Transmission of Cowpea Mild Mottle Virus by *Bemisia tabaci* in a Nonpersistent Manner

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ABSTRACT

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Individual *Bemisia tabaci* adults acquired cowpea mild mottle virus (CMMV) in 10 min and transmitted it within 5 min to soybeans. Starvation before acquisition had no effect upon transmission, but starvation after acquisition decreased transmission frequency. Irrespective of the length of acquisition, ability to transmit CMMV was retained in the whitefly for only four successive inoculation access periods of 5 min each, but adults that lost the ability to transmit the virus could reacquire and transmit the virus. CMMV was not detected by enzyme-linked immunosorbent assay in adults that had been given acquisition access periods of 1–8 hr.

Cowpea mild mottle virus (CMMV) on peanut in India has been recorded by Iizuka et al (8). CMMV has been shown to be transmitted by the whitefly, *Bemisia tabaci* (8,9). Whitefly-borne viruses are known to be transmitted in a semipersistent or persistent manner (1,4,11,12,14). In this paper, we report the nonpersistent transmission of CMMV by *B. tabaci*.

MATERIALS AND METHODS

Virus. The virus was initially isolated from peanuts (8) but subsequently maintained in soybeans (*Glycine max* 'Hardee') by infesting healthy plants, at 2 wk intervals, with whiteflies that had been exposed to CMMV-infected soybean plants for 30 min.

Vector. The *B. tabaci* culture was maintained on cotton (*Gossypium hirsutum* 'Varalakshmi'), which was found to be immune to CMMV. Only adult whiteflies were used in transmission experiments. To ascertain that the colony was virus-free, four groups of 20 insects each were tested on soybean seedlings at 2-wk intervals.

Transmission tests. Whiteflies were collected with an aspirator and released onto caged infected soybean plants. The cages (7.5 × 2.5 cm) were made of polyvinylchloride, and the upper end was covered with muslin cloth. Whiteflies were released through a small hole in the cloth, that was closed with a cotton plug.

About 300 adults were employed for each acquisition and inoculation access period experiment. To study the persistence of CMMV in *B. tabaci* after various acquisition access periods, groups of whiteflies were each transferred serially to individual soybean seedlings with a 5-min inoculation access period at each transfer. To study the effect of starvation on transmission, groups of 200 adults were starved in the glass tube of the aspirator for various intervals before or after the acquisition access period. For determination of the number of whiteflies required for transmission, adults were allowed an acquisition access period of 1 hr and one or more adults were allowed a 1-hr inoculation access period.

Unless otherwise mentioned, groups of five whiteflies were each transferred to individual test plants, which were 1-wk-old soybean seedlings. All exposed plants in the various experiments were sprayed with phosphorodithionate and maintained in a screenhouse with weekly sprays of the insecticide. Plants that failed to show symptoms after 6 wk of observation were discarded. Selected plants that showed symptoms of CMMV were assayed for CMMV by enzyme-linked immunosorbent assay (ELISA).

ELISA. CMMV antiserum was produced at ICRISAT Center and had a titer of 1/500, as determined by the precipitin ring test. Healthy and peanut mottle virus (PMV)- and CMMV-infected soybean

Table 1. Effect of acquisition access period on the transmission of cowpea mild mottle virus by *Bemisia tabaci*

Acquisition access period	Plants infected/ plants exposed[a] (no.)	Plants infected (%)
5 min	0/30	0
10 min	6/30	20
20 min	14/20	70
30 min	18/22	81
1 hr	18/25	72
3 hr	16/20	80
6 hr	24/30	80
24 hr	23/30	76

[a] Groups of five adults were transferred to each soybean seedling and allowed a 1-day inoculation access period.

Table 2. Effect of inoculation access period on the transmission of cowpea mild mottle virus by *Bemisia tabaci*

Inoculation access period[a]	Plants infected/ plants exposed[b] (no.)	Plants infected (%)
2 min	2/30	7
5 min	18/36	50
10 min	18/30	60
20 min	25/30	83
30 min	21/30	70
1 hr	25/30	83
6 hr	21/30	70
12 hr	18/30	60
24 hr	24/30	80

[a] All insects were given a 30-min acquisition access period.

[b] Groups of five insects were placed in a cage with each plant.

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extracts were prepared in PBS (0.02 M phosphate, 0.15 M sodium chloride, and 0.003 M potassium chloride, pH 7.4) containing 0.05% Tween 20 and 2% PVP (polyvinylpyrrolidone, mol wt 40,000). Dilutions were based on the original weight of leaf tissue. Whitefly extracts were prepared by grinding 200–300 whiteflies (previously held at −13 C for 30 min) with a pestle and mortar in 0.2 ml of PBS containing Tween 20 and PVP.

The procedure adopted was similar to that described by Clark and Adams (3) and as modified by Lister (10). Coating gamma globulin was used at 2.5 μg/ml and alkaline phosphatase-conjugated gamma globulin at a 1/200 dilution. Each well of ELISA plates was filled with 0.2 ml of reagents. Globulin coating was done at 37 C for 3 hr; antigen solutions were incubated for 16 hr at 4 C and the conjugate for 3 hr at 37 C. *p*-Nitrophenyl phosphate was added and incubated at room temperature for 1 hr, and the reaction was terminated by adding 0.05 ml of 3.0 M NaOH. Absorbance at 405 nm was measured in a Gilford 250 spectrophotometer. All assays were done in triplicate.

RESULTS

In all experiments, infected soybean plants showed veinclearing and veinal necrosis of leaves followed by downward curling of leaves by 10 days after inoculation. Two weeks after inoculation, newly emerged leaves showed mosaic and puckering.

When a single adult was used, percent transmission of CMMV was 0–30%. Maximum transmission of about 90% was obtained using five insects or more; therefore, five adults were used in all subsequent experiments.

B. tabaci adults acquired CMMV in access periods of 10 min or more but not in 5 min (Table 1). A 2-min inoculation access period was adequate for transmitting the virus (Table 2). In serial 5-min transfers, the virus was retained for a maximum interval of 20 min (Table 3). Preacquisition starvation did not affect transmission (Table 4), but starvation for 1 hr or more after acquisition decreased transmission (Table 5).

Two hundred whiteflies from two independent experiments, that had lost the ability to transmit CMMV after 11 serial transfers of 5-min intervals and a 12th transfer of 2 days (Table 3) were given acquisition access of 30 min on infected soybean and 2-hr inoculation access on healthy soybean seedlings. Twenty groups of five insects each transmitted CMMV to 35 of 40 plants.

In three experiments, CMMV was detected by ELISA in soybean extracts diluted to 1/6,400 to 1/12,800. Extracts from healthy and PMV-infected soybean leaves gave no reaction. ELISA values for extracts from 200 whiteflies given a 1-hr acquisition access period or from 300 adults given 30-min, 1-, 6-, 24-, and 48-hr acquisition access periods were similar to

Table 3. Persistence of cowpea mild mottle virus in *Bemisia tabaci*

Acquisition access period	Group[a] no.	Serial number of plants inoculated[b] 1	2	3	4	5	6	7	8	9	10	11	12[c]
10 min	1	+	+	−	−	−	−	−	−	−	−	−	−
	2	+	−*	−	−	−	−*	−	−	−	−	−	−
	3	+	+	+	−	−	−	−	−	−	−	−	−
	4	+	−	−	−	−	−	−	−	−	−	−	−
	5	+	+	−	−*	−	−	−	−	−	−*	−	−
	6	+	+	−	−	−	−	−	−	−	−	−	−
	7	+	+	−	−	−	−*	−	−	−	−	−	−
	8	+	+	−	−	−	−	−	−*	−	−	−	−
	9	+	+	−	−*	−	−	−	−	−	−	−	−
	10	+	−	−	−	−	−	−	−	−	−	−	−
20 min	1	+	+	+	−	−*	−	−	−	−	−	−	−
	2	+	+	−	+	−	−	−	−	−	−	−	−
	3	+	+	−	−	−	−	−	−	−	−	−	−
	4	+	+	−*	+	−	−	−	−	−	−	−	−
	5	+	+	+	−	−	−	−*	−	−	−	−	−
	6	+	+	−	−	−	−	−	−	−	−	−	−
	7	+	−	−*	−	−	−	−	−	−	−	−	−
	8	+	+	−	−*	−	−	−	−	−	−	−	−
	9	+	−	−	−	−	−	−	−	−	−	−	−
	10	+	+	−	−*	−	−	−	−	−	−	−	−
30 min	1	+	+	+	−	−	−	−	−	−	−	−	−
	2	+	+	−*	−	−	−	−	−	−	−	−	−
	3	+	+	−	−*	−	−	−	−	−	−	−	−
	4	+	+	−	+	−	−	−	−	−	−	−	−
	5	+	−	+	−	−	−	−	−	−	−	−	−
	6	+	+	+	−	−	−	−	−	−	−	−	−
	7	+	+	−*	−	−	−	−	−	−	−	−	−
	8	+	+	+	−	−	−	−	−	−	−	−	−
	9	+	−	−	−*	−	−	−	−	−	−	−	−
	10	+	+	+	−	−	−	−	−	−	−	−	−
60 min	1	+	+	−*	−	−	−	−	−	−	−	−	−
	2	+	+	−	−	−	−	−	−	−	−	−	−
	3	+	+	−	−	−	−	−	−	−	−	−	−
	4	+	−	−	−	−*	−	−	−	−	−	−	−
	5	+	+	−	−	−	−	−	−	−	−	−	−
	6	+	−	−	−	−	−*	−	−	−	−	−	−
	7	+	+	−	−	−	−	−	−	−	−	−	−
	8	+	+	+	−	−	−	−	−	−	−	−	−
	9	+	+	−	−	−	−	−	−	−	−	−	−
	10	+	+	−*	−	−*	−	−	−	−	−	−	−

[a] Groups of five insects were placed in a cage with each plant.
[b] A 5-min inoculation access period was allowed on each plant.
[c] Insects were allowed a 48-hr inoculation access period to test for any semipersistent transmission. Although at the time of transfer five insects were added, after 3 days all five insects were recovered in only 50% of the groups. + = Plant infected; − = plant remained healthy during 8 wk of observation; * = CMMV antigen was not detected in plant extracts by enzyme-linked immunosorbent assay.

Table 4. Effect of a preacquisition starvation period on the transmission of cowpea mild mottle virus by *Bemisia tabaci*

Preacquisition starvation period[a]	Plants infected/ plants exposed[b] (no.)	Plants infected (%)
None	18/20	90
30 min	17/20	85
1 hr	16/20	80
2 hr	15/20	75
3 hr	22/30	73
4 hr	16/20	80
5 hr	17/20	85
6 hr	24/30	80

[a] After starvation all insects were given a 30-min acquisition access period an a 2-hr inoculation access period.
[b] Five adults were transferred onto each soybean seedling.

Table 5. Effects of a preinoculation starvation period on the transmission of cowpea mild mottle virus by *Bemisia tabaci*

Preinoculation starvation period[a]	Plants infected/ plants exposed[b] (no.)	Plants infected (%)
None	30/36	83
30 min	15/20	75
1 hr	3/18	16
2 hr	4/20	20
3 hr	4/20	20
4 hr	0/20	0
5 hr	3/20	15
6 hr	0.20	0

[a] All insects were given a 30-min acquisition access period and a 2-hr inoculation access period.
[b] Five adults were transferred onto each soybean seedling.

those of extracts from whiteflies that were not exposed to the virus.

DISCUSSION

These results show that CMMV was transmitted by *B. tabaci* in a nonpersistent manner. Iwaki et al (9) demonstrated that *B. tabaci* could acquire CMMV in acquisition and inoculation access periods as brief as 5 min. Our results and those reported by Iwaki et al (9) show conclusively that there is no latent period for CMMV in *B. tabaci*. Iwaki et al (9) showed that the virus was retained by the vector for about 1 hr. Our tests using a minimum inoculation access period of 5 min during each serial transfer showed a retention time of 20 min. From the ELISA tests, we concluded that the virus retained by the whitefly for a short time was in concentrations too low to be detected by ELISA. If the virus enters internal organs, it is unlikely that it plays any significant role in transmission.

We prefer to use the term "nonpersistent" because acquisition access periods longer than 20 min failed to increase the rate of transmission. The virus was retained for 20 min in the vector. The shortest retention period reported so far for any other whitefly-transmitted virus was 3 days (1). We were unable to demonstrate a latent period for CMMV. If one exists, it is far shorter (5 min or less) than any previously reported minimal latent period (4 hr) for a whitefly-transmitted virus (1). Because we were unable to determine the exact feeding periods, we presume that the whitefly adult has the ability to transmit the virus soon after acquisition. Therefore, the transmission characteristics observed are comparable to those of nonpersistent, noncirculative transmission by aphids (6). Preacquisition starvation, which increases the level of transmission of aphid-transmitted viruses (15), did not affect transmission of CMMV by the whitefly.

Sweet potato mild mottle virus (7), cucumber vein yellowing virus (5,13), and cucumber yellows virus (16) are other filamentous viruses transmitted by whiteflies. These viruses, however, are transmitted in a semipersistent manner. The majority of the carlaviruses are transmitted by aphids in a nonpersistent manner, and interestingly, CMMV, which shows several characteristics of carlaviruses (2,8,9; Rajeshwari, Reddy, Nolt, and Bharathan, *unpublished*), is also transmitted in a nonpersistent manner by *B. tabaci*.

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