

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

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Abstract

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.

Introduction

Diagnosis of sharka (plum pox) by leaf symptoms is easiest during June and the beginning of July, when most plum varieties show distinct symptoms under Dutch conditions. Usually, diffuse, chlorotic rings and flecks on the leaves can be seen. Depending upon the variety the leaf symptoms may disappear or become less distinct during summer. In contrast, fruit symptoms become visible during this period and, if specific, might be helpful for diagnosis.

Irregular grooves and pits on plum fruits have been considered the main symptom of sharka virus (*/*; */*; E/E; S/Ap) infection (Christoff, 1958; Darke, 1968; Schmid, 1968; Šutić, 1971). Little attention has been given to discolorations of the fruit surface, although their occurrence has been reported by Christoff (1958) and Jordović (1961). This is easy to explain because fruits become worthless when showing grooves and pits. However, the value of this severe fruit symptom for diagnosis of sharka is doubtful because similar symptoms occur on fruits of some plum varieties without sharka virus infection (Christoff, 1958; Schuch, 1961; Posnette and Ellenberger, 1963; Kegler et al., 1964). Recently, these 'pseudo-pox' symptoms were also reported from the Netherlands (van Oosten, 1971). It was thought necessary therefore to investigate the specificity of the symptoms on the fruits of the main varieties of plum grown in the Netherlands in order to assess their value for field diagnosis of sharka.

Materials and methods

Observations of fruit symptoms. Trees with and without sharka virus belonging to the main Dutch plum varieties were compared for fruit symptoms. Observations were made during the period 1968–1971. During the first year observations were made every 14 days between April and October, and every 7 days during the ripening period. Symptom expression was also followed on individually labelled fruits. Based on preliminary results (van Oosten, 1971) the observations in later years were concentrated during the 6 weeks before harvest of each variety.

The number of trees infected with sharka virus was restricted and for some varieties only one tree with fruits was available for research during one season. For most varieties the number of trees free from sharka virus was almost unlimited.

Occurrence of other viruses in plum trees examined. Plum trees known to be either infected with or free from sharka virus were also tested for other viruses by graft-indexing to peach seedlings in the glasshouse. Trees free from sharka virus were placed into groups of five trees, all of uniform appearance and either with or without 'pseudo-pox' on their fruits. This was based on symptoms during the 1970 season; 'pseudo-pox' was much less prevalent in 1971. Three peach seedlings were grafted per tree and they were observed for symptoms during 3–4 months.

The occurrence of sharka virus in fruits was tested by mechanical inoculation of sap to *Chenopodium foetidum* (van Oosten, 1970).

Inclusion bodies. Fruits were also examined for the presence of inclusion bodies as described earlier (van Oosten and van Bakel, 1970).

Results

Three types of symptom were observed on fruits of plum trees infected with sharka virus. These symptoms were compared with those on fruits of trees free from sharka virus.

Type 1. Irregular grooves and pits on the fruits together with browning of the underlying tissues of the fruit flesh.

This type was found on the fruits of several varieties, independently of sharka virus infection (Table 1 and 2; Plate 1, 2 and 3). Its occurrence was very dependent on the season. During any one season large differences in symptom expression were found for trees of the same variety planted in different orchards.

The grooves and pits could be found on the first well-grown fruits. Affected fruits ripened somewhat earlier and usually dropped before unaffected fruits became ripe. Sometimes, however, affected fruits remained on the tree.

Usually, trees free from sharka virus had only a few fruits showing grooves and pits, but on 'Warwickshire Drooper' (Plate 1a) and 'Zoete Kwets' (Plate 3a) and occasionally on 'Victoria' (Plate 2a) and 'Early Prolific' the symptom was quite common. Fruits of some varieties with green or yellow fruits showed vague dark lines and spots below the fruit surface, suggesting the beginning of grooving and pitting. Sectioning showed that these lines and spots were caused by browning of the fruit flesh 3–5 mm below the fruit surface. In 'Reine-Claude verte' this browning sometimes

Table 1. The occurrence of symptoms and inclusions in fruits of Dutch grown plum varieties with sharka virus infection.

Variety	Symptom ¹				
	grooves and pits	browning of fruit flesh	broad red bands	thin red rings and lines	cellular inclusions
<i>varieties with green or yellow fruits:</i>					
Mirabelle de Nancy	+	+	—	—	+
Ontario	+	+	—	—	+
Reine-Claude d'Oullins	+	+	—	—	+
Reine-Claude verte	+	+	—	—	+
Tonneboer	+	+	—	—	+
Warwickshire Drooper	+	+	—	—	+
<i>varieties with orange or red fruits:</i>					
Early Laxton	+	+	+	+	+
Fleuriana	+	+	+	—	+
Victoria	+	+	+	—	+
<i>varieties with dark red or purple fruits:²</i>					
Bleue de Belgique	+	+	+	—?	+
Brompton (rootstock)	—?	—?	+	+	+
Czar	—	—	+	+	+
Early Prolific	+	+	+	+	+
Opal	+	+	+	+	+
Reine-Claude d'Althan	+	+	+	+	+
Vroege Tolse	+	+	+	+	+
Wolters' Blauwe	+	+	—?	+	+
<i>damson plums with dark purple fruits:</i>					
Hauszwetsche	+	+	+	—	+
Italiaanse Kwets	+	+	+	—	+
Zoete Kwets	+	+	+	—	+

¹ +: found with certainty in one or more seasons; —?: not found, but observations made during only a single season; —: not found in observations during 2–4 seasons.

² Trees of the variety 'Monsieur bâtif', infected with sharka virus, were not available.

Tabel 1. Het voorkomen van symptomen op en insluitsels in de vruchten van in Nederland voorkomende pruimerassen, geïnfecteerd met het sharkavirus.

reached the skin, causing red spots (Plate 6). Pits and grooves were present only when a more severe browning reached the fruit surface. Sometimes, the entire flesh of the fruit below the grooves and pits was brown and necrotic, resulting in a brown spot on the stone.

The varieties that showed grooves and pits on the fruits when free from sharka virus also showed the symptom when infected, but the grooving and pitting was usually more severe and occurred on more fruits. Grooves and pits were never observed on fruits of the variety 'Czar', whether infected with sharka virus or not.

Type 2: broad, red bands, mostly sharply defined on one side and diffuse on the other side (Plate 2b and 4). Sometimes this discoloration is very irregular.

Type 3: thin, sharply defined red rings and lines (Plate 5). These occurred mostly on the lower side of the fruits.

Table 2. The occurrence of symptoms and inclusions in fruits of Dutch grown plum varieties without sharka virus infection.

Variety	Symptom ¹				
	grooves and pits	browning of fruit flesh	broad red bands	thin red rings and lines	cellular inclusions
<i>varieties with green and yellow fruits:</i>					
Mirabelle de Nancy	+	+	—	—	—
Ontario	±	+	—	—	—
Reine-Claude d'Oullins	±	+	—	—	—
Tonneboer	—?	+	—	—	—
Warwickshire Drooper	+	+	—	—	—
<i>varieties with orange or red fruits:</i>					
Early Laxton	+	+	—	—	—
Fleuriana	0	0	0	0	0
Victoria	+	+	—	—	—
<i>varieties with dark red or purple fruits:</i>					
Bleue de Belgique	—?	—?	—?	—?	—?
Brompton (rootstock)	0	0	0	0	0
Czar	—	—	—	—	—
Early Prolific	+	+	—	—	—
Monsieur hâtif	—	—	—	—	—
Opal	—	—	—	—	—
Reine-Claude d'Althan	+	+	—	—	—
Vroege Tolse	0	0	0	0	0
Wolters' Blauwe	—?	—?	—?	—?	—?
<i>damson plums with dark purple fruits:</i>					
Hauszwetsche	+	+	—	—	—
Italiaanse Kwets	0	0	0	0	0
Zoete Kwets	+	+	—	—	—

¹ + : found with certainty in one or more seasons; ± : weak symptoms found in one or more seasons; —? : not found in observations made during a single season; — : not found in observations during 2 or more seasons; 0 : no controls available due to the scarcity of the variety

Tabel. 2. Het voorkomen van symptomen op en insluitsels in de vruchten van in Nederland voorkomende pruimerassen, zonder het sharkavirus.

Both types of discoloration were seen in varieties with orange, red and purple fruits, but not in those with green or yellow fruits. Fruits from trees free from sharka virus never showed the symptoms of types 2 and 3 (Table 1 and 2). Some varieties showed both types of symptom ('Czar', 'Reine-Claude d'Althan'), whereas others mainly showed type 2 ('Opal', 'Victoria') or type 3 symptoms ('Early Prolific').

Fruits that are dark red or purple when ripe showed both types of discoloration only during 7–10 days at the beginning of the colouring. Later on, the discolorations were visible only when the skin was removed and held against the light. On fruits that are orange or red when ripe, like the main Dutch grown variety 'Victoria', the discoloration was visible 3–4 weeks before picking. Once, during the 1971 season, red bands were seen in early June on prematurely ripe fruits (about 1 cm long) of the variety

'Czar'. These fruits were all attacked by sawfly larvae and dropped from the tree before the end of June.

Inclusion bodies. Fruits infected with sharka virus and showing one of the 3 types of external symptom described, all contained inclusion bodies in their parenchyma cells. Bundles of needle-like inclusions were usually abundant in the cytoplasm (Plate 7) and only exceptionally were they difficult to find within 15 minutes. Rarely, needle-like inclusions were found in the nuclei and granular inclusions in the cytoplasm.

The small prematurely ripe fruits of the variety 'Czar' with red bands, also contained numerous bundles of needle-like inclusions. Small fruits without symptoms collected from the same infected trees did not contain inclusions. Symptomless nearly ripe fruits on trees infected with sharka virus were insufficiently examined in this respect and therefore no comment can be made in this report. Fruits of trees free from sharka virus, whether showing 'pseudo-pox' or not, did not contain inclusions.

Presence of viruses in the trees examined. In tests made in the spring, of 110 plum trees that were free from sharka virus, 99 proved to be infected with ring spot virus, 92 with dark green mottle virus and at least 55 with line pattern virus. Thin, small chlorotic rings, indicative of ring spot virus, appeared on the leaves of the peach indicator seedlings usually within two weeks after infection, and normally disappeared after a short time. Dark green, diffuse sunken round flecks, indicative of dark green mottle virus, appeared on the peach leaves some days later and remained visible for weeks. Observations on line pattern virus were limited because the first symptoms on the leaves of the peach indicators did not appear until three months after infection and the plants had to be discarded after four months. Thus, it is only possible to say that line pattern virus occurred in at least some of the five tested trees of every orchard under examination, independently of the occurrence of fruit symptoms.

Identical results with respect to these three viruses were obtained in tests of trees infected with sharka virus.

The presence of sharka virus in fruits with one or more of the types of symptom described was checked always by inoculating sap to *Chenopodium foetidum* or by microscope examination of parenchyma cells for inclusions.

Discussion

Descriptions in the literature of sharka-like symptoms or 'pseudo-pox' on fruits of trees free from sharka virus always refer to irregular grooves and pits (Christoff, 1958; Schuch, 1961; Posnette and Ellenberger, 1963; Kegler et al., 1963). This is a consequence of the opinion that grooves and pits on the plum fruits are the most important symptom of sharka virus. Evidence presented here shows that 'pseudo-pox' occurs commonly on several varieties under Dutch conditions. Christoff (1958) and Kegler et al. (1964) suggested that line pattern virus is the causal agent of 'pseudo-pox' Anon. (1967) suggested that the causal agent was dark green mottle virus. However, the occurrence of 'pseudo-pox' on fruits of plum varieties grown in the Netherlands could not be related to infection with either of these viruses because they both occurred in trees with and without 'pseudo-pox'. Although line pattern virus was found in 55 of 110 plum trees tested in spring, the observation period on peach seedlings was

usually too short to detect all infections and therefore it seemed likely, that a larger number of trees was actually infected with line pattern virus. In addition, unpublished data of van Katwijk (Plant Protection Service, Wageningen) indicated also a common occurrence of ring spot, dark green mottle and line pattern viruses in Dutch plum trees.

The idea that sharka virus may act synergistically with other commonly occurring viruses in causing symptoms in plum trees (van Oosten, 1971) may be supported by the fact that grooves and pits were usually more abundant and more severe when sharka virus was present. It is also possible that the different plum viruses stimulate a similar pathogenic process in the fruits, resulting in the same symptom (grooves and pits). The influence of the viruses involved may then also be considered as cumulative.

In a few varieties only trees infected with sharka virus showed grooves and pits on their fruits. Other viruses were also present in these cases.

Based on these data, the grooves and pits on plum fruits cannot be considered as specific for sharka virus and therefore the symptom is unreliable for diagnosis under Dutch conditions.

In accordance with previous observations (van Oosten, 1971) the red bands and the thin rings and lines were related directly to sharka virus infection. Fruits of trees free from sharka virus never showed these symptoms. Some varieties only showed the broad red bands, while others showed mainly thin red rings and lines. Sometimes both symptoms occurred together on one fruit when they could usually be easily distinguished and rarely was it not clear which symptom was involved. However, the data are insufficient to decide whether or not both types of discoloration are related. Experiments with plum trees infected only with sharka virus will be needed.

The use of discolorations greatly facilitated field diagnosis of sharka virus during summer. The discolorations of the fruits could be seen easily at the beginning of the ripening period. Especially on the variety 'Victoria', which has orange-red fruits, discolorations were visible during the 3–4 weeks before picking. On this variety, the diagnosis of sharka based on leaf symptoms could be replaced by diagnosis based on fruit symptoms, as the latter was more reliable.

The occurrence of brown spots on the fruit stones was found a valuable symptom for diagnosis of sharka virus (Jordović, 1961). The spots were thought to be caused by impregnation of some substances from the fruit flesh (Jordović and Janda, 1963). Brown spots present on the fruit stones of Dutch plum varieties were related to severe grooving and pitting and not to sharka virus infection.

Earlier, 3 types of inclusion body were reported in the cytoplasm and nucleus of plants infected with sharka virus (van Oosten and van Bakel, 1970). For plum fruits, only the bundles of needles in the cytoplasm of parenchyma cells proved to be of practical value for diagnosis of sharka. So far, mostly fruits with symptoms were examined for inclusions and therefore their value for routine diagnosis of sharka, including latent infections, is not clear. However, when fruits showed grooves and pits and no other external symptoms, the occurrence of inclusions was the only evidence of sharka virus.

Thus, the broad red bands and the thin red rings and lines on the fruit surface of plum fruits were the only specific symptoms of sharka virus. They could be used with certainty for field diagnosis of sharka disease. When fruits showed irregular grooves and pits, the presence of sharka virus must be checked by other symptoms on the fruits or on the leaves, or by examination for inclusions or by testing on indicator plants.

Samenvatting

Diagnose van sharka ('plum pox') met behulp van in- en uitwendige vruchtsymptomen

De diagnose van de sharkaziekte van de pruim is met behulp van de bladsymptomen goed mogelijk van begin juni tot ongeveer half juli. Daarna zijn de bladsymptomen bij de meeste rassen moeilijk of in het geheel niet meer te vinden. Daarom werd nagegaan in hoeverre vruchtsymptomen bruikbaar zijn voor een betrouwbare diagnose in de zomer.

Op vruchten van met het sharkavirus geïnfecteerde bomen werden drie typen symptomen waargenomen (Tabel 1): 1. brede, rood- of paarsachtige bandvormige schilverkleuring, meestal aan een zijde scherp en aan de andere zijde diffuus begrensd (Plaat 2b en 4); 2. dunne, scherp begrensde rood- of paarsachtige lijntjes en kringetjes, vaak op de onderzijde van de vrucht (Plaat 5); 3. onregelmatige lijn- en putvormige inzinkingen, waaronder bruin necrotisch vruchtvlees (Plaat 1b, 2b en 3b). Per vrucht kon meer dan een symptoomtype voorkomen. De symptomen verschenen pas 2-4 weken voor de rijping van de vruchten.

Zowel de bandvormige verkleuringen als de dunne lijntjes en kringetjes werden waargenomen op vruchten van met het sharkavirus geïnfecteerde bomen van rassen met oranje, rode en paarse vruchten, doch niet op vruchten van rassen met gele of groene vruchten. Deze verkleuringen van de vruchtschil werden niet waargenomen op vruchten van niet mét het sharkavirus geïnfecteerde bomen (Tabel 1 en 2).

De lijn- en putvormige inzinkingen werden waargenomen op vruchten van verscheidene pruimerassen, zowel met als zonder het sharkavirus (Tabel 1 en 2; Plaat 1a en b, 2a en b en 3a en b). Bij een aantal rassen bleek dit 'pseudo-pox' (inzinkingen op vruchten van bomen zonder het sharkavirus) vrij algemeen, zoals bij 'Warwickshire Drooper' (Plaat 1a), 'Zoete Kwets' (Plaat 3a) en incidenteel ook bij 'Victoria' (Plaat 2a) en 'Early Prolific'. De inzinkingen waren veelal alleen zichtbaar op de eerst rijpende vruchten, onafhankelijk van het voorkomen van het sharkavirus. Vruchten met dit symptoom vielen vaak vóór de rijping van de overige vruchten van de boom. De symptoomexpressie was op de vruchten van met het sharkavirus geïnfecteerde bomen vaak heviger dan op vruchten van niet met dit virus geïnfecteerde bomen, terwijl ook een groter aantal vruchten het symptoom toonden. Het is onmogelijk gebleken verschillen aan te geven tussen inzinkingen op vruchten van bomen die al dan niet met het sharkavirus waren geïnfecteerd. Alleen op vruchten van het ras 'Czar' werden nooit inzinkingen gevonden.

In de parenchymcellen van rijpende vruchten van sharkazieke bomen van alle onderzochte rassen werden voor het virus karakteristieke insluitsels gevonden. Deze insluitsels ontbraken in vruchten van niet met het sharkavirus geïnfecteerde bomen.

Voor de diagnose van sharka kan dus bij rassen met oranje, rood of paars kleurende vruchten gebruik worden gemaakt van de beide typen schilverkleuring. Indien vruchten worden gevonden met inzinkingen, dan kan de aanwezigheid van het sharkavirus alleen worden vastgesteld op grond van andere symptomen op de vruchten en de bladeren, door insluitselonderzoek of door toetsing op indicatorplanten.

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