African Crop Science Journal, Vol. 21, No. 2, pp. 161 - 172
Printed in Uganda. 
ISSN 1021-9730/2013 $4.00


# STYLET PENETRATION BEHAVIOURS OF FOUR *Cicadulina* LEAFHOPPERS ON HEALTHY AND MAIZE STREAK VIRUS INFECTED MAIZE SEEDLINGS

S. OLUWAFEMI and L.E.N. JACKAI[1]
Department of Crop Production, Soil and Environmental Management, Bowen University,
P.M.B. 284, Iwo, Nigeria
[1]GWC- Agricultural Experiment Station, College of Agricultural, Environmental and Natural Sciences,
Tuskegee University, Tuskegee, Alabama 36088, USA
**Corresponding author:** soluwafemi2000@yahoo.com

*(Received 20 November, 2012; accepted 11 April, 2013)*

## ABSTRACT

*Cicadulina* leafhoppers (Homoptera: Cicadellidae) are major pests of maize (*Zea mays* L. (Poacea) as they transmit maize streak virus (MSV), the most important virus of maize in Africa. The stylet penetration behaviours of four species (*C. arachidis*, *C. dabrowskii*, *C. mbila* and *C. storeyi*) were studied with an alternating current (AC) electrical penetration graph (EPG) monitor to understanding how feeding differs among the species that have different transmission efficiencies on healthy and streak-infected maize seedlings. The stylet penetration behaviours were significantly affected by the infection status of the host plants in six out of eight measured response variables. The vectors preferred feeding on healthy plants, to streak-infected plants as the insects spent more time on non-probing behaviours like resting or walking when on streak-infected hosts than on healthy plants. There were more pathway activities (salivation and searching for phloem cells) and frequency of probing was higher when feeding on streak-infected seedlings. This might indicate the times that the virus is picked up from infective tissues. Feeding from phloem cells, overall probing and probe mean (the average time per probe) were higher on healthy than streak-infected seedlings. Preference to feed on healthy seedlings will encourage spread of MSV disease. The four vectors differed significantly in five out of eight stylet penetration behaviours studied. *Cicadulina mbila*, an efficient vector, spent significantly more time than others in non-probing activities, least time feeding from phloem and overall probing. This behaviour will enhance its spread of MSV disease. Time spent on feeding in mesophyll and penetrating phloem (X-wave) was also significantly different among the four vectors. The efficiencies of *C. mbila* and *C. storeyi* in transmitting MSV, as compared to the other two species, may also be linked to longer pathway activities and shorter probe mean although the effects were not statistically significant. Time spent on pathway activities followed expected ranking of the vectors' transmission efficiencies. Longer time for active feeding, searching for phloem cells and salivation would encourage efficient acquisition and inoculation of the virus.

*Key Words*: *Cicadulina* spp., electrical penetration graph, MSV, *Zea mays*

## RÉSUMÉ

La *Cicadelle* (Homoptera: Cicadellidae) est une importante peste du maïs (*Zea mays* L. (Poacea) transmitant à la plante le virus de la striure (MSV) réputé le plus important du maïs en Afrique. Le comportement de la pénétration du stylet de quatre espèces (*C. arachidis*, *C. dabrowskii*, *C. mbila* et *C. storeyi*) était étudié avec un moniteur de courant alternant (AC) de graphique de pénétration (EPG) afin de comprendre comment l'alimentation diffère parmi les espèces d'efficacité de transmission différente sur la santé et les plants infectés de maïs. Les comportements de la pénétration du stylet étaient significativement affectés par le degré d'infection des plants hôtes en terme de réponse dans six variables mesurées sur huit. Les vecteurs ont préférés se nourir sur les plantes en bonne santé que sur celles affectées par le virus. Il y avait plus d'activités (salivation et recherche de cellules du phloème) et

la fréquence de sondage était plus élevée lorsque le vecteur se nourrissait sur les plants infectés. Ceci pourrait indiquer les moments pendant lesquels le virus est trouvé dans les tissus infectés. La nutrition sur les cellules de phloème, en terme de moyenne de temps per sonde était en tout plus élevée sur les plants en bonne santé que sur celle infectée par la striure. La préférence de se nourir sur les plants sains pourra encourager la propagation de la maladie du MSV. Les quatre vecteurs différaient significativement des cinq sur huit du point de vue du comportement de pénétration du stylet étudié. La *Cicadulina mbila*, un vecteur efficace, a passé significativement plus de temps que les autres activités sondées et moins de temps d'alimentation sur le phloème. Ce comportement pourra promouvoir la propagation de la maladie du MSV. Le temps passé en se nourissant dans le mésophylle et la pénétration dans le phloème était aussi sognificativement different parmi les quatre vecteurs. L'efficacité de *C. mbila* et *C. storeyi* dans la transmission du MSV, en comparaison aux deux autres espèces, pourrait être aussi associée aux activités et le temps moyen de sondage bien que les effets n'étaient pas statistiquement significatifs. Le temps du déroulement des activités était en relation avec le ranking d'efficacité de transmission des vecteurs. Le temps le plus long d'alimentation active, l'exploration des cellules du phloème et la salivation pourraient promouvoir l'acquisition efficiente et l'inoculation du virus.

*Mots Clés*: *Cicadulina* spp., graphique de pénétration électrique, MSV, *Zea mays*

## INTRODUCTION

*Cicadulina* (Homoptera: Cicadellidae) leafhoppers are widely distributed in Africa, Indian Ocean islands and parts of Asia (Rose, 1978). Some *Cicadulina* species have been identified as vectors of Maize streak virus (MSV, genus *Mastrevirus*), which is the most important virus of maize (Thottappilly *et al*., 1993) and one of the most important factors reducing maize yields in sub-Saharan Africa (Bosque-Perez, 2000). MSV is transmitted in nature only by *Cicadulina* leafhoppers, in a persistent manner (Oluwafemi *et al*., 2007). The virus is acquired from the plant while feeding, passes through the gut wall into the haemolymph and is inoculated into the plant *via* the salivary glands during subsequent feeding. Virus acquisition feeding can be very rapid if the vectors ingest from mesophyll or phloem tissues in chlorotic areas of infected leaves.

There are 22 species of *Cicadulina*, 18 of which occur in Africa (Webb, 1987). Of these, ten have been confirmed to be vectors of MSV with varying transmission efficiencies: *C. arachidis* China, *C. bipunctata (=bipunctella*), *C. ghaurii* Dabrowski, *C. latens* Fennah, *C. mbila* Naude, *C. niger* China, *C. parazeae* Ghauri, *C. similis* China, *C. storeyi* China *(=triangula*) and *C. dabrowskii* Webb (Oluwafemi *et al*., 2007).

In 1964, D. L. McLean and M. G. Kinsey published an article in *Nature* that described their development of an electronic instrument that could record the feeding of pea aphid, *Acrythosiphon pisum* (Harris), on its host plant, *Vicia faba* L. This electronic monitoring system (EMS) (now termed AC electrical penetration graph {EPG}) became popular to study the stylet penetration (probing) behaviour of piercing and sucking insects in-situ, providing a clearer understanding of such feeding behaviours than any other technique (Backus, 1994; Walker, 2000).

The purpose of this study was to understand the stylet penetration behaviours of four species of *Cicadulina* leafhoppers on healthy or MSV diseased maize seedlings using AC- EPG, for better understanding of the effects of feeding behaviour on MSV transmission efficiency.

## MATERIALS AND METHODS

**Insects and plant materials.** The insects used in this study included *C. arachidis, C. dabrowskii, C. mbila* and *C. storeyi*. These four species have different transmission efficiencies, with *C. mbila* and *C. storeyi* being efficient vectors while the other two are inefficient (Oluwafemi *et al*., 2007). *Cicadulina arachidis* China, *C. dabrowskii* Webb, and *C. mbila* (Naude) were collected from different parts of Nigeria between 1997 and 1999 (Oluwafemi and Alegbejo, 2011) and were reared in screen houses at International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria where this study was carried out. Live adult leafhoppers were collected from the fields with aspirators (mouth-operated) and a sampling cage (Oluwafemi *et al*., 2007). The sampling cage consisted of four separate metal rods (1.5 m high)

that were fixed at the corners of a selected 1 $m^2$ sampling site (young maize plants or grasses around maize fields). The rods were covered by a tent of black cloth which had yellow netting on one side.

The cage was set up quickly so that all the insects at the site would be trapped (Dabrowski, 1983). Leafhoppers and other insects within the plot being sampled were attracted to the mesh side of the cage by sunlight. *Cicadulina* species were then selectively collected with an aspirator from the mesh. *Cicadulina storeyi* China that has been maintained for many years at IITA for purposes of MSV screening provided a fourth species. This old colony was usually renewed with addition of fresh field collections during the raining seasons.

The leafhoppers (except *C. dabrowskii*) were reared on seedlings of millet (*Pennisetum americanum* {Linn.} K. Schum). The seed was bought as a local variety from Ibadan and sown in 20 cm diameter plastic pots. Top soil from IITA soil-bin was used without addition of fertilisers. *Cicadulina dabrowskii* was reared on seedlings of *Setaria barbata* (Lam.) Kunth, on which it was usually collected. Seedlings of *S. barbata* were collected from around maize fields as a weed. The various *Cicadulina* populations were reared in wooden mesh cages (40 cm x 40 cm x 90 cm) inside screen houses.

The screen houses had no light or atmospheric control. Two weeks-old seedlings of open-pollinated, MSV-susceptible maize, variety Pool 16 (seeds were obtained from IITA Maize Programme) served as test plants. These were sown in 20 cm diameter plastic pots inside screen cages. The MSV diseased seedlings were produced by caging viruliferous adult *C. storeyi* (having fed on MSV diseased seedlings for 72 hr) from the IITA colony on the maize seedlings (10-12 days after sowing) for 48 hr. These seedlings were later kept separately in another screen house until symptoms of MSV disease were fully developed. Stylet probing activities were conducted on healthy (green) leaves or those showing fully developed chlorotic streak symptoms of MSV.

**EPG recording.** The AC system used for monitoring the feeding behaviour of the leafhoppers was earlier used to study the feeding behaviour of *C. storeyi* on maize (Mesfin and Bosque-Perez, 1998) and *Aphis craccivora* (Koch) on cowpea cultivars with different levels of aphid resistance (Mesfin *et al*., 1992). The equipment was constructed at the John Innes Institute (UK) using the design based on integrated circuits (Kinsey and McLean, 1987).

The leafhoppers were aspirated from the leaves of the host plant and starved in the aspirator for 1 hr before being tethered to the monitor. An insect was anaesthetised with carbon dioxide for about 10 seconds or placed on an ice-block. A gold wire 5 cm long and 25 µm diameter (M. Goodfellow, Cambridge Science Park, Cambridge, UK), was attached to the dorsal region of the prothorax, using silver conducting paint (Agar Scientific Ltd., Essex, UK).

The wired leafhopper was connected to the output terminal of the amplifier within a few minutes after recovery from anaesthesia. The circuit to the leaf was completed by means of a wire and alligator clip clamped to the stem of the maize plant at the base of the leaf on which the insect was placed. To avoid damage to the plant, several layers of silver foil were placed between the clip and the stem surface. Input to the insect was set at a frequency of 100 Hz and the substrate voltage was set at about 500 mV to give an appropriate response on the chart recorder. A potentio-metric strip chart recorder (Graphtec WR 7500R) for recording resistance changes was connected to the system and operated at a chart speed of 2 cm $min.^{-1}$ and at a sensitivity of 500 mV. Probing activities of the leafhopper caused voltage fluctuations in EPG outputs, which are represented as waveforms uniquely associated with probing in specific plant tissues. These waveforms were recorded with the strip chart recorder and were used to determine the occurrence of different activities and in computing the time spent for each activity. The EMS was set up on a table inside a secluded office and the experiment was run at room temperature ($22 \pm 2^{o}$ C). A single adult female insect of each species was used for each host type in a given occasion so that the 10 replicates of each combination formed sets over time. This blocking (by time) of the sets of treatment

combinations was accounted for in subsequent statistical analysis.

A "probe" is defined here as the duration from the insect first placing a drop of saliva on the plant surface, followed immediately by the stylet penetrating the tissue and continuing until the stylet was withdrawn. "Probing activity" or "Probing behaviour" constitutes several such probes with varying periods of "non-probing activities or "baseline (B)" (such as walking, resting) between. Terminology for probing waveforms is adjusted slightly here, compared with previous papers on *C. mbila* probing to conform to new standardisation of terms for EPG waveforms (Backus *et al*., 2005). Pathway Phase (previously termed "Salivation") represents the secretion of sheath and watery saliva from the stylets, stylet movement through plant cell as well as presumed tasting and other stylet activities enroute to an ingestion cell. A particularly prominent waveform within Pathway Phase which consisted of several rapid, high amplitude peaks at the beginning of each probe was defined as initial salivation.

In the new terminology, Pathway Phase also includes the very important X waveform, which represents penetration of phloem sieve elements and accompanying sap sampling/tasting behaviours (Mesfin *et al*., 1995). However, for purposes of statistical analysis to determine the time until reaching phloem, we will use the term pathway herein to denote all early stylet penetration behaviours (such as initial salivation), except X waveform. Instead, the X waveform durations and frequencies will be measured and analysed separately.

Ingestion phase consists of two waveforms previously correlated with salivary sheaths. Phloem ingestion (Ip) (occurring presumably in sieve elements; Mesfin *et al*., 1995) is also preceded by X waveform. Thus, the X wave is a landmark for phloem ingestion. Non-sieve element ingestion (Im) is not preceded by X waveform, and was correlated with salivary sheaths in mesophyll (Mesfin *et al*., 1995).

**Data collection and analysis.** Measured response variables of leafhopper probing behaviour evaluated on maize were (i) total number of probes, (ii) waveform duration per probe, (iii) waveform duration per baseline, non-probing event and (iv) total time spent in all probing activities (probing duration per probe). The insect-plant interactions of individual female adult *Cicadulina* species were recorded for 3 hr on the second leaf of healthy maize seedlings and youngest fully expanded leaf with MSV symptoms of streak-infected (with severe chlorotic symptoms) maize (var. Pool 16) seedlings.

There were 10 replicate insects for each combination of species (*C. arachidis, C. dabrowskii*, *C. mbila* and *C. storeyi*) and host (healthy, MSV-infected). The 10 replicates for each treatment combination were set up as 10 blocks over time. There were 80 observations in all. The mean number of activities and time spent in each activity were determined. Duration of each activity was calculated by measuring the length of the strip-chart graph containing the waveforms corresponding to each activity. The length was divided by two, as the chart moved at a speed of 2 cm $min^{-1}$.

Data were analysed using two-way analysis of variance (ANOVA) to consider the main effects and interaction between the four *Cicadulina* species; and the two types of host-plant status (healthy or streak-infected). The Statistical Analysis System (SAS) software version 6.12 (SAS, 1999) was used for analysis. Least Significant Differences (LSD) at the P= 0.05 were used to separate pairs of means of particular interest. No transformation of data were required to satisfy the assumptions of ANOVA.

## RESULTS

Typical results of the waveforms associated with salivation, ingestion and non-probing activities produced by the four *Cicadulina* spp. on maize seedlings are presented in Figure 1. There was no interaction between host and species ($P > 0.104$). However, there was a main effect of host, species and of both factors independently for the eight response variables (Tables 1 and 2).

The means of all probing behaviours on the host plant status are presented in Table 1. The probing behaviours of the leafhoppers were significantly ($P< 0.014$) affected by the infection status of the host plants (Table 1) in six out of

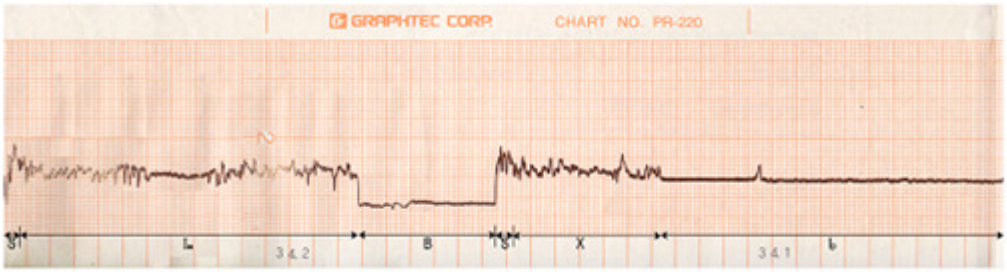


Figure 1. Typical waveforms produced by *Cicadulina mbila* while feeding on maize seedlings var. Pool 16 at the second leaf stage. S- salivation, X- sieve element penetration, Im- ingestion from mesophyll (non- phloem) tissue, Ip- Ingestion from phloem tissue, B- Non- probing.

TABLE 1. Means, Standard Error of the Difference (SED) between means, degrees of freedom (df) and Least Significant Difference (LSD) and probability (Pr> F) values on host plant status. All values are time (min) except for frequency of probing data

| Feeding behaviour[á] | Host plant status | | SED (63 df) | LSD (5%) | Pr> F |
|---|---|---|---|---|---|
| | Healthy | Streak | | | |
| Non-probing | 18.74 | 31.66 | 5.392 | 10.774 | 0.012 |
| Pathway | 5.48 | 12.12 | 1.964 | 3.925 | 0.001 |
| Mesophyll | 23.22 | 23.60 | - | - | 0.949 |
| Xwave | 54.45 | 51.93 | - | - | 0.842 |
| Phloem | 128.29 | 106.50 | 8.613 | 17.209 | 0.014 |
| Overall Probing | 161.23 | 148.34 | 5.363 | 10.716 | 0.017 |
| Frequency of probing | 4.52 | 11.99 | 1.634 | 3.265 | <0.001 |
| Probe mean | 48.56 | 16.25 | 9.245 | 18.471 | 0.001 |

[á] All measures are in minutes except for frequency of probing; - Main effect not significant

TABLE 2. Means, Standard Error of the Difference (SED) between means, degrees of freedom (df) and Least Significant Difference (LSD ) and probability (Pr> F) values on four *Cicadulina* species. All values are time (min) except for frequency of probing data

| Feeding behaviour[á] | *Cicadulina* species | | | | SED (63 df) | LSD (5%) | Pr> F |
|---|---|---|---|---|---|---|---|
| | *C. arachidis* | *C. dabrowskii* | *C. mbila* | *C. storeyi* | | | |
| Non-probing | 11.19 | 23.83 | 42.76 | 23.04 | 7.626 | 15.040 | 0.001 |
| Path way | 8.21 | 5.45 | 8.69 | 12.40 | - | - | 0.105 |
| Mesophyll | 31.58 | 11.73 | 26.18 | 15.83 | 8.192 | 16.368 | 0.005 |
| Xwave | 8.09 | 2.95 | 3.90 | 6.34 | 1.790 | 3.577 | 0.023 |
| Phloem | 114.34 | 136.06 | 96.39 | 122.79 | 12.181 | 24.337 | 0.016 |
| Overall probing | 168.82 | 156.17 | 137.25 | 156.97 | 7.585 | 15.154 | 0.001 |
| Frequency of probing | 8.24 | 7.02 | 9.06 | 8.20 | - | - | 0.930 |
| Probe mean | 37.53 | 47.74 | 17.53 | 26.28 | - | - | 0.117 |

[á] All measures are in minutes except for frequency of probing; - Main effect not significant

eight stylet penetration behaviours studied. The insects spent more time (31.66 min *vs.* 18.74 min) on non-probing behaviours while on streak-infected host than on healthy plants (P= 0.012). More time was spent (12.12 min vs. 5.48 min, P= 0.012) in pathway activities and frequency of probing was higher (11.99 vs. 4.52, P< 0.001) when feeding on streak-infected seedlings. However, time spent during feeding from phloem cells (128.29 min vs. 106.50 min, P= 0.014), average time per probe (probe mean) (48.56 min vs. 16.25 min, P=0.001) and time spent for probing (overall probing) (161.23 min vs. 148.34 min, P= 0.017) were higher on healthy maize seedlings than streak-infected maize seedlings.

The means of all probing behaviours of the individual *Cicadulina* species are presented in Table 2. The four vectors differed significantly (P<0.023) in five out of eight stylet penetration behaviours studied. *Cicadulina mbila* spent significantly (P= 0.001) more time in non-feeding activities (e.g. resting, walking) than all other three species. *Cicadulina dabrowskii* spent very short time, which was significantly different (P= 0.005) from the other three species, feeding from mesophyll cells. Time spent by the vectors to penetrate phloem cells before ingestion from phloem (X-waveform) was also significantly different (P=0.023) among species. *Cicadulina arachidis* spent longest time; while *C. dabrowskii* spent the shortest. There was also significant variation among the vectors in the time they spent ingesting from phloem sieve elements (P=0.016) and overall probing (P=0.001). *Cicadulina dabrowskii* spent the longest time ingesting from phloem, while *C. mbila* spent the shortest time. *Cicadulina arachidis* had the longest overall probing duration per insect followed by *C. dabrowskii* and *C. storeyi*. *Cicadulina mbila* spent the shortest time which was significantly different (P<0.05) from the other three.

The effects of host-status on the individual *Cicadulina* species are illustrated in Figures 2 to 6. The frequencies of probes made by all four species were higher on streak-infected plants than on healthy maize plants (Fig. 2). Figures 3 and 4 demonstrate the wide variation in the stylet penetration behaviours of *C. arachidis* and *C. dabrowskii* when feeding on healthy or diseased maize seedlings, compared to marginal changes for *C. mbila* and *C. storeyi* (Figs. 5 and 6). *Cicadulina arachidis* and *C. dabrowskii* probed more on diseased maize plants, but the probing frequencies of the other two species on the different hosts were not as different.

## DISCUSSION

This study shows that the infection status of the maize plants on which *Cicadulina* leafhoppers fed significantly affected their feeding behaviours. Physiological changes in MSV-infected maize plants may alter plant amino acid

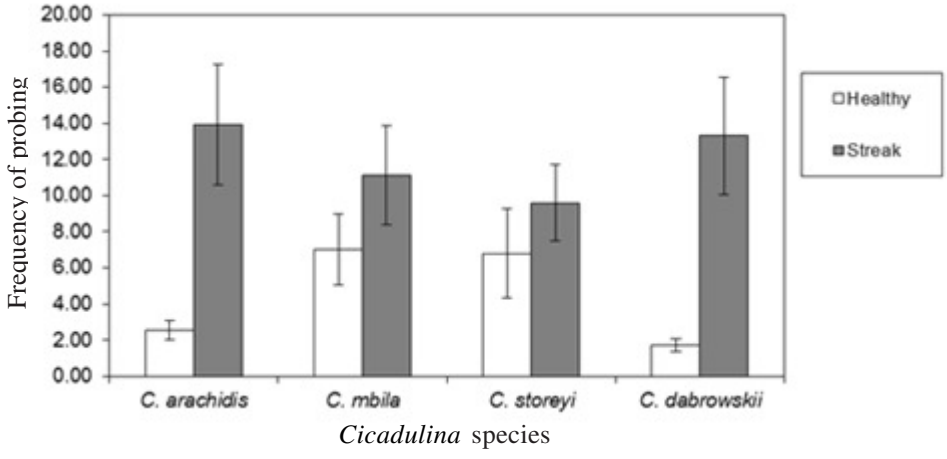


Figure 2. Frequency of probes made by four *Cicadulina* species while feeding on healthy and streak-infected maize seedlings within a 3 hr feeding access period. Error bars represent ±SE.

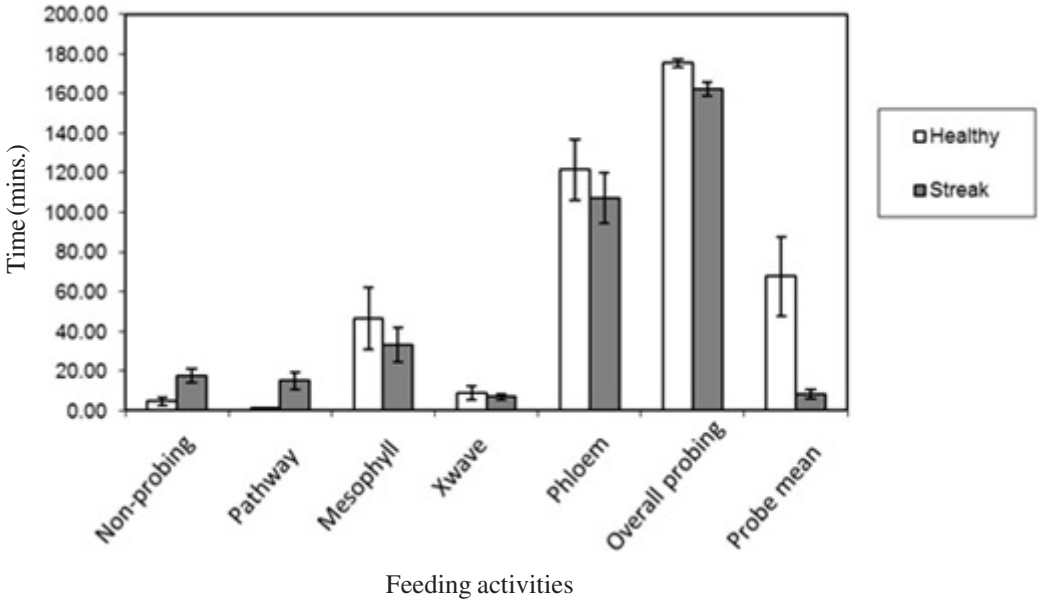


Figure 3. Mean waveform and probing durations per insect and per probe for *C. arachidis*, within a 3 hr feeding access period on healthy and streak-infected maize seedlings. Error bars represent ±SE.

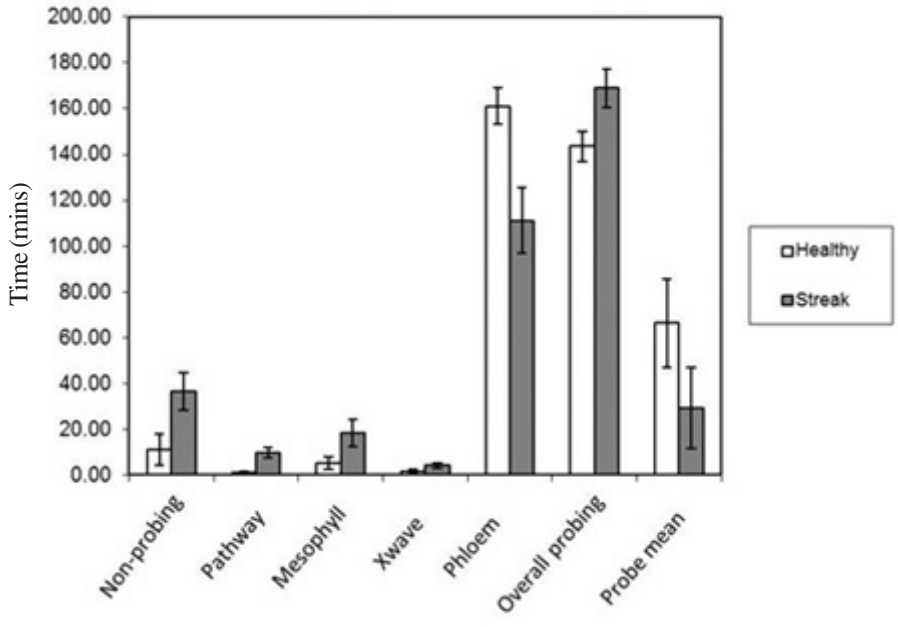


Figure 4. Mean waveform and probing durations per insect and per probe for *C. dabrowskii*, within a 3 hr feeding access period on healthy and streak-infected maize seedlings. Error bars represent ±SE.

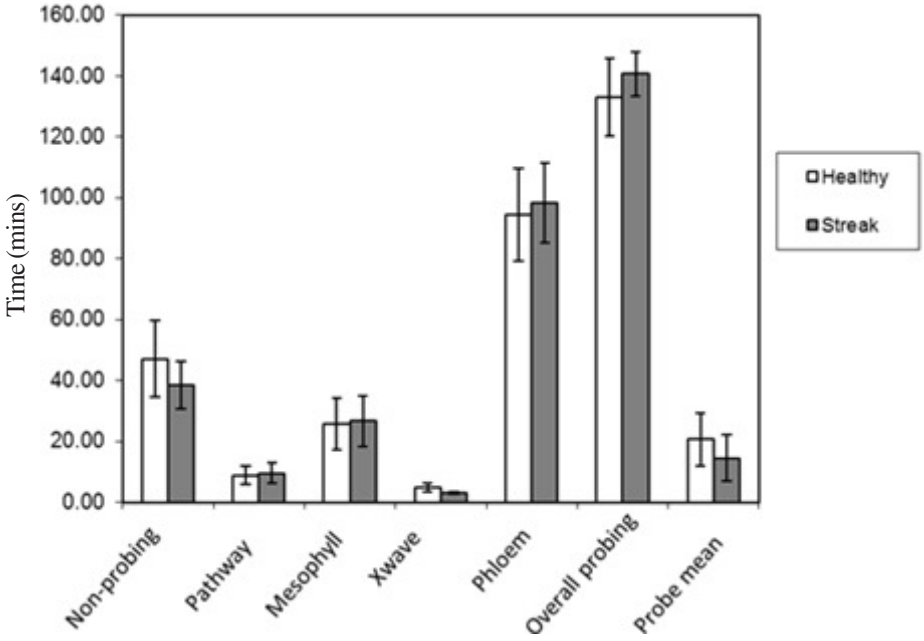


Figure 5. Mean waveform and probing durations per insect and per probe for *C. mbila*, within a 3 hr feeding access period on healthy and streak-infected maize seedlings. Error bars represent ±SE.

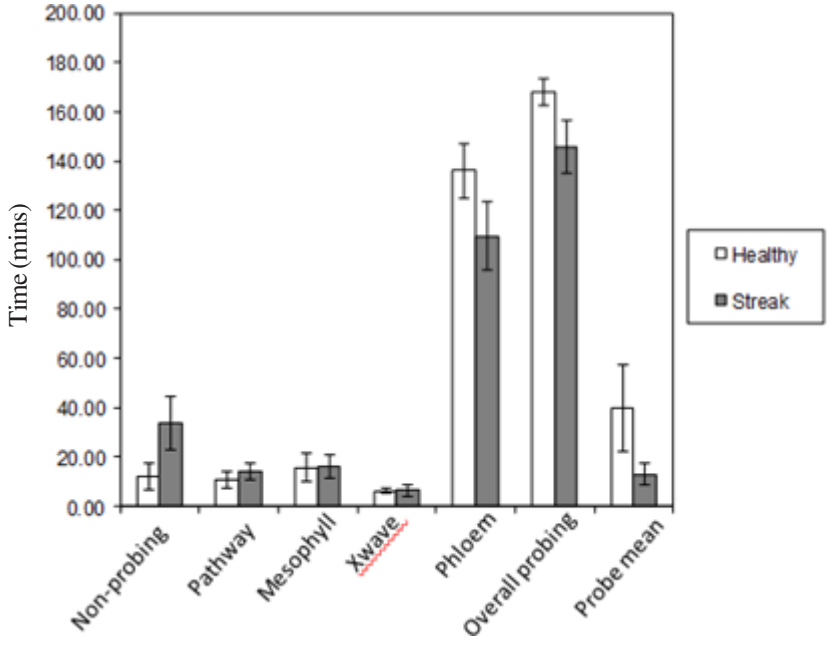


Figure 6. Mean waveform and probing durations per insect and per probe for *C. storeyi*, within a 3 hr feeding access period on healthy and streak-infected maize seedlings. Error bars represent ±SE.

composition and this in turn may induce the insects to change their feeding behaviours on the plants. The insects spent more time on non-probing activities, like resting and walking when on infected host plants. Since MSV is transmitted in a persistent manner, once disease has fully developed in a maize seedling, the viruliferous leafhopper would prefer feeding on healthy seedlings and, thereby spread the disease. Time spent on pathway activities was two times higher on streak-infected than on healthy host plants.

The insects also probed twice as frequently when on streak-infected hosts. This might indicate times the virus is picked up from infective tissues. The time spent feeding from phloem cells in infected maize seedlings was significantly shorter compared to healthy seedlings. This implies preference for green healthy tissues as compared with chlorotic, diseases ones. This report agrees with that of Mesfin and Bosque-Perez (1998), who showed that infection with MSV had effects on the feeding behaviours of *C. storeyi* as the percentage of time spent on phloem feeding was significantly shorter on virus-infected plants compared with healthy ones. Our study provides the information for the other three species of *Cicadulina*. Preference to feed on healthy seedlings will encourage spread of MSV disease because virus-carrying leafhoppers have sufficient time to inoculate ingested virus into healthy plants during feeding.

This study also shows that there are significant variations (five out of eight) in stylet penetration (probing) behaviours among four *Cicadulina* species that transmit MSV (*C. mbila, C. storeyi, C. dabrowskii* and *C. arachidis*) while feeding on maize seedlings. *Cicadulina mbila*, an efficient vector, spent significantly more time than others in non-probing activities, least time feeding from phloem and overall probing. This behaviour will enhance its spread of MSV disease as it inoculates more plant cells with the virus during the frequent sampling and tasting of plant cells' contents. Time spent feeding in mesophyll and penetrating phloem (X-wave) was also significantly different among the vectors. The efficiencies of *C. mbila* and *C. storeyi* in transmitting MSV may also be linked to pathway activities and probe mean; although the effects were not statistically significant (LSD 5% ). Time spent on pathway activities followed expected ranking of the vectors' transmission efficiencies. Longer time for active feeding, searching for phloem cells and salivation would encourage efficient acquisition and inoculation of the virus. The average time spent by the efficient vectors per probe (probe mean) was shorter than those by the inefficient vectors. This would encourage spread of disease as the efficient vectors are not engrossed with feeding on one spot.

Our results show that there are significant differences among the vectors in time spent to feed from phloem. *Cicadulina dabrowskii*, the most inefficient vector (Oluwafemi *et al.,* 2007), spent the longest time feeding from phloem sieve tubes. This might imply that spending more time ingesting from phloem sieve tubes does not promote transmission efficiency. Our results also show that the time spent probing (overall probing) by *C. arachidis*, was highest (168.82 min) and significantly more than that of *C. mbila* (an efficient vector) (137.25 min). This implies that *C. arachidis* spent more time feeding, not moving around or resting. The inefficient vectors (*C. arachidis* and *C. dabrowskii*) also had higher average time per probe. They seemed to feed more passively; while the efficient vectors were more active in stylet penetration behaviours. Lett *et al.* (2001) distinguished active ingestion in cells from passive ingestion in sieve tubes. Our study indicates that the inefficient vectors feed more passively from sieve tubes.

In this study, pathway includes salivation (both before and during location of phloem by the stylet). This study shows that salivation was not significant among the four vectors. However, *C. storeyi* and *C. mbila* had longer pathway durations than *C. arachidis* and *C. dabrowskii* indicating longer salivation and searching for phloem. This might enhance the abilities of *C. storeyi* and *C. mbila* to inoculate maize seedlings with MSV, as they spend longer times salivating and searching for phloem cells. It has been suggested that virus inoculation into maize by *Cicadulina* is associated with injection of saliva into phloem cells (Kimmins and Bosque-Perez, 1996). The pathway data actually followed the expected order of transmission efficiency: *C. storeyi* > *C. mbila* > *C. arachidis*> *C. dabrowskii* (Oluwafemi *et al*., 2007).

The "probe mean" data which shows that *C. storeyi* and *C. mbila* had shorter mean probe times as compared to *C. arachidis* and *C. dabrowskii* might imply that shorter mean probe times may lead to more efficient MSV transmission as the efficient vectors are not engrossed with feeding from one spot. Shorter time in stylet penetration and longer time in non-probing activities like walking around would encourage spread of disease. Pathway and probe mean data might suggest that *C. storeyi* and *C. mbila* are more efficient in transmitting MSV to healthy maize than *C. arachidis* and *C. dabrowskii* because they salivate / inoculate viruses into phloem for longer durations and have shorter mean probe times.

Lett *et al*. (2001) referred to previous reports (Storey, 1938; Harrison, 1985; Reynaud, 1988) and concluded that the feeding of *C. mbila* in non-vascular cells, although shorter than from sieve tubes, is long enough to allow efficient virus acquisition. The three hour period during which the stylet penetration behaviours of these vectors were studied is higher than published minimum acquisition access feeding (AAP) or minimum inoculation access feeding (IAP) periods for these vectors. Storey (1938) reported minimum AAP of 15 sec and minimum IAP of 5 min for *C. mbila*; Zagre (1983) reported minimum AAP of 30 sec and minimum IAP of 2 hr for *C. storeyi*; Asanzi (1991) reported minimum AAP of 15 min and minimum IAP of 1 hr for *C. arachidis*.

We thus conclude that higher transmission efficiencies of *C. mbila* and *C. storeyi* might be linked to longer pathway and shorter time per probe. Although there were significant differences in time taken to penetrate phloem cells (X-wave form), this did not follow a pattern that might be linked to virus transmission efficiency. This might imply that transmission efficiency is not linked to the time of penetrating phloem cells. According to Mesfin *et al.* (1992), slight differences in feeding behaviour (insect-plant interaction) are likely to explain differences in vector efficiency, especially among closely related vector species. This is because much depends on the localisation of the virus in the host plants and the cells where the vectors feed (Mesfin *et al*., 1992). Vector specificity of plant viruses depends on such factors as ability of the virus to multiply in an insect, ability to pass through the gut wall into the body cavity or ability to enter or survive in salivary glands (Bawden, 1964). Our study indicates the need to look at other factors involved in the transmission abilities of leafhoppers, especially the hypothesis that transmission of MSV by *C. mbila* is a genetic trait (Storey, 1932). It has been demonstrated that transmission of MSV by *C. storeyi* fitted basic Mendellian genetics (Oluwafemi, 2006). Future research will attempt to use molecular markers to compare these vectors of MSV.

## ACKNOWLEDGEMENT

We thank Dr. P.G. Markham of John Innes Centre, Norwich Research Park, UK, for useful guide about handling the feeding monitor and the interpretation of the waveform patterns. Dr. E. A. Backus, USDA Agricultural Research Service of Parlier, California, USA, reviewed the earlier manuscript. The contributions of Stephen Powers, Biomathematics and Bioinformatics Department, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK, in the analysis of this revised version is gratefully acknowledged. This research was financed by IITA as part of staff development programme of the principal author for his PhD thesis.

## REFERENCES

Asanzi, M.C. 1991. Studies of epidemiology of maize streak virus and its *Cicadulina* leafhopper vectors in Nigeria. PhD Thesis, The Ohio State University, Columbus, OH, USA.

Backus, E.A. 1994. History, development, and applications of the AC electronic monitoring system for insect feeding. pp. 1-51. In: History, development, and application of AC Electronic insect feeding monitors. Ellsbury, M.M., Backus, E.A. and Ullman, D.L.). Proceedings Entomological Society of America, Thomas Say Publications in Entomology, Maryland, USA.

Backus, E.A., Habibi, J., Yan, F. and Ellersieck, M. R. 2005. Stylet penetration by adult *Homalodisca coagulata* on grape: Electrical Penetration Graph waveform characterization,

tissue correlation, and possible implications for transmission of *Xylella fastidiosa*. *Annals of the Entomological Society of America* 98 (6): 787-813.

Bawden, F.C. 1964. Transmission by animals while feeding. pp. 110-147. In: Plant viruses and virus diseases, (4th Edition). Bawden, F.C. (Ed.). Ronald Press Company, New York, USA.

Bosque-Perez, N. A. 2000. Eight decades of maize streak virus research. *Virus Research* 71: 107-121.

Dabrowski, Z.T. 1983. Identifying and collecting *Cicadulina* for maize streak resistance screening. *IITA Research Briefs* 4 (4): 2-3.

Harrisson, B.D. 1985. Advances in geminivirus research. Annual Review of *Phytopathology* 23: 55-82.

Kimmins, F.M. and Bosque-Perez, N.A. 1996. Electrical penetration graphs from *Cicadulina* spp. and the inoculation of a persistent virus into maize. *Entomologia Experimentalis et. Applicata* 80: 46-49.

Kinsey, B.R. and McLean, D.L. 1987. Versatile electronic measurement system for studying probing and feeding behaviour of piercing and sucking insects. *Annals of the Entomological Society of America* 80: 118-129.

Lett, J.M,, Granier, M., Grondin, M., Turpin, P., Molinaro, F. 2001. Electrical penetration graphs from *Cicadulina mbila* on maize, the fine structure of its stylet pathways and consequences for virus transmission efficiency. *Entomologia Experimentalis et Applicata* 101: 93-109.

McLean, D.L. and Kinsey, M.G. 1964. A technique for electronically recording aphid feeding and salivation. *Nature* 202: 1358-1359.

Mesfin, T. and Bosque-Perez, N. A. 1998. Feeding behaviour of *Cicadulina storeyi* China (Homoptera: Cicadellidae) on maize varieties susceptible or resistant to maize streak virus. *African Entomology* 6: 185-191.

Mesfin, T., Den Hollander, J. and Markham, P.G. 1995. Feeding activities of *Cicadulina mbila* (Hemiptera: Cicadellidae) on different host-plants. *Bulletin of Entomological Research* 85: 387-396.

Mesfin, T., Thottappilly, G. and Singh, S.R. 1992. Feeding activities of *Aphis craccivora* (Koch) on cowpea cultivars with different levels of aphid resistance. *Annals of Applied Biology* 121: 493-501.

Oluwafemi, S. 2006. Genetic variation among active and inactive transmitters of maize streak virus within a population of *Cicadulina storeyi* China (Homoptera: Cicadellidae). *African Journal of Biotechnology* 5(8): 590-596.

Oluwafemi, S., Jackai, L. E.N. and Alegbejo, M. D. 2007. Comparison of transmission abilities of four *Cicadulina* species vectors of Maize streak virus from Nigeria. *Entomologia Experimentalis et. Applicata* 124:235-239.

Oluwafemi, S. and Alebgejo, M..D. 2011. Diversity, abundance and host range of *Cicadulina* species (Homoptera: Cicadellidae) vectors of *Maize streak virus* in Nigeria. *Moor Journal of Agriculture* 12 (1): 11-21.

Reynaud, B. 1988. Transmission des virus de la striure, du stripe et de la mosaique du mais par leurs vecteurs, *Cicadulina mbila* (Naude, 1924) et *Peregrinus maidis* (AShmead, 1980) (Homoptera). Approches biologique, genetique et epidemiologique de la relation vecteur-virus-plante. PhD Thesis, Universte des Sciences et Techniques du Langeedoc, Montpellier, France.

Rose, D.J.W. 1978. Epidemiology of maize streak disease. *Annual Review of Entomology* 23: 259-282.

SAS. 1999. Statistical Analysis System, version 8.2. SAS Institute Inc, Cary, NC, USA.

Storey, H.H. 1932. The inheritance by an insect vector of the ability to transmit a plant virus. *Proceedings of the Royal Society of London (Series B)* 112: 46-60.

Storey, H. H. 1938. Investigation of the mechanism of the transmission of plant viruses by insect vectors II. The part played by puncture in transmission. *Proceedings of the Royal Society of London (Series B)* 125: 455-477.

Thottappilly, G., Bosque-Perez, N.A. and Rossel H.W. 1993. Viruses and virus diseases of maize in tropical Africa. *Plant Pathology* 42: 494-509.

Walker, G.P. 2000. Beginner's guide to electronic monitoring. Principles and applications of electronic monitoring and other techniques in the study of Homopteran feeding behaviour. Walker, G.P. and Backus, E.A. (Eds.), pp. 14-40. Entomological Society of America, Lanham.

Webb, M.D. 1987. Species recognition of *Cicadulina* leafhoppers (Hemiptera: Cicadellidae): Vectors of pathogens of Graminae. *Bulletin of Entomological Research* 77: 683-712.

Zagre, M.B. 1983. Studies on maize streak virus and its transmission characteristics by the leafhopper vector *Cicadulina triangula*. Master's thesis, Universite Nationale du Benin, Cotonu, Benin.