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The identification of three new viruses isolated from Wisteria and Pisum in The Netherlands, and the problem of variation within the potato virus Y group

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Abstract

Three new legume diseases in The Netherlands are described: *Wisteria* vein mosaic, pea necrosis, and pea leafroll mosaic. In particle size and morphology and in host reaction the virus isolates resembled bean yellow mosaic virus (BYMV), but they were readily distinguishable in several test plants.

In recent years several new legume viruses related to BYMV and bean common mosaic virus have been described. Besides, more and more viruses of the potato virus Y group are proving to be naturally infectious to legumes, e.g. lettuce mosaic virus, beet mosaic virus, watermelon mosaic virus, and even turnip mosaic virus, all of which are somehow related to BYMV. To investigate the nature and degree of these relationships, the virus isolates causing the three new legume diseases were compared with a normal strain of BYMV and with pea mosaic virus, clover yellow vein virus, cowpea aphid-borne mosaic virus, two isolates of beet mosaic virus, and lettuce mosaic virus.

They were all found to have several hosts and symptoms in common. The differences observed showed a range of gradations only. Unexpectedly, BYMV was found to infect 17 out of 20 non-legumes tested. The *Wisteria* isolate and lettuce mosaic virus did not produce inclusion bodies, whereas all others did. Often nucleoli were very much enlarged or contained crystals. The pea necrosis isolate produced many nucleolar crystalline needles.

Cross-protection tests were of little help in determining mutual relationships.

Antisera prepared against the *Wisteria* isolate, the pea necrosis isolate, and BYMV, and an antiserum against bean common mosaic virus, revealed definite relationships, but also substantial differences.

By using the electron microscope the three new isolates were indistinguishable from BYMV, whereas the particle lengths of two isolates of beet mosaic virus were considerably shorter. The isolate of pea mosaic virus had much longer (840 mμ) and more rigid particles.

Thus, the more that known viruses are studied in detail, and the more new viruses and strains are described, the more borderlines supposed to exist between the different viruses of a morphological group disappear. The fading away of biological borderlines as described here, throws new light on the intergrading serological relationships between viruses of a morphological group as reported in the literature. Thus, extreme variation of viruses may make it impossible to define a species concept for viruses. Borderlines have to be drawn arbitrarily. The incitants of *Wisteria* vein mosaic, pea necrosis, and pea leafroll mosaic are here considered different viruses, although closely related to bean yellow mosaic and bean common mosaic viruses.

Introduction

In continuation of the author's work on legume viruses in The Netherlands investigations were made on some newly discovered diseases, viz. a vein mosaic of *Wisteria floribunda*, a severe necrosis of *Pisum sativum*, and a peculiar leaf narrowing or leafroll of the same host.

Table 1. Recently described viruses closely related to bean yellow mosaic virus (BYMV) and bean common mosaic virus (BCMV), listed chronologically.

Bean western mosaic virus	Skotland and Burke (1961)
Red clover necrosis virus	Zaumeyer and Goth (1963)
Passion fruit woodiness virus	Taylor and Kimble (1964)
	Teakle and Wildermuth (1967)
Cowpea aphid-borne mosaic virus	Brandes (1964)
	Lovisolo and Conti (1966)
Clover yellow vein virus	Hollings and Nariani (1965)
	Gibbs et al. (1966b)
Pea leafroll virus	Musil (1966)
	Kvičala and Musil (1967)
Pea seed-borne mosaic virus	Inouye (1967)
Lupin mottle virus	Hull (1968)
Peanut mottle virus	Kuhn (1965)
	Schmidt and Schmelzer (1966)

Tabel 1. Onlangs beschreven virussen die nauw verwant zijn aan bonescherpmozaïekvirus en bonerolmozaïekvirus, chronologisch gerangschikt.

When diagnosis of these diseases was attempted, it was soon found that all three inciting viruses had elongated particles approximately 750 mμ long, as well as several other features in common with bean yellow mosaic virus. However, they differed appreciably in some respects from bean yellow mosaic virus as described in the literature, especially in their ability to infect various non-legumes.

In the last decade several virus isolates differing in a similar degree from bean yellow mosaic virus (BYMV) and bean common mosaic virus (BCMV), or having an intermediate position, have been described as separate viruses (Table 1). In addition, an increasing number of other viruses of the potato virus Y group with flexuous threads of the same or nearly the same size have been found infectious to legume crops under natural conditions: lettuce mosaic virus (Ainsworth and Ogilvie, 1939), beet mosaic virus (Quantz, 1958), and watermelon mosaic virus (Inouye, 1964) all three infecting pea, and turnip mosaic virus (Inouye and Inouye, 1964) infecting peanut.

All these viruses are now known to be related serologically and in several other respects, but the nature and degree of these relationships are still rather obscure. This leads to complications in the identification of the viruses concerned, especially of supposed "new viruses" or deviating strains of known viruses.

Basically, we are dealing with the problem of variation of plant viruses and its bearing on classification and identification, which is of considerable scientific as well as practical importance.

This paper describes the three new legume diseases and their incitants with a view to illustrating the problem of virus variation, and with reference to the literature, to arriving at a conclusion pertaining to the identity (= individuality) of the three incitants.

Description of the three new diseases

Wisteria vein mosaic. In some places in The Netherlands plants of *Wisteria floribunda* and *W. sinensis* show a distinct variegation. The symptoms vary from an irregularly

Fig. 1. Leaves of *Wisteria floribunda* with symptoms of natural infection by *Wisteria* vein mosaic virus (Wis 2B).

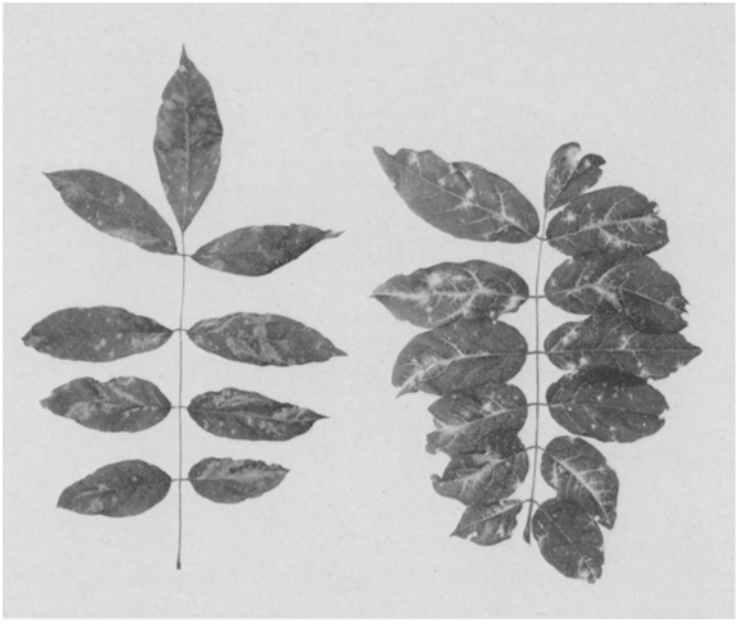

Fig. 1. Bladeren van Wisteria floribunda met symptomen van natuurlijke infectie met het nerfmozaïekvirus van Wisteria (Wis 2B).

Fig. 2. *Pisum sativum* 'Koroza', 14 (left) and 24 days (right) after inoculation with pea necrosis virus (E 178).

Fig. 2. Pisum sativum 'Koroza' 14 (links) en 24 dagen (rechts) na inoculatie met het erwtenecrosevirus (E 178).

distributed bright yellowing or clearing of the finer veins to a diffuse flecking or mottling. The vein yellowing often starts from the main veins and occurs in small asteroid spots or in ring and line patterns (Fig. 1). Growth reduction in discoloured areas often leads to leaf malformation. Plants do not seem to suffer from infection.

Since 1965 from six out of ten diseased *W. floribunda* plants and from three out of five *W. sinensis* plants virus isolates could be easily transmitted mechanically to a number of herbaceous hosts. The isolates did not seem to differ and the oldest (Wis 2B) was studied in detail.

Somewhat similar symptoms have been described in the literature from the U.S.A. (Brierley and Lorentz, 1957) and from Italy (Lovisolo, 1960, 1968; de Beni, 1964) without identification of their causes. Since 1966 the incitant of one such disease has been studied by Conti and Lovisolo (personal communication, Lovisolo, 1968, and Conti and Lovisolo, 1970) in Italy, and their isolate (Wis Ital) has been included in my investigations. In the Botanical Garden, Průhonice near Prague, Czechoslovakia, in 1967 I observed exactly the same symptoms. Thus the disease seems to be quite widespread.

Pea necrosis. Another virus (coded E 178) was isolated in 1964 from a single pea plant of a breeding line. The plant, obtained from Mr N. Hubbeling, showed a regular greyish stem discoloration and some leaf chlorosis. After mechanical transmission several pea varieties reacted in a similar way, usually dying prematurely (Fig. 2). Several such necrosis diseases have been recorded under the somewhat confusing name "pea streak", Symptomatologically, the majority are indistinguishable, but some eight different viruses with distinct epidemiologies have been reported as their incitants. The new necrosis disease was soon found to differ from all eight (Bos, 1969b) and to be characterized by most unusual inclusion bodies (Bos, 1969a; Bos and Rubio-Huertos, 1969).

Pea leafroll mosaic. The third disease was first observed by Messrs Hubbeling and Huyberts in the pea varieties 'Wyola', 'Olympic', and a Dutch breeding line in field trials. The symptoms consist of leaf narrowing and downward rolling, sometimes accompanied by a faint mosaic or mottling (Fig. 3). Such plants are somewhat reduced in size. Plants grown in the greenhouse by Hubbeling and Huyberts (unpublished) from the same seed as that used in the field also turned out to contain the virus. The virus isolate (designated E 210) may be related to two viruses described independently and incompletely under the confusing name pea leafroll virus in Czechoslovakia (Musil, 1966) and as pea seed-borne mosaic virus in Japan (Inouye, 1967).

Materials and methods

The virus isolates just described as well as some other viruses and isolates included in this study are listed in Table 2 together with some data on their origin.

The viruses were maintained in plants in the greenhouse at about 20 °C. For safety the isolates were also stored in a cold room in infected leaf material desiccated over calcium chloride to prevent mutation or contamination For purification and for most other tests the viruses were multiplied in 'Koroza' peas or in *Nicotiana clevelandii*. Biological properties were studied as outlined by Bos et al. (1960). Details on the techniques applied are given in the sections concerned.

Fig. 3. *Pisum sativum* 'Koroza' 1 month after inoculation with pea leafroll mosaic virus (E 210); right, healthy control.

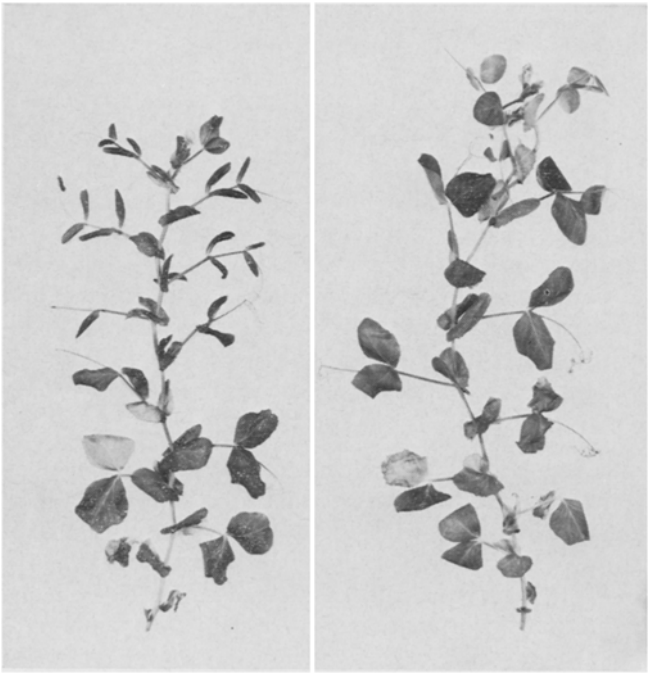

Fig. 3. Pisum sativum 'Koroza' 1 maand na inoculatie met het erwterolmozaïekvirus (E 210); rechts gezonde controle.

Identification of the new isolates

The incitants of the new diseases, viz. Wis 2B, E 178, and E 210, were easily transmitted through sap, and several non-legumes were infected. Very striking was the local susceptibility of cucumber cotyledons to E 178. In particle size and morphology the three isolates were soon found to resemble BYMV in form and size. They were therefore carefully compared with a typical strain of BYMV (BYMV B 25) and pea mosaic virus obtained from peas (PMV E 198) another virus closely related to BYMV (cf. Bos, 1964), as well as with some other viruses of the potato virus Y group listed under materials and methods and known to be able to infect legumes.

Host range and symptoms

To investigate the host reactions of most of the smaller plant species, such as peas, eight plants contained in two pots, and of the larger plants, two or three planted separately, were inoculated. Back inoculations were made with average samples of inoculated and non-inoculated leaves separately. Two or three plants of *Chenopodium amaranticolor* or *C. quinoa* and sometimes four or eight pea plants were used as indicator hosts. Several species were tested twice or more times.

Results of host range studies are assembled in Table 3. Symptoms will be summarized and illustrated here only so far as they are of differential meaning. Even though such

Table 2. Viruses and virus isolates involved in these investigations.

Code	Virus or isolate	Isolate provided by
Wis 2B	isolate from *Wisteria* to be identified (disease here described as *Wisteria* vein mosaic)	present author
E 178	isolate from necrotic peas to be identified (disease here described as pea necrosis)	present author
E 210	isolate from peas with leaf narrowing and leafroll to be identified (disease here described as pea leafroll mosaic)	N. Hubbeling, Institute of Phytopathological Research, Wageningen
BYMV B 25	bean yellow mosaic virus, normal strain from bean	present author
PMV E 198	bean yellow mosaic virus, yellow pea mosaic strain from pea	N. Hubbeling
CAMV	cowpea aphid-borne mosaic virus, type strain (Lovisolo and Conti, 1966)	O. Lovisolo, Laboratorio di Fitovirologia applicata del CNR, Torino, Italy
ClYVV	clover yellow vein virus, type strain (Hollings and Nariani, 1965)	M. Hollings, Glasshouse Crops Research Institute, Littlehampton, England
Wis Ital	undescribed virus from *Wisteria floribunda*	O. Lovisolo
BMV-SVP	beet mosaic virus from pea	N. Hubbeling
BMV-IPO	beet mosaic virus from pea	present author
LMV	lettuce mosaic virus from lettuce	A. C. van der Giessen, Institute for Horticultural Plant Breeding, Wageningen

Tabel 2. Bij dit onderzoek betrokken virussen en virusisolaten.

differential host reactions greatly depend on conditions, they remain of considerable importance in virus identification as will be further outlined in the discussion. Therefore some detail is inevitable here.

Since all isolates were able to infect French beans and peas, some have been tested on a number of varieties of these hosts (Table 4 and 5).

Phaseolus vulgaris, the dwarf French bean variety 'Bataaf', was susceptible to all virus isolates tested but BMV-IPO. With several isolates infection remained local, however. In bean varietal tests infection spectra were found to differ (Table 4).

In most bean varieties BYMV B 25 produced epinasty of inoculated primary leaves and chlorotic or necrotic local lesions often extending into the venation (Fig. 4, above, right). This symptom was followed by systemic mottle or mosaic, leaf curling or malformation and often by stunting (Fig. 4 below, right), and mottle and malformation of pods. In some varieties systemic leaf deformations were strikingly reminescent of infection by bean common mosaic virus (BCMV) (Fig. 5). With PMV E 198 local lesions were chlorotic (in 'Topcrop') or necrotic (in 'Pinto') or mainly showed up as green rings in yellowing leaves (in 'Bataaf'). Systemic symptoms were very mild and consisted of a yellowish stippling in most varieties or a similar stippling plus faint mottling in 'Pinto', whereas in 'Bataaf', 'Flits', and 'Imuna' infection remained local only, or were severe and consisted of systemic necrosis in 'Dubbele Witte'.

With E 178 beans exhibited severe reactions, local lesions usually being necrotic, mostly extending into the veins (Fig. 4 above, middle). Top leaves later showed curling, rugosity, yellowing, or a fine mosaic with systemic chlorotic or necrotic lesions or irregular veinal necrosis, usually followed by defoliation and premature death (Fig. 4 below, middle). Pods, if formed, commonly were mottled.

Wis 2B infected all bean varieties tested. Small necrotic local lesions were formed in 'Bataaf' and 'Beka' only (Fig. 4 above, left). Non-inoculated trifoliate leaves showed a very faint mottling or an inconspicuous chlorotic flecking or stippling (Fig. 4 below, left).

Table 3. Survey of results of host range studies (for explanation of symbols see bottom of table).

	Wis 2B	E 178	E 210	BYMV B 25	PMV E 198	ClYVV	CAMV	Wis Ital	BMV-SVP	BMV-IPO	LMV
Legumes											
Cassia tora	– –*	– –		–*–*							
Crotalaria spectabilis	S	L S		L S							
Lathyrus odoratus	L S	S*		s	l S						
Phaseolus vulgaris 'Bataaf'	L S	L S	l –	L S	(L) –	L S	L S	L S	L –	–*–*	(1) –
Pisum sativum 'Koroza'	S	S	S	S	S	S	S	S	S	L S	S
Trifolium incarnatum	l S	S*	– –	S*	S	–*–	S	l –*	– –	(1) –*	– –
T. pratense	– –	– (S)		S	–	–*–*	–	– –*	– –	– –*	– –
T. repens	– –	S	– –	–*–	–	S	S	– –*	– –	– –*	– –
Vicia faba 'Driemaal wit'	L S	L*S	S	S	S	S	S	L S	L S	– –*	– –
V. sativa	S	S*		S*	S						
Vigna sinensis 'Black'	– –*	– –		l S	– –						
'Black eye'	-	l –		l S	– –						
Legumes species tested/species susceptible	7/12	9/12	3/5	10/12	3/3	4/6	5/6	4/6	3/6	2/6	2/6
Non-legumes											
Beta vulgaris	– –*	– –*	– –*	– –*		– –*	– –*	– –*	l S	l S	– –*
Callistephus chinensis	l s	l/L –		l –							
Chenopodium album	L S	L –	L ?	L S		L –	L –	L –	L S	L S	L S
C. amaranticolor	L –	L –	L –	L S	L –*	L –	L –	L –	L*S*	L S*	L S
C. quinoa	L S	L S	L S	L –		L (S)	L –	L S	L*S*	L S*	L S
Cucumis sativus 'Gele tros'	– –*	L –	– –*	– –*	– –*	– –*	– –*	– –*	– –*	– –*	– –*
Cucurbita pepo	– –*	–*–*		–*–*	–*–*						
Datura stramonium	–*–*	– –*		– –*							
Gomphrena globosa	L –	L –	l s	L –	L –	(L)	L –	l –	L –	l/L(+)	L –
Nicotiana clevelandii	l S	L S	L S	L S	l S*	l S	l S	l S	– S	l S	l S
N. debneyi	(1) –	L –		l –	l –	–	L –				
N. glutinosa	– –	l –	– –*	l –		(1) –	l –	– –*	– –*	– –*	– –*
N. rustica	– –	l –	– –*	l –		– –*	l s	– –*	l –	– –	–
N. tabacum 'White Burley'	L/l –	L –	– –*	L –	l –*	L –	L*–*	l –	– –*	– –*	– –*
'Samsun'	l –	L ?		l –	L –*						

Petunia hybrida	L –	– –	– –*	L –	– –*	l –	L S	l –	– –	– –	
Physalis floridana	– –	– –	l –	l –	– –*	– –*	l S	– –*	– –	– –*	– –*
Spinacia oleracea 'Noorman'	l –	L s	l –	L s	l s	l –*	l S	+ –*			l s
Tetragonia expansa	L –	L –	L –	L –	l –*	L –	L –	+ –*	(+) –	+ –	l s
Zinnia elegans	l –	l –	– –	l –							
Non-legume species tested/ species susceptible	13/12	15/20	8/15	17/20	8/12	10/15	13/15	9/15	9/13	7/13	7/13

Explanation of symbols: l = latent local infection; s = latent systemic infection; L = visible local infection; S = visible systemic infection; – = no infection as tested by back inoculation; –* = no symptoms, no check by back inoculation; + = infection as proved by back inoculation, but no clear symptoms; () symbol in parentheses = in other case(s) no infection.

Tabel 3. Overzicht van de resultaten van het waardplantonderzoek.

Table 4. Bean varietal reaction (for explanation of symbols see Table 3).

Bean varieties	Virus isolates						
	Wis 2B	E 178	E 210	BYMV B25	PMV E 198	BMV-SVP	LMV
Bataaf	L S	L S	l –	L S	L –	L*–	l
Beka	L ?	L S	l S/s	L S		L –	l –
Dubbele Witte Zonder draad	– –					– –	
	S*	L*S*	– –	L*S*	L*S*	– –	l –
Flits	S*	L*S*	– –	L*S*	L –	–*–	– –
Imuna	– –	–*–*	l –	S*	L –	L –	– –
Pinto	– –	L S	l –	L S*	L S	– –	L –
Prelude	S*	S*	L*–	L*S*	L S	– –	
Processor	S*	L*S*	– –	S*	L*S*	L –	–*–
Sanilac	L*S*	L*S*	–*–	–*	L*S*	–*–	L –
Topcrop	– –	L S	–	L S	L S	l –	
Troef	S*	L*S*	–*–	L*S*	L*S*	L –	– –
Widusa	S*	L*S*	– –	L*S*	L*S*	L*–	– –

Tabel 4. Reactie van bonerassen.

French beans as a whole were strikingly resistent to E 210. ClYVV in 'Bataaf' is mainly characterized by some rugosity and systemic leaf curling and vein chlorosis followed by mottling and sometimes some stem necrosis.

CAMV evoked many necrotic local lesions in 'Bataaf', later radiating into the venation and followed by some systemic veinal necrosis. Wis. Ital had a similar effect but systemic veinal necrosis led to extensive patterns of necrotic veins in yellow leaf areas.

With LMV a few inconspicuous etched local rings have bean observed in 'Sanilac'. In 'Pinto' there was some local dark green coloration along the veins.

BMV-SVP produced a local infection in 6 out of 10 varieties tested (Table 4). A varying number of discrete necrotic local lesions were formed.

Table 5. Pea varietal reaction (for explanation of symbols see Table 3).

Pea varieties	Virus isolates						
	Wis 2B	E 178	E 210	BYMV B 25	PMV E 198	BMV-SVP	LMV
Big Ben	S*	S*	S*	S*	S*	S	l S
Early Perfection	–	– –	S*	– –	– –	S*	l s
Koroza	S	S	S	S	– S	L*S*	l S
New Era	s	– –	–	– –	– –	L S	l S/s
New Line Perfection	– –	–	–	–	– –	– –	
New Season	S	S		S	– S	– s	l S
Perfected Wales	– –	– –	–	– –	– –	L S	l s
Victory Freezer	S*	S*	S*	S*	– S*	S*	
Vitalis	S	S	S*	S*	– S*	S*	
Wisconsin Perfection	s	– –	– –	– –	– –	S*	l s

Tabel 5. Reactie van erwterassen.

Fig. 4. *Phaseolus vulgaris* 'Beka' (above) and 'Bataaf' (below) 18 and 24 days, respectively, after inoculation with *Wisteria* vein mosaïc virus (Wis 2B, left), pea necrosis virus (E 178, middle), and bean yellow mosaic virus (B 25, right).

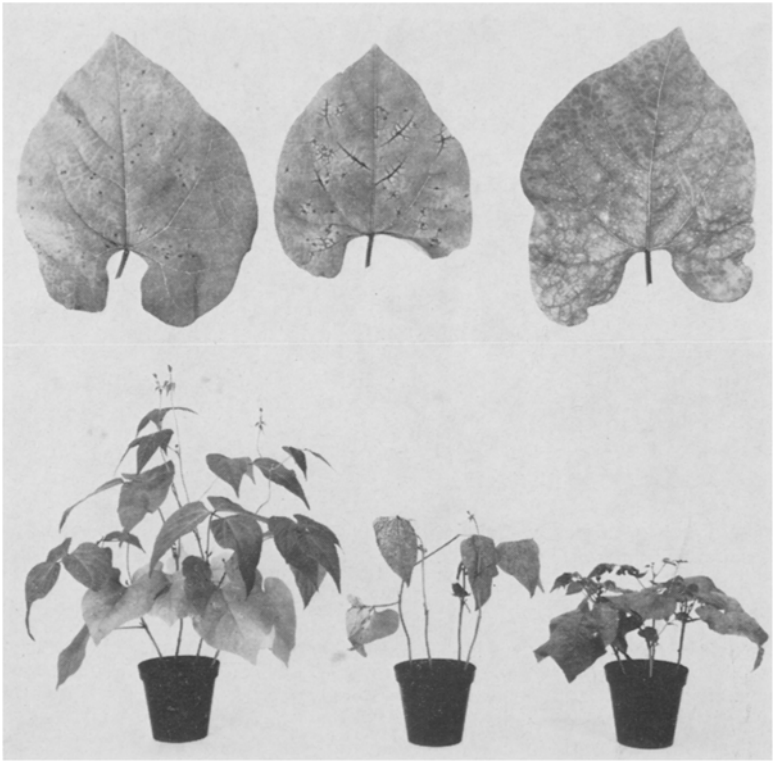

Fig. 4. Phaseolus vulgaris 'Beka' (boven) en 'Bataaf' (onder) respectievelijk 18 en 24 dagen na inoculatie met het nerfmozaïekvirus van Wisteria (Wis 2B, links), erwtenecrosevirus (E 178, midden) en bonescherpmozaïekvirus (B 25, rechts).

Pea, *Pisum sativum* 'Koroza', reacted to all virus isolates. With BYMV B 25 and PMV E 198 an ephemeral vein chlorosis started 7–9 days after inoculation and was soon followed by a mosaic in top leaves, which was greenish with B 25 and bright an yellow with E 198 (Fig. 6 B, C).

Wis 2B produced a weak vein chlorosis 11–14 days after inoculation followed by a faint interveinal chlorotic spotting or mottling (Fig. 6 A). Sometimes some stem necrosis was involved. 'New Era' and 'Wisconsin Perfection', immune to BYMV B 25, E 178, and E 210, were found to be susceptible (Table 5). In 'Koroza' E 178 caused much more drastic reactions (Fig. 2) starting with veinal chlorosis in 6–10 days. This was 1–2 weeks later followed by withering of the inoculated leaves, curling of the top leaves, greyish brown stem discoloration and premature death of the plants. The spectrum of susceptible varieties was similar to that of BYMV B 25 (Table 5).

E 210 incited a faint vein chlorosis in 'Koroza' and 'Rondo' about 1 week after inoculation. It was 10–14 days later followed by a very striking leaf rolling and leaf narrowing (Fig. 3) accompanied by a very inconspicuous mosaic. Its varietal spectrum was somewhat similar to that of E 178 and B 25 although symptoms were also produced in 'Early Perfection'. Peculiarly enough all pea varieties were infected with another isolate of the virus (isolate 6717 from Mr Hubbeling) later included in our experiments.

Fig. 5. *Phaseolus vulgaris*, from left to right 'Bataaf', 'Pinto', and 'Topcrop', 8 weeks after inoculation with bean yellow mosaic virus (B25).

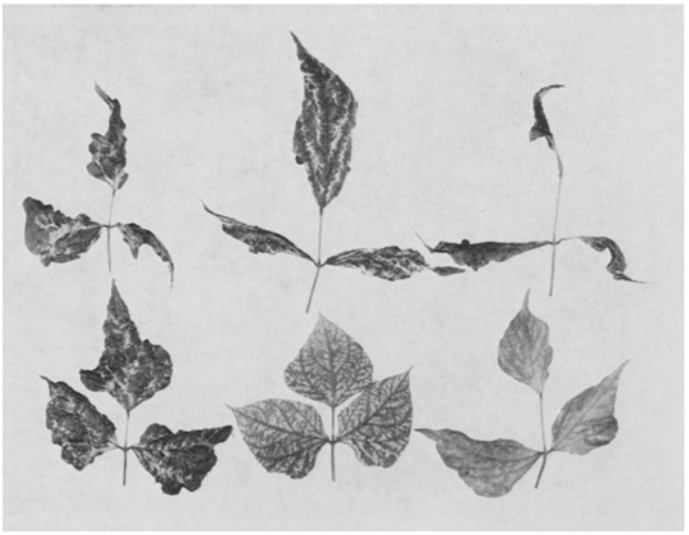

Fig. 5. Phaseolus vulgaris, v.l.n.r. 'Bataaf', 'Pinto' en 'Topcrop', met rolmozaïekverschijnselen 8 weken na inoculatie met bonescherpmozaïekvirus (B25).

Symptoms in 'Koroza' produced by ClYVV did not differ appreciably from those of E 178 although Hollings and Nariani (1965) reported *P. sativum* not to be infected. Plants died within a month. 'Victory Freezer' reacted similarly and 'Early Perfection' and 'Wisconsin Perfection' turned out to be immune.

With CAMV mottling and some wilting occurred in 'Koroza' and 'Victory Freezer'. This is in contrast to results of Lovisolo and Conti (1966) finding *P. sativum* to become latently infected, but this latency was found only in 'Laxton' (Lovisolo, personal communication).

Wis. Ital. induced vein chlorosis, mosaic, some stem necrosis and premature death in 'Koroza' and 'Victory Freezer'. 'Eearly Perfection' and 'Wisconsin Perfection' were immune.

Of 'Koroza' peas inoculated with BMV-IPO only few plants were infected. Sometimes veinal necrosis and wilting of inoculated leaves appeared in about eight days. In another 10 days necrotic stem streaking, top necrosis and later a necrotic spotting of pods followed. BMV-SVP was considerably more infectious and virulent to peas. In 'Koroza' in 10 days many small but later enlarging necrotic local lesions developed followed by wilting and premature death of the plants in 10 more days. This isolate easily infected the other varieties of Table 5 also. Here, initial irregular vein chlorosis and curling in top leaves preceded necrosis of veins and stems and premature death.

With LMV a very faint mottling was produced in 'Koroza' (Fig. 6D). In other varieties symptoms were very mild or absent although all varieties tested contained much virus.

Broad bean, *Vicia faba* 'Driemaal Wit', was susceptible to all isolates but BMV-IPO and LMV. Symptoms were highly diagnostic (Fig. 7). Both BYMV B 25 and PMV E 198 produced vein chlorosis in about 8 days, followed by a sharp-cut mosaic which was very bright for E 198. Symptoms of E 210 resembled those in pea and mainly consisted of leaf rolling and narrowing. These could be very pronounced and were usually accompanied by a mild mosaic.

All other isolates reacted in a completely different way. Wis 2 B produced chlorotic to dark-centered ring-like local lesions, in 1–2 weeks. The rings were especially prominent in yellowing leaves. Top leaves showed diffuse chlorotic spots which were often scarce. No growth reduction was evident.

With E 178 the first symptoms consisted of many necrotic local lesions 1–2 mm in diameter, in ageing leaves contrasting by a greenish colour. Systemic symptoms started with a slight speckling or yellow mottling soon followed by wilting and death of top leaves and necrotic stem streaking in 2–3

Fig. 6. *Pisum sativum* 'Koroza' with systemic symptoms 3 weeks after inoculation with Wis 2B (A), BYMV B 25 (B), PMV E 198 (C), and LMV (D).

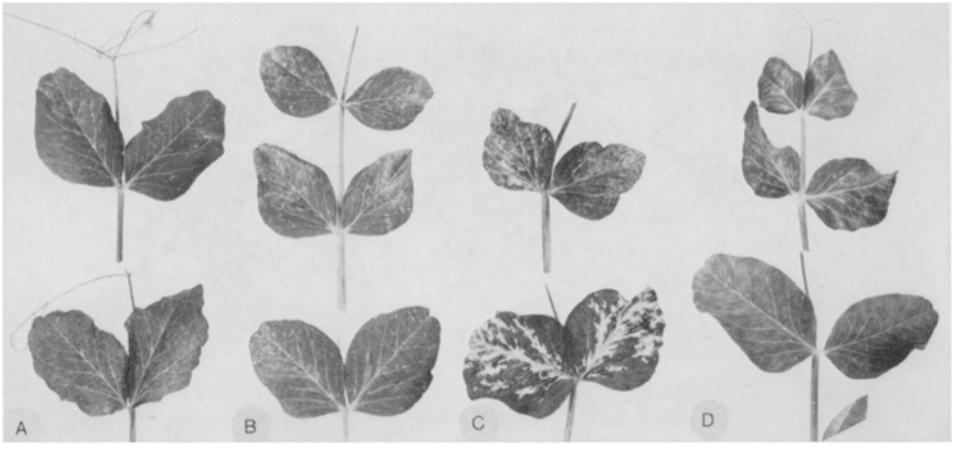

Fig. 6. Pisum sativum 'Koroza' met systemische symptomen drie weken na inoculatie met Wis 2B (A), BYMV B 25 (B), PMV E 198 (C) en LMV (D).

Fig. 7. *Vicia faba* 'Driemaal Wit' with systemic symptoms of Wis 2B (A), E 210 (B), PMV E 198 (C), and BMV-SVP (D), 6. 4, 3, and 4 weeks after inoculation, respectively.

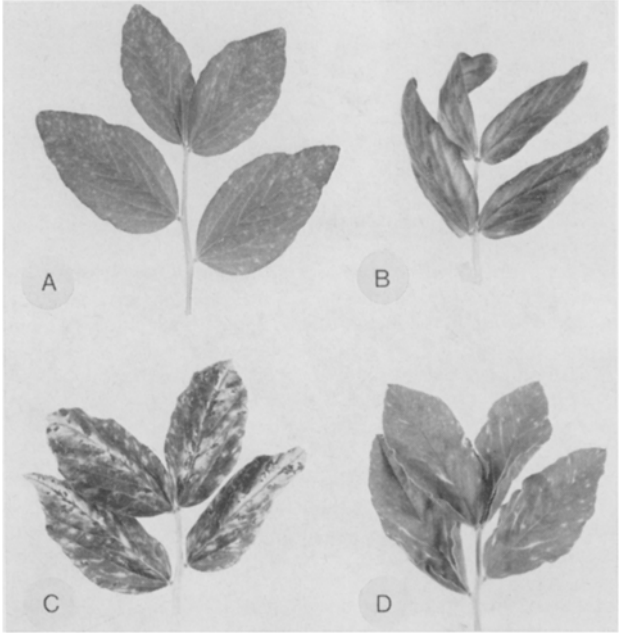

Fig. 7. Vicia faba 'Driemaal Wit' met systemische symptomen van Wis 2B (A), E 210 (B), PMV E 198 (C) en BMV-SVP (D), respectievelijk 6, 4, 3 en 4 weken na inoculatie.

Fig. 8. *Vicia faba* 'Driemaal Wit', 12 (left) and 30 days (right) after inoculation with E 178.

Fig. 8. Vicia faba 'Driemaal Wit', 12 (links) en 30 dagen (rechts) na inoculatie met E 178.

weeks after inoculation (Fig. 8). Usually plants died prematurely. Now and then they recovered somewhat with a severe malforming mottling.

ClYVV, though reported by Hollings and Nariani (1965) not to be infectious to broad bean, easily infected our variety, producing necrotic local lesions and a systemic mottle.

CAMV induced large vague concentric chlorotic local lesions and diffuse systemic spots somewhat resembling the effect of Wis 2B.

Symptoms evoked by Wis Ital did not differ appreciably from those of Wis 2B.

With BMV-SVP a low percentage of plants became infected. These showed necrotic local lesions soon causing the younger inoculated leaves to wither, and a few systemic chlorotic to yellow spots leading to some leaf distortion (Fig. 7D).

Beta vulgaris was found to be susceptible to the two isolates of beet mosaic virus only. The same held for BYMV B 25 except in one experiment using *Chenopodium amaranticolor* and spinach as virus sources whereafter a latent local infection could be demonstrated (for details see the section concerned).

Clovers, especially white and red clover, generally were hard to infect. In *Trifolium pratense* BYMV B25 sometimes caused a bright vein mosaic whereas E 178 incited some irregular vein chlorosis from which the plants soon recovered. In *T. repens* with E 178 systemic chlorotic streaks and rings on the leaves were produced, whereas ClYVV easily established infection and produced a rather bright vein mosaic.

Three *Chenopodium* species were susceptible to all isolates tested. Symptoms of some isolates were characteristic but other ones evoked intermediate syndromes.

In *C. amaranticolor* both BYMV B25 (Fig. 9 right) and PMV E 198 produced yellow local lesions at the earliest in 6 days. They usually extended into the venation and were followed by a more or less systemic invasion of the plant, characterized by systemic yellow veinal lesions often leading to leaf curling. Plants frequently recovered later on; then virus could no more be isolated. Inoculated leaves were soon cast.

With Wis. 2B (Fig. 9 left) the first local lesions were produced in 9–13 days. In 1–2 more weeks they turned to yellow flecks. In yellowing leaves they often developed into striking red rings with a desiccated centre. No systemic infection followed. The same held for E 178 (Fig. 9 middle) where lesions were more necrotic and remained smaller.

Fig. 9. *Chenopodium amaranticolor* 20 (above) and 22 days (below) after inoculation with Wis 2B (left), E 178 (middle) and BYMV B 25 (right); above, inoculated leaves.

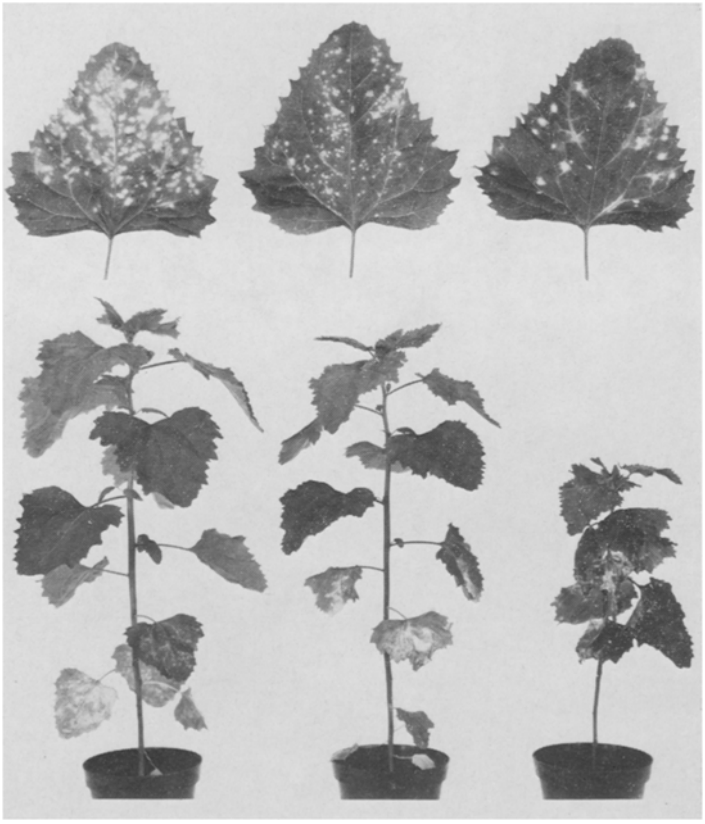

Fig. 9. Chenopodium amaranticolor 20 (boven) en 22 dagen (onder) na inoculatie met Wis 2B (links, E 178 (midden) en BYMV B 25 (rechts); boven, geïnoculeerde bladeren.

E 210 only produced many small chlorotic local lesions with a dry centre.

With CAMV local lesions were remarkably clear and large, in yellowing leaves 2–3 mm in diameter, desiccated and surrounded by a brownish red margin.

Local lesions of ClYVV resembled those of CAMV but were less bright and slightly smaller. With Wis Ital they were vague and resembled those of Wis 2B.

Both isolates of BMV caused tiny local necrotic rings with a dry centre, soon followed by a severe curling of young leaves and plant tops, and by top necrosis.

LMV produced small chlorotic local lesions in 6 days. Later these turned yellow and their centre desiccated. They were then followed by a systemic reaction somewhat resembling BYMV-infection. After a while virus could no more be recovered from top leaves, however.

In *C. quinoa* small chlorotic to yellow local lesions, turning into green rings in yellowing older leaves, were formed by BYMV B25, Wis 2B, E 210, ClYVV, and LMV. Such lesions tended to enlarge. Thus with E 210 in yellowing inoculated leaves green line patterns developed. With E 178, CAMV, and BMV-SVP local lesions were smaller, consisted of necrotic pin-points or small rings with a desiccated centre. With BMV such lesions were sometimes formed in 5 days. Systemic infections followed with most isolates except with BYMV B25 and CAMV. With Wis 2B, E 178, ClYVV, and Wis Ital often systemic chlorotic spots were formed. Plants infected with Wis 2B and E 178 soon recovered from sys-

Fig. 10. *Chenopodium quinoa* with systemic symptoms of E 210 (above) and LMV (below), 19 and 16 days after inoculation, respectively.

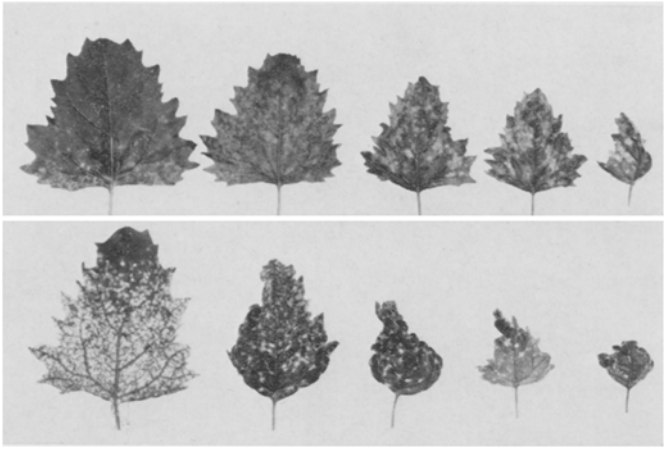

Fig. 10. Chenopodium quinoa met systemische symptomen van E 210 (boven) en LMV (onder) respectievelijk 19 en 16 dagen na inoculatie.

Fig. 11. *Gomphrena globosa* with local lesions: (A) 24 days after inoculation with LMV, (B) 19 days, and (C) 33 days after inoculation with CAMV.

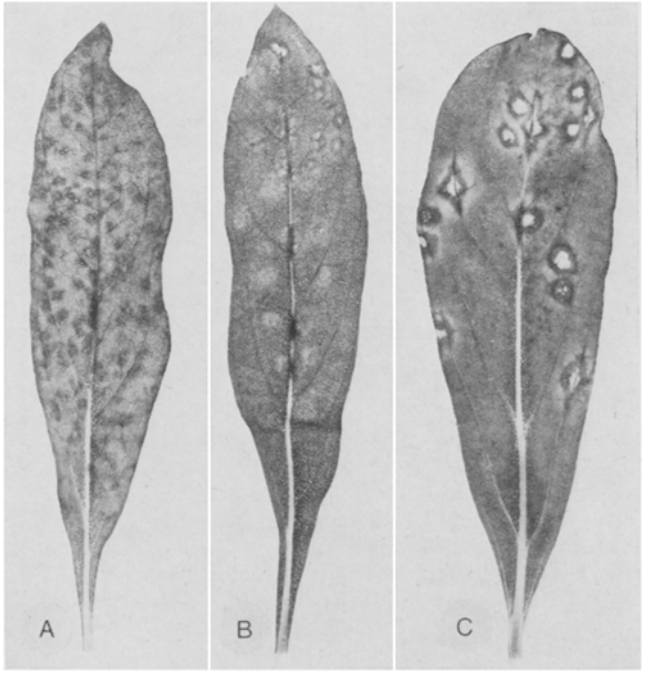

Fig. 11. Gomphrena globosa met lokale lesies: A, 24 dagen na inoculatie met LMV; B, 19 dagen en C, 33 dagen na inoculatie met CAMV.

temic invasion. Then virus could no more be isolated from top leaves. E 210 and LMV incited a severe systemic reaction (Fig. 10). With E 210 systemic symptoms rather consisted of a coarse mottling to mosaic and some leaf deformation whereas with LMV systemic speckling was more pronounced and yellow and leaf malformation was serious. Both isolates of BMV induced a severe yellowing and curling of top leaves and subsequent necrosis and curving of stem tops as early as 10 days after inoculation, usually followed by early plant death. Sometimes necrotic tops were overgrown by axial sprouts.

In cucumber, *Cucumis sativus* 'Gele Tros', infection was obtained several times with E 178 only. Pin-point chlorotic local lesions were formed in cotyledons in about 1 week, their diameter soon increasing to 2 mm (see Fig. 13B). From such cotyledons pea, broad bean, and *C. amaranticolor* plants were easily infected. Non-inoculated foliage leaves did not contain the virus.

Gomphrena globosa was easily infected with all isolates. Local reactions usually were mild consisting of a few reddish local lesions, often appearing some weeks after inoculation. With LMV, however, many small reddish rings with a dry centre appeared after 10 days (Fig. 11A). With CAMV such rings were much more pronounced and larger, up to 5 mm wide (Fig. 11 B, C). Back inoculation from non-inoculated symptomless top leaves only yielded results with E 210, Wis Ital, and LMV. With the latter two isolates the results were poor. For infection of pea plants, *Gomphrena* was found to be a much better virus source than *C. quinoa* (see also the section on influence of the source of inoculum).

Nicotiana clevelandii was highly susceptible to all isolates. Local reactions varied from none to a mild mottling or yellow flecking. With Wis Ital local lesions were chlorotic and distinct and with E 178 they were 1–2 mm wide, often necrotic and numerous (Fig. 13 C). Systemic symptoms varied also. They could hardly or not be observed with E 210 and LMV. A slight mottling occurred with BYMV B25. Sparse chlorotic spots to large rings were formed by Wis 2B (Fig. 12 B) and Wis Ital. This spotting to flecking was severe with E 178, CAMV and BMV-SVP (Fig. 12C–E); here top leaves often were small and malformed and plants severely stunted.

In *N. debneyi* most of the isolates tested produced a latent local infection only. With E 178, however, a striking reaction has been observed about a month after inoculation when leaves turned yellow and many large green rings developed (Fig. 13A). These later turned necrotic.

Fig. 12. Systemically infected top leaves of *Nicotiana clevelandii* 1 month after inoculation with Wis 2B (B), E 178 (C), CAMV (D), and BMV-SVP (E); A, healthy control.

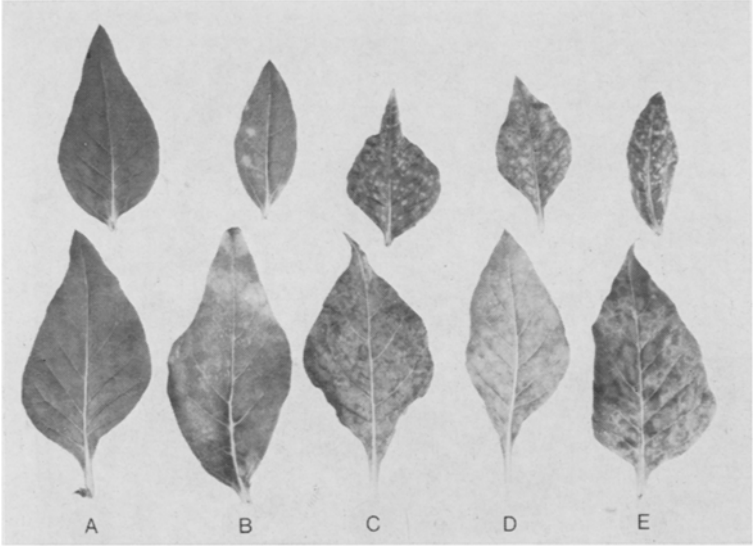

Fig. 12. Systemisch geïnfecteerde topbladeren van Nicotiana clevelandii 1 maand na inoculatie met Wis 2B (B), E 178 (C), CAMV (D) en BMV-SVP (E); A, gezonde controle.

Fig. 13. Local reaction to E 178 in *Nicotiana debneyi* 30 days after inoculation (A), *Cucumis sativus* 'Gele tros' 16 days (B), *N. clevelandii* 8 days (C), *N. tabacum* 'White Burley' 30 days (D), and *N. tabacum* 'Samsun' 12 days after inoculation (E).

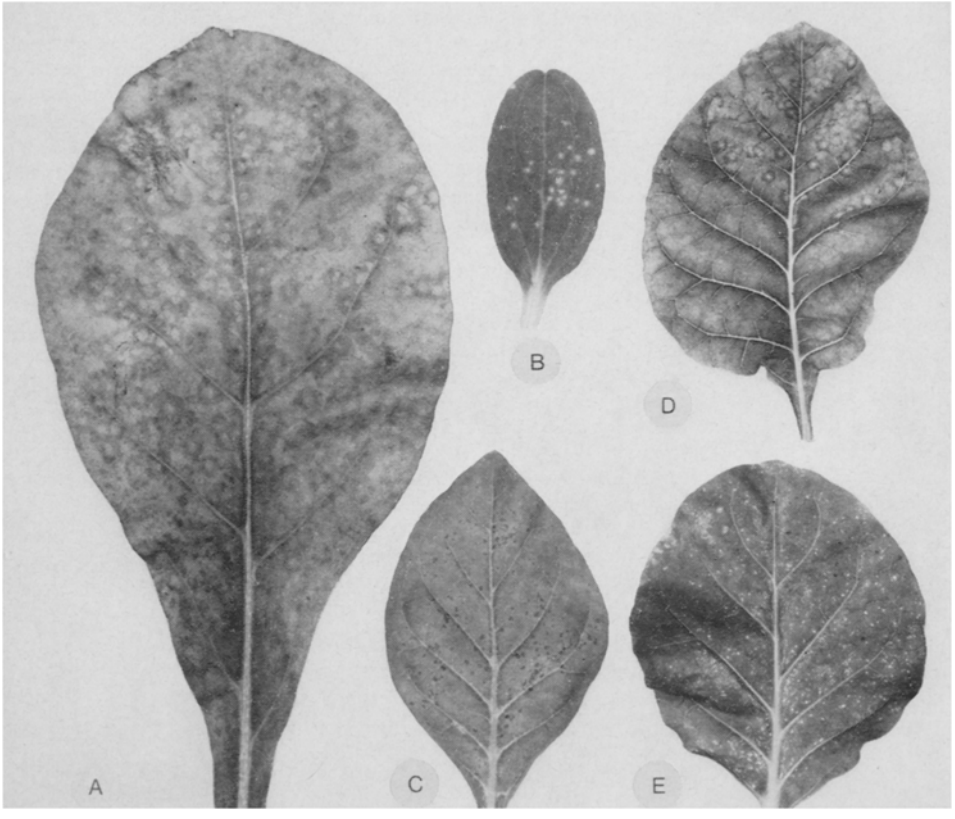

Fig. 13. Lokale reactie op E 178 in Nicotiana debneyi 30 dagen na inoculatie (A), Cucumis sativus 'Gele tros' 16 dagen (B), N. clevelandii 8 dagen (C), N. tabacum 'White Burley' 30 dagen (D) en N. tabacum 'Samsun' 12 dagen na inoculatie (E).

N. rustica assumed a latent local infection with BYMV B25, E 178, and BMV-SVP only, whereas CAMV gave a latent systemic infection as well.

All isolates, except E 210, BMV, and LMV, were able to infect *N. tabacum* 'White Burley' locally. BYMV B25 and especially E 178 (see Fig. 13D) induced a few chlorotic local lesions turning into green rings in ageing leaves. 'Samsun' tobacco readily reacted to E 178 in 6 days by producing numerous desiccated later somewhat concentric chlorotic local lesions (Fig. 13E). With PMV E 198 also many small but more vague local lesions were formed.

Petunia hybrida reacted locally to BYMV B25 with numerous green spots, to Wis 2B with green rings of 1-4 mm, and to CAMV with a few small lesions. In all instances lesions appeared only when leaves turned yellow. ClYVV and Wis Ital merely gave a latent local infection, whereas CAMV induced a pronounced breaking of flower colours (Fig. 14).

Physalis floridana was locally susceptible to E 210, BYMV B 25, and CAMV without producing symptoms. However, the latter isolate also caused systemic infection resulting in a striking stunting.

On *Spinacia oleracea* 'Noorman' all isolates gave a usually latent local infection. With B 25, however, faint chlorotic local lesions were formed and with E 178 many small etched later desiccating lesions. E 178, BYMV B 25, PMV E 198, CAMV, and LMV went systemic. With exception of CAMV,

Fig. 14. Flower colour breaking in *Petunia hybrida* 2 months after inoculation with CAMV; left, healthy control.

Fig. 14. Bloemkleurbreking in Petunia hybrida 2 maanden na inoculatie met CAMV; links, gezonde controle.

systemic infections were inapparent. From the literature spinach is known as a natural host of beet mosaic virus (Hoggan, 1933; Fujisawa et al., 1967).

Tetragonia expansa turned out to be locally susceptible to all isolates. With several isolates more or less distinct chlorotic local rings of varying diameters were formed.

Wisteria floribunda seedlings grown in the greenhouse and inoculated with Wis 2B, E 178, and BYMV B 25 (two plants each) only reacted to Wis 2B (one out of two) in about 5 weeks. Symptoms were bright and resembled those of natural infection. Virus could easily be recovered from top leaves. Seedlings of *W. sinensis* reacted to Wis 2B (one out of two) with a vague vein mosaic and leaf malformation and to BYMV B 25 (one out of four) with some chlorotic spotting. Virus has been recovered in both cases.

Inclusion bodies

In strips of petiole or stem epidermis inclusion bodies were stained with a solution of phloxine and methylene blue according to the method of Christie. Further data on techniques and some preliminary results as well as details of inclusions of E 178 have been published already (Bos, 1969a; Bos and Rubio-Huertos, 1969).

With both BYMV B 25 and PMV E 198 large amounts of granular cytoplasmic inclusions have been observed. The nucleoli were slightly enlarged and angular with B 25.

Although pea, broad bean, and *N. clevelandii* with distinct symptoms of Wis 2B were repeatedly tested at various times after inoculation only rarely were small islands of cells with faint granular inclusions found. The Italian isolate (Wis Ital), however, induced many granular inclusions in pea, although the nuclei seemed normal.

With E 178 in all plant species tested granular inclusions were less striking or absent. Nucleoli were very much enlarged, however, and commonly provided with unusual and radiating crystalline needles. Ultrathin sections of these needles did not contain complete virus particles (Bos and Rubio-Huertos, 1969).

Broad-bean plants infected with E 210 contained small granular cytoplasmic inclusions and slightly enlarged, sometimes angular nucleoli.

ClYVV, in addition to producing amorphous granular cytoplasmic inclusions as reported for *N. clevelandii* by Hollings and Nariani (1965), irregularly enlarged nucleoli of pea and broad bean.

CAMV caused many granular inclusions in pea and sparse groups in *Petunia*. The nucleolus was outlined somewhat more than normal.

With BMV–SVP sometimes vague granular cytoplasmic inclusions were observed in beet leaves and now and then nucleoli were somewhat irregularly enlarged. In pea, however, nucleoli were much enlarged, well-defined and sometimes "sprouting".

In pea plants showing faint mosaic symptoms after infection with LMV no inclusions were found.

Influence of source of inoculum and test plant

A variable factor determining the success of virus transmission, and thus greatly influencing the results of host-range studies reported before, is the source of inoculum and the test plant used. This effect of virus donor-acceptor combination was clearly demonstrated in four experiments.

Four groups of two young beet plants and one plant of *Chenopodium amaranticolor* were inoculated separately with BYMV B 25 from *C. amaranticolor*, *Gomphrena*, pea, and spinach. In all cases reaction of *C. amaranticolor* was typical of B 25. In beet, however, no symptoms were produced after back-inoculation form inoculated beet leaves on to *C. amaranticolor* 16 days later. A reasonable number of local lesions were obtained only from those beet plants that had been inoculated from *C. amaranticolor*, whereas those inoculated from spinach produced a few local lesions only. Later systemic symptoms in the indicator plants were typical of B 25.

The other three experiments have been summarized in Table 6. Here it was impossible to infect 'Koroza' peas with BYMV B 25 when using sap from *C. album*, *C. amaranticolor*, and spinach, and it was difficult to infect broad bean from *C. amaranticolor*, although in all cases the sap was clearly infectious to *C. amaranticolor*. A similar result was obtained with E 178, when testing pea varieties for their susceptibility to LMV, one series of plants were inoculated from systemically infected *C. quinoa* plants and another series from locally infected *Gomphrena* plants. Simultaneously inoculated *Gomphrena* plants demonstrated that the *Gomphrena* inoculum contained much less virus than the *C. quinoa* inoculum. Upon back inoculation from the pea varieties, however, the first inoculum turned out to have been much more infectious to pea than the latter one. The variety 'New Era', which was not infected from *C. quinoa*, readily produced the virus upon inoculation from *Gomphrena*.

Persistence of infectivity in expressed sap

Pea sap, containing B 25, E 178, and E 210, and sap of *Nicotiana clevelandii*, containing Wis 2B, was treated in the conventional way and tested for infectivity by inoculation on to *C. amaranticolor*, two plants per treatment. E 178 and BYMV B 25 were tested simultaneously. Results are summarized in Table 7.

Cross-protection tests

Mutual relationships between most of the isolates were investigated by testing their cross-protecting ability in various combinations in a number of hosts. Plants not showing evidence of infection with the protecting virus were discarded before super-

Table 6. Effect of virus donor–acceptor combination on result of sap transmission.

Virus	Virus donor	Virus acceptor	Results of visual examination or of back inoculation
BYMV			visual examination:
B 25	*C. album*	*P. sativum* 'Koroza'	0/7[1]
	top leaves with systemic symptoms	*C. amaranticolor*	2/2 reasonable number of l.l.[2] + systemic reaction
	C. amaranticolor	*P. sativum* 'Koroza'	0/12
	locally and systemically infected	*V. faba*	1/8
	leaves	*C. amaranticolor*	3/3 ∞ l.l + syst.[3]
		C. quinoa	2/2 ∞ l.l
	Spinacia oleracea 'Noorman'	*P. sativum* 'Koroza'	0/4
	a. symptomless inoculated leaves	*C. amaranticolor*	2/2 ∞ l.l. + syst.
	b. symptomless top leaves	*P. sativum* 'Koroza'	0/8
		C. amaranticolor	2/2 ∞ l.l. (plants discarded early)
E 178	*C. amaranticolor*	*P. sativum*	0/6
	leaves with local lesions	*C. amaranticolor*	2/2 reasonable number of l.l.
		C. quinoa	1/1 reasonable number of l.l.
	C. amaranticolor	*P. sativum* 'Koroza'	0/8
	leaves with local lesions	'Rondo'	0/8
		C. amaranticolor	3/3 reasonable number of l.l.
LMV	*C. quinoa*	*Gomphrena globosa*	visual: 2/2: 151 l.l.
	top leaves	*P. vulgaris* 'Bataaf'	local back inoculation onto *C. quinoa:*
		P. sativum	1/2: 11 l.l. + syst.
		'Big Ben'	2/2: 16 l.l. + syst.
		'Early Perfection'	2/2: 17 l.l. + syst.
		'Koroza'	2/2: 13 l.l. + syst.
		'New Era'	0/2
		'New Season'	2/2: 30 l.l. + syst.
		'Perfected Wales'	2/2: 36 l.l. + syst.
		'Wisconsin Perfection'	2/3: 33 l.l. + syst.
		'Wyola'	0/2
	Gomphrena globosa	*Gomphrena globosa*	visual: 2/2: 23 l.l.
	inoculated leaves	*P. vulgaris* 'Bataaf'	local back inoculation onto *C. quinoa:*
		P. sativum	1/2: 1 l.l. + syst.
		'Big Ben'	2/2: 73 l.l. + syst.
		'Early Perfection	2/2: ∞ l.l. + syst.
		'Koroza'	2/2: ∞ l.l. + syst.
		'New Era'	2/2: 43 l.l. + syst.
		'New Season'	2/2: 6 l.l. + syst.
		'Perfected Wales	2/2: 44 l.l. + syst.
		'Wisconsin Perfection'	2/2: 81 l.l. + syst.
		'Wyola'	0/2

[1] Number of plants reacting out of number of plants tested
[2] l.l. = local lesions
[3] Syst. = systemic reaction

Tabel 6. Invloed van de combinatie virusdonor/virusacceptor op het resultaat van overdracht met sap.

Table 7. Results of infectivity tests in expressed sap.

	Wis 2B	E 178	E 210	BYMVB25
Dilution				
0	50	∞	∞	∞
10	91	∞	∞	∞
100	13	∞	∞	∞
1,000	1	26	11	85
10,000	0	4	2	26
100,000	0	1	0	3
Thermal inactivation (°C)				
45	50	∞	∞	∞
50	9	24	30	∞
55	5	2	6	± 100
60	0	0	4	18
65	0	0	3	0
70	0	0	0	0
75				
80				
Ageing in vitro (days)				
1	62	55	75	∞
2	1	3	16	∞
3	0	2		∞
4	0	2	17	125
6	0			
7		0	1	28

Tabel 7. Resultaten van infectieproeven met uitgeperst sap.

inoculation. Depending on virus–host combination the effect of super-inoculation was tested visually in case of distinct symptoms, by means of back-inoculation, and sometimes by checking for the presence of characteristic inclusion bodies. Further details on the experiments and results are summarized in Table 8.

The table reveals that systemic necrosis and inclusion bodies typical of E 178 in broad bean plants were absent in such plants after previous infection with BYMV B 25 (Exp. 3a). In one experiment with pea plants a similar systemic necrosis of E 178 did not occur in the majority of plants systemically infected with BYMV B 25 (Exp. 8a). Symptoms in *Nicotiana clevelandii* singly infected with E 178 were severe, but mild after super-inoculation of plants previously infected with BYMV B 25, suggesting a slight protecting effect by B 25 (Exp. 13).

A retardation of about 2 weeks of necrosis produced by ClYVV in pea was obtained by a previous infection with BYMV B 25 (Exp. 7).

Unexpectedly, LMV protected *C. quinoa* plants against CAMV (Exp. 11b), whereas the reverse was observed in *N. clevelandii* (Exp. 14 g and 15). Peculiarly enough, in Exp. 12, *C. quinoa* plants systemically infected with LMV were easily infected locally with CAMV, whereas from the inoculated leaves the LMV could no more be recovered.

Thus in the majority of instances there was no evidence of any cross protection, even in one case BYMV B 25 and the closely related PMV E 198 (Exp. 6). Here, however, the time interval of 8 days could have been too short, because in another test (Exp. 9) BYMV protected aginst PMV.

Virus purification

In co-operation with Mr D. Z. Maat, IPO., Wageningen, some work was done on purification, mainly to prepare antisera against BYMV B 25, Wis 2B, and E 178 and to obtain good antigens to these and some other isolates for serological tests. No antiserum has been prepared against E 210 because this isolate was included in the investigations at a later date.

'Koroza' peas were harvested 11–13 days after inoculation, i.e. 2–3 days after vein chlorosis started, and were stored in a cold room for at least one night.

Partially purified preparations were made using a modification of the ether-carbon-tetrachloride clarification method of Wetter (1960), followed by two cycles of differential centrifugation (1 h 30,000 rpm in rotor R 30 Spinco model L and 20 min 6000 rpm in a Phywe Eispirouette refrigerated centrifuge). The finally resulting slightly opalescent suspensions, upon studying in the electron microscope, turned out to be reasonably clean and to contain many particles of characteristic size and morphlogy. In such preparations often considerable aggregation and breakage occurred.

Initially, differential centrifugation was followed by sucrose density gradient centrifugation (1.5 h 24,000 rpm Rotor SW 25.1). This always resulted in one wide zone with a clearly defined upper and a diffuse lower edge, whereas much virus sedimented to the bottom of the tube as was proved by resuspension and testing for infectivity. This is easily explained by the aggregation of virus particles as seen in the electron microscope. Thus rate zonal centrifugation on a sucrose gradient was found to be unsuitable in further purifying the virus as no clearly visible separation of virus and normal plant proteins was obtained and too much virus was lost in the sediment.

In one experiment, BYMV B 25, Wis 2B, E 178, and PMV E 198 were purified from inoculated leaves of 'Samsun' tobacco. Reasonable amounts of virus were obtained with E 178 only. More extensive tests were done with *Nicotiana clevelandii*. Leaf material was treated according to the same method as described for pea. In the first experiment plants were harvested 23–29 days after inoculation and final preparations tested onto *C. amaranticolor*. Good amounts of virus were obtained with BYMV B 25 and E 178, whereas the concentration was low with Wis 2B, E 210, and PMV E 198. In a second experiment more tests were included. Results were poor with Wis 2B, E 210, and BMV-SVP, exceptionally good with CAMV, and reasonable with the other isolates.

Infectivity of such virus suspensions from pea kept in the refrigerator, persisted for quite some time. With E 178 *C. amaranticolor* plants could still be infected after 17 days whereas pea plants escaped infection. After 67 days of storage Wis 2B still caused many local lesions in *C. amaranticolor* and pea plants were also easily infected. BYMV B 25 was highly infectious after 36 days of storage, clearly infectious after 65 days, but after 7 months infection failed.

Serology

Antisera were made and serological tests were performed by Mr D. Z. Maat. He prepared antisera against Wis 2B, E 178, and BYMV B 25 having maximum homologous titers of 8192, 4096, and 256, respectively. In the last experiment a 10 years old antiserum against BCMV was included. This antiserum originally had a homologous titer of 4096. Antigens had been prepared as described above. Antibodies against normal plant proteins were removed by absorption of antisera with healthy plant sap, or more

Table 8. Summary of cross-protection tests.

Experiment No.	Plant species	Protecting virus	Challenge virus	Time interval in days	Effect of challenge virus on super-inoculated plants			Cross protection
					systemic symptoms[1]	inclusions	back inoculation[2]	
1	*Vicia faba*	BYMV B25	Wis 2B	17	(local symptoms) only			−
2		Wis 2B	BYMV B25	16	11/14(14/19)	+	*C. amaranticolor* a +	−
3a		BYMV B25	E 178	22	0/8(13/18)	−		+
b		Wis 2B	E 178	22	8/13(13/18)			−
c		Wis 2B	B25	22	5/6(17/17)			−
4a		BYMV B25	E 210	29	10/16(13/16)		*C. quinoa* p 3/4[3]	−
b		E 210	BYMV B25	29	1/10(16/16)		*C. amaranticolor* p 4/4	−
c		E 210	PMV E198	29	3/6(16/16)		*C. amaranticolor* p 4/4 + *V. faba*	−
5a	*Pisum sativum* 'Koroza'	Wis 2B	E 178	12	+			−
b		BYMV B25	E 178	12	+			−
6		BYMV B25	PMV E198	8	16/16(15/15)			−
7		BYMV B25	ClYVV	16	9/10[4](12/12)			±
8a		BYMV B25	E 178	13	2/12(8/8)	−		+
b		BYMV B25	E 210	13	14/16(7/7)			−
9		BYMV B25	PMV E198	12	2/16(16/16)		*Pisum sativum* p 2/4	+
10	*Petunia hybrida*	CAMV	BYMV B25	20			*C. amaranticolor* s 3/5 (4/5)	−
11a	*Chenopodium quinoa*	LMV	BMV-SVP	17	3/3(4/4)			−
b		LMV	CAMV	17	(4/4)		*C. amaranticolor* + *Gomphrena* s 0/4	+
c		E 210	BYMV B25	17	(4/4)		*C. amaranticolor* + *V. faba* s 4/4	−
d		E 210	LMV	17	4/4(4/4)			−
e		E 210	CAMV	17	4/4(4/4)		*C. amaranticolor* + *Gomphrena* s 4/4	−
12		LMV	CAMV	19	(5/5)		*C. amaranticolor* + *C. quinoa* s 5/5[5]	−
13	*Nicotiana clevelandii*	BYMV B25	E 178	30	4/4[6](4/4)		*C. amaranticolor* + *P. sativum* s 4/4	−

14a	E 178	CAMV			*Gomphrena* s 2/2 (1/2)	—
b	E 178	BMV-SVP			*C. quinoa* s 2/2 (1/1)	—
c	E 178	LMV			*C. quinoa* s 2/2 (1/1)	—
f	CAMV	E 178			*P. sativum* s 2/2 (1/1)	—
g	CAMV	LMV			*C. quinoa* s 0/2 (1/1)	+
h	BMV-SVP	E 178			*P. sativum* s 2/2 (1/1)	—
j	BMV-SVP	CAMV			*Gomphrena* s 2/2 (1/2)	—
k	LMV	E 178			*P. sativum* s 2/2 (1/1)	—
m	LMV	CAMV		2/2(1/2)	*Gomphrena* s 2/2 (1/2)	—
15	CAMV	LMV	20		*C. quinoa* s 1/5	+

[1] Number of plants showing characteristic symptoms of challenge virus over number of plants showing symptoms of the protecting virus on the moment of super inoculation. In brackets number of plants showing symptoms of the challenge virus after inoculation with this virus only, over plants inoculated.

[2] Back inoculation onto test plants listed; a = average sample of all plants super-inoculated, p = average sample per pot containing four plants, s = plants tested separately.

[3] Plants or pots found to contain virus over plants or pots tested. In brackets number of plants or pots of plants inoculated with the challenge virus virus only found to contain virus over plants or pots tested.

[4] Super-inoculated plants showed necrosis and premature death characteristic of ClYVV, but death occurred about 2 weeks later than with plants only inoculated with ClYVV.

[5] Remarkably enough, from plants super-inoculated with CAMV, LMV could no more be recovered as judged by absence of systemic symptoms in *C. quinoa* plants used for back inoculation, whereas plants, inoculated from *C. quinoa* plants with LMV only, produced severe systemic symptoms.

[6] Symptoms in super-inoculated plants were less clear than in plants inoculated with E 178 alone.

Tabel 8. Samenvatting van de premunitieproeven.

Table 9. Summary of five serological tests; homologous titres are in italics.

Experiment No.	Antigen	Antiserum			
		Wis 2B	E 178	BYMV B 25	bean common mosaic virus
1	Wis 2B	*1024*	–	–	
	BYMV B 25	64	64	*128*	
2	E 178	4	*1024*	1	
	BYMV B 25	256	16	*256*	
3	Wis 2B	*4096*	–	–	
	E 178	–	*256*	–	
	BYMV B 25	64	64	*64*	
4	Wis 2B	*8192*	?	–	
	E 178	?	*4096*	?	
	BYMV B 25	128	64	*256*	
5	Wis 2B	*1024*	–	–	4
	E 178	16	*1024*	–	16
	BYMV B 25	64	4	*64*	4
	E 210	4	–	–	–

Tabel 9. Overzicht van vijf serologieproeven; homologe titers zijn cursief gezet.

effectively with highly concentrated preparations of normal plant proteins for the antisera againstWis 2B, E 178, and BYMV B 25. The micro-precipitin test under paraffin oil was used.

A survey of some preliminary experiments is given in Table 9. The results indicate that the three viruses are related mutually as well as to BCMV but that there are considerable differences. E 210 showed a distant relationship to Wis 2B.

Electron microscopy

Preparations of purified suspensions for the electron microscope were shadow-cast or negatively stained with PTA 2%, pH 6.5. For determining particle lengths negative staining of crude sap was applied exclusively and tobacco mosaic virus (TMV) was used as an internal standard. Such preparations were made by intensively chopping with a razor blade about 30 mm^2 of virus-containing leaf together with 5 mm^2 of old almost yellow leaf of TMV-diseased 'White Burley' tabacco in five or six drops of PTA on a microscope slide. One drop of slightly green liquid was then transferred with a glass capillary to a carbon-reinforced formvar-coated copper grid. After half a minute the excess of liquid was removed by carefully touching the edge of the grid with a small piece of filter paper. The grids were then viewed in a Philips EM 300 electron microscope at a magnification of about $\times 10{,}000$ at the screen with an additional binocular $\times 10$ and photographed at a magnification of about $\times 11{,}000$. Catching more than ten flexuous particles per picture was rare. With several preparations it was hard to get four into one photograph.

Particles were later measured directly from the negatives under a binocular microscope at a magnification $\times 10$ with a micrometer eye piece. Many particles were sufficiently rigid to warrant a reasonable size estimation. Measuring was done in classes

Tabel 10. Survey of particle length measurements.

Virus isolate and host	Number of preparations	Number of photographs	Micrometer classes[1]				Total number of TMV particles	Micrometer classes[1]													Total number of flexuous particles	Peak length[2]
			28	30	32	34		66	68	70	72	74	76	78	80	82	84	86	88	90	92	
Wis 2B in pea	4	48	1	*257*	*387*	2	647					5	45	91	*171*	45	5					362	774
E 178 in pea	6	28		36	*179*	21	236		3	3	2	20	20	25	*54*	20	11					141	750
E 210 in pea	6	24	1	68	*238*	7	314							11	*71*	46	10	2				140	752
BYMV B 25 in pea	4	29	1	72	*218*	8	299							12	45	*73*	38	9	2			179	771
in C. amaranticolor	2	14		13	*65*	8	86									6	5	19	*26*	*37*	19	113	834
PMV E 198 in pea	2	13	7	*231*	*199*	2	439									4	14	*53*	*41*	23	4	139	841
BMV-SVP in pea	1	19		7	*110*	5	122			3	1	8	*34*	*27*	14	3						90	722
in C. quinoa	1	17		*48*	*69*	2	119			4	19	44	*97*	48	29	2	1					244	739
BMV-IPO in pea	1	18		22	*70*	5	97	1	9	18	8	12	*35*	24	18	1						126	712
LMV in pea	3	25		46	*270*	16	332								17	*52*	*63*	14				146	778

[1] Peak classes are printed un italics.

[2] Peak length is calculated on the basis of TMV length. For example in case of E 178, TMV measures 32 classes (300 mμ). Then one class is 9.37 mμ, and E 178 is 80 × 9.37 = 750 mμ long.

Tabel 10. Overzicht van de deeltjeslengtemetingen.

of about 9.5 mμ, depending on the exact magnification of the electron microscope, and later grouped into classes of about 19 mμ to simplify recording of results in Table 10. These data were collected during a period of four months. Figures on a given virus isolate were only added if the curves for TMV were in close agreement. TMV particles were found to be very uniform and to yield sharp peaks varying one micrometer class of about 9.5 mμ in length at maximum during the given period. Thus, variation in particle length due to variation in magnification of the electron microscope and to other mechanical factors did not exceed 3%. The application of crude TMV preparations as an internal standard was found extremely simple and easy.

Measuring of purified preparations was usually found unreliable because of particle breakage and aggregation. These results are therefore omitted from Table 10. In general, length distribution of long flexuous particles is rather wide making calculation of average or mean lengths risky. Width of the curves may be partly due to incorrect size determination because of flexibility, but often rather rigid particles lying side by side clearly differed in length, thus visibly demonstrating a more intrinsic variation in length.

Peculiarly, the length of BYMV B 25 was found to vary with the host. Using the same host (pea) Wis 2B, E 178, E 210, BYMV B 25, and LMV did not differ appreciably, their peaks usually being around 750–775 mμ (Table 10, last column). This is slightly more than 750 mμ usually assumed in the literature for BYMV. Both isolates of BMV, with peaks varying from 712–730 mμ, were considerably shorter as is in agreement with observations by Brandes (1964).

The length of PMV E 198 (with a peak of 841 mμ) was unusual as has been found in several other preparations not recorded here. A similar length was estimated for some other "pea mosaic" virus isolates. Moreover, PMV E 198 and the other isolates all had a remarkably rigid appeareance thus clearly differing from BYMV B 25 in length as well as in morphology.

Discussion

The originally observed syndromes of pea necrosis and pea leafroll mosaic could easily be reproduced in pea plants by inoculation with the isolates E 178 and E 210, respectively (code references are listed in Table 2). Similarly, in *Wisteria floribunda* grown from seed, vein mosaic indistinguishable from natural infection could be incited by inoculation with Wis 2B and the virus could later be recovered from systemically infected leaves of such a plant, in contrast to its absence in non-inoculated seedlings. Thus the causal relationship between the virus isolates studied and the diseases described is beyond doubt.

When first studied separately, Wis 2B, E 178, and E210 seemed to be quite distinct from each other as well as from the generally accepted image of BYMV. Their actions on pea, bean, and especially on broad bean were quite different and the ability to infect several non-legumes was striking. Upon careful comparison with B 25, an isolate of BYMV, however, they were found to have several features in common. BYMV B 25 was able to infect locally 17 out of 20 non-legumes tested. In four of these, infection became systemic. There are some earlier reports on infection of non-legumes by BYMV. They have often been overlooked and are therefore summarized in Table 11. In my experiments *Beta vulgaris, Callistephus chinensis, Nicotiana debneyi, N. rustica, Petunia*

Table 11. Non-legumes reported to be susceptible to BYMV; data arranged chronologically.

Author	Natural or artificial infection	Plant species	Systemic or local infection
McWhorter et al. (1947)	natural	*Gladiolus*	S
Thomas and Zaumeyer (1953)	artificial	*Nicotiana rustica*	L
		Nicotiana sylvestris	L
		Nicotiana tabacum	L
Van Koot et al. (1954)	natural	*Freesia*	S
Hollings (1959)	artificial	*Amaranthus caudatus*	L
		Chenopodium amaranticolor	L
		Gomphrena globosa	L
		Phytolacca americana	L
Zschau (1961) (from yellow lupin)	artificial	*Chenopodium amaranticolor*	L
		Gomphrena globosa	L
Zaumeyer and Goth (1963) (from red clover)	artificial	*Nicotiana tabacum* 'Samsun'	L
		Chenopodium amaranticolor	S
Blaszczak (1965) (from red clover)	artificial	*Chenopodium amaranticolor*	L
		Zinnia elegans	L
		Citrullus vulgaris	L
Kovachevsky (1966, 1968)	natural	*Chenopodium album*	S
		Cirsium arvense	S
		Papaver somniferum	S
Teakle and Wildermuth (1967)	artificial	*Chenopodium album*	L
		Chenopodium amaranticolor	L
		Gomphrena globosa	L
		Nicotiana clevelandii	S
		Nicotiana tabacum	L

Tabel 11. Niet-vlinderbloemigen, die volgens de literatuur vatbaar zijn voor bonescherpmozaïekvirus; gegevens chronologisch gerangschikt.

hybrida, *Physalis floridana*, and *Tetragonia expansa* have been detected as new hosts, locally susceptible. Systemic susceptibility of spinach *(Spinacia oleracea)* might have a practical aspect as well. Natural infection of this plant species by BMV has also been reported (Hoggan, 1933; Fujisawa et al., 1967). In spite of comparable host ranges, however, several other plant species such as *C. amaranticolor* and *C. quinoa* reacted differentially. Extremely striking was the effect of E 178 on cucumber, *Nicotiana debneyi*, 'Samsun' tobacco, and spinach.

With ClYVV, CAMV, and Wis Ital host range variation did not differ basically. Wis Ital closely resembled Wis 2B, differing from the latter in causing a rather extensive local vein necrosis in French bean instead of small necrotic local lesions, and by producing many granular inclusion bodies in pea. CAMV differs from the other viruses in becoming systemic in *Nicotiana rustica* and in causing clear-cut systemic symptoms in *Petunia hybrida*, *Physalis floridana*, and spinach.

The ability of BYMV B 25 to infect several non-legumes does not necessarily mean that Wis 2B, E 178, and E 210, as well as ClYVV and CAMV, should all be considered strains of BYMV. This is because in general, the host ranges and symptomatology of BMV and LMV studied here, and those of other viruses already described

as distinct entities, though having related particle size and morphology, in the range of differences show a gradation only. For example, Hollings (1959) in studying host reactions and in vitro properties, found that lettuce mosaic virus and pea mosaic virus shared many properties. The lettuce virus (Ainsworth and Ogilvie, 1939) and beet mosaic virus (Quantz, 1958; my own investigations) have been reported to cause severe necrotic symptoms in pea crops, whereas watermelon mosaic virus produces a widespread mottle in pea in Japan (Inouye, 1964). Another virus of the group that is able to infect a legume naturally is turnip mosaic virus, which infects peanuts (Inouye and Inouye, 1964). With BMV-SVP and LMV I easily succeeded in getting local infection in several bean varieties and systemic infection in various pea varieties. BMV-SVP even produced in broad bean systemic symptoms somewhat intermediate between those of Wis 2B and E 178. In this respect artificial infection of beet plants with B 25 is of special importance. Recently, Schmelzer (1967) even found potato virus Y and tobacco etch virus to infect systemically most species tested of the legume genera *Trigonella* and *Melilotus*.

Breaking of flower colours, caused in my experiments by CAMV in *Petunia*, closely resembled the effect of turnip mosaic virus on this plant (e.g. Stace-Smith and Jacoli, 1967) of BYMV in gladiolus (McWhorter et al., 1947) and in freesia (van Koot et al., 1954). The symptom is quite well-known for other viruses of the potato virus Y group, e.g. for anemone mosaic virus, tulip mosaic virus, and iris mosaic virus in their main hosts.

Seed transmission is another character often used to differentiate viruses such as BYMV on the one hand and BCMV and soybean mosaic virus on the other. Seed transmission of CAMV in cowpea led Lovisolo and Conti (1966) to conclude that their virus was closely related to bean common mosaic virus (BCMV). The weight of this distinction is very relative, however, since BYMV is now known to be seed-transmitted in yellow lupin (Corbett, 1958). Thus lupin mosaic, for some time supposed to be due to a special lupin mosaic virus (Mastenbroek, 1942), is now generally accepted to be caused by BYMV, although recently Hull (1968) considered a lupin mottle virus, newly isolated from garden lupin, to be slightly distinct from BYMV. BYMV is also known to be seed-transmitted in broad bean, although in low percentage only (Quantz, 1954). Other viruses in Table 1 that have been reported to be seed-borne are cowpea aphid-borne mosaic virus (Lovisolo and Conti, 1966), pea leafroll virus (Musil, 1966), and pea seed-borne mosaic virus (Inouye, 1967); it is also well-known for lettuce mosaic virus in lettuce and for soybean mosaic virus in soybean.

BYMV is also generally accepted to be easily distinguished from BCMV by the wide host range of the first virus. The latter virus, like soybean mosaic virus, had long been supposed to be almost restricted to one species. Quantz (1961) and Galvez (1963), however, showed the host ranges of BCMV and soybean mosaic virus to be much wider than previously indicated. Recently I was able to recover two strains of BCMV from inoculated leaves of *Chenopodium quinoa*, *Gomphrena globosa*, and *Nicotiani clevelandii*, and in the latter case from non-inoculated top leaves as well (unpublished). Many workers on legume viruses will know that it is sometimes hard to distinguish between the symptoms of BYMV and BCMV in bean (cf. Fig. 5). As early as 1948 Grogan and Walker regarded the viruses as closely related because they found them to give partial or sometimes complete cross-protection in bean. A partial cross-protection between both viruses was also found by Quantz (1961), who

also pointed to a close resemblance between BCMV and soybean mosaic virus in host range, symptoms, and even bean varietal reaction.

Cytoplasmic granular inclusions are increasingly found to be characteristic of the entire potato virus Y group. The production of intranuclear abnormalities does not seem to be an exclusive prerogative of BYMV and tobacco etch virus either, since I found them with BMV as well as with most other isolates studied.

Thus, all the isolates and viruses studied here differ only slightly within the full range of effects on the host plants. They have many hosts in common such as French bean, pea, *Chenopodium* spp., *Gomphrena*, *N. clevelandii*, spinach, and *Tetragonia expansa*.

Attention should be drawn here to the relative meaning of data on host reactions. In my experiments ClYVV and CAMV produced systemic symptoms in 'Koroza' peas, although Hollings and Nariani (1965) reported *Pisum sativum* not to be infected by ClYVV and Lovisolo and Conti (1966) found the species to acquire latent infection with CAMV. Such discrepancies, of course, greatly depend on the varieties tested, as is evident in Tables 4 and 5. The influence of test plant variability, even in species not normally differentiated into varieties, was clearly demonstrated by Demski (1968). He found that out of six sources of *Chenopodium album* tested, only one was fit for assaying for watermelon mosaic virus.

Therefore, standardization of host ranges and the varieties involved – or at least the recording of details concerning types and varieties used – as well as standardization of conditions is essential, as stressed earlier (Bos et al., 1960). Although several of our host-range tests were repeated in different seasons, the average effect of the various isolates did not vary too much. Even host reactions of Wis Ital determined here, agreed closely with those observed by Conti and Lovisolo (1970) in Italy.

Another important factor in determining host susceptibility is the quality of inoculum used to test prospective hosts. This quality depends on the nature and age of the virus source and on the time interval between inoculation and testing. Experiments listed in Table 6 strikingly support the findings of others (e.g. Hollings, 1966), that mechanical virus transmission may succeed from one species to a second and fail to a third one, although the last-named may eventually turn out to be susceptible upon inoculation from another source. Thus, Schmelzer (1959) overcame difficulties in transmitting BMV from beet plants by first inoculating *Chenopodium foetidum*. This emphasizes the need for care in checking symptomless plants for the presence of virus. Difficulties involved can never be completely overcome since not all combinations virus-donor receptor can be tested. It may be concluded, however, that upon careful testing, the host ranges of many viruses will certainly turn out to be much wider than hitherto supposed.

In bean and pea varietal reaction tests (Table 4 and 5) considerable differences were detected between various isolates, such as BYMV B 25 and E 210. However, when reactions to all isolates are taken into consideration, here also there is a gradation in the range of differences. For quite some time the supposed inability of pea mosaic virus to infect *Phaseolus* beans has been an accepted criterion for considering this virus a distinct entity. This was found not to hold by Schroeder and Provvidenti (1966), and all our virus isolates tested infected beans, although with varying degrees of success (Table 4). The fact that resistance in pea (especially Perfection-type varieties) to isolates of BYMV and pea mosaic virus was conditioned by the same genotype (Bar-

ton et al., 1964; Cousin, 1965) was considered another argument that pea mosaic virus is a strain of BYMV. In this connection Wis 2B, E 178, and E 210 showed some relationship to BYMV B 25, whereas LMV and BMV-SVP easily infected Perfection-type peas. Peculiarly, another "strain" of pea leafroll mosaic virus indistinguishable symptomatologically from E 210 in pea easily infected a series of Perfection-type varieties.

Another phenomenon widely used in studying virus relationships is cross-protection. In our experiments (Table 8) a more or less clear protection of plants by isolate BYMV B 25 against E 178 was observed in a number of tests (Exp. 3a, 8a) and was beyond doubt. Indications of such a protection by BYMV B 25 against ClYVV (Exp. 7) could be concluded from a retardation of necrosis. Unexpectedly, there existed a cross protection between LMV and CAMV in most experiments (11b, 14g, 15) although the number of plants investigated was low. Amazingly, in one experiment with *C. quinoa* (Exp. 12) LMV did not protect against CAMV but was completely suppressed by the challenge virus as judged by back-inoculation to *C. quinoa*. In most other combinations, even of E 210, PMV E 198, and BYMV B 25, no signs of cross protection have been observed, with the exception only of the tests in Exp. 9 between BYMV B 25 and PMV E 198 in pea.

To evaluate the weight of these observations, the results of cross-protection tests reported in the literature, using strains of BYMV or closely related viruses are summarized in Table 12. This table reveals that closely related strains often do not cross protect, whereas complete protection has been observed with viruses hitherto considered to be different entities, such as soybean mosaic virus, BCMV and BYMV. It should be stressed here that negative results do not mean much, as has been pointed out by Köhler (1964), because with mosaic viruses penetration of the protecting virus is often incomplete. Since some of the viruses found to cross-protect are generally accepted to be distinct entities, such as soybean mosaic virus, BCMV, and BYMV, cross protection could not help solving our identification problem. Similar problems have been met with for tobacco etch virus, potato viruses Y and A, and henbane mosaic virus by Bawden and Kassanis (1941, 1945) and Schmelzer et al. (1960).

The first report on serological relationships between different viruses of the potato virus Y group, viz. between BYMV and BCMV, is that of Beemster and van der Want (1951). In 1960 Bercks (1960a, 1961) proved the existence of so-called distant serological relationships between BYMV, beet mosaic virus, and even potato virus Y when using high titre antisera. Later, these relationships were extended to almost all other members of this group by the same author and others.

Our serological tests so far were rather incomplete. However, differences between Wis 2B, E 178 and BYMV B 25 were quite distinct. They were related mutually as well as to BCMV. Since against each isolate only one antiserum was prepared and antisera produced by different rabbits against one and the same virus may differ considerably in revealing distant relationships (cf. Bercks, 1966), no one can say whether the differences found here are small or large. Moreover, Bercks (1960b) has found clear differences between some strains of BYMV and one isolate of BCMV. Further, there seems to exist no sharp borderline between close and distant serological relationships (cf. Wetter, 1965; Bercks, 1966; van Regenmortel, 1966).

Electron microscopy did not reveal identifiable differences between Wis 2B, E 178, E 210, BYMV B 25, and LMV, although E 178 and Wis 2B particles tended to be more flexible than the others. BMV was easily distinguished because of its shorter particle

Table 12. Literature survey of cross-protection tests with strains of BYMV or closely related viruses.

Author	Protecting virus	Challenge virus	Test plant	Proof of super infection	Cross protection
Grogan and Walker (1948a)	BCMV	BYMV	*Phaseolus vulgaris*	syst. symptoms	practically complete
			Phaseolus vulgaris	back inoc.	practically complete
	BYMV	BCMV	*Phaseolus vulgaris*	syst. symptoms	partial
				back inoc.	partial
				seed transm.	partial
Grogan and Walker (1948b)	BYMV	BYMV pod distorting strain	*Phaseolus vulgaris*	syst. symptoms	practically complete
	BCMV	pod distorting strain	*Phaseolus vulgaris*	syst. symptoms	practically complete
Thomas and Zaumeyer (1953)	BYMV	BYMV severe yellow mosaic strain	*Phaseolus vulgaris*	syst. symptoms	none
	BCMV	yellow mosaic strain	*Phaseolus vulgaris*	syst. symptoms	retardation
	BYMV severe yellow mosaic strain	BYMV necrotic lesion strain	*Phaseolus vulgaris*	loc. symptoms	none
Zaumeyer and Fischer (1953)	BYMV	BYMV necrotic lesion strain	*Phaseolus vulgaris*	loc. symptoms	almost complete
				syst. back inoc.	virus recovered
	BCMV	necrotic lesion strain	*Phaseolus vulgaris*	loc. symptoms	complete or almost
				syst. back inoc.	complete, virus recovered
Goodchild (1956)	PMV[1]	BYMV	*Vicia faba*	back inoc.	complete
Corbett (1957)	BYMV	BYMV	*Crotalaria spectabilis*	syst. symptoms	complete
Skotland and Burke (1961)	BCMV	BWMV[2]	*Phaseolus vulgaris*	back inoc.	virus recovered
	BWMV[2]	BYMV	*Phaseolus vulgaris*	back inoc.	virus recovered
Zschau (1961)	Lup M V[8]	Lup MV	*Vicia faba*	back inoc.	partial
Quantz (1961)	SBMV[4]	BCMV	*Phaseolus lathyroides*	loc. symptoms	clear reduction
	BYMV	BCMV	*Phaseolus vulgaris* ("Schalentest")	loc. symptoms	clear reduction
	BYMV	SBMV	("Schalentest")	loc. symptoms	clear reduction
Zaumeyer and Goth (1963)	BYMV type strain	RCNV	*Phaseolus vulgaris*	syst. symptoms	complete
	BYMV pod distorting strain	RCNV[5]	*Phaseolus vulgaris*	syst. symptoms	complete

[1] PMV = pea mosaic virus
[2] BWMV = bean western mosaic virus
[3] Lup. MV = Lupine mosaic virus
[4] SBMV = soybean mosaic virus
[5] RCNV = red clover necrosis virus

Tabel 12. Literatuuroverzicht van premunitieproeven met stammen van bonescherpmozaïekvirus of nauw verwante virussen.

length. A complicating factor seems to be the unexpected influence of host species on particle length of BYMV B 25, as was earlier found for BYMV by Taylor and Smith (1968) (in legumes 742–756 mμ and in *C. amaranticolor* 794–800 mμ). Extremely striking was the great length (about 840 mμ) and rigidity of virus particles of my pea mosaic virus isolates especially PMV E 198. A somewhat similar difference between BYMV (about 750 mμ) and pea mosaic virus (about 790–800 mμ) was found by Taylor and Smith (1968), who nevertheless concluded that they were strains of one virus. A less pronounced difference in particle length between a sorghum isolate (784 mμ) and a surgarcane isolate (753 mμ) of the sugarcane mosaic virus (von Wechmar and Hahn, 1967) and between this virus (uneven length 650–750 mμ) and maize dwarf mosaic virus (775 mμ) (Taylor and Pares, 1968) was also not considered to be sufficient to warrant the isolates concerned being regarded as different viruses. However, the great length and rigidity of PMV E 198 necessitates reconsideration of the identity of this pea mosaic virus isolate, and of the bearing of particle length on virus classification.

Thus in conclusion, it seems as if biologically as well as in other respects, borderlines, for long supposed to exist between the members of a certain morphological group, fade (1) the more such viruses are studied in detail, (2) the more strains of these viruses are distinguished, and (3) the more related viruses are described. Finally, all criteria for drawing borderlines, for delimiting taxonomic units within the morphological groups are failing. There is increasing evidence that this does not only hold for bean yellow mosaic virus and its close associates, but for other viruses of the potato virus Y group as well. For instance, Hollings (1957), in describing anemone mosaic virus, found the virus to resemble closely turnip mosaic virus, whereas no cross protection could be demonstrated.

An example studied in more detail later is the so-called tobacco etch virus group closely related to potato virus Y. Within this group varying degrees of cross protection exist (Schmelzer et al., 1960) and serological relationships are of gradual nature (Bartels, 1964). Recent discussions, referred to above, on relationships between maize dwarf mosaic virus, sugarcane mosaic virus etc. are of the same character. In recent years similar situations have been found with rod-shaped viruses, like TMV and its relatives (e.g. van Regenmortel, 1967), and with beetle-transmitted polyhedral viruses, like cowpea mosaic virus etc. (e.g. Gibbs et al., 1966a).

Here it should be emphasized also that serology is of relative importance in classifying viruses, not only because of intergrading degrees of relationships, but also because we now know that genetically viruses are polycystronic. That means that e.g. TMV nucleic acid strands may contain about twenty different genes, of which only one or two determine the amino acid configuration, size, and shape of the protein coat. Moreover, serological behaviour is determined by a small part of the total number of amino acid residues only (e.g. van Regenmortel, 1967). Thus, in studying the so-called intrinsic virus characters only a small part of the total genetic information is covered. In this connection Gibbs et al (e.g. 1966a) have advocated the determination of base composition to differentiate further the group of beetle-transmitted polyhedral viruses. However, *base sequence* instead of base composition is of major importance in determining virus behaviour. This sequence, reflected in virus behaviour, so far *can only be studied biologically*. This leads to the conclusion that biological characters remain of considerable importance in identifying viruses.

Results of the research reported here clearly indicate that biologically as well as in intrinsic characters viruses are extremely variable, and this conclusion greatly undermines the hope for a successful Linnaean classification. Like bacteria, viruses seem to reproduce asexually exclusively or preponderantly. Consequently, each mutant if originating or arriving under proper selective conditions may give rise to a new biotype. This may, e.g., explain the extreme variation of soil-borne viruses like tobacco rattle virus, the isolates of which may completely lack mutual serological affinity. So, we may never be able to define a virus species. Theoretically the only way out would be a widening of the species concept to a morphological group because so far no serological or other affinities have been found between such groups. In that case variation within a group or "species" would be somewhat comparable with variation within several fungus species as demonstrated by the existence of "formae", "formae speciales", and "physiological races." Slight differences in length within a group could be covered by the term "forma" – e.g. the difference between potato virus Y (730 mμ) and BYMV (750 mμ) – and "physiological" differences by the term "race" or preferably "strain", Then, however, there would be only few plant "viruses" and an abundance of strains within each "species". Complicated names with suffixes for "forma" and "strain" would result and greatly obstruct written and verbal communication. Moreover, this would not reduce the problem concerning the delimitation of taxonomic units used in practice.

Consequently, borderlines, which are inevitable if "storage and retrieval" of data on virus diseases and their control are to be facilitated, have to be drawn arbitrarily (cf. also Gibbs and Harrison, 1968). Since the differences between the incitants of *Wisteria* vein mosaic, pea necrosis, and pea leafroll mosaic, and BYMV and BCMV are not inferior to those between the latter two viruses and between the "viruses" closely related to tobacco etch virus, it is proposed that the first three should be considered different viruses.

Relationships between pea leafroll mosaic virus and the "seed-borne pea leafroll virus" (Musil, 1966) and "pea seed-borne mosaic virus" (Inouye, 1967) will have to be studied further. Independently of my investigations, Conti and Lovisolo (1970) have supported the individuality of their Italian *Wisteria* vein mosaic virus and its close relationship to the Dutch isolate. Their cross-protection tests between the Italian isolate and BYMV were also negative.

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Samenvatting

De identificatie van drie nieuwe virussen geïsoleerd uit Wisteria en Pisum in Nederland en het probleem van de variabiliteit binnen de aardappel-Y-virusgroep

Drie nieuwe in Nederland voorkomende ziekten van vlinderbloemigen worden beschreven, te weten *Wisteria*-nerfmozaïek, dat vrij algemeen bij de sierplant blauwe regen voorkomt (Fig. 1), erwtenecrose, slechts éénmaal geconstateerd (Fig. 2), en een met zaad overgaand, waarschijnlijk niet zeldzaam erwterolmozaïek (Fig. 3). In deeltjesgrootte en -vorm leken de betrokken virusisolaten op bonescherpmozaïekvirus, maar ze konden in verscheidene toetsplanten gemakkelijk worden onderscheiden.

In de laatste jaren zijn talrijke "nieuwe virussen van vlinderbloemigen" beschreven die verwant zijn aan het bonescherpmozaïekvirus en het bonerolmozaïekvirus (Table 1). Bovendien blijkt dat een toenemend aantal virussen uit de aardappel-Y-virusgroep, zoals slamozaïekvirus, bietemozaïekvirus, watermeloenemozaïekvirus en zelfs een knollemozaïekvirus ("turnip mosaic virus"), in staat is onder natuurlijke omstandigheden vlinderbloemigen aan te tasten. Op de een of andere wijze zijn al deze virussen verwant aan het bonescherpmozaïekvirus. Om een inzicht te krijgen in de aard en mate van deze verwantschappen zijn de drie nieuw ontdekte isolaten vergeleken met een normale stam van het bonescherpmozaïekvirus en met erwtemozaïekvirus en verder met "clover yellow vein virus", "cowpea aphid-borne mosaic virus", twee isolaten van het bietemozaïekvirus en slamozaïekvirus (Tabel 2). Ze bleken alle ettelijke waardplanten en symptomen gemeen te hebben (Tabel 3, Fig. 4–14), waaronder erwte- en bonerassen (Tabel 4 en 5). Onderlinge verschillen waren slechts van graduele aard. Talrijke niet-vlinderbloemigen reageerden op de te identificeren isolaten, vooral op het erwtenecrosevirus (o.a. Fig. 13). Geheel onverwacht werd gevonden dat zelfs de normale stam van het bonescherpmozaïekvirus 17 van de 20 getoetste niet-vlinderbloemigen kon infecteren. Met moeite ging het virus lokaal over op biet, en spinazie werd zelfs systemisch geïnfecteerd. Bij nader inzien blijken in de literatuur meer verspreide meldingen van infectie van niet-vlinderbloemigen voor te komen (Tabel 12). De vorming van celinsluitsels en zelfs van vergrotingen van de nucleolus is niet beperkt tot bonescherpmozaïekvirus en tabaks-etsvirus, een andere vertegenwoordiger uit de Y-virusgroep. Ze werden alleen niet gevonden bij het *Wisteria*-virus en bij slamozaïekvirus. Het erwtenecrosevirus veroorzaakte vergrote en zeer opvallend van talrijke naalden voorziene nucleoli.

Gegevens uit de literatuur en uit dit onderzoek over het niet vatbaar zijn van bepaalde plantesoorten zijn van betrekkelijke waarde, omdat de resultaten afhankelijk zijn van het ras of type van de getoetste plantesoort en de omstandigheden, alsmede van de kwaliteit van het inoculum en van de combinatie donor-acceptor (virusbron-toetsplant) (Tabel 6).

De gevonden verschillen in bestendigheid van het infectievermogen in uitgeperst sap waren voor enkele isolaten slechts gering (Tabel 7). Premunitieproeven bleken nauwelijks te helpen bij het bepalen van onderlinge verwantschappen (Tabel 8 en 12).

De in samenwerking met de heer D. Z. Maat bereide antisera tegen de isolaten uit *Wisteria* en necrotische erwt en de normale stam van het bonescherpmozaïekvirus, alsmede een beschikbaar antiserum tegen bonerolmozaïekvirus toonden het bestaan van duidelijke verwantschappen, maar ook van niet geringe verschillen aan (Tabel 9).

In de elektronenmicroscoop waren de drie nieuwe virusisolaten niet van bonescherpmozaïekvirus te onderscheiden terwijl beide isolaten van bietemozaïekvirus belangrijk korter waren (Tabel 10). Merkwaardigerwijs had het erwtemozaïekisolaat vrijwel rechte deeltjes van aanzienlijk grotere lengte (840 mμ).

Uit dit onderzoek en uit gegevens uit de literatuur kan worden geconcludeerd dat naarmate de bekende virussen meer in detail worden bestudeerd en meer nieuwe virussen en virustammen worden beschreven de grenzen die men lange tijd veronderstelde te bestaan tussen verschillende virussen van een morfologische groep geleidelijk vervagen. Het hier vooral beschreven vervagen van biologische grenzen werpt nieuw licht op het reeds langer bekende bestaan van graduele serologische verwantschappen tussen virussen van een morfologische groep. Hiermee zitten we midden in het probleem van de variabiliteit van de virussen dat ook geldt voor de meer intrinsieke viruseigenschappen. De laatste zijn overigens ook al van betrekkelijke waarde voor de identificatie van virussen, omdat slechts een deel van de totale hoeveelheid genetische informatie van invloed is op deeltjesvorm, deeltjesgrootte en serologische eigenschappen. Biologische eigenschappen zullen daarom van waarde blijven voor de identificatie van virussen.

Virussen schijnen zich overwegend of uitsluitend ongeslachtelijk te vermeerderen of vermeerderd te worden. Daardoor kan iedere mutant, indien ontstaan of terechtgekomen onder selectieve omstandigheden leiden tot een nieuw biotype. Door de enorme variabiliteit der virussen zal het vermoedelijk onmogelijk zijn ooit een soortsbegrip voor virussen te omschrijven. Toch moeten uit praktische overwegingen kunstmatige grenzen worden getrokken. Daar de geconstateerde verschillen tussen de verwekkers van de drie nieuwe ziekten en het bonescherpmozaïekvirus niet onderdoen voor die tussen laatstgenoemd virus en bonerolmozaïekvirus en tussen de nauw aan tabaks-etsvirus verwante virussen, worden de nieuwe verwekkers als aparte virussen beschouwd, hoewel ze onderling en aan het bonescherpmozaïekvirus nauw verwant zijn.

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