Virus-vector relationships and the role of whiteflies, *Bemisia tabaci*, and farmer practices in the spread of cassava brown streak viruses

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ABSTRACT

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Cassava brown streak disease (CBSD) is arguably the most dangerous current threat to cassava, which is Africa's most important food security crop. CBSD is caused by two species of cassava brown streak viruses (CBSVs). The role of cassava whiteflies and farmer practices in the spread CBSVs was investigated in a set of field- and laboratory-based experiments. *Cassava brown streak virus* (CBSV) was acquired and transmitted by *Bemisia tabaci* quickly (5-10 min each for virus acquisition and inoculation), and the virus was retained for up to 48 h when feeding on cassava. Maximum mean virus transmission (60%) was achieved using 20-25 viruliferous whiteflies per plant that were given acquisition and inoculation periods of 24 h each. Experiments mimicking the agronomic practices, such as cassava leaf picking, or the use of contaminated tools for making cassava stem cuttings did not show the transmission of CBSV. Screenhouse and field experiments in Tanzania showed that the maximum spread of CBSVs occurred next to spreader rows, and that the rate of spread decreased with increasing distance from the source of inoculum. The disease spread systematically in the field up to a maximum of 17 meters in a cropping season. These results collectively indicate that CBSVs are transmitted by *B. tabaci* semi-persistently, but for only short distances in the field. This implies that spread over longer distances is due to movements of infected stems or cuttings used for planting material. These findings have important implications for developing appropriate management strategies for CBSD.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a woody shrub that produces tuberous roots which are consumed as a staple in much of sub-Saharan Africa (SSA). As well as being the main source of dietary calories for a large proportion of the rural and urban populations in SSA, cassava roots have an industrial use in the production of animal feed, starch, paper and bio-fuel (Nassar and Ortiz 2007). The food security and livelihood benefits of cassava are, however, negatively affected by biotic constraints, of which the two most important are the virus diseases – cassava mosaic disease (CMD) and cassava brown streak disease (CBSD). CBSD currently has major impacts on production in eastern and southern African countries (Hillocks and Jennings 2003; Legg et al. 2011, 2015). Until recently, CBSD was endemic only in the low altitude areas of Kenya, Malawi, Mozambique and Tanzania (Hillocks and Jennings 2003; Storey 1936, 1939) where the disease was reported to cause reductions of up to 70% in tuberous root yield of susceptible cultivars (Hillocks et al. 2001). As well as having direct deleterious effects on the growth of cassava plants, the disease causes necrosis of affected roots, making them unfit for consumption or marketing, and thus affecting food security (Legg et al. 2014). The continental significance of CBSD increased greatly from 2004, when the first reports were made of epidemics in mid-altitude areas of Uganda (Alicai et al. 2007). In subsequent years, further outbreaks were reported from other countries in the Great Lakes region of East and Central Africa, including western Kenya, north-western Tanzania, Rwanda, Burundi and Democratic Republic of Congo (Bigirimana et al. 2007; Legg et al. 2011; Mahungu et al. 2003; Mulimbi et al. 2012). The disease has potential to spread from the mid-altitude regions of East and Central Africa to the neighboring cassava-growing areas in southern and West Africa, and eventually to much of SSA with devastating consequences (Legg et al. 2014, 2015).

CBSD is caused by two distinct species of single-stranded RNA (ssRNA) viruses: *Cassava brown streak virus* (CBSV) and *Ugandan cassava brown streak virus* (UCBSV), (genus *Ipomovirus*, family *Potyviridae*), which are together referred to as cassava brown streak viruses (CBSVs) (Mbanzibwa et al. 2009; Monger et al. 2010; Winter et al. 2010) . Earlier work on the transmission of CBSVs showed that they can be graft-transmitted from cassava to cassava (Ogbe et al. 2006) and mechanically-transmitted from cassava to a number of herbaceous hosts (Lister 1959). In addition, it was suggested that CBSVs spread naturally in the field through the vectoring activity of insects, in particular two whitefly species; *Bemisia tabaci* (Gennadius) (Bock 1994; Storey 1939) and *Bemisia afer* (Priesner & Hosny) (Hemiptera: Aleyrodidae), which were abundant in some areas where CBSD incidences were high (Bock 1994; Munthali 1992). Subsequent transmission studies with both species of whitefly and with some species of aphid, however, were unsuccessful (Bock 1994; Lennon et al. 1986).

The first evidence of CBSV transmission by an insect vector, the whitefly *B. tabaci*, was obtained in our earlier laboratory studies (Maruthi et al. 2005), which was later confirmed (Mware et al. 2009). However, virus transmission patterns were inconsistent in both of these studies, and the low rate of transmission observed could not explain the high rate of spread of CBSVs seen in the field. The lack of correlation between laboratory studies and field observations has led to speculation that CBSVs may also be spread by other means, such as through contact between diseased and healthy plants, through tools contaminated during the process of cassava harvesting, and/or in the process of harvesting cassava leaves for use as a vegetable.

The aim of this study was therefore to investigate these possibilities; to determine if CBSVs can be transmitted by contaminated tools or during the process of leaf picking, as well as to characterize the transmission characteristics of CBSVs by *B. tabaci*. The findings from these studies will provide critical guidance for the development and implementation of control strategies to address what is currently one of Africa's biggest crop production threats.

MATERIALS AND METHODS

Cassava varieties, virus isolates and whitefly colonies used in the study. Two CBSD-susceptible cassava varieties (var.) – Albert and TMS 60444 – were grown from stem cuttings and confirmed to be free from CBSVs by reverse transcription polymerase chain reaction (RT-PCR) (Abarshi et al. 2010, 2012). These were used as target plants for virus inoculations. Two virus isolates – UCBSV from Kabanyoro, Uganda and CBSV from Naliendele, Tanzania – described previously were used in virus transmission experiments (Mohammed et al. 2012). Virus-free plants of two other cassava var. – Kiroba and Kaleso – were also used to test the efficiency of virus transmission by whiteflies. Another cassava var. Ebwanateraka infected with either CBSV or UCBSV provided the source of viruses. The colony of cassava whiteflies, *B. tabaci*, used in this study was collected originally from Uganda and maintained subsequently in the quarantine insectary facilities of NRI in the UK (Maruthi et al. 2001). This colony was confirmed to belong to the species sub-Saharan Africa1 - sub-group1 (SSA1-SG1) based on mitochondrial cytochrome oxidase I gene sequences.

Virus-indexed tissue culture plantlets of a var. Kiroba, shown to be free of CBSVs using RT-PCR, were hardened off in an insect-proof screenhouse in Kibaha, Pwani Region, Tanzania. These plants were subsequently used to establish the CBSD spread trials in the field and screenhouse, as described below. Field-grown CBSD-affected plants of the same variety were obtained from field experiments at Kibaha for use as the spreader blocks in each of these trials, and *B. tabaci* whiteflies used in this experiment were similarly obtained from field-grown plants.

Verifying the transmission of CBSV by cassava whiteflies. Initial CBSV transmission experiments by whiteflies involved a combination of using long periods of virus acquisition access (AAP) and inoculation access (IAP) of up to five days and using high whitefly numbers to increase the probability of virus transmission. Whiteflies were collected from the colony and allowed to feed for four days on CBSD-affected cassava plants of var. Ebwanateraka. Viruliferous whiteflies were then collected and released in two groups of either 20-25 or 50-100 insects on each target plant for five days to inoculate the virus. In another experiment, whiteflies emerging from the nymphal stage on diseased plants were used for transmitting CBSV to the healthy plants (Table 1). All inoculated plants were enclosed individually in insect-proof bread bags to prevent cross-contamination. Plants were kept in an insectary (28 ± 5 ºC) for symptom development and tested for CBSV infection by RT-PCR (Abarshi et al. 2010, 2012) three months after virus inoculation.

Determining the mode of transmission of CBSV by cassava whiteflies. Transmission experiments were initiated to investigate potential non-persistent, semi-persistent and persistent modes of CBSV transmission by whiteflies. To verify the non-persistent mode of transmission,

whiteflies were given three relatively short AAP of 5-10 min, 30 min and 1 h on a CBSV-affected cassava plant of var. Ebwanateraka. 20-25 viruliferous *B. tabaci* were introduced to each target plant for a 48 h IAP.

To investigate the semi-persistent mode of transmission, whiteflies were given a longer AAP of 24 h and 48 h on diseased plants, after which viruliferous insects were transferred to healthy plants for a 48 h IAP. Finally, to verify the persistent mode of transmission, whiteflies that had been introduced to healthy plants in the semi-persistent experiment were collected and re-released onto a new batch of healthy plants for 48 h.

Determining virus acquisition, inoculation and retention times in whiteflies. For testing AAP, whiteflies were allowed to feed on CBSV-infected cassava var. Ebwanateraka for 5-10 min, 30 min, 1 h, 4 h, 24 h and 48 h. Other whiteflies tested had emerged from the nymphal stage on infected plants. For each category of AAP, 20-25 viruliferous whiteflies were transferred to 25 healthy plants of var. Albert for 48 h IAP.

The methodology used to estimate IAP was similar to that of AAP except that the time given for whiteflies to inoculate the virus varied and included the following time periods: 5-10 min, 30 min, 1 h, 4 h, 24 h, 48 h and up to death (which was on average 15 days). Each category of whiteflies was given a 48 h AAP on diseased cassava plants prior to inoculation.

To determine the retention of CBSV by whiteflies, insects were given a 24 h AAP on diseased cassava plants after which they were transferred to healthy cassava plants for an IAP of 24 h or

48 h. The surviving insects from the 24 h or 48 h IAP plants were then collected and re-released on to a new batch of healthy cassava plants for a further 48 h to verify if whiteflies retain CBSV following feeding on healthy plants.

Determining other virus-vector transmission parameters. CBSD produces typical chlorotic symptoms on older leaves at the bottom of infected plants while the younger leaves are either symptom-free or only show early symptoms of the disease (vein clearing but not yellowing). How this affects virus acquisition and subsequent transmission by the whiteflies was not known. To investigate this, groups of whiteflies were confined for a 48 h AAP on mature symptomatic leaves at the bottom of the plant, or on younger leaves showing early signs of CBSD symptoms. Viruliferous insects were collected and then allowed to feed freely on healthy plants of var. Albert for 48 h for virus inoculation to determine the effect of leaf age on virus transmission. The transmission efficiencies of CBSV and UCBSV were also compared using 20-25 whiteflies per plant that were given a 48 h AAP and IAP each.

Transmission of CBSVs to different cassava varieties. Three cassava var. – Albert, Kiroba and Kaleso – were inoculated with CBSV or UCBSV by whiteflies to validate the whitefly transmission method for varieties with contrasting levels of resistance to CBSD. Albert is susceptible to CBSD, Kiroba is tolerant with delayed expression of root symptoms, and Kaleso is resistant with no root symptoms but with mild leaf symptoms (Maruthi et al. 2014). Thirty plants of each variety were each inoculated with 20-25 viruliferous whiteflies that were given an AAP and IAP of 24 h each.

Sap-inoculation of CBSVs. Cassava plants of var. Albert and TMS60444 were each inoculated with sap extracted from either CBSV- or UCBSV-infected cassava plants (Mohammed et al. 2012). A total of 120 plants were inoculated in this experiment which contained three replications with 10 plants in each replication for each virus species (3 replications x 10 plants x 2 varieties x 2 virus species = 120). Plants inoculated with buffer alone served as controls. The efficiency of sap transmission of UCBSV and CBSV was determined by assessing the presence or absence of the virus by RT-PCR.

Transmission of CBSVs by leaf picking. Shoots of cassava plants containing tender leaves are picked/ snapped by women in some countries of SSA for use as a leafy vegetable. We mimicked this process by picking leaves alternately between virus-infected and virus-free plants of three-month-old var. Albert and TMS60444. This was done in an attempt to transmit the virus from diseased to healthy plants by hands that become contaminated with plant sap in the process of leaf picking. Similar to the above experiments, a total of 120 plants were used in the experiment and tested for virus infection by RT-PCR after six months. Leaf picking between healthy plants served as a control.

Transmission of CBSVs by contaminated tools. Farmers use machetes for cutting stems of cassava plants to produce stem cuttings for planting material. We imitated this process by alternately cutting stems of virus-infected and virus-free cassava plants of var. Albert and TMS60444 using a pair of secateurs. A single cut to the stem of an infected stem was followed by a cut to the stem of a healthy plant of the same variety. Following this process, 30 cuttings were made for each variety and virus type in a three replicate experiment, giving a total of 120

inoculated plants. Ten plants of each variety cut between virus-free plants only were used as a control. Virus infection status of the plants was tested by RT-PCR after six months.

Transmission of CBSVs by grafting. Three month old plants of var. Albert and TMS60444 were graft-inoculated (Mohammed et al. 2012) to compare the transmission efficiency of UCBSV and CBSV in comparison with other non-vector transmission methods tested in this study. The experiment was repeated three times with a total of 120 plants graft-inoculated for each variety. Ten plants of each variety were graft-inoculated with healthy scions as controls. The efficiency of transmission of UCBSV and CBSV by this method was determined after a period of six months by RT-PCR. All plants used in the laboratory study were kept in controlled environment (28 ± 5 ºC, 60% relative humidity, 14 h light:12 h dark) for symptom development and examined for virus infection by RT-PCR (Abarshi et al. 2010, 2012) three months after virus inoculation.

Screenhouse simulation of CBSD spread. A 20 m x 8 m insect-proof screenhouse, at Kibaha Research Station, Kibaha, Tanzania, was used to establish an experiment that aimed to simulate field-based spread of CBSD. In one half of the screenhouse, a spreader plot of CBSD-infected cuttings (var. Kiroba) was planted in the soil using a spacing of 0.5 m x 0.5 m. Once these plants had sprouted, CBSD-free cuttings obtained from virus-indexed tissue culture plants of var. Kiroba were planted in 10 litre pots in the second half of the screenhouse. These were arranged in four blocks of 60 plants each, at increasing distances from the spreader, with block 1 closest to the spreader, and block 4 furthest away. Each block was further divided into four replicates, each of which comprised three rows of five plants. Plants within replicates were spaced at 0.5 m x 0.5

m, whilst there were 1 m gaps between replicates and between blocks. The central rows of each block were 2 m (block 1), 4 m (block 2), 6 m (block 3) and 8 m (block 4) distant from the closest row in the spreader plot.

Four weeks after the potted test plants had been planted (4 WAP), >1000 field-collected adult *B. tabaci* were introduced to the central part of the spreader plot that was most distant from the blocks of test plants. Whiteflies were introduced directly to the infected spreader plants at this position, and were subsequently able to move freely from plant to plant and through the screenhouse. From 4 WAP, and at approximately weekly intervals, CBSD symptom presence/absence, CBSD severity and whitefly abundance were recorded for all test plants as described previously. Whiteflies were also counted on the 18 plants making up the row of the spreader plot that was adjacent to the 2 m block of test plants.

B. tabaci population increase on the spreader plot began to produce physical damage to spreader plants from 13 WAP, so these plants were cut back to 15 cm above ground level (ratooned) and allowed to re-sprout. This action had the additional intended effect of encouraging movement of whiteflies from the spreader to the test plots. Record taking resumed approximately one month after ratooning, and was continued for an additional five weeks.

Field transmission of CBSVs. A field experiment was established at Kibaha Research Station, Kibaha, Coast Region, Tanzania, in order to examine the spatio-temporal pattern of CBSD spread into initially CBSD-free plants. Tissue culture derived plants of var. Kiroba were hardened off in an insect-proof screenhouse before being planted out in the field as stem cuttings

in an experimental trial. The trial comprised one 50-plant 'spreader' plot and five 20-plant test plots. All plots were planted at the standard spacing of 1 m x 1 m. The spreader plot was planted with 10 rows of five plants each, and cuttings used for this plot were obtained from CBSD-infected parent plants. Each of the five test plots was made up of four rows of five plants, and there was a spacing of 2 m between all plots. One test plot was adjacent to the spreader. Other test plots were situated on the distal side of the first test plot with respect to the spreader, and at increasing distances from it (2 m from spreader, 7 m, 12 m, 17 m and 22 m).

The spreader plot was planted one month before the test plots, in order to encourage vector spread from the spreader to the neighbouring test plots. Starting at two months after test plot planting (2 MAP), records were taken for all test plot plants of the presence/absence of foliar CBSD symptoms, the severity of those symptoms and numbers of the whitefly vector, *B. tabaci*. Severity was assessed using the standard 1-5 scoring system in which '1' corresponds to symptom-free, '2' to the mildest symptoms and '5' the most severe symptoms (Hillocks et al. 2001; Hillocks and Jennings 2003). Whitefly abundance was assessed by counting the number of adult *B. tabaci* on the top five leaves of each plant. Data were recorded up to 6 MAP.

RESULTS

Verifying the transmission of CBSV by whiteflies. Initial experiments to verify the transmission of CBSV by whiteflies involved using long virus AAP and IAPs of up to five days as well as using low and high numbers of whiteflies. The highest rate of virus transmission was recorded (53.0%) when 50-100 insects that had up to five days each AAP and IAPs were used in the experiments (Table 1). A slightly lower rate of transmission was achieved (40.0%) when 50-

60 whiteflies that emerged from CBSD-affected cassava plants inoculated each target plant. The efficiency of transmission was further reduced (to 30.0%) when only 20-25 insects were used. Although the highest rate of transmission was achieved using a large number of insects (50-100), we used 20-25 whiteflies in subsequent experiments to prevent feeding damage to the test plants caused by high whitefly numbers.

Investigating the mode of CBSV transmission by whiteflies. Whiteflies that had an AAP of 5-10 min were able to acquire and transmit CBSV to 12.0% of inoculated plants. Whiteflies that had 30 min and 1 h AAP transmitted CBSV to 20.0% and 16.0% of the plants, respectively (Table 2). The rate of transmission increased to 25.0% and 40.0% with the increase in AAP to 24 h and 48 h, respectively. Viruliferous whiteflies that were previously fed on healthy cassava plants for 24 h or 48 h did not transmit CBSV to the second batch of healthy cassava plants, indicating that whiteflies lost the virus within 24 h after virus acquisition (Table 2).

Determining optimum AAP, IAP and retention of CBSV in whiteflies. This experiment reconfirmed that CBSV can be acquired within 5-10 min of whitefly feeding on CBSD-affected plants (Table 3). The maximum rate of transmission (45.0%) was achieved at 24 h AAP, although this was not significantly different from those that had AAPs of 1 h, 4 h, and 48 h. Whiteflies were also able to transmit CBSV within 5-10 min (IAP) of feeding on a diseased plant (Table 3). Maximum transmission (60.4%) was achieved when feeding for 24 h.

In the experiment to determine the retention of CBSV by the vector, whiteflies were given a 24 h AAP on CBSD-affected cassava plants. None of the viruliferous whiteflies fed on healthy

cassava plants for 48 h and subsequently transferred to a new batch of healthy cassava plants transmitted CBSV, again confirming that whiteflies lost the ability to transmit the virus by 48 h after acquisition.

Effect of leaf age, virus species and cassava varieties on virus transmission. Whiteflies that fed on younger leaves with no or early symptoms of CBSD achieved a slightly higher rate of transmission (36.3%) compared to those fed on older but fully symptomatic leaves (28.5%). In the experiment conducted to compare the transmission efficiencies of the two viruses, the rate of CBSV transmission (40.0%) was slightly higher than that of UCBSV (34.5%), although this difference was not statistically significant. The rate of transmission also varied when cassava varieties differing in disease resistance levels were challenged by whitefly inoculations. Maximum infection levels (56.6%) were seen on the susceptible var. Albert by CBSV while none of the resistant var. Kaleso plants were infected by UCBSV (Table 4). Differences in transmission of the CBSVs to the three varieties were statistically significant ($F = 29.7$; $P < 0.001$). Infection of both var. Albert and Kiroba was greater than that for var. Kaleso.

Verifying non-vector transmission of CBSVs. CBSV, but not UCBSV, was transmitted at low levels by sap-inoculation from infected cassava to virus-free cassava plants (Table 5). Up to eight weeks was required for CBSD symptom expression on the sap-inoculated plants.

In the experiment conducted to verify the transmission of CBSVs by leaf picking, none of the tested plants from var. Albert and TMS 60444 expressed CBSD symptoms for the two viruses. All plants were also negative for CBSVs when tested by RT-PCR (Table 5).

Similarly, none of the plants showed CBSD symptoms six months after planting in the experiment conducted to verify the transmission of CBSVs by contaminated secateurs. The viruses were also not detected by RT-PCR in these plants (Table 5).

In contrast, inoculation of viruses by grafting produced maximum infection levels. CBSV was transmitted with 100% efficiency to both varieties while the efficiency of UCBSV transmission was slightly lower at 77-80% (Table 5). The time taken for symptom expression between the viruses also varied. Plants infected with CBSV expressed symptoms in 1-2 weeks, while the UCBSV-infected plants took 4-5 weeks. All the symptomatic plants tested positive for virus infection by RT-PCR, and the asymptomatic and control plants tested negative.

Screenhouse simulation of CBSD spread.

i. Whitefly abundance. Whiteflies were first recorded from test plots one week after their introduction, but over the course of the first four weeks of records (4-7 WAP) spread to reach block 4, which was most distant from the spreader (Figure 1). By 8 WAP a strong abundance gradient was established running from block 1 to block 4, and this was maintained up to 11 WAP. Overall whitefly abundance declined just before the spreader plot was ratooned (13 WAP), but then increased again from 18 WAP up to the final three weekly records (20-22 WAP). ANOVA results demonstrated a clear gradient in whitefly abundance at 18 WAP running from block 1 to 4 (Table 6; $F = 10.0$, $P < 0.001$, df = 15), but there were no significant differences between blocks by the time of the final data record at 22 WAP ($F = 1.1$, $P = 0.38$, df = 15).

ii. CBSD incidence. The first symptoms of CBSD in test plants were recorded in block 2 at 8 WAP (Figure 2). CBSD was restricted to blocks 1 and 2 (maximum distance 4 m) up to 13 WAP. Incidences increased greatly in all blocks following the ratooning of the spreader – from 18 WAP onwards. There were strong gradients in the incidence of CBSD from the nearest to the furthest blocks away from the spreader from 18-20 WAP, after which the disease became more generally distributed. Statistically significant gradients were seen in CBSD incidences for both the 18 WAP and 22 WAP datasets (Table 6).

It was evident both from the graphical representation of the data (Figures 1-2) and the statistical analyses (Table 6) that gradients in whitefly abundance corresponded with those for CBSD incidences. In order to examine this further, Pearson's correlation analyses were run to relate mean whitefly abundances to CBSD incidences for corresponding plots, using both the 18 WAP and 22 WAP datasets (Table 7). The strongest correlation was obtained with whiteflies at 18 WAP and CBSD at 22 WAP. In addition, there was a strongly significant linear regression relationship between whitefly abundance at 18 WAP and CBSD incidence four weeks later (CBSD = 0.28 + 0.018 WF; $F = 24.0$, $P < 0.001$, $r^2 = 0.63$). Taken together, the results demonstrated that CBSD spreads through an array of cassava plants following dispersal through those plants of the *B. tabaci* whitefly vector, and that clear and steep gradients in the spread of both are apparent during this process.

Field transmission of CBSVs.

i. Whitefly abundance. The number of whiteflies ranged from 5 to 15 through the entire plot when recording started at 4 WAP, with the exception of the most distant plot from the spreader in which whitefly abundance was generally lower for the duration of the experiment (Figure 3). The numbers increased steadily and reached a maximum of 70 adults per plant by 6 WAP. They then decreased gradually reaching almost zero in the period from 10-12 WAP due to a prolonged dry period. The whitefly numbers never subsequently recovered and on average numbered 1-2 insects per plant for the duration of the experiment (22 WAP).

ii. CBSD incidence. The first symptoms of CBSD on test plants were recorded at 4 WAP, 2 m and 7 m from the spreader plot (Figure 4). Incidences of CBSD appeared at 17 m from the spreader plot starting from 7 WAP. The first symptoms at 12 m from the spreader plot were observed at 8 WAP. There was a strong gradient of declining CBSD incidence from the test plot nearest to the spreader plot (2 m) to the plot that was 12 m from the spreader. This gradient was sustained from 7 WAP to the end of the experiment at 22 WAP. Disease incidences were generally low at 12 and 17 m from the spreader, and not recorded at 22 m.

DISCUSSION

Research into CBSD and its causal viruses (CBSV and UCBSV) has increased greatly since the spread of the disease was reported into previously unaffected parts of East Africa (Alicai et al. 2007). However, the mechanisms of transmission of these viruses remain poorly characterized. Our results respond to several of the key questions on transmission and epidemiology. Initial experiments confirmed that CBSV can be transmitted by *B. tabaci* adults under laboratory conditions. The rate of transmission, however, was moderate (maximum 53%) even when using high whitefly numbers (50-100 per plant) and with prolonged acquisition and inoculation access

periods of up to five days, or when using whiteflies that had emerged from CBSD-affected plants. These results were, however, similar to previous findings (Maruthi et al. 2005; Mware et al. 2009) and further confirmed the generally moderate efficiency of CBSV transmission by *B. tabaci*. Experiments investigating the time required for virus acquisition revealed that CBSV can be acquired within 5-10 min of feeding on diseased plants, although the rate of transmission achieved from this short AAP was low (12%). Increasing the AAP to 24 h resulted in a significantly increased transmission efficiency (45%), although efficiency of transmission was more-or-less the same for all AAPs between 1 h and 48 h. The shortest time period used (5-10 min) for IAPs resulted in 19% infected plants, confirming that CBSV can be both acquired and inoculated in very short periods of time. Notably, the combination of an AAP of 48 h with an IAP of 24 h resulted in 60% of plant infections, which represents a relatively high level of transmission efficiency. When viruliferous whiteflies are placed on uninfected host plants for 24 h or 48 h, and then transferred to a further set of uninfected hosts plants for 48 h, no infections result. This suggests that *B. tabaci* whiteflies do not retain CBSV for long after leaving infected plants. Put together, our results indicate that CBSV is semi-persistently transmitted by *B. tabaci*. In comparison, the persistently transmitted begomoviruses that co-infect cassava in Africa required a similar inoculation period (5-10 min) but much longer acquisition (minimum 3.5 h), latent (minimum 3.5 h) and retention periods (9 days) (Dubern 1994). The transmission of CBSV, by contrast, seems to be comparable to other whitefly-transmitted ipomoviruses such as *Squash vein yellowing virus* (SqVYV) in the USA (Webb et al. 2012) and *Cucumber vein yellowing virus* (CVYV) in the Middle-East (Harpaz and Choen 1965; Mansour and Al-Musa 1993). SqVYV was acquired and transmitted in 30 min with moderate transmission efficiency (50%) using 25-35 whiteflies per plant at 24 h AAP and 24 h IAP. Whiteflies' retention of

SqVYV declined rapidly after they were removed from infected plants (infection rate dropped from 76% to 20% after 1 h) and they lost the ability to transmit the virus completely within 8–24 h (Webb et al. 2012). Transmission of CVYV was also moderately efficient. Virus acquisition and inoculation occurred within 10-20 min, but required 30–35 whiteflies to reach a maximum transmission rate of 80%. Persistence in the vector was also short, with a dramatic decrease in transmission from 81 to 14% after 2 h (Harpaz and Choen 1965). Similar results were obtained using another isolate of CVYV in the 1990s (Mansour and Al-Musa 1993), indicating that regardless of the geographical location, the different whitefly species used in transmission experiments or the host plants they infect – ipomoviruses are generally transmitted with only moderate efficiency by their whitefly vectors and are only retained for short periods after the removal of the vector from an infected host. There could be several reasons for the moderate transmission efficiency of ipomoviruses, including the presence or absence of endosymbiotic bacteria such as *Hamiltonella*, which has been shown to influence transmission rates for other whitefly/virus pathosystems (Gottlieb et al. 2010). Neither the species from the *B. tabaci* cryptic species complex used in the transmission of SqVYV or CVYV, nor their endosymbiont infection status, were reported. However, the absence of *Hamiltonella* in the *B. tabaci* Mediterranean (MED) species made it an inefficient vector of the geminivirus *Tomato yellow leaf curl virus* (Gottlieb et al. 2010). *Hamiltonella* was also absent in the cassava whitefly species SSA1-SG1 used in this study (Ghosh et al. 2015), which might partly explain the moderate rates of CBSV transmission achieved using this species. Another reason for the moderate transmission of CBSV by the whiteflies could be due to the low quantities of virus present in infected plants. Studies have shown the widely varying titres of CBSVs that occur in different cassava varieties, both in laboratory and field conditions (Kaweesi et al. 2014; Maruthi et al. 2014). The var.

Ebwanateraka used as the main virus source plant in this study generally exhibited severe symptoms of CBSD, but its virus titre is not known and should be investigated in future studies.

Experiments comparing the transmission of the two CBSD-causing viruses – CBSV and UCBSV – showed that both were transmitted to the susceptible var. Albert, tolerant var. Kiroba and resistant var. Kaleso, although at differing efficiencies. UCBSV was only transmissible to Albert and Kiroba, but not to Kaleso. This could be due to the relatively mild nature of the virus and low virus quantities in infected plants (Mohammed et al. 2012; Winter et al. 2010). CBSV in comparison was transmitted to all three varieties with different efficiencies, including the resistant var. Kaleso, confirming that whiteflies play a significant role in virus spread in the field irrespective of the variety that is grown. Experiments confirmed that neither leaf picking nor the use of contaminated tools for cutting stems resulted in CBSV transmission. It is therefore concluded that neither of these widespread practices contribute to the epidemiology of CBSD in the field, as had been suspected by some researchers. Circumstantial evidence further confirms this finding, since leaf picking is practiced in some regions of East Africa and not in others, and there is no apparent association between the incidence of CBSD and the prevalence of leaf picking. Similarly, if stem cutting resulted in transmission, significant increases in incidence might be anticipated even in areas where whiteflies are infrequent, which does not match with field data (Jeremiah et al. 2015; Legg et al. 2011).

CBSV was poorly transmitted by mechanical inoculation of sap extracted from diseased cassava leaves, while UCBSV was not transmitted at all, further indicating that this might be to do with the relatively low titres in infected plants or mild nature of the virus. Graft transmission of both viruses, however, resulted in 100% transmission, indicating that grafting remains the most reliable way of transmitting the two viruses under laboratory conditions.

Epidemiology experiments run in both confined screenhouse and open field conditions in coastal Tanzania showed that CBSD spread along a clearly-defined gradient from CBSD-diseased spreader plots. The gradient of spread was relatively steeper in the screenhouse, probably since whiteflies were initially introduced from only one side (in the spreader plot) and wind speeds were lower. In both experiments there was a clear association between the abundance of *B. tabaci* whiteflies (known to be SSA1-SG3 in coastal Tanzania) and new CBSD infections, both in space and through time. Over the eight months that data were recorded in the field experiment, the furthest distance that CBSD infections were recorded from the spreader plot was 17 m. Both of these experiments emphasize the relatively short distances over with CBSVs are spread – a result which is strongly congruent with the semi-persistent transmission mechanism described from the laboratory-based experiments.

The results of our experiments present a consistent picture for the pattern of transmission of CBSVs by the whitefly vector – *Bemisia tabaci*. The semi-persistent transmission characteristic of these viruses fits well with regional-level epidemiological data (Legg et al. 2011), which have shown the contrasting spread characteristics of the CBSD and CMD pandemics. The pandemic of severe CMD spread through East and Central Africa as an advancing 'front', in which super-abundant cassava *B. tabaci* moved together with cassava mosaic geminivirus (CMG) species mixtures that caused severe CMD. By contrast, CBSVs did not move together with these whitefly populations, but were apparently picked up and rapidly spread at locations where CBSD already occurred, or where it had been inadvertently introduced through infected planting material. As well as helping to explain how these cassava virus pandemics are spreading, knowledge of the semi-persistent transmission mechanism also allows us to design appropriate and effective control strategies. The relatively poor retention of CBSVs by *B. tabaci*, and

associated short gradients of spread, mean that isolation is likely to be more effective in preventing infection from neighboring virus sources than it would be for the CMGs. This effect is strengthened by the fact that there are currently no known alternative hosts for CBSVs other than *Manihot glaziovii* (Muel.-Arg.) (Mbanzibwa et al. 2011), unlike the CMGs, for which a much greater range of alternative hosts has been documented (Ogbe et al. 2006). By far the greatest threat of long-distance spread of CBSVs comes from the inadvertent carriage by people of stems or stem cuttings of infected cassava. This is a much greater problem for CBSD than it is for CMD, since the symptoms of CBSD are cryptic and inconspicuous, while those of CMD are readily recognized. This highlights the importance of applying rigorous phytosanitary standards when multiplying and disseminating cassava germplasm obtained from regions affected by CBSD. New programmes aimed at boosting cassava production and promoting the safe exchange of cassava germplasm will be strengthened through being fully cognisant of the contrasting virus transmission and field spread characteristics of the viruses that cause CBSD and CMD.

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References

Abarshi, M. M., Mohammed, I. U., Jeremiah, S. C., Legg, J. P., Kumar, L., Hillocks, R. J., and Maruthi, M. N. 2012. Multiplex RT-PCR assays for the simultaneous detection of both RNA and DNA viruses infecting cassava and the common occurrence of mixed infections by two cassava brown streak viruses in East Africa. J. Virol. Methods 179:176–184.

Abarshi, M. M., Mohammed, I. U., Wasswa, P., Hillocks, R. J., Holt, J., Legg, J. P., Seal, S. E., and Maruthi, M. N. 2010. Optimization of diagnostic RT-PCR protocols and sampling procedures for the reliable and cost-effective detection of Cassava brown streak virus. J. Virol. Methods 163:353–359.

Alicai, T., Omongo, C. A., Maruthi, M. N., Hillocks, R. J., Baguma, Y., Kawuki, R., Bua, A., Otim-Nape, G. W., and Colvin, J. 2007. Re-emergence of cassava brown streak disease in Uganda. Plant Dis. 91:24–29.

Bigirimana, S., Barumbanze, P., Ndayihanzamaso, P., Shirima, R., and Legg, J. P. 2011. First report of cassava brown streak disease and associated *Ugandan cassava brown streak virus* in Burundi. New Dis. Rep. 24:26.

Bock, K. R. 1994. Studies on cassava brown streak virus disease in Kenya. Trop. Sci. 34:134–145.

Dubern, J. 1994. Transmission of African cassava mosaic geminivirus by the whitefly (*Bemisia tabaci*). Trop. Sci. 34:82-91.

Gottlieb, Y., Zchori-Fein, E., Mozes-Daube, N., Kontsedalov, S., Skaljac, M., Brumin, M., Sobol, I., Czosnek, H., Vavre, F., Fleury, F., and Ghanim, M. 2010. The transmission efficiency of *Tomato yellow leaf curl virus* by the whitefly *Bemisia tabaci* is correlated with the presence of a specific symbiotic bacterium species. J. Virol. 84:9310–9317.

Ghosh, S., Bouvaine, S., and Maruthi, M. N. 2015. Prevalence and genetic diversity of endosymbiotic bacteria infecting cassava whiteflies in Africa. BMC Microbiol. 15:93. Published online.

Harpaz, I. and Cohen, S. 1965. Semipersistent relationship between cucumber vein yellowing virus (CVYV) and its vector, the tobacco whitefly (*Bemisia tabaci* Gennadius) Phytopathology Z. 54:240-248.

Hillocks, R. J., and Jennings, D. L. 2003. Cassava brown streak disease: a review of present knowledge and research needs. Int. J. Pest Manage. 49:225–234.

Hillocks, R. J., Raya, M., Mtunda, K., and Kiozia, H. 2001. Effects of brown streak virus disease on yield and quality of cassava in Tanzania. J. Phytopathology 149:1–6.

Jeremiah, S. C., Ndyetabula, I. L., Mkamilo, G. S., Haji, S., Muhanna, M. M., Chuwa, C., Kasele, S., Bouwmeester, H., Ijumba, J. N., and Legg, J. P. 2015. The dynamics and environmental influence on interactions between cassava brown streak virus disease and the whitefly, *Bemisia tabaci*. Phytopathology 105:646-655.

Kaweesi, T., Kawuki, R., Kyaligonza, V., Baguma, Y., Tusiime, G., and Ferguson, M. E. 2014. Field evaluation of selected cassava genotypes for cassava brown streak disease based on symptom expression and virus load. Virology J. 11:216. Published online.

Lister, R. M. 1959. Mechanical transmission of cassava brown streak virus. Nature 183:1588-1589.

Legg, J. P., Jeremiah, S. C., Obiero, H. M., Maruthi, M. N., Ndyetabula, I., Okao-Okuja, G., Bouwmeester, H., Bigirimana, S., Tata-Hangy, W., Gashaka, G., Mkamilo, G., Alicai, T., and Kumar, P. L. 2011. Comparing the regional epidemiology of the cassava mosaic and cassava brown streak pandemics in Africa. Virus Res. 159:161–170.

Legg, J. P., Kumar, P. L., Makeshkumar, T., Tripathi, L., Ferguson, M., Kanju, E., Ntawuruhunga, P., and Cuellar, W. 2015. Cassava virus diseases: biology, epidemiology, and management. Adv. Virus Res. 91:85-142.

Legg, J. P., Somado, E. A., Barker, I., Beach, L., Ceballos, H., Cuellar, W., Elkhoury, W., Gerling, D., Helsen, J., Hershey, C., Jarvis, A., Kulakow, P., Kumar, L., Lorenzen, J., Lynam, J., McMahon, M., Maruthi, G., Miano, D., Mtunda, K., Ntawuruhunga, P., Okogbenin, E., Pezo, P., Terry, E., Thiele, G., Thresh, M., Wadsworth, J., Walsh, S., Winter, S., Tohme, J., and Fauquet, C. 2014. A global alliance declaring war on cassava viruses in Africa. Food Secur. 6:231–248.

Lennon, A. M., Aiton, M. M. and Harrison, B. D. 1986. Cassava viruses from South America. Report of the Scottish Crops Research Institute, Dundee, UK, p. 167.

Mahungu, N. M., Bidiaka, M., Tata, H., Lukombo, S., and N'luta, S. 2003. Cassava brown streak disease-like symptoms in Democratic Republic of Congo. Roots 8:8–9.

Mansour, A. and Al-Musa, A. 1993. Cucumber vein yellowing virus; host range and virus vector relationships. J. Phytopathology 137:73-79.

Maruthi, M. N., Bouvaine, S., Tufan, H. A., Mohammed, I. U., and Hillocks, R. J. 2014. Transcriptional response of virus-infected cassava and identification of putative sources of resistance for cassava brown streak disease. PLoS ONE 9:e96642.

Maruthi, M. N., Colvin, J. and Seal, S. 2001. Mating compatibility, life-history traits, and RAPD-PCR variation in *Bemisia tabaci* associated with the cassava mosaic disease pandemic in East Africa. Entomol. Exp. Appl. 99:13-23.

Maruthi, M. N., Hillocks, R. J., Mtunda, K., Raya, M. D., Muhanna, M., Kiozia, H., Rekha, A. R., Colvin, J., and Thresh, J. M. 2005. Transmission of Cassava brown streak virus by Bemisia tabaci. J. Phytopathology 153:307-312.

Mohammed, I. U., Abarshi, M. M., Muli, B., Hillocks, R. J., and Maruthi, M. N. 2012. The symptoms and genetic diversity of cassava brown streak viruses infecting cassava in East Africa. Adv. Virol. 2012:Article ID 795697. Published online.

Monger, W. A., Alicai, T., Ndunguru, J., Kinyua, Z. M., Potts, M., Reeder, R. H., Miano, D. W., Adams, I. P., Boonham, N., Glover, R. H., and Smith, J. 2010. The complete genome sequence of the Tanzanian strain of Cassava brown streak virus and comparison with the Ugandan strain sequence. Arch. Virol. 155:429–433.

Mbanzibwa, D. R., Tian, Y. P., Mukasa, S. B., and Volkonen, Y. P. T. 2009. *Cassava brown streak virus* (*Potyviridae*) encodes a putative Maf/HAM1 pyrophosphatase implicated in reduction of mutations and a P1 proteinase that suppresses RNA silencing but contains no HC-Pro. J. Virol. 83:6934–6940.

Mbanzibwa, D. R., Tian, Y. P., Tugume, A. K., Mukasa, S. B., Tairo, F., Kyamanywa, S., Kullaya, A., and Valkonen, J. P. T. 2011. Simultaneous virus-specific detection of the two cassava brown streak-associated viruses by RT-PCR reveals wide distribution in East Africa, mixed infections and infections in *Manihot glaziovii*. J. Virol. Methods 171:394–400.

Mulimbi, W., Phemba, X., Assumani, B., Kasereka, P., Muyisa, S., Ugentho, H., Reeder, R., Legg, J. P., Laurenson, L., Weekes, R., and Thom, F. E. F. 2012. First report of *Ugandan cassava brown streak virus* on cassava in Democratic Republic of Congo. New Dis. Rep. 26:11.

Munthali, D. C. 1992. Effect of cassava variety on the biology of *Bemisia afer* (Priesner & Hosny) (Hemiptera: Aleyrodidae). Ins. Sci. Appl. 13:459–465.

Mware, B., Narla, R., Amata, R., Olubayo, F., Songa, J., Kyamanywa, S., and Ateka, E. M. 2009. Efficiency of cassava brown streak virus transmission by two whitefly species in coastal Kenya. J. Gen. Mol. Virol. 1:40–45.

Nassar, N. M. A., and Ortiz, R. 2007. Cassava improvement: challenges and impacts. J. Agric. Sci. 145:163-171.

Ogbe, F. O., Dixon, A. G. O., Huges, J. d'A., Alabi, O. J., and Okechukwu, R. 2006. Status of cassava begomoviruses and their new natural hosts in Nigeria. Plant Dis. 90:548-553.

Storey, H. H. 1936. Virus diseases on East African plants. VI. A progress report on the studies of the diseases of cassava. East Afr. Agric. J. 2:34–39.

Storey, H. H. 1939. Report of the plant pathologist. East African Agricultural Research Station Reports for 1939. Empire Cotton Growing Corporation. p.9.

Webb, S. E., Adkins S., and Reitz, S. R. 2012. Semipersistent whitefly transmission of *Squash vein yellowing virus*, causal agent of viral watermelon vine decline. Plant Dis. 96:839-844.

Winter, S., Koebler, M., Stein, B., Pietruszka, A., Paape, M., and Butgereitt, A. 2010. The analysis of cassava brown streak viruses reveals the presence of a distinct virus species causing cassava brown streak disease in East Africa. J. Gen. Virol. 91:365-376.

Table 1. Initial experiments to verify the whitefly, *B. tabaci*, as the vector of CBSV

No. of whiteflies used to inoculate each plant	AAP	IAP	No. of plants infected/inoculated	% transmission achieved
20-25	4 days	5 days	7/20	30.0
50-100	4 days	5 days	14/26	53.0
50-60	Whiteflies emerging from CBSD-infected cassava plants	5 days	4/10	40.0

Table 2. Investigating the mode of CBSV transmission by the cassava whitefly, *B. tabaci*

Mode of transmission tested	No. of whiteflies per plant	AAP	IAP	No. of plants infected/ inoculated	% transmission achieved
Non-persistent mode of transmission	20-25	5-10 min	48 h	3/25	12.0
	20-25	30 min	48 h	5/25	20.0
	20-25	1 h	48 h	4/25	16.0
Semi-persistent mode of transmission	20-25	24 h	48 h	5/20	25.0
	20-25	48 h	48 h	8/20	40.0
Persistent mode of transmission	10-20	24 h	48 h + 48 h	0/15	0
	7-20	48 h	48 h + 48 h	0/15	0

Table 3. Determining AAP and IAP of CBSV in the cassava whitefly, *B. tabaci*

	Determining AAP for CBSV on cassava[A]		Determining IAP for CBSV on cassava[B]	
Time period	Total no. of plants infected/ inoculated	% infected plants	Total no. of plants infected/ inoculated	% infected plants
5-10 min	4/25	16.0	6/31	19.3
30 min	8/25	32.0	7/33	21.2
1 h	10/25	40.0	8/39	20.5
4 h	6/15	40.0	13/35	37.1
24 h	9/20	45.0	29/48	60.4
48 h	6/15	40.0	6/15	40.0

[A]Viruliferous whiteflies were given a standard 48 h IAP for testing different AAPs

[B]Viruliferous whiteflies were given a standard 48 h AAP for testing different IAPs

Table 4: Comparison of transmission of UCBSV and CBSV on three cassava varieties by whitefly inoculations

	No. of cassava plants infected/ inoculated for UCBSV			No. of cassava plants infected/ inoculated for CBSV		
Variety	Albert	Kiroba	Kaleso	Albert	Kiroba	Kaleso
Replication 1	5/10	4/10	0/10	6/10	6/10	0/10
Replication 2	3/10	3/10	0/10	6/10	5/10	0/10
Replication 3	7/10	6/10	0/10	5/10	3/10	1/10
Total	15/30	13/30	0/30	17/30	14/30	1/30
% infected plants	50.0	43.3	0	56.6	46.6	3.3

Table 5: Summary of non-vector modes of transmission verified for UCBSV and CBSV

Treatment	Control – No. of plants infected/ inoculated		Time taken for first plant developing symptoms (week)		No. of plants +ve for virus by RT-PCR/ no. tested		Efficiency of virus transmission (%)	
	For UCBSV	For CBSV	UCBSV	CBSV	UCBSV	CBSV	UCBSV	CBSV
Transmission of CBSVs by extracted sap								
Albert	0/10	0/10	-	8	0/30	5/30	0	16.6
TMS 60444	0/10	0/10	-	7	0/30	7/30	0	23.3
Transmission of CBSVs by leaf picking								
Albert	0/10	0/10	-	-	0/30	0/30	0	0
TMS 60444	0/10	0/10	-	-	0/30	0/30	0	0
Transmission of CBSVs by contaminated tools								
Albert	0/10	0/10	-	-	0/30	0/30	0	0
TMS 60444	0/10	0/10	-	-	0/30	0/30	0	0
Transmission of CBSVs by graft-inoculation								
Albert	0/10	0/10	5	2	23/30	30/30	77	100
TMS 60444	0/10	0/10	4	1	24/30	30/30	80	100

Table 6: CBSD incidence and *B. tabaci* whitefly abundance in a screenhouse at Kibaha Research Station, Tanzania

Distance from spreader (m)	CBSD incidence (%) 18 WAP	CBSD incidence (%) 22 WAP	Whitefly abundance 18 WAP	Whitefly abundance 22 WAP
2	65.0a (7.4)	83.3a (4.3)	28.0a (5.5)	71.3a (42.8)
4	18.3b (5.0)	55.0b (4.2)	9.0b (4.4)	132.4a (79.4)
6	6.7b (4.7)	23.3c (9.6)	3.9b (2.2)	54.2a (13.1)
8	8.3b (6.3)	26.7c (7.2)	2.3b (1.3)	17.4a (2.8)

Means compared using the Holm-Sidak procedure. Values with different letters were significantly different at the $P = 0.05$ level. Values in brackets are standard errors. WAP – weeks after planting.

Table 7: Pearson's correlation analyses relating *B. tabaci* abundance with CBSD incidence for the 16 test plots (four per block) within the screenhouse trial, Kibaha, Tanzania

Comparison	R	P	n
Wf 18 WAP vs CBSD 18 WAP	0.77	0.0006 ***	16
Wf 22 WAP vs CBSD 22 WAP	0.29	0.27 ns	16
Wf 18 WAP vs CBSD 22 WAP	0.80	0.0002 ***	16

***P = highly significant, at 0.001 level

Captions for figures:

Figure 1, Maruthi, *Phytopathology*, Spatio-temporal distribution of *Bemisia tabaci* whiteflies on initially CBSD-free cassava plants under screenhouse conditions, Kibaha, Tanzania. Values in boxes are mean numbers of adult *B. tabaci*

Figure 2, Maruthi, *Phytopathology*, Spatio-temporal spread of CBSD into initially CBSD-free cassava plants under screenhouse conditions, Kibaha, Tanzania. Values in the boxes are percent CBSD incidence

Figure 3, Maruthi, *Phytopathology*, Spatio-temporal distribution of *Bemisia tabaci* whiteflies on initially CBSD-free cassava plants in the field, Kibaha, Tanzania. Values in boxes are mean numbers of adult *B. tabaci*

Figure 4, Maruthi, *Phytopathology*, Spatio-temporal spread of CBSD into initially CBSD-free cassava plants in the field, Kibaha, Tanzania. Values in the boxes are percent CBSD incidence

Figure 1, Maruthi, *Phytopathology*, Spatio-temporal distribution of *Bemisia tabaci* whiteflies on initially CBSD-free cassava plants under screenhouse conditions, Kibaha, Tanzania. Values in boxes are mean numbers of adult *B. tabaci*

		Date of Observation (weeks after planting)														
		4	5	6	7	8	9	10	11	12	13	18	19	20	21	22
Distance from spreader	8 m	0	0	0	0.1	3.3	6.1	7.9	13.7	31.6	8.4	2.3	13.9	20.1	28.1	17.4
	6 m	0	0	0	0	2.8	5.0	7.8	18.6	28.9	4.1	3.9	14.8	51.7	46.6	54.2
	4 m	0	0	0.1	0.2	16.4	16.9	26.9	80.4	63.2	22.5	9.0	31.3	80.1	92.9	132.4
	2 m	0	0.6	0.3	2.6	34.0	26.7	68.2	103.6	42.5	42.8	28.0	58.6	102.6	68.1	71.3
	Spreader	1.2	1.5	8.6	49.7	79.2	119.1	160.9	88.1	48.1	43.5	51.2	82.1	62.8	46.4	74.0

Figure 2, Maruthi, *Phytopathology*, Spatio-temporal spread of CBSD into initially CBSD-free cassava plants under screenhouse conditions, Kibaha, Tanzania. Values in the boxes are percent CBSD incidence

		Date of Observation (weeks after planting)														
		4	5	6	7	8	9	10	11	12	13	18	19	20	21	22
from	8 m	0	0	0	0	0	0	0	0	0	0	8.3	8.3	8.3	23.3	26.7
	6 m	0	0	0	0	0	0	0	0	0	0	6.7	16.7	16.7	23.3	25.0

	4 m	**0**	**0**	**0**	**0**	**5.0**	**5.0**	**5.0**	**5.0**	**5.0**	**5.0**	**18.3**	**21.7**	**35.0**	**55.0**	**55.0**
	2 m	**0**	**0**	**0**	**0**	**0**	**6.7**	**6.7**	**6.7**	**8.3**	**11.7**	**65.0**	**75.0**	**81.7**	**83.3**	**83.3**

Figure 3, Maruthi, *Phytopathology*, Spatio-temporal distribution of *Bemisia tabaci* whiteflies on initially CBSD-free cassava plants in the field, Kibaha, Tanzania. Values in boxes are mean numbers of adult *B. tabaci*

		Date of Observation (weeks after planting)														
		4	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**18**	**19**	**20**	**21**	**22**
Distance from spreader	**22 m**	0.94	2.22	5.6	0.28	0.24	0.06	0.06	0	0.12	0.24	0.44	0.06	0	0.06	0.11
	17 m	12.0	23.0	70.0	3.7	1.0	0.9	0	0	0	0.9	0.8	1.1	0.7	1.0	0.9
	12 m	14.5	24.7	48.5	9.4	1.8	0.44	0	0	0	0.38	0.56	1.4	0.63	0.5	1.5
	7 m	9.8	16.0	33.0	5.7	0.6	0.9	0.1	0.1	0.1	0.3	1.2	0.1	0.1	1.4	2.0
	2 m	5.4	10.0	36.0	4.7	1.7	0.9	0	0	0.1	0.1	1.1	1.1	0.7	0.9	1.1
	Spreader	11.6	33.9	64.0	13.5	1.3	0.53	0	0	0	0.29	0.24	3.4	2.3	1.3	0.75

Figure 4, Maruthi, *Phytopathology*, Spatio-temporal spread of CBSD into initially CBSD-free cassava plants in the field, Kibaha, Tanzania. Values in the boxes are percent CBSD incidence

		Date of Observation (weeks after planting)														
		4	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**18**	**19**	**20**	**21**	**22**
Distance from spreader	**22 m**	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0
	17 m	0	0	0	8	0	10	5	0	0	0	0	0	0	0	0
	12 m	0	0	0	0	5.6	5.6	5.6	17	5.6	12	5.6	11	5.9	5.9	5.9

	7 m	**12**	**5.9**	**5.3**	**11**	**17**	**17**	**28**	**28**	**11**	**11**	**39**	**39**	**39**	**39**	**39**
	2 m	**11.1**	**33.3**	**15**	**25**	**45**	**47.4**	**68.4**	**52.6**	**42.1**	**57.9**	**63.2**	**68.4**	**52.6**	**68.4**	**73.7**