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The host ranges, classification and identification of eight persistent aphid-transmitted viruses causing diseases in legumes

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Abstract

Seventy one seed-lines representing 23 species of papilionoid legumes and 17 species of non-legumes were collected and distributed to four countries; Australia, New Zealand, the Netherlands and the U.S.A. In each of these countries plants were grown from the seeds and their susceptibility to a total of eight persistent aphid isolates transmitted viruses was assessed. The viruses were a strain of beet western yellows (BWYV) from *Glycine max* in Illinois, legume yellows (LYV) in California and a virus in Michigan (MiAV) from *Medicago sativa,* from *Pisum sativum* causing leaf roll and top yellows in New Zealand (PeLRV-NZ) and the Netherlands (BLRV), isolates of subterranean clover red leaf from New Zealand (SCRLV-NZ) and Tasmania (SCRLV-T), and subterranean clover stunt (SCSV) from Tasmania.

The relationships between the eight viruses as indicated by their host reactions were assessed using computer classification techniques. SCRLV-NZ and SCRLV-T were the most similar. They had moderately wide host ranges that included some non-legumes. A second group comprized BWYV and PeLRV-NZ. These were typical of most beet western yellows virus strains in that they infected *Brassica napus, Capsella bursa-pastoris* and *Stellaria media.* MiAV and BLRV also formed a pair. They generally induced severe symptoms on the hosts which they infected and had host ranges confined to legumes except that BLRV also infected *Claytonia perfoliata* and *Erodium* spp. The relationships of LYV and SCSV were not consistent. They paired together in some classifications, but SCSV sometimes grouped with the SCRLV isolates. Both had host ranges confined to legumes, caused severe symptoms in most hosts and were often difficult to recover from affected plants. LYV had some affinities with BLRV and MiAV.

The tests indicated a set of test plants which were most useful for propagating and identifying persistent aphid-transmitted viruses from legumes. Two, *P. sativum* cv. Onyx and *Trifolium subterraneum* cv. Bacchus Marsh were susceptible to all isolates. Ten others distinguished between the isolates and were *Arachis hypogea, Beta vulgaris, C. bursa-pastoris, G. max* cv. Shiro-

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tsurunoko, *Gomphrena globosa, Lactuca sativa, Lens esculenta* cv. 179307, *M. sativa* cv. Washoe, *Phaseolus vulgaris* cv. Canadian Wonder and *Trifolium hybridum.*

Additional keyword: luteovirus.

Introduction

The luteoviruses have only recently been defined as a discrete group by the International Committee on Taxonomy of Viruses (ICTV) (Fenner, 1976) even though this group is economically most important because of the great losses caused by its members in a range of crops (Duffus, 1977). Luteoviruses cause yellowing or reddening symptoms and are transmitted by aphids in a persistent manner, though they do not replicate in aphids. They have small isometric particles which are about 25 nm in diameter. They have only been found in phloem tissue and this is probably the reason why luteovirus particles occur in very small concentrations (ng/l) in plant sap extracts. As a result, only recently have some been obtained in sufficient quantities to permit them to be properly characterized, and particles of different luteoviruses to be compared.

The ICTV now recognizes 15 definitive, 3 tentative and another 16 possible luteoviruses (Matthews, 1982). Some of these have been isolated from legumes in various parts of the world, and among the most fully characterized are beet western yellows virus (BWYV) and soybean dwarf virus (SDV) (Duffus, 1972; Tamada, 1977). Most luteoviruses are less well known and many have been placed with the luteoviruses merely on account of the symptoms which they induce and because they are transmitted by aphids in a persistent manner.

Quarantine barriers, geographical isolation and a lack of resources are likely to prevent for some time the full characterization and serological comparison of the particles of many such luteoviruses. Therefore we started in 1977 to investigate the value of host range studies for comparing luteoviruses and similar viruses from legumes. Seeds of suitable test plant species and cultivars were exchanged and plants grown from these in four different countries were used to determine the host ranges of eight persistent aphid-transmitted viruses isolated from legumes. This paper reports the results of those tests, which have been used to classify the viruses and to indicate a set of twelve test plants that can be used to identify them.

The use of host range information to classify viruses has clear limitations that are worth some comment. The host range of a virus and the symptoms it induces in susceptible hosts seem to be among the most mutable characters; mutation of even a single nucleotide can dramatically change its virulence (Wittman and Wittman-Liebold, 1966). If two virus isolates have the same host range and produce the same symptoms in all susceptible hosts, it is likely that they are isolates of the same virus; the more species tested the more certain is that conclusion. However if two isolates have different host ranges or give different symptoms, then not only is it clear that they are different, but experience has shown that one cannot deduce how unrelated they are; host range and symptoms are of limited value for taxonomic predictions, though two virus isolates that differ in virulence but not host range are often found to be strains of one virus. Thus classifications based on host range information are most likely to be 'artificial' and may not reflect evolutionary relationships. However we believe that some inferences of natural relationships may be cautiously drawn from our classifications based on luteovirus host ranges because, as we report, the groupings obtained

correlate well with the few studies on serological relationships between the particles of these viruses.

Material and methods

Details of the eight virus isolates we tested are listed in Table 1. Five are definitive luteoviruses, namely:

BLRV bean leaf roll virus
BWYV beet western yellows virus
LYV legume yellows virus
SCRLV-NZ subterranean clover red leaf virus – New Zealan
SCRLV-T subterranean clover red leaf virus – Tasmania

and three are possible luteoviruses whose particles have either not been visualized or been properly characterized, namely:

MiAV Michigan alfalfa virus
PeLRV-NZ pea leaf roll virus – New Zealand
SCSV subterranean clover stunt virus.

Seventy-one different seed-lines representing 23 species of the Papilionoideae (Polhill, 1981) and 17 non-leguminous species were used in the host range studies. These are listed in Table 2. They were selected on the basis of published and unpublished records of their reactions to inoculation with various luteoviruses isolated from legumes and from other plant families.

Seed of each line used in our tests originated from one seed-lot. These were used to produce seedlings in insect-screened glasshouses under conditions of high natural light intensity. Groups of viruliferous aphids were placed on young seedlings for 2-3 days in attempts to infect them. The total time of the acquisition and transmission feeds was never less than 4 days. With few exceptions, ten or more plants were inoculated with each isolate. Similar seedlings used as controls, were exposed to aphids that had not acquired virus. Virus recovery (back-) tests were done using sensitive indicator lines (Table 1) whenever inoculated plants failed to develop symptoms, so that immune and resistant hosts could be differentiated from tolerant ones.

The aphids used in each inoculation and recovery test comprized a random mixture of alate and apterous nymphs and adults. The numbers in each test varied according to the aphid species, 5-10 for *Aulacorthum solani,* 10-15 for *Aphis craccivora,* and more than 20 for those isolates which involved using *Acyrthosiphon pisum* and *Myzus persicae.* The conditions of the tests resulted in transmission rates of more than 95% between the propagation and indicator back-test hosts.

Records were kept on the proportion of plants of each line which displayed symptoms and on the proportion from which each isolate was recovered in back-tests. These data were analysed using programs in the TAXON P3 library of the CSIRONET Cyber computer; Canberra (MCAN), Euclidean (MSED) and Gower (MGOW) metrics were compared for their usefulness in measuring relationships (Burr, 1968; Lance and Williams, 1967). These were displayed by the flexible agglomerative hierarchical methods (SAHN; Lance and Williams, 1966) and by principal co-ordinates analysis (PCOA: Gower, 1966). The classifications were correlated with the attributes used by the GCOM and BACRIV programs (Lance et al., 1968). Identification keys were computed by the KEY program of Dallwitz (1974).

Table 1. Details on the eight persistent aphid-transmitted virus isolates from legumes.

Virus	Acronym	Source/ origin	Vector	Propagation host	Indicator (test) host	Place of testing	Reference
bean leaf roll virus	BLRV	lucerne, the Netherlands	*Acyrthosiphon pisum*	pea cv. Onyx	pea cv. Onyx	Wageningen, the Netherlands	Ashby and Huttinga, 1979
beet western yellows virus	BWYV	soybean, Illinois, U.S.A.	*Myzus persicae*	shepherd's purse	shepherd's purse	Salinas, California, U.S.A.	Duffus and Milbrath, 1978
legume yellows virus	LYV	alfalfa, California U.S.A.	*A. pisum*	broad bean	sub-clover cv. Geraldton	Salinas, California, U.S.A.	Duffus, 1979
Michigan alfalfa virus	MiAV	alfalfa, Michigan, U.S.A.	*A. pisum*	broad bean cv. Broad Improved Long Pod	broad bean cv. Broad Improved Long Pod	East Lansing, Michigan, U.S.A.	Thottappilly et al., 1977
pea leaf roll virus – New Zealand	PeLRV-NZ	pea, New Zealand	*M. persicae*	sub-clover cv. Bacchus Marsh	sub-clover cv. Bacchus Marsh	Lincoln, New Zealand	Wilson, and Close, 1973
subterranean clover red leaf virus – Nieuw Zealand	SCRLV-NZ	white clover, New Zealand	*Aulacorthum solani*	pea cv. Onyx	sub-clover cv. Bacchus Marsh	Lincoln, New Zealand	Ashby et al., 1979
subterranean clover red leaf virus – Tasmania	SCRLV-T	white clover, Tasmania	*A. solani*	sub-clover cv. Mt Barker	sub-clover cv. Mt Barker	Hobart, Tasmania, Australia	Johnstone, 1978
subterranean clover stunt virus	SCSV	broad bean, Tasmania	*Aphis craccivora*	sub-clover cv. Mt Barker	sub-clover cv. Mt Barker	Hobart, Tasmania Australia	Johnstone, 1978

Table 2. The host ranges of the eight persistent aphid-transmitted isolates from legumes.

Host number	Host	Virus							
		BLRV	BWYV	LYV	MiAV	PeLRV-NZ	SCRLV-NZ	SCRLV-T	SCSV
	Legumes								
1	*Arachis hypogea*	+[1]	+(N)	+(N)	+	+(N)	−	−	−
2	*Astragalus sinicus*	+	nt	nt	+	nt	+(N)	+(N)	+
3	*Cicer arientinum*	+	+	+	+	+	−	−	−
4	*Dolichos lablab (Lablab purpureus)*	−	−	−	+(N)	−	−	−	−
5	*Glycine max* cv. Koganejiro	+	+(N)	+(N)	+	−	+	+	+
6	cv. Shirotsurunoko	−	+(N)	−	−	+	+	+	+
7	*Lathyrus odoratus*	+	−	−	+(N)	nt	−	−	−
8	*Lens esculenta* cv. Chilean	+	+	+	+	+	+	+(N)	+(N)
9	cv. Tekoa	+	+(N)	+	+	+	+	+	−
10	cv. 178940	+	+(N)	+	+	+	+	+	+
11	cv. 179307	−	+(N)	+	+	+	−	+(N)	−
12	*Lupinus albus* cv. Ultra	−	+(N)	+(N)	+	+	+	+	−
13	*Lupinus cosentini*	+	+(N)	+(N)	−	+	+	+	−
14	*Medicago hispida*	+	+	+	+	+	+	+	+
15	*Medicago sativa* cv. Du Puits	+	−	−	+(N)	−	+	−	−
16	cv. Saranac	+	−	−	+(N)	+	+	−	−
17	cv. Wairu	+	−	−	+	+	+	−	−
18	cv. Washoe	+	−	−	+(N)	+	+	−	−
19	*Melilotus alba*	−	−	−	+(N)	+	−	−	−
20	*Melilotus indica*	−	−	−	−	+	+	−	+
21	*Phaseolus vulgaris* cv. Apollo	+	−	−	+	+	+	+	+
22	cv. B019	−	−	−	+	+	+	+	+
23	cv. Canadian Wonder	−	−	−	+	−	−	+	+
24	cv. Galatin 50	nt	−	−	−	+	+	+	+

Table 2. (Continued).

Host number	Host	Virus							
		BLRV	BWYV	LYV	MiAV	PeLRV-NZ	SCRLV-NZ	SCRLV-T	SCSV
25	*Phaseolus vulgaris* cv. Pinto 111	+	−	−	+	nt	−	−	−
26	cv. Red Mexican 37	−	−	−	+(N)	nt	−	−	−
27	cv. Tendergreen	+	−	−	+	nt	+	+	+
28	cv. Topcrop	+	−	nt	+	nt	+	+	+
29	cv. 526	nt	nt	nt	nt	nt	+	+	+
30	*Pisum sativum* cv. Frosty	+	+(N)	−	−	+	+	−	+
31	cv. Jade	+	+(N)	−	+	+	+	+	+
32	cv. Koroza	+	+(N)	−	−	+	−	+	+
33	cv. Laxton's Superb	+	+(N)	+	+	nt	+	+	+
34	cv. Lord Chancellor	+	+(N)	−	−	nt	+(N)	+(N)	+
35	cv. Onyx	+	+(N)	+	+	+	+	+	+
36	cv. Puke	+	−	+	−	nt	+(N)	+(N)	+
37	cv. Rondo	+	+(N)	+	+	nt	+(N)	+(N)	+
38	cv. Victoria	+	+	+	+	+	+	+	+
39	*Trifolium hybridum*	+	−	−	+	+	+(N)	+(N)	−
40	*Trifolium incarnatum*	+	+	+	+	+	+	+	+
41	*Trifolium pratense*	−	+(N)	−	+(N)	+	−	−	−
42	*Trifolium repens*	+(N)	+(N)	−	+	+	+(N)	+(N)	−
43	*Trifolium subterraneum* cv. Bacchus Marsh	+	+	+	+	+	+	+	+
44	cv. Tallarook	+	+	+	+	+	+	+	+
45	cv. Woogenellup	+	+	+	nt	+	+	+	+
46	*Vicia faba* cv. Beryl	+(N)	+(N)	+	+	nt	+	+	+
47	cv. Cole's Dwarf Prolific	+	+(N)	+	+	nt	+	+	+
48	cv. Compacta	+	+	+(N)	+	nt	+	+	+

49	cv. Extra Early Seville	−	+(N)	+	+	+	+	+	+
50	cv. Primo	−	+	+(N)	+	nt	+(N)	+	+
51	cv. Staygreen Origineel	+	−	+	+	nt	+	+	+
52	cv. Triple White	+	+(N)	+	+	nt	+	+	+
53	*Vicia sativa*	+	+(N)	−	+	+	+	+	+
54	*Vigna unguiculata*	+(N)	+(N)	−	+(N)	−	+(N)	+(N)	−
	Non-Legumes								
55	*Amaranthus tricolor-splendens*	nt	−	−	−	nt	nt	nt	nt
56	*Beta vulgaris*	−	−	−	−	+	+	+	−
57	*Brassica napus*	−	+	−	−	+	−	−	−
58	*Capsella bursa-pastoris*	−	+	−	−	+	−	−	−
59	*Claytonia (Montia) perfoliata*	+	+	−	−	+	−	+(N)	−
60	*Datura stramonium*	nt	−	−	−	−	−	−	−
61	*Erodium cicutarium*	+(N)	+	−	nt	nt	+	+	−
62	*Erodium moschatum*	+(N)	−	−	−	nt	+	+	−
63	*Gomphrena globosa*	−	+(N)	−	−	−	+(N)	+(N)	−
64	*Lactuca sativa*	−	+	−	−	+(N)	−	+	−
65	*Linum usitatissimum*	−	−	−	−	−	−	−	nt
66	*Malva parviflora*	nt	+(N)	−	−	nt	+(N)	−	−
67	*Rumex obtusifolius*	−	−	−	−	−	−	−	−
68	*Senecio vulgaris*	−	+	−	−	+	−	+(N)	−
69	*Stellaria media*	−	+	−	−	+	−	−	−
70	*Tetragonia expansa*	−	−	−	−	nt	+	−	−
71	*Zinnia elegans*	−	+	−	−	nt	+	+	−

[1] + and − indicate infected and non-infected, respectively; (N) indicates no symptoms on infected plants; nt indicates not tested.

Results

The results were analyzed to obtain various types of information. Firstly simple qualitative differences in the responses of different seed-lines were assessed, then the quantitative results were used both to assess the relatedness of the virus isolates based on their host ranges, and to devise strategies for identifying the viruses.

Qualitative differences. The intensity of the symptoms induced by each isolate was usually positively correlated with the proportion of plants which developed symptoms. This was particularly apparent in the tests using *Phaseolus vulgaris, Pisum sativum* and *Vicia faba* seed-lines, as several cultivars of each were included in the tests. Some seed-lines were either immune or more resistant or tolerant to particular isolates and hence are obvious candidates for use in breeding or selection programs, whereas others which were particularly susceptible or sensitive were suitable for diagnostic work. For example most cultivars of *Lens esculenta, P. sativum* and *Trifolium subterraneum* were susceptible to most of the virus isolates and showed clear symptoms, and hence were useful for propagation and indexing; *P. sativum* cv. Onyx and *T. subterraneum* cv. Bacchus Marsh were particularly suitable for such purposes.

Relatedness based on host range. Classifications of the virus isolates were computed using as attributes either the complete quantitative data set, namely the proportion of plants infected by each isolate, or a restricted binary data set (Table 2) consisting of records on whether or not any plant of a particular seed-line proved to be susceptible to each virus. The classifications produced using the two data sets agreed in that all eight isolates were indicated to be clearly different from each other but that, in both, the two SCRLV isolates grouped together as did BWYV with PeLRV-NZ and BLRV with MiAV. LYV had some affinities with BLRV and MiAV.

The binary data (susceptibility of the 71 seed-lines) were then analyzed in more detail because they are perhaps less likely to be influenced by factors that vary from one experiment to another, such as plant susceptibility, than are the quantitative data.

They were classified using Euclidean, Gower and Canberra metrics, and then further classified after compression by pooling the responses of all species in each genus (40 attributes; genera), treating either susceptibility or insusceptibility as the dominant attribute. All the resulting classifications were closely similar and examples are illustrated in two forms in Figs 1 and 2.

In all these classifications of binary data the two SCRLV isolates paired as before, as did BWYV with PeLRV-NZ and BLRV with MiAV. The position of the other two isolates was less certain, SCSV grouped with the SCRLV isolates in some classifications, but in others it paired with LYV. There was no clear constancy in the relationships of these pairs.

Analysis of the dendrogram in Fig. 1 by the group comparison program of TAXON indicates which host responses contributed most to its branching pattern. For example the BWYV/PeLRV-NZ pair differed from the other six isolates in that they infected such non-legumes as *Brassica napus, Capsella bursa-pastoris* and *Stellaria media.* At a lower level in the dendrogram, the three subterranean clover viruses differed from the BLRV/LYV/MiAV group in being able to infect *G. max* cv. Shirotsurunoko but not *Arachis hypogea, Cicer arientinum* and *P. vulgaris* cv. Pinto 111. The key hosts respon-

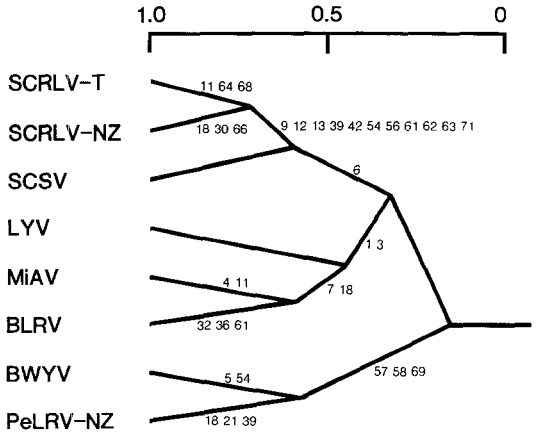

Fig. 1. Dendrogram illustrating an hierarchical agglomerative classification of eight virus isolates using binary susceptibility data of the responses of 71 seed-lines; squared Euclidean distance metric, flexible sorting strategy. On each branch in the classification are given the numbers (Table 2) of the seed-lines that were susceptible to the virus or viruses it represents; the same species were insusceptible to the virus or viruses attached to the adjoining branch. For acronyms of viruses see text.

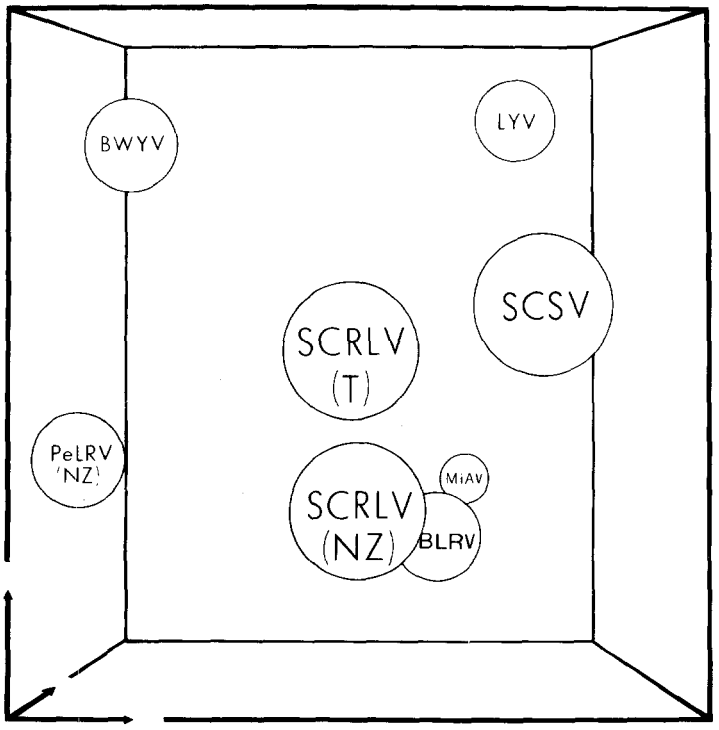

Fig. 2. A pseudo three-dimensional diagram illustrating a principal co-ordinates analysis of the measures of similarity upon which Figure 1 is based; MSED and PCOA programs of the CSIRONET TAXON P3 library.

sible for branching of the dendrogram are shown in Fig. 1; the numerals on the branches refer to host numbers (see Table 2) and indicate that those hosts were infected by the viruses represented on that branch but were not infected by viruses on the corresponding branch from which it diverged.

The two SCRLV isolates were the most similar we studied. They had moderately wide host ranges that included several non-legumes and caused disease in most cultivars of *L. esculenta, P. vulgaris, P. sativum* and *V. faba.* Alsike *(T. hybridum)* and white clover *T. repens)* were infected without displaying symptoms and red clover *(T.pratense)* was not infected.

BWYV and PeLRV-NZ formed a second group. They had wide host ranges, infected more non-legumes than the SCRLV viruses, and were transmitted by *M. persicae.*

A third group consisted of BLRV and MiAV. These generally induced severe symptoms on their more restricted ranges of hosts which were confined to legumes, apart from BLRV infecting *C. perfoliata* and *Erodium* spp.

The remaining isolates, LYV and SCSV, which sometimes grouped together in the classifications, had the fewest numbers of hosts and these were exclusively legumes. They generally induced very severe disease symptoms but were often difficult to recover from affected plants. Although LYV was originally isolated from *M. sativa* (Table 1), this isolate failed to infect the four alfalfa cultivars used in these tests. It had some affinities with BLRV and MiAV.

Identification. The binary data set was also analyzed by the KEY program, which seeks the minimum set of diagnostic tests (and their replicates) required to distinguish between the isolates. An ideal set of diagnostic hosts, which successively divided the isolates into two equal groups, would require only three identificatory steps (2^3) to distinguish between 8 isolates.

The KEY program allows weighting of the value of different attributes, in this instance, host susceptibility. An initial unweighted analysis gave the key in Table 3. It can be seen for example that PeLRV-NZ can be distinguished from all seven other isolates by its ability to infect *B. vulgaris, M. sativa* cv. Washoe and *Melilotus alba.* It is noteworthy that *B. vulgaris* and *E. moschatum* were the only non-legume species in this key, even though the primary division of the classification dendrogram was determined by non-legumes (Fig. 1).

Table 4 shows a key obtained by weighting in favour of non-legumes; on a scale of 0 to 10 their weighting was 7 compared with 5 for the leguminous hosts. It shows that non-legumes could not alone distinguish between the eight isolates; legumes were required to distinguish between LYV, MiAV and SCSV.

Indicator set. We have considered the overall results on which Table 2 is based and the computer outputs on classification and identification in order to select a group of hosts which seem most useful for the propagation, classification and identification of luteoviruses infecting legumes. Our choice was also guided by our assessments of their ease of use in greenhouses, the reliability of establishing infections in them and on the intensity of symptoms produced by them in response to inoculation with the isolates.

A total of twelve hosts was selected (Table 5) of which two *(P. sativum* cv. Onyx and *T. subterraneum* cv. Bacchus Marsh) were sensitive to all isolates. The remaining ten

Table 3. Diagnostic key based on unweighted host responses.

Virus isolate	Identification steps			
	1	2	3	4
PeLRV-NZ	16+[1,2] or 17+ or 18+	6+ or 20+ or 56+	1+ or 3+ or 11+ or 15− or 19+	
SCRLV-NZ			1− or 3− or 11− or 15+ or 19−	
MiAV		6− or 20− or 56−	11+ or 12+ or 19+ or 22+ or 23+	
BLRV			11− or 12− or 19− or 22− or 23−	
SCRLV-T	16− or 17− or 18−	39+ or 56+ or 62+		
BWYV		39− or 56− or 62−	6+ or 30+ or 31+ or 32+ or 34+	1+ or 3+ or 12+ or 13+ or 20−
SCSV				1− or 3− or 12− or 13− or 20+
LYV			6− or 30− or 31− or 32− or 34−	

[1] Seed-lines corresponding to host numbers are shown in Table 2.
[2] + and − indicate respectively, infected and not infected by the virus isolate.

Table 4. Diagnostic key with weighting in favour of non-legumes.

Virus isolate	Identification steps			
	1	2	3	4
SCRLV-T	59+ [1,2]	22+ or 56+	5+ or 57− or 58− or 63+ or 69−	
PeLRV-NZ			5− or 57+ or 58+ or 63− or 69+	
BWYV		22− or 56−	57+ or 58+ or 62− or 63+ or 69+	
BLRV			57− or 58− or 62+ or 63− or 69−	
SCRLV-NZ	59−	56+ or 62+ or 63+ or 66+ or 70+		
MiAV		56− or 62− or 63− or 66− or 70−	13− or 21+ or 22+ or 23+ or 27+	12+ or 15+ or 16+ or 17+ or 18+
SCSV				12− or 15− or 16− or 17− or 18−
LYV			13+ or 21− or 22− or 23− or 27−	

[1] Seed-lines corresponding to host numbers are shown in Table 2.
[2] + and − indicate respectively, infected and not infected by the virus isolate.

Table 5. Reactions of the eight persistent aphid-transmitted viruses from legumes on twelve selected host plants.

Virus	Universal hosts		Differentiating legumes						Differentiating non-legumes			
	Pisum sativum cv. Onyx	*Trifolium subterraneum* cv. Bacchus Marsh	*Arachis hypogea*	*Glycine max* cv. Shirotsu-runoko	*Lens esculenta* cv. 179307	*Medicago sativa* cv. Washoe	*Phaseolus vulgaris* cv. Canadian Wonder	*Trifolium hybridum*	*Beta vulgaris*	*Capsella bursa-pastoris*	*Gomphrena globosa*	*Lactuca sativa*
BLRV	+[1]	+	+	−	−	+	−	+	−	−	−	−
BWYV	+	+	+	+	+	−	−	−	−	+	+	+
LYV	+	+	+	−	+	−	−	−	−	−	−	−
MiAV	+	+	+	−	+	+	+	+	−	−	−	−
PeLRV-NZ	+	+	+	+	+	+	−	+	+	+	−	+
SCRLV-NZ	+	+	−	+	−	+	−	+	+	−	+	−
SCRLV-T	+	+	−	+	+	−	+	+	+	−	+	+
SCSV	+	+	−	+	−	−	+	−	−	−	−	−

[1] + and − indicate respectively, infected and not infected by the virus isolate.

are six legumes and four non-legumes, and these differentiate between the viruses; they are *A. hypogea, B. vulgaris, C. bursa-pastoris, G. max* cv. Shirotsurunoko, *Gomphrena globosa, Lactuca sativa, L. esculenta* cv. 179307, *M. sativa* cv. Washoe, *P. vulgaris* cv. Canadian Wonder and *T. hybridum.*

Effects of vector species and botanical classification on host ranges. The known vectors of each isolate, the prime vectors which were used in our tests and the known non-vectors of the isolates are listed in Table 6. The prime vectors are all polyphagous; *M. persicae* has the most extensive host range followed in order by *A. solani, A. pisum* and *A. craccivora. A pisum* and *A. craccivora* do not infest non-leguminous species in nature and may have fed poorly on the non-legume test plants used in our tests. The facts that LYV, MiAV, BLRV and SCSV had host ranges confined almost exlusively to legumes may have therefore reflected merely the feeding preference of their vectors rather than an inability of these isolates to multiply in non-legume species.

Table 6. Vectors of the eight persistent aphid-transmitted viruses isolated from legumes.

Virus	Prime vector	Other vectors	Non-vectors
BLRV	*Acyrthosiphon pisum*	*Macrosiphum euphorbiae* *Megoura viciae* *Myzus persicae*	no reports
BWYV	*M. persicae*	*Aulacorthum solani;* eight other spp. not normally associated with legumes (Duffus, 1973)	no reports
LYV	*A. pisum*	*A. solani*	*Acyrthosiphon kondoi* *Acyrthosiphon pelargonii* *Aphis craccivora* *Dactynotus sonchi* *M. euphorbiae* *M. persicae*
MiAV	*A. pisum*	*A. craccivora* *A. solani* *M. persicae*	*Aphis fabae*
PeLRV-NZ	*M. persicae*	*A. pisum*	*A. craccivora*
SCRLV-NZ	*A. solani*	*A. pisum* *M. euphorbiae*	*M. persicae*
SCRLV-T	*A. solani*	*Aulacorthum (Neomyzus) circumflexum*	*A. kondoi* *A. pisum* *A. craccivora* *M. euphorbiae* *M. persicae*
SCSV	*A. craccivora*		*A. kondoi* *A. pisum* *A. solani* *M. euphorbiae* *M. persicae*

The 23 legume species and 17 non-legume species used in our host range studies were a rather biased taxonomic sample, having been selected primarily from previous reports on host reactions to inoculation with luteoviruses isolated from legumes. They represented less than one third of the papilionoid legume tribes (Polhill, 1981). There was no evidence that any of the isolates had a preference for test species within particular tribes, or for test species that represented particular sub-groups of the Crassinucelli and Tenuinucelli (Young and Watson, 1970), other than Leguminosae. This was confirmed by classifying the seed-lines by their virus responses. There was no correlation between the classifications obtained and the generally accepted taxonomic relationships of the species and cultivars.

Discussion

When the studies reported here were started in 1977, BWYV was the only virus we studied whose particles had been purified and characterized. Since then the particles of an additional four of the isolates have been purified, visualized and at least partially characterized, namely BLRV (Ashby and Huttinga, 1979), LYV (Duffus, 1979) and the two isolates of SCRLV (Ashby and Kyriakou, 1982; Johnstone *et al.*, 1982).

Serological studies reported in the recent descriptions of these viruses confirm what is indicated by the biological host range and vector studies reported here. The two isolates of SCRLV are the most closely related and quite distinct form both BLRV and BWYV. These latter two viruses are also clearly distinct from each other, though all have some serological relationship to one another and to other members of the luteovirus group (Ashby and Kyriakou, 1982; Rochow and Duffus, 1981; Johnstone *et al.*, 1982).

The results from the host range studies indicated that PeLRV-NZ is probably a strain of BWYV. It does not have close host range affinities with European isolates of BLRV as was previously suggested in reports from New Zealand (Smith, 1966; Wilson and Close, 1973) because of the large number of species which it infects including non-legumes. BWYV is now known to be common and widespread both in New Zealand (Kyriakou *et al.*, 1983) and Tasmania (Duffus and Johnstone, 1982; Johnstone and Duffus, 1984). The BWYV from an Illinois soybean included in the tests reported here was atypical of most BWYV isolates in that it failed to infect *B. vulgaris.*

The relationships of SCSV have not been determined nor have its virions been characterized despite several serious independent attempts by persons skilled in handling luteoviruses (R.I.B. Francki, P.L. Guy, G.R. Johnstone, J.W. Randles and P.M. Waterhouse – personal communications). Its vectors, host range and symptoms make it quite distinct from any other isolates which we studied. The prime vector *(A. craccivora)* is a member of the Aphidini tribe of the Aphidinae whereas the vectors of the other seven isolates are from the Macrosiphini tribe.

It had been thought that MiAV was closely related to BLRV from Europe (Thottappilly *et al.*, 1977). They both induce symptoms on *C. arietinum, L. esculenta, P. vulgaris, P. sativum, T. incarnatum* and *V. faba* and have similar vector species relationships. However we found MiAV infected more legumes, including *Dolichos lablab, Melitotus* species, *T. pratense* and several cultivars of *P. vulgaris* and *V. faba.* It also generally infected a greater proportion of plants within cultivars which were hosts to both viruses. However it was most closely related to BLRV and appears to be a particularly virulent strain of this virus with a relatively wide host range. BLRV and MiAV

are closely related serologically (G. Thottappilly, unpublished information).

Four persistent aphid-transmitted viruses infecting legumes which were not included in our tests were groundnut rosette assistor (GRAV) (Hull and Adams, 1968). Indonesian soybean dwarf (ISDV) (Iwaki *et al.*, 1980), milk vetch dwarf (MVDV) (Inouye *et al.*, 1968) and soybean dwarf (SDV) (Tamada, 1973). The first three of these may form a natural group together with SCSV as all have *Aphis* spp. as their prime vectors, *A. craccivora* for GRAV, MVDV and SCSV, and *A. glycines* for ISDV. Particles resembling luteovirions were recently observed trapped on electron microscope grids coated with various luteovirus antisera that had been incubated with sap extracts from GRAV-infected plants (Casper *et al.*, 1983) but the other three have not been characterized serologically nor have their virions been visualized. Their host ranges are confined almost exclusively to legumes apart from MVDV which can infect *Datura stramonium, Nicotiana* spp. and *Spinacia oleracea.* GRAV and ISDV have very few host species. The symptoms of MVDV closely resemble those of SCSV and these are not typical of most luteovirus syndromes.

SDV is transmitted by *A. solani* and its reported host range is limited to the Papilionaceae. The host range closely resembles that of the two SCRLV isolates except that SDV also infects *A. hypogea* and *T. pratense.* However, SDV, unlike the SCRLV isolates, does not infect non-legumes (Tamada, 1977). Ashby and Kyriakou (1982) reported that SCRLV and SDV were indistinguishable in serological tests. The symptoms of the yellowing strain of SDV in soybean and *Trifolium* spp. closely resemble those of SCRLV.

It is suggested that the set of 12 hosts which we have recommended for the propagation, classification and identification of luteoviruses from legumes is probably the best available for those purposes from among the 71 hosts that were included in our tests. This set also classifies and identifies the luteoviruses recorded from legumes which were not included in our tests. Seeds of these host plants are available on request (J.W.A., G.R.J.).

Since these host range tests were completed many new luteovirus isolates from legumes have been studied at Christchurch, Hobart and Salinas in more limited host range tests using some of the differential hosts recommended from this report. These isolates, numbering several hundred in total, have represented BLRV, BWYV, LYV and SCRLV. These new isolates have reacted consistently in the differentials, matching serological specifities, and we therefore have confidence in the reproducibility of our results. In addition the groupings based on host range are consistent with groupings based on the results of serological tests, namely:

SCRLV-NZ and SCRLV-T are very close serologically (Johnstone et al., 1982);

BWYV and PeLRV-NZ are very close serologically (Kyriakou et al., 1983);

BLRV, LYV and MiAV are very close serologically (J.E. Duffus and G. Thottappilly, unpublished data; Hampton, 1983).

Furthermore, the epidemiologies of the members of each of these groups are similar; SCRLV perennates mostly in white clover in Australasia and spreads with *A. solani* to annual legumes, BLRV perennates in lucerne and spreads to annual legumes with *A. pisum* and the BWYV types spread in the field mostly with *M. persicae* and occur commonly in brassica, composite and legume crops and weeds. These three groupings appear to be natural and the various isolates should probably be considered as strains of these groups (Ashby and Johnstone, 1984).

There are many points to consider when undertaking host range studies on luteoviruses using aphid vectors and an indicator set. Some details on general procedures which should produce satisfactory results, and on the precautions which need to be heeded, are set out as an Appendix to this paper.

Appendix

Recommended procedures for host range studies on luteoviruses infecting legumes

Aphid cultures. The species of each culture needs to be properly identified. Assistance with identification can be obtained, if necessary, from the Commonwealth Institute of Entomology, London, England. Care must also be taken to ensure that biotypes of a species are not selected which are ineffective as vectors (Stubbs, 1955). Continuous culture of aphid species in greenhouses can also lead to the selection of non-transmitting strains (G.R. Johnstone, unpublished data).

The cultures to be used in transmission studies must be virus-free. These can be conveniently set up by taking nymphs born overnight from adult females caged on layers of Parafilm membrane that sandwich a 20% sucrose solution.

Plants which are satisfactory for rearing the various vector species are:
broad bean, lentil and pea for *A. pisum;*
broad bean and cowpea for *A. craccivora;*
D. stramonium and *Geranium dissectum* for *A. solani;*
radish *(Raphanis sativus)* for *M. persicae.*

The plant species must be grown well and maintained in an insect-free environment until required for use in vector cultures. Care must be taken to ensure that vector cultures do not become contaminated with parasites, pathogens or predators.

Rapid aphid multiplication is favoured by diurnal fluctuations in temperature which avoid extremes of minima (< 10 °C) and maxima (> 30 °C). Avoiding high humidities, providing for good plant growth through adequate nutrition and illumination, and maintaining an air flow through the culture cages also assist in maintaining vigorous, healthy colonies.

Choice of propagation and indicator hosts. These can only be determined by experiment. The species from which an isolate is first obtained is often not the most suitable for use in greenhouses as a propagation and indicator host. Plants which are satisfactory as indicator hosts may not be good as propagation hosts, and vice versa. Hosts which were satisfactory for our different isolates included *C. bursa-pastoris, P. sativum* (cv. Onyx), *T. subterraneum* (cvs. Bacchus Marsh, Geraldton or Mt Barker) and *V. faba* (cvs. Broad Improved Long Pod, Coles Dwarf Prolific or Triple White).

Production of propagation and indicator hosts. Propagation and indicator hosts must be maintained in an insect-free environment. It is essential that they be grown well as the symptoms of luteovirus infections can easily be confused with those of premature senescence, faulty nutrition, root rots, water-logging, unfavourable climatic factors and drought (Duffus, 1977). Plants are therefore best grown in a medium which can be sterilized and in which the nutrient status and moisture content can be

carefully controlled; the University of California has developed one such system (Baker, 1957) but several others have also been documented.

The symptoms of luteovirus infections are easily masked. A high light intensity is necessary for good symptom development and supplementary lighting in the greenhouse can be useful. Strong vegetative growth is also very important for symptom expression and this necessitates planting seedlings, such as those of *C. bursa-pastoris* and *T. subterraneum*, at a very early age so that their growth is not slowed and so that they are not induced to flower.

Conditions of the tests. The incubation periods of luteoviruses in their aphid vectors generally have minima of 12-24 h and the minimum acquisition access and inoculation feeding times are generally about 30 minutes. The proportion of plants that become visibly infected, and the intensity of symptoms which develop is increased by lengthening the duration of both the acquisition access and inoculation feeding times. Involving many aphids rather than a few in each test also improves transmission rates and symptom expression.

Our experiences suggest that the acquisition access and inoculation feeding times should ideally be 2-3 days each, that neither should be less than 24 h and that the total transmission cycle should be at least 4 days. *A. solani* has toxic saliva which can easily damage young seedlings and it is therefore preferable not to use more than about 10 nymphs of this species on young plants. At least 15 individuals should be used for the other vector species.

The plants must be completely freed from aphids before returning them to the greenhouse following completion of the transmission tests. The persistence of various aphicides on (and in) plants varies greatly and it is often more convenient to select one from among those which do not persist for long, particularly if recovery tests are contemplated within 3 to 4 weeks. Aphicides such as maldison (syn. malathion) persist on plants in greenhouses much longer than on field plants. The most effective and least persistent aphicide available commercially is probably mevinphos but this is considered to be too dangerous for use in greenhouses. Dichlorvos and the pyrethrins are satisfactory alternatives.

Number of tests. A proportion of apparent failures to transmit luteoviruses is not uncommon. It is recommended that a total of ten seedlings of each species be used when undertaking host range studies. These should be inoculated as young as possible and observed over a period up to 16 weeks following inoculation.

Inoculated plants which remain healthy should be checked for the presence of latent infections by attempting recoveries from them to young seedlings of a sensitive indicator species, using at least two indicators per test plant.

Seed-lines of shepherd's purse can vary greatly in the intensity of symptoms they produce following inoculation with BWYV. This is probably a common phenomenon and samples of seed from our suggested indicator set can therefore be made available (J.W.A. or G.R.J.) as their seedlings are sensitive to the isolates we have studied. It is important that both known positives are included in transmission tests as well as negative controls to ensure that the test conditions are satisfactory.

Samenvatting

Waardplantenreeksen, classificatie en identificatie van acht door bladluizen op persistente wijze overgebrachte virussen van vlinderbloemigen

Eenenzeventig zaadmonsters van 23 soorten van de Papilionaceae en 17 andere soorten werden verzameld en naar laboratoria in Australië, Nieuw Zeeland, Nederland en de Verenigde Staten van Amerika gezonden. In elk van deze landen werden de uit de zaden verkregen planten op hun vatbaarheid voor acht door bladluizen op persistente wijze overgebrachte virussen getoetst. Het betrof de volgende virussen: 'beet western yellows virus' (BWYV) van *Glycine max* uit Illinois, 'legume yellows virus' (LYV) uit Californië, een virus van *Medicago sativa* uit Michigan (MiAV), twee virussen die bladrol en topvergeling in *Pisum sativum* veroorzaken, één uit Nieuw Zeeland (PeLRV-NZ) en één uit Nederland (BLRV), isolaten van het 'subterranean clover red leaf virus' uit Nieuw Zeeland (SCRLV-NZ) en Tasmanië (SCRLV-T) en 'subterranean clover stunt virus' (SCSV).

De verwantschap tussen de acht genoemde virussen, zoals blijkend uit de reacties van de verschillende waardplanten, werd vastgesteld met behulp van een computerprogramma voor classificatie.

SCRLV-NZ en SCRLV-T vertoonden de meeste verwantschap. Beide hadden een tamelijk grote waardplantenreeks, waaronder enkele soorten die niet behoorden tot de vlinderbloemigen.

BMYV en PeLRV-NZ vormden een tweede groep. Zij waren typisch voor de meeste stammen van het 'beet western yellows virus' in zoverre zij de toetsplanten *Brassica napus, Capsella bursa-pastoris* en *Stellaria media* konden infecteren.

MiAV en BLRV vormden eveneens een paar. Zij veroorzaakten meestal verschillende typen symptomen op de vatbare waardplanten. De waardplantenreeks was beperkt tot de Leguminosae. Alleen kon BLRV ook *Claytonia perfoliata* en *Erodium* spp. infecteren.

De mate van verwantschap tussen LYV en SCSV was niet eenduidig. Uit bepaalde eigenschappen zou tot onderlinge verwantschap besloten kunnen worden, uit enkele andere echter zou meer een verwantschap tussen SCSV en SCRLV worden vermoed. De waardplantenreeks van LYV en SCSV was beperkt tot de Leguminosae; beide veroorzaakten hevige symptomen in hun waardplanten en konden moeilijk worden geïsoleerd uit geïnfecteerde planten. LYV vertoonde enige verwantschap met BLRV en MiAV.

Op grond van de resultaten van de proeven kon een groep van toetsplanten worden samengesteld, die zeer bruikbaar is voor de virusvermeerdering en voor de identificatie van de genoemde acht virussen. Twee van de toetsplanten, nl. *P. sativum* en *Trifolium subterraneum* cv. Bacchus Marsh bleken vatbaar voor alle isolaten. Tien andere, nl. *Arachis hypogea, Beta vulgaris, C. bursa-pastoris, G. max* cv. Shirotsurunoko, *Gomphrena globosa, Lactuca sativa, Lens esculenta* cv. 179307, *M. sativa* cv. Washoe, *Phaseolus vulgaris* cv. Canadian Wonder en *Trifolium hybridum* zijn geschikt voor onderscheiding van alle genoemde isolaten.

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