerst in Nederland waargenomen. Dit heeft ertoe geleid, het virus en de ziekte ook in Nederland te bestuderen. In dit onderzoek werd bijzondere aandacht besteed aan de diagnose van de ziekte en de vraag of waardplanten van het pathogeen in de natuurlijke vegetatie voorkomen.

Uit plantmateriaal, waarvan op grond van symptomen werd verwacht dat het geïnfecteerd was met het sharkavirus, werd een virus geïsoleerd dat kon worden geïdentificeerd als het sharkavirus. De gevonden bladsymptomen bleken overeen te komen met die welke in de literatuur zijn vermeld, maar bij de vruchtsymptomen was dit niet geheel het geval. De 'groeven en putten' werden waargenomen op vruchten van met het sharkavirus geïnfecteerde bomen van verschillende rassen. Evenwel bleken deze verschijnselen ook voor te komen op vruchten van een aantal rassen zonder sharka. In de literatuur vat men de groeven en putten op pruimevruchten wel samen onder de naam 'pseudopox' als het sharkavirus niet aanwezig is. Opmerkelijk was het voorkomen van twee typen schilverkleuring op de vruchten van sharka-zieke bomen van rassen met rode of paarse vruchten. Op vruchten van bomen zonder een infectie met het sharka virus werden deze typen schilverkleuring nooit waargenomen. Geconcludeerd werd dat de (specifieke) schilverkleuringen betrouwbaar zijn te gebruiken voor de diagnose, maar de (niet specifieke) groeven en putten echter niet.

De kruidachtige toetsplanten *Chenopodium foetidum* en *Nicotiana clevelandii* werden op hun bruikbaarheid voor de diagnose van sharka onderzocht. Alleen *C. foetidum* voldeed: in het voorjaar werden positieve reacties verkregen wanneer extracten van jonge bladeren of uitlopende knoppen als inoculum werden gebruikt. In de zomer was dit alleen het geval als met het sap uit rijpe vruchten werd geïnoculeerd.

Ranunculus arvensis en *Nicandra physaloïdes* werden beschreven als mogelijke nieuwe toetsplanten.

In met het sharka virus besmette planten werden gemakkelijk herkenbare insluitsels gevonden. Deze ontbraken in planten die vrij waren van dit virus. De insluitsels waren talrijk in parenchymcellen van rijpende vruchten van zieke pruimebomen. Het voorkomen van insluitsels bleek vooral nuttig bij het onderscheiden van 'groeven en putten' als sharka-symptoom en als 'pseudopox'.

Tegen het sharka virus werden antisera bereid met een titer van 4096. Met behulp van de microprecipitatietoets kon in het voorjaar het sharka virus worden aangetoond in perzik, pruim en abrikoos. De betrouwbaarheid van deze toets was echter vrij gering. Na begin juni werden in het geheel geen positieve reacties meer verkregen.

In inoculatieproeven bleken veel meer soorten als waardplant van het sharka virus te kunnen fungeren dan uit de desbetreffende literatuur kon worden afgeleid. Van de 180 in het onderzoek betrokken kruidachtige plantesoorten, behorend tot 28 families, bleken 60 soorten, behorend tot 8 families, vatbaar voor het sharka virus. Enkele zeer algemene onkruiden werden systemisch door het virus geïnfecteerd (*Lamium amplexicaule, Lamium purpureum, Vicia sativa ssp. angustifolia*). In kasproeven werd het virus met behulp van *Myzus persicae* overgedragen van perzik en pruim naar *Lamium amplexicaule* en omgekeerd. Deze resultaten wijzen op de mogelijke betekenis van wilde, kruidachtige planten in de epidemiologie van de ziekte.

Insluitsels konden in *Nicotiana clevelandii* pas circa 2 weken na infectie met het virus worden waargenomen. De eerste, naaldvormige insluitsels bevonden zich in de celkern. Pas 5 - 7 dagen nadien werden grotere bundels naalden in het cytoplasma aangetroffen, terwijl omstreeks dat moment de kerninsluitsels weer verdwenen. Nog weer 5 dagen later vormden zich granulaire insluitsels in het cytoplasma. In het cytoplasma bleven de naaldvormige en granulaire insluitsels maandenlang zichtbaar.

Met de elektronenmicroscoop werden in ultradunne coupes schoepenradvormige insluitsels ('pinwheels'), lamellaire aggregaten en kristallijne structuren in het cytoplasma waargenomen, terwijl in de kern overeenkomstige kristallijne structuren bleken voor te komen. Verondersteld werd, dat deze kristallijne structuren identiek zijn aan de naaldvormige insluitsels, die met de lichtmicroscoop in de kern en het cytoplasma waren waargenomen.

Met de elektronenmicroscoop werden ook insluitsels gezien in extracten van bladeren van *Nicotiana clevelandii*. Deze insluitsels vertoonden 2 strepingen onder een vaste hoek van 80 graden.

Een bevredigende zuivering van het sharka virus werd verkregen met behulp van Triton X-100, differentiëel centrifugeren en centrifugeren in een suikergradiënt. Opbrengsten van 20 - 45 mg virus per kg blad werden verkregen. De virusdeeltjes waren in gezuiverde suspensies gemiddeld 764 nm lang. De dikte werd in ultradunne coupes op 16 nm bepaald. Deze en andere eigenschappen wijzen er op dat het virus terecht kan worden ingedeeld bij de aardappel-Y-virusgroep.

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Abstracts from papers

The isolation of sharka (plum pox) virus from leaves and fruits of plum with herbaceous plants

From leaves and fruits of plum trees with apparent sharka symptoms, a virus was transmitted to *Chenopodium foetidum* and *Nicotiana clevelandii*. *C. foetidum* reacted with yellow-ochre lesions upon inoculation. *N. clevelandii* sometimes became infected. The infection was symptomless and thus *N. clevelandii* was not useful as test plant. The virus was transmitted from *C. foetidum* to *N. clevelandii* by inoculation of sap and from *N. clevelandii* to peach seedlings and vice versa by the aphid species *Myzus persicae*. It was shown that the isolated virus was sharka virus. Another still unidentified virus was usually transmitted from plum to *C. foetidum* but caused no symptoms on this test plant.

Herbaceous host plants for the sharka (plum pox) virus

A number of 75 species belonging to 18 families were tested for their susceptibility and sensitivity to the sharka virus of plum using sap from infected *Nicotiana clevelandii* leaves; 27 species out of 6 families were found to be new hosts. Only *Ranunculus arvensis* may serve as a new test plant. Common weeds and garden plants were among the newly found host plants. *Lamium amplexicaule* and *Zinnia elegans* became systemically infected. In the glasshouse the virus was transmitted by *Myzus persicae* from peach seedlings to *L. amplexicaule* and vice versa. If transmitted in the field as easily as in the glasshouse, elimination of the virus might be very difficult.

Further information about the herbaceous host range of sharka (plum pox) virus

A hundred and eighty species belonging to twenty-eight families were tested for their susceptibility and sensitivity to the Sharka virus of Plum, obtained from Sharka-infected *Nicotiana clevelandii* leaves. Sixty species out of eight families were found to be new hosts. Only *Ranunculus arvensis* and *Nicandra physaloides* may serve as new test plants. Common weeds and garden plants were among the newly found host plants. *Lamium amplexicaule, L. purpureum, Zinnia elegans, Vicia sativa* spp. *angustifolia, Nicotiana exigua* and *N. megalosiphon* became systemically infected. In the greenhouse the virus was transmitted by *Myzus persicae* from peach seedlings to *L. amplexicaule* and vice versa. If transmission in the field is as easy as in the greenhouse, elimination of the virus may be very difficult.

The diagnostic value of [symptoms on and] inclusion bodies in the fruits of plum trees infected with sharka virus

On the fruits of Sharka-diseased trees, four types of symptom were seen which were independent of each other, viz. purple pits and irregular grooves, oval pale flecks, thin sharply defined red lines and rings, and band-like discoloration. Symptoms similar to the former two types of symptom were found also on fruits of Sharka-free trees. The latter two types of symptom were found on Sharka-diseased trees only. Which of these symptoms is the true Sharka symptom is difficult to say. An explanation of the occurrence of several external symptoms on the fruits of one tree is that these may be a synergistic effect of Sharka virus on the expression of symptoms of other viruses occurring commonly in Plum.

In parenchyma cells of the fruits of Sharka-diseased trees only, cytoplasmic and intranuclear inclusion bodies were found.

The external symptoms on the fruits can be used for diagnosis when the full range of symptoms is known for every variety with and without Sharka virus infection. The internal symptoms, viz. the inclusion bodies, seem to be specific. Therefore they could be of value for diagnosis of Sharka virus disease. The inclusions could be helpful for differentiation between Plum Pox and pseudo-Pox fruit symptoms.

Diagnosis of sharka (plum pox) by internal and external fruit symptoms

Depending upon the variety, fruits of plum trees infected with sharka virus may show grooves and pits, red bands and thin red rings and lines. The latter two types of symptom were only found on fruits that become orange, red or purple during ripening. On fruits of trees free from sharka virus these discolorations were never observed and therefore these symptoms are diagnostic for sharka virus. In several varieties the grooves and pits, previously thought to be the main symptom produced by sharka virus on plum fruits, were observed more or less frequently on fruits of trees free from sharka virus. Therefore, this symptom was unreliable for diagnosis of sharka virus under Dutch conditions.

Inclusions were present in parenchyma cells of fruits of all varieties, when infected with sharka virus. They may be helpful for diagnosis when external symptoms are not conclusive.

Purification of plum pox (sharka) virus with the use of Triton X-100

Plum pox virus was purified by adding up to 5% non-ionic detergent Triton X-100 to extracts clarified by low-speed centrifugation. After stirring for 1/2 h, the suspensions were subjected to 2 cycles of differential centrifugation followed by sucrose density-gradient centrifugation. Purity of the product was confirmed by electron microscopy and equilibrium density-gradient centrifugation in CsCl. The virus sedimented in the analytical ultracentrifuge as a single peak with a sedimentation coefficient of about 170 S at infinite dilution. Virus so purified showed an absorption spectrum with a minimum at 247 nm and a maximum at 263 nm. The modal length of the virus particles in purified preparations was 764 nm. Antiserum prepared had a titer of 4096.

Inclusion bodies in plants infected with sharka (plum pox) virus

Sharka virus was found to give rise to the formation of inclusion bodies in nucleus and cytoplasm of host cells, as is known for several other viruses of the potato virus Y group. In inoculated *Nicotiana clevelandii* needle-shaped inclusion bodies were found loosely distributed in the nucleus 10 days after the first external symptoms appeared. In the cytoplasm, bundles of needles and granular inclusions arose 14 and 18 days, respectively, after external symptoms became visible. The intranuclear needles disappeared short-

ly before or after the first appearance of granular cytoplasmic inclusions.

Inclusion bodies abound in parenchyma cells of fruits from sharka-diseased plum trees, but they did not occur in fruits from sharka-free trees, with or without pseudo-pox symptoms. Thus, inclusion bodies can be of value in the diagnosis of sharka and be of great help in differentiating between plum pox and pseudo-pox.

Additional data on the ultrastructure of inclusion bodies evoked by sharka (plum pox) virus

Electron microscopy of ultrathin sections of leaves of *Nicotiana clevelandii* infected with sharka virus revealed several types of cytoplasmic inclusions. Pinwheels and lamellar aggregates were frequent. Pinwheels showed a central core with a threadlike structure in the middle. Lamellar aggregates showed a striation with a periodicity of 55 Å on their surface and they were associated with the endoplasmic reticulum. Irregular crystalline structures were found less frequently. Microbodies were common in the cytoplasm of sharka virus infected *N. clevelandii* plants, but they also abounded in healthy controls.

Nuclear inclusions were present only for short periods after infection.

In leaf extracts irregularly shaped inclusions were found. They had a regular striation of 55 Å. Sometimes nearly parallel stripes were seen on their surface, always at an angle of 80 degrees with the striation.