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Comparison of transmission abilities of four *Cicadulina* species vectors of maize streak virus from Nigeria

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Abstract

Four *Cicadulina* species [*Cicadulina arachidis* China, *Cicadulina dabrowskii* Webb, *Cicadulina mbila* (Naude), and *Cicadulina storeyi* China (Homoptera: Cicadellidae)] found during field surveys in 1997–1999 across five ecological zones in Nigeria were reared in screenhouses and females were used in a study to compare their abilities to transmit Maize streak virus (Geminiviridae: genus *Mastrevirus*) from maize to maize using the susceptible variety Pool 16. The results showed that for both acquisition access feeding periods (AAP) and inoculation access feeding periods (IAP), transmission efficiencies decreased in the following order: *C. storeyi* > *C. mbila* > *C. arachidis* > *C. dabrowskii*. The transmission efficiencies of these vectors increased with longer feeding periods, as the means of susceptible test plants that showed *Mastrevirus* symptoms for both acquisition and IAPs were higher for 24 and 48 h than for 1 and 2 h for all four species studied. The epidemiological implications of these differences in transmission abilities are discussed.

Introduction

Maize streak viruses of the genus *Mastrevirus* (MSV) are transmitted in a persistent manner by several species of leafhoppers in the genus *Cicadulina* (Homoptera: Cicadellidae). A vector insect is able to retain and transmit the virus throughout its lifespan. There are 22 species of *Cicadulina*, 18 of which occur in Africa (Webb, 1987). Of these, nine have been reported to be vectors of MSV: *Cicadulina arachidis* China, *Cicadulina bipunctata* (= *bipunctella*) (Melichar), *Cicadulina ghaurii* Dabrowski, *Cicadulina latens* Fennah, *Cicadulina mbila* (Naude), *Cicadulina niger* China, *Cicadulina parazeae* Ghauri, *Cicadulina similis* China, and *Cicadulina storeyi* (= *triangula*) China (Storey, 1925, 1936; Fennah, 1959; Rose, 1962; Dabrowski, 1985, 1987; Okoth & Dabrowski, 1987; Webb, 1987; Okoth et al., 1988; Reynaud, 1988; Efron et al., 1989; Thottappilly et al., 1993; Birgirwa et al., 1995). The minimum latent period in *C. mbila* is 6–12 h (Storey, 1925, 1928). The minimum latent period in *C. storeyi* (reported as *C. triangula*) is 14–18 h and a median latent period (LP50) is 16–20 h (Okoth et al., 1988). Asanzi (1991) reported LP50 values of 14–24 h and 12–14 h for *C. arachidis* and *C. ghaurii*, respectively. Storey (1925, 1938) demonstrated that *C. mbila* can acquire MSV from infected plants within 15 s and transmit it in 5 min. Zagre (1983) found a minimum acquisition access period (AAP) of 30 s and transmission after a 2-h inoculation access period (IAP) for *C. storeyi*. Asanzi (1991) found minimum AAPs of 15 min and 1 h for *C. arachidis* and *C. ghaurii*, respectively, and a minimum IAP of 1 h for both species. Generally, the transmission efficiencies of *Cicadulina* species increase with increasing duration of acquisition and inoculation feeding.

Storey (1932) showed that *C. mbila* populations are composed of active and inactive vectors. Other species have also been reported to vary in their ability to transmit MSV. Earlier reports from Nigeria indicated that populations of *C. mbila* and *C. storeyi* have more active transmitting individuals than those of *C. ghaurii* and *C. arachidis* (Okoth et al., 1988; Effron et al., 1989; Asanzi et al., 1995). In all species, the percentage of active transmitters among females was 2–3 times higher than among males. It was also demonstrated that the percentage of active transmitters could be increased in the population through selection. Factors affecting the epidemiology of MSV disease in a

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given region include the population density of the vectors and their species composition. To compare the transmission efficiencies of four vector species that were prevalent in Nigeria, this study was undertaken to ascertain the epidemiological significance of their natural populations.

Materials and methods

Field surveys were undertaken in 1997–1999 across five ecological zones in Nigeria to collect *Cicadulina* leafhoppers. Natural populations of *C. arachidis*, *Cicadulina dabrowskii*, and *C. mbila* were reared in insect-proof cages inside screenhouses at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. An IITA population of *C. storeyi* that has been reared for several years and used for artificial inoculation of MSV in breeders' trials was used as the fourth test species. The procedure of Mesfin et al. (1992) was followed for the transmission studies. Only female individuals were used in the transmission studies.

Acquisition access feeding

Leafhoppers that had not fed before on MSV-infected plants were confined within polyvinylchloride (PVC) tubes on symptomatic maize plants or excised leaves for a given period to acquire the virus while feeding. This period was designated the AAP. To ensure that leafhoppers being used for transmission had not already acquired MSV, the following negative check experiment was carried out. The insects were caged for 24 h on healthy maize seedlings (10–14 days old) prior to acquisition access feeding. After the insects were removed, the maize seedlings were protected from other insects by adding 0.1 g of granular insecticide, Furadan® (active ingredient carbofuran; FMC Corporation, Philadelphia, PA, USA) at the leaf whorls and plant base. The maize seedlings were thereafter kept in wooden cages in the screenhouse. The seedlings were observed for symptom developments for 2 weeks. If the seedlings showed streak symptoms, the leafhoppers were regarded to have been in contact with MSV-infected plants and were not used for subsequent experiments. Transmission experiments for which such leafhoppers had been used were consequently disregarded and repeated.

Groups of 100 non-viruliferous *C. arachidis*, *C. dabrowskii*, *C. mbila*, and *C. storeyi* were confined on MSV-infected maize seedlings for 1, 2, 24, and 48 h to acquire MSV while feeding. Female insects were then transferred singly with an aspirator to healthy maize seedlings for a uniform IAP of 48 h. Seven- to 10-day-old seedlings of the MSV-susceptible, open-pollinated maize variety Pool 16 were used as test plants. Twenty insects were used for each AAP and the experiments were replicated four times (over time).

Inoculation access feeding

Female leafhoppers that had fed on MSV-infected plants were confined within PVC tubes on healthy seedlings of test plants for a given period to inoculate the test plant with the virus while feeding. This period was designated the IAP.

Groups of 100 non-viruliferous *C. arachidis*, *C. dabrowskii*, *C. mbila*, and *C. storeyi* were confined on MSV-infected maize seedlings for 48 h to acquire MSV while feeding. Females were then transferred singly with an aspirator to healthy maize seedlings for 1, 2, 24, and 48 h IAPs. Seven- to 10-day-old seedlings of the MSV-susceptible, open-pollinated maize variety Pool 16 were used as test plants. Twenty insects were used for each IAP and experiments were replicated four times.

Statistical analysis

The data obtained were log transformed and a general linear model (GLM) analysis of variance was used to analyse the data using SAS software version 6.12 (SAS, 1999). Student-Newman-Keuls (SNK) was used as the post hoc multiple comparison test.

Results

The four *Cicadulina* species tested differed significantly in their MSV-transmission ability (GLM: P = 0.0001; Table 1). Transmission efficiency varied significantly between AAPs (GLM: P = 0.0001; Table 1). Transmission efficiency differed significantly between the four values of AAP tested. There was a significant interaction between AAP and species. Likewise, the four species differed significantly (GLM: P = 0.0001; Table 2) in their MSV-transmission ability and different IAPs resulted in different transmission efficiencies (GLM: P = 0.0001; Table 2).

Source	d.f.	Sum of squares	Mean square	F-value	P-value
Replicates	3	0.137	0.046	1.81	0.1596
Treatments	3	3.327	1.109	43.98	0.0001
Species	3	9.267	3.089	122.50	0.0001
Treatments*species	9	3.270	0.363	14.41	0.0001
Error	45	1.135	0.025		

Table 1 General linear model (GLM) of *Mastrevirus* (MSV) transmission abilities of four *Cicadulina* species, given four acquisition access feeding periods (AAP) and a 48-h inoculation access feeding period (IAP), and replicated four times (20 adults per treatment)

Table 2 General linear model (GLM) on the *Mastrevirus* (MSV) transmission abilities of four *Cicadulina* species, given four inoculation access feeding periods (IAP) and a 48-h acquisition access feeding period (AAP), and replicated four times (20 adults per treatment)

Source	d.f.	Sum of squares	Mean square	F-value	P-value
Replicates	3	0.271	0.090	2.42	0.0788
Treatments	3	4.591	1.530	40.87	0.0001
Species	3	3.512	1.171	31.227	0.0001
Treatments*species	9	0.615	0.068	1.82	0.0900
Error	45	1.685	0.037		

Pooled over the four vector species, the mean number of plants that were infected with MSV during the 24- and 48-h AAPs were not significantly different from each other, but were different from the results obtained during the 1- or 2-h AAPs (Table 3). The mean number of plants that showed MSV symptoms during 1- and 2-h IAPs were not significantly different from each other, but were different from the results obtained during 24 or 48 h IAPs. There was also a significant difference between the results of 24 and 48 h inoculation access feeding durations (Table 4).

The mean numbers of maize plants that showed MSV symptoms for the four *Cicadulina* species are presented in Table 5. The results for AAP and IAP show decreasing transmission efficiency in the following order: *C. storeyi* > *C. mbila* > *C. arachidis* > *C. dabrowskii.* The transmission efficiencies increased with longer feeding duration as the mean number of susceptible test plants that showed MSV symptoms for both AAPs and IAPs were higher for 24 and 48 h than 1 and 2 h for each of the four species.

Table 3 Mean number out of 20 maize test plants that showed *Mastrevirus* (MSV) symptoms when females of four *Cicadulina* species were given varied acquisition access feeding periods (AAP) and a 48-h inoculation access period

Acquisition access period (h)	Log (mean)[1]
48	0.682a
24	0.690a
2	0.358b
1	0.151c

[1]Means followed by the same letter are not significantly different at P<0.05 (Student-Newman-Keuls test).

Table 4 Mean number out of 20 maize test plants that showed *Mastrevirus* (MSV) symptoms when females of four *Cicadulina* species were given a 48-h acquisition access period and varied inoculation access feeding periods (IAP)

Inoculation access period (h)	Log (mean)[1]
48	1.008a
24	0.819b
2	0.411c
1	0.380c

[1]Means followed by the same letter are not significantly different at P<0.05 (Student-Newman-Keuls test).

Discussion

All four *Cicadulina* species studied were able to transmit MSV. This is the first report that adds *C. dabrowskii* to the list of MSV vectors in Nigeria and throughout Africa. Our results show that confining the vectors for 48 h acquisition feeding would not significantly increase their efficiency compared to 24 h (Table 3). However, there were significant differences when the vectors were confined for 1, 2, or 24 h (Table 3). This indicates that more virus particles are being acquired with longer feeding times. Boulton & Markham (1986) showed that the concentration of maize streak virus acquired by *C. mbila* increases with AAP. A longer AAP would also result in higher vectoring efficiency because it would surpass virus latency within the vectors. Previous workers have reported the latent periods of different *Cicadulina* species to range between 6 and 24 h (Storey, 1925, 1928; Okoth et al., 1988; Asanzi, 1991). These reports show that a longer AAP would not only imply more virus acquisition but more time for replication of the virus within the vector to become available for transmission, as MSV is transmitted in a persistent manner.

Table 5 Mean number out of 20 maize test plants that showed *Mastrevirus* (MSV) symptoms when females of four *Cicadulina* species were given varied acquisition (AAP) and inoculation access feeding (IAP) periods

	Log (mean)[1]	
Species	AAP	IAP
Cicadulina storeyi	0.869a	1.002a
Cicadulina mbila	0.722b	0.736b
Cicadulina arachidis	0.413c	0.449c
Cicadulina dabrowskii	−0.124d	0.431c

[1]Means followed by the same letter within a column are not significantly different at P<0.05 (Student-Newman-Keuls test).

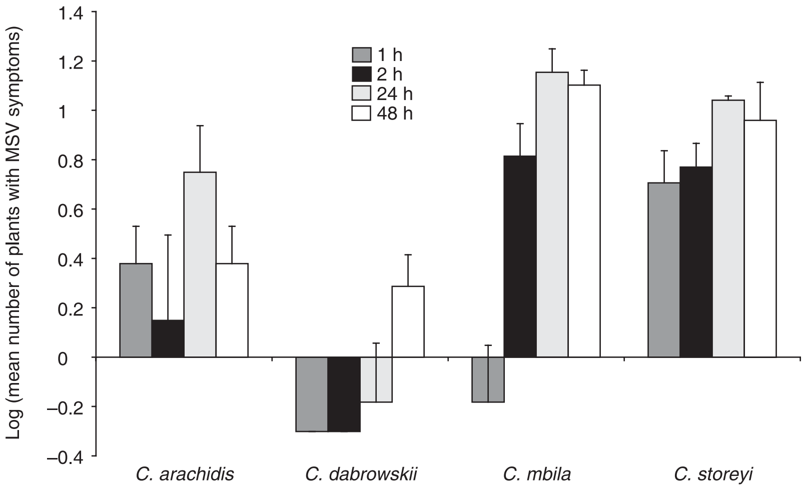

Figure 1 Mean number out of 20 test plants that showed *Mastrevirus* (MSV) symptoms when females of four *Cicadulina* species were given variable virus acquisition access periods (1, 2, 24, and 48 h) and a 48-h inoculation access period.

Our results also show that the vectors were better able to infect a susceptible host plant when confined on it for longer periods (Table 4). Short inoculation access feeding of 1 or 2 h gave significantly lower transmission of MSV compared to 24 h, while 48 h was also significantly higher than 24 h. For transmission of a virus by leafhoppers after they have acquired it from infected plants to be successful, the virus must pass through some parts of the alimentary tracts into the haemolymph, reach the salivary glands, and then be injected into healthy plants with the salivary secretions while the insects feed (Sinha, 1969). With longer feeding time, a viruliferous *Cicadulina* would achieve more success in inoculating a healthy host with MSV.

This study further shows significant differences in the transmission abilities of the vectors. The results for both AAPs and IAPs showed that *C. storeyi* was the most efficient vector (Table 5), whereas *C. storeyi* and *C. mbila* were more efficient in transmitting MSV than *C. arachidis* and *C. dabrowskii*. MSV-transmission efficiency studies carried out by Asanzi et al. (1995) showed *C. mbila*, *C. storeyi*, and *C. ghaurii* to be equally efficient while *C. arachidis* was reported as inefficient. This study confirms the latter in that *C. arachidis* is a poor vector of MSV. One possible explanation for the differences in transmission abilities of these vectors might be in their feeding/probing activities while on the host plants. This will be the subject of a separate article (S Oluwafemi, unpubl.).

The transmission efficiencies of the four vectors increased with longer feeding time (Figures 1 and 2). This is in agreement with a previous report by Asanzi et al. (1995) that transmission efficiency generally increased with longer duration of inoculation feeding and in some instances increased also with longer acquisition access feeding. The four vectors followed this rule with respect to IAP (Figure 2). More work will be done in the future on the two poor vectors (*C. arachidis* and *C. dabrowskii*) especially on *C. arachidis*

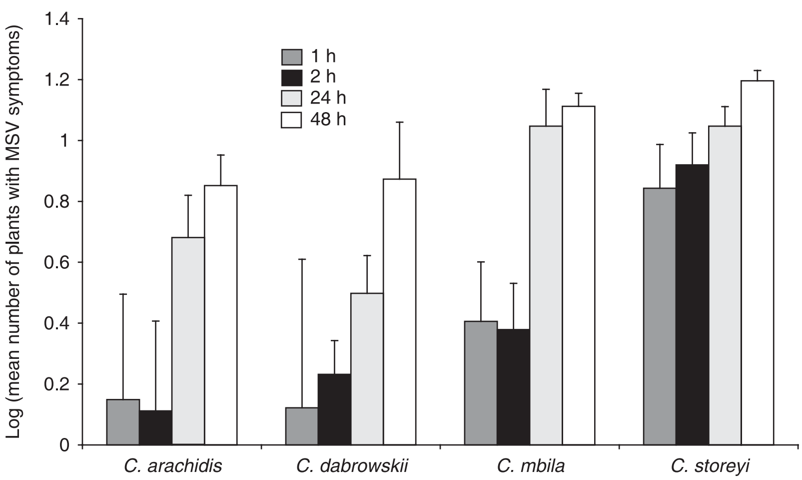

Figure 2 Mean number of plants that showed *Mastrevirus* (MSV) symptoms when females of four *Cicadulina* species were given variable virus inoculation access periods (1, 2, 24, and 48 h) and a 48-h acquisition access period.

because of the significant difference between the 24- and 48-h AAP (Figure 1).

Maize streak virus epidemics are likely to occur in years during which weather conditions allow vector survival and population build-up and in locations where maize-competent strains of the virus are present in grass hosts (Bosque-Perez & Buddenhagen, 1999). The present study underscores the importance of the vectors' species composition in the epidemiology of MSV. Field surveys show that although *C. mbila* is the most prevalent species, it was usually found in association with other species. In some locations, other species were more abundant than *C. mbila* (S Oluwafemi, unpubl.). A location with higher abundance of vectors with poor transmission abilities would be more suitable for maize cultivation than those where *C. storeyi* and *C. mbila* are prevalent. This probably explains previous findings that there is not necessarily a direct relationship between incidence of MSV disease in maize and density of leafhoppers (Reynaud, 1988).

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