The Relationship Between Feeding and Virus Retention Time in Beetle Transmission of Plant Viruses

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Supported by the U.S. Department of Agriculture, CRGO, under agreement 89-37153-4559 (SEA, Competitive).
Approved for publication by the Director of the Arkansas Agricultural Experiment Station.
Accepted for publication 25 May 1994.

ABSTRACT

Wang, R. Y., Gergerich, R. C., and Kim, K. S. 1994. The relationship between feeding and virus retention time in beetle transmission of plant viruses. Phytopathology 84:995-998.

Three beetle vectors of plant viruses, the Mexican bean beetle (*Epilachna varivestis*), the bean leaf beetle (*Cerotoma trifurcata*), and the spotted cucumber beetle (*Diabrotica undecimpunctata howardi*), were used to study retention of southern bean mosaic virus (SBMV). After bean leaf beetles acquired SBMV from virus-infected bean leaves, they were viruliferous for less than 5 days unless they did not feed, in which case they were viruliferous for longer periods. To test the hypothesis that virus retention time is determined mainly by feeding activity that depletes the amount of ingested virus, Mexican bean beetles, bean leaf beetles, and spotted cucumber beetles were held at 5–7 C for 1, 2, 3, or 4 wk without feeding after virus acquisition from SBMV-infected leaves. The virus transmission rate and pattern after removal of beetles from storage after starvation were similar to the control beetles that were not subjected to starvation, suggesting that postacquisition feeding influences plant virus retention by leaf-feeding beetles. Beetles lost viruliferousness over a 4-wk period if they fed on healthy plants during that period.

Additional keywords: Chrysomelidae, Coccinellidae, Coleoptera, hemolymph.

Retention time is an important factor in determining temporal patterns of plant virus dissemination and overwintering in viruliferous vectors. The length of time that a virus is retained by leaf-feeding beetles varies (4,6,11,14,17–19). Retention increases with an increase in consumption of the virus-infected tissue (3,10). In an unconfirmed preliminary report (6), the Mexican bean beetle, *Epilachna varivestis* Mulsant, transmitted cowpea severe mosaic comovirus for only 1 or 2 days, whereas the bean leaf beetle, *Cerotoma trifurcata* (Forster), transmitted the same virus efficiently for 6 days, and in some cases, transmission was recorded after 8 days. Apparently under diapausing conditions the virus may be retained much longer. Freitag (4) obtained viruliferous western striped cucumber beetles, *Acalymma trivittatum*, from overwintering weeds not susceptible to squash mosaic comovirus.

Freitag (4) first recovered squash mosaic comovirus from the hemolymph of western striped cucumber beetles and western spotted cucumber beetles, *Diabrotica undecimpunctata undecimpunctata*, after virus acquisition from infected tissue. Since then, other beetle-transmissible viruses, such as cowpea mosaic comovirus (15), the cowpea strain of southern bean mosaic sobemovirus (CP-SBMV) (12,16), and the type strain of SBMV (13), have been found in the hemolymph of the bean leaf beetle, spotted cucumber beetle, *D. u. howardi* Barber, and striped cucumber beetle, *A. vittatum*. The hemolymph of the bean leaf beetle is a reservoir for CP-SBMV, and ingested CP-SBMV appears very rapidly in the hemolymph of bean leaf beetles (12,16). Based on these reports, beetle hemolymph was assumed to be the site where ingested viruses were retained during prolonged retention times (5,8).

Some plant viruses efficiently transmitted by beetles are not circulative in their vectors (20). Specifically, bean pod mottle comovirus (BPMV) and the type strain of SBMV are not found in the hemolymph of viruliferous Mexican bean beetles, and BPMV is not found in the hemolymph of viruliferous spotted cucumber beetles and bean leaf beetles. Thus, it appears that the beetle hemocoel does not serve as a reservoir for at least some of the plant viruses vectored by beetles. Therefore, for these virus–beetle combinations, the digestive system must serve as the site of long-term retention.

The purpose of this research was to investigate the relationship between postacquisition feeding and virus retention time in the Mexican bean beetle, the bean leaf beetle, and the spotted cucumber beetle.

MATERIALS AND METHODS

Virus. SBMV was selected because it is transmitted efficiently by the three species of beetles used in this study (5,7). The virus was propagated in bean (*Phaseolus vulgaris* L. 'Black Valentine') and purified by the methods described by Gergerich et al (9).

Beetles. Mexican bean beetles were reared in the greenhouse on *P. vulgaris* cv. Pinto. During the growing season, bean leaf beetles and spotted cucumber beetles were collected in the field from soybean, bean, and squash and maintained on detached, primary leaves of Pinto bean. The leaves were changed daily for more than 1 wk before the beetles were used. During the winter, spotted cucumber beetles were purchased from French Agricultural Research, Inc. (Lamberton, MN).

Retention of SBMV. Beetles were starved for 24 h before acquisition access. Individual beetles were given access to SBMV-infected bean leaves in a petri dish in the laboratory at room temperature for 24 h. Each beetle was watched to determine the beginning of the acquisition period. The beetles were individually labeled and followed throughout the remainder of the experiment.

After virus acquisition, single beetles were placed on individual Black Valentine bean seedlings at the primary leaf stage in small cages for a 24-h inoculation access period. The beetles were transferred daily to bean seedlings for 10 days. Test plants were grown in the greenhouse, and 2 wk later, all plants were tested for virus infection by the Ouchlerlony double-diffusion test. Forty beetles of each species were used for each treatment. Five beetles of each species that had not acquired virus were used as controls. The experiment was repeated twice.

The relationship between virus entry into the hemocoel and retention time was studied. Hemolymph of bean leaf beetles and

spotted cucumber beetles was collected after 24-h virus acquisition and prior to placement on the test plants. The presence of the virus in the hemolymph was determined by enzyme-linked immunosorbent assay as described by Wang et al (20). Forty beetles of each species were used for each treatment. The hemolymph of eight beetles fed virus-free bean leaves was used as a negative control, and the hemolymph of eight beetles injected with purified SBMV (5 μg of purified virus in 5 μl of 0.01 M phosphate buffer, pH 7.2) was used as a positive control.

Virus transmission by beetles after starvation. The influence of postacquisition feeding on virus retention time was examined. After virus-acquisition access, described above, representatives of the three species of viruliferous beetles were placed in empty petri dishes in a moisturized container and held at 5–7 C for 1, 2, 3, or 4 wk. After starvation, beetles were transferred to Black Valentine bean seedlings (1 beetle per plant) for a 24-h inoculation access period. Beetles that acquired SBMV and that were used immediately served as controls. Thirty beetles were used for each treatment. The experiment was repeated twice. Data were subjected to analysis of variance, and means were separated by Duncan's new multiple range test (SAS Institute Inc., Cary, NC).

Virus retention in beetles after starvation. Viruliferous Mexican bean beetles, bean leaf beetles, and spotted cucumber beetles were held in empty petri dishes in a moisturized container and kept at 5–7 C in a cold room after the acquisition access period as mentioned above. Two weeks later, they were serially transferred to Black Valentine bean seedlings (1 beetle per plant) for 24-h inoculation access periods for 10 days. Similarly, viruliferous Mexican bean beetles held in an empty petri dish in a moisturized container at room temperature (22–25 C) for 2 wk were used

TABLE 1. Patterns of serial transmission of southern bean mosaic virus (SBMV) by the Mexican bean beetle

	Days after virus acquisition[x]									
No. of beetles[w]	1	2	3	4	5	6	7	8	9	10
4	–[y]	–	–	–	–	–	–	–	–	–
6	+	–	–	–	–	–	–	–	–	–
1	–	+	–	–	–	–	–	–	–	–
44	+	+	–	–	–	–	–	–	–	–
15	+	+	+	–	–	–	–	–	–	–
6	+	+	+	+	–	–	–	–	–	–
2	+	+	–	+	–	–	–	–	–	–
1	+	–	+	+	–	–	–	–	–	–
1	+	–	–	+	–	–	–	–	–	–
Transmission (%)[z]	93.8	87.5	27.5	12.5	0	0	0	0	0	0

[w] Combined data from two experiments.
[x] Beetles were given an acquisition access period of 24 h on SBMV-infected bean leaves.
[y] – = No virus infection; + = virus infection. All beetles fed every day.
[z] Number of beetles that transmitted SBMV/number of beetles tested × 100.

TABLE 2. Patterns of serial transmission of southern bean mosaic virus (SBMV) by the bean leaf beetle

		Days after virus acquisition[w]									
	No. of beetles[v]	1	2	3	4	5	6	7	8	9	10
Beetles without virus in the hemolymph[y]	11	–[x]	–	–	–	–	–	–	–	–	–
	24	+	–	–	–	–	–	–	–	–	–
	10	+	+	–	–	–	–	–	–	–	–
	6	+	+	+	–	–	–	–	–	–	–
	5	+	–	+	+	–	–	–	–	–	–
	3	+	+	–	+	–	–	–	–	–	–
	2	+	*	+	+	+	*	*	*	–	–
	1	*	+	*	+	+	–	–	*	–	–
	1	+	*	*	*	+	+	*	*	*	*
	1	+	*	+	*	*	+	+	–	*	–
	1	+	+	*	*	*	+	+	#	#	#
	1	+	*	*	*	*	*	–	+	*	–
	1	*	+	*	*	*	*	*	+	–	–
	1	*	*	+	*	*	*	*	*	+	–
	1	+	*	*	*	*	*	*	*	*	+
Transmission (%)[z]		79.7	31.9	20.3	15.9	5.8	4.3	2.9	2.9	1.4	1.4
Beetles with virus in the hemolymph	2	–	–	–	–	–	–	–	–	–	–
	3	+	–	–	–	–	–	–	–	–	–
	1	+	+	–	–	–	–	–	–	–	–
	1	+	–	+	–	–	–	–	–	–	–
	2	+	+	–	+	–	–	–	–	–	–
	1	–	+	*	+	+	*	+	*	*	–
	1	+	*	*	*	*	+	*	+	*	+
Transmission (%)[z]		72.7	36.4	9.1	27.3	9.1	9.1	9.1	9.1	0	9.1
Total transmission (%)[z]		78.8	32.5	18.8	17.5	6.3	5.0	3.8	3.8	1.3	2.5

[v] Combined data from two experiments.
[w] Beetles were given an acquisition access period of 24 h on SBMV-infected bean leaves.
[x] – = No virus transmission; + = virus transmission; * = no beetle feeding on this day; # = beetle dead. Only beetles with virus transmission longer than 4 days after virus acquisition have * or #.
[y] Hemolymph of beetles was collected after 24-h virus acquisition and prior to placement on the test plants.
[z] Number of beetles that transmitted SBMV/number of beetles tested × 100.

TABLE 3. Patterns of serial transmission of southern bean mosaic virus (SBMV) by the spotted cucumber beetle

	No. of beetles[v]	Days after virus acquisition[w]									
		1	2	3	4	5	6	7	8	9	10
Beetles without virus in the hemolymph[y]	37	–[x]	–	–	–	–	–	–	–	–	–
	24	+	–	–	–	–	–	–	–	–	–
	2	+	+	–	–	–	–	–	–	–	–
	1	+	+	+	–	–	–	–	–	*	–
Transmission (%)[z]		42.2	4.7	1.6	0	0	0	0	0	0	0
Beetles with virus in the hemolymph	10	–	–	–	–	–	–	–	–	–	–
	4	+	–	–	–	–	–	–	–	–	–
	1	+	+	–	–	–	–	–	–	–	–
	1	+	+	+	+	–	–	*	–	–	–
Transmission (%)[z]		37.5	12.5	6.3	6.3	0	0	0	0	0	0
Total transmission (%)[z]		41.3	6.3	2.5	1.3	0	0	0	0	0	0

[v] Combined data from two experiments.
[w] Beetles were given an acquisition access period of 24 h on SBMV-infected bean leaves.
[x] – = No virus transmission; + = virus transmission; * = no beetle feeding on this day. Only beetles with virus transmission longer than 2 days after virus acquisition have the *.
[y] Hemolymph of beetles was collected after 24-h virus acquisition and prior to placement on the test plants.
[z] Number of beetles that transmitted SBMV/number of beetles tested × 100.

TABLE 4. Transmission of southern bean mosaic virus (SBMV) after starvation of viruliferous beetles

Starvation time (wk)[y]	Transmission (%)		
	Mexican bean beetle	Bean leaf beetle	Spotted cucumber beetle
0	93.8 A[z]	78.8 A	41.3 A
1	90.9 A	76.7 A	39.7 A
2	90.4 A	77.2 A	43.8 A
3	81.4 B	70.4 A	38.8 A
4	92.4 A	75.8 A	37.5 A

[y] Time at 5–7 C after a 24-h virus acquisition period on SBMV-infected bean leaves. Thirty beetles were used in each treatment, and the experiment was repeated twice.
[z] Values in a column followed by the same letter are not significantly different at the 0.01 level according to Duncan's new multiple range test.

in serial transmission tests for 10 days. Beetles that had acquired SBMV but that had not been subsequently starved were used as controls. Thirty beetles were used for each treatment. The experiment was repeated twice.

RESULTS

Retention of SBMV. Retention times of SBMV in the three species of beetles used in this study were very different. In general, Mexican bean beetles and spotted cucumber beetles retained SBMV for shorter periods than did bean leaf beetles (Tables 1–3). For example, Mexican bean beetles and spotted cucumber beetles only remained viruliferous for 4 days after 24-h acquisition from virus-infected bean leaves (Tables 1 and 3). Bean leaf beetles retained SBMV up to 10 days after virus acquisition, although transmission rates were low after 5 days (Table 2). Only those bean leaf beetles that did not feed every day were viruliferous for more than 4 days.

About 16% of bean leaf beetles and 25% of spotted cucumber beetles had SBMV present in their hemolymph 24 h after virus acquisition from infected plants (Tables 2 and 3). Similar transmission rates and retention times were obtained from bean leaf beetles and spotted cucumber beetles that had virus in the hemolymph and beetles without virus in the hemolymph (Tables 2 and 3).

Virus transmission rates and retention time by beetles after starvation. Without postacquisition feeding, beetles remained viruliferous for up to 4 wk after virus acquisition (Table 4). No significant difference ($P = 0.01$, Duncan's new multiple range test) in transmission efficiency existed among different time treatments within one species of beetle (except for 3 wk of starvation of the Mexican bean beetle). In contrast, significant differences ($P = 0.01$) existed in the transmission efficiency among the three species of beetles.

After 2 wk of starvation (5–7 C), the trend of SBMV transmission rates and retention times of Mexican bean beetles, bean leaf beetles, and spotted cucumber beetles were the same for both starved and unstarved beetles (Fig. 1). Similar results were obtained with Mexican bean beetles starved at room temperature as with those starved at 5–7 C (Fig. 1A).

DISCUSSION

Previous studies of retention time of beetle-transmissible viruses used the same experimental design (i.e., after acquisition access, active beetles were transferred daily to healthy host plants [4,6, 11,14,17–19]). In this manner, the longest demonstrated retention time was the 17-day retention of squash mosaic virus by western striped cucumber beetles (4). However, this does not explain why some beetles remain viruliferous over winter (4).

Berger et al (1,2) demonstrated that the efficiency of virus transmission by aphids was greatly increased by immobilization treatment with nitrogen or argon gases at 25 C or cold treatment at 6 C after acquisition access. They also demonstrated that potyvirus retention times in aphids were much longer with this treatment. They concluded that the increased virus transmission efficiency and the prolonged retention time resulted only when solid surface-probing behavior of the aphid was avoided. In our experiments, beetles starved at 5–7 C were not immobilized, and occasional slow walking was observed. The work reported here demonstrates that even after 4 wk of starvation in the cold, virus transmission efficiency did not decline compared with that of unstarved beetles (Table 4). Actually, this kind of starvation is very similar to field conditions during the winter, when viruliferous overwintering beetles have been found (4). The beetle species used in this study overwinter in the field as adult beetles under diapausing conditions (5,8). The similar results obtained with Mexican bean beetles starved for 2 wk at room temperature as with those starved at 5–7 C (Fig. 1A) suggest that viruliferous beetles may retain viruses during the interval between crops in the same growing season.

Different species of beetles may have very different feeding habits. For example, the Mexican bean beetle and spotted cucumber beetle are voracious feeders in laboratory experiments. The bean leaf beetle, however, feeds more intermittently on bean under laboratory conditions, and often does not feed for several days. In this experiment, only those bean leaf beetles that did not feed every day retained SBMV for more than 5 days (Table 2). In

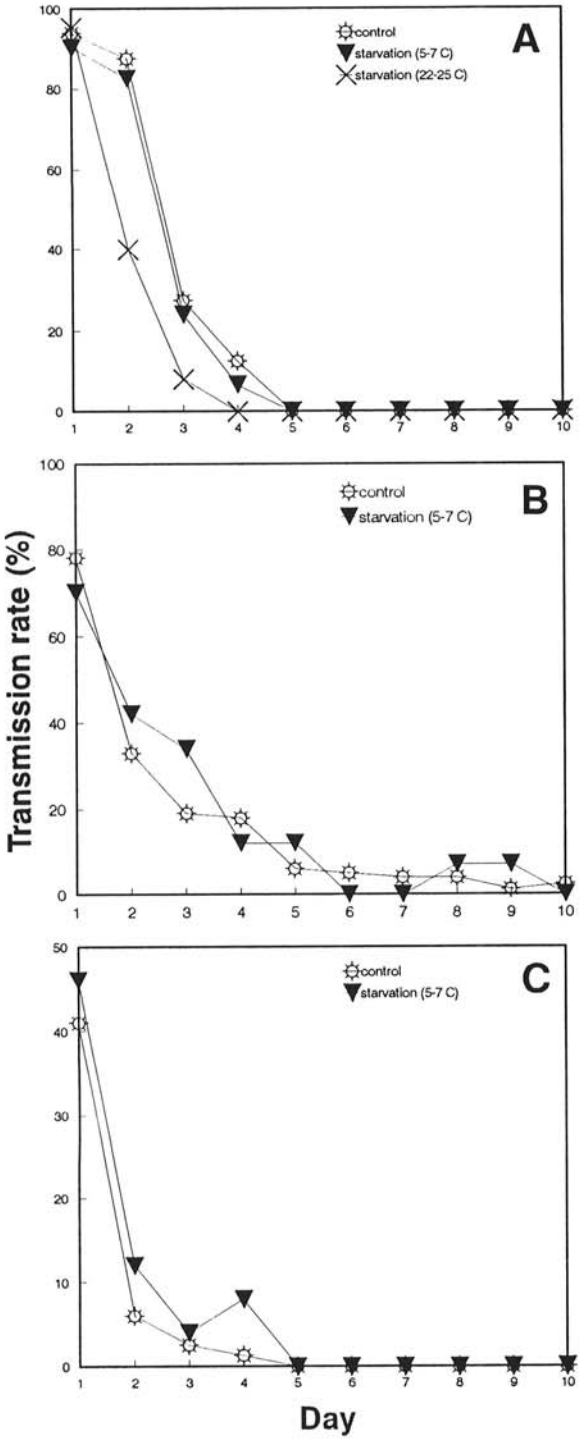

Fig. 1. Transmission rates and retention times of southern bean mosaic virus (SBMV) by three species of beetles after 2 wk of starvation. **A,** Mexican bean beetle; **B,** bean leaf beetle; and **C,** spotted cucumber beetle. Beetles that had acquired SBMV but that were not starved were used as controls. Data in this figure are the averages from two experiments.

contrast, Mexican bean beetles and spotted cucumber beetles that fed daily lost transmission ability within 4 days (Tables 1 and 3). This further indicates that postacquisition feeding reduces virus retention time and that apparent differences in retention time by different beetle vectors are related to their feeding habits and not to the presence of the ingested virus in the hemocoel of beetle vectors.

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