Survival of Bean Pod Mottle and Cowpea Mosaic Viruses in Beetles following Intrahemocoelic Injections

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ABSTRACT

Bean leaf beetles, *Cerotoma trifurcata*, transmitted bean pod mottle virus (BPMV) and cowpea mosaic virus (CPMV) after injection of purified virus into the hemocoel. The transmission rate by injected beetles was considerably less than transmission by beetles that acquired the virus by an acquisition feed. BPMV and CPMV were recovered from hemolymph after injection of virus, although CPMV was recovered at a significantly higher rate than BPMV. There is an indication that BPMV is inactivated in bean leaf beetle hemolymph at a faster rate than CPMV or the cowpea strain of southern bean mosaic virus.

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Additional key word: transmission.

Several polyhedral plant viruses are transmitted by plant-feeding beetles of the family Chrysomelidae (7). Included are four viruses capable of being transmitted by bean leaf beetles: bean pod mottle virus (BPMV); cowpea mosaic virus (CPMV); southern bean mosaic virus; and cowpea strain of southern bean mosaic virus (CP-SBMV) (7). Freitag (2), Slack & Scott (5), and Slack & Fulton (4) reported the recovery of virus from the hemolymph of beetle vectors. Slack & Fulton (4) suggested that most beetle-transmitted viruses could be recovered from the hemolymph of beetle vectors, but that BPMV seemed to be an exception. Because Arkansas CPMV and BPMV are serologically related and have many similar characteristics, they were utilized for comparative purposes in these studies to extend the evaluation of virus retention and transmission following injection of virus into the hemocoel.

MATERIALS AND METHODS.—*Viruses.*—BPMV and CPMV were purified for injection by the chloroform-butanol method (6), and both were suspended in 0.01 M phosphate buffer, pH 7.2.

Beetles.—Bean leaf beetles (*Cerotoma trifurcata* Forst.) were collected from soybean fields in the Arkansas River Valley near Van Buren and held en masse on cowpea [*Vigna sinenis* (Torner) Savi 'Monarch'], soybean [*Glycine max* (L.) Merr. 'Lee'], or bean [*Phaseolus vulgaris* (L.) 'Black Valentine'] until used. BPMV occurs commonly in soybeans from this area, and it is possible that beetles could be carrying this virus and could transmit it to test plants. To reduce this possibility, individual beetles were given a 3-day feed on Lee soybean plants prior to their use in tests. We observed that viruliferous beetles generally transmit BPMV during the first 3 days of feeding even though the virus may be retained and transmitted for longer periods. The 3-day test of field-collected beetles showed that 24 of 609 beetles, or 3.9%, were carrying BPMV from the field. Such viruliferous beetles are not included in the results. Although it is possible that this test did not totally eliminate viruliferous beetles, the number not detected would be too small to affect the interpretation of the results. CPMV virus was never detected in soybeans in the field in the general area from which beetles were collected. Occasional random contamination was never recorded, and this would have been readily detected in indexing the test plants. Plants from all experiments were indexed for virus by the Ouchterlony gel diffusion test (3).

Injections.—Beetles were injected with purified virus by a procedure similar to that described by Slack & Scott (5). Generally, beetles were given a 3 µliter dosage of virus preparation, but there was often leakage of hemolymph from body joints and cut wing areas during injection which made it impossible to

calculate the exact amount of virus any single beetle retained. After injection, the beetles were placed onto individual seedlings in cages for test feeding and later bleeding. Beetles injected with BPMV were fed on Lee soybean or Black Valentine bean and those injected with CPMV were fed on Monarch cowpea.

Hemolymph recovery.—Hemolymph from injected beetles was recovered by the method of Slack & Scott (5). Two types of hemolymph tests were made: (i) Beetles were injected with virus and placed onto plants for 24 hr or longer before bleeding; or (ii) beetles injected with BPMV were held in vials without feeding for shorter time intervals prior to bleeding.

Hemolymph from beetles injected with BPMV was assayed for virus by rubbing the hemolymph onto primary leaves of Carborundum-dusted Lee soybean or Black Valentine bean seedlings and hemolymph from beetles injected with CPMV was assayed in a similar fashion on Monarch cowpea seedlings.

Transmission by feeding.—Bean leaf beetles were fed on primary leaves of Monarch cowpea infected with CPMV and on leaves of Black Valentine bean infected with BPMV for virus acquisition. Individual beetles were placed in petri dishes with a single infected leaf. Twenty-four hours later, single beetles that had fed on the BPMV-infected leaves were placed on Lee soybean seedlings, and those that had fed on the CPMV-infected cowpea leaves were placed on Monarch cowpea seedlings, one beetle/seedling. The beetles were then serially transferred to healthy plants daily for 10 days.

Specific infectivity.—Specific infectivity of several samples of purified BPMV and CPMV was determined. Virus samples were diluted to equal optical density (OD) (usually 0.02 A_{260}) with 0.01 M phosphate buffer, pH 7.2, and compared by inoculating opposite half-leaves of six Pinto bean plants, *Phaseolus vulgaris* (L.). No differences in the specific infectivities of the two viruses could be demonstrated when samples of equal OD were tested.

RESULTS.—*Transmission.*—A small percentage of bean leaf beetles transmitted BPMV after intrahemocoelic injection (Table 1). The rate was considerably less than the rate of transmission by beetles given 24-hr acquisition feed on BPMV-infected leaves (Table 2).

Bean leaf beetles also became viruliferous after injection of CPMV into the hemocoel. About 23% of the beetles transmitted CPMV within 2 days after injection (Table 1), significantly less than the rate of transmission of CPMV after feeding on infected cowpea leaves (Table 2).

Recovery of virus from hemolymph.—BPMV was obtained from hemolymph of 10 of 66 beetles 1 day after injection (Table 3) when Black Valentine bean was used as the assay host for the virus. The virus was recovered from only 1 of 28 beetles the second day after injection, indicating rapid loss of BPMV in the hemolymph. When Lee soybean was used as the assay host 24 hr after injection, BPMV was recovered from the hemolymph of only 1 of 30 beetles, and no virus was recovered from 20 beetles bled the 2nd day after injection.

An attempt was made to detect differences in rates of recovery of BPMV from the hemolymph at several time intervals after injection of purified BPMV (average OD = 24.6). Hemolymph was tested on Black Valentine bean seedlings. There was no essential difference in the rate of recovery of BPMV from hemolymph at 1, 2, 3, 5, and 10 hr after injection; the recovery rate was similar to that of 1 day after injection (Table 3).

CPMV was readily recovered from the hemolymph after injection of the virus into the hemocoel. Virus was recovered from hemolymph of 13 of 31 beetles assayed 1 day after injection (Table 3), which is similar to the rate of recovery reported for CPMV from bean leaf beetle hemolymph after a 24-hr feeding on CPMV-infected plants (4). Thirty-three of 71 beetles yielded CPMV from their hemolymph on the 2nd day. This was followed by a rapid drop in rate of recovery, with no virus recovered from 40 beetles bled the 5th day after injection of CPMV.

DISCUSSION.—These results indicate that BPMV and CPMV can be transmitted by feeding after virus has been acquired through intrahemocoelic injection. This is similar to the results obtained by Slack & Scott (5) with CP-SBMV although the rate of transmission is lower than that of source-fed beetles, particularly in the case of BPMV.

One possible mechanism of transmission by injected beetles is passage of virus from hemolymph

TABLE 1. Transmission of bean pod mottle virus (BPMV) and cowpea mosaic virus (CPMV) by bean leaf beetles after injection of the virus into the hemocoel

Virus	OD averages[b]	Test host	Transmission[a] 1	2	3	4	5	6
BPMV	21.1	Lee soybean	1/36[c]	3/26	1/17	1/10	1/14	0/17
		Black Valentine bean	6/39	1/29	0/14	0/15	0/10	0/5
CPMV	25.2	Monarch cowpea	1/28	12[d]/52	6/64	0/60	1/25	

[a] Days after injection; data for each day represent a compilation of results from several separate experiments.
[b] Optical density (OD), A_{260}, concentration of purified virus used for injection.
[c] Numerator is number of beetles that transmitted; denominator is number that fed.
[d] Transmitted during first 48 hr after injection.

TABLE 2. Transmission of bean pod mottle virus (BPMV) and cowpea mosaic virus (CPMV) by bean leaf beetles after a 24-hr acquisition feed on virus-infected plants[a]

Virus	Transmission[b]									
	1	2	3	4	5	6	7	8	9	10
BPMV	13/56[c]	18/54	5/52	0/45	2/48	1/48	2/47	0/49	0/47	0/42
CPMV	51/54	39/50	34/56	14/44	5/52	4/46	2/50	0/43	0/47	0/46

[a] Beetles fed on BPMV-infected Black Valentine bean or CPMV-infected Monarch cowpea and test fed on Lee soybeans and Monarch cowpea, respectively.
[b] Days after virus acquisition.
[c] Numerator is number of beetles that transmitted; denominator is number that fed.

TABLE 3. Recovery of bean pod mottle virus (BPMV) and cowpea mosaic virus (CPMV) from hemolymph of bean leaf beetles after injection of virus into the hemocoel

Virus	OD averages[b]	Assay host	Virus recovery[a]				
			1	2	3	4	5
BPMV	22.1	Lee soybean	1/30[c]	0/20			
		Black Valentine bean	10/66	1/28			
CPMV	25.6	Monarch cowpea	13/31	33/71	6/37	2/53	0/40

[a] Days after injection; data for each day represent a compilation of results from several separate experiments.
[b] Optical density (OD), A_{260}, concentration of purified virus used for injection.
[c] Numerator is number of beetles from which virus was recovered; denominator is number bled.

through the gut lining followed by movement to the mouth when beetles regurgitate in feeding. Another possible mechanism of virus transmission that would involve the hemolymph could be through the process of reflex bleeding. Some beetles possess the phenomenon of reflex bleeding (8), in which blood may leak from limb joints, mouth, and base of the elytra. It is conceivable that hemolymph containing virus could leak from mandibles of the beetle during feeding, and result in transmission of the virus.

CPMV can be recovered from the hemolymph of injected beetles at a higher percentage and for a longer period of time than can BPMV. Some differential reaction on BPMV and CPMV that results in a quicker inactivation of BPMV or its elimination from the hemolymph is apparently occurring within the beetle. Longer survival of CPMV in the hemolymph is not due to a greater infectivity than BPMV, since the two viruses have similar specific infectivities. The fact that BPMV is recovered from hemolymph of only a small percentage of injected beetles and disappears quickly, and the failure to recover the virus in the hemolymph of source-fed beetles (4), suggest a reaction of the insect against BPMV.

An immunological response is a possible reaction of insects which might eliminate virus from the hemolymph (1). No evidence exists for a specific serological reaction against a foreign protein such as the coat of a virus, but little work has been done in this area. Beetles used in this study are most commonly associated with BPMV in the field and perhaps, by a process of evolution, the beetles have developed a specific reaction to BPMV that results in its elimination from the hemolymph.

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