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The relationships between pea necrosis virus and bean yellow mosaic virus

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Abstract

A standard pea necrosis virus isolate (PNV-E178) and two isolates resembling PNV (Kow14 and E242) were fully compared with bean yellow mosaic virus type strain B25 (BYMV-B25). PNV-E178 and PNV-like isolates Kow14 and E242 resembled each other and the earlier described pea necrosis strain of BYMV in their reaction on pea, but differed from BYMV strains studied so far in inclusion bodies, and in their reaction in cucumber.

Serologically, PNV isolates E178 and E242 were closely related to each other and both showed a more distant relationship to BYMV-B25. PNV isolate Kow14 was serologically intermediate between PNV and BYMV-B25, but was hardly infectious to *Phaseolus* beans.

E242 and, to a lesser extent, also Kow14 were considered strains of the pea necrosis virus, which is closely related to BYMV, but apparently not more so than bean common mosaic virus, pea seed-borne mosaic virus, clover yellow vein virus and some other members of the potyvirus group.

The lack of well-definable borderlines between the different taxonomic entities unavoidably leads to problems in diagnosing (identifying) intermediate isolates.

Introduction

A number of viruses are able to cause necrotic streaking and premature death in peas *(Pisum sativum)*, e.g. some viruses of the potyvirus group: lettuce mosaic virus (Ainsworth and Ogilvie, 1939), beet mosaic virus (Quantz, 1958), a special pea necrosis virus (PNV-E178) (Bos, 1969, 1970) and pea necrosis strains of bean yellow mosaic virus (BYMV) (Bos et al., 1974). When identifying the pea necrosis strains of BYMV, the latter authors still considered the PNV, although closely related to BYMV (Bos, 1969), to be a distinct virus because of its slightly wider host range, serological differences, and peculiar inclusion bodies. Another virus isolate necrotic on pea (Kow14), differed in that nearly all bean cultivars *(Phaseolus vulgaris)* were immune, and it needed further identification.

Recently, a new isolate was obtained from necrotic peas (E242) and this had some features in common with PNV, as well as with Kow14. We have now tried to further characterize Kow14 and E242 and compared them with PNV and BYMV-B25, as described earlier, and reconsidered the distinction between the latter two viruses.

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Materials and methods

Isolates and maintenance. Isolates B25 (BYMV), E178 (PNV), Kow14 and the two other BYMV isolates included in the serological tests (viz. the pea yellow mosaic isolate E198 and pea necrosis isolate E221 of BYMV) were the same as of Bos et al. (1974). Isolate E242 originated from necrotic peas in a breeder's nursery. For the present study, B25, E178 and Kow14 were reactivated from the collection of viruses maintained in desiccated leaf material.
Inclusion bodies were studied by light microscopy in epidermal strips stained with 1% phloxine and 1% methylene blue in Christie's solution (Bos and Rubio-Huertos, 1969).

Virus purification, antiserum preparation and serological tests. Of the isolates B25, E198, E221 and Kow14 purified preparations were still available from previous experiments (Bos et al., 1974). So were the antisera to B25, E198 and to the English isolates of BYMV (No 226) and pea mosaic virus (PMV) (No 227), both obtained from Dr M. Hollings, Littlehampton, U.K. For antiserum preparation and for serological tests PNV-E178 and the isolates E242 and Kow14 were purified according to the method described below.

Homogenization was performed in a Waring blendor, the plant material, buffer and organic solvents being chilled at 3 °C. Centrifuging at low speed was at 8000 rpm during 10 min in a Sorvall RC2-B centrifuge, the rotor used depending on the quantity of material. To sediment the virus, preparations were centrifuged in a Beckman ultracentrifuge at 15000 rpm during 1.5 h (rotor 21 or 30) or, when containing sucrose after sucrose-gradient centrifuging, at 25000 rpm during 2 h (rotor 30). For sucrose-gradient centrifuging 1 ml suspension was layered on a linear gradient of 10–40% sucrose and centrifuged at 25000 rpm (rotor SW27) during 1.5 h. Zones with virus were isolated from sucrose gradients with an ISCO density-gradient fractionator. Before concentrating, the sucrose-containing fractions were diluted at least 1:1 with distilled water or buffer. As a buffer, McIlvaine's phosphate-citric acid buffer, 0.18 M, pH 7, was used. For homogenization and for resuspending the first high-speed sediment, 0.1% thioglycolic acid and 0.02 M sodium diethyldithiocarbamate were added.

Infected plants of *Pisum sativum* 'Koroza' were harvested 7–10 days after inoculation. Every 100 g of plant material was homogenized together with 300 ml of buffer, 50 ml of chloroform and 50 ml of carbon tetrachloride. After centrifuging at low speed the clear supernatant was ultracentrifuged and the sedimented virus resuspended in about 35 ml of buffer. The procedure of centrifuging at low and high speed was repeated and the final sediment resuspended in 1 ml of buffer. Then, after centrifuging at low speed, sucrose-gradient centrifuging was applied, the material being subjected to this procedure twice, in between concentrating the virus again to 1 ml. The final zones of 6 gradient tubes, originating from 600 g of plant material were concentrated to 3 ml, mixed with 3 ml of glycerol, and stored at –20 °C.

For immunization with E178, E242 and Kow14, rabbits were given two successive intravenous injections of 3 ml each (3-day interval). An emulsion of 3 ml of virus and 3 ml of Freund's incomplete adjuvant was injected intramuscularly two weeks later. Bleeding was begun 2–3 weeks after the final injection. If further immunization was

Table 1. Summary of host range tests.

Legumes	B25	E178	Kow14	E242
Phaseolus vulgaris				
'Bataaf'	L S	L S	l* –	L S
'Topcrop'	L S	L S	L* –	L S
'Double White Princess'	L S	L S*	L* –	L S
'Dark Red Kidney'	L S	L S	L* –	L S
'Imuna'	L S	–* –	L –	– –
'Jubila'	L S	L S		L S
'Great Northern 123'	L S	– –	L –	– –
'Amanda'	L S	– S*	L –	– –
'Jolanda'	L S	–* –	L –	– –
Pisum sativum				
'Cobri'	– S	L* S	–* S	L S
'Dik Trom'	– S	L* S	L S	L S
'Koroza'	L S	L S	L S	L S
'Rondo'	– S	L S	L S	L S
'Mignon'	– –	– –	– –	– –
'Juwel'	– –	– –	– –	– –
'Relonce'	– –	– –	– –	– –
Trifolium incarnatum	– S	– S	– S	– S
Trifolium pratense	– S*	– –*	– –*	– –
Trifolium repens	– –	– S*	– –*	– –
Vicia faba 'Compacta'	(L) S	L S	L S	L S
Non-legumes				
Ammi majus	L S	L S	L S	L S
Chenopodium amaranticolor	L S	L –	L –	L –
Chenopodium quinoa	L (S)	L S	L –	L S
Chenopodium murale	L –	L –	L –	L –
Cucumis sativus 'Gele Tros'	– –	L –	L –	L –
Gomphrena globosa	l –	l –	l –*	l –
Nicotiana clevelandii	– S	– S	– S	– S
Nicotiana debneyi	l –	L (s)	– –	L –
Nicotiana glutinosa	– –	– –	– –	– –
Nicotiana megalosiphon	l s	L S	L s	L S
Nicotiana rustica	l –	–* –	– –	l –
Nicotiana tabacum				
'White Burley'	l –	l* –	–* –	L –
'Bel 61-10'	– –	l –	l –	l –
Petunia hybrida	l –	l –	–* –	l –
Spinacia oleracea 'Noorman'	L s	L s	L* –	L –
Tetragonia expansa	L –	L –	L –	L –
Zinnia elegans	–* –	L* –	l –	L –

l = latent local infection; L = visible local infection; s = latent systemic infection; S = visible systemic infection; – = no infection as tested by back inoculation; ( ) symbol in parentheses = in other case(s) no infection; * = results slightly differing from those of Bos et al. (1974).

Tabel 1. Samenvatting der waardplantproeven.

necessary, additional intravenous injections were administered.

As a serological test 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 8 h at room temperature.

Results

Host range and symptoms. The results of host range tests are given in Table 1. Those obtained with B25, E178 and Kow14 agree quite well with those reported earlier for these isolates by Bos et al. (1974). There are slight differences, indicated with an asterisk, which could very well be explained by differences in conditions, e.g. due to season.

The four pea *(Pisum sativum)* cultivars susceptible to B25, and producing mosaic with this isolate, all contracted severe necrosis after initial vein chlorosis in some 10 days after inoculation with the other isolates, and died prematurely. Some cultivars reacted with necrotic local lesions. The BYMV resistant cultivars Mignon, Juwel and Relonce were immune to all four isolates.

The nine bean *(Phaseolus vulgaris)* cultivars tested were all sensitive to B25. In Table 1 they have been arranged according to their sensitivity, the last three being more tolerant. With E178 and E242 systemic symptoms were produced in the more BYMV sensitive cultuvars. Local symptoms consisted of chlorotic or necrotic vein patterns, or of green patterns later showing up in yellowing inoculated leaves. Systemic symptoms usually followed late and erratically, and consisted of chlorotic spots or chlorotic and necrotic vein patterns (Fig. 1) and irregular stem or petiole necrosis often leading to

Fig. 1. *Phaseolus vulgaris* 'Topcrop' with local (right) and systemic symptoms (left) 24 days after inoculation with pea necrosis virus (E178).

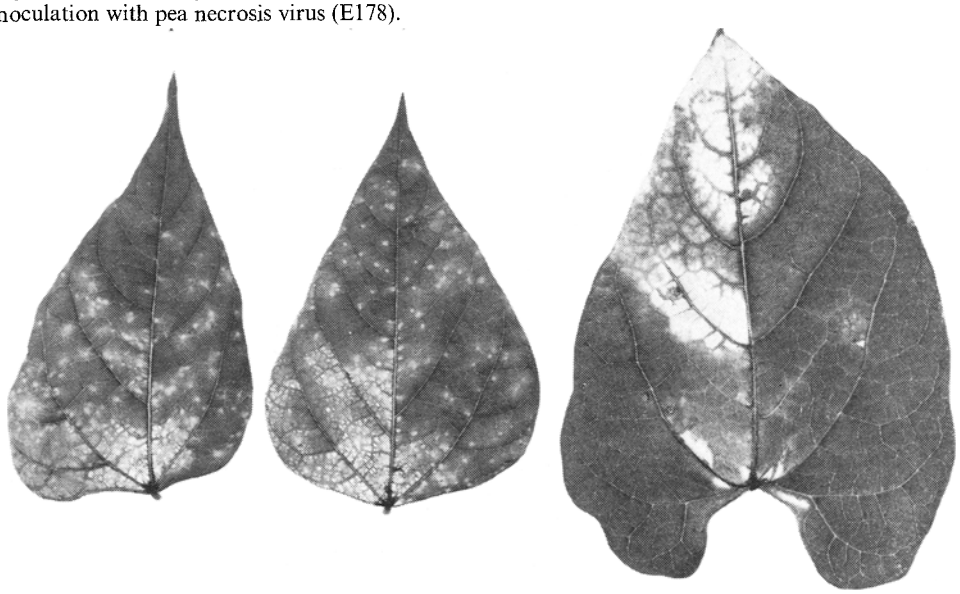

Fig. 1. Phaseolus vulgaris 'Topcrop' met lokale (rechts) en systemische symptomen (links) 24 dagen na inoculatie met het erwtenecrosevirus (E178).

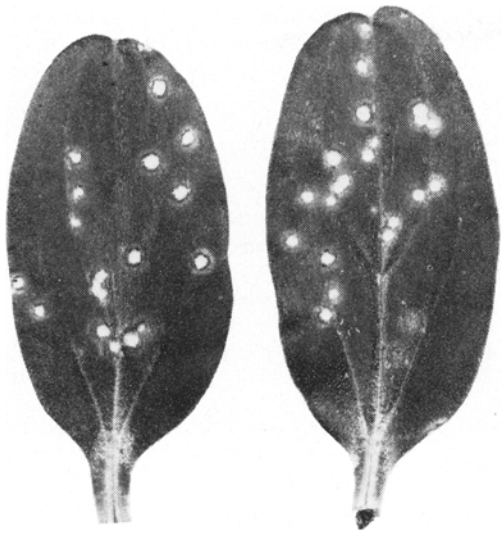

Fig. 2. *Cucumis sativus* 'Gele Tros' with local lesions 18 days after inoculation with E242.

Fig. 2. Cucumis sativus 'Gele Tros' met lokale symptomen 18 dagen na inoculatie met E242.

Fig. 3. *Nicotiana megalosiphon* with systemic symptoms 22 days after inoculation with E242.

Fig. 3. Nicotiana megalosiphon met systemische symptomen 22 dagen na inoculatie met E242.

defoliation. With Kow14, this time most cultivars reacted with a few necrotic local lesions but none of the cultivars became systemically infected and this also held for 'Double White Princess' being highly sensitive to all isolates of BYMV (Bos et al., 1974). Symptoms of E242 were much more severe than those of E178.

Broad bean *(Vicia faba)* 'Compacta' reacted to all four isolates. With B25 a distinct systemic mosaic was formed, whereas the other isolates produced chlorotic, sometimes necrotic local lesions and systemic spotting and mottling.

In *Chenopodium amaranticolor* all isolates but B25 remained local. In *C. quinoa* E242 resembled E178, both going systemic. B25 sometimes also caused systemic symptoms. The virus that could then be recovered from tip leaves more easily went systemic when again transferred to *C. quinoa*, but still produced symptoms characteristic of B25 on bean and pea.

In *Cucumis sativus* 'Gele Tros' all isolates but B25 produced chlorotic, later dry to necrotic local lesions and these were greatest in number with E242 (Fig. 2).

Nicotiana megalosiphon produced clear chlorotic local lesions to all isolates but B25. With E242 numerous of such lesions were formed and systemic symptoms consisted of a chlorotic spotting or many conspicuous small chlorotic rings (Fig. 3).

Ammi majus, an umbelliferous species, readily reacted to all isolates. Local lesions were particularly discrete and numerous with E242.

The reactions of plant species that may be of help as differential hosts are summarized in Table 3.

Inclusion bodies. All four isolates produced inclusion bodies that were readily detectable in all plant species tested: pea, broad bean, and *Ammi majus*. In all species they were characteristic of the isolate concerned. With B25 striking granular cytoplasmic inclusions were prevalent, nuclei and nucleoli apparently were normal. E242 and Kow14 greatly resembled each other in producing granular cytoplasmic inclusions and striking nucleolar enlargements often nearly filling the entire nucleus. With E178 the granular inclusions were indistinct or absent, nucleoli were more or less enlarged and typical crystalline radiating needles were those described earlier (Bos and Rubio-Huertos, 1969).

Serology. Results of serological experiments are summarized in Table 2. Serologically, E178 and E242 were nearly identical. They differed considerably from B25. With the antiserum to the pea mosaic strain E198 differences were less. The new preparations of Kow14 resembled BYMV isolates B25 and E198 much more closely than the old preparation (Bos et al., 1974). According to the reactions with the new preparations Kow14 seems to be more closely related to BYMV and its strains than are E178 and E242. A clear difference between B25 and E198 on the one hand and E178 and E242 on the other is also shown by reaction of the antisera prepared against the latter two.

Discussion

In host range and symptoms the four isolates compared had many features in common. However, this does not necessarily mean that they are identical or strains of one virus since many members of the potyvirus group share hosts and symptoms (e.g. Bos, 1970). Results of these biological tests are summarized in Table 3, employing the differential hosts as used by Bos et al. (1974). With these hosts, PNV-E178 could be easily differentiated from the BYMV-B25 as well as from its pea mosaic and pea

Table 2. Summary of microprecipitin tests. Homologous titres are in italics.

Antigens	Antisera									Normal serum
	B25		E198		BYMV	PMV	E178	Kow14	E242	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Hollings	Hollings				
B25	*4096*	*1024*	1024	1024	64	1024	16	1024	64	–
E198	4096	1024	*1024*	*1024*	256	1024	64	1024	64	–
E221	1024	1024	1024	1024	256	4096	64	1024	64	–
E178	1	4	256	256	–	256	*1024*	64	1024	–
Kow14[1]	16	64	16	16	16	256	256	256	1024	–
Kow14[2]	4096	1024	1024	256	256	256	64	*1024*	64	–
		4096[4]		1024[4]			256[4]	*1024*[4]	256[4]	–
Kow14[3]		1024[4]		1024[4]			64[4]	1024[4]	1024[4]	–
E242	1	4[4]	256	256[4]	16	1024	256[4]	256[4]	*256*[4]	–
Healthy concentrated pea sap	–	–	–	–	–	–	–	–	–	–

– no reaction; [1] Preparation of Bos et al., 1974; [2]Preparation at pH 7 in 1975; [3] Preparation at pH 9 in 1975; [4] Titres obtained in a separate test using the same antiserum preparations.

Tabel 2. Overzicht van de micro-precipitatieproeven. Homologe titers zijn cursief weergegeven.

Table 3. Differentiation between the B25 strain of bean yellow mosaic virus and the three isolates of pea necrosis virus studied.

	Bean yellow mosaic virus	Pea necrosis virus		
	B25	E178	Kow14	E242
Phaseolus vulgaris				
'Double White Princess'	L S[1]	L S[2]	L –	L S[1]
'Great Northern 123'	L S	– –	L –	– –
Pisum sativum				
'Koroza'	L S[4]	L S[3]	L S[5]	L S[3]
'Juwel'	– –	– –	– –	– –
Vicia faba 'Compacta'	(L) S	L S	L S	L S
Chenopodium amaranticolor	L S	L –	L –	L –
Chenopodium quinoa	L[6] (S)	L[7] S	L[6] –	L[7] S
Cucumis sativus	– –	L –	L –	L –
Nicotiana debneyi	l –	L (s)	– –	L –

For legends see also Table 1.
[1] Tip necrosis; [2] Delayed tip necrosis; [3] Severe necrosis; [4] Green mosaic; [5] Severe necrosis (sometimes yellow mosaic); [6] Chlorotic; [7] Necrotic.

Tabel 3. Onderscheid tussen de B25-stam van het bonescherpmozaïekvirus en de drie isolaten van het erwtenecrosevirus.

necrosis strains, among others E221 (Bos et al., 1974: Table 6). E221 and other isolates of the pea necrosis strain of the BYMV have not been included in the present biological tests. In inclusion bodies, host range and symptoms (N.B. no local lesions in cucumber) they are more closely related to BYMV than to PNV, although in symptoms in pea and tobaccos they closely resemble PNV (Bos et al., 1974). Kow14 and E242 resemble E178 in their wider host range among non-legumes than BYMV, in producing severe necrosis in peas and in causing striking nucleolar enlargements in infected cells, although E178 was the only one producing intranuclear needles protruding from the surface of the nucleolus. Kow14 was more extreme in not causing systemic symptoms in any of the bean cultivars tested.

Serologically, E242 also appeared to be very closely related to PNV-E178 and both differed considerably from B25 and slightly less from a pea yellow mosaic strain of the BYMV (E198), E242 can therefore be considered a strain of PNV. Kow14 seemed some sort of intermediate between PNV (E178 and E242) and BYMV (B25 and E198), and will be regarded as a deviant strain of PNV. Using Kow14 as an antigen, the question arises why the 1975 preparations differ so much from the 1974 preparation in their reactions with several of the antisera (especially those to B25, E198 and BYMV-Hollings), the reactions of the 1974 preparation being much more in agreement with Bos et al. (1974: Table 5). The virus seems to have changed in its antegenic properties. According to the typical inclusion bodies produced, contamination with BYMV was unlikely. Moreover, the differences mentioned are not caused by the buffers used for purification. This can be concluded from Table 2. One new preparation of Kow14 was purified according to the method used in earlier studies (Bos et al., 1974), applying tris buffers at pH 9. The reactions of this preparation did not deviate much from that purified according to the present method using

McIlvaine's phosphate-citric acid buffer at pH 7. With the latter buffer particles of E178 showed less fragmentation and yields were better than with tris buffers at pH 9.

Serological relationships as found between BYMV and PNV are common within the potyvirus group (e.g. Bercks, 1960, for a number of strains of BYMV and one of bean common mosaic virus (BCMV). BYMV and PNV may therefore very well be considered as distinct viruses. We have earlier pointed out that lettuce mosaic virus, watermelon mosaic virus, soybean mosaic virus, clover yellow vein virus, cowpea aphid-borne mosaic virus and pea seed-borne mosaic virus are other members of the same morphological group all being more or less closely related (Bos, 1970; Bos et al., 1974). This holds for their biological properties as well as for serology. Recently, Koenig and Givord (1974) found that within the turnip yellow mosaic virus group (tymovirus group) there is a continuous range of serological relationships. They again demonstrated how antisera from different rabbits and from different bleedings of the same rabbit showed considerable differences in the degrees of serological relationships revealed. Hence, study of relationships with single antisera, as practised by most authors describing new viruses, is inadequate.

Similarly, within the potyvirus group there is a continuum of various types all having relatively wide host ranges with overlapping characters. Borderlines will therefore have to be drawn arbitrarily, and they will not be sharp-cut between the artificially defined taxonomic entities. This will continue to create problems when diagnosing intermediate isolates. For a further discussion of the problem of virus variation see also Bos (1970).

Note added in proof

Recently, some experiments were made by the junior authors together with Dr K. Lindsten, Uppsala, Sweden, to compare the pea necrosis virus isolates (PNV) studied in this publication with two Swedish isolates of clover yellow vein virus (ClYVV) and the type strain of that virus. It was found that ClYVV is more closely related to bean yellow mosaic virus than suggested originally by Hollings and Nariani (1965; Ann. appl.Biol. 56: 99–109). PNV and ClYVV may have sufficient features in common to consider them as strains of a single virus. More information will be published soon.

Samenvatting

De verwantschap tussen erwtenecrosevirus en bonescherpmozaïekvirus

Een aantal virussen kan in erwten ernstige afstervingsverschijnselen doen ontstaan, zoals een eerder beschreven erwtenecrosevirus (PNV-E178) en een erwtenecrosestam van het bonescherpmozaïekvirus (BYMV). Twee nieuwe erwtenecrose-isolaten (Kow14 en E242) werden bestudeerd en hun verwantschap met het erwtenecrosevirus (PNV) en het bonescherpmozaïekvirus (BYMV) gaf aanleiding tot een nieuw onderzoek naar de relatie tussen de twee laatstgenoemde virussen.

In hun reactie op erwt en boon leken PNV-E178, Kow14 en E242 veel op elkaar en op de erwtenecrosestam van BYMV. Ze verschilden echter van de mozaïekstam (B25) van dit virus in de geproduceerde celinsluitsels, vooral wat betreft de sterke vergroting der nucleoli en bij E178 bovendien door de opvallende uitstaande kristal-

naalden in de celkern. Ook zijn de eerste drie isolaten duidelijk minder pathogeen voor *Phaseolus*-bonen (Fig. 1) dan de bonemozaïekstam en de erwtegeelmozaïekstam van het BYMV. Op deze plantesoort doet Kow14 slechts een gering aantal lokale vlekken ontstaan. Verder worden *Nicotiana*-soorten gemakkelijker door de drie isolaten aangetast (Fig. 3) dan door BYMV en ontstaan in komkommerzaadlobben lokale lesies (Fig. 2 en Tabel 1). Deze laatste worden ook niet veroorzaakt door de erwtenecrosestam van het BYMV, hoewel ook deze vrij gemakkelijk *Nicotiana*-soorten aantast (o.a. het nu in de serologieproef gebruikte isolaat E221).

Serologisch zijn PNV-E178 en E242 nauw aan elkaar verwant en zijn beide minder nauw verwant aan B25 (Tabel 2). Kow14 neemt serologisch een tussenpositie in, hoewel dit isolaat in biologisch opzicht meer een uiterste is omdat het nauwelijks infectieus is voor *Phaseolus*-bonen.

E242, en hoewel minder verwant, ook Kow14, worden nu beschouwd als stammen van het erwtenecrosevirus (Tabel 3). Dit virus is weliswaar vrij nauw verwant aan het bonescherpmozaïekvirus, maar niet nauwer dan het bonerolmozaïekvirus, het erwterolmozaïekvirus, het nerfvergelingsvirus van klaver en enkele andere leden van de aardappelvirus-Y-groep.

Het klaarblijkelijk ontbreken van scherpe grenzen tussen de verschillende taxonomische eenheden leidt onvermijdelijk tot moeilijkheden bij de beschrijving en herkenning van intermediaire isolaten(tussenvormen).

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