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A biologically highly deviating strain of red clover vein mosaic virus, usually latent in pea (Pisum sativum), and its differentiation from pea streak virus

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Abstract

From pea plants (*Pisum sativum*) with necrotic stem streaking a virus (E207) was isolated and readily transmitted by sap to all 30 pea cultivars tested. In most of these infection was latent. *Trijolium incarnatum*, *T. repens* and *Vicia faba* sometimes reacted with systemic symptoms. Local lesions were rarely formed in two *Chenopodium* spp., *Phaseolus vulgaris* and *V. faba* and more often in *C. album*, *C. amaranticolor*, *C. quinoa* and *Gomphrena globosa*. The other 6 hosts of the 32 plant species tested in total did not produce symptoms.

Pea 'Koroza' and *V. faba* 'Compacta' reacted differentially to the virus and to two strains of red clover vein mosaic virus (RCVMV: RK31 and P42) and to Wisconsin pea streak virus (WPSV) used for comparison.

Its ageing in vitro was 3–5 days, thermal inactivation point 60–65 °C and dilution end-point 10^3–10^4. The virus was inefficiently transmitted in the non-persistent manner by *Acyrthosiphon pisum* and *Aphis fabae*.

In cross-protection tests the virus was found to be closely related to the two strains of RCVMV, but not to WPSV. The latter two viruses proved to be distantly related.

The four virus isolates were purified from pea by clarification with diethyl ether and carbon tetrachloride, followed by differential centrifugation and sucrose-gradient centrifugation in a zonal rotor. Sedimentation coefficients were 156 for E207, 159 for RCVMV-RK31, 163 for RCVMV-P42 and 160 for WPSV. E207 and WPSV were more stable than RK31 and P42.

Serologically E207 could not be distinguished from both strains of RCVMV, whereas it differed considerably from WPSV, potato virus S and chrysanthemum virus B.

In crude sap of pea plants, E207 and WPSV occurred in extremely high concentration and could be rapidly diagnosed with the electron microscope. They could easily be distinguished in particle length (circa 670 and 630 nm, respectively), even in mixed preparations. RK31 and P42 occurred in much lower concentrations, but were indistinguishable from E207 in particle lengths.

It is concluded that E207 is a new highly deviating strain of RCVMV. The results obtained here further support the distinction between the red clover vein mosaic virus and pea streak virus.

Introduction

In 1967 the senior author obtained some plants of an unidentified pea line (*Pisum sativum*) from a breeding station near Wageningen. The plants were normal in growth

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habit and pod formation, but the stems showed intense superficial greyish streaking, the top leaves were slightly curled, and the main veins and adjacent tissue were diffusely chlorotic.

A seemingly new virus, coded E207, was iolated. In preliminary experiments it showed relationship to the American and/or Wisconsin pea streak virus (PSV), a devastating pathogen not yet known to occur in this country, and to the red clover vein mosaic virus (RCVMV, cryptogram R/1:*/5:E/E:S/Ap), earlier reported here from pea and red clover (Hagedorn et al., 1959).

We have studied the virus in detail because of its potential practical importance and because the relationships between PSV and RCVMV required further clarification.

Results of investigations on inclusion bodies and intracellular localization of the viruses will be published separately (Bos and Rubio-Huertos, in preparation).

Materials and methods

Virus isolates. For comparison with E207 two strains of RCVMV were used. RCVMV-RK31 had been isolated from field-grown red clover in the Netherlands during 1958. It closely resembles the RK5 described by Hagedorn et al. (1959). RCVMV-P42 was provided by Dr R. W. Goth, Beltsville, USA. It had been described by Zaumeyer et al. (1964) as a new streak-producing strain isolated from peas in Maryland, USA, which caused severe symptoms in pea, *Vicia faba* and other hosts.

A Wisconsin isolate of PSV, stated to be the "Wisconsin pea streak virus" (WPSV), was sent by Dr D. J. Hagedorn, Madison, Wisc., USA.

For serology an isolate of potato virus S (PVS) from potato 'IJsselster' provided by Dr J. A. de Bokx, Wageningen, and chrysanthemum virus B (CVB) isolated from the chrysanthemum variety 'Migoli' were also used.

Maintenance, propagation and assay plants. The legume viruses were maintained and propagated in 'Koroza' or 'Rondo' peas, but were also stored in leaf material desiccated over $CaCl_2$ (Bos, 1969) to allow returning to original inoculum. 'Koroza' peas were usually employed as test plants, because they reacted differentially to the four isolates (see Host range and symptoms). *Vicia faba* 'Compacta' turned out to be a useful local-lesion indicator host for WPSV.

PVS was purified from secondarily infected 'IJsselster' potato plants. CVB was obtained from chrysanthemum plants of the cultivar 'Migoli'.

Biological experiments. Tests with plants were generally performed at 18–24 °C in an insect-proof greenhouse regularly treated with insecticides, muslin shaded during summer and illuminated with fluorescent tubes (TL) during winter. Sap inoculations were made with 0.01 *M* phosphate buffer pH7, using the forefinger to rub carborundum-dusted leaves. Persistence of infectivity in expressed sap was determined according to Bos et al. (1960).

For aphid transmission, virus-free aphid cultures were obtained from the Entomology Department, where they were maintained on *Vicia faba.* The insects were starved for half an hour at minimum, and then left for 15 min on virus-infected pea plants. They were then transferred to healthy pea test plants 10 per plant, where they were

allowed to feed for $^1/_2$ h and then killed with an insecticide or transferred to another series of plants where they were left overnight.

Virus purification. Viruses were purified as follows. In a Waring blendor 200 g of leaves were homogenized together with 300–400 ml 0.18 *M* phosphate-citric acid buffer solution (pH7) containing 0.1% thioglycollic acid, 100 ml diethyl ether and 100 ml carbon tetrachloride. The homogenate was centrifuged for 10 min at 7,500 g, and the supernatant then centrifuged for 1 h at 80,000 g. The sediment was resuspended in 70 ml of the buffer mentioned to which sometimes 1 or 2% Triton X-100 was added against aggregation. The suspension was allowed to stand for 1 h and was then centrifuged at 7,500 g during 10 min; thereafter another high-speed centrifugation was given (1–$1^1/_2$ h at 80,000 g). For rate-zonal centrifugation, the sediment was resuspended in 10 ml 0.18 *M* phosphate-citric acid buffer (pH7) and for determination of sedimentation coefficients in 5 ml distilled water. After being kept overnight preparations were centrifuged 10 min at 7,500 g. As far as possible, temperatures were between 0 and 5°C. The method varied only slightly among the different viruses. Because of their instability, RCVMV isolates for determination of sedimentation coefficients were worked up as quickly as possible and partial purification and determination finished within 12 h. In some cases, PVS was purified as mentioned by de Bokx (1969). For CVB instead of 0.18 *M* buffer, 0.03 *M* was used. The buffer contained 0.5% Na_2SO_3 instead of thioglycollic acid.

For antiserum preparation viruses were generally further purified by rate-zonal centrifugation in a zonal rotor as described by Maat and Vink (1971), but centrifuging lasted for 1 instead of 2 h. Final preparations (in 0.18 *M* buffer) were mixed with glycerol (1 : 1) and stored at –20°C. In a few cases partially purified preparations of E207 and PVS were centrifuged on a column of 20% sucrose (de Bokx, 1969). For CVB filtration through a column of granulated agar was applied (Ackers and Steere, 1967). In preliminary experiments, E207 has also been purified by differential centrifugation followed by rate-zonal centrifugation in a swing-out rotor or by centrifugation through 20% sucrose, and by chromatography according to Venekamp and Mosch (1964).

Antiserum preparation. Rabbits were injected with purified virus preparations. In general immunization was started with 2 intravenous injections with an interval of 2 days. Two weeks later an emulsion of equal parts of virus and Freund's incomplete adjuvant was injected intramuscularly. After 2–3 more weeks bleeding was started. If further immunization was necessary additional intravenous and intramuscular injections were given simultaneously. Antisera to CVB and carnation latent virus were kindly supplied by Ir D. H. M. van Slogteren, Lisse (see Hakkaart et al., 1962).

Serological test methods. Serological tests were performed according to the microprecipitin test under paraffin oil. Dilution series (factor 4) of antisera and antigens were prepared with saline containing 0.05% NaN_3. The purified virus preparations showed much aggregation; so they were treated as follows. To 2 ml virus (containing 50% glycerol) 1 ml normal serum and 1 ml saline (containing 0.05% NaN_3) were added and the mixture sonified for 15–30 sec using a Kerry Vibrason cell disruptor, probe diameter 0.9 cm, output 50–100 Watt.

Controls for reactions with normal plant proteins were made with the Ouchterlony double-diffusion test. Moreover, in some cases the micro-precipitin test was applied using clarified or partially purified and concentrated preparations of healthy plants. In experiment 1 only the antisera against CVB and carnation latent virus were absorbed with healthy plant material. In experiment 2 all antisera were absorbed with highly concentrated preparations of healthy pea plants.

Determination of sedimentation coefficients. Sedimentation coefficients were determined with a Spinco model E analytical ultracentrifuge using Schlieren optics. A series of dilutions of partially purified preparations in 0.002 *M* phosphate-citric acid buffer (pH7) was run at 27,690 rpm. With E207 and WPSV temperature was held at 20 °C and with RCVMV at 10 or 16 °C. Photographs were made at intervals of 4 min. A graphical method (Markham, 1960) was used to calculate the sedimentation coefficients. Values found were extrapolated to zero concentration. For samples run at 10 or 16 °C, values were corrected for the viscosity of water to those at 20 °C.

Electron microscopy. Preparations of purified suspensions were shadowcast or, usually, negatively stained with PTA 2% pH 6.5. For determining particle lengths, negative staining was used only, and preparations were made according to the chopping method employing TMV as an internal standard (Bos, 1970). Particles were measured directly in negatives at about × 5,000 or 10,000 under a low-power binocular microscope with objective lens × 1 and a micrometer eyepiece × 12.5.

Further details on the techniques applied are given in the sections concerned.

Host range and symptoms

Host range experiments with E207 were repeated several times and in different seasons. Per experiment usually 2–8 plants of each species were inoculated, the number depending on plant size. Back-inoculations were mostly done onto peas, one average leaf sample per host tested. To study the impact of temperature, some species were tested in climatic rooms at 17 °C (12 ° during the night period) and 23 ° (17 ° during the night), respectively. The more important hosts were also inoculated with RK31, P42 and WPSV for comparison. In additional tests a number of pea and bean cultivars have been investigated for their reaction to E207 and the bean cultivars to RK31 as well.

Results of host range tests with E207 are given in Table 1. The comparison of E207 with RK31 in bean cultivars is summarized in Table 2. The reactions of more important species are described below.

Pisum sativum. In 'Koroza' peas after about 2 weeks the inoculated lower leaves often showed some local desiccation or necrosis sometimes starting as lesions of about 1 mm diameter, followed by a withering of these leaves. In the third week the higher leaves usually showed a faint veinal chlorosis (Fig. 1: A, B) and some downward curling of the lamina, but these symptoms often disappeared. Typical was the necrotic stem streaking (Fig. 1: A, B) produced about 3 weeks after inoculation or later. After another 4 weeks this was the only obvious symptom left. At 17 °C necrosis in inoculated leaves developed slower, but eventually stem streaking was much more severe than at 23 °C or in the greenhouse (Fig. 1: A, B). Al 28 other pea cultivars tested were found to be susceptible. Most showed no signs of infection, but the virus could mostly be easily recovered. In a few varieties like 'New Era'

Table 1. Results of visual and back-inoculation tests with E207 on test plants (positive tests/tests performed).

Plant species	Symptoms		Results of back-inoculation		Summary[1]
	local	systemic	local	systemic	
Chenopodium album	2/2	0/2	2/2	0/1	L –
Chenopodium amaranticolor	3/3	0/3	2/2	0/2	L –
Chenopodium ambrosioides	0/1	0/1	0/1		– –*
Chenopodium bonus-henricus	0/1	0/1	0/1		– –*
Chenopodium capitatum	0/1	0/1	0/1		– –*
Chenopodium glaucum	1/1	0/1	0/1		(L) –*
Chenopodium hybridum	1/1	0/1	0/1		(L) –*
Chenopodium quinoa	3/3	0/3	2/2	0/2	L –
Cucumis sativus 'Gele Tros'	0/2	0/2	0/2	0/2	– –
Glycine max 'Hamney'	0/1	0/1	1/1	0/1	1 –
Glycine max 'Illen'	0/1	0/1	1/1	0/1	1 –
Glycine max 'Tokio Black'	0/1	0/1	1/1	0/1	1 –
Gomphrena globosa	3/3	0/3	3/3		L –*
Lathyrus odorata	0/1	0/1	0/1	0/1	– –
Lycopersicon esculentum	0/1	0/1	0/1	0/1	– –
Medicago sativa	0/1	0/1	0/1	0/1	– –
Nicotiana benthamiana	0/1	0/1	1/1	0/1	1 –
Nicotiana clevelandii	0/2	0/2	1/2	0/2	(1) –
Nicotiana debneyi	0/1	0/1	0/1	0/1	– –
Nicotiana glutinosa	0/2	0/2	0/2	0/2	– –
Nicotiana hybrida	0/1	0/1	0/1	0/1	– –
Nicotiana rustica	0/1	0/1	0/1	0/1	– –
Nicotiana tabacum 'White Burley'	0/2	0/2	0/2	0/2	– –
Petunia hybrida	0/1	0/1	0/1	0/1	– –
Phaseolus vulgaris 'Bataaf'	1/4	0/4	1/3	2/4	1(L)s
Pisum sativum 'Koroza'	+	+	+	+	L S
Spinacia oleracea 'Noorman'	0/1	0/1	1/1	0/1	(l) –
Tetragonia expansa	1/3	0/3	2/3	0/3	L/1 –
Trifolium incarnatum	0/3	1/3	1/1	3/3	1 S/s
Trifolium pratense	0/3	0/3	1/2	2/3	1 s
Trifolium repens	0/3	1/3	2/3	2/3	1 S/s
Vicia faba 'Compacta'	1/1	0/1	1/1	1/1	L S
Vicia faba 'Driemaal Wit'	2/2	1/2	2/2	2/2	L S/s
Vicia sativa	0/1	0/1	1/1		1 –*
Vigna sinensis 'Ramshorn Black Eye'	0/1	0/1	0/1	0/1	– –

[1]L = local symptoms
1 = symptomless local infection
S = systemic symptoms
s = symptomless systemic infection
* = no back inoculation
() = reaction or symptoms questionable
– = no symptoms and no virus demonstrated by back-inoculation

Tabel 1. Resultaten van een aantal visuele beoordelingen en terugtoetsingen met E207 op een reeks van proefplanten.

(Fig. 2) leaf symptoms resembled those of 'Koroza' or were somewhat more severe (B, C). Such plants were slightly stunted (E).

The other virus isolates always produced striking and characteristic symptoms in 'Koroza' and 'Rondo' peas. In 'Koroza', P42 usually produced pin-point or 1 mm wide ring-like necrotic local lesions in 7–9 days. With WPSV sometimes many small necrotic lesions were observed in yellowing leaf sections. In all instances, the inoculated leaves soon withered.

Systemic symptoms in 'Koroza' were quite differential. With RK31 they always consisted of a slight

Fig. 1. Necrotic stem streaking and slight vein chlorosis in 'Koroza' peas caused by E207; A, in climatic room at 17 °C (12 °C during the night); B, in the greenhouse; C, healthy control; 3–4 weeks after inoculation.

Fig. 1. Necrotische stengelstreping en zwakke nerfchlorose veroorzaakt door E207; A, in klimaatkamer bij 17 °C (12 °C gedurende de nacht); B, in de kas; C, gezonde controle; 3–4 weken na inoculatie.

stunting of the stem tip and a striking vein chlorosis and leaf curling or downward rolling (Fig. 3). Sometimes some stem necrosis occurred. With P42, however, such a stem necrosis always occurred and soon extended irregularly into the leaf veins, causing irregular yellowing of plant parts leading to an early halt of plant growth and often to top necrosis and in most cases to early plant death. In other pea cultivars, initial symptoms often were more reminiscent of RK31 but later necrosis always dominated and plants usually died prematurely. With WPSV irregular vein yellowing and vein banding or vein mosaic always occurred and this was accompanied by leaf malformation (Fig. 4). Later stem necrosis usually led to premature plant death. 'Rondo' generally reacted less severely to systemic infection with these isolates.

Phaseolus vulgaris. In 'Bataaf' beans with E207 in one out of four experiments some local veinal necrosis was observed in the inoculated primary leaves, and in another experiment virus could be recovered from symptomless primary leaves. In two out of four experiments the back inoculations from tip leaves were also successful (Table 1). Some other varieties were therefore carefully tested for susceptibility and sensitivity to E207 and this time also to RCVMV-RK31.

Fig. 2. Vein chlorosis and leaf rolling in 'New Era' (B, C) associated with plant stunting (E) some over 5 weeks after inoculation with E207; A, healthy leaf; D, healthy plant.

Fig. 2. Nerfchlorose en bladrolling bij 'New Era' (B, C) samengaand met achterblijvende groei (E) ongeveer 5 weken na inoculatie met E207; A, gezond blad; D, gezonde plant.

Results are recorded in Table 2. This shows that 7 out of 11 bean cultivars tested (with 'Bataaf' included: 8 out of 12) acquired latent local infection with E207. Five of these were locally susceptible to RK31 also. After back inoculation from the latter to pea, symptoms were highly characteristic of RK31. 'Bataaf' bean with systemic infection by E207 remained an exception among bean varieties.

Vicia faba. 'Driemaal Wit' was only tested with E207 and a few plants reacted with some small green rings in yellowing inoculated leaves. Virus could be recovered from symptomless top leaves. In 'Compacta' most plants showed a few vague local rings in chlorotic areas of inoculated leaves. Although going systemic, the virus did not produce definite systemic symptoms.

Fig. 3. Vein chlorosis, leaf curling and tip stunting in 'Koroza', 18 days after inoculation with RCVMV-RK31; left, healthy control.

Fig. 3. Nerfchlorose, bladkrulling en gedrongen topgroei in 'Koroza', 18 dagen na inoculatie met RCVMV-RK31; links, gezonde controle.

Fig. 4. Vein yellowing, vein banding and vein mosaic with leaf malformation and stem streaking in 'Koroza', $2^1/_2$ weeks after inoculation with WPSV.

Fig. 4. Nerfvergeling en nerfband- tot nerfmozaïek plus bladmisvorming en stengelstreping in 'Koroza', $2^1/_2$ week na inoculatie met WPSV.

Table 2. Reaction of cultivars of *Phaseolus vulgaris* to E207 and RCVMV-RK31.

Cultivar		E207: back-inoculation to				RK31: back inoculation to pea 'Koroza' for symptoms	
		pea 'Koroza'[1]		*Chenopodium*[2]			
		symptoms	EM[3]	*amaranti-color*	*quinoa*		
Double White	L[4]	−	+	−	+	−	0/4
	S[4]	−		−	−		
Flits	L	+	++	−	−	−	0/3
	S	−	−	−	−		
Imuna	L	?	++	−	−	+	3/3
	S	−	−	−	−		
Pinto UI 114	L	−	−	−	−	−	0/8
	S	not tested					
Pinto 724026	L	−	−	−	−	−	0/8
	S	not tested					
Prelude	L	−	−	−	−	−	0/4
	S	not tested					
Processor	L	+	+	+	+	+	3/4
	S	−	−	−	−		
Red Kidney	L	+	++				
		+	+++	−	−	+	3/3
	S	−	−	−	−		
Sanilac	L	+	+				
		−	−	−	−	−	0/4
	S	−		−	−		
Top crop	L	+	+++	−	−	+	2/3
	S	−		−	−		
Troef	L	+	+++	+	+	+	1/3
	S	−		?	−		

[1]Always 1 pot of peas each with four plants was used.
[2]Always 2 pots with one plant each of each *Chenopodium* species was used.
[3]Because with E207 the pea reaction was hard to evaluate quantitatively on the basis of symptoms (in contrast to RK31, last column), average samples of pea test plants were checked in the electron microscope (EM) 20 days after inoculation; + few particles, +++ abundant particles observed.
[4]L = inoculated bean leaves tested; S = tip bean leaves tested.
[5]Number of pea test plants reacting out of plants tested.

Tabel 2. Reactie van rassen van Phaseolus vulgaris op E207 en RCVMV-RK31.

Both isolates of RCVMV as well as WPSV were able to induce local lesions in 'Compacta' (Fig. 5: A, B, C). With WPSV they could be observed after 4 days and started as numerous chlorotic lesions. In lower leaves they soon developed into distinctive necrotic rings with a desiccated centre (C). RK31 and P42 could also produce local lesions but at least 5 days later and the lesions were more irregular in size and shape. With RK31 they were more concrete and dark green (A), with P42 ring-like and sometimes somewhat necrotic (B). In both strains of RCVMV their production was highly unreliable in contrast to WPSV.

With both isolates of RCVMV and with WPSV most 'Compacta' plants inoculated showed systemic symptoms (Fig. 5: D, E, F). With RK31 (D), they were mildest, consisting of a vague vein chlorosis and a slight leaf curling. With P42 they were severe, mainly due to stem necrosis leading to a

Fig. 5. *Vicia faba* 'Compacta' with local lesions of RK31 (A), P42 (B) and WPSV (C), 15, 12 and 7 days after inoculation, respectively, and with systemic symptoms of RK31 (D), P42 (E) and WPSV (F), 3 weeks after inoculation.

Fig. 5. Vicia faba 'Compacta' met lokale lesies van RK31 (A), P42 (B) en WPSV (C), respectievelijk 15, 12 en 7 dagen na inoculatie, en met systemische symptomen van RK31 (D), P42 (E) en WPSV (F), 3 weken na inoculatie.

considerable growth reduction or even death. In surviving plants tops were stunted and top leaves slightly curled. With WPSV (F), the inoculated leaves were soon cast and top leaves showed a slight vein chlorosis and a striking leaf curling and rolling, whereas stems showed a striking necrotic streaking.

Trifolium incarnatum (crimson clover), *T. pratense* (red clover) *and T. repens* (white clover) E 207, as well as RK 31 and P 42, could be recovered from all three clover species (Table 1); WPSV could not from red and white clover. E 207 only produced indistinct vein mosaic in a few leaves of white clover and a faint variegation in some crimson clover leaves. With all other isolates usually only a few of the clover plants inoculated showed syptoms, and then in a few leaves only.

In crimson clover with RK31 some vein chlorosis to vein mosaic was observed; with P42 there was considerable yellowing and with WPSV some leafrolling and a slight chlorotic leaf striping. In red clover a characteristic bright yellow vein mosaic, as often described in the literature, was incited by RK31. The abnormality, however, was always distributed irregularly. With P42, a few leaves showed necrosis of veins and petioles as well. In white clover a vein mosaic was produced both by RK31 and P42.

Chenopodium amaranticolor. With E207 often, but very erratically, many vague chlorotic local lesions were produced 9–14 days after inoculation, gradually becoming more clear and $^{3}/_{4}$–1 mm wide in another week (Fig. 6, below), later, in yellowing leaves, standing out as whitish lesions with a greenish border. In a climatic room the lesions remained small pinpoints at 17 °C, and tended to

Fig. 6. *Chenopodium quinoa* (top) and *C. amaranticolor* (below) with local lesions $3^{1}/_{2}$ weeks after inoculation with E207.

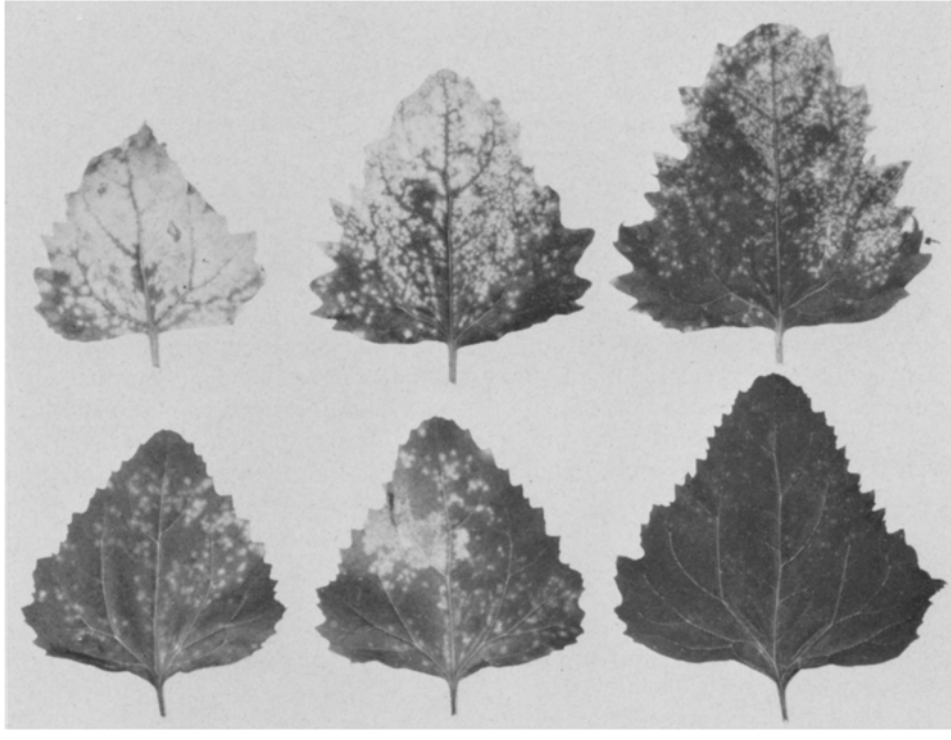

Fig. 6. Chenopodium quinoa (boven) en C. amaranticolor (beneden) met lokale lesies $3^{1}/_{2}$ week na inoculatie met E207.

enlarge up to 3 mm in diameter after 29 days at 23 °C. Neither both strains of RCVMV nor WPSV produced visible local lesions.

Chenopodium quinoa. With E207 after circa 10 days numerous small chlorotic local lesions were often formed (Fig. 6, top), about one month after inoculation standing out in yellowing leaves with a greenish border. RCVMV and WPSV did not produce any visible reaction. The effect of temperature was as in *C. amaranticolor.*

Gomphrena globosa. E207 often incited a moderate number of clearcut local lesions $1^1/_2$–4 mm wide with a dry centre and a reddish-brown edge. They were sometimes concentric. With WPSV more but smaller local lesions were obtained and they first appeared after 11 days. With RK31 no, and with P42 a few local lesions were observed, which did not show up before the 14th day.

Virus source and assay plants

Table 1 shows that the host range of E207 is limited. Moreover, in most susceptible species, even when infection became systemic, symptoms were absent and the virus could not always be recovered.

Under normal greenhouse conditions symptom production in the few local-lesion hosts available, such as *Chenopodium amaranticolor*, *C. quinoa* and *Gomphrena globosa*, was highly unreliable (e.g. see Table 2). In climatic rooms symptom expression seemed better at 23° than at 17 °C, but even then these hosts now and then failed when pea plants reacted with systemic symptoms. Results with some other *Chenopodium* species listed in Table 1 were even poorer or negative.

Because 'Red Kidney' beans had been found useful for bio-assaying potato virus M (Hiruki, 1970), a number of *Phaseolus* cultivars were tested. Remarkably enough 8 out of 12 were locally susceptible without reacting visibly, however (Table 2).

So far, the only reliable test plant for E207 remains *Pisum sativum* ('Koroza' and 'Rondo') itself. Here, again local reaction is unreliable, leaving the retarded systemic stem streaking and faint vein chlorosis the only symptoms to rely upon.

Pea plants have also proved the only good systemic host for virus propagation. Back inoculations, from tip leaves every 4 days, starting one week after inoculation, in one experiment with 'Koroza' and another with 'Admiral' did not lead to local lesions in *C. quinoa* until on the 25th and 21st day after inoculation, respectively. Then, many lesions were produced. Thus, virus multiplication slowly starts but final concentration is high. This was confirmed by many electron microscope observations of crude sap. During the first three weeks only very few particles could be observed, whereas later on particles were abundantly present (Fig. 10) even in many symptomless cultivars.

Persistence of infectivity in expressed sap

Since no good local-lesion host was available, the results of a few tests performed can only be considered of provisional value. In these tests *Gomphrena globosa* was used as an indicator.

After 0–3 days of storage, sap was infective but after 5 days no lesions were formed, indicating an *ageing in vitro* between 3 and 5 days.

The *thermal inactivation point* was found between 60 and 65 °C.

In the two experiments performed, sap was infective at dilutions up to 1000, but not at 10,000 or higher, indicating a *dilution end-point* between 1000 and 10,000.

Aphid transmission

Two provisional experiments were performed with *Acyrthosiphon pisum*, *Aphis fabae* and *Myzus persicae*. In both experiments 80 aphids per species were fed on two pots of pea plants each. In the first experiment there were questionable symptoms in several pea plants fed upon by the first two aphid species. In peas back-inoculated from the plants inoculated by *A.pisum* E207 symptoms were produced and many particles could be observed with the electron microscope. Back inoculation from peas with *A.fabae* gave questionable symptoms. In the second trial one plant out of 8 with *A.pisum* and 1 out of 8 with *A.fabae* showed symptoms. The one plant with *A.pisum* contained many virus particles, as did the pea plants back-inoculated from this one. With *M.persicae* in both experiments no transmission could be demonstrated. In the second experiment particles could not be detected with the electron microscope.

No symptoms were observed in pea plants fed upon overnight after the first $^1/_2$-hour test feeding on other pea plants.

The experiments were greatly hampered by absence of clear symptoms and lack of a good test plant for back inoculations. Reactions on *C.quinoa* and *Gomphrena* this time were greatly unreliable.

Tentative conclusions are that the virus could be transmitted in a non-persistent manner by *Acyrthosiphon pisum* and *Aphis fabae* only, and that transmission rates seem low.

Cross protection

Pisum sativum was the only species available for cross-protection tests. In part of the experiments 'Koroza' was used because of its more flushy growth. 'Rondo' had the advantage of reacting less severely to WPSV when used for protection. Always 4 pots with 4 plants each were taken per treatment. Super-inoculated plants were tested visually for infection with the challenge virus, and they were often back-inoculated, one average sample of tip leaves per pot. Of the control pots always one average sample was tested.

A survey of the experiments and their results is given in Table 3. Often results were unsatisfactory because of poor symptom expression in plants super-inoculated late to allow sufficient systemic infection with the protecting virus, especially with E207. Then, plants had already reached some stage of maturation further enhanced by the protecting virus even when the plants remained green. This would even have influenced super inoculation with non-related viruses. Of course, back inoculation of one average sample per pot of 4 plants is a rather coarse test, because some plants might have escaped protective infection, although plants were judged visually before super inoculation. Nevertheless some unmistakable observations could be made.

RK31 clearly protected against P42 as evidenced by absence of clear P42 symptoms after super inoculation, and by absence of virus in 2 out of 4 pots (exp. 2 and 4), and a poorer inoculum in the other two pots when back-inoculated. However, the protection did not seem complete.

Table 3. Cross-protection tests.

Experiment number and host plant used	Protecting virus	Challenge virus	Time interval in days	Direct effect challenge virus	Back inoculation for challenge virus			Protection
					days after super inoculation	test plants	pots proving positive	
1. Koroza	E207	RK31	16 and 23	−	33 and 26	Koroza	4/4	strong but not complete
	0	RK31		+				
2. Koroza	E207	P42	21	+[1]	7 and 18[3]	Koroza	0/4 4/4	some (absence local
	0	P42		+[2]	do.	do.	1/1 1/1	reaction, delay of systemic symptoms)
	E207	WPSV	21	+	7 and 21	*V.faba* 'Compacta'	4/4 4/4	none
	0	WPSV		+	do.	do.	1/1 1/1	
	RK31	WPSV	10 and 15	?	18 and 13	*V.faba* 'Compacta' and *Gomphrena*	4/4	some to none
	0	WPSV		+	do.	do.	1/1	
	RK31	P42	10 and 15	−	18 and 13	Koroza	2/4[4]	incomplete
	0	P42		+	do.	do.	1/1	
3. Rondo	WPSV	RK31	18	+				some?
	0	RK31		+				
	WPSV	P42	18	+[1]				some?
	0	P42		+[2]				
4. Rondo	E207	P42	21	+	29	Koroza	4/4	some (slight delay
	0	P42		+	do.		1/1	of systemic symptoms and less severe syptoms after back inoculation)
	E207	WPSV	21	+	29	*V.faba* 'Compacta' and *Gomphrena*	4/4	none
	0	WPSV		+	do.	do.	1/1	
	RK31	WPSV	18	?	21	do.	4/4	none
	0	WPSV		+	do.	do.	1/1	
	RK31	P42	18	?	21	Koroza	2/4[4]	not complete
	0	P42		+	do.	do.	1/1	
	WPSV	RK31	18	?	32	Rondo	?	
	0	RK31		+	do	do.	?	

[1]No local lesions by P42, systemic symptoms developing slower.
[2]Many local lesions by P42.
[3]Back inoculation from super-inoculated and tip leaves, respectively.
[4]The two pots reacting produced no local lesions and weaker symptoms than with P42 alone.

Tabel 3. Premunitieproeven.

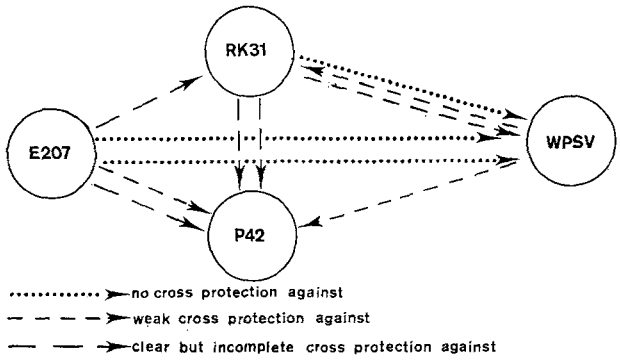

Fig. 7. Relationships between the four virus isolates as revealed by cross-protection tests in pea.

Fig. 7. Verwantschap tussen de vier virusisolaten zoals bleek uit de premunitieproeven in erwt.

There may have been some protection by WPSV against RK31 (exp. 3) because only 4 out of 16 plants infected with WPSV produced RK31 symptoms, whereas 15 out of 16 control plants did. Here again, poor infection by RK31 of plants previously infected by WPSV might have been due to poor growth caused by WPSV. Similarly, with WPSV symptoms of P42 developed somewhat slower, and no local lesions were formed.

There was no protection by E207 against WPSV, but E207 protected to some extent against RCVMV. Challenge inoculation in 'Koroza' with P42 (exp. 2) did not produce necrotic local lesions on E207 plants in contrast to healthy plants showing many. Back inoculation from E207 leaves one week after super inoculation with P42 did not reveal P42. Tip leaves of these plants later showed clear P42 symptoms, and 18 days after super inoculation the virus could easily be recovered from these tip leaves. So, super infection was clearly hampered and considerably delayed. With 'Rondo' this delay in symptom expression of P42 was only slight (exp. 4). Here, symptoms in pea after back inoculation with E207 + P42 were less severe than from P42 alone, suggesting a lower concentration of P42 in the first case. Thus, protection against P42 by E207 is not complete. This protection was more pronounced against RK31 (exp. 1). RK31 did not produce symptoms after super inoculation. Only one out of 15 pea plants was later found to contain RCVMV inclusion bodies. These were then abundant in RK31 controls. Back inoculation from all 4 pots was positive, however.

Not too much weight should be given to weak cross-protecting effects observed. The protecting virus usually produced a general growth reduction which could naturally reduce infection velocity with most other viruses.

The results of cross-protection tests are finally summarized in Fig. 7, suggesting a close relationship between RK31 and P42, a slightly less close kinship of these two to E207 and a very distant relationship between these three and WPSV.

Virus purification

Preliminary purification experiments with E207 resulted in preparations containing numerous elongated particles. Fig. 8 shows electron micrographs of preparations purified with differential centrifugation followed by centrifugation through 20% sucrose (A, B) or rate-zonal centrifugation in a swing-out rotor (C). The results obtained with the chromatographic procedure were less satisfactory.

A comparison of the results of rate-zonal centrifugation in the zonal rotor of par-

Fig. 8. Electron micrographs of E207 virus already obtained after preliminary purification; A, B, negative staining; C, shadow casting. Magnification × 30,000.

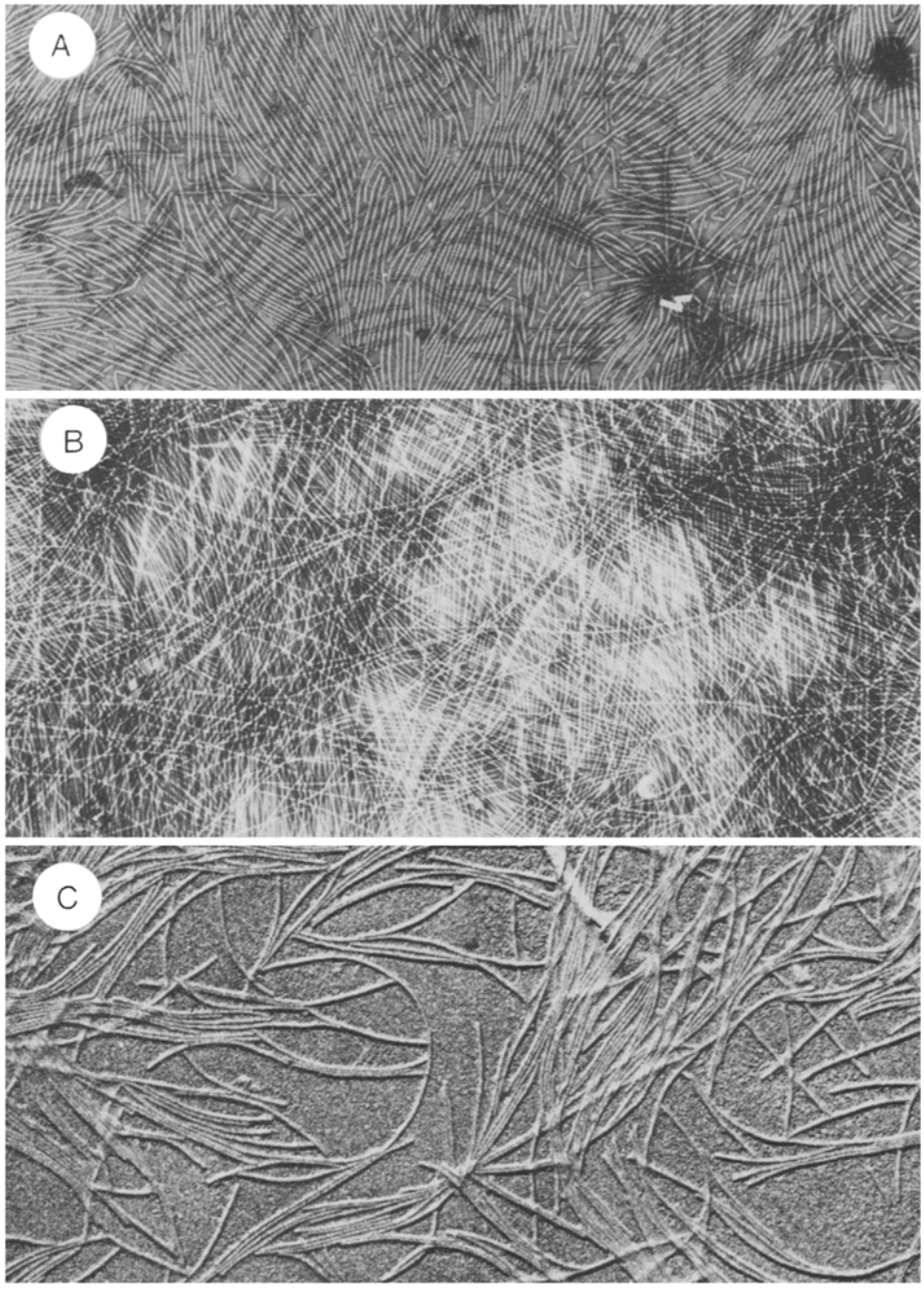

Fig. 8. Elektronenmicroscopische foto's van E207-virus reeds verkregen na voorlopige zuivering; A, B, negatieve kleuring; C, schaduwing. Vergroting 30.000 ×.

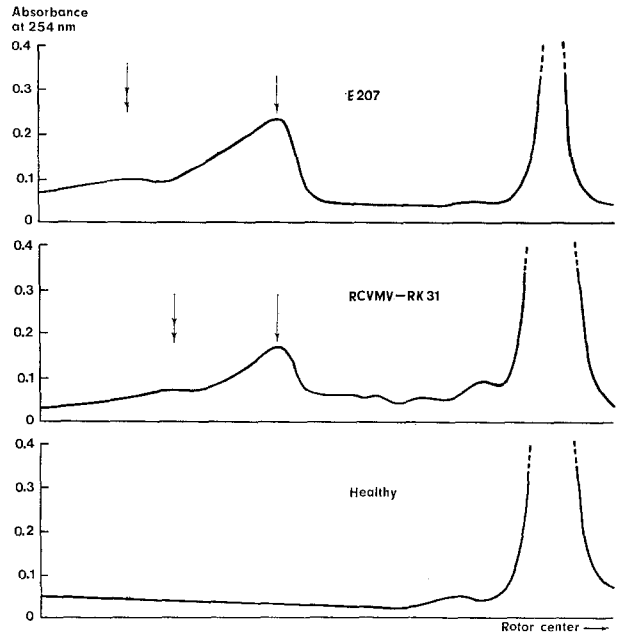

Fig. 9. Comparison of the results of rate-zonal centrifugation in a zonal rotor of partially purified preparations of E207, RCVMV-RK31 and of sap from healthy pea plants.

Fig. 9. Vergelijking van de resultaten van snelheidszone-centrifugering in een zonerotor van gedeeltelijk gezuiverde preparaten van E207, RCVMV-RK31 en van sap van gezonde erwteplanten.

tially purified preparations of E207, RCVMV-RK31 and of an extract of healthy pea plants, prepared in the same way, is given in Fig. 9. The picture of WPSV was similar to that of E207. In the analytical ultracentrifuge RCVMV-P42 sedimented in more components, as did RCVMV-RK31 in rate-zonal centrifugations as well in the analytical ultracentrifuge. The nature of the extra components has not been studied. In the one experiment made with RCVMV-P42, no clear picture of the sedimentation in a sucrose gradient was obtained because of technical trouble. Fractions indicated with a single and a double arrow in Fig. 9, respectively, contained monomers and dimers (end to end) of the virus as appeared from electron micrographs. In general, antisera were prepared with fractions indicated by the single arrows.

Sedimentation coefficients

Values found in 0.002 *M* buffer are 156 for E207, 160 for WPSV and 159 for RCVMV-RK31. These are the averages of 2 experiments. The values in the 2 experiments did not differ more than 2 units. In the one experiment performed with RCVMV-P42 a sedimentation coefficient of 163 was found. Both RCVMV isolates were very unstable at 20 °C and therefore had to be run at lower temperatures.

Serology

The results of two serological tests are given in Table 4. In experiment 1 only the antisera to CVB and carnation latent virus were absorbed with extracts from healthy plants. In experiment 2 all antisera were absorbed. The results obtained indicate a very close serological relationship between E207 and the RCVMV isolates. The reaction of E207 with PVS antiserum in experiment 1 may be due to reaction with normal

Table 4. Summary of the results of two serological experiments with E207 and some other viruses of the PVS-group.

Antigens	Titers of the antisera of										
	E207		RCVMV-RK31		WPSV	PVS		CVB	Carnation latent virus	Normal serum	
Test No	1	2	1	2	2	1	2	1	1	1	2
E207	1024	4096	1024	1024	16	16	–	–	–	–	–
RCVMV-RK31	256	4096	256	1024	16	<4	1	–	–	–	–
RCVMV-P42		4096		1024	16		1				–
WPSV		1		16	4096		–				–
PVS	16	16	256	64	–	1024	4096	4	4	±	–
CVB	1	1	–	–	–	16	64	64	–	–	–
Healthy controls:											
Pea	–	–	–	–	–	4	–	–	–	–	–
Potato	–	+	–	+	+	–	+	–	–	–	+
Chrysanthemum	–	–	–	–	–	–	–	–	–	–	–

+ non-specific reactions with certain serum dilutions
– no reaction

Tabel 4. Overzicht van de resultaten van twee serologieproeven met E207 en enkele andere virussen van de aardappelvirus-S-groep.

plant material. This reaction was not reproduced in experiment 2 where E207 did not react at all with antiserum to PVS. So, clear differences between E207 and RCVMV do not appear from data in Table 4. In a few cases non-specific reactions were recorded (indicated as ± or +). The reaction of PVS with normal serum in experiment 1 occurred in the highest serum dilutions only, and differed from the other reactions in type of precipitate formed. In experiment 2 this reaction did not occur whereas the other reactions were almost the same as in experiment 1. The reaction in experiment 2 of all antisera with sap from healthy potato plants was only with the lowest serum dilutions and did not occur in other experiments not mentioned here and otherwise giving comparable results. Moreover, the normal serum did not react with the virus preparations suggesting the titers presented to have resulted from specific virus-antibody reactions. WPSV, PVS and CVB clearly differed from E207 and RCVMV. Several of the relationships demonstrated have been published earlier (see Hakkaart et al., 1962, and General discussion).

Tomlinson and Walkey (1967) and Koenig (1969) sonified turnip mosaic virus and members of the potato virus X group. In spite of very intensive treatments, no changes in antigenic properties of potato virus X could be found. Likewise, in our experiments short ultrasonic treatments to dissolve virus aggregates did not appreciably alter homologous antiserum titers.

Electron microscopy

Many chop preparations with E207 have been made. In pea during the first three weeks after inoculation always only few virus particles could be observed. However,

Fig. 10. Electron micrographs of crude sap of E207-infected pea plants: A, with TMV-containing tobacco sap, applied as an internal standard for length determination; × 33,000; B, pea sap only, × 35,000.

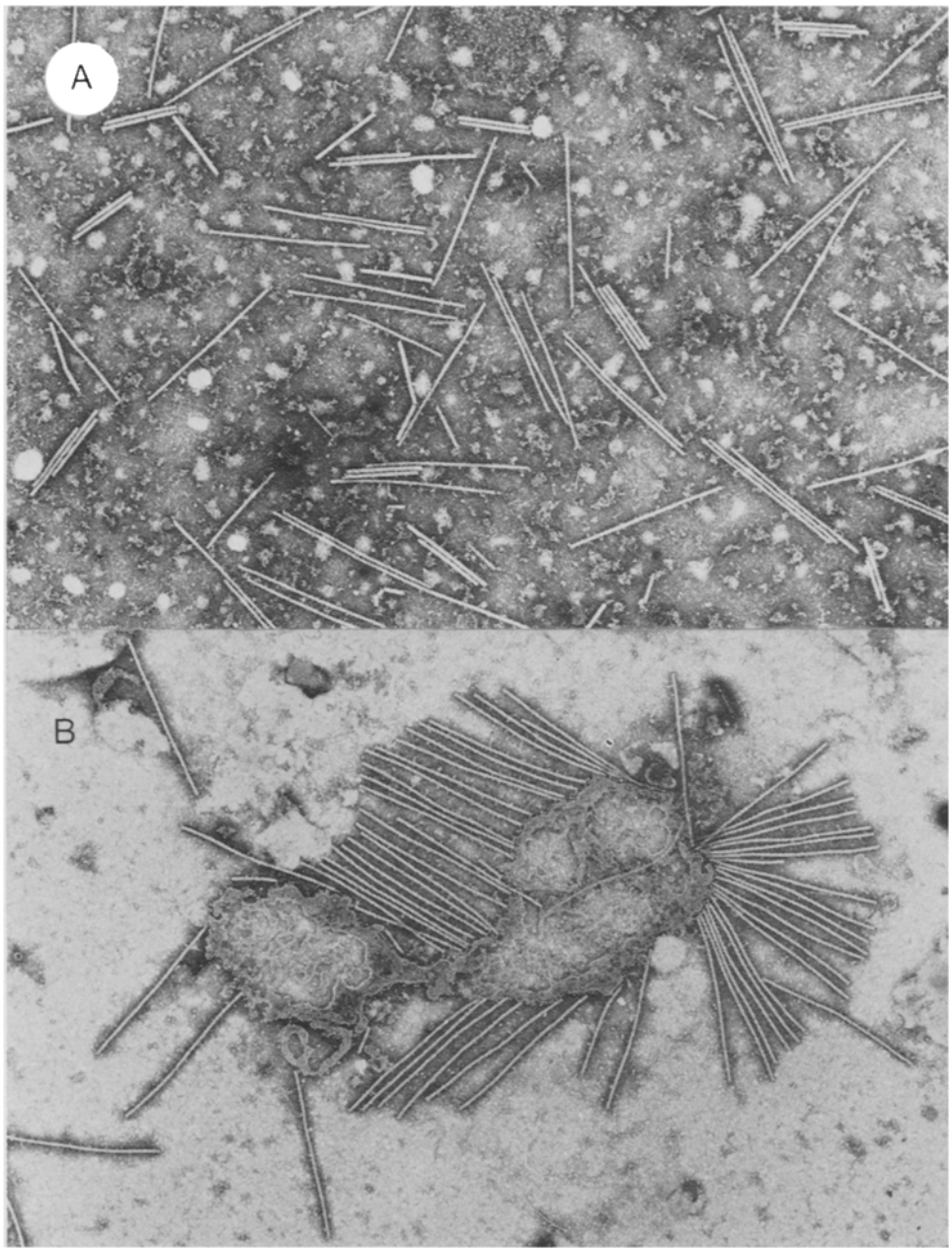

Fig. 10. Elektronenmicroscopische foto's van ruw sap van erwt geïnfecteerd met E207: A, met TMV-houdend tabakssap, toegevoegd als interne standaard voor lengtemetingen; 33.000 ×; B, alleen erwtesap, 35.000 ×.

from then on they occurred abundantly and could be most easily detected (Fig. 10A). The particles tended to spread evenly through the preparation, but sometimes lay side-by-side. They were rarely aggregated end-to-end. They also often occurred in groups with their extremities attached to organelles (Fig. 10B). Sometimes extensive mats or crowds of particles were observed as if dispersing from a dense accumulation.

In our experiments electron microscopy turned out to be the easiest and most reliable way to test plants for infection with E207.

When lying free, the E207 particles were straight (Fig. 10A) or slightly curved. When situated near other particles they proved slightly flexible (Fig. 10B), but soon broke when further bent, as demonstrated in concentrated purified preparations (Fig. 8A). Pictures of such concentrated preparations exhibited an extremely pronounced mutual repulsion of particles. When adhering to the film on the grid the particles rather broke than came close to each other. At high magnification several particles showed a central core.

With WPSV the concentration and distribution of virus particles in crude sap was completely like with E207, although those of WPSV appeared earlier.

In contrast, concentration with RCVMV (RK31 and P42) was always low. In morphology, these isolates did not differ from E207 and WPSV.

Particle measurements led to rather narrow distribution curves. Crude tobacco sap with TMV was used as an internal standard. Results are summarized in Table 5. E207 seemed slightly longer than RK31 and P42, but in a series of measurements all three were about 670 nm long. WPSV, however, was distinctly shorter, viz. about 630 nm.

When E207 and WPSV were present in one preparation, they yielded distribution curves with two clearly separate peaks. There was not much overlapping so that most individual particles could easily be recognized by length as E207 or WPSV. The same held for a mixture of WPSV and RK31 (Fig. 11).

Table 5. Length measurements of virus particles.

Experiment number and date	E207			WPSV			RCVMV-RK31			RCVMV-P42		
	total number of particles		calculated length (nm)	total number of particles		calculated length (nm)	total number of particles		calculated length (nm)	total number of particles		calculated length (nm)
	E207	TMV		WPSV	TMV		RK31	TMV		P42	TMV	
1. 7–10–'68	207	41	688									
2. 20–3–'69	397	40	662									
3. 24–6–'69	94	49	678									
	62	47	672									
4. 19–11–'69							90	107	663			
5. 3–2–'71										114	92	679
6. 24–2–'71	177	24	652	186	19	627				25	31	674
	103	51	663	286	77	633						
7. 18–3–'71	66	38	679	155	29	624	197	235	672	125	26	673
8. 24–9–'71	54	75	675	92	37	631	49	33	656			
9. 26–10–'71	294	220	673							33	77	675

Tabel 5. Lengtemetingen van virusdeeltjes.

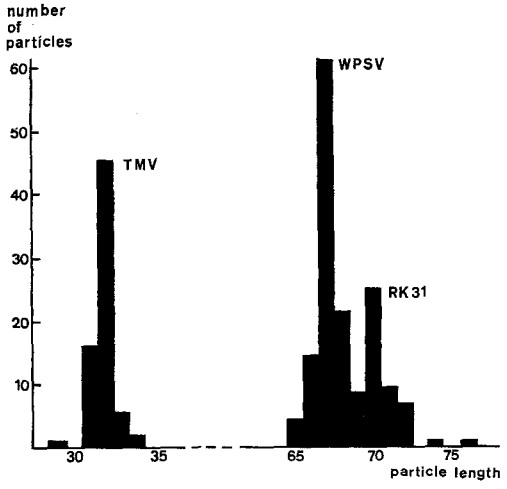

Fig. 11. Length distribution curve of virus particles in crude sap of pea plant simultaneously infected by WPSV and RK31 and mixed with tobacco sap containing TMV as internal standard.

Fig. 11. Lengteverdeling van de virusdeeltjes in ruw sap van erwteplant gelijktijdig geïnfecteerd met WPSV en RK31 en gemengd met tabakssap dat TMV bevat als interne vergrotingsstandaard.

General discussion

The E207 virus undoubtedly belongs to the potato virus S group on the basis of its particle length and shape, its limited host range and its persistence of infectivity in expressed sap (cf. Gibbs, 1969). Like some other representatives, e.g. carnation latent virus, hop (latent) mosaic virus, *Passiflora* latent virus, potato viruses S and especially M, and the recently described artichoke latent virus (Majorana and Rana, 1970), the E207 virus is often latent in spite of its high concentrations in infected plants.

Research on the virus has been greatly hampered by lack of a reliable local-lesion host. The high virus concentration of E207 as revealed by electron microscopy seems to conflict with the low dilution end-point of 1000–10,000. The latter may be due to the low sensitivity of the test plants used. With WPSV Kim and Hagedorn (1959) found dilution end-points of over 10^6. There, however, *Chenopodium amaranticolor* is a highly sensitive test plant (Rosenkranz and Hagedorn, 1964).

The main similarities and differences between E207 and the two isolates of RCVMV and WPSV, two other legume representatives of the S-group, have been summarized in Table 6. They have to be discussed in some detail.

Stem streaking produced by E207 in the few pea cultivars that are sensitive, especially in 'Koroza', is reminiscent of pea streak virus. An isolate of this virus (WPSV) and E207, when compared, have many other features in common, such as extremely high concentration in infected plants both in crude sap (this publication) and in ultrathin sections (Bos and Rubio-Huertos, in preparation).

However, E207 and WPSV are different viruses because of absent cross-protection (Table 3), distant serological relationship (Table 4) and definite differences in particle lengths (Table 5 and Fig. 11).

E207 also differs in a number of aspects from RCVMV (RK31 and P42), e.g. by its symptoms in pea, absence of crystalline inclusion bodies highly characteristic of RCVMV (Bos and Rubio-Huertos, in preparation) in spite of its high concentration, and in its high concentration in chop-preparations. During purification E207 was

Table 6. Condensed comparison of the four virus isolates.

	E207	RK31	P42	WPSV
reaction in pea	usually latent, sometimes stem streaking and slight vein chlorosis	conspicuous leaf curling and vein chlorosis; plant stunting	leaf curling and vein chlorosis soon followed by severe necrosis, yellowing and early plant death	often latent, usually severe stem streaking and premature death
reaction in *Vicia faba* 'Compacta'	sometimes a few l.l., latent systemic infection	often l.l., usually vein chlorosis	often l.l., systemic leaf curling and sometimes plant death	regularly many necrotic l.l. and systemic necrosis leading to plant death
reaction in *Gomphrena*	sometimes l.l.			regularly many l.l.
inclusion bodies in pea (epidermal strips, light microscopy)[1]	none	conspicuous intracytoplasmic crystals	conspicuous intracytoplasmic crystals, not observed in case of early necrosis	finely granular often diffuse intracytoplasmic bodies
ultrathin sections, electron microscopy[1]	extensive layered accumulations of virus particles	crystals of virus particles	(insufficiently studied)	extensive layered accumulations of virus particles
virus concentration in chop-preparations	abundant particles	few particles	few particles	abundant particles
particle length (nm)	652–688	656–677	658–679	624–633
virus stability in purification	high	low (tendency to aggregation)	low (tendency to aggregation)	high
sedimentation coefficient	156	159	163	160
serology	← indistinguishable →			only distantly related to the other three
cross protection	strong but incomplete against RK31 and P42, absent against WPSV	incomplete against P42, doubtful against WPSV		doubtful against RK31 and P42

[1]Data from Bos and Rubio-Huertos (in preparation)

Tabel 6. Samenvattende vergelijking van de vier virusisolaten.

much more stable than RK31 and P42, as was WPSV, and its sedimentation pattern differed. When determining sedimentation coefficients, RK31 and P42 rapidly aggregated at 20°C. This agrees with the finding of Varma et al. (1970) that RCVMV is rather labile and easily aggregates. This tendency towards aggregation might also explain the formation of crystalline inclusions, and that it shows up less easily in dip or chop preparations. In contrast, E207 particles tend to stay apart as is evident in electron micrographs of crude and purified preparations (Fig. 8 and 10). But E207 protects pea plants to a considerable extent against both RK31 and P42. Moreover, the three isolates could not be distinghuished serologically (Table 4). The close biological relationship between E207 and RCVMV is further supported by the almost equal bean varietal reaction of E207 and RK31 (Table 2).

The close relationships between the Dutch RK31 and the American P42 in particle length, host range, cross protection, serology, low virus concentration in crude sap preparations and production of crystalline inclusions (Bos and Rubio-Huertos, in preparation), further corroborate the earlier conclusion by Zaumeyer et al. (1964) that P42 is a strain of RCVMV. In pea and broad bean, however, P42 is a very virulent necrosis-producing strain.

Varma et al. (1970) reported the sedimentation coefficient of their RCVMV to be 161. This is in between the values we found for the RK31 (159) and P42 (163) isolates. But E207 (sedimentation coefficient 156) does not differ more from RK31, than does P42 from RK31 in this respect, whereas the value for WPSV (160) is very close to those of the RCVMV isolates.

Our finding of a central hole in E207 particles agrees with a similar report for RCVMV particles by Varma et al. (1970). These data are new for the potato virus S group.

Evidently, E207 represents another highly deviating strain of the red clover vein mosaic virus, latent in most pea cultivars as well as in broad bean. It is also distinct in its extremely high concentration in infected plants, ist high stability and absence of crystalline inclusions. In connection with the strain differentiation of RCVMV it would be very important to have more information on the degree of relationship between RCVMV and muskmelon vein necrosis virus. Recently this virus, causing disease in some *Cucumis* species, was found to infect several legumes and to be related serologically to RCVMV (Freitag and Milne, 1970).

The clear distinction made here between E207 with RK31 and P42 as strains of RCVMV on the one hand and WPSV on the other now leads us to the problem of the many viruses inducing necrotic streaking in pea and described in the literature: alfalfa mosaic virus, bean red node virus, bean yellow mosaic virus, beet mosaic virus, broad bean vascular wilt virus, cucumber mosaic virus, lettuce mosaic virus, pea early browning virus, pea necrosis virus, tobacco ringspot virus and tomato spotted wilt virus. Under certain conditions they can cause symptoms in peas hardly or completely indistinguishable, but they can now be easily differentiated on the basis of differential host reactions and particle morphology and size.

Considerable confusion still exists, however, as to the exact identity of the streak inciting viruses belonging to the potato virus S group. This even holds for "pea streak virus" in the USA. Normal strains of RCVMV, since long known to incite the stunt disease of peas (Hagedorn and Hanson, 1951), not rarely produce stem necrosis and streaking and premature plant death (Quantz, 1958) or do so when occurring in com-

plex with bean yellow mosaic virus (Schroeder et al., 1959). The P42 strain of RCVMV, described by Zaumeyer et al. (1964) in Maryland, produces a much more radical necrosis than do the conventional isolates of "pea streak virus". Some viruses specified by their authors to be "pea streak virus", such as the Oregon isolate 331 of Ford (1966), certainly are RCVMV serologically and in particle length (651 nm). It is confounding when Ford and Baggett (1965), studying reactions of plant introduction lines of pea to some important viruses, list the P42 strain of RCVMV as "pea streak virus". The authoritative CMI/AAB Descriptions of Plant Viruses even add to the confusion by listing among the selected synonyms of red clover vein mosaic virus the name pea streak virus 1, although a question mark was added (Varma, 1970).

The first to make an important advance in clearing up the situation were Wetter and Quantz (1958) and Wetter et al. (1962) in Germany, studying the identity and relationships of "Steinkleevirus" of *Melilotus albus* described by Quantz and Brandes (1957) and "Stauchevirus der Erbse" described by Quantz (1958). They carefully studied these viruses for biological properties, serology and particle length as compared with a number of American viruses, *viz.* an isolate of RCVMV (Hagedorn and Hanson, 1951), Wisconsin pea streak virus and I5 pea streak virus from Idaho (Kim and Hagedorn, 1959) and Idaho pea streak virus (Zaumeyer and Patino, 1959). The German authors could clearly distinguish between pea streak virus and red clover vein mosaic virus on the basis of particle lengths of 619 and 654 nm., respectively. (Note that their length ratio 619 : 654 equals a calculated ratio of 630 : 667 which corresponds to the differences we found between WPSV and our RCVMV strains). The PO pea streak virus of Kim and Hagedorn (1959) differed in having spherical particles. This virus was later proved to be broad bean vascular wilt virus (Schroeder and Provvidenti, 1970).

Serologically the isolates around 654 nm compared by Wetter et al. (1962) were closely related and the same held for those around 619 nm (Wisconsin, I5 and Idaho pea streak viruses). Between viruses 654 and 619 nm long there was a distant serological relationship only. The serological differences we have now found between the three strains of RCVMV and WPSV are of the same degree as those found by Wetter et al. (1962). The latter authors found clear symptom differences between PSV and RCVMV in a few hosts only. In this respect, strains of RCVMV found later, such as P42 of Zaumeyer et al. (1964) and our E207, may even differ to a greater extent. *Thus, here electron microscopy and serology are more important than symptomatology to distinguish the two viruses.* Our results further corroborate the differentiation between PSV and RCVMV. For the RK31 and P42 strains of RCVMV, light microscopy of crystalline inclusions (see also Bos and Rubio-Huertos, in preparation), and for the E207 strain of RCVMV and for PSV the checking of crude sap with the electron microscope seem to be the most reliable and rapid diagnostic tests.

Within the pea streak virus also various strains may exist. Kim and Hagedorn (1959) found the I5 strain to differ considerably from the Wisconsin pea streak virus in ageing in vitro (4–7 and 50–60 days, respectively), whereas the first was aphid-transmitted and the second not. Serologically they were closely related. Those authors found an MS pea streak virus from Minnesota to differ in many ways such as serology, although the elongate particles seemed of comparable size. The relationships of these 'true' pea streak viruses to the original pea streak virus 1 of Zaumeyer (1938) can not be determined because the original virus is no more available (see Wetter and

Quantz, 1958), and the same holds for the New Zealand pea streak virus of Chamberlain (1939).

The discovery of the new pea latent strain of RCVMV remains of considerable practical importance. Sofar no immunity was found in pea (30 cultivars tested). The virus occurs in extremely high concentrations even when symptomless. Like with potato virus M such a latent virus can be easily distributed widely unnoticed, although with a non-vegetatively propagated crop and possibly inefficient insect vectors chances may be less than in e.g. potatoes. Lack of host plants reacting with local or other characteristic symptoms will make discovery highly improbable when testing field-grown plants with indicator plants. As with potato viruses S and M (de Bokx, 1969) and chrysanthenum virus B (Hakkaart, 1969) electron microscopy seems to be the most reliable way of testing for infection.

Such more or less latent viruses, when occurring in complex with other viruses, may cause severe syndromes. Examples are RCVMV plus bean yellow mosaic virus inducing severe streak in peas (Schroeder et al., 1959), and lily symptomless virus plus a member of the potato virus Y group together causing severe necrosis ('bruinkringerigheid') in lilies (C. J. Asjes, personal communication).

In many respects the E207 strain of RCVMV can act as a characteristic model of the potato virus S group.

Samenvatting

Een biologisch sterk afwijkende stam van het nerfmozaïekvirus van rode klaver, die doorgaans latent voorkomt in erwt, en zijn verschil met het erwtestrepenvirus

Uit erwteplanten met oppervlakkige stengelnecrose (Fig. 1) werd een gemakkelijk met sap overgaand virus (code E207) geïsoleerd dat alle 30 getoetste erwterassen kon aantasten en daarin dan in hoge concentratie maar meestal latent voorkwam.

Het onderzoek werd bemoeilijkt door het ontbreken van een betrouwbare lokalelesie-toetsplant.

Slechts 18 van de 32 getoetste plantesoorten bleken vatbaar te zijn (Tabel 1); uit slechts 7 daarvan kon het virus in de niet-geïnoculeerde bladeren worden aangetoond, echter in de meeste gevallen slechts zo nu en dan. Slechts enkele erwterassen, w.o. 'Koroza', reageerden met systemische verschijnselen (Fig. 1 en 2) en soms ook *Trifolium incarnatum*, *T. repens* en *Vicia faba*. Zelden ontstonden lokale symptomen in enkele *Chenopodium*-soorten, *Phaseolus vulgaris*, *Tetragonia expansa* en *Vicia faba*. Vrij vaak, maar onbetrouwbaar werden lokale lesies gevormd in *Chenopodium album*, *C. amaranticolor*, *C. quinoa* (Fig. 6) en *Gomphrena globosa*.

Het virus werd in verschillende opzichten vergeleken met 2 stammen (RK31 en P42) van het nerfmozaïekvirus van rode klaver (RCVMV) (Fig. 3, Tabel 2) en met Wisconsin pea streak vi us (WPSV, Fig. 4), de twee op vlinderbloemigen voorkomende vertegenwoordigers uit de aardappelvirus-S-groep. Erwten reageerden differentiërend op de vier virusisolaten (Fig. 1, 3 en 4). *Vicia faba* 'Compacta' vormde lokale lesies met RK31 en P42, en reeds na enkele dagen met WPSV (Fig. 5: A, B, C) en vertoonde daarna systemische symptomen (Fig. 5: D, E, F).

Het virus werd geïnactiveerd in uitgeperst sap bij bewaring tussen 3–5 dagen, bij

warmtebehandeling tussen 60 en 65 °C, bij verdunning tussen 1000 en 10.000. Hierin komt het overeen met de meeste leden van de aardappelvirus-S-groep.

Overdracht door *Acyrthosiphon pisum* en *Aphis fabae* op non-persistente wijze leek weinig efficiënt.

E207 beschermde erwteplanten nagenoeg volledig tegen de beide RCVMV-stammen maar niet tegen WPSV. RK31 beschermde voorts in zekere mate tegen P42 en er was slechts een zeer zwakke onderlinge bescherming tussen RK31 en P42 enerzijds en WPSV anderzijds (Fig. 7).

Zuivering vond in het algemeen plaats d.m.v. klaring met ether en tetrachloorkoolstof gevolgd door differentiële centrifugering en centrifugeren in een suikergradiënt in een zone-rotor (Fig. 9). Het nieuwe erwtevirus leverde fraaie preparaten op (Fig. 8). In de analytische ultracentrifuge werden de volgende sedimentatie-coëfficienten bepaald: E207, 156; RCVMV-RK31, 159; RCVMV-P42, 163; WPSV, 160.

Bereide antisera hadden een titer van respectievelijk 4096 (E207), 1024 (RCVMV-RK31) en 4096 (WPSV). Met deze antisera werd een vrijwel volledige serologische gelijkheid van E207 en de beide stammen van RCVMV aangetoond, terwijl het duidelijk verschilde van WPSV, aardappelvirus S (PVS) en chrysantevirus B (CVB) (Tabel 4).

Elektronenmicroscopisch zijn E207 en WPSV beide in ruw sap gemakkelijk aantoonbaar (Fig. 10) en komen in zeer hoge concentraties voor, RK31 en P42 echter in geringe hoeveelheden. E207 en beide RCVMV-vormen verschillen niet duidelijk in lengte (gemiddeld 670 nm), terwijl WPSV aanzienlijk korter is (ongeveer 630 nm) (Tabel 5). Het laatste kan zelfs in mengpreparaten gemakkelijk worden onderscheiden (Fig. 11).

Op grond van de verkregen gegevens (Tabel 6), vooral van premunitie, serologie en deeltjeslengte, moet worden geconcludeerd dat E207 een, weliswaar zeer afwijkende, stam van het nerfmozaïekvirus van rode klaver is.

In de literatuur bestaat er nog steeds veel verwarring rond de relatie nerfmozaïekvirus van rode klaver en erwtestrepenvirus ("pea streak virus"). De hier verkregen resultaten dragen bij tot een verdere differentiatie tussen de twee virussen.

Potentieel betekent de nieuwe stam van het nerfmozaïekvirus in rode klaver een gevaar door zijn latente wijze van voorkomen en het de aandacht ontsnappen bij het op de conventionele wijze toetsen van erwtemonsters met indicatorplanten. Elektronenmicroscopie is hier een zeer waardevolle toetsmethode.

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