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Similarity of clover yellow vein virus and pea necrosis virus

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Abstract

There still is confusion concerning the relationships between clover yellow vein virus (ClYVV), pea necrosis virus (PNV) and bean yellow mosaic virus (BYMV). Therefore, three Swedish isolates of ClYVV and its type strain have now been compared with three isolates of PNV. A bean mosaic isolate and three pea necrosis isolates of BYMV have been used for reference.

Based on host range tests, serology, and light microscope studies of inclusion bodies, ClYVV and PNV isolates are now considered to be strains of one virus, with the first name having priority.

ClYVV (including the original PNV) especially differs from BYMV in its ability to infect white clover, to produce local lesions on cucumber cotyledons (at least two cultivars), to go systemic in *Chenopodium quinoa* (the two local selections used at Wageningen and at Uppsala), to be rather virulent on *Nicotiana clevelandii*, and to provoke extensive nucleolar enlargements in its host cells. Serologically the two viruses are more or less distinct.

Introduction

In a number of papers on the identification of strains of bean yellow mosaic virus (BYMV) and of some closely related viruses such as pea necrosis virus (PNV) (Bos, 1969a, 1970; Bos et al., 1974; Beczner et al., 1976), it has been stressed that within the potyvirus group overlapping characters make it difficult to draw well-defined borderlines between viruses. They all share more features than previously supposed and de-amphasis of the various differences would necessitate lumping, for example, of all virus types which cluster around BYMV (Bos, 1970).

It has also been stressed that within a morphological group unambiguous classification seems impossible and that taxonomic entities may have to be defined arbitrarily. This complicates diagnosing closely related viruses or intermediate isolates. Examples are the interrelationships among clover yellow vein virus (ClYVV) incompletely described by Hollings and Nariani (1965), PNV and BYMV. Recent investigations on Swedish isolates of ClYVV (Lindsten et al., 1976) have indicated that PNV may be more closely related to ClYVV than suggested by the original data on the latter virus (see also Hollings and Stone, 1974). Therefore, we have now further investigated ClYVV and PNV isolates at Wageningen and Uppsala.

Data from the literature

ClYVV was originally described as having flexuous particles, shorter than those of the potyvirus group, not infecting pea and broad bean, and not being related serologically

to BYMV (Hollings and Nariani, 1965). Gibbs et al. (1966) determined the virus to be ca. 750 nm long and reported a distant serological relationship to BYMV and some other members of the potyvirus group (see also Varma et al., 1970). In a more definite description, Hollings and Stone (1974) claimed that there are no consistent differences in host ranges and symptoms among the many described strains of the potyviruses in Leguminosae, but that ClYVV is most readily distinguished by serological tests.

Early during this continuing characterization of ClYVV, Bos (1969a, 1970) described PNV. The latter virus was found to be serologically related to BYMV, but it exhibited a wider host range among non-legumes, and induced necrotic reactions on pea and broad bean and unique intranuclear abnormalities, particularly the production of crystalline needles protruding from the enlarged nucleolus (Bos, 1969a, b; Bos and Rubio-Huertos, 1969).

Although ClYVV was found to be able to infect pea and broad bean (in contrast to Hollings and Nariani's (1965) findings), it differed from PNV in several test species, and PNV was found to be clearly related serologically to BYMV (Bos, 1970). ClYVV was also found to produce striking nucleolar enlargements, but no protruding needles typical of PNV (E178) were formed (Bos, 1969b). Granular cytoplasmic inclusions but no such nucleolar enlargements had been found by Hollings and Nariani (1965) after staining with trypan blue.

Later, Bos et al. (1974) studied pea mosaic and pea necrosis isolates of BYMV and still found PNV to differ sufficiently from the latter to maintain its distinction as a separate virus. Beczner et al. (1976) thereafter described two other strains of PNV from peas isolated in the Netherlands (E242) and in Poland (Kow14), showing considerable variation of PNV.

In the meantime, ClYVV had also been identified from white clover in eastern and western Canada (Pratt, 1968, 1969) and in most Southeastern states of the USA (Barnett and Gibson, 1975). The latter authors found their ClYVV isolate not to react to their BYMV antiserum, but BYMV reacted with ClYVV antiserum. Jones (1974), studying a virulent virus from beans in Ohio, found this virus to be closely related serologically to Barnett's ClYVV, but also related to BYMV and tends to consider ClYVV nothing but a serotype of BYMV.

Recently, Lindsten et al. (1976) studied five virus isolates from white clover and six isolates from gladiolus in Sweden. Standard strains of BYMV and all BYMV isolates from gladiolus were not infectious to white clover, whereas ClYVV and the white clover isolates were. Isolates from white clover were closely related serologically to ClYVV and more distantly to BYMV strains and isolates from gladiolus. Pratt (1969) and Lindsten et al. (1976) have stressed that the few data from the literature on infection of white clover by BYMV (Houston and Oswald, 1953; Kreitlow et al., 1957; Kreitlow and Hunt, 1958; Thomas and Zaumeyer, 1953) most probably are due to the fact that the authors were unknowingly dealing with ClYVV.

Materials and methods

Isolates and maintenance. ClYVV-H is the type strain originally described by Hollings and Nariani (1965). ClYVV isolates L80/75, L51/75, and L32/75 have been described by Lindsten et al. (1976). The PNV isolates E178, E242 and Kow14 are by Bos (1969a)

and by Beczner et al. (1976). BYMV-B25 is a bean yellow mosaic type strain (Bos, 1970). E221, GM1 and GM2 are isolates of the pea necrosis strain of BYMV (Bos et al., 1974; Lindsten et al., 1976). All but the Swedish isolates have been maintained in the senior author's virus collection in plant material dried and stored over $CaCl_2$.
Techniques of study, including serological tests and study of inclusion bodies, were those described in previous papers (Bos, 1970; Bos et al., 1974). For the present host range studies, mainly those species and cultivars were used that had earlier been found to react differentially (Bos et al., 1974; Beczner et al., 1976; Lindsten et al., 1976).

Results

Host range and symptoms

The results of host range tests carried out at Wageningen and at Uppsala are summarized in Table 1. At Wageningen the results with isolates of PNV and BYMV agreed quite well with those reported earlier (Bos et al., 1974; Beczner et al., 1976). Slight differences found may be due to differences in season or to low numbers of plants inoculated. The results obtained at Uppsala are in good agreement with those of Lindsten et al. (1976).

Phaseolus vulgaris. In 'Bataaf', one of the cultivars most sensitive to BYMV, all isolates but Kow14 produced symptoms in most inoculated plants. Local reactions either consisted of necrotic or chlorotic local lesions or of spreading necrotic lesions followed by necrotic vein patterns. Systemic symptoms consisted of severe vein necrosis and leaf malformation (B25) including top necrosis (E242) and plant death (ClYVV-H) or of yellow leaf flecking with irregular patterns of necrotic veins with E221 and more intensively with L80/75 and L32/75, and with E178 leading to premature leaf casting.

In 'Double White Princess', another cultivar sensitive to BYMV, B25 and three ClYVV isolates all caused a few necrotic local lesions, developing into necrotic vein patterns with B25 and ClYVV-H. With B25, L80/75 and L32/75 this was followed by systemic vein necrosis, some yellowing and leaf curling, and sometimes by top necrosis. With ClYVV-H plants died prematurely. With BYMV-E221 and all three PNV isolates no obvious symptoms were produced and virus could not be recovered, except with E242.

The rather BYMV-resistant cultivars 'Great Northern 123' and 'Jolanda' were immune to most isolates. In 'Great Northern 123', E221 was the only isolate to produce a few small necrotic local lesions. In previous tests (Bos et al., 1974; Beczner et al., 1976) B25 had caused both local and systemic symptoms in this cultivar. In 'Jolanda' both B25 and E221 produced many small chlorotic local lesions later changing into green rings when primary leaves turned yellow. Two out of eight plants with E221 produced small systemic stipples. The same held for two plants inoculated with ClYVV-H, one of which had shown green rings in inoculated leaves.

'Bonita' reacted to all BYMV isolates with necrotic local lesions. The ClYVV isolates and E178 caused usually no such lesions but necrosis in the veins, and produced top wilting and top necrosis. With E178 systemic necrosis was fastest, leading to plant death within one to two weeks.

Pisum sativum 'Koroza' readily reacted with a distinct green mosaic to BYMV-B25. All other isolates, including E221 (an isolate of the pea necrosis strain of BYMV), rapidly produced severe stem and leaf necrosis and premature death soon after initial vein chlorosis (Fig. 1). 'Juwel' and a cultivar apparently mislabeled as 'Perfected Wales', immune to BYMV, were so to all isolates tested.

'English sword' reacted to the ClYVV isolates and E178 with identical and very characteristic symptoms including top wilting, top necrosis and premature death (Lindsten et al., 1976). Also the pea necrosis strains of BYMV, viz. E221, GM1 and GM2, caused top wilting and gradually also necrosis, but this usually appeared much later. BYMV-B25 produced vein clearing and green mosaic.

Table 1. Summary of test plant reactions at Wageningen (W) and Uppsala (U).

Test plants		Virus isolates										
		clover yellow vein virus				pea necrosis virus			bean yellow mosaic virus			
		H	L32/75	L51/75	L80/75	E178	E242	Kow14	B25	E221	GM1	GM2
Legumes												
Phaseolus vulgaris												
'Bataaf'	W	LS	LS		LS	LS	LS	––	LS	LS		
'Bonita'	U	LS	LS	LS		LS			LS	LS	LS	LS
'Double White Princess'	W	LS	LS		LS	––	–S	––	LS	––		
'Great Northern 123'	W	––	––		––	––	––	––	––	L–		
'Jolanda'	W	LS	––		––	––	––	––	L–	LS		
Pisum sativum												
'English Sword'	U	S	S	S		S			S	S	S	S
'Juwel'	W	––	––		––	––	––	––	––	––		
'Koroza'	W	–S	–S		–S	–S	–S	–S	–S	–S		
'Perfected Wales'[1]	W	––	––		––	––	––	––	––	––		
'Swedish Sword'	U	S	S	S		S			S	S	S	S
Trifolium hybridum	U	S	S	S		S			S	S	S	S
Trifolium repens												
'Cultuur'	W	–*S	–*S		–*S	–*s	–*S	–*S	–*–	–*–		
'Lena'	U	S	S	S		S			–*–	–*–	–*–	–*–
'Nora'	U	S	S	S		S			–*–	–*–	–*–	–*–
Vicia faba												
'Compacta'	W	–*S	–*S		–*S	LS	–S	LS	–S	–S		
'Freya'	U	S	S	S		S			S	S	S	S
Non-legumes												
Chenopodium amaranticolor	W	L–	L–		L–	L–	L–	L–	LS	L–		
Chenopodium quinoa	W	LS	LS		LS	LS	LS	L–	L–	L–		
	U	LS	LS	LS		LS			L–	L–	L–	L–
Cucumis sativus												
'Gele Tros'	W	L–	L–		L–	L–	L–	L–	––	––		
	U	L–	L–	L–		L–			––	––	––	––
'Rhensk Druv'	U	L–	L–	L–		L–			––	––	––	––
Nicotiana clevelandii	W	–*S	LS		LS	LS	–*S	–*S	–*S	–*(S)	–*S	
	U	S	S	(S)	S	(S)			s	S	S	S
Nicotiana megalosiphon	W	LS	LS		LS	L(S)	L–	L–	––	(L)–		
	U	S	S	S		S			––	––	––	––

W = observations at Wageningen
U = observations at Uppsala
L = local symptoms
S = systemic symptoms
l = latent local infection
s = latent systemic infection
( ) = symbol in parentheses = sometimes no infection or no symptoms
– = no infection as tested by back inoculation
–* = no symptoms, but not tested by back inoculation

[1] Apparently mislabeled seed lot since 'Perfected Wales' is susceptible to BYMV.

Tabel 1. Samenvatting van de toetsplantreacties te Wageningen(W) en Uppsala (U).

Fig. 1. Necrosis in 'Koroza' pea, 20 days after inoculation with ClYVV-L32/75 (left) and PNV-E178 (right).

Fig. 1. Necrose in 'Koroza' erwt, 20 dagen na inoculatie met ClYVV-L32/75 (links) en PNV-E178 (rechts).

Vicia faba 'Compacta' reacted to B25 with vein chlorosis and green mosaic. Isolate E221 induced initial necrotic stem streaking soon followed by plant death (Fig. 2, D). With the PNV isolates, except E242, some necrotic local lesions were produced and most plants died prematurely (Fig. 2, C). With E178 in some plants a malforming flecking, leaf curling and plant stunting were observed. This also occurred in all plants with E242. All three isolates of ClYVV tested on 'Compacta' (Fig. 2, A and B) induced different symptoms in fewer plants. Symptoms did not appear within ca. 13 days after inoculation, and with L80/75 only after 5 weeks. Symptoms consisted of a usually sparse chlorotic stippling in tip leaves; in yellowing leaves such spots often developed into green rings (Fig. 2. A). With the type strain (H) symptoms were more intense and of a vein mosaic type (Fig. 2. B).

The symptoms on 'Freya' were rather variable for all isolates, except BYMV-B25 which always caused a typical green mosaic without necrosis. Vein necrosis and stem necrosis were rather common with the ClYVV isolates and E178, but also with the pea necrosis strains of BYMV.

Trifolium hybridum was tested only at Uppsala. All isolates tested infected 'Svea', but the ClYVV isolates and E178 produced more severe symptoms including leaf curling, dwarfing and frequently also brown reddish discoloration.

Trifolium repens. In 'Cultuur' sparse symptoms of vein mosaic or vein banding were produced in some of the plants with all isolates of ClYVV (Fig. 3) and PNV-E242. Virus could be recovered by back inoculation from plants inoculated with E178 (earlier, symptoms similar to those of ClYVV had also been observed; Fig. 3 and Bos, 1970) but not from those with both BYMV isolates. Systemic symptoms were obtained in 'Nora' and 'Lena' with the ClYVV isolates and with E178. Back inoculations from plants inoculated with all BYMV isolates were always negative.

Fig. 2. Chlorotic stippling (A, B) and systemic necrosis (C, D) in *Vicia faba* 'Compacta', 20 days after inoculation with ClYVV-L32/75 (A), ClYVV-H (B), PNV-E178 (C) and BYMV-E221 (D; an isolate of the pea necrosis strain of BYMV).

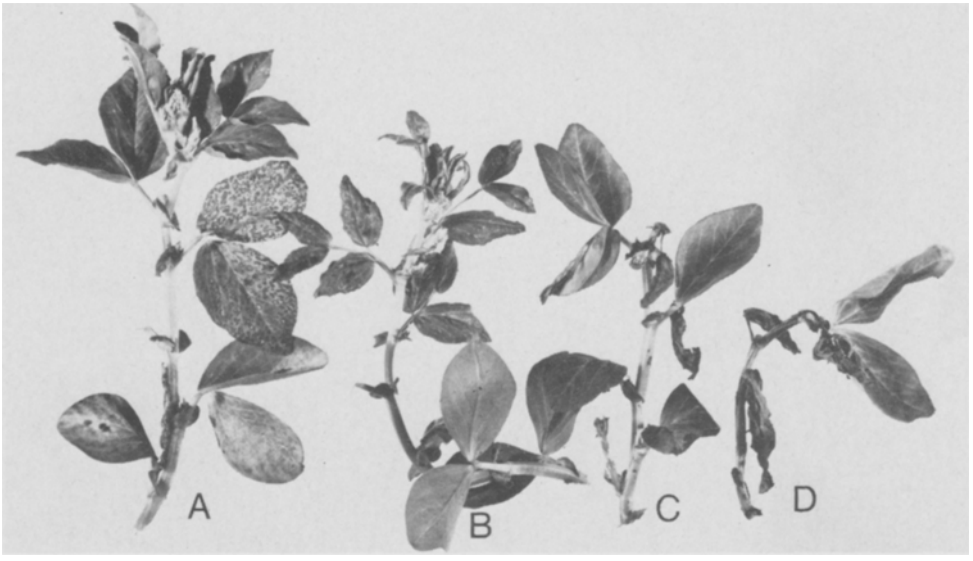

Fig. 2. Chlorotische stippeling (A, B) en systemische necrose in Vicia faba 'Compacta' (C, D), 20 dagen na inoculatie met ClYVV-L32/75 (A), ClYVV-H (B), PNV-E178 (C) en BYMV-E221 (D, een isolaat van de erwtenecrosestam van het BYMV).

Fig. 3. Vein mosaic and vein banding in white clover after inoculation with PNV-E178 (above) and ClYVV-H (below).

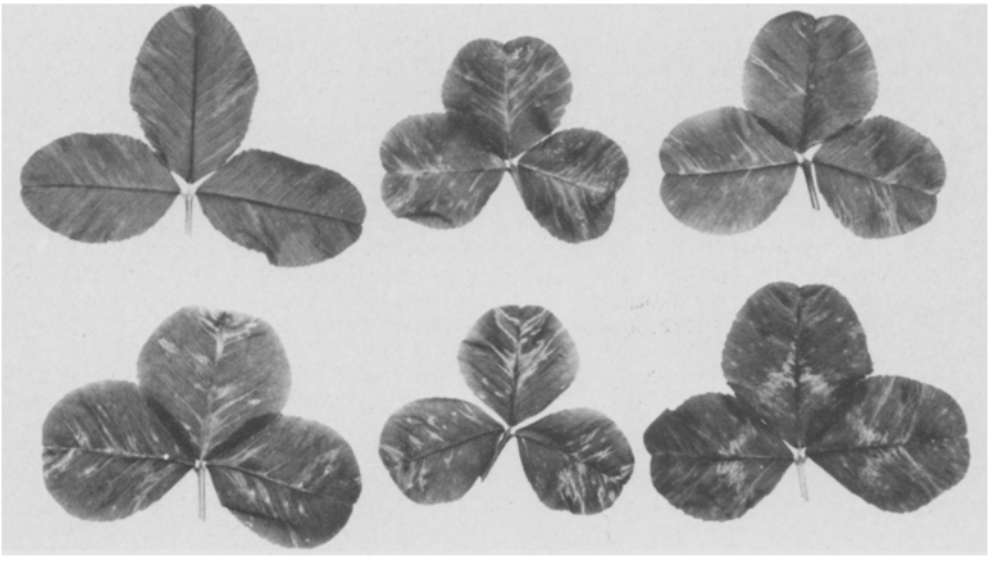

Fig. 3. Nerfmozaïek en nerfbandmozaïek in witte klaver na inoculatie met PNV-E178 (boven) en ClYVV-H (onder).

Fig. 4. Chlorotic spotting and leaf malformation in *Chenopodium quinoa*, 17 days after inoculation with ClYVV-H (A) and PNV-E178 (B) and -E242 (C); D healthy control.

Fig. 4. Chlorotische vlekking en bladmisvorming in Chenopodium quinoa, 17 dagen na inoculatie met ClYVV-H (A) en PNV-E178 (B) en -E242 (C); D gezonde controle.

Chenopodium amaranticolor at Wageningen always reacted with systemic irregular vein patterns and leaf distortion to B25 only. In *C. quinoa* a systemic chlorotic spotting was produced by all isolates of ClYVV and PNV except Kow14, being severe with H, E178 and E242, and especially malforming with H (Fig. 4). Similarly, at Uppsala, only the ClYVV isolates and E178 caused systemic symptoms in *C. quinoa*. These were conspicuous and malforming with ClYVV-H and E178. ClYVV-L51/75, a deviating isolate (Lindsten et al., 1976), produced slight deformation only.

Cucumis sativus. Both at Wageningen and Uppsala 'Gele tros' reacted to all isolates but those of BYMV with chlorotic, later desiccating local lesions, and the same held for 'Rhensk druv' at Uppsala. However, when inoculum from *N. clevelandii* was used the inoculation frequently failed under Swedish conditions.

In *Nicotiana clevelandii* BYMV-B25 and E221 hardly produced any mottling and at Uppsala B25 usually remained latent. ClYVV-L80/75 and especially L32/75 caused many local lesions, which were more etched with L80/75 and thoroughly necrotic with L32/75. All ClYVV isolates, including H, produced a fine systemic mottling and leaf curling which was especially severe with L32/75, there associated with severe plant stunting (Fig. 5). With L51/75, however, mottling and malformation was very slight. With the PNV isolates, non-inoculated leaves showed small scattered chlorotic spots and rings and slight growth retardation.

N. megalosiphon. At Wageningen numerous local lesions were caused in 4 to 5 days. These were small and necrotic with ClYVV-L80/75 and L32/75, or showed the appearance of etched rings with ClYVV-H and PNV-E178 and E242. With PNV-Kow14 and BYMV-E221 they were rather chlorotic

Fig. 5. Mottling and malformation in *Nicotiana clevelandii*, 20 days after inoculation with ClYVV-L32/75 (A) -L80/75 (B) and PNV-Kow14 (C).

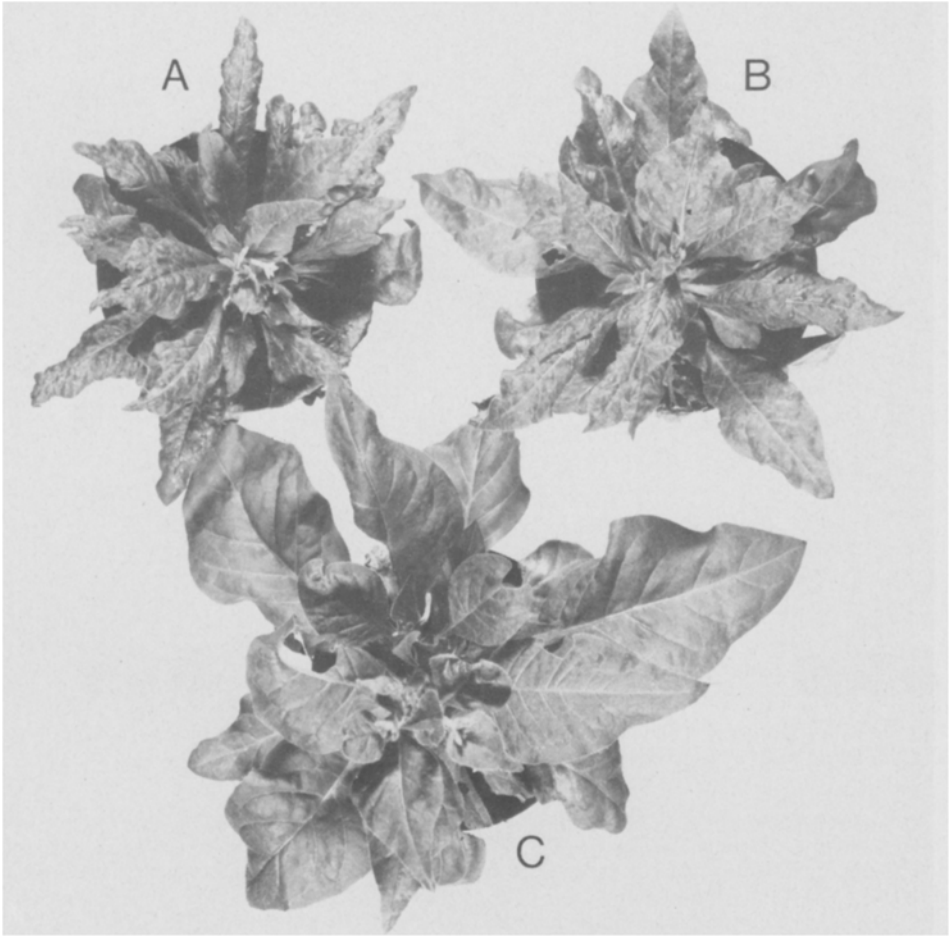

Fig. 5. Vlekkerigheid en misvorming in Nicotiana clevelandii, 20 dagen na inoculatie met ClYVV-L32/75 (A), -L80/75 (B) en PNV-Kow14 (C).

and no clear reaction was observed with B25. Systemic symptoms were observed with the ClYVV isolates only. They consisted of plant stunting and leaf curling and, with L80/75 and L32/75, some vein mosaic. At Uppsala this species also became easily infected with the ClYVV isolates and E178, but the other isolates did not show any symptoms and back inoculations from non-inoculated leaves were negative.

Inclusion bodies

Granular cytoplasmic inclusions were readily detectable in stained epidermal stem and petiole strips of pea plants with all isolates of ClYVV and PNV. Nucleolar enlargements were of comparable size with all three ClYVV isolates tested at Wageningen and PNV-Kow14. They were extremely large with PNV-E242, sometimes

Fig. 6. Granular cytoplasmic inclusions and nucleolar enlargement in epidermal cells of 'Koroza' pea, 10–13 days after inoculation with PNV-E242. Bar represents 10 μm.

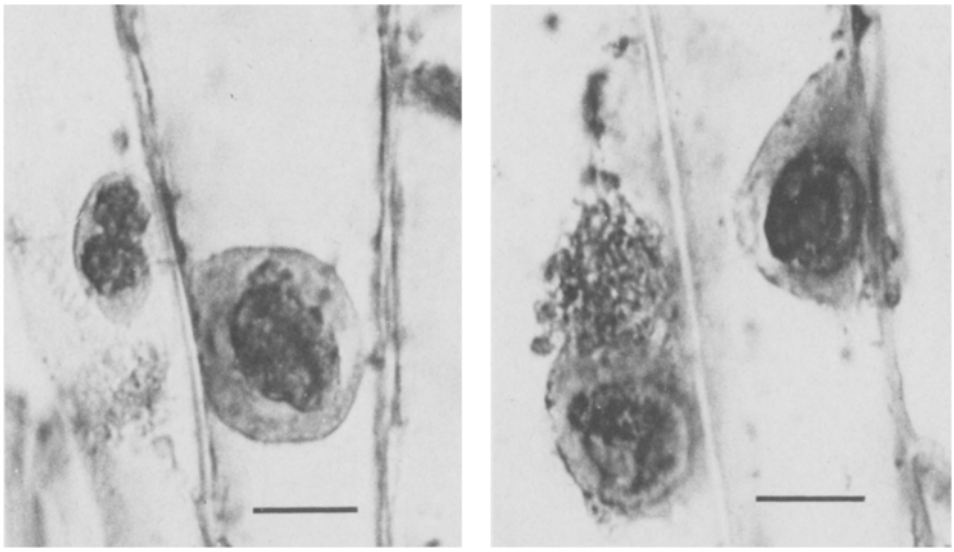

Fig. 6. Granulaire cytoplasma-insluitsels en vergroting van nucleoli in epidermiscellen van 'Koroza' erwt, 10–13 dagen na inoculatie. Staaf geeft 10 μm weer.

nearly filling the entire nucleus (Fig. 6) (see also Beczner et al., 1976), and smaller and usually provided with radiating needles with E178 (Bos, 1969a, b, 1970). The cytoplasmic and intranuclear inclusions characteristic of E242 were also easily found in plants of *V. faba*, *N. clevelandii* and *N. megalosiphon* infected with that isolate. With BYMV-B25 and BYMV-E221 (the pea necrosis-inducing strain of BYMV) the nucleoli were only slightly enlarged or apparently normal, as reported earlier (Bos et al., 1974; Beczner et al., 1976).

Serology

The results of serological microprecipitin tests using purified antigens (BYMV-B25 and PNV isolates) and clarified sap of *N. clevelandii* (ClYVV isolates) are presented in Table 2. The results clearly indicate a close serological relationship between all three isolates of ClYVV and the E178 and E242 isolates of PNV, and showed a distant relationship to BYMV-B25, whereas Kow14 occupies an intermediate position, as reported earlier (Beczner et al., 1976). Titres obtained with Hollings' antiserum support the close relationships between ClYVV and PNV isolates, but reaction titres were low as compared with the reaction obtained with healthy sap.

Discussion

The present results both at Wageningen and Uppsala corroborate the distinction between PNV and BYMV, including its pea necrosis isolate E221, in serology, host

Table 2. Summary of serological microprecipitin tests; homologous titres are in italics.

Antigens	Antisera								
	BYMV-B25		PNV-E178		ClYVV-Hollings		ClYVV-Sweden		normal serum
	exp. 1	exp. 2	exp. 1	exp. 2	exp. 1	exp. 2	exp. 1	exp. 2	
BYMV-B25	*1024*	*256*	16	16	–	–	16	16	–
PNV-E178	4		*256*		16		256		–
PNV-E242	–	–	64	64	64	16	1024	256	–
PNV-Kow14	256		64		64		256		–
ClYVV-Hollings		16		64		*64*		256	–
ClYVV-Sweden 32/75	4	4	256	64	16	16	1024	256	–
ClYVV-Sweden 80/75	64	4	256	64	64	64	1024	256	–
Nicotiana clevelandii, healthy	4	–	–	–	16	16	16	4	–

Tabel 2. Samenvatting van de serologische microprecipitatieproeven; homologe titers zijn cursief weergegeven.

reaction, especially local reaction in cucumber cotyledons, and in inclusion bodies as earlier found by Bos (1970), Bos et al. (1974), and Beczner et al., (1976). In Sweden, E221 was now also found to closely resemble two of the BYMV isolates from gladiolus, viz. GM1 and GM2.

On the other hand, upon closer examination, the ClYVV isolates investigated share many features with PNV. They do cause local lesions in cotyledons of cucumber and produce striking nucleolar enlargements different from those of BYMV. ClYVV isolates do not produce the abundance of needles protruding from the enlarged nucleolus, but in this respect E178 seems unique, even within PNV. Systemic symptoms in *N. clevelandii* are usually more severe with ClYVV than with PNV and weak or often absent with BYMV and the same holds for *N. megalosiphon*, although B25 had earlier been recovered by back inoculation from symptomless inoculated and tip leaves of this species. In broad bean 'Compacta', ClYVV isolates less easily produce systemic symptoms and they then consist of a more or less intense chlorotic spotting, whereas PNV isolates produce a systemic necrosis and/or severe chlorotic flecking and leaf distortion and plant stunting.

In general, the PNV isolates share many features with those of ClYVV studied, and the differences found do very well fall within the range of variation of a single virus. Differences between the original PNV isolates and those of ClYVV do not exceed those found between isolates of PNV (Beczner et al., 1976) or those of ClYVV (Lindsten et al., 1976) and between the bean mosaic, pea yellow mosaic and pea necrosis strains of BYMV (Bos et al., 1974). Hence, it is now concluded that we are dealing with strains of one virus and the name *clover yellow vein virus* should have priority.

ClYVV seems to have features in common with the severe strain of BYMV

(BYMV-S) described by Thomas and Zaumeyer (1953). That strain also causes severe necrosis in peas and certain bean cultivars. It is not infectious to 'Great Northern U.I. 123' bean and goes systemic in white clover. It has been reported to cause local lesions in some *Nicotiana* species (Thomas and Zaumeyer, 1953) and to go systemic in certain Cucurbitaceae (Provvidenti and Uyemoto, 1973). It has even been found to naturally affect some non-legumes, such as *Proboscidea jussieui* (Provvidenti and Schroeder, 1972), summer squash *(Cucurbita pepo)* (Provvidenti and Granett, 1974). Although distinct from regular BYMV, the BYMV-S also on serological grounds is still considered a strain of BYMV by Granett and Provvidenti (1975). However, these authors have used chemically degraded antigens. Shepard et al. (1974), also working with chemically dissociated antigens, have earlier demonstrated similar serological cross reactions between several biologically widely different viruses of the potyvirus group.

For a discussion of the weight of the differences between PNV and thus of ClYVV on the one hand and BYMV on the other, see the last two paragraphs of the discussion by Beczner et al. (1976). We have also taken into consideration that ClYVV has been included in the CMI/AAB Descriptions of Plant Viruses as a separate entity (Hollings and Stone, 1974). A more detailed comparison of ClYVV and BYMV-S seems very worth-while. We would like to reemphasize that the taxonomy of these viruses is not merely an academic problem. Especially their biological differentiation, notably in host range, has an important bearing upon their epidemiological potentialities.

Samenvatting

Overeenkomst tussen klavergeelnervigheidsvirus en erwtenecrosevirus

Het blijkt moeilijk te zijn om binnen de potyvirusgroep scherpe grenzen te trekken tussen taxonomische eenheden bijv. in de 'zwerm' van virussen rond het bonescherpmozaïekvirus. Zo bestaat er nog steeds verwarring over de verwantschap tussen het klavergeelnervigheidsvirus (ClYVV), het erwtenecrosevirus (PNV) en het bonescherpmozaïekvirus (BYMV). Daarom zijn nu in gelijktijdig onderzoek in Nederland en Zweden drie Zweedse isolaten van het ClYVV en de type-stam ervan vergeleken met drie isolaten van het PNV, terwijl een bonemozaïekisolaat en drie erwtenecrose-isolaten van BYMV als standaard werden gebruikt.

Op grond van waardplantonderzoek (Tabel 1, Fig. 1 tot en met 5), serologie (Tabel 2) en bestudering van celinsluitsels met de lichtmicroscoop (Fig. 6) worden de ClYVV- en de PNV-isolaten nu alle beschouwd als stammen van eenzelfde virus, waarbij de naam klavergeelnervigheidsvirus (ClYVV) internationaal prioriteit heeft.

ClYVV (inclusief de oorspronkelijk als PNV beschreven stammen) verschilt vooral van BYMV in zijn vermogen om witte klaver te infecteren (Fig. 3), om in tenminste twee cultivars van komkommer lokale lesies op de zaadlobben te doen ontstaan, om in *Chenopodium quinoa* (twee verschillende in Wageningen en Uppsala gebruikte selecties) systemisch te worden (Fig. 4A–C), om nogal virulent te zijn op *Nicotiana clevelandii* (Fig. 5) en om in zijn waardcellen opvallende vergrotingen van de nucleoli te veroorzaken (Fig. 6). ClYVV en BYMV zijn ook serologisch min of meer verschillend (Tabel 2).

References

Barnett, O. W. & Gibson, P. B., 1975. Identification and prevalence of white clover viruses and the resistance of *Trifolium* species to these viruses. Crop Sci. 15: 32–37.

Beczner, L., Maat, D. Z. & Bos, L., 1976. The relationships between pea necrosis virus and bean yellow mosaic virus. Neth. J. Pl. Path. 82: 41–50.

Bos, L., 1969a. Some problems in the identification of a necrosis virus of pea (*Pisum sativum* L.). Proc. 6th Conf. Czechosl. Plant Virologists, Olomouc, Sept. 1967. Czechosl. Acad. Sci., Prague: 253–262.

Bos, L., 1969b. Inclusion bodies of bean yellow mosaic virus, some less known closely related viruses, and beet mosaic virus. Neth. J. Pl. Path. 75: 137–143.

Bos, L., 1970. 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. Neth. J. Pl. Path. 76: 8–46.

Bos, L., Kowalska, Cz. & Maat, D. Z., 1974. The identification of bean mosaic, pea yellow mosaic and pea necrosis strains of bean yellow mosaic virus. Neth. J. Pl. Path. 80: 173–191.

Bos, L. & Rubio-Huertos, M., 1969. Light and electron microscopy of cytoplasmic and unusual nuclear inclusion bodies evoked by a virus from necrotic peas. Virology 37: 377–385.

Gibbs, A. J., Varma, A. & Woods, R. D., 1966. Viruses occurring in white clover (*Trifolium repens* L.) from permanent pastures in Britain. Ann. appl. Biol. 58: 231–240.

Granett, A. L. & Provvidenti, R., 1975. Partial purification and serological relationship of three strains of bean yellow mosaic virus. Ann. appl. Biol. 81: 413–415.

Hollings, M. & Nariani, T. K., 1965. Some properties of clover yellow vein, a virus from *Trifolium repens* L. Ann. appl. Biol. 56: 99–109.

Hollings, M. & Stone, O., 1974. Clover yellow vein virus. C.M.I./A.A.B. Descr. Pl. Viruses 131: 4 pp.

Houston, R. & Oswald, J. W., 1953. The mosaic virus disease complex of Ladino clover. Phytopathology: 271–276.

Jones, R. T., 1974. Purification, biological and physical properties and serology of bean yellow mosaic virus isolates from soybean, navy bean and clover. Diss. Ohio State Univ.: 198 pp. (Diss. Abstr. Intern. 35, Nr. 8, 1955: order nr. 75: 3108).

Kreitlow, K. W. & Hunt, O. J., 1958. Effect of alfalfa mosaic and bean yellow mosaic viruses on flowering and seed production of Ladino white clover. Phytopathology 48: 320–321.

Kreitlow, K. W., Hunt, O. J. & Wilkins, H. L., 1957. The effect of virus infection on yield and chemical composition of Ladino clover. Phytopathology 47: 390–394.

Lindsten, K., Brishammar, S. & Tomenius, K., 1976. Investigations on relationship and variation of some legume viruses within the potyvirus group. Meddn St. VäxtskAnst. 16: 289–322.

Pratt, M. J., 1968. Clover viruses in eastern Canada in 1967. Can. Pl. Dis. Surv. 48: 87–90.

Pratt, M. J., 1969. Clover yellow vein virus in North America. Pl. Dis. Reptr 53: 210–212.

Provvidenti, R. & Granett, A. L., 1974. Sweet violet, a natural host of bean yellow mosaic virus. Pl. Dis. Reptr 58: 155–156.

Provvidenti, R. & Schroeder, W. T., 1972. Natural occurrence of bean yellow mosaic virus in *Proboscidea jussieui*. Pl. Dis. Reptr 56: 548–550.

Provvidenti, R. & Uyemoto, J. K., 1973. Chlorotic leaf spotting of yellow summer squash caused by the severe strain of bean yellow mosaic virus. Pl. Dis. Reptr 57: 280–282.

Shepard, J. F., Secor, G. A. & Purcifull, D. E., 1974. Immunochemical cross-reactivity between the dissociated capsid proteins of PVY group plant viruses. Virology 58: 464–475.

Thomas, H. R. & Zaumeyer, W. J., 1953. A strain of yellow bean mosaic virus producing local lesions on tobacco. Phytopathology 43: 11–15.

Varma, A., Gibbs, A. J. & Woods, R. D., 1970. A comparative study of red clover vein mosaic virus and some other plant viruses. J. gen. Virol. 8: 21–32.

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