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MICROBIOLOGY, PARASITOLOGY & VIROLOGY | RESEARCH ARTICLE

Whitefly species efficiency in transmitting cassava mosaic and brown streak virus diseases

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Abstract: Whiteflies are vectors of plant viral diseases. The rate of disease transmission by whiteflies on cassava continue to present a complex of efficiency in relation to species diversity. An experiment was carried out to access the period required for viruliferous whitefly to feed on cassava plant for it to transmit both cassava mosaic and brown streak diseases (CMD and CBSD) as well as the number of whiteflies required for transmission of the pathogens. The whitefly species used in study were *Bemisia tabaci*, *Trialeuroides vaporariorum* and *Aleurodicus dispersus* in plant cages. The result showed that a minimum period of 6 h was required for whitefly to feed and transmit the viral diseases. Only *B. tabaci* species was capable of transmitting CMD, whereas for CBSD all the species under experimentation transmitted the disease. Higher density of whitefly led to higher transmission of diseases. The findings here highlight the complex transmission rate of these two diseases of cassava by different whitefly species.

Subjects: Agriculture & Environmental Sciences; Botany; Plant & Animal Ecology; Zoology; Entomology

Keywords: whitefly species; cassava brown streak, cassava mosaic; transmission rate

1. Introduction

The whitefly insect has associations with almost 600 different species of plant which comprise a large number of both cultivated and non-cultivated as well as both annual and perennials crops (Bedford, Briddon, Markham, Brown, & Rosell, 1992; Brown, Frohlich, & Rosell, 1995; Martin, Mifsud, &

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Mr Moffat K. Njoroge works for Ministry of Agriculture and Livestock in Kitui County. He is currently training on Master of Science in Crop Protection at the University of Nairobi. He has carried out a greenhouse study on rate of viral disease transmission by different whitefly species at KALRO Katumani Research Centre where Dr Daniel Mutisya supervised his research activity. At present is the contact extension person on Crops in Kitui County. The other two co-authors Dr D. Miano and Dr C. Kilalo are his Msc supervisors of his thesis proposal from University of Nairobi.

PUBLIC INTEREST STATEMENT

Whiteflies are not true flies, but are small and white looking insects on most vegetative plants. The whitefly feeds on the plant leaf tissue by sucking cell sap. Besides being minor pests, these insects are major disease vectors on most cultivated crops. In the present work, three whitefly species were evaluated in enclosed cages to find out which one spreads most two major viral diseases namely cassava mosaic and brown streak. The cassava brown streak disease causes severe root rot and huge loss of yield once it fully establishes in a cassava field. On the other hand the cassava mosaic disease causes relatively lower yield loss in comparison to the former depending on field incidence and vector presence. In the present study results, the common whitefly species, *Bemisia tabaci* was found to spread the two diseases on cassava.

Rapisarda, 2000; Naranjo, Cañas, & Ellsworth, 2009). Whiteflies are major vectors of viral diseases, such as cassava mosaic disease (CMD) and cassava brown streak disease which can reduce yield by up to 40% and at times up to 100% (Legg & Fauquet, 2004). *Bemisia tabaci* (Genn.) is one of the major vectors of cassava mosaic *begomoviruses* (CMBs) and cassava brown streak viruses (CBSVs) which are causative agents of CMD and cassava brown streak diseases (CBSD), respectively (Legg et al., 2011; Maruthi et al., 2005). Studies on *B. tabaci* reveal convincing evidence of at least 35 cryptic species with extensive genetic diversity and which show diverse behaviour concerning host plant preference, oviposition, ecological adaptation as well as virus dissemination (Ahmed, De Barro, Ren, Greeff, & Qiu, 2013; De Barro, Trueman, & Frohlich, 2005). Even in the same genetic group such as *B. tabaci*, different subclades can differ in important aspects of biology such as virus transmission, fecundity and mating ability (Habibu et al., 2012). The mode of transmission of plant viruses can either be classified as persistent, semi persistent or non-persistent depending on time required for the vector to acquire the ability to transmit virus and length of time a specific vector retains the ability. For whiteflies, *ipomovirus* are transmitted in non-persistent mode (Hollings, Stone, & Bock, 1976) whereas *criniviruses*, *carlaviruses* and *closteroviruses* are transmitted in semi-persistent mode and *begomoviruses* in a persistent mode (Duffus, Larsen, & Liu, 1986; Goodman & Bird, 1978; Horn et al., 2011). There is also evidence of transovarial passage of *begomoviruses* to progeny and lateral transmission among the adult whiteflies in sex-related manner (Ghanim & Czosnek, 2000; Ghanim, Morin, Zeidan, & Czosnek, 1998).

The altitude above sea level and abundance of whiteflies on cassava plant has no correlation to incidences of CBSD and CMD (Njoroge, Kilalo, Miano, & Mutisya, 2016). Hence, the need for further studies on species involvement in transmission of the two diseases on cassava. The objective of the present study was to elucidate virus transmission rate by three greenhouse mass reared species, *B. tabaci*, *Trialeuroides vaporariorum* and *A. dispersus* in staggered feeding for 2, 6, 12 and 24 h on virus-free tissue culture plants.

2. Materials and methods

2.1. Whitefly colony establishment

Tomatoes *Lycopersicon esculentum* Mill, pumpkin (*Cucurbita maxima* (L.) and *Tephrosia purpurea* (L.) are reported hosts of whiteflies (Saraf, Al-Musa, & Batta, 1985; Mutisya and Miano, unpublished). These were used for mass rearing purposes of the whitefly species at KALRO Katumani greenhouse and KALRO Kiboko substation. Ten seedlings of tomatoes, five seedlings of pumpkins and five seedlings of *T. purpurea* were established in plastic containers filled with sandy loam soil and followed careful recommended agronomic practices of plant growth requirements. Nitrogen fertilizer (calcium ammonium nitrate) was also applied as top dressing nutrient to increase leaf growth and enhance egg laying by whiteflies. A temperature of around 28°C and relative humidity of 30–50% was maintained by opening and closing of greenhouse as well as watering to encourage optimum growth of the host plants.

The pupal stage of whiteflies were collected from the field and identified to species level before introduction on plants in the pots. The whiteflies were let to develop to adults on the disease-free plants. The disease-free adult whiteflies were isolated in a cage for eight weeks to have a non-viruliferous colony of whiteflies before start of the experimentation (Lapidot, 2007; Mutisya and Miano, unpublished).

2.2. Diseased cassava plants establishment

Cassava cuttings obtained from field diseased material of cassava brown streak disease and one obtained from KALRO Katumani field infected with CMD were planted in plastic containers and placed in cages for establishment. Ad lib watering was done to prevent plant withering and defoliation. Disease-free cassava cuttings were also added to the cages with brown streak infected plants and whiteflies introduced to ensure there was enough inoculum source on the vectors. Some other disease-free cassava cuttings of variety TM-14 were obtained from KALRO-NARL which was verified

through use of RT-PCR method. The variety TM-14 is known to be highly susceptible to both mosaic and brown streak diseases in Kenya. Some 200 cuttings of TM-14 variety were then established in a greenhouse at KALRO Katumani in isolated cages and free of whitefly infestation where they were planted in plastic containers filled with sterilized soil mixed with sand and compost manure at a ratio of 3:2:1. They were then watered until they sprouted and after 2 weeks, about 10 g of nitrogen fertilizer per plant pot was added to enhance vegetative growth.

2.3. Transmission studies

Adult whiteflies which of 3–4 days old were used in the studies as they are known to be active and able to transmit cassava *gemiviruses*. A total of 162 disease-free plantlets were randomly isolated for the transmission studies under insect rearing cages each measuring 45 × 45 × 45 cm and laid in a completely randomized design in a greenhouse.

The non-viruliferous whiteflies were then introduced to diseased plants to feed for 48–72 h according to Mware et al. for acquisition of disease virus and then trapped in standard plastic Petri dishes and introduced to feed on disease-free cassava. The number of whiteflies introduced in disease-free cassava was varied from 5, 10 and 20 and the feeding period (inoculation) on disease-free cassava was also varied from 2, 6, 12 and 24 h. This was replicated for three times on both mosaic and brown streak disease for each of the three whitefly species. The disease symptoms were then monitored and recorded from 21st to 91st day at 7 days interval. To ensure optimum feeding, the whiteflies were disturbed by gently shaking the plantlets to ensure they did not aggregate on top of the leaves, while feeding. After the feeding period was over, the plants were removed, and whiteflies shaken off and sprayed with imidacloprid to kill all the insects. Thereafter the plantlets were cut back, added nitrogen (10 g/plant pot) and watered until leaves emerged and then monitored for another 42 days for disease symptoms observation.

3. Results

3.1. Species pathogen transmission rate

It was observed that only *B. tabaci* species were capable of transmitting both CBSD and CMD diseases, whereas for the CBSD all species were able to transmit the disease but at different rates and with different numbers of whiteflies introduced (Figure 1). Species *B. tabaci* and *T. vaporariorum* had similar ability to transmit CBSD at 50% plant incidence during the observation time period. *Aleurodicus dispersus* had least incidence of 30% CBSD incidence.

As the results indicated, the transmission of CMVD was significant ($p < 0.05$) by viruliferous *B. tabaci* species when fed on healthy plants for 6 h at $44 \pm 16\%$ followed by when fed for 24 h at 22 ± 31 and at $11 \pm 16\%$ when same species was fed for 12 h. All other species did not transmit the disease irrespective of time of feeding (Table 1). On the other hand *B. tabaci* had CBSD transmission of $22 \pm 16\%$ for all time variations of 6, 12 and 42 h. Species *T. vaporariorum* had CBSD incidence of 22 ± 16 , 11 ± 16 and $33 \pm 27\%$ for 6, 12 and 24 h. Similarly *A. dispersus* had CBSD incidence of $11 \pm 16\%$ for all time variations of 6, 12 and 24 h.

Figure 1. Viral disease transmission incidence of three whitefly species on cassava plants.

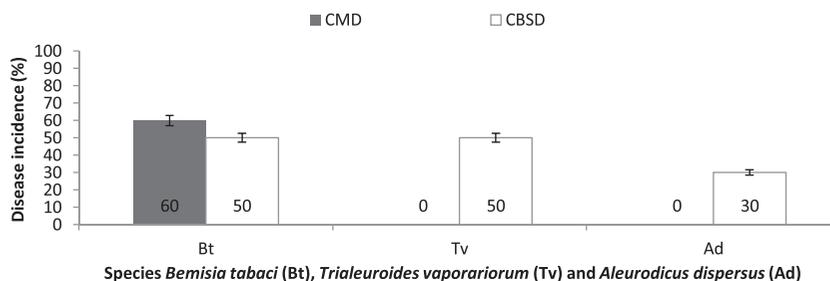


Table 1. Mean number (\pm SD) of CMD and CBSD incidences in relation to feeding period and whitefly species

Feeding time (Hrs)	Whitefly species	CMD (%)	CBSD (%)
2	<i>B. tabaci</i>	0b	0b
2	<i>A. dispersus</i>	0b	0b
2	<i>T. vaporariorum</i>	0b	0b
6	<i>B. tabaci</i>	44 \pm 16a	22 \pm 16ab
6	<i>A. dispersus</i>	0b	11 \pm 16ab
6	<i>T. vaporariorum</i>	0b	22 \pm 16ab
12	<i>B. tabaci</i>	11 \pm 16b	22 \pm 16ab
12	<i>A. dispersus</i>	0b	11 \pm 16ab
12	<i>T. vaporariorum</i>	0b	11 \pm 16ab
24	<i>B. tabaci</i>	22 \pm 31b	22 \pm 16ab
24	<i>A. dispersus</i>	0b	11 \pm 16ab
24	<i>T. vaporariorum</i>	0b	33 \pm 27a

Notes: Similar lower case letters denote insignificant ($p > 0.05$) mean value of CMD and CBSD incidences upon different hours of feeding (SNK at 5% level).

3.2. Species disease transmission efficiency

Species *B. tabaci* showed increases of whitefly numbers leading to higher efficiency of CMD transmission incidence of 8 \pm 14, 17 \pm 11 and 33 \pm 33% scored for 5, 10 and 20 individual insects (Table 2). Closely related incidence of CBSD was observed where *B. tabaci* had 8 \pm 14, 17 \pm 11 and 25 \pm 14% for 5, 10 and 20 respective individuals. On the same CBSD *T. vaporariorum* had 17 \pm 16% incidence for both 5 and 10 individuals and 17 \pm 29% when increased to 20 individuals. The species *A. dispersus* did not transmit CBSD at 5 and 10 individuals introduced on the plants. When increased to 20 individuals, *A. dispersus* increased transmission efficiency of CBSD to incidence of 17 \pm 16%.

3.3. Feeding rate and disease correlation

There was no correlation observable between increased whitefly exposure duration on cassava and rate of disease pathogen transmission of CMD. Highest pathogen transmission occurred at 5 and 24 h exposure periods at 66% disease incidence on the leaves (Figure 2). A transmission rate of 33% was noted for 5 and 12 h exposure feeding periods. The least period of feeding period of two hours showed no CMD symptoms on leaves. The results showed no relationship between exposure period and transmission rate.

Table 2. Mean number (\pm SD) of CMD and CBSD incidences in relation to number of whitefly and inoculant species

Whitefly numbers	Whitefly species	CMD incidence (%)	CBSD incidence (%)
5	<i>B. tabaci</i>	8 \pm 14a	8 \pm 14a
5	<i>A. dispersus</i>	0b	0b
5	<i>T. vaporariorum</i>	0b	17 \pm 16a
10	<i>B. tabaci</i>	17 \pm 11a	17 \pm 16a
10	<i>A. dispersus</i>	0b	0b
10	<i>T. vaporariorum</i>	0b	17 \pm 16a
20	<i>B. tabaci</i>	33 \pm 33a	25 \pm 14a
20	<i>A. dispersus</i>	0b	17 \pm 16a
20	<i>T. vaporariorum</i>	0b	17 \pm 29a

Notes: Similar lower case letters denote insignificant ($p > 0.05$) mean value of CMD and CBSD incidences upon different hours of feeding (SNK at 5% level).

Figure 2. Relationship between whitefly exposure period and CMD incidence for different species.

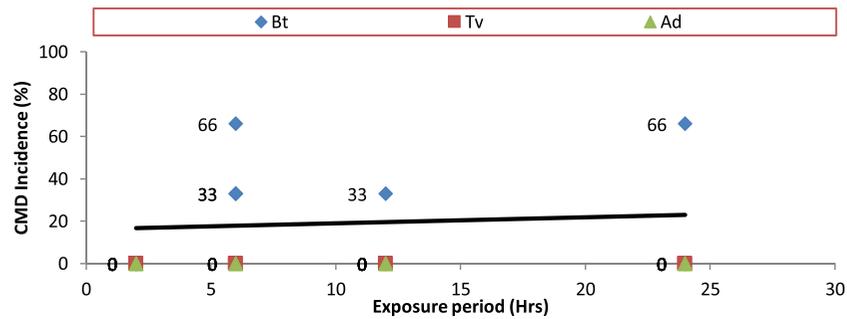


Figure 3. Relationship between number of whitefly and CBSD incidence for different species during transmission studies.

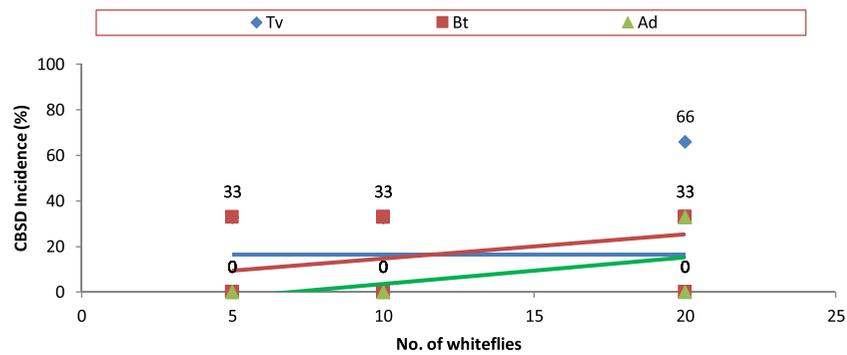
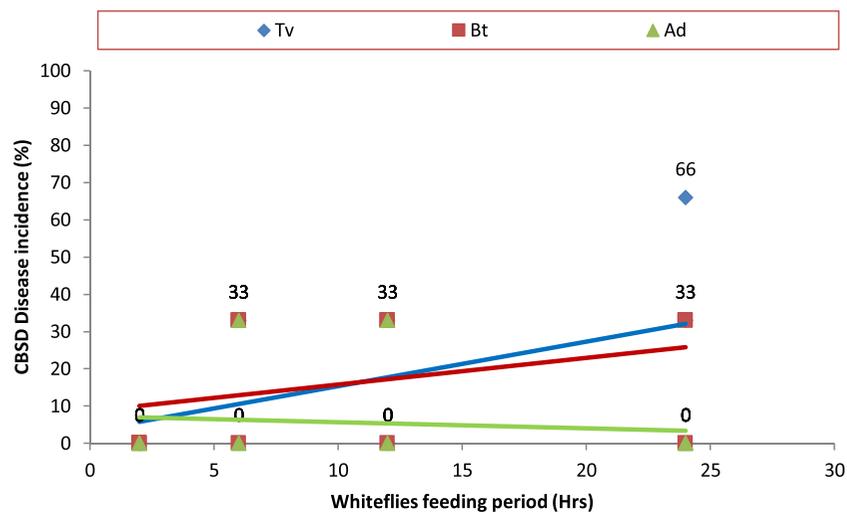


Figure 4. Relationship between whiteflies feeding period and CBSD incidence for different species.



There was similarly no correlation between number of whiteflies introduced of (*T. vaporariorum*, *B. tabaci* and *A. dispersus*) and CBSD percentage incidence (Figure 3). Likewise the analysis showed no positive correlation between period of feeding for whiteflies species of *T. vaporariorum*, *B. tabaci* and *A. dispersus* (Figure 4).

4. Discussion

As the study results showed it is only *B. tabaci* species which was found capable of transmitting the CMD. Moreover, the results were also in contrast to earlier work done by Hillocks (2000) who reported rapid expansion of CMD being closely associated to whitefly population specifically *B. tabaci*. Hence, from the results it was noted that increasing the number of *B. tabaci* did not lead to higher disease incidence in contrast to results from work done by Maruthi et al. (2005) who reported CMD disease incidence being positively correlated to number of whiteflies in an area. This can be clarified that it

would depend on the whitefly earlier disease acquisition level and persistence in the insect body. It was also noted that for effective transmission of the virus, the pest would require a minimum of sufficient feeding period. This therefore calls for concerted effort in management of *B. tabaci* as the main vector of CMD disease and ensuring the source of inoculum is eradicated as the pest transmits the disease pathogen.

For CBSD, the results showed all whitefly species were capable of transmitting the viral disease pathogen in agreement with earlier work done by Maruthi et al. (2005) and Alicai et al. (2007). This is also the first time to report *T. vaporariorum* being able to transmit the CBSD disease. Lapidot (2007) has reported *T. vaporariorum* transmitting tomato yellow leaf curl virus in similar rates as *B. tabaci*. The rate of transmission was found to be 17% for *B. tabaci*, 17% for *T. vaporariorum* and 6% for *A. dispersus* in comparatively lower time rates. Mware et al. reported lower transmission rate for *A. dispersus* and high level by *B. tabaci*. In reference to time period required for transmission, 6 h was the minimum time period required for all whitefly species pathogen transfer to plant tissue. For the number of whitefly species required for transmission, it was noted that this varied with species and conditions. All the tested whitefly species did not show difference on transmission rate even as the numbers were increased, indicating that it all depended on whether the insect had disease pathogen or not. The present results therefore calls for good management practices to be put in place for all whitefly species to arrest the spread of the disease to susceptible plants in crop production systems.

The biology of the whitefly is such that it purely relies on the leaf texture and nutrient level on phloem cell tissue of the host plant (Backus, Cline, Ellerseick, & Serrano, 2007). The virus on the leaf tissue is reported to influence more feeding of the whitefly on specific crop species (Fereses & Moreno, 2009). One such classical relationship is the tomato leaf yellow curl virus (TLYCV) and *B. tabaci* species (Moreno-Delafuente, Garzo, Moreno, & Fereses, 2013). As the level of the virus increase on the plant leaf tissue the *B. tabaci* feeds more and moves out to spread the disease to other less infected similar plants within the vicinity (Backus et al., 2007; Czosnek & Rubinstein, 1997; Shah, Zhang, & Liu, 2015). As it would be expected of the increased feeding of the vector more eggs are laid and juvenile development increases with the virus presence on the leaf tissue. The present study objective was mainly on transmission rate of virus diseases by the virulent whitefly adults among the three species. Nevertheless, field spatial occurrence of *B. tabaci* on cassava mosaic infected plants has been reported showing increased disease incidence where the whitefly species is common (Martin et al., 2000; Njoroge et al. 2016).

In conclusion, it has been noted that CMD which is caused by viruses in Begomovirus family and transmitted in persistent manner is spread by *B. tabaci* species only, whereas CBSD caused by virus in Ipomovirus family and transmitted in semi persistent manner is spread by different species of whiteflies (Hull, 2002). This calls for more research on mode of transmission and biochemical analysis for these species.

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Competing Interests

The authors declare no competing interest.

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