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Tobacco streak virus in sunflower (Helianthus annuus)

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Abstract

Tobacco streak virus (TSV) was isolated from a plant of sunflower *(Helianthus annuus)* showing severe necrosis and chlorosis in the leaves. The virus was identified as TSV by serology and, to some extent, by host range. The type of symptoms varied with the host plant in which the virus had been propagated in successive transfers. Test plants inoculated with the virus propagated in *Nicotiana rustica* produced symptoms which very much differed from those brought about by the virus from either *N. clevelandii* or *Chenopodium amaranticolor.*

The significance of the host-mediated variation in symptoms is discussed.

Additional keywords: Chenopodium amaranticolor, host-mediated variation, *Nicotiana clevelandii, Nicotiana rustica.*

Introduction

In the summer of 1980 a number of plants of sunflower *(Helianthus annuus)* in the garden around the Laboratory of Virology were affected by a serious disease, characterized by severely curled leaves with chlorosis and necrosis. In some cases one half of a leaf (or even the whole leaf) was so necrotic that it looked scorched (Fig. 1). Often a strong downcurling of the bracts of the involucre was observed.

In initial experiments a small number of test plants were inoculated with sap from the leaves of a diseased sunflower. Only one plant of *Gomphrena globosa* developed a whitish necrotic local lesion with a reddish-violet border on one leaf, suggesting the presence of a virus. This reaction in only one test plant was possibly due to a low concentration of the pathogen in the rather old sunflower plants. The local lesion was cut out, ground with water and inoculated onto 10 plants of *G. globosa.* This time a larger number of local lesions appeared on the leaves which were used as starting material for identification of the virus. Chop preparations for the electron microscope, made from symptom-showing leaves of sunflower and *G. globosa,* did not reveal the presence of virus-like particles.

Sunflower has been reported to be naturally infected by a number of viruses. Traversi (1949) described a serious disease of sunflowers in Argentina which she presumed to be caused by a virus. The symptoms of this disease consisted of a mosaic which, in a later stage of infection at a favourable temperature, was followed by necrosis and swollen veins. Battu and Phatak (1965) recorded the presence of a sap-transmissible

Fig 1. *Helianthus annuus* with severe necrosis, chlorosis and leaf deformation.

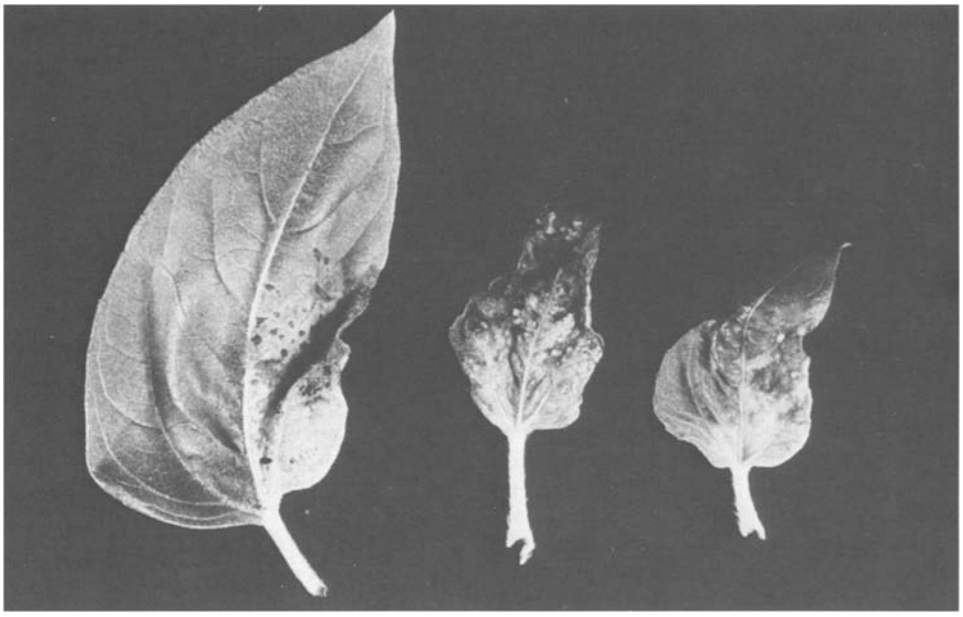

Fig. 1. Helianthus annuus met sterke necrose, chlorose en bladmisvorming.

agent in mosaic-diseased sunflowers in India. Sunflower mosaic virus described by Smith (1972) induced a mild mosaic and mottling in the leaves in the early stage of infection, and severe necrosis of leaves and stems as the disease progressed. On the basis of the presence of pinwheels in infected sunflower leaves, Arnott and Smith (1967) assumed that sunflower mosaic virus belongs to the potato virus Y group. Orellana and Quacquarelli (1968) isolated cucumber mosaic virus from mosaic-diseased sunflower plants in the USA. This virus caused hardly any necrosis in the leaves, but sometimes it induced narrow light-brown streaks on the petioles and stems. Russell et al (1975) reported that chlorotic symptoms on sunflowers in England were caused by a representative of the beet western yellows virus group. In 1977, Gupta and Gupta isolated three viruses from sunflower in India which caused mosaic, yellow rings and yellow specks, respectively. The first two viruses could be transmitted by sap and dodder, but the last one only by grafting. None of the three viruses caused necrosis in sunflower.

The aim of the present study was to identify the virus isolated from sunflower at Wageningen.

Material and methods

Virus transmission in sap, host range studies and determination of persistence of infectivity in crude sap were performed in the conventional ways, with carborundum 600 mesh as an abrasive and water as diluant.

In host range tests, plants of 23 species were inoculated either with diluted crude sap of symptom-showing leaves of *Chenopodium amaranticolor, Nicotiana clevelandii* or *N. rustica,* or with purified virus suspensions. The following cultivars were used: *Beta vulgaris* 'Groeningia', *Cucumis sativus* 'Lange Gele Tros', *Dahlia variabilis* 'Cactus', *H. annuus* 'Sungold', *Lycopersicum esculentum* 'Moneymaker', *N. tabacum* 'Samsun NN', *N. tabacum* 'White Burley', *Phaseolus vulgaris* 'Noordhollandse Bruine', *Solanum melongena* 'Lange Violette', *Vigna unguiculata 'California Blackeye', Zinnia elegans* 'Persian Carpet'. For comparison, a purified suspension of tobacco streak virus (TSV) was used, which was kindly provided by Dr L. van Vloten-Doting, Department of Biochemistry, State University of Leyden. This was the TSV-WC isolate described by Fulton (1970). After 25 days, the non-inoculated, symptomless leaves and the inoculated leaves of symptomless plants were tested for virus by return inoculation to *B. vulgaris, C. amaranticolor, Cyamopsis tetragonoloba, Datura stramonium, H. annuus, N. benthamiana, N. clevelandii, N. tabacum 'Samsun NN'* and *Z. elegans. Chenopodium amaranticolor, C. quinoa* and *G. globosa* were used as assay plants in experiments on properties of the virus in crude sap. Because of the rapid inactivation of the virus in expressed sap, fresh virus extracts were made for each temperature treatment, to determine the thermal inactivation point. About 15 min elapsed from the time of extraction to inoculation.

In transmission experiments with *Myzus persicae,* aphids starved for 30 min were given an acquisition access period of 10 min on symptom-showing leaves of either *N. clevelandii* or *N. rustica* or *C. amaranticolor* (10-20 aphids per plant). Thereafter, these aphids were transferred to young plants of *N. clevelandii, N. rustica* and *C. amaranticolor,* respectively, for an inoculation feeding period of about 90 min. For experiments on transmission in the persistent manner, aphids were given an acquisition access period of 7 days whereafter they were transferred to healthy plants for inoculation feeding for another period of 7 days. From these plants the aphids were transferred to another series of healthy plants. After 7 days they were killed with an insecticide.

In dodder transmission experiments shoots of *Cuscuta subinclusa* established on infected plants of *N. benthamiana, N. clevelandii, N. rustica* or *C. amaranticolor* were trained onto healthy plants of these species.

For seed transmission experiments about 50 seeds of diseased plants of *N. rustica* were sown.

All test and assay plants were grown in sterilized soil in the glasshouse at 20-27 °C.

In the first experiments, the virus was purified from leaves of infected plants of *N. clevelandii* by the method described by Fulton (1967) for TSV. In later experiments, the method of Van Vloten-Doting (1975) was used for the infected leaves of *C. amaranticolor, N. clevelandii, N. rustica* and *V. unguiculata.* The optical density of purified virus preparations was measured in a Zeiss spectrophotometer or in a Gilford 2400-2 self-recording spectrophotometer. Absorbance values were not corrected for light scattering. Virus concentrations were estimated using an extinction coefficient $E_{260}\ ^{0.1\%}_{1\ cm}$ of 5.1, the value given for TSV by Fulton and Potter (1971).

The virus was examined electron-microscopically in crude sap preparations and in purified suspensions. For crude sap preparations, symptom-showing leaves of *C. amaranticolor, G. globosa, N. clevelandii* and *N. rustica* were chopped. The carbon-reinforced formvar-coated copper grids were floated on a drop of sap from the chopped material for 15 sec and then transferred to a drop of 3% glutaraldehyde for fixa-

tion. After 5 min the specimen was stained with 2% uranyl acetate or 2% potassium phosphotungstate (PTA) for 1 min. Purified virus suspensions were directly placed on carbon-reinforced formvar-coated copper grids for 1 to 2 min and then stained with 2% uranyl acetate or 2% PTA. The specimens were examined with a Siemens Elmiskop 1 or Siemens Elmiskop 101 electron microscope. For measurements of the virus particles, fixed catalase crystals with a lattice spacing of 8.6 ± 0.22 nm (Wrigley, 1968) were used as an internal size standard. For serology a rabbit was injected intravenously with a virus suspension of 1 mg ml^{-1} purified from *N. clevelandii.* After 12 days an intramuscular injection with a mixture containing 2 ml of a virus suspension of 5 mg ml^{-1} and 2 ml of Freund's complete adjuvant followed. Twelve days later a second intramuscular injection with the same amount of virus was given. After another period of 12 days the blood was collected.

In case of the virus from *N. rustica,* a purified suspension of 0.5 mg ml^{-1} was injected into a rabbit intracutaneously. Twelve days after injection the titre of the antiserum was found to be low and two more intramuscular injections were given, each with 2 mg ml^{-1} virus, on the 16th and 36th day after the first injection, respectively. On the 48th and 62nd day blood was collected. The Ouchterlony agar-gel double-diffusion test was applied. The concentration of the agar was 1% in saline containing 0.05% sodium azide.

Antisera to the following viruses were provided by Ing. D.Z. Maat, Research Institute for Plant Protection at Wageningen, along with their homologous titres as given in parentheses: apple mosaic (3), *Arabis* mosaic (512), cherry leaf roll (512), lilac ring mottle (256), *Prunus* necrotic ringspot (16), spinach latent (32), strawberry latent ringspot (1024), tobacco ringspot (1024), tobacco streak (isolate from soybean (64) and from dahlia (titre not known), tomato black ring (256) and tomato ringspot (1024). For the serological tests purified virus and virus-containing sap of the following, locally (L) or systemically (S)-infected leaves were used: *G. globosa* (L), *H. annuus (S), N. benthamiana* (S), *N. clevelandii* (S), *N. rustica* (S), *N. tabacum* 'White Burley' (L and S) and *Z. elegans* (S). Sap of healthy plants of the same species served as controls.

Results

Reaction of test plants and host range. Some of the test plants, inoculated with sap from *G. globosa* leaves with a large number of papery-white necrotic local lesions with a red border, showed symptoms reminiscent of those described for the type strain of TSV (Fulton, 1971). *N. tabacum* 'Samsun NN' and 'White Burley', for instance , displayed whitish necrotic ringspots, line patterns and oak-leaf patterns in the inoculated leaves as well as in the systemically infected ones. Later the plants recovered to some extent and younger leaves were without chlorosis or necrosis but very often they showed morphological deviations like narrowing, prominent venation and dentate instead of entire margins. Moreover, the flowers had a split corolla showing separated petals. Inoculated cotyledons or leaves of *C. tetragonoloba* produced the typical pin-point, dark-brown necrotic lesions, but this symptom was not very consistent. *N. rustica* reacted with local and systemic chlorotic ringspots and systemic line patterns. One out of three inoculated plants of *C. amaranticolor* got systemically infected with chlorotic (ring)spots and vein yellowing in the newly formed leaves and

malformation and epinasty of the top leaves. However, in a number of test plants the predominant type of symptoms proved to be dependent on the plant in which the virus had been propagated. Differences in symptoms due to different source-hosts were especially striking when the virus was propagated in either *C. amaranticolor* or *N. clevelandii* or *N. rustica.* The virus isolated from these hosts is henceforth referred to as Ca-subisolate, Nc-subisolate and Nr- subisolate, respectively. The results of host range tests with these subisolates are presented in Table 1.

When the Nc-subisolate, obtained from *N. clevelandii* leaves with local and systemic line patterns and fine necrotic etching (Fig. 2) was used as inoculum, symptoms on the test plants were comparable with those described by Fulton (1971) for the type strain of TSV. *H. annuus* produced brown necrotic local lesions with a chlorotic halo and systemic chlorosis, net necrosis, crinkle and vein yellowing (Fig. 3).

When the Nr-subisolate, obtained from systemically infected leaves of *N. rustica* showing one-sided necrosis (similar to that caused by tomato spotted wilt virus in the same host), leaf deformation, scorching (Fig. 4) and shot-holes was used as inoculum, the symptoms on test plants were quite different from those caused by the Nc-subisolate. These differences were especially obvious in *C. sativus, D. stramonium, H. annuus, L. esculentum, N. benthamiana, N. glutinosa, N. rustica, N. tabacum* 'Samsun NN' and 'White Burley', and *V. cylindrica.* The typical 'streak' symptoms in tobacco were generally missing and instead very big, solid, dark-brown necrotic local lesions were formed, followed by strong necrosis of stems and top leaves leading to the death of the plant. The newly formed leaves of *D. stramonium* and *N. rustica* were strongly

Fig. 2. Leaves of *Nicotiana clevelandii* with necrotic lesions, ringspots and line patterns after inoculation with tobacco streak virus propagated in *N. clevelandii.* From left to right: Two locally infected leaves and one leaf with systemic symptoms.

Fig. 2. Bladeren van Nicotiana clevelandii met necrotische lesies, kringvlekken en figuurpatronen na inoculatie met tabaksstrepenvirus, vermeerderd in N. clevelandii. Van links naar rechts: Twee lokaal-geïnfecteerde bladeren en een blad met systemische symptomen.

Table 1. Comparison of reactions of test plants to inoculation with a virus from *Helianthus annuus* (HV), propagated in *Chenopodium amaranticolor* (Ca-subisolate), *Nicotiana clevelandii* (Nc-subisolate) and *Nicotiana rustica* (Nr-subisolate) with those of a number of isolates of tobacco streak viruses (TSV) as reported in literature.

Test plant	Symptoms	HV Ca-subisolate Sunflower[a]	HV Nc-subisolate Sunflower[a]	HV Nr-subisolate Sunflower[a]	TSV WC Tobacco[a]	TSV Bell pepper[b]	TSV Dahlia[c]	TSV Soybean[d]	TSV Tobacco[e]	TSV Tobacco[f]	TSV red-node strain Bean[g]	TSV pea strain Pea[h]	TSV SB 10 Potato[i]	TSV Pathotype I and II (cowpea and sweet clover resp.)[j] I	TSV Pathotype I and II (cowpea and sweet clover resp.)[j] II
Beta vulgaris	L	NL,NL (conc)	ChRSp, ChSp, NRSp NSp	ChSp, NL, NRSp	NRSp	- -					- -	- -			
	S	-	- -	-	-	- -					- -	- -			
Chenopodium amaranticolor	L	NL(pp)	ChSp,NL NL(pp)	NL(pp)	NL(pp)	NL	NL(pp)	ChSp					-	ChSp	ChSp
	S	ChRSp, ChSp,Ep, Ldf,LN, VY,WB	ChSp,Ep Ldf,N, NSp VY, YSP	-	-	Ldf,Mot	ChSp,Dst	N					AN,Ldf St	Ldf,St	Ldf
Chenopodium quinoa	L	NL	NL,NRSp (conc)	NL,NRSp	NL	NL	NL,VN	NSp					NSP	NL	NL
	S	AN	AN,Ch, ChSp, Ldf,Wlt	-	Ch,N,Rec	Ldf,Mot	AN,Ch	N					AN,St	AN,St	Ldf,Mot
Cucumis sativus	L	-	ChSp	NL(pp)	ChRSp ChSp, NL(pp)	-		ChSp			ChSp	- -		- -	-
	S	-	Mos,Mot YSp	-	Mos,Mot, St,YSp	Mos,VC		Mos,St			Mot,YSp	- -		- -	Mot,St,Y
Cyamopsis tetragonoloba	L	-	NL(pp)	NL(pp)	NL(pp)			NSp			NL	- -		NL	NL
	S	-	- -	- -	-		-				-	- -		-	-
Dahlia variabilis	L	-	Ch,NL NRSp	NL	Ch,NL, NRSp										
	S	-	AN, Ldf	Ep,N,Sco	AN, ChSp,Ldf										
Datura stramonium	L	-	ChLP, ChRSp (conc)NL, NRSp	ChRSp(conc) NRSp,NL NRSp	ChRSp (conc), NSp	Mot		- -	NL	Mot	NRSp	-	Ldf		

	S	-	BL,Ch, ChRSp (conc) Et,Ldf	ChRSp(conc) ChSp,Ep,Et, Ldf,Mos,Mot NN,NRSp,Rag	Ch,Et, NRSp	Mot		-	NSp.Rag, Rec,St	Mot	Mot,NSp	Mot, YSp	St		
Gomphrena globosa	L	NL,NRSp Oak(N)	NL,NRSp Wth	NL,NRSp (conc)	NL,NRSp			NSp				NL	NSp	NL	NL
	S	Ep,N, NLP,NSp YSp	AN,Ch,N, Rec,St, Wlt	-	N,Oak(N)			-				-	Ldf,NRSp	Mos,St	- -
Helianthus annuus	L	NL	Ch,NL, NN	NL	NL,NN			NSp							
	S	-	Ch,Cr, NN,VY	AN,ChSp, Ldf,NSp, Sco,St	Ch,Sco, YSp			+							
Lablab niger	L	-	ChSp, NRSp, NSp	NRSp	NSp		NRSp	NSp							
	S	-	-	-	-		-	-							
Lycoper-sicum esculentum	L	-	Ch,NSp	NSp	Ch,NRSp, NSp	-	-	NSp		- -	- -				
	S	-	N	N	AN	Mos,NSp	-	+		- -	- -				
Nicotiana bentha-miana	L	-	ChSp, NRSp (conc)	ChSp,NRSp, NSp	NRSp NRSp (conc)										
	S	-	BV,Ch, Cr, Mot, NN	AN,Ldf,Rec SN,VN,Wlt	BV,Ch, Cr, Ldf,N, NN										
Nicotiana clevelandii	L	-	NL,NLP NRSp, NRSp (conc), NSp	NN,NRSp (conc),NSp	NLP, NRSp,Str	Mos							-		
	S	-	AN,Et, LN,Mot, NG,NLP, NN,NRSp, St,VY	AN,BV Ldf,NL, St,Wlt	AN,LN, NLP,Rec	Mos							+		

Table 1. Continued

Test plant	Symptoms	HV Ca-subisolate Sunflower[a]	HV Nc-subisolate Sunflower[a]	HV Nr-subisolate Sunflower[a]	TSV WC Tobacco[a]	TSV Bell pepper[b]	TSV Dahlia[c]	TSV Soybean[d]	TSV Tobacco[e]	TSV Tobacco[f]	TSV red-node strain Bean[g]	TSV pea strain Pea[h]	TSV SB 10 Potato[i]	TSV Pathotype I and II (cowpea and sweet clover resp.)[j] I	II
Nicotiana glutinosa	L	-	ChRSp, ChSp, NL,NRSp	-	Ch,NL, NRSp, NRSp (conc) NSp	NSp	- -	Oak(N)	NL,NRSp (conc)	-	- -	YSp			
	S	-	ChSp,Et, Mot	-	Ch,Cr, Mos,Mot, NSp	AN,N	- -	Mos,St	NSp,Rec, YSp	Mot,N	- -	Ldf,Mot, NSp			
Nicotiana rustica	L	-	ChRSp	NRSp, NRSp(conc) NSp,Oak(N)	-	NLP, NRSp, NSp			-		NL	-			
	S	-	ChLP, ChRSp ChSp, NLP, SC	ChSp,Et, Ldf,N,NLP, Rag,Sco	ChLP, ChRSp, Et,NLP, Oak(N)	NLP, NRSp, NSp,SC			AN,NLP, Rec,Sco, St,VC, VN		-	ChSp			
Nicotiana tabacum	L	-	NLP, NRSp, Str	NL	NLP, NRSp, Oak(N)	NLP, NRSp, NSp	ChRSp	NL,NSp, NRSp	NRSp, VN	NLP, NRSp, NSp, Oak(N)	NL,NRSp	ChSp	-	- -	- -
	S	-	Dt,N,NL, NLP,Rec, SC	AN,SN, VN	BV,Dt, NLP,NN Rec	Dt,LN, NLP, NRSp, NSp,SC	NLP, NSp,Rec	Dt,N Rec.SC	AN,Dt, LN,NLP, NRSp, Rec,SN, VN	N,Rec, VC	NLP, NRSp, Oak(N), Rec,St	ChRSp (conc), St,YSp	Ldf,Mos Mot,St	- -	- -
Nicotiana tabacum 'Samsun NN'	L	-	NLP, NRSp, Oak(N), Str	NL,Oak(N)	NLP, NRSp, Str	NL,NLP, NRSp		NL,NRSp, NRSp (conc) Oak(N)							
	S	-	Dt,Mot, N,NLP, Rec, SC,Str	AN,NSp, SN,VN	BV,Dt, Ldf,NN, Rec	Dt,LN, NLP, NRSp, NSp,Rec		NRSp, NRSp (conc), Oak(N),SC							
Petunia hybrida	L	NL	NL	-	NL	-		-			- -	NL			
	S	-	Ch,Cr, Ldf,NRSp, NSp	-	Mos,Mot	ChLP,Mos		+			- -	Mot			

Phaseolus vulgaris	L	NSp	ChSp,Ep, NSp	NSp	ChRSp, NRSp,VN	NL		NRSp, VN	NL,NN, NRSp, VN		NL,VN	NRSp, NSp,VN	VN	-
	S	-	Ep,Mos, Mot,VB, YSp	-	Mos,Mot	AN,N		N,St	-		Dw,Mot, Rec,RN, St	ChRSp, Ldf,Mot, VN,YSp	AN,St	Mos,St
Solanum melongena	L	-	- -	-	NL,NRSp (conc)					- -				
	S	-	- -	-	N					- -				
Verbesina encelioides	L	-	- -	-	-		- -							
	S	-	- -	-	Ldf,Mot, NRSp,NSp		- -							
Vigna cylindrica	L	-	Ch,Ep, NRSp	-	NRSp,NSp VN		NRSp							
	S	-	Ch,Ldf, Mos,SN	-	-		-							
Vigna unguiculata	L	-	Ch,Ep, NRSp,VN	NL,VN	Ch,NSp, VN	-		NRSp VN			N	NRSp,	NL,VN	- -
	S	-	AN,Ch, Ep,NSp	-	NSp	AN,Mos, N		N,St			N	Mot	AN	- -
Zinnia elegans	L	NSp	NSp	NRSp,NSp	NSp	-					- -	+		
	S	AN,Ch, Ep,Ldf	AN,Ch, Ep,Ldf, Rec,Wlt	AN,Ep,Sco, Wlt	AN,Ep, Rec,Wlt	Mos,NSp					- -	- -		

AN = apical necrosis; BL = blistering; BV = black veins; Ch = chlorosis; ChLP = chlorotic line pattern; ChRSp = chlorotic ringspots; ChRSp(conc) = concentric chlorotic ringspots; ChSp = Chlorotic spots; Cr = crinkling; Dst = distortion; Dt = dentation; Dw = dwarfing; Ep = epinasty; Et = etching; L = local; LDf = leaf deformation; LN = leaf narrowing; Mos = mosaic; Mot = mottling; N = necrosis; NG = negative geotropy; NL = necrotic lesions; NL(conc) = concentric necrotic lesions; NL(pp) = pin-point necrotic lesions; NLP = necrotic line patterns; NN = net necrosis; NR = necrotic rings; NRSp = necrotic ringspots; NRSp(conc) = concentric necrotic ringspots; NSp = necrotic spots; Oak(N) = necrotic oak-leaf patterns; Rag = ragged or shot-hole appearance; Rec = recovery; RN = red node; S = systemic; Sc = split corolla; Sco = scorched appearance; SN = stem necrosis; St = stunting; Str = streak; Vb = vein banding; VC = vein clearing; VN = veinal necrosis; VY = vein yellowing; Wlt = wilting; Wth = withering; YSp = yellow speck and spots; WB = witches' broom-like symptoms.

+ = symptomless infection; - = no symptoms; - - = no infection; blank = not reported.

[a]This paper; [b]Gracia and Feldman (1974); [c]Brunt (1968); [d]Fagbenle and Ford (1970); [e]Berkeley and Phillips (1943); [f]Johnson (1936); [g]Thomas and Zaumeyer (1950); [h]Patino and Zaumeyer (1959); [i]Salazar et al. (1982); [j]Kaiser et al. (1982).

Tabel 1. Subisolaten van een virus uit Helianthus annuus (HV) vergeleken in hun reacties op toetsplanten met isolaten van het tabaksstrepenvirus (TSV), zoals vermeld in de literatuur.

Fig. 3. Leaves of *Helianthus annuus* with systemic net necrosis, chlorosis and vein yellowing after inoculation with tobacco streak virus propagated in *N. clevelandii.*

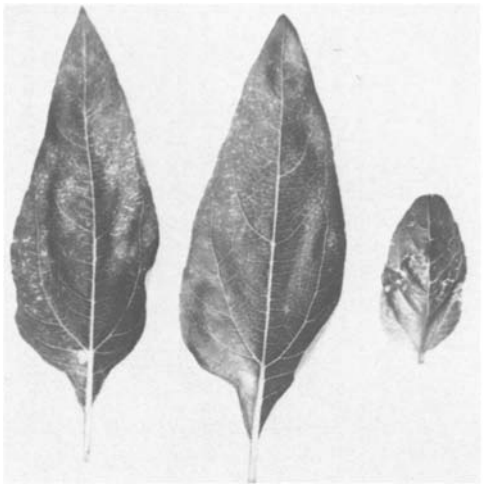

Fig. 3. Bladeren van Helianthus annuus met systemische netnecrose, chlorose en nerfvergeling na inoculatie met tabaksstrepenvirus, vermeerderd in N. clevelandii.

Fig. 4. Systemically infected leaves of *Nicotiana rustica* with deformation, severe necrosis ('scorching'), and one-sided necrosis (leaf on the right) after inoculation with tobacco streak virus propagated in *N. rustica.*

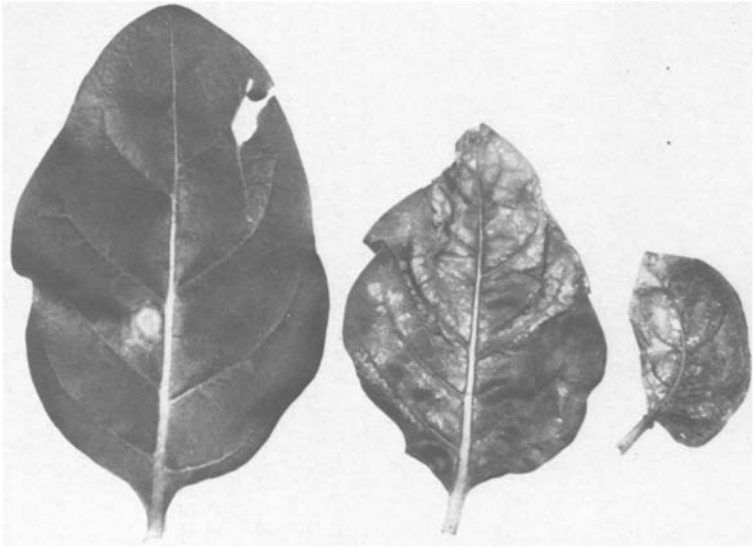

Fig. 4. Systemisch geïnfecteerde bladeren van Nicotiana rustica met misvorming, sterke necrose ('verschroeiing') en eenzijdige necrose (rechterblad) na inoculatie met tabaksstrepenvirus, vermeerderd in N. rustica.

Fig. 5. Top leaves of a plant of *Helianthus annuus* with severe necrosis ('scorching'), deformation, net necrosis and chlorosis after inoculation with tobacco streak virus propagated in *N. rustica*.

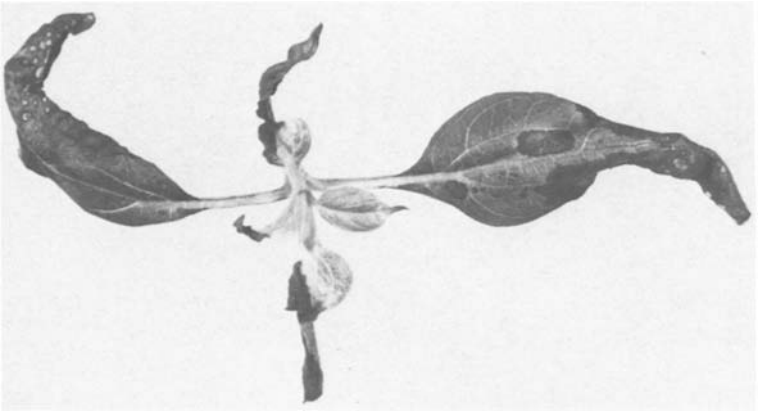

Fig. 5. Topbladeren van een Helianthus annuus plant met hevige necrose ('verschroeiing'), misvorming, netnecrose en chlorose na inoculatie met tabaksstrepenvirus, vermeerderd in N. rustica.

malformed and displayed shot-holes, giving them a ragged appearance. The middle leaves of plants of the latter species were so necrotic that they appeared to be scorched. Scorching of the leaves was also prominent in *H. annuus* (Fig. 5) and strongly resembled one of the striking symptoms displayed by the original diseased sunflower plants in the garden. Occasionally we observed a 'White Burley' tobacco plant with very light 'streak' symptoms upon inoculation with the Nr-subisolate. In a later stage of infection this plant showed dentation of the newly formed leaves.

With the Ca-subisolate, obtained from systemically infected leaves of *C. amaranticolor* showing chlorotic spots, epinasty, vein yellowing and narrowing a much smaller number of test plants got infected than in case of the Nc- and Nr-subisolates.

There were no qualitative differences in reactions of the test plants to the three subisolates whether crude sap or purified suspensions were used as inoculum.

The above-mentioned effect of the host plants proved to be reversible. When the Nr-subisolate was propagated in *N. clevelandii* through a number of successive transfers, the virus caused symptoms typical of the Nc-subisolate. Similarly, the Nc-subisolate propagated in *N. rustica* brought about symptoms characteristic of the Nr-subisolate. As the Ca-subisolate usually did not infect *N. clevelandii* it was more difficult to prove the same for this subisolate. However, once we succeeded in getting one *V. unguiculata* plant infected with the Ca-subisolate and using this plant as inoculum we could infect plants of *N. clevelandii*. Through further propagation in *N. clevelandii* we obtained a virus similar to the Nc-subisolate.

Aphid transmission. Neither of the three virus subisolates was transmitted by *M. persicae*.

Table 2. Comparison of the persistence of infectivity in crude sap of a virus from *Helianthus annuus* (HV) with that of a number of isolates of tobacco streak virus (TSV) as reported in literature.

Virus designation	Propagation host	Diluant used	DEP[1]	TIP[1] (°C)	LIV[1] (h)	Reference
HV	*Chenopodium amaranticolor*	Water	1000-10 000	50-58	2-4	This paper
HV	*Nicotiana clevelandii*	Water	500-1000	55-60	24-28	This paper
HV	*Nicotiana rustica*	Water	1000-100 000	54-56	12-26	This paper
TSV	*Chenopodium quinoa*	Phosphate buffer	1000-10 000	55-57.5	48-96	Brunt (1968)
TSV-SB10	*Chenopodium quinoa*	Water	100	55	2	Salazar et al. (1982)
TSV *Rubus* strain	*Cucumis sativus*	Phosphate buffer + mercapto-ethanol	32	56-60	NRp	Converse (1972)
TSV	*Nicotiana tabacum*	Water + sodium sulphite	NRp[2]	53	<24	Berkeley and Phillips (1943)
TSV	*Nicotiana tabacum*	Phosphate buffer + sodium sulphite	15 625-78 125	60-65	9-72	Costa and Carvalho (1961)
TSV	*Nicotiana tabacum*	Water	100	50-55	12-24	Costa et al. (1940)
TSV	*Nicotiana tabacum*	Water	1280-1560	56-60	36	Gracia and Feldman (1974)
TSV	*Nicotiana tabacum*	Water	20-30	53	24-36	Johnson (1936)
TSV pea strain	*Phaseolus vulgaris*	Water	2000-4000	62-64	>27	Patino and Zaumeyer (1959)

TSV red-node strain	*Phaseolus vulgaris*	Water	500-1000	56-58	24-48	Thomas and Zaumeyer (1950)
TSV-B	*Phaseolus vulgaris*	Water	500-1000	54-56	<24	Thomas and Zaumeyer (1950)
TSV	*Vigna unguiculata*	Phosphate buffer + sodium sulphite	640	58	24	Fagbenle and Ford (1970)

[1] DEP = dilution end-point; TIP = thermal inactivation point; LIV = longevity in vitro.
[2] NRp = not reported.

Tabel 2. Vergelijking van de infectiositeit van een virus uit Helianthus annuus in ruw sap met die van een aantal isolaten van tabaksstrepenvirus (TSV), zoals vermeld in de literatuur.

Dodder transmission. Virus was transmitted only from *N. benthamiana* plants infected with the Nc-subisolate to healthy plants of the same species.

Persistence of infectivity in crude sap. The dilution end-point, thermal inactivation point and longevity in vitro of the three subisolates are presented in Table 2.

Virus purification. Purification according to the method described by Fulton (1967) gave a very low yield of the Nc-subisolate. This method has not been tried for the other two subisolates. Better results were obtained with the method of Van Vloten-Doting (1975) especially in case of the Nc-subisolate, although the virus yields varied greatly with the season. Yields ranged from 0.9 mg to 40 mg virus per 100 g leaf material, with the lower amounts in March, April and May and the higher ones in June till September. Purification of the Ca-subisolate was performed from February till September and gave virus yields ranging from 1 mg to 7.9 mg per 100 g leaf material. In this case no seasonal influences were observed. The latter was also the case with the Nr-subisolate which gave yields ranging from 0.32 mg to 4.23 mg per 100 g leaf material.

Purified preparations of the three subisolates had an absorption maximum at 260 nm and a minimum at 240 nm. The A_{260}/A_{280} ratio of each subisolate varied from 1.51 to 1.60 with a mean ratio of 1.56.

Seed transmission. None of the *N. rustica* plants raised from seeds from diseased plants of the same species was infected.

Electron microscopy. Chop preparations of *C. amaranticolor, N. clevelandii* and *N. rustica* displayed a few isometric virus-like particles of about 30 nm in diameter. The purified Ca-, Nc- and Nr-subisolates contained isometric virus particles ranging in diameter from 25 nm to 36 nm, with main peaks in the histograms at c. 27, 31 and 35 nm.

Serology. The antisera prepared to the Nc- and Nr-subisolates had homologous titres of 64. All three subisolates reacted only with antiserum to TSV and were serologically indistinguishable from each other and from the dahlia and soybean isolates of TSV, as the gels showed confluent precipitin lines and lack of spur formation. However, with the antiserum to the soybean isolate of TSV there was also a strong non-specific reaction due to appreciable quantities of antibodies to host proteins in this antiserum. Non-specific reactions both with sap from healthy plants and with that from infected plants were also observed with some of the other antisera tested and their precipitin lines fused.

Discussion

From the results of serological tests, persistence of infectivity in crude sap and, to some extent, from reactions of test plants in host range studies, it is clear that the virus isolated from a diseased sunflower plant in the garden is TSV. A complicating factor in the identification of the virus was that the reactions of the test plants to the virus depended to a great extent on the plant in which the virus was propagated. When the

inoculum was from infected *N. rustica* in which the virus had been propagated continuously, symptoms on the test plants were much different from those obtained when *N. clevelandii* was used for propagation of the virus, even suggesting the possibility of infection by another virus. However, when the virus was purified from these different propagation hosts it proved to be identical in serological, electron microscopical and some physical properties, which had been determined. Regarding its symptomatology none of the subisolates thus obtained was identical to any of the TSV-isolates described in literature (Table 1), although the Nc-subisolate had much in common with Fulton's TSV-WC-isolate.

Tobacco streak virus, the type member of the Ilarviruses, is a multicomponent virus with a tripartite genome (Fulton, 1981). In general, the proportion of the nucleoprotein components of TSV is strain-specific (Fulton, 1967), but in certain host plants the usual proportion may be altered (Fulton and Potter, 1971). Lister and Bancroft (1970) reported that different component ratios could be obtained for one strain by varying extraction procedure or host. The propagation-host-specific differences in symptoms, which we have found with TSV from sunflower, might be explained by differences in nucleoprotein component ratios. Host passage effects are well known in plant virology and believed to be based on selection of mutants (Yarwood, 1979).

It is possible that mutations occurred after isolation of the virus from the original lesions in *G. globosa* so that the three different subisolates result from selection of mutants thus formed. However, the possibility cannot be excluded that the different subisolates are formed by differential multiplication of the nucleoprotein components in the respective hosts.

We may conclude that diagnosis of a disease caused by TSV is difficult on the basis of symptomatology alone and sometimes even impossible.

The properties of TSV in crude sap vary greatly too (Table 2). From the data provided by the various authors, it is clear that this variation cannot simply be attributed to the fact that in some cases the virus had been stabilized with anti-oxidants prior to experimentation and in other cases not, as suggested by Fulton (1981).

To be sure about the identity of the virus, one must resort to serology. However, negative results of serological tests should be interpreted with caution, as it is known that some strains of TSV do not cross-react with each other's antiserum (Converse, 1972). Moreover, we found that in many instances sap from symptom-showing leaves did not give a visible reaction with its homologous antiserum in agar-gel double-diffusion tests. This might be due to a too low virus concentration in these leaves. Therefore, the more sensitive ELISA-test may yield better results, as has been shown for potatoes infected with TSV (Salazar et al., 1982).

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Samenvatting

Tabaksstrepenvirus in zonnebloem (Helianthus annuus)

Tabaksstrepenvirus (TSV) werd geïsoleerd uit zonnebloem *(Helianthus annuus)* die sterke necrose en chlorose van de bladeren vertoonde. De identiteit van het virus werd vastgesteld op grond van serologische reacties en, tot op zekere hoogte, de symptomatologie. Het type symptoom op de toetsplanten bleek echter sterk afhankelijk te zijn van de plant waarvan het inoculum afkomstig was. Was het virus verschillende malen achtereen vermeerderd in *Nicotiana rustica* dan waren de symptomen op de toetsplanten zeer verschillend van die, welke werden veroorzaakt door virus vermeerderd in *N. clevelandii* of *Chenopodium amaranticolor.* De betekenis van deze door de waardplant bewerkstelligde variatie in symptomen wordt besproken.

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