Journal of General and Molecular Virology Vol.1 (4), pp. 040-045, December, 2009
Available online at http://www.academicjournals.org/jgmv

Full Length Research Paper

Efficiency of cassava brown streak virus transmission by two whitefly species in coastal Kenya

B. Mware[1, 2], R. Narla[1], R. Amata[2], F. Olubayo[1], J. Songa[2], S. Kyamanyua[4], and E. M. Ateka[3]*

[1]Department of Plant Science and Crop Protection, Faculty of Agriculture, University of Nairobi. P. O. Box 30197-00200 Nairobi, Kenya.
[2]Kenya Agricultural Research Institute/National Agricultural Research Laboratories, P. O. Box 14733, 00800 Nairobi, Kenya.
[3]Department of Horticulture, Jomo Kenyatta University of Agriculture and Technology. P. O. Box 62000-00200 Nairobi, Kenya.
[4]Makerere University Kampala-Uganda.

Accepted 17 November, 2009

The efficiency of cassava brown streak virus (CBSV) transmission by *Bemisia tabaci* (Gennadius) (Hemiptera: *Aleyrodidae*) and spiraling whitefly (*Aleurodicus dispersus)* Russell (Hom, Aleyrodidae) was determined. The transmission utilized field collected adult whitefly populations fed on (allowed 48 h acquisition access feeding period (AAP)) on CBSD (cassava brown streak virus disease) symptomatic leaves before transfer onto clean recipient plants. In subsequent transmission experiments, adult whitefly numbers of each species were varied per plant to determine the effect of whitefly numbers on the rate of CBSV transmission. CBSV was transmitted by *B. tabaci* allowed 48 h AAP on CBSD infected cassava leaves at a higher rate of 40.7% compared to that of *A. dispersus* at 25.9%. This work reports for the first time the transmission of CBSV by *A. dispersus*. A likely biological property of CBSV reported here for the first time is its ability to be transmitted by two whitefly species belonging to two different genera (*Bemisia* and *Aleurodicus)*. Management of CBSD therefore needs to focus on the control of the two whitefly species to reduce the chances and rates of infection and disease spread.

Key words: *Aleurodicus dispersus, Bemisia tabaci*, CBSD, spiraling whitefly, transmission efficiency.

INTRODUCTION

Cassava brown streak disease (CBSD) first described in Tanzania (Storey, 1936) attacks cassava leading to root weight losses of up to 70% in susceptible cultivars (Hillocks et al., 2001). The disease is caused by cassava brown streak virus (CBSV), an *Ipomovirus* in the family Potyvirideae (Monger et al., 2001b) a virus that is graft-transmissible from cassava to cassava (Storey, 1936) and is mechanically transmitted from cassava to a number of herbaceous hosts (Lister, 1959). An earlier report pointed out that CBSV is insect-transmitted and that the most probable vector is the whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) (Storey, 1939; Bock, 1994). More recent work by Maruthi et al. (2004) reported *B. tabaci* exhibiting low CBSV transmission rates ranging from 20 - 22%. The low rates of natural spread (Storey, 1939; Bock, 1994; Maruthi et al., 2004 and (Mware et al., 2009) are inconsistent with the high incidences of CBSD observed in the field surveys of up to 64%, (Alicai et al., 2007). The high incidences observed in the field could be due to a wide range of vectors responsible for transmission and accumulation of the virus through continuous use of same planting material year in year out by farmers. Successful CBSV transmission by *B. tabci* has been reported (Maruthi et al., 2004 and Mware et al., 2009), but this does not preclude the possibility that under suitable conditions, *Aleurodicus dispersus* whose population has been directly correlated with CBSD incidence may also transmit the virus (Mware et al., 2009). *A. dispersus* is an emerging pest infesting cassava in coastal Kenya and may also

*Corresponding author. E-mail: eateka@agr.jkuat.ac.ke.

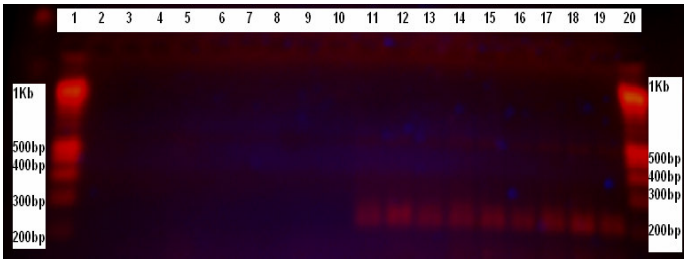

Figure 1. Shows diagnostic bands from CBSV infected plants and none for healthy plants used as controls in the transmission experiment. Lanes 2 - 8: healthy plants (controls), 9: negative control (water), 11, 13 - 17, 19: CBSV infected plants and 12, 18: positive control.

Plate 1. Spiraling whitefly feeding on CBSV-infected cassava leaves within a falcon tube to acquire CBSV before transfer onto recipient plants.

transmit CBSV (Mware et al., 2009). Prior to the transmission trials, *A. dispersus* populations were observed to be highest on lower mature CBSD symptomatic leaves during a whitefly collection survey in Kilifi, Malindi, Lunga lunga and Msambwueni within coastal cassava growing regions. Its population was mostly high on lower mature leaves (Mare et al., 2009).

Although *B. tabaci* is already reported to transmit CBSV, its efficiency to transmit the virus has not been determined. Furthermore, the fact that high populations of *A. dispersus* seemed to coincide with resurgence of CBSD in Coastal Kenya prompted transmission trials to elucidate this relationship. The objective of this study was to determine the transmission efficiency of the two whitefly species.

MATERIALS AND METHODS

Collection of cassava cultivars and whiteflies

CBSV susceptible cassava cultivars were identified during diagnostic surveys in Western Kenya (Mware et al., 2009). They included MM96/5280 and MM96/4466 which are most preferred by farmers due to their high yield, are early maturing (12 months), sweet, are consumed fresh and are resistant to cassava mosaic geminiviruses (CMGs). The cuttings of the cultivars were collected, established within an insect-proof glasshouse to ensure the absence of the CBSV. All plant materials were subjected to reverse transcriptase polymerase chain reaction (RT-PCR) to confirm freedom from CBSV infection as described by Monger et al. (2001) (Figure 1). The materials were then kept in whitefly exclusion cages (0.3 mm mesh) prior to the start of the transmission experiments.

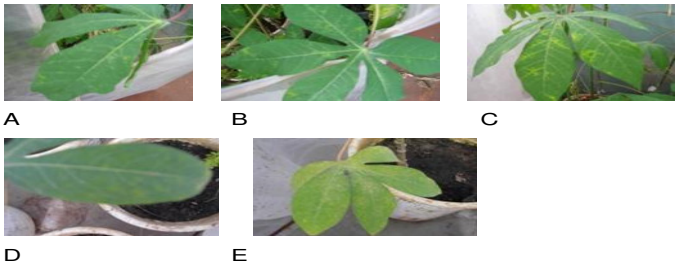

Plate 2. Showing symptoms in vector-inoculated plants of cultivar MM96/5280 (A, B, C) and MM96/4466 (D, E) Leaf inoculated with CBSV by *B. tabaci* showing CBSD early vein clearing and feathering after 26 days.

CBSV transmission tests

Virus transmission tests were done according to the protocols developed by Maruthi et al. (2004), with some modifications. Adult whiteflies were collected directly from CBSV infected cassava in the field then immediately transferred to recipient plants. However, in another treatment adult whitefly populations were allowed 48 h access acquisition feeding time under no choice confinement (in Falcon tube cages; plate 1) on CBSV infected cassava plants followed by an access inoculation feeding period of 48 h on recipient plants within 0.3 mm mesh cages.

The first transmission trial involved an adult whitefly population collected from CBSV infected cassava leaves in the field and then allowed a 48 h inoculation access feeding period within 1 x 1 x 1.5 m whitefly tight cages. Three cages (9 recipient plants per cage) were set up for each species in the first and second experiments (a total of 27 recipient plants were used in each experiment).

In the second trial, a mesh cage and modified falcon tube cages were used to confine CBSV cassava plants and leaf petioles, respectively for 48 h acquisition access feeding period (Plate 2). Specifically colonies of the adult whitefly species were allowed 48 h acquisition access feeding period on CBSV infected cassava leaves then transferred onto 9 recipient plants of cultivar MM96/5280 for 48 h inoculation access feeding period. The set up had 9 target plants replicated three times (27 recipient plants) for each whitefly species. Approximately 30 adult whiteflies were confined within the modified clip cages in which a single leaf was introduced with petiole undetached from the main recipient plant. The whiteflies were then eliminated by spraying with an insecticide (Brigade) and CBSD symptom development monitored specifically on the inoculated leaves and the entire recipient plant for 26 - 60 days. A control cage for each whitefly species was set up with 3 CBSV-free cassava plants not infested with whitefly since the population collected were from diseased plants in the field. The experiments were repeated three times for each whitefly species. The rate of transmission was determined as a proportion of infected target plants expressed as a percentage of the total number of plants tested.

Efficiency of transmission by whiteflies

To determine the transmission efficiency of the two whitefly spe-

Table 1. CBSV transmission probability by adult whitefly species allowed 48 h acquisition access feeding period and those not allowed the 48 h acquisition access feeding period.

Directly collected adult whiteflies without 48 h AAP									
Experiment	***Bemisia tabaci***				***Aleurodicus dispersus***				
Replicate	**Recipient plants**	**Probability**			**Replicate**	**Recipient plants**	**Probability**		
		Exp 1	**Exp 2**	**Exp 3**			**Exp 1**	**Exp 2**	**Exp 3**
1	9	0.01	0	0.012	1	9	0.003	0	0.01
2	9	0.003	0.01	0	2	9	0	0.003	0
3	9	0.003	0	0.01	3	9	0.012	0	0
Probability		(0.005)	(0.002)	(0.006)			(0.005)	(0.001)	(0.001)
Adult whiteflies allowed 48 h AAP on CBSV- infected plants									
Experiment	***Bemisia tabaci***				***Aleurodicus dispersus***				
Replicate	**Recipient plants**	**Probability**			**Replicate**	**Recipient plants**	**Probability**		
		Exp 1	**Exp 2**	**Exp 3**			**Exp 1**	**Exp 2**	**Exp 3**
1	9	0.012	0.033	0.012	1	9	0.01	0	0.012
2	9	0.01	0.01	0.024	2	9	0.003	0.003	0.017
3	9	0.024	0.012	0.017	3	9	0.012	0.017	0.024
Probability		(0.014)	(0.016)	(0.017)			(0.007)	(0.006)	(0.017)

Note: Probability calculated as $P = 1-(1-I)^{1/K}$ where I is the proportion of CBSV infected recipient plants and K is the number of whitefly adults per plant.

cies, viruliferous populations of each were introduced on leaves of several recipient CBSV free cassava plants. In addition, the effect of whitefly numbers on transmission efficiency was also assessed using 1, 5, 15 and 30 adult whiteflies of each species on target plants. The colonies of the two whitefly species were given 48 h acquisition feeding period on CBSD symptomatic leaves then transferred onto 4 recipient plants of cultivar MM96/5280 for an inoculation feeding period of 48 h within modified clip cages made from falcon tubes. The 1, 5, 15, 30 adult whiteflies were confined within the modified clip cages in which a single leaf was introduced with petioles undetached from the main recipient plant. Four cages were set up for the (1, 5, 15, 30) different whitefly populations of each whitefly species. The whiteflies were eliminated by spraying with an insecticide (Brigade) after inoculation. CBSD symptom development was monitored on inoculated leaves and on entire recipient plants. Virus transmission efficiency was calculated as the proportion (in percentage) of the total number of plants infested with viruliferous whitefly species that became infected. Comparisons of virus transmission efficiency were made using probability estimates of transmission by a single whitefly (Gibbs and Gower, 1960; Ng and Perry, 1999).

RESULTS

Transmission of CBSV by *B. tabaci* and *A. dispersus*

In the experiments with falcon caged *B. tabaci* (given 48 h AAP) in KARI-Mtwapa 33 of 81 test plants (40.7%) developed CBSD symptoms whereas the mass fed (collected and introduced on diseased plants within cages without 48 h AAP) had 17 of 72 test plants (23.6%) developing CBSD symptoms. The control plants were not infected (Figure 1)

A total of 18 cassava plants var. MM96/5280 exposed to *A. dispersus* (7) and *B. tabaci* (11) collected from CBSV-infected cassava plants in the field without 48 h AAP, developed CBSD symptoms. CBSV was transmitted more efficiently when adult whiteflies were allowed 48h CBSV infected cassava plants then when not allowed 48 h acquisition access feeding. For instance, a transmission rate greater than 35% was achieved by *B. tabaci* population allowed a 48 h AAP as compared to a population not allowed 48 h AAP which was lower than 20% (Table 1).

Similarly *A. dispersus* also transmitted CBSV efficiently when given a 48 h AAP than when not allowed acquisition access feeding (Table 1). It is noteworthy here that the field collected adult whiteflies populations were directly transferred onto recipient plants immediately after collection without 48 h AAP (simulating a field scenario).

Effectiveness of *B. tabaci* over *A. dispersus* as a vector was shown by higher rates of transmission by both populations allowed 48 h AAP and those not allowed 48 h AAP. CBSV was transmitted at higher rate by *B. tabaci* adult whiteflies (40.7%) fed on CBSV infected cassava leaves for 48 h whereas the spiraling whitefly had an overall CBSV transmission rate of 25.9%. Symptom took a long time (26 - 60 days) to appear in inoculated plants and none of the recipient plants showed symptoms until after 26 days.

Table 2. CBSV transmission rate by a single *B. tabaci* and *A. dispersus.*

Adult whitefly species per pant										
Experiment	***Bemisia tabaci***					***Aleurodicus dispersus***				
Replicate	**Recipient plants**	**1**	**5**	**15**	**30**	**Recipient plants**	**1**	**5**	**15**	**30**
1	4	1(25.0)	2(50)	3(75)	4(100)	4	0	1(25)	2(50)	3(75)
2	4	0	1(25)	2(50)	3(75)	4	0	2(50)	2(50)	4(100)
3	4	1(25.0)	0	2(50)	3(75)	4	0	1(25)	1(25)	2(50)
4	4	0	2(50)	3 (75)	2(50)	4	0	0	1(25)	2(50)
		(12.5)	(31.2)	(62.5)	(75)		(0)	(25)	(37.5)	(68.8)

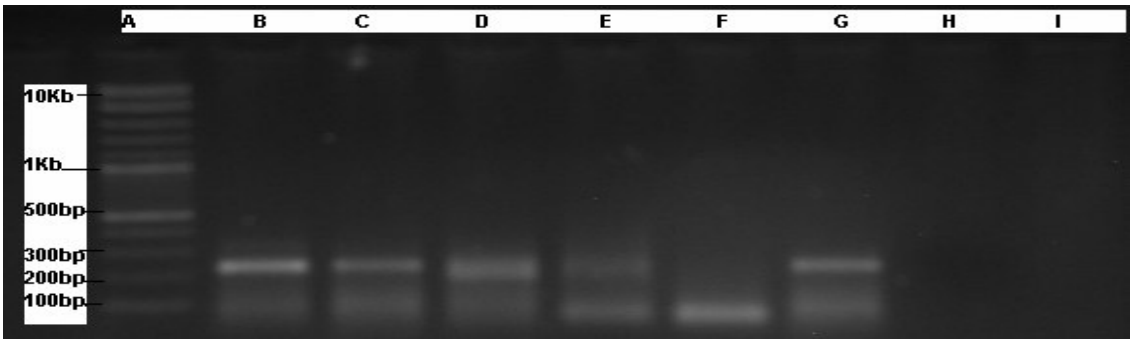

Figure 2. CBSV diagnostic bands following amplification of cDNA obtained from plants inoculated using *B. tabaci* (B and C) and *A. dispersus* (D and E). A is a DNA marker, whereas H and I are negative controls and G is a positive control.

Transmission efficiency

Efficiency of transmission differed among the two whitefly species examined in this study. The most efficient transmission was observed with *B. tabaci* (Table 2), following a 48 h AAP on CBSV-infected cassava plants and a 48 h IAP. CBSV was transmitted (Figure 2) at low rates by individual whiteflies of *B. tabaci* with 12.5% (2/16) transmission. In addition, *B. tabaci* transmitted at highest efficiency when 30 whiteflies per plant were used in transmission experiments compared to when 1, 5 or 15adults were used per plant. On the other hand *A. dispersus* was less efficient at transmitting CBSV (Fisher, $P < 0.0001$) (Table 2). However, unlike *B. tabaci,* transmission was not observed with individual spiraling whiteflies over the course of experiments due to adverse conditions that lead to high mortality. Non-choice feeding on CBSD symptomatic leaves within falcon cages led to greater efficiency of transmission by both species.

Figures in parenthesis are percentage infection per replicate.

Transmission was scored as the number of infected target plants over total number of plants tested. Experiments were repeated 4 times using 4 plants for each whitefly species.

DISCUSSION

Cassava brown streak virus was transmitted (Figure 1) with different efficiencies by both *B. tabaci* and *A. dispersus* with *B. tabaci* being more efficient (Table 1 and 2). However, the transmission rates achieved were low compared to the field recorded CBSD incidences of up to 64%, (Alicai et al., 2007). The low transmission rates of CBSV by the two whitefly species may be due to technical difficulties in the transmission protocols such as high temperatures within confined cages (mass mortality) and high humidity levels in falcon tubes. Environmental conditions may adversely affect transmission (Maruthi et al., 2004). For instance, high humidity within the clip cages lead to mass mortality of *A. dispersus* although B. *tabaci* was able to survive despite the humid conditions.

The feeding behaviour of adult *B. tabaci* on cassava plants seems to greatly influence CBSV transmission. More than 90% of adult *B. tabaci* feed on the top five leaves of cassava plants in the field (Maruthi et al., 2004b), whereas the most obvious CBSD symptoms and presumably higher virus titres develop in the lower leaves. The transmission mechanism employed here tried to overcome this challenge by allowing *B. tabaci* to feed on the most symptomatic leaves of field-grown cas-

sava using clip cages (no choice feeding), thus providing ready access to virus for whiteflies. This resulted into higher efficiency of transmission than in mass feeding.

The transmission of CBSV by adult whitefly populations when both species were not allowed 48 h AAP, demonstrates the ability of the vectors to acquire the virus and naturally transmit it under field conditions. Up to 1.7% in a population of the adult *B. tabaci* whiteflies had been shown to be infective when collected in heavily infected cassava fields in Ivory Coast then transferred to young test seedlings of cassava (Fargette et al., 1990). During this trial the whiteflies were collected from CBSV-infected cassava and also from non-choice feeding then immediately transferred on to the recipient plants. In both cases, transmission occurred meaning that adults which had acquired the virus did not loose the ability to transmit it during the transfer, suggesting that both vectors may not require a latent period before they can transmit the virus after acquisition. Different modes of virus transmission have been characterized depending on the retention time, sites of retention, and internalization of virions by vectors (Andret-Link and Fuchs, 2005). Non-persistent viruses are retained by their vectors for less than a few hours whereas semi persistent viruses are retained for days, weeks, or even years. Viruses in these two categories are acquired from infected plants and inoculated within seconds or minutes to recipient plants. In addition, they do not require a latent period, e.g. time interval between acquisition and transmission, and do not replicate in the vector (Andret-Link P. and Fuchs M., 2005). Further work need therefore to focus on categorizing the mode of CBSV transmission by the vectors involved and the specificity of the transmission relationship.

Transmission of plant virus by a single *B. tabaci* has been reported previously such as for cotton leaf curl virus (Kirkpatrick, 1931), tomato yellow leaf curl virus (Mehta et al., 1994) and tobacco leaf curl virus (Aidawati et al., 2002). In most cases, the efficiency of transmission increased as the number of adult *B. tabaci* was increased. A similar result was achieved from this experiment when CBSV was transmitted by a single *B. tabaci* adult. The adult whiteflies per pant greatly influenced the transmission efficiencies achieved as it was observed that transmission rates increased with increase in whitefly numbers used. The ability of *B. tabaci* to transmit CBSV also seemed to be affected by the inoculation and acquisition feeding periods. When 48 AAP was allowed there was higher percent transmission rate achieved by both the adult whitefly species unlike when the whiteflies were not allowed the 48 AAP.

One remarkable biological property of CBSV is its ability to be transmitted by two different whitefly vectors belonging to two genera (*Bemisia* and *Aleurodicus*). This is however not very unusual for a whitefly-transmitted virus. Earlier studies have demonstrated that tomato chlorosis virus (ToCV) is transmitted with equal efficiency by both *Trialeurodes abutilonea* and *B. tabaci* biotype B, members of two different genera (*Trialeurodes* and *Bemisia*, respectively), and was achieved using individual whiteflies of either vector (Wintermantel and Wisler, 2006). Moreover, both *B. tabaci* biotype A and *Trialeurodes vaporariorum* can transmit ToCV, but single insect transmission was not observed with either of these vectors over five independent experiments (Wintermantel and Wisler, 2006).

These findings report for the first time the ability of spiraling whitefly to transmit CBSV and may explain its contribution in the spread of CBSD in cassava growing areas in coastal Kenya. High whitefly populations in the fields, comprising B. tabaci and A. dispersus may be correlated with the high CBSD incidences observed. The results of the investigations on ability of the insects collected from the infected cassava field to acquire and transmit the virus increases the understanding of the role the whitefly species play in the spread of CBSD.

Management options need to focus on the control of the vectors in addition to other control measures.

ACKNOWLEDGEMENT

We thank the Eastern African regional Research Network for Biotechnology, Biosafety and Biotechnology Policy Development BIO-EARN for financial support.

REFERENCES

Aidawati N, Hidayat SH, Suseno R, Sosromarsono S (2002). Transmission of an Indonesian isolate of *tobacco leaf curl virus* (Geminivirus) by *Bemisia tabaci* Genn. (Hemiptera: Aleyrodidae). Plant Path. J. 18: 231-236.

Alicai T, Omongo C A, Maruthi MN, Hillocks RJ, Baguma Y, Kawuki R, Bua A, Otim-Nape GW, Colvin J (2007). Re-emergence of Cassava Brown Streak Disease in Uganda. Plant Disease 91: 1-24.

Andret-Link P, Fuchs M (2005). Transmission Specificity of Plant Viruses by Vectors. J . Plant Path. 87: 153-165.

Bock KR (1994). Studies on cassava brown streak virus disease in Kenya. Trop. Sci. 34: 134–145.

Hillocks RJ, Raya MD, Mtunda K, Kiozia H (2001). Effects of brown streak virus disease on yield and quality of cassava in Tanzania. J. Phytopathol. 149: 389–394.

Lister RM (1959). Mechanical transmission of cassava brown streak Virus. Nat London 183: 1588–1589.

Maruthi MN, Hillocks RJ, Rekha AR, Colvin J (2004). Transmission of Cassava brown streak virus by whiteflies. In: Sixth International Scientic Meeting of the Cassava Biotechnology Network– Adding Value to a Small-Farmer Crop, 8-14 March 2004, CIAT, Cali, Colombia p. 80.

Mehta PJ, Wyman JA, Nakhla, MK Maxwell DP (1994). Transmission of *tomato yellow leaf curl geminivirus* by *Bemisia tabaci* (Homoptera: Aleyrodidae). J. Econ Entomology 87:1291-1297.

Monger WA, Seal S, Isaac AM, Foster GD (2001a). Molecular characterization of the Cassava brown streak virus coat protein. Plant Pathol. 50: 527–534.

Monger WA, Seal S, Cotton S, Foster GD (2001b). Identification of different isolates of cassava brown streak virus and development of a diagnostic test. Plant Pathol. 50: 768–775.

Mware BO, Ateka EM, Songa JM, Narla RD, Olubayo F, Amata R, (2009). Transmission and distribution of cassava brown streak virus

disease in non coastal cassava growing areas of Kenya. J. App. Biosci. 16: 864– 870.
Storey HH (1936). Virus diseases of East African plants. VI-A progress report on studies of the disease of cassava. East Afr. Agric. J. 2: 34–39.
Storey HH (1939). Report of the Plant Pathologist. East Afr. Agric. Res. Station Rep.
Wintermantel WM, Wisler GC (2006). Vector specificity, host range, and genetic diversity of Tomato chlorosis virus Plant Dis. 90: 814-819.
William M. Wintermantel, United States Department of Agriculture–Agricultural Research Service, Salinas CA, Gail C, Wisler, Department of Plant Pathology, University of Florida, Gainesville.