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Insect transmission of okra mosaic virus in the Ivory Coast

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SUMMARY

The Ivory Coast and Nigerian strains of okra mosaic virus (OMV) were transmitted by the flea-beetle *Podagrica decolorata*, a serious pest of okra in the southern Ivory Coast. The Ivory Coast strain was also transmitted by the orthopteran, *Zonocerus variegatus*. The Ivory Coast strain was acquired faster than it was inoculated by *P. decolorata*. When groups of five beetles were given acquisition and inoculation access periods of 24 and 48 h, respectively, 60% of the okra test plants were infected. OMV-carrying *P. decolorata* remained infective for up to 6 days. The virus was readily detected in extracts of crushed beetles that had fed on infected plants for 20 h. The beetle was also able to transmit to and from plants of *Hibiscus sabdariffa* and *Corchorus olitorius*; as a food source it preferred *C. olitorius* to okra or *H. sabdariffa*. The beetle is active throughout the year, and presumably can spread OMV at any time between plants of these species. A considerable and unexplained decrease in frequency of transmission was observed in experiments done in the rainy season. The revised cryptogram of OMV is R/1:*/32:S/S:S/Ve/Cl.

INTRODUCTION

Okra (*Hibiscus esculentus* L., Malvaceae) crops in field and garden plots around the Adiopodoumé Research Station in the southern Ivory Coast are almost invariably infected with okra mosaic virus (OMV), a tymovirus which was first described from the Ivory Coast (Givord & Hirth, 1973). The known natural vectors of tymoviruses are all leaf-eating beetles in the group Chrysomelidae (Markham & Smith, 1949; Freitag, 1941; Dale, 1954; Gibbs, Hecht-Poinar, Woods & McKee, 1966; Jankulowa, Huth, Wittmann & Paul, 1968), except for the vector of scrophularia mottle virus which is a weevil (Curculionidae) (Weidemann, 1973). In Nigeria, three chrysomelid species are reported to be vectors of OMV: *Podagrica sjostedti* Jac., *P. uniforma* Jac. and *Syagrus calcaratus* F. (Lana & Taylor, 1976). This paper describes the transmission of Ivory Coast and Nigerian isolates of OMV by *Podagrica decolorata* Duvivier (Chrysomelidae: Halticinae) to okra and other natural host plants (Givord, 1978); we also report transmission of the virus by *Zonocerus variegatus* L. (Orthoptera: Pyrgomorphidae).

MATERIALS AND METHODS

Viruses and plants. The Ivory Coast (OMV-CI) isolate (Givord & Hirth, 1973) and Nigerian (OMV-Nig) isolate (Givord, 1977) of OMV were maintained in okra cv. Clemson Spineless. They were transmitted by grinding infected leaves with 0·01 M sodium phosphate buffer, pH 7·0, and applying the extract with the finger to Celite-dusted leaves of healthy test plants. All experiments were with isolate OMV-CI unless otherwise stated.

Okra, *Corchorus olitorius* L. and *Hibiscus sabdariffa* L. were grown from seed and were kept

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in gauze houses. For inoculation either mechanically or by insects, okra and *H. sabdariffa* seedlings were used at the cotyledon stage. *C. olitorius* seedlings, which have small cotyledons and primary leaves, were used when they had eight to 10 leaves. After inoculation by insects, seedlings were sprayed weekly with insecticide.

Insects. Most experiments were with adults of *P. decolorata* but another halticid beetle, *Nisotra dilecta* Dalm., and larvae of the orthopteran *Z. variegatus*, were used in some tests. The beetles were collected by aspirator from crops of okra, and some also from *C. olitorius* and *H. sabdariffa*, near the Research Centre. To ensure that they were not infective at the start of an experiment, beetles were kept after collection for 8 days in an insect-proof cage containing healthy okra plants, which were changed daily.

Transmission experiments. Beetles were tested for infectivity either singly or in groups of five. When tested singly, each beetle was caged on an okra seedling inside a clear plastic tube (7·5 × 2·5 cm) with a gauze top. When tested in groups of five, each group was caged on two seedlings inside a plastic cylinder (20 cm high, 9 cm diameter) with a gauze top and a gauze-covered side vent. An aspirator was used to transfer the beetles. In all experiments control batches of okra plants kept in the same conditions as treated plants remained healthy. At the end of the inoculation period live insects were counted and then killed.

RESULTS

Observations on Podagrica decolorata *and other insects*

Podagrica decolorata is 3–4 mm long, ellipsoidal in shape and has thread-like antennae, bright brown elytra, and a lighter brown head and thorax. Like other halticid beetles, its hind femurs are greatly enlarged for jumping, a feature that accounts for the common name 'flea beetle'.

In the Ivory Coast, *P. decolorata* is a serious pest of okra and occurs also on *C. olitorius* and *H. sabdariffa*. It feeds by making a succession of small holes in the leaves, then attacks the buds and stems and kills the plants. This commonly happened in our experiments when access periods were longer than 48 h. The insect is active throughout the year and, unlike flea-beetles in temperate areas, apparently has no seasonal cycle of reproduction. The larvae, which are subterranean, were never observed in the field and all beetles collected for the experiments were adult. Although copulations were very often observed in cages (Table 4), attempts to rear *P. decolorata* were unsuccessful. In one instance 100 eggs were laid in artificial soil cracks. After incubation on wet filter paper in Petri dishes for 6–7 days, 20 larvae were obtained; these were kept with okra rootlets in Petri dishes but only four reached the next larval stage before dying.

In two experiments, 10 male and 10 female insects were allowed to feed simultaneously for 24 h in separate cages on two okra seedlings each with two cotyledons and one primary leaf. The leaf areas eaten by the male and female insects were 222 and 242 cm^2, respectively, in the first experiment, and 246 and 252 cm^2, respectively, in the second experiment; these differences were not significant.

Nisotra dilecta is another halticid beetle common on *H. sabdariffa* and sometimes found on okra. In size, shape and colour it resembles *P. decolorata* except for the colour of the elytra which are shining dark blue. Several species of the genus *Podagrica* and *N. dilecta* are well-known pests of *Gossypium* sp., *Hibiscus* sp., okra and other species of Malvaceae, and of *Corchorus* sp. (Tiliaceae) in tropical Africa (Kranz, Schmutterer & Koch, 1977). Many other chrysomelid species were found while collecting beetles from crops of okra, *H. sabdariffa* and *C. olitorius*, but they were not sufficiently numerous to test as vectors of OMV.

The orthopteran *Z. variegatus* L. is a well-known pest of cassava. On one occasion, this insect was observed in large numbers feeding voraciously in an okra field near a cassava crop in the month of December. The insects were collected and tested for ability to transmit OMV.

Transmission experiments with Podagrica decolorata

Tests for presence of OMV in leaves of source plants. Tests were made to find out at what time after inoculation with OMV-CI plants could be used as sources of virus in insect transmission experiments. The leaves were ground in sodium phosphate buffer and the extracts assayed by inoculating serial dilutions to five okra seedlings. Extracts made 15 days after inoculation from leaves of okra, *C. olitorius* and *H. sabdariffa* with obvious symptoms were infective at dilutions up to 10^{-6}, 10^{-5} and 10^{-5}, respectively. Later, infected plants of all three species became symptomless. Nevertheless, extracts from okra and *C. olitorius* leaves 2 months after inoculation were still infective at dilutions up to 10^{-4} and 10^{-2}, respectively; in contrast, leaf extracts from *H. sabdariffa* were non-infective 1 month after inoculation.

In subsequent experiments source plants were used within 14 days after inoculation and were stripped of old and symptomless leaves, so that the beetles fed only on young leaves showing obvious symptoms.

Effect of duration of acquisition and inoculation access periods. Table 1 shows that increasing the acquisition access time from 0·5 to 2 h had little effect on transmission of OMV-CI but increasing the time to 4 h doubled the frequency of transmission. Transmission was poor when the beetles were allowed inoculation access times of only 0·5 to 4 h. Maximum frequency of transmission was by beetles allowed 24 or 48 h on infected plants and 48 or 72 h on test plants. Increasing the inoculation access time from 24 to 48 h had no effect on the number of transmissions except in one experiment.

Much lower rates of transmission (from 2 to 8%) were obtained in similar experiments done in the rainy season with acquisition access times of 48 or 72 h and an inoculation access time of 48 h.

Retention of the virus in the vector. Table 2 shows that *P. decolorata* retained ability to transmit OMV-CI for up to 6 days after an acquisition access time of 24 h.

In Expts 4 and 5 (Table 2), the beetles surviving after the last inoculation access period were kept in the same conditions as for the main part of the experiment, except that okra seedlings

Table 1. *Transmission of OMV-CI from okra to okra by* Podagrica decolorata *allowed different acquisition and inoculation access times*

Acquisition access time (h)	Inoculation access time (h)	No. plants infected/no. tested*	Mean no. insects surviving†
0·5	24	9/50 (18%)	3·6
0·5	48	9/58 (16%)	3·6
1	24	10/57 (18%)	
1	48	11/60 (18%)	4·2
2	24	12/52 (23%)	
2	48	12/59 (20%)	4·4
4	24	24/60 (40%)	
4	48	21/55 (38%)	3·5
24	24	18/59 (31%)	
24	48	35/58 (60%)	3·0
48	72	17/27 (63%)	1·0
24	0·5	2/60 (3%)	5·0
24	1	0/60 (0%)	4·9
24	2	9/60 (15%)	4·8
24	4	6/57 (11%)	4·5

* The beetles were starved for 4 h before the acquisition feed; they were tested in groups of five per two test plants.

† Mean no. beetles out of five still living at the end of the experiment.

Table 2. *Transmission of OMV-CI by* Podagrica decolorata *after serial daily transfers on okra seedlings*

Expt. no.*	Acquisition access time (h)	No. plants infected/no. tested on days indicated†												
		1	2	3	4	5	6	7	8	9	10	11	12	13
1	24	5/28	2/28	0/26	0/26									
		(3·4)	(2·9)	(2·0)	(1·0)									
2	24	12/29	6/30	1/30	1/30									
		(4·5)	(4·3)	(3·5)	(3·3)									
3	24	12/30	5/22	1/17	1/12	0/8	0/6	0/4	0/2					
4	48	25/61	3/37	0/24	0/18	0/12	0/9	0/6	0/4	0/3	0/2	0/2		
		(2·8)	(2·9)	(3·6)	(3·7)	(3·5)	(2)	(3·5)	(3·5)	(3)	(5)	(3)		
5	24	17/74	9/72	2/64	1/29	1/26	1/21	0/16	0/13	0/10	0/10	0/8	0/7	0/6
		(2·6)	(3·3)	(3·2)	(3·4)	(3·9)	(3·5)	(3·7)	(3·6)	(4·6)	(4·2)	(4·5)	(4·0)	(4·5)

* In Expts 1 and 2 the insects were starved for 4 h before the acquisition feed, in Expts 3, 4 and 5 there was no pre-acquisition starvation. The beetles were tested in groups of five per two test plants.

† Figures in parentheses are the mean number of insects out of five still living at the end of the inoculation periods; no counts were made in Expt. 3.

were changed only every 2 or 3 days. The last beetles died 49 and 25 days respectively after the beginning of the experiments.

Detection of virus in P. decolorata *extracts.* Beetles were allowed to feed for 20 h on infected okra seedlings and were then anaesthetised with diethyl ether. The beetles were washed with alcohol and then with water to remove any virus contaminating the outside of the insects, and the thorax and abdomen were then separated using a fine nylon thread. The thoraces were ground with 0·01 M sodium phosphate buffer, pH 7·0, the abdomens were ground without buffer, and each extract was inoculated manually to healthy okra seedlings, using Celite abrasive. All of 50 plants inoculated with the extracts were infected.

Transmission of a Nigerian strain of OMV. No transmissions of OMV-Nig were obtained in three experiments done in the rainy season, but in three experiments done in the dry season using acquisition and inoculation access periods of 24 h or 48 h, the number of plants infected were three out of 10, three out of eight and seven out of 59, respectively.

Effect of source and test plant species. Table 3 shows that *P. decolorata* transmitted OMV-CI from okra to the cultivated species *Hibiscus cannabinus*, *H. sabdariffa* and *C. olitorius* and *vice versa.* These experiments were done in the rainy season and, although frequencies of

Table 3. *Transmission of OMV-CI by* Podagrica decolorata *between different host species*

Source plant*	Test plant	No. test plants infected/no. tested	Mean no. insects surviving†
Okra	*H. sabdariffa*	1/36	2·8
Okra	*H. cannabinus*	1/40	2·5
Okra	*C. olitorius*	5/26	4·3
C. olitorius	Okra	6/52	2·9
H. cannabinus	Okra	1/58	3·8
H. sabdariffa	Okra	1/55	3·7

* Acquisition and inoculation access times were 48 h; the experiments were done in the rainy season using five insects and two seedlings per cage.

† Mean no. insects out of five still living at the end of the inoculation period.

transmission were low, they were much higher when *C. olitorius* was used either as source or test plant than when the other species were used.

Three other natural host species, *Urena lobata* L. (Malvaceae), *Borreria intricans* Hepper (Rubiaceae), which are common weeds in crops in southern Ivory Coast, and the shoots of a stump of *Blighia welwitschii* Hiern-(Radlk) (Sapindaceae) (Givord, 1978), were also used as source and test plants, but again the experiments were done in the rainy season and very few transmissions were obtained.

Table 4. *Host preferences of* Podagrica decolorata *among three natural hosts of OMV-CI*

		P. decolorata	
Cage no.	Host species compared*	Presence on species (expressed as % of total observations)†	No. copulations observed
1	Okra	13	0
	C. olitorius	66	22
2	Okra	32	2
	C. olitorius	30	3
3	Okra	13	6
	C. olitorius	55	8
4	Okra	47	16
	H. sabdariffa	20	0
5	Okra	42	12
	H. sabdariffa	20	0
6	Okra	52	12
	H. sabdariffa	20	2
7	*C. olitorius*	48	5
	H. sabdariffa	24	0
8	*C. olitorius*	52	18
	H. sabdariffa	21	8
9	*C. olitorius*	57	14
	H. sabdariffa	22	1

* Ten beetles were caged on each pair of plants.

† The location of the beetle was noted four times a day for 7 days; presence on species (%)

$$= \frac{\text{no. times insect found on the species} \times 100}{\text{total no. observations}}$$

(when not recorded as present on either plant the insect was on the cage wall or on the soil).

Tests for host preferences of P. decolorata. An experiment on the host preference of *P. decolorata* was made using cylindrical plastic cages 40 cm high and 16 cm in diameter; the top of each cage and two lateral windows were covered with gauze. Plants of okra, *C. olitorius* and *H. sabdariffa* were planted in pairs in all possible combinations. The plants were of similar height and leaf area, and each had several leaves. Each combination was replicated three times (nine cylinders). Ten *P. decolorata* were placed in each cage. The location of the insects was recorded at 08.00, 10.30, 15.30 and 18.00 hours every day for 7 days. The results (Table 4) showed that *P. decolorata* prefers C. olitorius to *H. esculentus* or *H. sabdariffa*, and *H. esculentus* to *H. sabdariffa*.

Transmission experiments with other species

In two experiments done in the rainy season no transmission of OMV-CI was obtained using adults of the beetle *N. dilecta*. The acquisition and inoculation access periods were 24 and 48 h, respectively. The beetles were tested in groups of five per two seedlings.

In one experiment with the orthopteran *Z. variegatus* one nymph was used per two test plants and allowed acquisition and inoculation access periods of 4 and 3 h, respectively; two out of 20 plants were infected.

DISCUSSION

Our results suggest that *P. decolorata* acquired OMV-CI faster than it transmitted it. The shortest inoculation and acquisition access periods tested (0·5 h) gave very low frequencies of transmission (3–18%) whereas the longer periods (24–72 h) gave transmission frequencies up to 63%. The latter result and the retention of OMV-CI in *P. decolorata* for 6 days resemble those obtained by Lana & Taylor (1976) for the transmission of a Nigerian isolate of OMV by the beetles *P. sjostedti* and *P. uniforma*. These authors also obtained transmission of a Nigerian isolate by the whitefly *Bemisia tabaci* Genn. but Givord, Pfeiffer & Hirth (1972) failed to transmit an Ivory Coast strain of OMV with this insect.

OMV was recovered from the crushed thoraces and abdomens of beetles that had fed on virus-infected plants. In other work (L. Givord and K. Van Beek, unpublished data), salivary glands were observed in the head of *P. decolorata* (Gabe, 1968). This is contrary to Smith's (1965) report that beetles have no salivary glands. Walters (1969) stated that viruses retained by beetle vectors for prolonged periods of time were transmitted as a result of regurgitation during feeding. Obviously much more is involved than a simple contamination of beetle mouthparts during feeding, as has been shown on other occasions (Walters, 1969; Filton & Scott, 1977): further research on the mechanism of transmission of OMV by *P. decolorata* is necessary.

Transmission of OMV by *P. decolorata* was very inefficient in all experiments done in the rainy season; these observations were repeated in 1976, 1977 and 1978. Other experimental conditions were identical to those prevailing in the dry season. Symptoms on mechanically inoculated plants appeared a little more slowly in the rainy season than at other times and were a little less obvious, although 100% transmission was achieved. In all transmission experiments the beetles acquired virus from leaves with well-developed symptoms and there was obvious feeding damage; however, no comparisons were made of the feeding activity of the beetles in the rainy and dry seasons. The temperature is slightly lower in the wet than in the dry season but the main difference is the marked decrease in the amount of sunshine. Gibbs *et al.* (1966) observed that dulcamara mottle virus was transmitted by the potato flea-beetle *Psylliodes affinis* Paykull in autumn and spring but not in summer; however, these studies were made in a temperate climate with well defined seasons and beetle reproductive cycles, conditions very different from those obtaining in the Ivory Coast. Further research is necessary to find the reason for the inefficient transmission of OMV in the Ivory Coast in the rainy season.

Although the host preference experiment suggested that *P. decolorata* prefers *C. olitorius* to okra or *H. sabdariffa*, our observations show that this is apparently not always so in the field. Nevertheless this result could explain why in the rainy season OMV was transmitted more efficiently from *C. olitorius* to okra and from okra to *C. olitorius* than between okra and *H. sabdariffa* or *H. cannabinus*.

Several viruses transmitted by beetles can also be transmitted by other biting insects; OMV is transmitted by at least one orthopterous insect (*Z. variegatus*), as is turnip yellow mosaic virus (Markham & Smith, 1949). However, *P. decolorata* is the most important vector in southern Ivory Coast because it is common in okra fields throughout the year. It also feeds on the other natural hosts of OMV, both cultivated plants and weeds. The damp tropical climate allows okra

to be grown without interruption throughout the year; commonly the new okra sprouts appear while the old plants, which are often virus-infected and still covered in vector insects, remain undestroyed in the field. Even when the ground is well tilled, the insect takes refuge among the wild plants at the edges of the fields, or on *C. olitorius* and *H. sabdariffa* in neighbouring gardens. *C. olitorius* plants are often left in the ground for 2 yr and can maintain the cycle of infection between successive okra crops. Natural transmission of OMV possibly occurs also by simple contact of the leaves aided by the wind, animals or man, because the virus is readily transmissible by mechanical inoculation.

The fourth pair of the cryptogram of okra mosaic virus published in Givord & Koenig (1974) can be completed as follows: R/1:*/32:S/S:S/Ve/Cl.

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