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The identification of bean mosaic, pea yellow mosaic and pea necrosis strains of bean yellow mosaic virus

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Abstract

Twelve virus isolates from pea, broad bean, red clover and yellow lupin have been compared with the B25 strain of bean yellow mosaic virus (BYMV-B25), the E198 strain of pea mosaic virus (PMV-E198) and the pea necrosis virus (E178), which were described earlier (Bos, 1970).

On the basis of host ranges, symptoms and bean and pea varietal reactions most isolates could be classified into three groups, representatives of which did not differ appreciably serologically. These groups were considered to be typical *bean yellow mosaic virus* isolates (E212, L1, B25), *pea yellow mosaic strain* isolates of BYMV (E198, E204, Kow28) and *pea necrosis strain* isolates of BYMV (E197, E199, E221). From these results and from a survey of literature it is concluded that PMV is only a strain of BYMV.

The pea necrosis virus (E178), described earlier as a distinct entity, is still considered a different virus. A severe pea necrosis isolate (Kow14) resembled E178 in many respects and was also more distantly related serologically to the BYMV isolates tested. Four other virus isolates from pea and broad bean (E196, Vf15, Vf18 and Vf30) could not yet be identified. Lettuce mosaic virus (LMV) was found to be serologically rather closely related to BYMV.

Results of cross-protection tests were erratic, and particle length measurements were no help in differentiating the strains and viruses studied.

Introduction

Recently, Bos (1970) identified three new viruses closely related to bean yellow mosaic virus (BYMV; cryptogram */*:*/*:E/E:S/Ap). He then pointed to the problem of variation within the potyvirus group, especially among viruses related to BYMV. In the present study more attention is paid to this variation. A number of virus isolates, with similar particle size and shape, but inducing green mosaic, yellow mosaic or necrosis in pea respectively, are compared with the B25 strain of BYMV, the pea mosaic isolate E198, and the pea necrosis virus (E178) described earlier (Bos, 1970), and further investigated to ascertain whether they are strains of one virus or separate viruses. An important question is whether pea mosaic virus, as an entity distinct from BYMV, does exist.

Certain isolates studied here and some other members of the potyvirus group are currently being biophysically characterized in our department. Part of this work has already been published (Huttinga, 1973; Huttinga and Mosch, 1974).

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Review of the literature

Around 1930 several mosaic diseases were described in various crops and their incitants differentiated primarily on the basis of symptoms and host ranges. In legumes, bean yellow mosaic virus (BYMV) was one of the first to be rather well identified (Pierce, 1934). Since the work of Doolittle and Jones (1925), Pierce (1935) and Murphy and Pierce (1937) it has been generally accepted that (common) pea mosaic virus (PMV) is a distinct entity, also causing mosaic in broad bean (Böning, 1927; Merkel, 1929) and in red clover (Zaumeyer and Wade, 1935), and 'sore shin' in yellow lupin (Chamberlain, 1935). For more details on the older literature see e.g. Weiss (1945), Goodchild (1956a) and Zschau (1961).

One of the main distinctions between BYMV and PMV was that the latter virus was not able to infect *Phaseolus* beans. However, every now and then it was reported that PMV could infect beans (Zaumeyer and Wade, 1935; Johnson and Jones, 1937) although it was much less pathogenic to bean than normal BYMV (Schroeder and Provvidenti, 1966; Taylor and Smith, 1968). It was also found that 'Perfection' type peas are resistant (immune) to BYMV (Pierce, 1934; Hagedorn, 1951; Yen and Fry, 1956) as well as to PMV (Pierce, 1935), and that resistance to both viruses was conditioned by the same recessive gene (Barton et al., 1964; Cousin, 1965).

Serologically, Goodchild (1956b) and Taylor and Smith (1968) could not distinguish between BYMV and typical isolates of PMV. Bercks (1960) found an isolate from pea resembling PMV to differ serologically but to be closely related to three typical BYMV isolates and only distantly to a bean common mosaic virus (BCMV) isolate. Varma and Gibbs (1967) and Hull (1968) could serologically distinguish their BYMV and PMV isolates. The first authors considered them to be different viruses. Tapio (1970) found no appreciable differences between her BYMV and PMV and an isolate of BCMV.

So far, cross protection tests with these viruses have been rather erratic (Goodchild, 1956b; Hull, 1968; Bos, 1970).

The general confusion arising from vague distinction between BYMV and PMV is further exemplified by publications on mosaic of yellow lupin. The disease has been ascribed to a special lupin mosaic virus (Mastenbroek, 1942) to BYMV (Corbett, 1958; Zschau, 1961; Blaszczak, 1963) and to PMV (Ksiazek, 1962 a, b).

Infection of pea with normal BYMV is usually causing a green mosaic (e.g. Hagedorn and Walker, 1950) differing from the striking yellow mosaic common for PMV. In a few instances severe necrosis in pea has been ascribed to BYMV (Zaumeyer and Goth, 1963: red clover necrosis strain of BYMV; Cousin, 1969: a strain of BYMV), to PMV isolates (Tapio, 1970), or to a special pea necrosis virus (Bos, 1969a, 1970), but it should also be remembered that some eight different viruses have been reported to cause necrotic 'streak' in pea (cf. Bos, 1969a).

Materials and methods

Isolates and maintenance. A number of virus isolates in our collection of viruses stored in desiccated infected leaf material over $CaCl_2$, and previously found to resemble BYMV and PMV in host reaction and particle morphology, as well as three apparently related isolates from pea (Kow14), red clover (Kow28) and yellow lupin (L1), all

Table 1. List of virus isolates studied.

Code	Original host	Symptoms or references
B25	French bean	see Bos (1970) (bean yellow mosaic virus)
E178	pea	see Bos (1970) (pea necrosis virus): necrosis
E196	pea	mosaic, some stem and pod necrosis
E197	pea	severe stem and tip necrosis
E198	pea	see Bos (1970) (pea mosaic virus): yellow mosaic
E199	pea	yellow mosaic
E204	isolated from E199	
E212	pea 'Supcovert'	vein necrosis, weak discoloration of stems and pods
E221	pea, breeding line	mosaic
Kow14	pea	mosaic
Kow28	red clover	mosaic
L1	yellow lupin 'Bas'	stunting and leaf narrowing
Vf15	broad bean	mosaic, pods with necrotic spots and flecks
Vf18	broad bean 'Express'	mosaic
Vf30	broad bean	mosaic
LMV	lettuce	see Bos (1970) (lettuce mosaic virus)
PVY^N	potato 'Record'	provided by Dr J. A. de Bokx (potato virus Y^N)

Tabel 1. Lijst van bestudeerde virusisolaten.

from Poland, were selected for study. For comparison the isolates B25 and E198, pea necrosis virus (E178) and lettuce mosaic virus (LMV), described earlier (Bos, 1970), were included. PVY^N was added in the serological tests as a more distant member of the potyvirus group. All isolates are listed in Table 1.

Virus purification. B25, E198, E221, Kow14, Kow28 and LMV were purified from *Pisum sativum* 'Koroza' and PVY^N from *Nicotiana tabacum* 'Samsun NN' by the method described by Huttinga (1973), who kindly provided us with preparations of E221, Kow14 and Kow28. For antiserum preparation and/or serological testing B25, E198, LMV and PVY^N were further purified by rate zonal centrifugation in a zonal rotor as described by Maat and Vink (1971), but centrifuging lasted for 1 h instead of 2 h, and phosphate-citric acid buffer was replaced by tris-HCl buffer at pH 9. The final preparations were mixed with glycerol (1:1) before storage at -20 °C.

Antiserum preparation. Rabbits were given two successive intravenous injections (2 day interval). An emulsion of equal parts of virus and Freund's incomplete adjuvant was injected intramuscularly two weeks later. Bleeding was begun 2–3 weeks after the final injection. If further immunization was necessary, additional intravenous and/or intramuscular injections were administered.

Serological method. The micro-precipitin test under paraffin oil was employed, using purified or partially purified virus preparations. Dilution series of antisera and antigens were prepared with saline, containing 0.05% NaN_3. Antisera were absorbed with concentrated extracts from non-inoculated pea plants to eliminate antibodies to host components. Reactions were recorded after 24 h at room temperature. Antisera

Table 2. Summary of host range tests.

	I			II											
				a			b					c			
	B25	E212	L1	E198	E204	Kow28	E197	E199	E221	E178	Kow14	E196	Vf15	Vf18	Vf30
legumes															
Lathyrus odoratus	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Lupinus albus 'Kali'	-^{x}S	LS	LS	-^{x}S	(L)S	-^{x}S	LS	LS	LS	(L)S	(L)S	LS	LS	LS	(L)S
Lupinus angustifolius 'Obornicki'	-^{x}S	-^{x}S	-^{x}S	-^{x}S	-^{x}S	-^{x}S	-^{x}S	-^{x}S	-^{x}S	-^{x}S	-^{x}S	-^{x}S	-^{x}S	-^{x}S	-^{x}S
Phaseolus vulgaris 'Bataaf'	LS	LS	LS	LS	LS	(L)(S)	(L)s	L(S)	(L)s	LS	--	L(S)	L-	LS	(L)-
'Top Crop'	LS	LS	LS	ls	LS	(L)S	L-	Ls	LS	LS	--	LS	Ls	LS	l-
'Double White Princess'	LS	LS	LS	LS	LS	LS	L-	L-	L-	L-	--	LS	LS	LS	(L)(S)
'Red Kidney'	LS	LS	LS	(L)S	LS	L-	L(S)	L(S)	L-	LS	l-	ls	L-	L-	--
'Fana'	LS	LS	ls	LS	LS	(L)-	L-	L-	L-	L-	--	l-	l-	l-	l-
'Pinto 111'	LS	LS	LS	L-	L-	L-	L-	L-	L-	L-	L-	L-	L-	L-	L-
'Imuna'	LS	LS	lS	L-	L-^{x}	L-^{x}	L-^{x}	L-^{x}	L-^{x}	L-^{x}	L-^{x}	L-^{x}	L-^{x}	L-^{x}	L-^{x}
'Jubila'	LS	LS	LS	l-	l-	l-	L-	L-	L-	LS	l-	L-	l-	l-	--
'Prince'	LS	LS	ls	--	L-	(L)-	L-	L-	--	--	--	L-	L-	L-	--
'Great Northern 123'	LS	LS	LS	L-	L-	L-	L-	L-	L-	--	L-	L-	L-	L-	L-
'Amanda'	LS	LS	-S	L-	l-	L-	L-	L-	L-	--	L-	L-	L-	l-	L-
'Jolanda'	Ls	L-	LS	L-^{x}	L-^{x}	L-	L-^{x}	L-^{x}	L-^{x}	L-^{x}	L-^{x}	L-^{x}	L-^{x}	L-^{x}	L-^{x}
Pisum sativum 'Cobri'	-^{x}S	-^{x}S	-^{x}S	-^{x}S	-^{x}S	-^{x}S	-^{x}S	-^{x}S	-S	-^{x}S	LS	-^{x}S	-^{x}S	-^{x}S	-^{x}S
'Dik Trom'	-^{x}S	-^{x}S	-^{x}S	-^{x}S	-^{x}S	-^{x}S	-^{x}S	-^{x}S	-S	-^{x}S	LS	-^{x}S	-^{x}S	-^{x}S	-^{x}S
'Koroza'	-^{x}S	-^{x}S	-^{x}S	-^{x}S	-^{x}S	-^{x}S	(L)^{x}S	(L)S	(L)S	(L)S	LS	-^{x}S	-^{x}S	-^{x}S	-^{x}S
'Rondo'	-^{x}S	-^{x}S	LS	-^{x}S	-^{x}S	-^{x}S	(L)S	(L)S	LS	(L)S	LS	L^{x}S	-^{x} S	-^{x}S	-^{x}S
'Mignon'	-^{x}-	-^{x}-	-^{x}-	-^{x}-	-^{x}-	-^{x}-	-^{x}-	-^{x}-	-^{x}-	-^{x}-	--	-^{x}-	-^{x}-	-s	-^{x}-
'Juwel'	-^{x}-	-^{x}-	-^{x}-	-^{x}-	-^{x}-	-^{x}-	-^{x}-	-^{x}-	-^{x}-	-^{x}-	--	-^{x}-	-^{x}s	-s	-^{x}-
'Relonce'	-^{x}-	-^{x}-	-^{x}-	-^{x}-	-^{x}-	-^{x}-	-^{x}s	-^{x}-	-^{x}-	-^{x}-	--	-^{x}-	-^{x}-	-s	-^{x}-
Trifolium incarnatum	-^{x}S	-^{x}S	-^{x}S	-^{x}S	-^{x}S	-^{x}S	-^{x}S	-^{x}S	-^{x}S	-^{x}S	-^{x}S	-^{x}S	-^{x}S	-^{x}S	-^{x}S
Trifolium pratense	-^{x}-	-^{x}-		-^{x}S	-^{x}s	L^{x}S	-^{x}-	-^{x}s	-^{x}s	-^{x}-	-^{x}s	Ls	LS	-s	-^{x}-
Trifolium repens	-^{x}-	-^{x}-		-^{x}-	-^{x}s	-^{x}-	-^{x}-	-^{x}s	-^{x}-	-^{x}-	-^{x}s	-^{x}-	-^{x}-	-^{x}s	-^{x}-
Vicia faba 'Compacta'	-^{x}S	L^{x}S	-^{x}S	-^{x}S	-^{x}S	-^{x}S	(L)^{x}S	(L)^{x}S	(L)^{x}S	L^{x}S	L^{x}S	L^{x}S	-^{x}S	-^{x}S	-^{x}S
non-legumes															
Chenopodium amaranticolor	LS	LS	LS	L-	L-	L-	L-	L-	L-	L-	L-	L-	L-	L-	L-
Chenopodium quinoa	L-	L-	LS	L-	L-	L-	L-	L-	L-	LS	L-	L-	L-	L-	L-
Cucumis sativus 'Gele Tros'	--	--	--	--	--	l-	--	l-	l-	L-	L-	l-	l-	l-	--
Gomphrena globosa	L-	L-		Ls	Ls	L-	L-	L-	--		Ls	L-	L-	--	L-
Lactuca sativa 'Attractie'	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
'Portato'	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Nicotiana clevelandii	-^{x}S	-^{x}s	-^{x}s	-^{x}S	-^{x}-	-^{x}-	-^{x}l	-S	-S	-^{x}S	-^{x}S	-^{x}S	-^{x}-	-S	-^{x}-
Nicotiana glutinosa	--	--		l-	l-	l-	-^{x}-	l-	l-		--	l-	--	--	--
Nicotiana tabacum 'White Burley'	L-	l-	l-	L-	l-	l-	Ls	ls	L-	L-	L-	L-	--	ls	--
Petunia hybrida	l-	--		l-	--	--	--	--	l-		l-	l-	--	--	--
Spinacia oleracea 'Noorman'	(L)s	LS		l-	--	l-	LS	ls	LS		--	LS	ls	ls	--

l = latent local infection L = visible local infection - = no infection as tested by back inoculation

s = latent systemic infection S = visible systemic infection -^{x} = no symptoms, no check by back inoculation

() symbol in parentheses = in other case(s) no infection

Tabel 2. Samenvatting van het waardplantenonderzoek.

against English isolates of BYMV (No 226) and PMV (No 227), both obtained from gladiolus, were kindly supplied by Dr M. Hollings.

Results

Host range and symptoms. The results of host range tests are summarized in Table 2. Since pea and French bean were found helpful to differentiate the isolates, a number of cultivars were tested. Results of these tests are also included in Table 2.

In this table the isolates have been grouped on the basis of their type of reaction in pea and French bean. Group I easily infected systemically all bean varieties tested, usually causing mosaic. With group II several bean varieties were immune or showed a local reaction (often necrotic local lesions) only, whereas systemic symptoms, if produced (mainly with IIa), were a weak systemic spotting. The isolates of group I always caused a mild green mosaic on susceptible peas. Group II could be subdivided mainly on the basis of symptoms in pea: bright mosaic (IIa), necrosis (IIb), and green mosaic (IIc). This greatly corresponded with the type of reaction in broad bean (with the exception of Vf18 and Vf30 of IIc). Two members of group I (B25 and L1) were the only isolates regularly going systemic in *C. amaranticolor*. There was no clear correlation between the grouping and susceptibility and type of symptoms in the other test plants. The plant species and cultivars which are especially useful to 'key out' the respective groups have also been mentioned, and their reactions summarized in Table 6.

Some details will now be given on the reaction of certain differential hosts.

The pea (*Pisum sativum*) cultivars Cobri, Dik Trom, Koroza and Rondo reacted to all isolates. With some isolates necrotic local lesions were produced. With the isolates of group I and IIc vein chlorosis was followed by an often inconspicuous green mosaic (Fig. 1A). In IIa the mosaic was always bright and yellow (Fig. 1B), reducing plant and leaf size. In IIb, however, these pea cultivars reacted with a drastic necrotic reaction. Initial systemic vein chlorosis was followed by yellowing, wilting and withering of inoculated leaves, curling of tip leaves, browning of stems and premature death (Fig. 3). With E221 internodes were also shortened. Kow14 usually killed the plants in 3–4 weeks.

The cultivars Juwel, Mignon and Relonce did not react to any of the isolates. With Vf18 all three attained a latent systemic infection, as was the case with E197 in 'Relonce' and with Vf15 in 'Juwel'.

All 12 French bean cultivars (*Phaseolus vulgaris*) tested were susceptible to the 3 isolates of group I. In Table 2 the bean cultivars have been arranged according to their sensitivity to BYMV, the last three ('GN. 123', 'Amanda' and 'Jolanda') being known as more or less resistant. In most cultivars, especially the sensitive ones, severe symptoms were produced by group I, with the exception of L1 in 'Fana'. 'Jolanda' did not become systemically infected with E212. Symptoms usually consisted of epinasty and chlorotic and sometimes necrotic local lesions in primary leaves, followed by systemic mottle or mosaic, leaf curling or malformation and often by stunting.

Most isolates of the other groups (IIa, b, and c) only produced systemic infection in the more BYMV-sensitive cultivars, with the exception of E178 causing systemic symptoms in 'Jubila', and Kow14 not going systemic in any bean cultivar. With isolates of IIa and c, symptoms, if produced, were a more or less distinct mosaic or spotting, and a more severe curling, yellowing or mosaic associated with systemic chlorotic or necrotic lesions or irregular vein necrosis usually followed by defoliation and premature plant death with IIb. With this group local lesions tended to be more necrotic than with the other groups.

Thus, isolates of all groups are able to infect certain bean cultivars. However, those of group II are clearly less agressive to bean and those of IIa also less virulent to susceptible bean cultivars.

'Double White Princess' was especially helpful in distinguishing between the groups. With all members of group I chlorotic local lesions were soon followed by severe systemic necrosis leading to

Fig. 1. *Pisum sativum* 'Rondo' with green mosaic (A) and yellow mosaic (B) 24 days after inoculation with E212 (A) and Kow28 (B); C, healthy control.

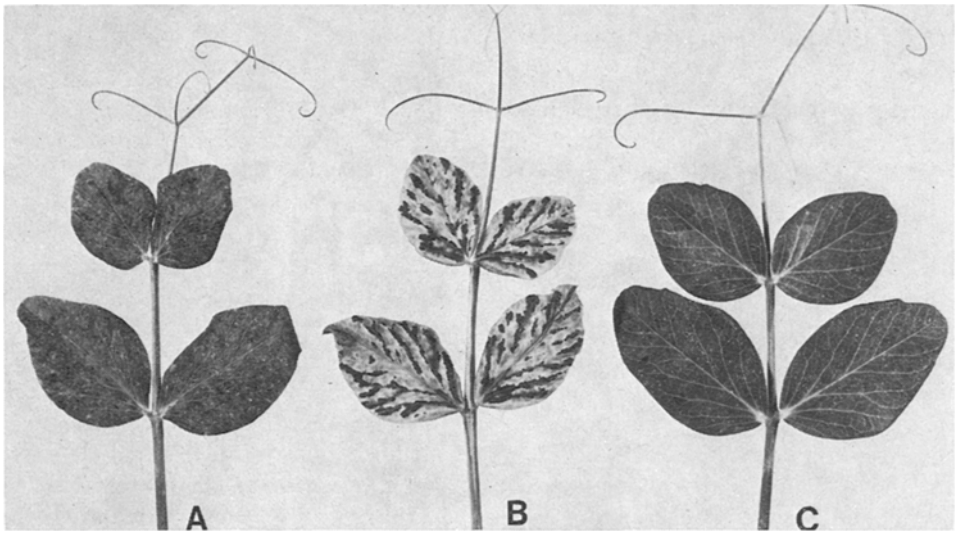


Fig. 1. Pisum sativum 'Rondo' met groen mozaïek (A) en geel mozaïek (B) 24 dagen na inoculatie met E212 (A) en Kow28 (B); C, gezonde controle.

killing of the tips of the plants. With IIa local lesions tended to be more necrotic, and mainly consisted of small pieces of vein necrosis or necrotic veinbanding, gradually followed by some chlorotic systemic spotting and malformation and in winter at a later stage by tip necrosis. With IIb local lesions were like those of IIa but slightly more necrotic and no systemic infection followed. With IIc reactions were like those of IIa.

Broad bean (*Vicia faba*) 'Compacta' was sensitive to all isolates. Chlorotic local lesions were produced by E212 (I) and E196 (IIc) and necrotic local lesions by all members of group IIb. Systemic symptoms were a green mosaic in groups I and IIc (but Vf18 and Vf30), a bright yellow green mosaic in IIa, and in IIb vein chlorosis and curling of top leaves was followed by top and stem necrosis, leaf wilting and premature death (Fig. 2) or sometimes some partial recovery by production of side shoots with severe leaf malformation and mottling.

Red and white clover (*Trifolium pratense* and *T. repens*) were hard to infect, but crimson clover (*T. incarnatum*) was susceptible to all isolates and always reacted with mosaic symptoms.

Symptoms in *Lathyrus odoratus* did not coincide with the grouping of isolates. With E197, E199, Kow14, and E196 the inoculated leaves dried out and were cast, and plants usually died prematurely. With E212, B25, Kow28, E198, E204 and Vf15 a rather weak mosaic and with E221, Vf18 and Vf30 a green mosaic was produced.

In white lupin (*Lupinus albus*) 'Kali', 11 isolates produced small necrotic local lesions on inoculated cotyledons and foliage leaves. Systemic infections first caused vein chlorosis, small necrotic lesions in tip leaves and later tip and stem necrosis and early plant death with all isolates, but those of group IIa, Kow14 and Vf30. Lateron symptoms usually were milder, consisting of leaf chlorosis and narrowing and growth reduction.

Lupinus angustifolius 'Obornicki' was highly sensitive to all isolates. In 2–3 weeks plant stunting and wilting of tip leaves was followed by stem necrosis and plant death. Kow14 and E178 differed in causing chlorosis in tip leaves followed by leaf dropping and yellowing and extreme leaf narrowing of tip leaves. With Vf15 a shock reaction was followed by some recovery with leaf narrowing and plant stunting.

Fig. 2. *Vicia faba* 'Compacta' 14 days after inoculation with E221. One plant shows a retarded systemic infection.

Fig. 2. Vicia faba 'Compacta' 14 dagen na inoculatie met E221. Eén plant vertoont een vertraagde systemische infectie.

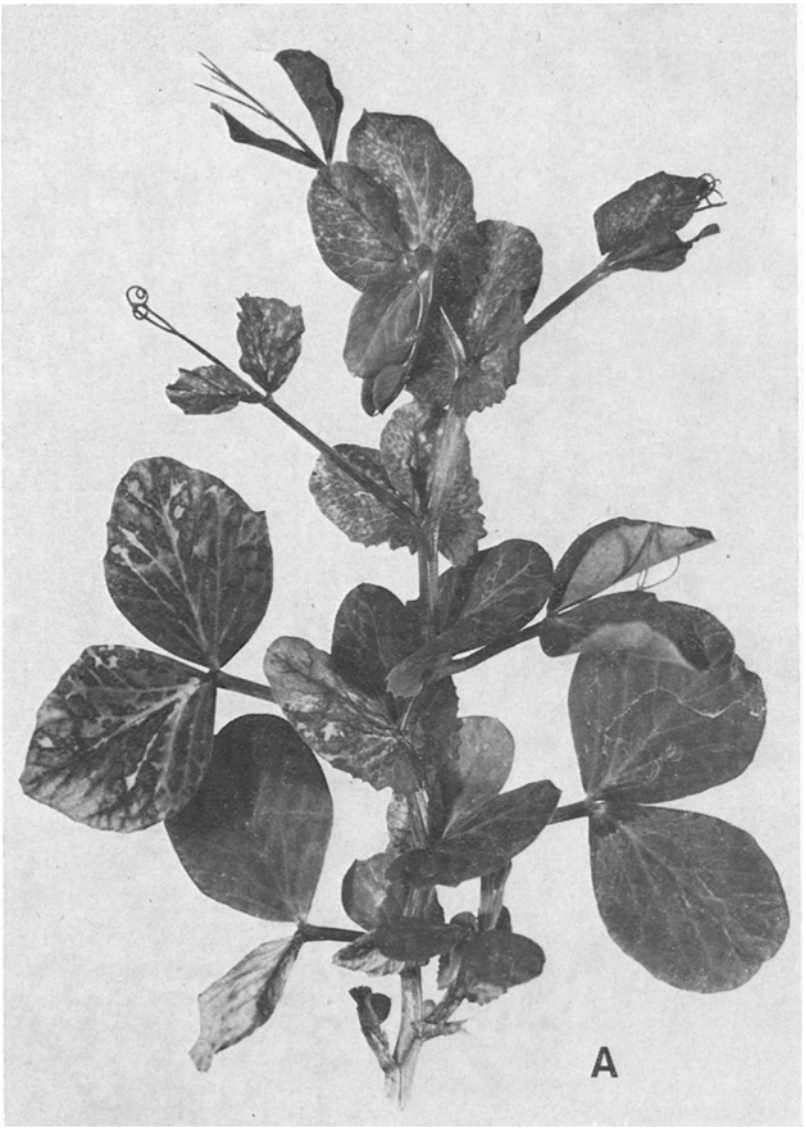
In *Chenopodium amaranticolor* all isolates produced yellow and/or necrotic local lesions in about a week. Only with L1 and B25 and often with E212 a systemic vein mosaic and leaf distortion followed; then local lesions tended to enlarge irregularly and to extend into the venation. With L1 systemic reaction was very severe (Fig. 4). In *C. quinoa* all isolates gave yellow local lesions. With E178 systemic yellow spotting (cf. also Bos, 1970) and with L1 more severe systemic vein mosaic and leaf malformation followed. With E212 sometimes systemic symptoms were produced.

In *Cucumis sativus* 'Gele tros', Kow14 and E178 were the only isolates producing local symptoms. With Kow14, these consisted of numerous chlorotic spots whereas with E178 few small, often pinpoint chlorotic lesions were formed (Bos, 1970: Fig. 13B). Six isolates gave a latent local infection. Plants were immune to group I.

Nicotiana tabacum 'White Burley' was locally susceptible to most isolates but Vf15 and Vf30. A few vague chlorotic local lesions were found with some members from all groups and all of group IIb except E199. In this group two isolates gave a latent systemic infection as did Vf18. In *N. glutinosa* most members of IIa and b and E196 gave a latent infection only. *N. clevelandii* easily contracted systemic infection with most isolates, often producing more or less pronounced mottling and some stunting, but rather severe symptoms with all members of IIb, except E197 giving a latent infection only. According to Bos (1970) numerous necrotic local lesions were formed with E178.

In *Spinacia oleracea* 'Noorman' few vague chlorotic local lesions were found with some isolates from most groups. Systemic symptoms, if produced, were indistinct.

Fig. 3. *Pisum sativum* 'Koroza' with severe necrosis 22 (A) and 31 days (B) after inoculation with E197.



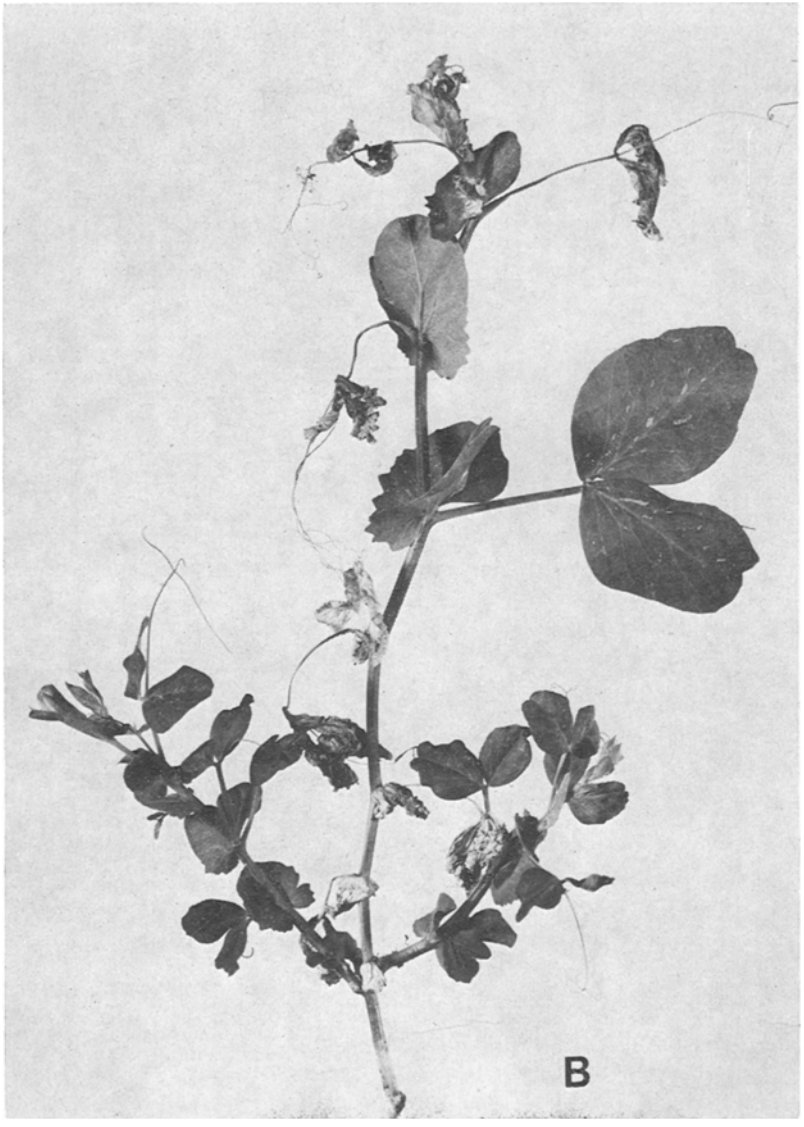


Fig. 3. Pisum sativum 'Koroza' met ernstige necrose 22 (A) en 31 dagen (B) na inoculatie met E197.

Fig. 4. *Chenopodium amaranticolor* (A) and *C. quinoa* (B) with systemic symptoms 21 days after inoculation with L1.

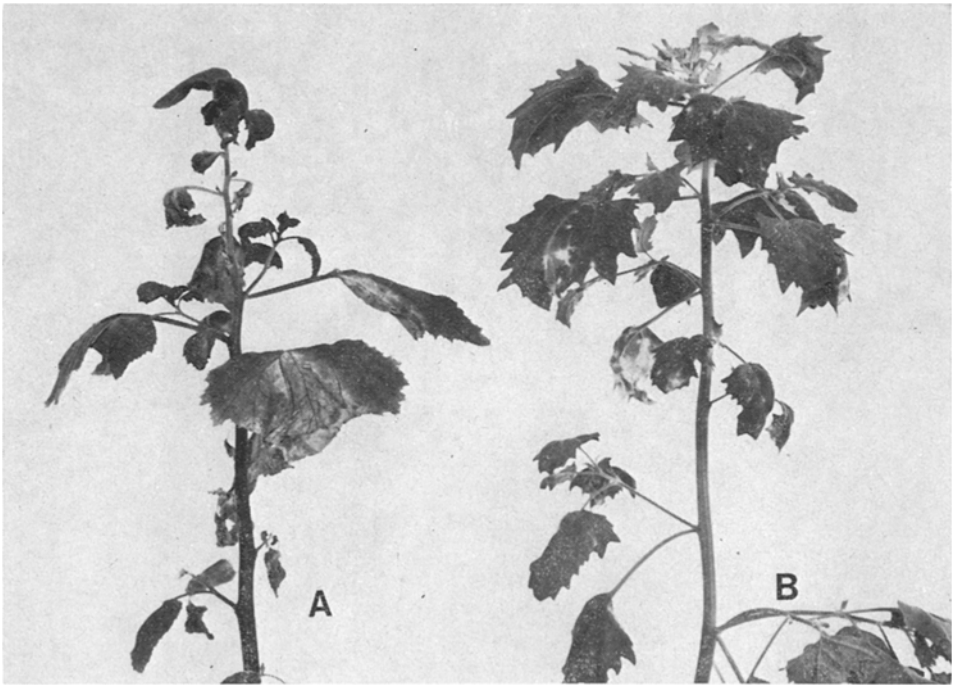


Fig. 4. Chenopodium amaranticolor (A) en C. quinoa (B) met systemische symptomen 21 dagen na inoculatie met L1.

Inclusion bodies. All fifteen isolates induced formation of granular cytoplasmic inclusions typical for BYMV (Bos, 1969b, 1970), which were rapidly detectable by using phloxine and methylene blue in Christie's solution. In addition to these structures, isolate Kow14 induced nucleolar angularity and enlargement. Isolate E178 induced characteristic crystalline needles radiating from the enlarged nucleolus into the nucleoplasm.

Cross-protection tests. Mutual relationships between some isolates were investigated by testing their cross-protecting ability in various combinations. As challenge virus, isolates were chosen which could be easily recognized by their symptoms (yellow mosaic with E198 and Kow28, and necrosis with Kow14 and E221), or which could easily be detected by back inoculation. Further details on the experiments and the results are summarized in Table 3.

In two instances (exp. III4 and IV5), characteristic symptoms of the challenge virus were not produced in any of the plants super-inoculated, but in exp. IV5 the challenge virus could easily be detected in all 9 plants back-inoculated. Similarly, Kow14 did not show up with symptoms in most of the plants super-inoculated (exp. II2, 3, 4) but it could be easily detected in all plants tested by back inoculation. So, in all these instances protection was a suppression of symptoms only, perhaps because of a

Table 3. Summary of cross-protection tests.

Experiment number	Pea cultivar	Protecting virus	Challenge virus	Time interval in days	Effect of challenge virus on super-inoculated plants: systemic symptoms[1]		back inoculation[2]		Cross protection
I,1	Rondo	Kow28	E212	10	–3	(16/16)	*Phaseolus vulgaris* p 2/4,	s 8/16	8/16+[4]
2		E212	Kow14		11/16	(16/16)	pea from 5 plants without symptoms of Kow28	s 1/5	4/16+
II,1	Koroza	E212	E198	9	10/13	(13/13)	pea	s 0/3	3/13+
2		E212	Kow14		4/13	(12/12)	cucumber	s 2/6	
							pea	s 6/6[5]	0/13±[6]
3		B25	Kow14		4/13	(12/12)	cucumber	6/9	
							pea	9/9[5]	0/13±
4		E198	Kow14		7/13	(12/12)	cucumber p 3/4, pea	s 12/12	0/13±
III,1	Koroza	Vf15	E198	10	16/16	(16/16)			0/16–
2		E198	Vf15		–	(16/16)	*Lupinus albus*	s 6/9	3/9 +
3		E197	E198		16/16	(16/16)			0/16–
4		E198	E197		0/16	(16/16)			16/16+
IV,1	Rondo	E196	E198	10	16/16	(16/16)			0/16–
2		E198	E196		–	(16/16)	pea s 6/8, *L. albus* s 5/9, *V. faba*	8/8	–
3		E196	E221		16/16	(16/16)			0/16–
4		B25	E221		12/16	(16/16)	pea from 3 plants	s 0/3	4/16+
5		E198	E221		0/16	(16/16)	pea from 9 plants s 9/9, *V. faba*	s 9/9	0/16±
V,1	Rondo	Vf15	E221	9	10/16	(16/16)	pea and *V. faba*	s 2/6	4/16+
2		E212	E221		16/16	(16/16)			0/16–
3		Kow28	E221		11/15	(16/16)	pea and *V. faba*	s 1/4	3/15+
4		Kow28	Vf15		–	(16/16)	*P. vulgaris*	s 0/12	
							L. albus	s 8/12	4/12+
5		Vf15	Kow28		16/16	(16/16)			0/16–

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

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

[3] Presence of challenge virus not to be judged visually.

[4] Number of plants free of challenge virus as judged by symptoms and/or back inoculation over number of plants super-inoculated.

[5] Tests for inclusion bodies characteristic of Kow14 revealed these in all 6 and 9 plants tested.

[6] ± means retarded or weakened symptom production by challenge virus.

Tabel 3. Samenvatting van de premunitieproeven.

suppression of virus multiplication. In exp. II, 2, III, III2, IV4, and V1, 3, 4 a positive cross protection was obeserved in part of the plants.

In 'Koroza' plants of exp. III3 showing necrosis after protective infection with E197, plants super-inoculated with the yellow isolate E198 later produced new outgrowth with bright yellow mosaic characteristic of E198 instead of necrosis. In the

Table 4. Survey of particle length measurements.

Virus isolates		Host	Nr. of preparations	Nr. of photographs	Micrometer classes[1]				Total number of TMV particles	Micrometer classes[1]							Total number of flexuous particles	Peak lenght[2]
					28	30	32	34		78	80	82	84	86	88	90		
I	B25	*V. faba*	1	17		*23*	*40*	2	65		7	*25*	*23*	14	1		70	795
	E212	*V. faba*	2	18	6	*84*	*54*	5	149	2	5	22	*22*	25	4	1	81	820
	E212	pea	1	26		*106*	*166*	3	275			13	*24*	*32*	11	1	111	810
	L1	pea	1	2		*6*	*11*		17				*3*	*3*			6	810
IIa	E198	*V. faba*	1	15	2	*85*	*43*		130			3	25	*56*	13	1	98	840
	E198	pea	1	11	5	*96*	40	1	142			25	*31*	27	11		94	830
	E198	pea	1	21		*68*	*90*	2	160			4	28	*50*	13		95	830
	Kow28	pea	1	34		*88*	*187*	1	276			2	11	*72*	23		108	820
IIb	E221	pea	2	25	1	*46*	*110*	9	166		1	3	6	*20*	*25*	8	60	830
	E221	pea	1	5		*11*	*12*		23					*3*	*8*		11	840
	E221	pea	1	18	3	*70*	*110*	3	186		1	5	11	*21*	*35*	7	80	815
	Kow14	pea	1	20	3	*47*	*83*	4	137		9	22	*29*	*42*	19	1	122	815
	Kow14	pea	1	18		*38*	*115*		154				5	*31*	*20*	1	57	825

[1] Peak classes are printed in italics.

[2] Peak length is calculated on the basis of TMV length. Final figures are rounded off to multiples of 5.

Tabel 4. Overzicht van de deeltjeslengtemetingen.

reverse experiment (III4) no necrotic symptoms of E197 were produced in plants protected by E198.

Thus, although cross protection was suggested in several instances during these experiments, results were very erratic and a 100 % cross protection was never obtained. Where members of group I were challenged by those of groups IIa and IIb, or the reverse, 8 of 10 tests showed indication of protection. Where isolates of these three groups were tested against IIc isolates, 4 out of 9 tests indicated some protection. These results might suggest that members of groups I, IIa and IIb are more closely related mutually than any of these to isolates of group IIc.

Electron microscopy. Particles were measured in crude sap preparations as described earlier, using TMV as an internal standard (Bos, 1970). Both for TMV and for the isolate to be measured peak lengths in micrometer classes were estimated, taking curve shapes into account. Assuming TMV to be 300 nm long, peak lengths of the isolates were then calculated and rounded off to multiples of 5. Results are listed in Table 4. The isolates of group I were slightly shorter than those of groups IIa and b. Isolate B25 was shortest. Earlier (Bos, 1970) B25 in pea was c. 770 nm and E198 c. 840 nm long.

Serology. Results of serological experiments are summarized in Table 5. They indicate a very close relationship between B25, E198, E221 and Kow28. Different from these, but still rather closely related are Kow14 and LMV (lettuce mosaic virus). The latter two only show a distant interrelationship. The PVY^N isolate was only distantly related to LMV.

Table 5. Summary of serological microprecipitin tests; homologous titres are in italics.

Antigens	Antisera					
	B25	E198	BYMV Hollings	PMV Hollings	LMV	normal serum
B25 (1)	*4096*	1024	256	16384	64	–[2]
E198 (IIa)	16384	*4096*	256	16384	64	–
Kow28 (IIa)	4096	1024	256	1024	256	–
E221 (IIb)	16384	4096	1024	16384	256	–
Kow14 (IIb)	64	64	64	1024	1	–
LMV	256	256	(1)[1]	256	*1024*	–
PVY^N	–[2]	–	–	–	4	–
Healthy concentrated pea sap	–	–	–	–	–	–

[1] Reaction not clear.
[2] No reaction.

Tabel 5. Samenvatting van de serologische microprecipitatieproeven; homologe titers zijn cursief weergegeven.

Conclusions and discussion

Although nature and severity of symptoms depend on conditions, the tentative grouping in Table 2 on the basis of host ranges and symptoms turned out to be a reliable one. The groups I, IIa and IIb can be easily distinguished with a limited number of differential hosts as summarized in Table 6. Serologically and in inclusion bodies produced, the groups hardly differed, with the exception of E178 and Kow14. So, but for the latter two, all I, IIa, and IIb isolates are considered to belong to one virus.

The green mosaic isolate from pea (E212) and the seed-transmitted isolate from yellow lupin (L1) together with B25 (group I) typically represent *bean yellow mosaic virus*. It causes systemic symptoms in most bean cultivars (including top necrosis in 'Double White Princess') and an often inconspicuous green mosaic in pea and broad bean. In contrast to all other isolates, these isolates usually produce systemic symptoms in *C. amaranticolor*, being severe with L1. The lupin isolate further differed in not giving systemic symptoms in 'Fana' and 'Prince' but especially doing so in 'Jolanda'. It may resemble the lupin isolates of Zschau (1961), Blaszczak (1963), and Ksiazek (1962a, b).

The two pea yellow mosaic isolates (E198 and E204) and Kow28 from red clover

Table 6. Differentiation between our isolates of bean mosaic, pea yellow mosaic and pea necrosis strains of bean yellow mosaic virus, pea necrosis virus and pea seed-borne mosaic virus (for explanation of symbols see Table 2).

Differential hosts	Bean yellow mosaic virus			Pea necrosis virus	Pea seed-borne mosaic virus
	bean mosaic strains (I)	pea yellow mosaic strains (IIa)	pea necrosis strains (IIb)		
Phaseolus vulgaris					
'Double White Princess'	LS (tip-necrosis)	LS (spotting or delayed tipnecrosis)	L–	L– (sometimes tip-necrosis)	–
'Great Northern 123'	LS	L–	L–	–	
Pisum sativum	–S	–S	(L)S	(L)S	S
'Koroza' (mosaic sensitive)	(green mosaic)	(yellow mosaic)	(severe necrosis)	(severe necrosis)	(leafrolling and narrowing)
'Juwel' (mosaic resistant)	–	–	–	–	S (leafrolling and narrowing)
Vicia faba 'Compacta'	(L)S (green mosaic)	lS (yellow mosaic)	(L)S (severe necrosis)	LS (local and systemic necrosis)	lS (leafrolling and narrowing)
Chenopodium amaranticolor	LS	L–	L–	L–	L–
C. quinoa	(L)S	L–	L–	LS	L (S)

Tabel 6. Onderscheid tussen onze isolaten van bone-, gele erwtemozaïek- en erwtenecrosestammen van het bonescherpmozaïekvirus, erwtenecrosevirus en erwtebladrolvirus (voor verklaring van de symbolen zie Tabel 2).

(group IIa), all causing striking yellow mosaic in pea and broad bean and mild symptoms only in the more BYMV-sensitive *P. vulgaris* cultivars are considered representing the *pea yellow mosaic strain of bean yellow mosaic virus*.

The three pea isolates (E197, E199, E221), causing characteristic and usually severe whole plant necrosis in pea and broad bean and a hypersensitivity reaction in most *P. vulgaris* cultivars (group IIb), are now described as the *pea necrosis strain of bean yellow mosaic virus*. The red clover necrosis virus of Zaumeyer and Goth (1963), killing peas, broad beans and certain bean cultivars, and Cousin's (1969) necrotic pea isolate may be closely related.

The pea necrosis virus E178, and the pea necrosis isolate Kow14, tentatively grouped in IIb, obviously occupy a special place. E178 more easily produces systemic symptoms in a number of bean cultivars, whereas nearly all are immune to Kow14. In this respect and in their inclusions, they clearly differ from all other isolates as well as from each other. They also differ from the other isolates in producing local lesions in cucumber and in not causing systemic necrosis in *Lupinus angustifolius*. They were also less closely related serologically to the BYMV isolates. These results corroborate the earlier description of E178 as a special pea necrosis virus (Bos, 1970). Kow14 needs further study.

The isolates of Group IIc also require further investigation. They resemble normal BYMV in their green mosaic on pea, but are like the pea mosaic strain in their limited reaction on *P. vulgaris*. Very strikingly, Vf18 gave a latent systemic infection in all three BYMV resistant pea cultivars tested.

For a simple differentiation between the strains of BYMV and pea necrosis virus discussed here and the pea seed-borne mosaic virus (E210 of Bos, 1970) see Table 6.

The pea yellow mosaic isolates described here, evidently are characteristic of what is usually designated as pea mosaic virus (PMV). Previous evidence that PMV is indeed infectious to *P. vulgaris* (e.g. Schroeder and Provvidenti, 1966; Taylor and Smith, 1968; Tapio, 1970) is further supported by our present results. However, PMV is much less pathogenic to *P. vulgaris*.

Like Goodchild (1956b), Bercks (1960), Taylor and Smith (1968) and Tapio (1970), working with BYMV and PMV, we did not find appreciable serological differences between our bean mosaic, pea yellow mosaic and pea necrosis isolates of BYMV (Table 5). In contrast, Varma and Gibbs (1967) and Hull (1968) found differences between their isolates of BYMV and PMV. These differences did not exceed, however, those obtained by Bercks (1960) between his strains of BYMV. Similarly, in our experiments Hollings's BYMV antiserum reacted differently from ours with LMV antigen. Moreover, differences in ratios of heterologous to homologous titres found with different antisera may also be due to differences between rabbits injected (van Regenmortel and von Wechmar, 1970).

So we feel the most plausible conclusion is *that PMV constitutes a pea yellow mosaic strain of bean yellow mosaic virus and that no special pea mosaic virus exists.* Striking was the rather close serological relationship between the BYMV isolates tested and lettuce mosaic virus. LMV has recently been found to cause natural disease in peas (Provvidenti, 1973), but it differs from BYMV in easily going to BYMV resistant 'Perfection'-type peas (Bos, 1970; Provvidenti, 1973). Peculiarly enough resistance in peas to watermelon mosaic virus is conditioned by the same genotype as that to BYMV (Schroeder and Provvidenti, 1971).

In our experiments *C. amaranticolor*, showing systemic symptoms to any bean isolate of BYMV was useful in distinguishing between bean and pea strains of BYMV. Hollings (1957a,) and Hull (1968) conversely reported that PMV became systemic and that BYMV remained local in this host. Varma and Gibbs (1967) reported that both were systemically infectious in this test plant. This disparity may be due to differences between local *C. amaranticolor* selections used, as observed earlier for *C. album* by Demski (1968). Thus, results obtained in different laboratories may not be directly comparable.

Here and in the earlier study (Bos, 1970), cross-protection tests (Table 3) have been found of little help. Closely related isolates often do not cross-protect, whereas more distant viruses do, e.g. BCMV and BYMV, and LMV and cowpea aphid-borne mosaic virus (see Bos, 1970). In spite of cross protection, Hollings (1957b) considered anemone mosaic virus to be distinct from turnip mosaic virus because of absence of serological relationship.

Determination of physical properties is also of little help. Particle length as determined by electron microscopy is too variable and all members of the potyvirus group seem to fall so much within a narrow length range, that it will be hard if not impossible to distinguish them by length. Concurrent research in our laboratory by Huttinga (1973) and Huttinga and Mosch (1974) has already shown that viruses of the potyvirus group (B25, E198, LMV and PVY^N) have sligthly different S-values and buoyant densities and the same molecular weight of their coat protein subunit. Some recent results (Huttinga, paper in preparation) have shown that variation among BYMV isolates may be greater than between different viruses of the potyvirus group.

Finally, the results of the present investigations have again demonstrated the diversity of BYMV and its very close relationship to several other viruses of the potyvirus group. In differentiating these viruses, serology and electron microscopy are of limited or no help, whereas other physical properties of the protein coat not necessarily parallel pathogenic differentiation. This is not astonishing in view of the relatively small part of the total genetic information of the virus involved in the protein coat. Since viruses are mainly of (practical) importance because of their pathogenicity, this character should have much weight in their classification, at least inside the morphological groups. Such a classification may also give a better idea of their evolution. In this respect, data obtained by molecular hybridization experiments demonstrating the degree of genetic relatedness between the nucleic acid strands of the virus isolates concerned, as discussed for the distinction between the severe and yellow strains of cowpea mosaic virus by Swaans and van Kammen (1973), may be of further help.

Samenvatting

De identificatie van bonemozaïek-, erwtegeelmozaïek- en erwtenecrosestammen van het bonescherpmozaïekvirus

Twaalf virusisolaten uit erwt, tuinboon, rode klaver en gele lupine werden vergeleken met de eerder beschreven (Bos, 1970) B25-stam van het bonescherpmozaïekvirus (BYMV), de E198-stam van het erwtemozaïekvirus (PMV) en het erwtenecrosevirus (E178) (Tabel 1).

Op grond van waardplantreeksen, symptomen en de reacties van bone- en erwterassen (Tabel 2) konden de meeste isolaten worden ingedeeld in drie groepen, waarvan vertegenwoordigers serologisch niet duidelijk verschilden (Tabel 5). Een groen erwteisolaat (E212) en een met zaad overgaand isolaat uit gele lupine (L1) bleken typische bone-isolaten van het *bonescherpmozaïekvirus*, dat duidelijke symptomen veroorzaakt in de meeste bonerassen en groen mozaïek in erwt (Fig. 1A) en tuinboon. In *Chenopodium amaranticolor* geven deze isolaten in tegenstelling tot alle andere getoetste isolaten gewoonlijk systemische symptomen, die met het lupineïsolaat zeer hevig zijn (Fig. 4A).

Twee erwtegeelmozaïekisolaten (E198 en E204) en een isolaat uit rode klaver (Kow28), die geelmozaïek in erwt en tuinboon doen ontstaan en slechts milde symptomen in een deel der op het bonescherpmozaïekvirus reagerende bonerassen, werden opgevat als behorend tot de *erwtegeelmozaïekstam* van het bonescherpmozaïekvirus.

Drie erwtenecrose-isolaten (E197, E199, E221), die necrose veroorzaken in erwt (Fig. 3) en tuinboon (Fig. 2) terwijl de bonerassen gewoonlijk overgevoelig bleken, werden beschreven als *erwtenecrosestammen* van het bonescherpmozaïekvirus.

De drie voor erwtemozaïek onvatbare erwterassen bleken immuun voor alle isolaten van het bonescherpmozaïekvirus behalve E197, dat een latente systemische infectie gaf in 'Relonce'.

Het eerder als een aparte eenheid beschreven erwtenecrosevirus (E178) gedroeg zich ook nu duidelijk verschillend van de bonescherpmozaïekvirusisolaten. Het isolaat Kow14, dat in erwt eveneens ernstige necrose veroorzaakte, leek op E178 in de necrose die werd teweeggebracht in erwt en tuinboon, in de lokale vlekken in komkommerzaadlobben en in het ontbreken van systemische necrose in *Lupinus angustifolius*, maar de meeste bonerassen waren onvatbaar voor Kow14. Het was ook serologisch minder nauw verwant aan de onderzochte bonescherpmozaïekvirusisolaten.

Vier andere virusisolaten uit erwt en tuinboon konden nog niet worden geïdentificeerd. Eén ervan (Vf18) vertoonde een unieke latente systemische infectie in alle drie mozaïekonvatbare erwterassen (Tabel 2).

Resultaten van de premunitieproeven (Tabel 3) waren wisselvallig en nergens werd een volledige bescherming verkregen. Deeltjeslengtemetingen (Tabel 4) bleken niet van nut bij de onderscheiding van de onderhavige stammen en virussen.

Uit de verkregen resultaten en uit een overzicht van de literatuur wordt tenslotte geconcludeerd *dat het erwtemozaïekvirus opgevat moet worden als een stam van het bonescherpmozaïekvirus*. Het laatstgenoemde virus is tamelijk nauw verwant aan het slamozaïekvirus, zoals met een antiserum tegen dit virus werd aangetoond.

De variabiliteit van het bonescherpmozaïekvirus en zijn relaties met een groep van nauw verwante virussen wordt verder besproken. Biologische eigenschappen van de onderhavige virussen hoeven niet samen te vallen met de 'lichamelijke' eigenschappen van hun deeltjes, inclusief de serologische. De virussen en hun stammen kunnen gemakkelijk worden onderscheiden met behulp van een beperkte reeks van differentiërende waardplantsoorten (Tabel 6). Een indeling van de betrokken virussen op grond van pathogeniteit is zowel van betekenis om praktische redenen, als voor het verkrijgen van inzicht in hun ontstaan.

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