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INTRASPECIES VARIABILITY IN TRANSMISSION EFFICIENCY OF STYLET-BORNE VIRUSES BY THE PEA APHID (*ACYRTHOSIPHON PISUM*)

M. JURÍK, V. MUCHA, V. VALENTA

Institute of Virology, Slovak Academy of Sciences, 809 39 Bratislava, Czechoslovakia

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Summary. — The efficiency of bean yellow mosaic virus (BYMV; *Potyvirus* group) transmission from pea to pea plants by 36 clones of the pea aphid (*Acyrthosiphon pisum*) was studied. The efficiency of the clones varied; it was not related to the aphid colour form (green, red or yellow) or the origin of the clone (host plant, geographic area). With alfalfa mosaic virus, the virus-vector relationships concerning transmission efficiency were, in general, similar to those found with BYMV. The behaviour of the clones to the circulative pea enation mosaic virus was, however, different.

Key words: *virus — vector relationships*; *aphid clones*; *plant viruses*; *transmission efficiency*

Introduction

Intraspecies variability in virus transmission was studied in several aphid species (Stubbs, 1955; Hinz, 1964; Rochow and Eastop, 1966; Kvíčala, 1967; Upreti and Nagaich, 1971; and others). In *Acyrthosiphon pisum*, these problems were investigated on both circulative (Bath and Chapman, 1966; Kvíčala, 1975; etc.) and stylet-borne (Sohi and Swenson, 1964; Tapio, 1970; Thottappilly *et al.*, 1977) viruses.

The aim of the present work was to investigate whether and to what degree clones of the green, red and yellow forms of *A. pisum* of different origin (host plant, geographic area) differ in their ability to transmit bean yellow mosaic virus (*Potyvirus* group). In some experiments, alfalfa mosaic and pea enation mosaic viruses were used for comparison. A preliminary account was presented (Jurík, 1976).

Materials and Methods

Viruses. Bean yellow mosaic virus (BYMV) isolate K was used throughout. It was obtained from a naturally infected pea (*Pisum sativum*) plant collected in Kalinčiakovo (south Slovakia). Alfalfa mosaic virus (AMV) isolates TrM6A and Me2B of Slovak origin were kindly supplied by Drs. M. Musil and J. Gallo from our Institute, respectively. The pea enation mosaic virus (PEMV) isolate used was obtained from naturally infected field peas in Central Slovakia (Žiar nad Hronom). The BYMV-K isolate was maintained by aphid transmission on red clover plants.

Table 1. Efficiency of BYMV transmission by clones of the green form of A. pisum

Clone	Locality	Host	Alt.	n	n_v	x	x_l	x_r
KN	Kližská Nemá	T.p.	I	93	34	36.6	26.8	47.2
Sl	Sládkovičovo	M.s.	I	82	21	25.6	16.6	36.4
Z-z	Želešice	M.s.	I	86	22	25.6	16.8	36.1
H-u	Hluboká nad Vl.	P.s.	III	124	19	15.3	9.5	23.0
Ko	Košariská	M.s.	III	21	3	14.3	3.0	36.3
S-z	Šenkvice	M.s.	I	57	7	12.3	5.1	23.7
F-z	Fačkov	M.s.	V	67	8	11.9	5.3	22.2
G	Gbelany	M.s.	III	51	11	21.6	11.3	35.3
Hl	Hlinsko	M.s.	IV	64	12	18.7	10.1	30.6
G_1	Gabčíkovo	M.s.	I	62	11	17.7	9.2	29.5
K-c	Kunovice	M.s.	IV	30	5	16.7	6.6	34.7
I	Ivanka pri D.	M.s.	I	65	9	13.8	6.5	24.7
Ro	Rožmberk	P.s.	III	32	3	9.4	2.0	25.0
Po	Pohořelice	M.s.	II	63	5	7.9	2.6	17.6
B	Buzica	M.s.	II	50	3	6.0	1.2	16.5
L	Ledenice	M.s.	III	53	3	5.7	1.2	15.7

Host: original host plant; T.p. = *Trifolium pratense*; M.s. = *Medicago sativa*; P.s. = *Pisum sativum*.
Alt.: altitude above sea level; I — 101—200 m; II — 201—300 m; III — 301—400 m; IV — 401—500 m; V — 500—600 m.
n = Total No. of aphids tested in 3 experiments.
n_v = No. of aphids which transmitted the virus.
x, x_l and x_r: transmission efficiency in % with lower (x_l) and upper (x_r) limits.

AMV and PEMV were maintained by passaging in pea (*Pisum sativum* cv. Raman) plants. Before used in the aphid experiments, the viruses were propagated in pea plants. Any individual comparative experiment was carried out with the same virus passage.

Aphids. Green, red and yellow forms of the pea aphid, *Acyrthosiphon pisum* (Harris), were collected on lucerne, red clover and pea crops in 28 localities in various parts of Czechoslovakia.

Table 2. Efficiency of BYMV transmission by clones of the red form of A. pisum

Clone	Locality	Host	Alt.	n	n_v	x	x_l	x_r
VL	Vyšný Lánec	M.s.	II	98	33	32.6	23.5	42.9
HS	Horná Streda	M.s.	I	34	11	32.3	17.4	50.5
K	Kunovice	T.p.	IV	22	7	31.8	13.9	54.9
H	Hurbanovo	M.s.	I	98	31	31.6	22.6	41.8
S-c	Šenkvice	M.s.	I	62	19	30.6	19.6	43.6
Z	Želešice	M.s.	I	44	13	29.5	16.8	45.2
HS_1	Horná Streda	M.s.	I	29	8	27.6	12.7	47.2
Tr	Třeboň	M.s.	IV	85	19	22.2	14.0	32.7
F	Fačkov	M.s.	V	120	27	22.5	15.4	21.0
P	Povina	T.p.	IV	32	6	18.7	7.2	36.4
Sl-z	Sládkovičovo	M.s.	I	62	6	9.7	3.6	19.9
M	Mosty u Jabl.	T.p.	IV	32	3	9.4	2.0	25.0
T	Terchová	T.p.	V	44	3	6.8	1.4	10.7
R	Rohovec	M.s.	I	60	4	6.7	1.8	16.2
Kl	Kľúčovec	M.s.	I	31	1	3.2	0.1	16.7
KN-c	Kližská Nemá	T.p.	I	34	1	2.9	0.1	15.3

For explanations see Table 1.

Table 3. Efficiency of BYMV transmission by clones of the yellow form of A. pisum

Clone	Locality	Host	Alt.	n	n_v	x	x_l	x_r
Tv	Tvořihráz	M.s.	III	87	23	26.4	17.5	37.0
Sl-z	Sládkovičovo	M.s.	I	85	21	24.7	16.0	35.2
To	Topoľovec	M.s.	I	63	12	19.0	10.2	30.9
S	Šenkvice	M.s.	I	113	9	8.0	4.0	14.7

For explanations see Table 1.

Aphid populations from each locality and from each host plant were carried through 3 parthenogenetic generations by transfer of individual females on fresh pea plants. Each population had originated from a single female. The progeny of a parthenogenetically multiplying female, without any sexual generation was considered to represent a clone.

Arrangement of experiments. With BYMV, second instar nymphs that had been starved for 2 hr, were placed in groups (8—12 aphids) on infected pea leaves, taken from plants 18—22 days after inoculation (at the maximum level of infectious virus — Jurík, 1976). After 5 min those aphids which were quiet for the whole time and had their stylets in feeding position were transferred to test plants (1 aphid per plant). The next day the aphids were killed by Arafosfotion and the plants kept in a greenhouse (21 ± 3° C) for 21—30 days. Experiments on aphid transmission of AMV were arranged similarly except that the pea plants were used as virus source 14 days after inoculation. With PEMV, 50 first instar nymphs of each test clone were allowed an acquisition feeding of 48 hr on infected pea plants and then transferred serially on to 8 pea plants within 12 days. The results were evaluated based on symptoms appearing on the test plants. Only with AMV, which may cause symptomless infection in pea plants, sap from the test plants was inoculated on primary bean (*Phaseolus vulgaris* cv. Perlička) leaves. Transmission efficiency was expressed as the per cent of nymphs that had transmitted the virus out of the total number of aphid nymphs tested. The results were evaluated statistically by the χ^2 test at 5 and 1% levels of significance.

Results

Bean yellow mosaic virus

Sixteen clones of the green form of *A. pisum* transmitted BYMV-K from pea to pea plants with an efficiency varying from 5.7 to 36.6% (Table 1).

Table 4. Efficiency of AMV transmission by A. pisum clones

AMV isolate	Aphid form	clone	I n	I n_v	I x	II n	II n_v	II x
Me2B	Green	KN	55	13	23.6			
	Green	B				77	3	4.0
	Red	VL	63	9	14.2			
	Red	R				56	3	3.6
	Yellow	S				27	2	7.4
TrM6A	Green	KN	25	7	28.0			
	Green	B				22	4	13.6
Total			143	29	20.0	182	12	6.5

I and II: results obtained with aphid clones that transmitted BYMV with a high (I) and low (II) efficiency.
n, n_v and x: see Table 1.

Table 5. Transmission efficiency of PEMV by selected clones of A. pisum

Aphid clone	Aphid form	Transmission efficiency (%) BYMV*	Transmission efficiency (%) PEMV
KN	Green	36.6	78
VL	Red	32.6	68
R	Red	6.7	70
S	Yellow	8.0	68
B	Green	6.0	94

* Data from Tables 1—3.

Sixteen clones of the red form of *A. pisum* transmitted the virus under similar conditions with an efficiency varying from 2.9 to 32.6% (Table 2). The four available clones of the yellow form of *A. pisum* transmitted the virus with an efficiency varying from 8.0 to 26.4% (Table 3). Disregarding the colour form, the transmission efficiency of individual clones varied in a broad range from 2.9 to 36.6%.

The results obtained with the green and red form were subjected to statistical evaluation; those concerning the yellow form were not included because of the low number of clones tested. A hypothesis of the same transmission efficiency of all clones of *A. pisum* was rejected at a 5% level of significance. This also applied to clones within the green and red form respectively. A comparison of the efficiences of the green and red clones revealed a significant difference between the two colour forms at a 5% but not at a 1% level of significance:

	n	n_v	x
red form	887	192	21.65
green form	949	165	17.39

As concerns the origin of the clones, the data summarized in Tables 1 and 2 show that several clones differing from one another in their transmission efficiency were obtained from the same host plant and the same locality. On the other hand, several clones with a similar transmission efficiency had originated from different host plants. For example clone KN

Table 6. Effect of virus source on the efficiency of BYMV transmission by A. pisum clones

Aphid clone	BYMV source *P. sativum*			*T. incarnatum*			*T. pratense*		
	n	n_v	x	n	n_v	x	n	n_v	x
VL	50	15	30.0	98	33	33.6	106	44	41.5
R	50	3	6.0	78	7	8.9	46	5	10.8

Test plant: *P. sativum* cv. Raman.
n, n_v, x: see Table 1.

Table 7. Effect of the test plant on the efficiency of BYMV transmission by A. pisum clones

Aphid clone	Test plant								
	P. sativum			*T. incarnatum*			*T. pratense*		
	n	n_v	x	n	n_v	x	n	n_v	x
VL	50	15	30.0	95	13	14.0	49	1	2.0
R	50	3	6.0	48	2	4.1	45	1	2.2

Source of BYMV: *P. sativum* cv. Raman.
n, n_v, x: see Table 1.

from red clover and clone Sl from lucerne transmitted BYMV with a high efficiency, while clones KN and KN-c from the same locality differed widely from one another. The altitude of the localities above the sea level within the range from 100—600 m apparently was in no relation to the transmission efficiency of the aphid clones. For example the highly efficient clones HS and K originated from localities situated 160 and 400 m above the sea level, respectively.

Alfalfa mosaic virus

The results obtained with 5 selected clones of *A. pisum* and two isolates of AMV are summarized in Table 4. Aphid clones which proved to be efficient vectors of BYMV (KN and VL) transmitted AMV with a mean transmission efficiency of 20%, while little efficient vectors of BYMV (clones B, R, S) transmitted AMV with a mean efficiency of 6.5%.

Pea enation mosaic virus

Two efficient vectors of BYMV (clones KN and VL) and three little efficient vectors of BYMV (clones B, R and S) were selected for these experiments, the results of which are summarized in Table 5. The efficiency of transmission of PEMV varied from 68 to 94%, there being no significant difference between the two groups of clones.

Effect of virus source and test plants on transmission efficiency

To test whether the transmission efficiency of aphid clones might be affected by the virus source and test plants, experiments summarized in Tables 6 and 7 were carried out. BYMV-K and a highly (VL) and a little (R) efficient clone of *A. pisum* were used in them. Acquisition and test feedings lasted 5 min and 24 hr, respectively.

Clovers (*Trifolium incarnatum* and *T. pratense*) proved to be better sources of virus than pea plants, but the general behaviour of the two aphid clones tested remained unchanged, i.e. clones VL and R transmitted BYMV-K with a high and low efficiency, respectively.

As concerns the effect of test plants, the experiments on red clover (*T. pratense*) could not be evaluated since this plant species showed an extremely low susceptibility to aphid transmission of BYMV-K. *T. incarnatum* proved to be less susceptible than *P. sativum*, but the difference between the two aphid clones tested as concerns their transmission efficiency was preserved.

Discussion

In the present experiments, 36 clones of *A. pisum* were compared as to their efficiency to transmit BYMV. Irrespective of the colour form (red, green and yellow), the transmission efficiency of the clones varied within wide limits (2.9—36.6%). In general, the red form was a better vector than the green form, the difference being significant at a 5% but not at a 1% level of significance. The transmission efficiencies of pea aphid clones found in the present experiments were comparable with those reported for BYMV and *A. pisum* clones from Finland (Tapio, 1970) and from the U.S.A. (Sohi and Swenson, 1964). The latter authors isolated two *A. pisum* biotypes differing in BYMV transmission.

The present findings, namely that the efficiency in transmitting BYMV by the clones of any colour form of *A. pisum* varied within wide limits, indicate that results obtained with single or only a few randomly picked-up aphid clones should be interpreted with extreme caution. Thus the conclusions by Kvíčala (1968) concerning pea mosaic virus (now considered to represent a strain of BYMV) transmission by a red and a green clone of *A. pisum* should be re-evaluated. This also applies to the reports on AMV transmission by Kvíčala (1970) and Beczner (1975).

The causes of the variations observed among the pea aphid clones remain obscure. They evidently were not connected with the colour form or the origin (geographic area, host plant) of the aphid clone. The general ability of the individual clones to virus transmission was not affected by the source of virus or the test plant. Important from the point of view of the variations among aphid clones appears the finding by Lim *et al.* (1977) of more virus attached to the stylets of an efficient biotype than to those of an inefficient biotype of *Macrosiphon euphorbiae*. It appears questionable, however, whether stylet morphology plays an important role in this respect, since Schmidt *et al.* (1974) found no substantial differences in stylet morphology of 10 aphid species.

Our comparative experiments on BYMV and AMV suggest that the transmission of any stylet-borne virus would follow a similar general pattern. But other mechanisms are involved in the transmission of circulative viruses. In our experiments, pea aphid clones transmitting stylet-borne viruses with a low efficiency transmitted PEMV with a moderate to high (68—94%) efficiency. We used another approach than Thottappilly *et al.* (1972), but their and our results do not contradict each other.

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