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Herbaceous host plants for the sharka (plum pox) virus

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Abstract

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.

Introduction

Hitherto little has been known about the host range of the sharka virus of plum (*Prunus domestica*). Knowledge of the full host range is of great importance, however. It may improve diagnosis by revealing useful indicator plants. It may also give insight into the natural host range and thus into the epidemiology of the disease, as the virus has been reported to be aphid-transmissible (Atanasoff, 1934; Christoff, 1958; Jordović, 1963; Kassanis and Šutić, 1965). Kassanis and Šutić reported that *Myzus persicae* transmit sharka virus in the non-persistent way. This has been confirmed by Kunze and Krczal (1968) and Van Oosten (1970).

Important woody indicator plants are some plum species which show typical sharka symptoms within one or two seasons after infection, and peach seedlings (*Prunus persica*) which react 3–8 weeks after infection (Kegler, 1963; Mischke, 1963; Šutić, 1963; Kegler et al., 1964; Van Katwijk, Plant Protection Service, Wageningen). Only two herbaceous hosts are known[1] and used as test plants, viz *Chenopodium foetidum* (Németh, 1963) and *Nicotiana clevelandii* (Kassanis and Šutić, 1965). However in our conditions *N. clevelandii* is not suitable as an indicator plant for sharka virus (Van Oosten, 1970). For that reason it was thought essential to have the disposal of more herbaceous hosts reacting rapidly and differentially. Therefore many plant species commonly used in plant virology were tested. Many common weeds were also included in this investigation to test them as potential natural hosts. This is of importance in answering the question how far herbaceous plants play a role in the epidemiology of sharka virus.

[1] During preparation of the manuscript Bode (1969) reported two plant families (Chenopodiaceae and Solanaceae) in which new herbaceous host plants were found.

Material and methods

Virus isolates
The isolation and identification of sharka virus was reported earlier (Van Oosten, 1970). In this investigation two isolates were used which were cultured on *Nicotiana clevelandii*, viz a Dutch isolate and a Jugoslavian one from the plum variety Požegača. The latter isolate was kindly provided by Mr Jordović and Mr Ranković, Institut za voćarstvo, Čačak, Jugoslavia.

Mechanical transmission
Tip leaves of *N. clevelandii* plants with sharka were crushed in a mortar containing a 0.01 *M* phosphate buffer pH 8, w/v = 1/5. Sometimes leaves of *C. foetidum* were also used. For plum leaves a buffer was used somewhat modified according to Kegler and Opel (1963), viz 0.02 *M* phosphate buffer pH 8, 0.03 *M* coffein and 0.015 *M* sodium-diethyldithiocarbamate, w/v = 1/3. Plants were dusted with carborundum 600-mesh and inoculated with the fingertip. Per plant species six young plants (4–6 leaves) were inoculated, while three control plants were inoculated with buffer only. The inoculated plants were observed during $2\frac{1}{2}$–4 weeks. Back inoculations were made to *C. foetidum* 2–3 weeks after inoculation. Sap from leaves of *C. foetidum* and *N. clevelandii* was assayed on *C. foetidum*, and infectivity was estimated by the local lesion method (latin square), one and two weeks after inoculation, respectively.

Aphid transmission
Young apterous *Myzus persicae* were starved for at least three hours and after an infection feeding period of 1–2 min on *N. clevelandii* leaves with the sharka virus were transferred to test plants. Control aphids were allowed to feed on healthy *N. clevelandii* leaves for 1–2 min. In each experiment six test plants and three control plants were used. After 24 h the plants were sprayed with Phosdrin to kill the aphids.

All experiments in mechanical and aphid transmission studies were repeated once or twice. Most of the experiments were done in glasshouses at a temperature of about 22 °C.

Electron microscopy
Dip preparations (Brandes, 1964) were made from leaves of some plant species showing symptoms after inoculation. The preparations were shadowed with palladium and investigated with a Siemens Elmiskop I (modified to a Ia) electron microscope.

Results

Mechanical transmission
From October 1968 to October 1969 75 plant species from 18 families were tested. The 27 species which became infected are summarized in Table 1. The following species were not susceptible:
Boraginaceae: *Myosotis sylvatica, Symphytum officinale*
Caryophyllaceae: *Stellaria graminea*
Chenopodiaceae: *Axyris amaranthoïdes, Chenopodium amaranticolor, C. bonus-henricus*

Table 1. Experimental herbaceous host plants of the sharka virus of plum

Host plant	Symptoms	Back inoculation to *C. foetidum*	
		L[1]	S[1]
Chenopodiaceae			
Chenopodium capitatum	chlorotic lesions after 14–16 days	+	–
C. foetidum	ochrous lesions, mostly with a necrotic centre after 5–8 days	+	–
C. foliosum	chlorotic lesions after 14–16 days	+	–
C. quinoa	no symptoms	+	–
Compositae			
Senecio viscosus	no symptoms	+	–
S. vulgaris	brownish necrotic lesions after 7–10 days	+	–
Zinnia elegans	occasionally systemic chlorotic lesions after 14–21 days	+	+
Labiatae			
Lamium amplexicaule	systemic chlorotic lesions after 14–16 days	+	+
Galeopsis segetum	no symptoms	+	–
Passifloraceae			
Passiflora foetida	occasional vein yellowing after 3–4 weeks	+	–
Ranunculaceae			
Ranunculus arvensis	yellow lesions after 7–10 days	+	–
Solanaceae			
Nicotiana affinis	faint chlorotic lesions after 12 days	+	–
N. clevelandii	necrotic lesions and rings after 4–6 days; a systemic chlorotic mottle and in winter necrotic spots after 8–10 days	+	–
N. debneyi	necrotic spots with a faint chlorotic edge after 4–7 days	+	–
N. glutinosa	no symptoms	+	–
N. rustica	no symptoms	+	–
N. sylvestris	chlorotic lesions after 9–12 days	+	–
N. tabacum 'Samsun'	chlorotic lesions after 7–9 days	+	–
N. tabacum 'Samsun NN'	chlorotic lesions after 7–12 days	+	–
N. tabacum 'White Burley'	chlorotic lesions after 5–7 days	+	–
N. tabacum 'turks landras'	chlorotic lesions after 12–14 days	+	–
N. tabacum 'Xanthi'	faint chlorotic lesions after 7–9 days	+	–
Solanum luteum	no symptoms	+	–
S. nigrum	no symptoms	+	–
S. polyadenium	no symptoms	+	–
S. rostratum	no symptoms	+	–
S. villosum	chlorotic rings and sometimes lines after 2–3 weeks	+	–

[1]L = from inoculated leaves; S = from tip leaves.

Tabel 1. Experimentele kruidachtige waardplanten van het sharka-virus van pruim.

Fig. 1. Leaf of *Ranunculus arvensis* showing yellow lesions 10 days after inoculation with sharka virus.
Fig. 2. Local lesions on a leaf of *Ranunculus arvensis* surrounded by a diffuse ring, about 18 days after inoculation with sharka virus.
Fig. 3. Leaf of *Nicotiana tabacum* 'White Burley' with many chlorotic lesions 6 days after inoculation with sharka virus.

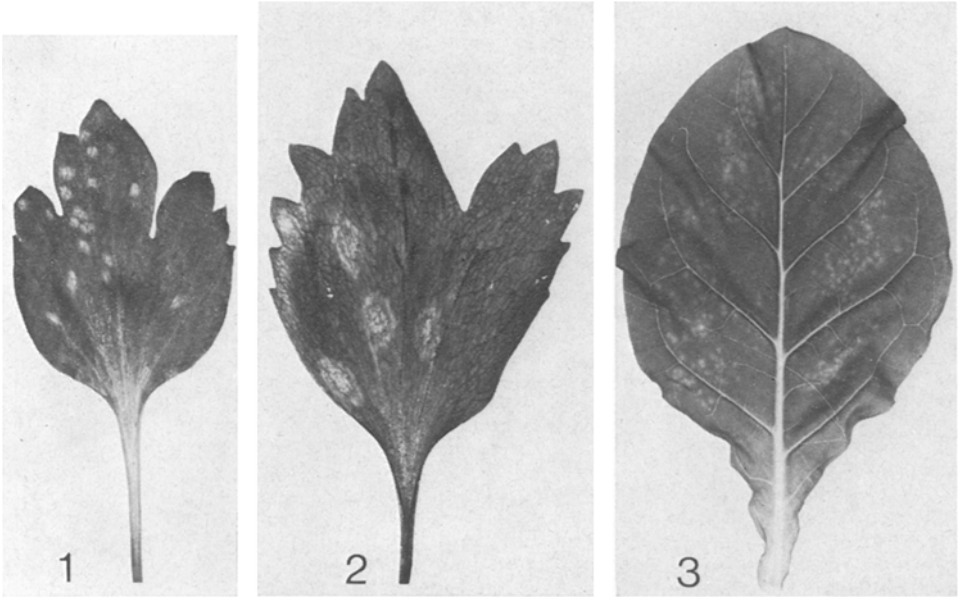
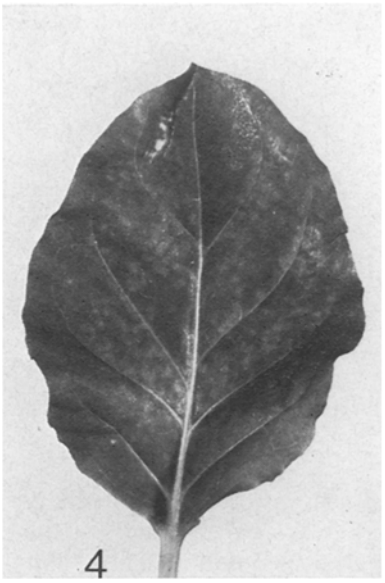


Fig. 1. Blad van Ranunculus arvensis met gele lokale lesies, 10 dagen na inoculatie met het sharka-virus.
Fig. 2. Lokale lesies op een blad van Ranunculus arvensis, omgeven door een diffuse kring, 18 dagen na inoculatie met het sharka-virus.
Fig. 3. Blad van Nicotiana tabacum 'White Burley' met vele chlorotische lesies, 6 dagen na inoculatie met het sharka-virus.

Compositae: *Bellis perennis, Centaurea jacea, Chrysanthemum parthenium, Erigeron annuus, Gnaphalium sylvaticum, Inula conyza, Senecio adonidifolius, S. elegans, S. fuchsii, S. jacobea, S. speciosus, Sonchus asper, S. oleraceus, Tanacetum vulgare, Taraxacum officinale*
Cucurbitaceae: *Cucumis sativus* 'Lange Gele Tros'
Geraniaceae: *Geranium dissectum, G. sylvaticum*
Gramineae: *Poa nemoralis*
Hypericaceae: *Hypericum perforatum, H. maculatum*
Labiatae: *Salvia officinalis*
Onagraceae: *Epilobium montanum*
Passifloraceae: *Passiflora edulis* 'Flavicarpa'
Plantaginaceae: *Plantago lanceolata, P. major, P. media*
Polygonaceae: *Rumex acetosa, R. acetosella*
Ranunculaceae: *Ranunculus acer, R. bulbosus, R. montanus*
Rosaceae: *Fragaria vesca, Potentilla recta*

Fig. 4. Leaf of *Nicotiana tabacum* 'Samsun' showing chlorotic lesions 8 days after inoculation with sharka virus.
Fig. 5. Leaf of *Solanum villosum* showing large chlorotic rings about 2 weeks after inoculation with sharka virus.



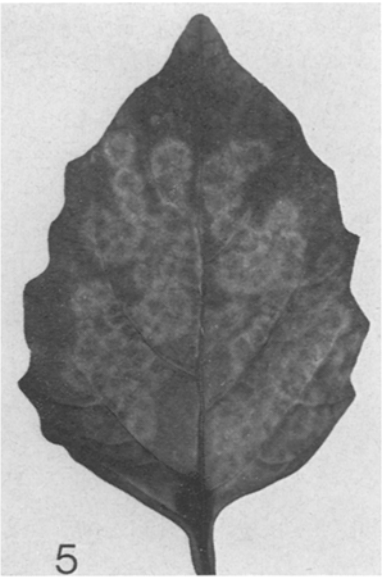


Fig. 4. Blad van Nicotiana tabacum 'Samsun' met chlorotische lesies, 8 dagen na inoculatie met het sharka-virus.
Fig. 5. Blad van Solanum villosum met grote chlorotische kringen, ongeveer 2 weken na inoculatie met het sharka-virus.

Scrophulariaceae: *Veronica officinalis, V. persica*
Solanaceae: *Capsicum annuum* 'Oranje Wonder', *Datura metel, D. stramonium, Solanum hirsutum, S. lycopersicum*
Urticaceae: *Urtica urens.*

Symptom expression on the leaves of the newly found hosts was mostly weak. The most distinct and rapid reaction was found on *C. foetidum, Ranunculus arvensis* (Fig. 1 and 2), *N. clevelandii, N. debneyi, N. tabacum* 'White Burley' (Fig. 3) and *N. tabacum* 'Samsun' (Fig. 4). When the first five species were inoculated with sap from leaves of sharka-diseased plum trees, only *C. foetidum* and *R. arvensis* showed a distinctive reaction. A clear local reaction was also found on *Solanum villosum* (Fig. 5), but symptoms appeared only after 2–3 weeks.

Some of the newly found hosts did not become infected or did not show symptoms after inoculation with sap from infected *C. foetidum* leaves. This may have been due to the lower concentration of the virus in *C. foetidum* sap, since sap from leaves of this plant had a lower dilution end point (10^3) than that from leaves of *N. clevelandii* (10^4).

C. foetidum, N. clevelandii, R. arvensis, S. villosum and *Zinnia elegans* were inoculated with sap from *N. clevelandii* leaves infected with the Jugoslavian isolate. All species proved to be susceptible.

Aphid transmission

Myzus persicae transmitted the virus in the glasshouse very well from peach seedlings to *N. clevelandii* and *Lamium amplexicaule*; all test plants became infected. When *N. clevelandii* was used as a donor plant of sharka virus, all peach seedlings became infected and showed distinct symptoms within 12–16 days. When *L. amplexicaule* was used as a donor, only two out of six peach seedlings became infected (sum of two experiments with three plants each).

Electron microscopy

Dip preparations from leaves of peach seedlings, *C. foetidum*, *R. arvensis*, *N. clevelandii* and *N. tabacum* 'White Burley' all contained the same type of virus particles (flexuous rods), mostly with a length between 600 and 800 nm. It was difficult to find virus particles in dip preparations from leaves of peach seedlings, while it was easy to find them in the tobacco species.

Discussion

The symptom expression on the leaves of many of the newly found experimental host plants after inoculation with sap from infected *N. clevelandii* leaves was mostly very weak.

Sap of infected plum or *C. foetidum* leaves seems to have lower inoculation potential than that of *N. clevelandii:* the plant species found to be new hosts did not show symptoms or did not become infected at all. Several investigators (Németh, 1963; Kegler et al., 1964; Săvulescu and Macovei, 1965) tested many plant species in this way, including some species which are now known to be host plants, but with negative results. Kassanis and Šutić (1965) worked with sap from infected *N. clevelandii* leaves, also without positive results. However none of these authors has made back inoculations to *C. foetidum*. Thus symptomless infections were not traced. Moreover, the presence of inhibitors in *C. foetidum* (Schmelzer, 1959) and plum (Fulton, 1966) may have influenced the transmission of sharka virus in those experiments. It is also possible that the virus concentration in sap from leaves of *C. foetidum*, which is lower than in sap from *N. clevelandii*, has played a role.

Among the newly found host plants of sharka virus only *Ranunculus arvensis* might be a useful test plant. The reaction of the primary leaves to inoculation was quick and distinct when sap from leaves of *N. clevelandii* or plum was used.

Five plant species became infected with the Jugoslavian as well as with the Dutch isolate. Some differences in symptom expression were found after inoculation with both isolates during summer. However a conclusion regarding the existence of possible strains might be drawn only after observation of the symptoms caused by the two isolates all the year round.

The finding that some weeds and garden plants are susceptible to sharka virus is of great importance. So far only two annual species, viz *Lamium amplexicaule* and *Zinnia elegans*, have become systemically infected in our tests. Thus, in nature these species might play a role in the epidemiology of the virus. In the glasshouse the virus was transmitted with the aid of *M. persicae* from peach seedlings to *L. amplexicaule* and vice versa. If the natural transmission of sharka virus by aphids is as easy as found in the glasshouse, the problem of eliminating sharka virus from infected areas might be

more complicated than expected. A rapid spread of the virus, thought to have been caused by aphids, has already been reported by Jordović (1965). Spread of sharka disease might be further facilitated by an even larger host range (weeds, garden plants and perhaps woody plants) of the virus than the one reported in the present paper.

Samenvatting

Kruidachtige waardplanten van het sharka-(plum pox-) virus

Uit 18 families werden 75 plantesoorten getoetst op hun vatbaarheid en gevoeligheid voor het sharka-virus van de pruim. De planten werden geïnoculeerd met het sap van geïnfecteerde topbladeren van *Nicotiana clevelandii*; 27 soorten uit 6 families bleken vatbaar voor het virus. Alleen *Ranunculus arvensis* is wellicht een bruikbare toetsplant. Onder de pas gevonden waardplanten van het sharka-virus bevinden zich ook enkele algemeen voorkomende onkruiden en tuinplanten. *Lamium amplexicaule* en *Zinnia elegans* werden systemisch door het virus geïnfecteerd. In de kas kon het virus met behulp van *Myzus persicae* worden overgebracht van perzikzaailingen naar *L. amplexicaule* en omgekeerd. Indien de overdracht in de natuur even gemakkelijk verloopt als in de kas, dan kan dit het uitroeien van het sharka-virus in besmette gebieden ernstig bemoeilijken.

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