"TRANSMISSION AND DISTRIBUTION OF CASSAVA BROWN STREAK VIRUS IN MAJOR CASSAVA GROWING REGIONS OF KENYA"

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Declaration

I declare that this thesis is my original work and has not been presented for the award of a degree in any other University.

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Dedication

To the Almighty God

For His provision, favour, strength, patience and endurance spirit throughout the period of my studies

To my parents (Mrs Ann Mware and Mr. Peter Mware), wife (Emily Law), and relatives (Dr. Amolo and Family)

For their never ending love, encouragement, relentless moral and financial support during my studies

I'm forever grateful

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List of abbreviations

AAP	Acquisition Acess feeding period
BIO-EARN	East African Regional Programme and Research Network for Biotechnology, Bio safety and Biotechnology Policy Development
CBSD	Cassava brown streak disease
CBSV	Cassava brown streak virus
cDNA	Complementary DNA
CGM	Cassava green mite
CIAT	International Centre for Tropical Agriculture
CMD	Cassava mosaic disease
CMGs	Cassava mosaic, Gemini viruses
DNA	deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
FAO	Food Agriculture Organisation
IAP	Inoculation Access feeding period
IITA	International Institute of Tropical Agriculture
MOA	Ministry of Agriculture
M-MLV RT	Moloney Murine Leukaemia Virus Reverse Transcriptase
PCR	polymerase chain reaction
RT-PCR	reverse transcriptase polymerase chain reaction
SPMMV	Sweet potato mild mottle virus

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Abstract

Cassava brown streak virus disease (CBSD) is a viral disease that remained endemic for a long period of time in Eastern Africa exhibiting a delimited distribution along the coastal low lands. However, today there are reports of CBSD out break and re-emergence in areas where it was absent or commonly observed as endemic. Despite the fact that CBSD has been confined along the coastal cassava growing districts for a long period, there is no information on its spread and occurrence in other cassava growing regions in Kenya. Its vectors of transmission have not been clearly studied in details in Kenya, although there is a report suggesting a whitefly species *Bemisia tabaci* to be transmitting the cassava brown streak virus.

A survey to determine the prevalence, incidence and severity of CBSD in relation to whitefly populations was conducted in selected cassava growing areas of Western, Nyanza, Eastern and Central provinces of Kenya. An additional survey was conducted to determine the occurrence, distribution and host range of Spiralling whitefly *Aleurodicus dispersus* in relation to CBSD incidence in major cassava growing districts of coastal Kenya. The adult whitefly populations were directly counted on 5 representative shoots per cassava plant. Cassava leaf samples from farmers' fields in the main cassava-growing districts were collected and subjected to reverse transcription-polymerase chain reaction (RT- PCR) for detection of cassava brown streak virus (CBSV).

Green house trial on the transmission of CBSV was also carried out using two susceptible farmer preferred cassava varieties and cassava pests within cages. Arthropod pest species (mites and insects) including cassava green mite *Mononychellus tanajoa* (Bondar), cassava mealy bug *Phenacoccus manihoti* (Matile-Ferrero), spiraling whitefly *Aleurodicus*

dispersus (Russels) and the whitefly *Bemisia tabaci* (Gennadius) were used in CBSV transmission experiments. Adults of the pests were given access to CBSV by introducing them in cages with CBSV-infected cassava plants for 48-h access acquisition feeding period (AAP). This was followed by transferring them to recipient virus free CBSD-susceptible cassava plants of MM96/5280 and MM96/4466 clones in net cages for access inoculation feeding period of 48-hours (IAP). Successful transmission was confirmed by detection of CBSV in leaf samples by RT-PCR.

CBSD incidence was highest in western Kenya (46-100%) but not detected in samples from Central and Eastern provinces. The results report and confirm the presence of CBSD for the first time in Nyanza and Western provinces indicating that the disease is no longer confined along coastal lowlands of Kenya. There was a significant and positive correlation ($r=+0.6977$, $p<0.001$) between number of adult whitefly vector, *Bemisia tabaci*. The emergence of spiraling whitefly having a wide host range and a significant and positive correlation ($r=+0.5189$, $p<0.001$) with CBSD incidence in coastal Kenya is reported for the first time.

Bemisia tabaci transmitted CBSV (40.7%) whereas *Mononychellus tanajoa* (Bondar), cassava mealybug *Phenacoccus manihoti* (Matile-Ferrero), did not transmit the virus at all. However, Spiraling whitefly also showed ability to transmit CBSV at low rate of 25.9%. These results and high incidence of cassava brown streak disease (CBSD) in the field significantly coinciding with increases in whitefly numbers; further support the evidence that *B. tabaci* is a vector of CBSV and a first report of the role of spiraling whitefly in spread of CBSD .

The results of the investigations on ability of the insects collected from the infected cassava field to acquire and inoculate the virus help us in understanding the role of the whitefly species in the development and spread of CBSD in field conditions. The implication of this important finding is that host-plant resistance to whitefly may be considered as a possible method of dual control for CBSD (Hillocks *et al.*, 2005). All these findings help in understanding the epidemiology of CBSD hence an input in development of disease management strategies.

CHAPTER ONE

1.1 Introduction

1.1.1 Economic Importance Production and Utilization of Cassava

Cassava (*Manihot esculenta* Crantz) is ranked among the top 10 most significant food crops produced in developing countries (Scott, 2000) and is a major source of carbohydrate for human consumption throughout the tropics (Fauquet *et al.*, 1990). Its adaptability to relatively marginal soils and erratic rainfall conditions, high productivity per unit of land and labor, the certainty of obtaining some yield even under the most adverse conditions and the possibility of maintaining continuity of supply throughout the year make this root crop a basic component of the farming systems in many areas of Africa (Arias *et al.*, 1994). The tropical root crop cassava is the third most important source of calories in the tropics, after rice and corn. It is grown by poor farmers, many of them women, often on marginal land. For these people, the crop is vital for both food security and income generation (Were *et al.*, 2004).

According to FAO estimates, 222 million tones of cassava was produced worldwide in the year 2006 (FAO, 2009) valued at approximately 14 billion US dollars. Africa accounted for 53%, Asia for 30%, and Latin America and the Caribbean for 17% of the total world production (FAO, 2009). In 2006, Nigeria produced 45 million tones making it the world's largest producer. In terms of area harvested, a total of 18.4 million hectares was planted with cassava throughout the world in 2006 leading to an average production of 12.1tonnes per hectare (FAO, 2009). It is one of the major staple crops in sub-Saharan Africa with a total production estimated at more than 90 million tones (FAO, 2001) valued at 6 billion US dollars.

In Kenya, cassava is produced mostly through subsistence farming as an important staple food in Western and Coastal regions of the country (MOA 1999; Mbwaka, 2000) although it is also

grown for food in eastern and central provinces. The crop is grown in over 77,502 ha with an annual production of about 841,196 metric tons in the four cassava growing regions in Kenya (FAO, 2007). Cultivation is mainly in western Kenya comprising of Nyanza and Western provinces (60%), Coast Provinces (30%) and Eastern (10%) (Crop crisis project, 2006)

Cassava is a food security (consumed in fresh or processed forms) as well as cash crop, used as animal feed and raw material for industrial uses such as starch and alcohol production (Wright, 1996). Ugwu and Ay (1992) classified cassava products into 9 groups as follows; cooked fresh roots, cassava flours (fermented and unfermented), granulated roasted cassava, granulated cooked cassava, fermented pastes, sedimented starches, drinks with cassava components, leaves (cooked as vegetables) and medicine indicating the extent of diversity in utilization. As a result the crop constitutes an important source of income in rural and often marginal areas (Were *et al.*, 2004).

1.1.2 Constraints to Cassava Production in Kenya

Diseases and pests are the major factors limiting cassava production. Cassava mosaic disease caused by cassava mosaic, Gemini viruses (CMGs) (Thresh *et al.*, 1998) and cassava brown streak disease caused by cassava brown streak virus (CBSV) (Monger *et al.*, 2001) are the major biotic constraints to cassava production in Kenya. However, Cassava bacterial blight, fungal leaf spot and Alternaria root rot also attack cassava although not as serious as CBSD and CMD in Kenya. CMD has been shown to cause up to 84% yield loss for plants raised from infected cuttings (Briant and Tudburry, 1940). Moreover studies have shown that yield losses induced by CMD range from 20% to 95% depending on the variety tested, time of infection and conditions of growth (Guthrie, 1988; Fargette *et al.*, 1988). CMD has been the most important constraint to cassava production with yield loss incurred in 1998 estimated at 15,000 tons valued at US$ 10 million in western Kenya (Obiero *et al.*, 2007).

CBSD is wide spread problem in coastal Kenya (Bock, 1994) that has been reported to cause up to 70% yield loss by reducing the roots sizes and causing pitting and constriction (Hillocks *et al.*, 2001). Root necrosis caused by CBSD accounts for the quantitative and qualitative reduction in total yields through the presence of necrotic lesions or discoloration of the root rendering them unpalatable and unmarketable (Nichols, 1950).The major pests are cassava green mite CGM (*Mononychellus tanajoa*), cassava mealy bug CM, (*Phenacoccus manihoti*), root mealy bug, and whitefly (*Bemisia tabaci, B. afer*).

1.1.3 Problem Statement and Justification

CBSD is the second most important constraint affecting cassava production in eastern Africa after CMD. It is a devastating disease that causes weight loss of root production (70%) and quality (Pheneas and James, 2007). This disease was primarily known as a disease of the coastal lowland cassava growing-regions of East Africa including shores of Lake Malawi (Pheneas and Legg, 2007). However, it was not until early 2007 when CBSD re-emergence and out break occurrence was reported at high incidences (64%) in higher areas such as central Uganda (Alicai *et al.*, 2007) when it became apparent that the disease was becoming more widespread beyond 1000m a.s.l.

In Kenya CBSD had been endemic along coastal cassava growing areas with most field surveys limited to the coastal region only and little attention to other major cassava growing regions of Kenya. Even at the coastal region, despite the effect of CBSD on root quality in both the local and improved cultivars no attention was given to the disease until 2000 when Thresh carried out a brief survey along the Lunga – Lunga, Malindi highway (Hillocks *et al.*, 2001). Detailed survey carried out in some parts of coastal province revealed the disease to be widespread (Bock, 1994; Munga and Thresh, 2002). However, certain parts of coastal region

like Taita Taveta district, which stands at a higher altitude than the rest of the coastal areas, have not been surveyed for CBSD incidence.

There is no reported work to date showing any detailed survey for CBSD spread and distribution in the other major cassava growing regions of Kenya such as Lake Victoria region (Western and Nyanza) bordering Uganda where the disease re-emergence and outbreak has been reported. Similarly CBSD occurrence in Eastern province which borders the coastal region where the disease is wide spread needs to be determined. This leaves a gap in information about the extent of its spread and distribution along side its overall epidemiology in Kenya. It was therefore necessary that a detailed CBSD survey be conducted particularly in non coastal cassava growing regions of the country such as Western, Nyanza, Eastern, Central provinces and parts of Coast province where such work had not been done. Since *B. tabaci* a whitefly sp has been reported as the vector of CBSV, the CBSD survey also determined the whitefly populations in relation to CBSD incidence in areas of study.

The CBSV transmission has been reported to occur naturally at low (20-22%) rates (Storey, 1939, Bock, 1994 and Maruthi *et al.,* 2004), inconsistent with the high incidences of CBSD observed in field surveys of up to 64%, (Alicai *et al.,* 2007). The few reports about CBSV transmission do not give adequate information about other pests or whitefly species that may be contributing to the high CBSD incidences in the field.

Where as several arthropod pests are known to infest cassava many of them have not been tested for their ability to transmit CBSV. It is therefore necessary to investigate whether other cassava pests such as cassava green mites, *Mononychellus tanajoa* (Bondar) cassava red mites *Tetranychus urticae* and mealy bug can acquire and transmit the cassava brown streak virus.

The spiraling whitefly (*Aleurodicus dispersus*) that infests cassava in coastal lowlands may possibly transmit CBSV (T.L Munga's personal communication, 2008). This communication had no direct evidence and needed a detailed and systematic study. In addition there has not been any survey conducted to report the emergence and occurrence of spiraling whitefly in coastal Kenya where it has been sighted. Its population dynamics, host range and distribution in relation to CBSD incidence in coastal cassava growing districts have to be determined in order to establish its efficiency as a vector. Based on this background the following objectives were developed and implemented to study "Transmission and distribution of Cassava brown streak virus and its vectors in major cassava growing regions of Kenya.

1.2 Objectives of the study

The overall objective of the study was to determine the transmission and distribution of Cassava brown streak virus in major cassava growing regions of Kenya. This was further developed into specific objectives as outlined below:

1. To establish CBSD occurrence and distribution in major non coastal cassava growing regions of Kenya
2. To establish the abundance, distribution and host range of spiraling whitefly (*Aleurodicus dispersus)* in relation to CBSD incidence in coastal Kenya.
3. To study the transmission of CBSV by *Bemisia tabaci* and determine other possible vectors of CBSV in Kenya
4. To determine and compare the transmission efficiencies of CBSV by *Bemisia tabaci* and other vectors of CBSV

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1.1 Constraints to cassava production in Africa

Cassava (*Manihot esculenta* Crantz) plays an important role in Africa's agricultural stage (CIAT, 2001). It has become one of the most important crops grown in the continent (Legg and Thresh, 2006) and considered as a valuable root crop in sub-Saharan Africa with an average consumption that exceeds 300 kg per person per year in some areas (CIAT, 2001). Poor yields in Africa have been attributed to a range of factors, but one of the most important is loss due to pests and diseases (Legg and Thresh, 2006). Some of the most severe disease problems, for example the cassava mosaic disease, are found in Africa (CIAT, 2001).

Economically important among the pests are the cassava green mite, *Mononychellus tanajoa* (Bondar) and the cassava mealy bug, *Phenacoccus manihoti* Matt.-Ferr., both of which were introduced inadvertently to the continent from South America in the early 1970s (Legg and Thresh, 2006). The most important diseases include cassava bacterial blight, *Xanthomonas campestris* f.sp. *manihoti*, also introduced from South America in the 1970s, and two virus diseases, cassava mosaic disease (CMD) and cassava brown streak disease (CBSD). The two latter diseases are thought to have risen from infection of cassava by viruses already present in the indigenous African flora (Legg and Thresh, 2006).

2.1.2 The viruses infecting cassava

Cassava is a vegetatively propagated crop whose virus diseases cause particular problems as they are carried from one crop cycle to the next through the cuttings used as planting material (Legg and Thresh, 2006). Without intervention, infection can therefore readily build up from one crop cycle to the next, particularly where there is also a significant level of transmission by

vectors (Legg and Thresh, 2006). There is greater diversity among viruses of cassava in Africa as there is in the South/Central American region of origin (Calvert and Thresh, 2002). Four virus genera have been described by Calvert and Thresh (2002). However, only two of these are of economic significance to cassava namely: *Cassava brown streak virus* (CBSV) (*Potyviridae*: *Ipomovirus*) and the group of cassava-infecting geminiviruses (*Geminiviridae*: *Begomovirus*) (Fauquet *et al.*, 2003).

2.2.0 Cassava Brown Streak Virus (CBSV)

2.2.1 Biology and Etiology

Since CBSD was first described, the causal agent was assumed to be a virus in the absence of a visible pathogen (Hillocks and Thresh, 2000). This was confirmed when the disease was sap-transmitted to a range of herbaceous indicator hosts by Lister (1959) and in later experiments by Bock and Guthrie (1976). Virus particles were then detected by electron microscopy in leaf samples showing typical CBSD symptoms. The particles were elongate, flexuous filaments 650–690 nm long (Lennon *et al.*, 1986) and contained 'pin-wheel' inclusions, typical of potyviruses (Harrison *et al.*, 1995). The exact etiology of the disease remained a matter of speculation until recent work by Monger *et al.* (2001), during which the coat protein gene of CBSV was cloned and sequenced. The genome of the CBSV was shown to comprise a single-stranded particle of RNA, enclosed within a protein coat. The particles are flexuous, filamentous with a clear modal length of 650nm (Monger *et al.*, 2001). Since the highest sequence identity of the CBSV coat protein core and SPMMV was 43.2% it was concluded that the virus is an Ipomovirus, from the family potyviridae with sweet potato mild mottle virus being its type member (Monger *et al.*, 2001).

2.2.2 Symptomatology of cassava brown streak disease

The symptoms due to CBSD include foliar chlorosis often in a characteristic pattern of feathering along the veins and sometimes stem lesions. Young green stem tissues show purple to brown lesions, which may appear on the exterior surface (Hillocks *et al.*, 1990). Highly susceptible varieties develop very conspicuous stem symptoms including leaves becoming necrotic, abscising and shoot dying back (Hillocks *et al.*, 2002). In extreme cases, stem necrosis results in shoot die back (Jennings, 1960).

CBSV also affects the swollen roots which develop a yellow dry, corky necrosis within the starch bearing tissues sometimes accompanied by pitting and distortion that is visible externally (Hillocks, 1997). Unlike cassava mosaic disease (CMD) with which it is often associated, symptoms of CBSD may be found also on the roots making the most severely affected roots unfit for consumption (Hillocks *et al.*, 2002).

2.2.3 Host range

CBSV occurs naturally on cassava (Storey, 1936) and its host range is extended to herbaceous hosts including *Manihot spp*, *Petunia hybrida*, *Datura stramonium*, *Nicotiana tabacum* and *N. glutinosa* (Lister, 1959; Jennings, 1960; Kitajima and Costa, 1964). The following plant species like *Nicotiana tabacum*, *N. rustica*, *N. glutinosa*, *N. debneyi*, *N. benthamiana*, *D. stramonium*, *P. hybrida*, *Chenopodium quinoa* and *C. amaranticolor* have been used as experimental hosts for CBSV (Thresh, 1996). Of these species, *N.debneyi* and *N. benthamiana* have proved to be the most useful experimental hosts (Bock, 1994; Monger *et al.*, 2001). Isolates inducing different symptoms in *N. benthamiana* have been shown to differ by up to 8% and 6% nucleotide amino acid levels respectively (Monger *et al.*, 2001b).

2.2.4 Distribution of cassava brown streak virus

Nichols (1950) noted that the distribution of CBSD is delimited by altitude. The disease is rarely found above altitudes of 1000m, although symptoms can be expressed at higher altitudes if infected cuttings are obtained from low altitude areas. Surveys conducted between 1996 and 2002 confirmed the presence of CBSD at high incidences in the coastal areas of Kenya (Munga and Thresh, 2002), Tanzania, Northern Mozambique and areas close to the shore of Lake Malawi both in Tanzania and Malawi (Legg and Raya 1998; Hillocks *et al.*, 2002). In 1999 CBSD was reported in Mozambique for the first time at alarmingly high incidences in some coastal districts of Nampula and Zambezia provinces (Hillocks *et al.*, 2002).

The pattern of distribution has been confirmed in Tanzania, Mozambique and Malawi with the highest average incidences occurring below 300m, moderate incidences between 300m and 600m and low incidences at altitude above 600m (Hillocks *et al*, 1999; Hillocks *et al*, 2002; Gondme *et al.*, 2002). The explanation for such a restricted pattern of distribution is hitherto unknown but is likely to be due to either the distribution of the vector or the distribution of alternative hosts in the natural vegetation or both. However, CBSD re-emergence and outbreak occurrence has been reported in land areas of central and eastern Uganda at high incidences of up to 64% (Alicai *et al.*, 2007). In Kenya CBSD has been found to be widely distributed in coastal region and showed a general incidence of the disease (Nichols, 1950, Bock, 1994).

2.2.5 Detection of cassava brown streak virus

Antisera are available for serological detection of CBSV. However, they are unreliable since they give erratic results and are of limited sensitivity in ELISA tests of CBSV infected cassava. A sensitive reverse transcription polymerase chain reaction procedure (RT-PCR) has been developed that can detect CBSV in young symptomless leaves of infected cassava (Monger *et al.*, 2001b).

2.2.6 Response of cassava varieties to cassava brown streake disease

Cassava varieties differ greatly in their sensitivity and response to infection by CBSV (Hillocks *et al.*, 2001). The growth and yield of sensitive varieties are severely affected in the form of extensive dieback of the stems, necrosis of the tuberous roots and rotting to such an extent that they are virtually worthless. Hillocks *et al* (2001) found out that CBSV can decrease root yields of the most sensitive varieties by 70% as well as induce necrosis of roots, which renders them unmarketable. Tolerant varieties are much less severely affected with little effect on root yield or quality. For instance Nachinvaya, a local cultivar in southern Tanzania had a form of tolerance in which leaf symptoms were produced but the development of root necrosis was so delayed to the extent that the full potential yield was obtainable (Hillocks *et al.*, 2001). Cultivar Kibandameno in coastal Kenya is very susceptible to CBSD to an extent of zero root yields when attacked at earlier stage or when planting materials are derived from diseased cuttings.

2.2.7 Transmission and spread of cassava brown streak virus

Storey (1936) demonstrated that CBSV is graft-transmissible, and that cuttings from affected plants gave rise to plants showing characteristic foliar symptoms of the CBSD. Thus the disease is readily introduced into newly planted areas through the use of infected planting material (Hillocks and Thresh, 2000). Lister (1959) demonstrated that CBSV could be transmitted mechanically to indicator plants and was able to return the isolate from an indicator plant to cassava and reproduce the symptoms of CBSD. Earlier studies showed that sap transmission to herbaceous hosts induced pinwheel inclusion similar to those associated with Potyviridae (Harrison *et al.*, 1995).

Storey (1939) believed that the disease was caused by an insect-borne virus and that the most likely vector was a whitefly (*Bemisia spp.*). Observations in field trials conducted in Tanzania indicated that considerable spread takes place between plants (Hillocks and Thresh, 2000)

without successfully determining the insect that transmitted CBSV from one plant to the next. In Kenya, Bock (1994) was also unable to transmit CBSV with *B. tabaci* (which is known to transmit CMGs) and six species of aphid. Moreover, Lennon *et al* (1986) also reported failure to transmit CBSV with the aphid *Myzus persicae* (Sulz). Two other species of whitefly (*Bemisia afer* Priesner and Hosny and *Aleurodicus dispersus* Russell) also infest cassava in Africa and have not been tested adequately as possible vectors (Hillocks and Thresh, 2000).

Bemisia afer occurs in East Africa, together with *B. tabaci*, reaching highest population densities in some of the areas where CBSD incidence is greatest (Robertson, 1987). *B. afer* was considered generally the less abundant whitefly species in the cassava growing areas of East Africa (Hillocks and Thresh, 2000). However, surveys in Malawi showed that *B. afer* was the predominant species on cassava in most parts of the country and may be the main vector of CMGs there (Munthali, 1992). Bock (1994) suggested that *B. afer* was the putative vector and recent progress on classification of the causal agent as an ipomovirus, again points to a whitefly vector (Hillocks and Thresh, 2000). All these uncertainties and inconsistent findings existed until recently, when Maruthi *et al* (2004) gave the first direct evidence of CBSV transmission by *B. tabaci*. Since this first report was achieved no supportive evidence of CBSV transmission by *B. tabaci* has been realized. However, field surveys in coastal Kenya (Penina and Ngure 2006 unpublished) have shown significant positive correlation between CBSD incidence and *B. tabaci* population.

2.2.8 Management of cassava brown streake disease

The basic approach to control of CBSD is selecting planting material from symptomless mother plants. The health of the stock needs to be maintained by continued selection and rouging of any infected cassava plants which appear at sprouting for areas of low disease pressure with little or no disease spread (Hillocks and Thresh, 2000). For areas of high disease

pressure, release of virus-free planting material needs to be combined with deployment of cultivars which exhibit tolerance to CBSD (Hillocks and Thresh, 2000). Local cultivars such as 'Nanchinyaya' in southern Tanzania and Nguzo in coastal Kenya which seem to be tolerant of CBSV infection and are slow to develop root necrosis, could be used (Hillocks and Thresh, 2000). Dissemination of the tolerant cassava varieties to farmers and campaign to inform them of availability of such varieties for CBSD management must be emphasized (Hillocks *et al.*, 2005). Whereas these measures can control the levels of CBSD, resistance to this disease needs to be a priority in cassava breeding for long term remedy (Hillocks *et al.*, 2005).

2.3.0 Arthropod pests of cassava

2.3.1 Whitefly

Whiteflies belong to the order Hemiptera and family Aleyrodidae (Mound and Halsey, 1978). They are minute usually inconspicuous plant bugs mostly found infesting the foliage of plants. They are so called because the adults are small, fly-like, often dull white in color. Adult whiteflies measure from 1 - 3 mm in length, are four-winged and fully mobile with a feeding rostrum and seven-segmented antennae. Forewing venation is reduced to a simple or once branched major vein (Martin *et al.*, 2000). Reproduction can be sexual or parthenogenesis. Unmated females (2N) produce male offspring (1N), and fertilized eggs yield female offspring (2N). Eggs are oviposited on the leaf or other plant surfaces (Mound and Halsey, 1978).

Whitefly eggs are borne on pedicels. The first instar larvae termed crawlers are mobile and can crawl a short distance to locate suitable feeding site. Once the first molt has taken place, however, the remaining three larval instars are sessile. The fourth instar is often referred to as a pupa. However, it is not a true pupa since feeding occurs in the first part of the stage and

transformation in to adult takes place in the last part without any pupal molt (Gill, 1990). Some species have dark spots on the wings, although these may not develop until a few hours after emergence, and a few species are not white. The Citrus Black fly, *Aleurocanthus woglumi*, has black wings and little wax, and several species of Aleurodicinae have patterned wings. An undescribed species of *Dialeurodes* on coffee in southern Nigeria has red wings, and *Bemisia giffardi* has very pale yellow wings (Mound and Halsey, 1978).

Most whiteflies species are monophagous (feed on only one host plant). However, a few species are oligophagous or polyphagous. Some whitefly species can be agricultural pests and vectors of plant viruses (Bink-Moenen *et al.*, 1990). They cause damage to plants by feeding on phloem sap and also excrete a sugar-rich substance called 'honeydew', which at times encourages the development of sooty mould fungi that reduce the plants' photosynthetic capability. When the attack occurs on 1, 6, 11 months old cassava crop yield loss of 5, 42 and 79% respectively do occur (Belloti *et al.*, 1983)

Presently, three whitefly species, *B. tabaci*, *T. vaporariorum* (West.), or *T. abutiloneus* (Haldeman), are known to be vectors of plant viruses, while *B. tabaci* is the most important, having been associated with more than 100 viral diseases in the tropics and subtropics (Nault, 1997).

2.3.2 Bemisia complex

Two species within the *Bemisia* genus, *B. afer* and *B. tabaci* contain several species, variants or biotypes. The *B. afer* group includes *B. berbericola* and *B. tuberculata*, while the *tabaci* complex includes only *tabaci* and *graminus* as well as several biotypes (Gill, 1994). It is typically polyphagous causing heavy losses on many crops in various agricultural systems and

in greenhouses (Oliveira *et al.*, 2001). Damage is caused primarily by phloem feeding by the nymphs and adults, phytotoxic disorders, and the transmission of plant viruses.

B. tabaci (Gennadius) (Homoptera: Aleyrodidae), is a widely distributed pest species colonizing many agricultural crop systems including greenhouses in both the tropics and sub tropics (Oliveira *et al.*, 2001). It is has been a major agricultural pest of field and horticultural crops world-wide since the early 1980s (Brown *et al.*, 1995; Oliveira *et al.*, 2001). It vectors begomoviruses, criniviruses, (*Lettuce infectious yellow virus, Cucurbit yellow stunting disorder virus, Lettuce chlorosis virus, Pumpkin yellow mosaic virus, Sweet potato sunken vein virus, Sweet potato chlorotic stunt virus* and *Tomato chlorotic virus* (transmitted by B and Q biotypes), Carla virus; *Cowpea mild mottle virus* and poty-like virus (*Squash yellow leaf curl virus*) and *Sweet potato mild mottle virus* and *Cassava brown streak virus* of genus Ipomovirus (Brown and Czosnek, 2001).

B. tabaci transmits cassava mosaic geminiviruses, the most damaging pathogens which threaten food security supply in many countries in Africa (Legg, 1994; Pita *et al.*, 1998). The *B. tabaci* has been also reported to transmit cassava brown streak virus at low rates inconsistent with high CBSD incidences recorded in field surveys (Maruthi *et al.*, 2004). It also transmits *Tomato yellow leaf curl virus* and *Okra leaf curl virus* (Burban *et al.*, 1992).

2.3.3 Spiraling whitefly (*Aleurodicus dispersus*)

Aleurodicus dispersus Russell (Hom, Aleyrodidae) is a highly polyphagous pest and a native of the Caribbean region and Central America (Russell, 1965). It has been known from a wide range of host plants, although it was not regarded as a pest (Waterhouse and Norris, 1989). Hosts reported so far include citrus, avocado, guava, plantain, banana, coconut, soybean, cassava and stone fruit (John *et al.*, 2006).

It is commonly known worldwide as 'spiraling whitefly' because it characteristically lays eggs in a typical spiral pattern (Kumashiro *et al.*, 1983). It was introduced and assumed pest status in the Canary Islands in 1962 and in Hawaii in 1978 (Paulson and Kumashiro, 1985), in American Samoa and Guam in 1981 (Firman, 1982) and then in most of the Pacific islands (Waterhouse and Norris, 1989). The whitefly later spread westwards into several regions including Africa (Akinlosotu *et al.*, 1993; M'Boob and van Oers, 1994; Neuenschwander, 1994), Asia (Anon, 1987; Wijesekera and Kudagamage, 1990; Kajita *et al.*, 1991; Wen *et al.*, 1994; Palaniswami *et al.*, 1995) and Australia (Carver and Reid, 1996; Lambkin, 1998).

Adults and nymphs of the spiraling whitefly cause damage by direct feeding on plant sap and when present in very large numbers can cause leaf fall, but even heavy infestations are insufficient to kill the plants. Spiraling whitefly has a high reproduction and dispersal rate (John *et al.*, 2006). It has been a major threat to the banana, tropical fruit trees, vegetable and ornamental industries in northern Queensland (John *et al.*, 2006). The copious white, waxy, flocculent material secreted by all the stages of the pest is readily spread by wind, creating nuisance. Honeydew excreted by the nymphs encourages growth of sooty mould on leaf surfaces, reducing the photosynthetic capacity of the plant (Kumashiro *et al.*, 1983).

Its extensive host range covers 481 host plants belonging to 295 genera and 90 families, including several vegetables, fruits, ornamentals and avenue trees (Srinivasa, 2000). In India, it has been reported on over 253 plant species belonging to 176 genera and 60 families (David and Regu, 1995; Palaniswami *et al.*, 1995; Prathapan, 1996; Ranjith *et al.*, 1996; Geetha *et al.*, 1998; Muralikrishna, 1999; Gajendra Babu and David, 1999; Mani and Krishnamoorthy, 1999a; Geetha *et al.*, 1999; Ramani, 2000; Srinivasa, 2000; Mariam *et al.*, 2000; Geetha and

Swamiappan, 2001a; Gopi *et al.*, 2001). The major host plants of economic concern in India are banana, guava, avocado, papaya, coconut, cucurbits, dahlia, gerbera, gladiolus, tomato, mulberry, tapioca and bell pepper, in addition to several species of shade trees in the urban environment. The families Fabaceae, Asteraceae, Malvaceae, Myrtaceae, Euphorbiaceae and Moraceae seem to contain the most species of host plants (Srinivasa, 2000; Geetha and Swamiappan, 2001a).

Wen *et al.* (1995) reported a loss in fruit yield of 80% in guava attacked by the pest for four months consecutively in Taiwan, but estimates of yield loss due to this pest are not available in many areas where this pest occurs. Ranjith *et al.* (1996) observed severe damage to many crops where as Geetha *et al.* (1998) observed severe incidence in a groundnut crop in Tamil Nadu. Heavy incidence of the whitefly caused yield reduction to an extent of 53.10% in tapioca (Geetha, 2000). Adverse impacts, such as longer larval duration, decreased food conversion, utilization and reduction in economic parameters of the cocoon, were noticed when the whitefly-infested mulberry leaves were fed to the silkworm, *Bombyx mori* (L.) (Lep., Bombycidae), due to reduced nutrition levels in affected leaves (Mariam, 1999; Ahamed *et al.*, 1999; Narayanaswamy *et al.*, 1999).

2.3.4 Transmission of plant viruses by whitefly species

Recent unprecedented upsurges in the populations of the whitefly *Bemisia tabaci* Gen. (Hemiptera: Aleyrodidae) have resulted in the emergence of virus diseases that are vectored by this insect (Moriones, 2003) (Table 1). Epidemics caused by begomoviruses (genus Begomovirus, family Geminiviridae), criniviruses (genus Crinivirus, family Closteroviridae) or ipomoviruses (genus Ipomovirus, family Potyviridae) are causing severe damage to vegetable crops worldwide and frequently are the limiting factor to production (Moriones, 2003). Differences in the efficiency or selectivity of virus transmission can affect the pattern of virus

dissemination during epidemics either in crop or in weed species. Specific interactions should occur between the virus and the vector for efficient transmission. In these interactions are involved both vector and virus determinants that are poorly understood at present for *B. tabaci* and *Aleurodicus dispersus*. Lessons from virus-vector relationships might probably help to design more efficient strategies to control virus epidemics (Moriones, 2003).

The modes of transmission of plant viruses are characterized into three types namely persistent , semi persistent and non persistent, mainly based on the time required for the vector to acquire the ability to transmit the virus (acquisition time), and length of time the vector retains that ability (retention time). Ipomoviruses are transmitted by whiteflies in a non persistent manner (Hollings *et al.*, 1976; Liao *et al.*, 1979), with acquisition and retention times of a few seconds and several minutes, respectively. For criniviruses, Carla viruses, and Closter viruses, whiteflies transmit pathogens in a semi persistent mode, acquiring the viruses in a few minutes and retaining the viruses for a few hours or days until they molt (Table 1) (Schaefer's and Terry, 1976; Duffus *et al.*, 1986; Horn *et al.*, 1986).

2.4. 1 Cassava green mite

The cassava green mite, *Mononychellus tanajoa* (Bondar) is an important exotic pest of cassava *(Manihot esculenta* Crantz) in the dry season throughout much of the African cassava belt (Yaninek, 1985). *M. tanajoa* was discovered on cassava near Kampala, Uganda in late 1971 (Lyon, 1973). It has since spread to at least twenty-seven countries in Africa causing damage estimated at 18-80% (Yaninek *et al.*, 1987). *M. tanajoa* is biologically similar (Yaninek, 1985) to other agronomically important tetranychids (Huffaker *et al.*, 1970; McMurtry *et al.*, 1970). The adult female lays fertilized female eggs and unfertilized male eggs. There are four active stages: a six-legged larva, two nymphal stages (protonymph and

deutonymph) and the adult stage. The active stages prefer to feed on the terminal parts of the plant, killing leaf cells and reducing photosynthesis (Bellotti and Schoonhoven, 1978).

2.4.2 Cassava mealy bug

The cassava mealy bug, *Phenacoccus manihoti*, was inadvertently introduced from South America in the early 1970s on infected planting materials. Over the years, *P. manihoti* spread throughout the cassava belt of Africa and caused considerable reductions in cassava yields. The cassava mealy bug gained the status of being the worst agricultural insect pest in the tropics (Herren, 1981). The damage the mealy bug caused in Africa has been as high as 80% of all losses in cassava root yield (Nwanze, 1982). Estimates of the portion of potential yield loss to cassava mealy bug differed among countries and years. In Ghana, for example, yield losses due to both cassava mealy bug and cassava green mite were estimated at 70%, which is 0.8 million tons of tubers (Korang-Amoakoh *et al.*, 1987).

2.4.3 Red spider mite

The red spider mite, *Tetranychus urticae* is one of many species of plant-feeding mites found in dry environments, and generally considered a pest. It is the most widely known member of the family Tetranychidae or Spider mites. Several workers have examined the effects of mite feeding damage on crop yields and on leaf photosynthetic rates (Andrews and LaPre', 1979; Allen, 1981; Sances *et al.*, 1981-1982; Smith and Mozingo 1983; Welter *et al.*, 1984; Hardman *et al.*, 1985).

T. urticae is extremely small, barely visible with the naked eye as reddish or greenish spots on leaves and stems; the adults measure about 0.5 mm long. It is extremely polyphagous and can feed on hundreds of plants, including most vegetables and food crops (peppers, tomato, potato, beans, corn, strawberry, cassava) and ornamental roses (Hardman *et al.*, 1985). It lays its eggs

on the leaves and poses a threat to host plants by sucking cell contents from the leaves, leaving very tiny, pale spots or scars where the green epidermal cells have been destroyed (Smith and Mozingo, 1983). Although the individual lesions are very small, commensurate with the small size of the mites, the frequently-observed attack of hundreds or thousands of spider mites can cause thousands of lesions and thus can significantly reduce the photosynthetic capability of plants, greatly reducing their production of nutrients, sometimes even killing the plants. This kind of feeding by the red spider mites could spread plant viruses, however it is considered of secondary importance (Smith and Mozingo, 1983).

Table1: Whitefly transmitted plant viruses (Jones, 2003)

Example species	Genus/family	Mode	Reference
Bean golden mosaic virus	Begomovirus/Geminiviridae	Persistent	Goodman and Bird, 1978.
Sweet potato mild mottle virus	Ipomovirus/Potyviridae	Non persistent	Hollings *et al.*, 1976; Liao *et al.*, 1979.
Lettuce infectious yellow virus	Crinivirus/Closteroviridae	Semi-persistent	Duffus *et al.*, 1986.
Sweet potato sunken vein virus	Closter virus/Closteroviridae	N/A*	Schaefers and Terry, 1976.
Cucumber vein yellowing virus	Potyviridae	Semi-persistent	Sela *et al.*, 1980.
Cowpea mild mottle virus	Carlavirus/Flexiviridae	Semi-persistent	Horn *et al.*, 1989.
Cassava mosaic virus	Begomovirus/Geminiviridae	Persistent	Goodman and Bird, 1978.
Cassava brown streak virus	Ipomovirus/Potyviridae	N/A*	Maruthi *et al.*, 2004.

N/A*: No information available

CHAPTER THREE

OCCURRENCE AND DISTRIBUTION OF CASSAVA BROWN STREAK DISEASE IN NON COASTAL CASSAVA GROWING REGIONS OF KENYA

3.1 Introduction

Cassava, *Manihot esculenta* Crantz is an important crop in Kenya, grown by many households for both food and income. Its cultivation in Kenya currently covers approximately 77,502 ha with an annual output of 841,196 tons (FAO, 2007). Approximately 60% of this production is from Western Kenya, 10% from Eastern and 30% from Coast province (Crop Crisis Control Project, 2006). It ranks among the three most important food crops besides maize and sorghum. Cassava production is constrained by many biotic factors of which cassava mosaic geminiviruses (CMGs) and cassava brown streak virus (CBSV) diseases are the major threats to its production in Kenya.

The cassava brown streak disease (CBSD) is caused by CBSV a member of the family Potyviridae and genus Ipomovirus (Monger *et al.*, 2001). The disease causes economic losses resulting from damage to the above ground plant parts through leaf chlorosis, stem lesions with complete die back as well as the spoilage of roots due to a dry corky necrotic rot on starch tissues (Hillocks *et al.*, 2003; Hillocks, 1999). CBSD has been reported to cause up to 70% yield loss by reducing the root sizes and by causing pitting and constriction on roots (Hillocks *et al.*, 2001). Root necrosis caused by CBSV accounts for the quantitative and qualitative reduction in total yields through the presence of necrotic lesions or discoloration of the roots rendering them unpalatable and unmarketable (Nichols, 1950).

Since CBSD's first description in northern Tanzania (Storey, 1936), it has been endemic in East African coastal cassava-growing areas from southern Kenya, through to the Zambezi river

in Mozambique, and occurs also, in some inland areas of Malawi and Uganda, up to altitudes of 1000 m (Nichols, 1950; Sauti and Chipungu, 1993; Hillocks *et al.*, 2002; Legg and Raya, 1998.) The pattern of distribution has been confirmed in Tanzania, Mozambique and Malawi with the highest average incidences occurring below 300m, moderate incidences between 300m and 600m and low incidences at altitudes above 600m (Hillocks *et al*, 1999; Hillocks *et al.*, 2002; Gondme *et al.*, 2002). The reasons for the restricted occurrence of CBSD, despite the wide spread distribution of *B. tabaci* its only reported vector (Maruthi *et al.*, 2004) throughout Africa and the considerable movement of cassava materials remain unknown (Alicai *et al.*, 2007). Although the occurrence of the disease has for long thought to be confined to altitudes below 800 m, it has now been realised that CBSD symptoms can be expressed at altitudes greater than 1000m when infected cuttings are planted (Alicai *et al.*, 2007). CBSD was endemic in Uganda from that time until 2004 when it re-emerged (Alicai *et al.*, 2007).

The emergence of CBSD in inland areas in the East African countries as is the case in Central Uganda has led to a shift in the general understanding that the disease was delimited in distribution mainly along the coastal low lands. Whereas CBSD is reported to be widespread in coastal Kenya (Thresh *et al.*, 2002), the extent of its occurrence and distribution in the whole country has not been determined. Since cassava is grown in altitudes higher than 1000m in Kenya where the known vector is widely distributed, it is likely that the disease may be spread to such altitudes (Eastern, Central and Western Kenya) where it has not been reported before. It is against this background that a CBSD survey in Kenya was conducted to determine the occurrence and distribution of this important disease.

3.3 Materials and Methods

3.3.1 Survey of CBSD in major non coastal cassava growing regions in Kenya

A survey of CBSD was conducted during the dry season of November 2006 and June-July 2007 covering major cassava growing areas of Central, Eastern, Nyanza and Western provinces of Kenya. The survey determined CBSD incidence in relation to whitefly (*Bemisia tabaci*) populations in major cassava growing districts of Eastern, Central, Western Kenya and areas bordering the shores of Lake Victoria region and Uganda where CBSD re-emergence has been reported (Alicai *et al.*, 2007). The survey area was divided into districts, which were further subdivided into divisions for sampling (Otim-Nape *et al.*, 2001). Information on farmer perceptions and knowledge of CBSD in relation to its spread, vector of transmission and management was recorded using a questionnaire (Appendix 3).

3.3.2 CBSD incidence and severity assessment

CBSD incidence assessment procedure involved stopping at regular predetermined intervals of about 2km to 5km between farmer fields along major motorable roads traversing each sampling location. The variation in the interval between the stops was necessitated by the availability of predominant farmer preferred cassava cultivars at 6-9 months stage of growth. An average of 10 farms was sampled per district with three districts being targeted in each province. However, in Central Kenya cassava was not commonly found in farmers' fields leading to few sampling units. The data determined during sampling included CBSD prevalence, incidence and the varieties grown by farmers. Visual CBSD incidence was estimated by identifying plants within a farm that showed foliar mosaic with a characteristic pattern of feathering along the veins and incidence expressed as a percentage of the total number of plants sampled per farm. A total of 30 plants were assessed along diagonals in each farmer field. The prevalence was recorded as the proportion in percentage of production units (farmer fields) in which the disease symptoms were observed. Severity of shoot symptoms was

determined following a scale of 1 to 5 (Hillocks *et al.*, 1996) where; 1- no apparent symptoms, 2- slight foliar mosaic, no stem lesions, 3- foliar mosaic, mild stem lesions no die back, 4- foliar mosaic and pronounced stem lesions no die back and 5- defoliation with stem lesions and pronounced die back. Whereas there was a wide range of varieties grown by farmers, the assessment of disease parameters was done on the predominant cultivars. Diseased and non diseased stem cuttings and leaf samples were collected for testing using reverse transcriptase polymerase chain reaction to confirm the absence or presence of CBSV.

3.3.3 *Bemisia tabaci* population assessment

The adult whitefly population was determined by direct count on representative shoots per cassava plant (Sseruwagi *et al.*, 2004). Due to preferential feeding and oviposition on top-most immature leaves by *B. tabaci* adults (Gameel, 1977; Ohnesorge *et al.*, 1980; Fargette, 1985), the five youngest apical leaves were examined at sampling time (Fargette, 1985). Each leaf was held by a petiole and gently inverted so that the adults present on the lower surface could be counted (Seif, 1981; Fargette, 1985; Fargette *et al.*, 1985; Fishpool *et al.*, 1995). The counting was done mainly in early morning when the adult *B. tabaci* were less active in their flight behaviour. Adult whitefly populations were determined by recording the number of adults on the five top most expanded leaves of a representative shoot on 30 cassava plants randomly selected along diagonals of each field.

3.3.4 Detection of cassava crown streak virus

The presence of Cassava brown streak virus in leaf samples was assayed essentially following the methods of Monger *et al.* (2001) with modifications. CBSD leaf samples from coast where CBSD is prevalent were collected to act as positive control whereas clean cassava leaves from western Kenya and plain clean water were used as negative control. The initial step involved CTAB extraction of RNA from cassava leaf samples collected from the farmers' fields. The

single stranded RNA was used to synthesize a complementary DNA (cDNA) following manufacturer's instruction using Moloney murine leukemia virus reverse transcriptase enzyme from Invitrogen and gene specific primers CBSV (F) and CBSV 11(R) (Monger *et al.*, 2001).

The complementary DNA was amplified in a 25-mL PCR reaction volume containing 19.05 µl RNase-Free Water, 2.5µl of 10x PCR buffer, 0.75 µl of 50 mm $MgCl_2$, 0.5µl dNTPs Premix , 0.5µl of each primer (CBSV 10 (F), CBSV 11 (R)), 1mL of cDNA sample and 0.2µ Taq polymerase (Promega UK Ltd, Southampton, UK). The PCR program was set at 94°C initial denaturation for 2 min followed by one loop of 3 segments; 94°C for 1 min (denaturation), 57°C for 1 min (annealing) and 72°C for 1 min (Extension), repeated for 30 cycles then held at 72°C for 10 min (Final extension). Products were separated by 1% agarose gel electrophoresis containing10mg/ml ethidium bromide in TAE buffer at 80 volts for 45 minutes and viewed under UV light against a 1kb DNA ladder (Promega). The cDNA bands appearing at 231 base pair were positive for CBSV presence.

3.3.5 Statistical analysis

The CBSD prevalence, incidence and *B. tabaci* populations' data collected were subjected to analysis of variance and the means compared by least significant difference test at P=0.05. CBSD incidence was correlated to the *B. tabaci* populations for each district surveyed for the disease occurrence.

3.4 Results

3.4.1 Symptomatology

Symptoms caused by CBSD were observed on the leaves, stems and roots (Plates 1, 2, 3, 4, 5 and 6). Foliar mosaic showing a characteristic pattern of feathering along the veins was observed in farmers' fields. For instance, Var MM96/5280 from Yala swamp in Siaya district had chlorosis appearing in roughly circular patches between the main veins with characteristic patterns of feathering along the veins (Plate 1). In certain areas such as Bungoma, Teso and Busia districts in western Kenya the symptoms caused by CBSD were expressed as chlorosis along the margins of the secondary veins and widened into chlorotic blotches (Plate 2).

There were also differences in the range of cassava cultivars encountered in the farmers' fields with varying CBSD incidences (Table 2). The cultivars appeared to have varying sensitivity or tolerance to CBSD. The variety MM96/5280 appeared most susceptible showing distinctive CBSD symptoms while Migyera and some local cultivars showed mild CBSD-like symptoms (Table 2). In some stems the symptoms occurred as purple lesions with characteristic concentric rings emanating from the stem nodes extending into a group of concentric rings on the stem (Plate 3).

Plate 1: Advanced foliar chlorosis and feathering on lower cassava leaves

Plate 2: Foliar chlorotic blotches on a detached cassava leaf (MM96/5280)

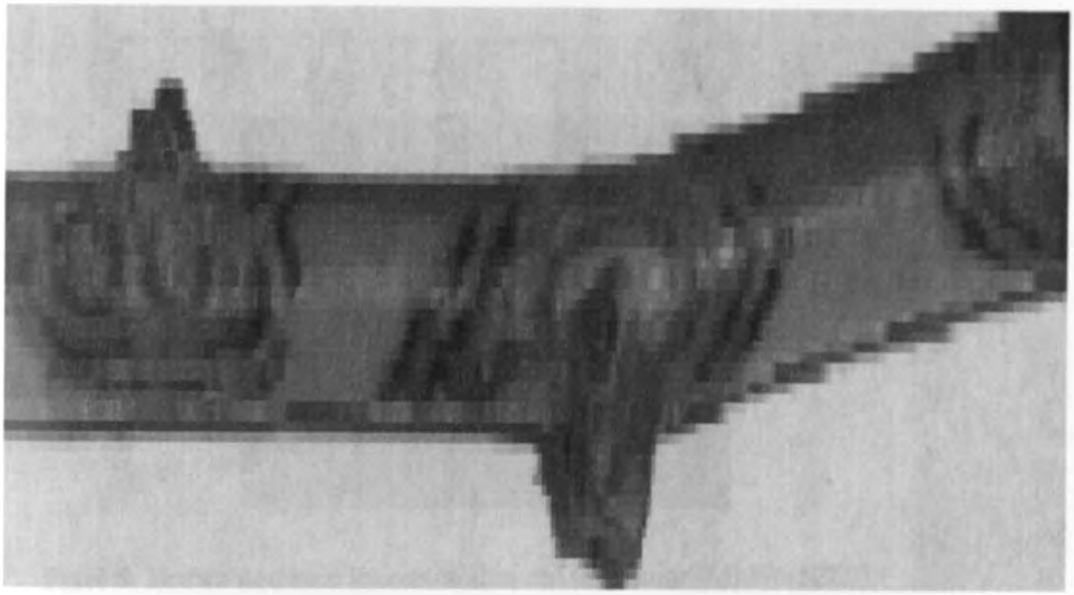

Plate 3: CBSD brown purplish lesions on cassava stem (MM96/5280). The concentric rings were suspected to be sign of a different disease (Concentric ring leaf spot disease) (Yala swamp-Siaya district)

Plate 4: Necrotic brown lesions on cassava root (MM96/5280)

Plate 5: Brown necrotic lesions within cassava stem (MM96/5280)

Plate 6: Necrotic brown lesions on cassava root leading to secondary infection (complete root rot) (MM96/5280)

3.4.2 Farmers' knowledge and perception on cassava brown streak disease

A majority (80%) of the farmers interviewed in western Kenya were not aware of the foliar mosaic and had no idea what caused such symptoms. In fact to the farmers the foliar mosaic and yellowing was normal leaf senescence and a sign of plant maturity. They could not tell whether these were symptoms of a disease and again farmers were unable to relate the disease spread with whitefly vectors. For some, it was the first time they were observing both the disease symptoms and the whiteflies. However, 5% of the farmers acknowledged they had observed the dry corky necrosis in the edible root tissues but could not relate it to the foliar mosaic. No deliberate disease or conscious CBSD management practice was being carried out by any of the visited farmers. Most of the farmers (65%) acknowledged that they sourced their planting material through exchange among themselves and from across the Busia-Uganda border. The rest of the farmers either sourced directly or indirectly (neighbour) from cassava seed multiplication programs by research institutions or non governmental organisation (World vision).

Table 2: CBSD severity and incidence on popular cultivars encountered in farmer fields in various cassava growing districts in Kenya

District	No of farms	Predominant Cultivar	Cassava brown streak disease Severity	Incidence (%)
Bondo	14	MM96/5280	2-4	100
Siaya	4	MM96/5280	2-3	100
Busia	7	MM96/5280	2-3	100
Teso	6	MM96/5280	2-2	100
Siaya	10	Migyera	1	0
Busia	6	Migyera	1-2	12.8
Teso	10	Migyera	1-2	21
Bungoma	1	Migyera	1-2	0.6
Embu	11	Muerisheri	1	0
Busia	1	MM96/4466	2-3	100
Busia	1	Nase4	2	3.3
Embu	10	Ndolo	1	0
Embu	10	Nguche	1	0
Busia	5	Magana	1-2	18
Busia	5	Ngungume	1	0
Bondo	3	Nyakatanegi	1	0
Siaya	7	Adhiambo lera	1	0

Note: 30 plants were sampled in each farm from which mean CBSD incidence was calculated for each cultivar. Cultivars MM96/5280 and MM96/4466 were most susceptible (100% incidence and severity >1

3.4.3. CBSD prevalence, incidence and severity.

CBSD was present in 5 districts (Western and Nyanza) out of the 11 districts visited. The highest (100%) prevalence of the disease was observed in Bondo district (Nyanza) whereas the lowest prevalence (0%) was recorded in Eastern province (Table 3). The disease was most prevalent in Western Kenya and seemed widespread being detected in samples collected from 5 out of six districts surveyed in this region. The disease was not present at all in Eastern and Central Kenya since the samples collected (had no foliar mosaic) and when tested by reverse transcriptase polymerase chain reaction, were CBSV free.

The data collected indicates high incidences in Bondo and Busia to moderate incidences in Teso and Siaya with considerable differences between the districts ($F_{[8, 74]} = 16.5$, $p<0.001$). The disease incidence was generally higher in Western Kenya unlike Eastern and Central Kenya where only slight faint foliar mosaic was observed. The overall CBSD mean incidence for western Kenya (Nyanza/Western) was 42.2% whereas Eastern/Central was at 0%.

The severity of CBSD was generally low in most of the areas surveyed standing at level 1 with no apparent foliar symptoms. However, severity was high in certain locations with Bondo having the highest (3) whereas Bungoma and Busia were at 2 (Table 3). The overall mean severity was low (1.4) in most of the districts. In western Kenya severity ranged from 1 to 3 while in Eastern and Central were generally at level 1 with a few plants having slight foliar mosaic (Table 3). CBSD symptom severity significantly ($P>0.001$) varied between the districts.

3.4.4 Abundance of whitefly (*B. tabaci*) on Cassava

The number of adult whitefly/plant differed significantly ($P>0.05$) between various districts (Table 3). Whitefly (mean) populations were highest (6.8) in Bondo per the 5 top most leaves

of each plant and lowest in Kirinyaga (0.5) (Table 3) with an overall mean of 1.6 per leaf. In western Kenya, the average whitefly population was at 2.6 per leaf in 5 districts surveyed (Table 3). Very low whitefly population was recorded with majority of fields having no whitefly at all in districts such as Kisii, Embu, Machakos, Mbere, Kirinyaga and Thika. However, high whitefly population in younger plants 3 to 4 months old with an average population of 5 adult whiteflies on each of the 5 top most leaves was recorded in some farms in Western Kenya (Table 3).

There was a significant ($r=+0.6977$, $p<0.001$) and positive correlation between the number of adult whitefly *Bemisia tabaci*, and CBSD incidence. The disease incidence was highest wherever whitefly population was observed to be abundant (Table 3). Similarly, there was a significant and positive correlation ($r=+0.8108$, $p<0.001$) between number of adult whiteflies, *B. tabaci*, and CBSD prevalence.

2.3.5 RT-PCR analysis

A total of 31 and 68 with symptoms and symptomless leaf samples respectively were tested for the presence of CBSV by RT-PCR. Eighty three percent (31) of the symptomatic leaves were found to be CBSV infected all of which were from Western Kenya. Seventeen percent of symptomatic (CBSD-like foliar symptoms) samples which were from Bungoma (1), Embu (2) and Kirinyaga (2) districts were CBSV free (Of the symptomless samples, seven percent (Busia (1), Teso (3) and Bondo (1)) tested positive for the presence of CBSV (Table 4).

Table 3: Prevalence (%), incidence (%), severity of CBSD and mean whitefly (*Bemisia tabaci*) counts in selected cassava growing districts in Kenya

Survey region		Cassava brown streak disease			*B. tabaci*
		Prevalence			
Province	**District**	**(%)**	**Incidence (%)**	**Severity**	**Mean counts**
Western	Busia	87.5	60^{b} (52.45)	2 (1-3)	1.7 ± 0.396
	Bungoma	20	6^{c} (6.58)	2 (1-2)	2.3 ± 0.396
	Teso	88	48^{b} (43.46)	1 (1-3)	2.92 ± 0.499
Nyanza	Bondo	100	93^{a} (84.49)	3(1-4)	6.77 ± 0.885
	Siaya	50	38^{b} (33.24)	1(1-3)	2.2 ± 0.712
	Kisii	0	0^{c}	1	0
Eastern	Embu	0	0^{c}	1	0.7 ± 0.367
	Machakos	0	0^{c}	1	0.5 ± 0.432
	Mbere	0	0^{c}	1	0
Central	Kirinyaga	0	0^{c}	1	0.5 ± 0.432
	Thika	0	0^{c}	1	0.7 ± 0.367

Note: Figures in parenthesis are severity range (severity column) and transformed % incidence (incidence column). Means followed by the same letter were not significantly different at 5% significant level.

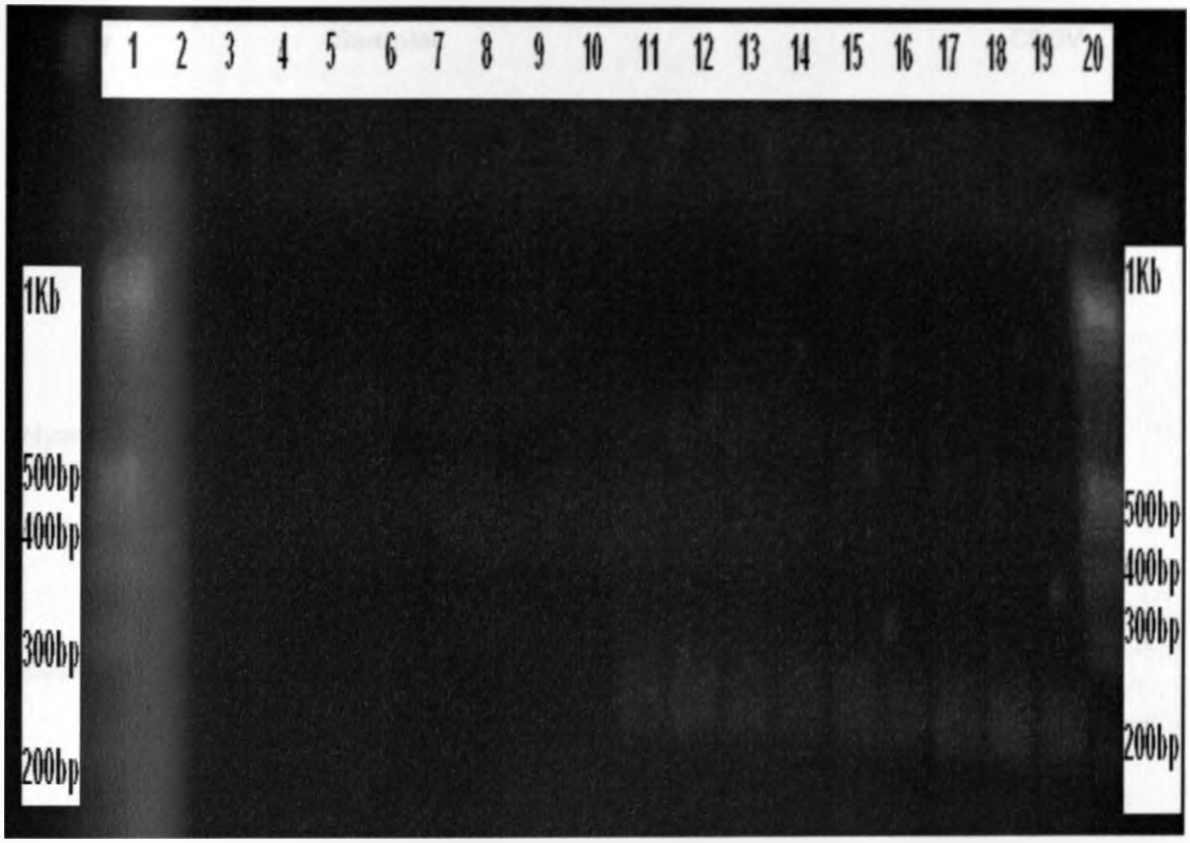

Fig 1: PCR gel electrophoresis showing diagnostic bands of CBSV complementary DNA synthesized from RNA extracted from cassava leaves. Lanes (1,20) 1Kb DNA ladder, (2-4) Central province samples , (5-8) Eastern province samples, (9) a negative control (water) , (10) CBSD free sample and (12) positive control. Lanes 11, 13-19 was samples from Western Kenya.

Table 4: RT-PCR test results showing CBSV presence in symptomatic and symptomless samples collected from 11 districts of Kenya.

Survey region	District	Samples tested	Symptomatic	Symptomless	CBSV presence
Western	Busia	10	5	5 (1)	6
	Bungoma	10	2	8	1
	Teso	12	3	9	6
Nyanza	Bondo	14	13	1(1)	14
	Siaya	10	4	6	4
	Kisii	2	0	2	0
Eastern	Embu	10	2	8	0
	Machakos	10	0	10	0
	Mbere	1	0	1	0
Central	Kirinyaga	10	2	8	0
	Thika	10	0	10	0
TOTAL		**99**	**31**	**68**	**31**

Note: Symptomatic were the samples that showed clear foliar mosaic with characteristic feathering along the veins whereas symptomless had no apparent symptoms. Figures in parenthesis indicate the number of leaf samples that had no apparent CBSD symptoms but tested positive for CBSV presence.

The districts (Bondo, Busia, Siaya Teso and Bungoma) where CBSV positive leaf samples were collected, border Uganda where the disease re-emergence and outbreak has been reported. CBSV was not detected in any of the samples collected from Eastern and Central provinces indicating absence of the virus within the farmers' plots covered during the survey in these two regions (Table 4). It was also observed that some samples that were visually symptom less during the survey were CBSV infected when tested by the RT-PCR. Overall a third (31.2%) of the cassava stem cuttings collected from various districts during the survey in all the regions and established in green house before being subjected to RT-PCR test were CBSV infected.

The RT-PCR results showed the presence of CBSD in samples from 5 districts surveyed in western Kenya similar to the visual symptom incidence recorded during the CBSD survey (Table 4 and Fig 1). Despite, some cassava leaf samples showing slight foliar mosaic in Eastern and Central provinces none of them tested positive for CBSD presence in the RT-PCR tests. The diagnostic bands of complementary DNA appeared at approximately 231 base pairs an indication of CBSV presence (Fig 1).

The absence of CBSV in samples from districts surveyed in central and eastern Kenya is further demonstrated by the absence of the diagnostic bands (lanes 2-10 of Fig 1). Different cassava cultivars were encountered in farmers' fields during the sampling some of which had very clear foliar CBSD symptoms (Western Kenya) and others have faint symptoms which could only tested by RT PCR for true identification.

3.5 Discussion

The foliar symptoms observed during this study were characteristic of CBSD and therefore indicated the presence of the disease in Western and Nyanza provinces. Seasonal effects and varietals variation might have lead to the symptom differences observed in this study. Nichol, (1950) highlighted variability of CBSD symptoms from season to season and in different varieties. In this study CBSD symptoms varied from one variety to another pointing to the varied cultivar reaction to the disease. An earlier study showed that different cassava cultivars have varied symptoms when infected with CBSD (Hillocks *et al.*, 1996).

The findings on farmer perception and knowledge of CBSD in Western Kenya indicate that the disease has not been known in the region and seems to have emerged recently. Since they do not recognise the disease, farmers have not employed any management practices for the disease. This may be of serious consequence as they continue using diseased planting materials have led to further spread of the disease. Farmers lack accurate information on the symptoms, cause, spread and management of the disease. This information regarding CBSD symptoms, identification and management practices need to be availed to farmers to avoid further losses inflicted by this disease.

The moderate to high prevalence of CBSD in all the districts surveyed in Western Kenya indicate how widely this disease is becoming distributed in the region. It is conceivable that the disease could be present in districts that were not surveyed but border the ones covered in this region. In fact it is no longer restricted to Yala swamp where, a significant 'outbreak' had been reported from a large multiplication site by Pheneas and Legg (2007).

The whitefly numbers were higher on apical shoots of younger (3-6 months old) cassava plants than on the older plants and leaves indicating a preferential feeding behaviour of *B. tabaci* adults that is a similar to an observation by Gameel (1977); Ohnesorge *et al* (1980) and Fargette (1985). Moreover adult *B. tabaci* were more common on certain varieties particularly the MM96 series (MM96/4466 and MM96/5280) unlike on Ngungume and Nyakatanegi demonstrating host preference. This observation could help in focusing cassava breeding strategies to include resistance to both whitefly and CBSD.

The positive correlation between CBSD incidence and the number of adult whiteflies indicate a considerable contribution of the whiteflies to the spread of the CBSV. It had been observed in Tanzania that considerable spread takes place between plants through vector transmission (Robertson, 1987 and Maruthi *et al.,* 2004). Furthermore, a second whitefly species, *B. afer* occurs in East Africa together with *B. tabaci*, reaching highest population densities in some of the areas where CBSD incidence is greatest (Robertson, 1987). Therefore the spread of the disease from Uganda into Kenyan districts bordering eastern Uganda such as Siaya, Busia and Teso by the whitefly species is plausible.

The RT-PCR test, done on leaf samples collected from farmer fields' confirmed the presence of CBSD in Western Kenya particularly in Bondo, Busia, Teso, Siaya and Bungoma districts. This finding supports the earlier report of out break occurrence of CBSD in a multiplication plot in Yala swamp of Western Kenya (Pheneas and Legg, 2007). An interesting observation was the fact that cultivars bred for tolerance (MM96/5280, I92/0427, MH95/0198 and MM96/3868- Alupe) against CMGs were the ones seriously affected by CBSD, which compounds the cassava production problem in western Kenya.

This reinforces the need to control/curb CBSD particularly in a region which contributes 60% of the total cassava production in Kenya.

The symptoms variation among cassava varieties points to cultivar sensitivity to CBSD. Thus there is need for a trial to study the reaction of farmer preferred cassava cultivars to CBSV particularly in Nyanza and Western provinces where the disease was present. This would help in identifying those locally adapted cultivars with low sensitivity to the disease such as low sensitivity to root necrosis. Such cultivars can be cleaned of virus and distributed to high risk areas such as Western and Nyanza regions of Kenya.

The study revealed for the first time that CBSD was prevalent and widely distributed in 5 districts of western Kenya. This compounds the losses already being caused by CMD because CBSD causes the edible cassava roots to become corky and inedible (Hillocks *et al* 2003, Hillocks, 1999). Once introduced into a field, as it is now in Nyanza and Western provinces the virus can spread rapidly. With yield losses of up to 70% percent of root yield having been recorded (Hillocks *et al.*, 2001) food security from cassava in this region is at risk of tremendous decline.

CHAPTER FOUR

SPIRALING WHITEFLY (*Aleurodicus dispersus*) OCCURRENCE, ABUNDANCE, DISTRIBUTION AND HOST RANGE IN ASSOCIATION WITH CBSD INCIDENCE IN COASTAL KENYA

4.1 Introduction

Aleurodicus dispersus Russell (Hom. Aleyrodidae) is a highly polyphagous pest and a native of the Caribbean region and Central America (Russell, 1965). It is commonly known worldwide as 'spiraling whitefly' because it characteristically lays eggs in a typical spiral pattern (Kumashiro *et al.*, 1983). It has been recorded on a wide host range covering 481 host plants belonging to 295 genera and 90 families, including several vegetables, fruits, ornamentals and avenue trees (Srinivasa, 2000). These include citrus, avocado, guava, plantain, banana, coconut, soybean, cassava and stone fruit (John *et al.*, 2006). Adults and nymphs of the whitefly cause damage by direct feeding on plant sap and when present in very large numbers can cause leaf fall, but even heavy infestations are insufficient to kill the plants (John *et al.*, 2006). Honeydew excreted by the nymphs encourages growth of sooty mould on leaf surfaces, reducing the photosynthetic capacity of the plant (Kumashiro *et al.*, 1983).

Wen *et al* (1995) reported a loss in fruit yield of 80% in guava attacked by the pest for four months consecutively in Taiwan, however, estimates of yield loss due to this pest are not available in many areas where this pest occurs. Ranjith *et al* (1996) observed severe damage to many crops where as Kerala and Geetha *et al* (1998) observed severe incidence in a groundnut crop in Tamil Nadu. Heavy incidence of the whitefly has caused yield reduction to an extent of 53.10% in tapioca (Geetha, 2000).

Despite the pest being a serious threat to crop production, information on its occurrence and distribution in sub Saharan Africa and cassava growing countries such as Kenya is lacking except for a survey report of 2004 in Tanzania (Pallangyo *et al.*, 2004). Since the reported hosts include cassava, understanding of the pest population dynamics and damage on cassava along side its distribution in cassava growing regions will help determine the extent of its impact on cassava production and aid in development of management strategies. A question about the inconsistency between vector transmission rates achieved under laboratory tests (22%) and those observed in field conditions (64%) still remains unanswered to date.

Moreover, re-emergence and resurgence of CBSD has been reported (Alicai *et al.*, 2007) a fact that cannot be explained by the low transmission rates achieved so far by tests conducted using *B. tabaci* alone. Moreover a spiraling whitefly-like pest has recently been observed on cassava in coastal region of Kenya (Munga *et al.*, 2008 personal communication). This unconfirmed report required a detailed survey and study to report emergence of this pest in Kenya. During whitefly spp collection for cassava brown streak virus transmission in 2007 this spp was predominantly present on cassava infesting the lower mature CBSD symptomatic leaves in Mtwapa (Kilifi), Msabaha (Malindi) and Msambweni. These together with uncertainty about the range of vectors transmitting cassava brown streak virus prompted a detailed study of this whitefly spp, its association with cassava and CBSV. It became necessary to specifically determine its occurrence, distribution and host range in coastal Kenya where it has been sighted.

4.2 Materials and Methods

4.2.1 Abundance of *Aleurodicus dispersus* in association with cassava brown streak disease incidence in major cassava growing regions of coastal Kenya

A survey was conducted to determine the occurrence, distribution and host range of *A. dispersus* against CBSD incidence in major cassava growing districts of coastal Kenya namely Kilifi, Malindi, Msambweni, Kwale, and Kaloleni (Fig 2). Locally adopted cassava varieties were identified in relation to CBSD incidence and severity correlated to adult whitefly (*A. dispersus*) abundance. The spiralling whitefly host range was determined by enlisting any plant in which the whitefly adults and eggs were present. The survey area was divided into districts, which were further subdivided into locations for sampling (adopted from Otim-Nape *et al.*, 2001). The sampling procedure involved stopping at regular predetermined intervals of about 2 to 5km between farmer fields along major motorable roads traversing each sampling location.

4.2.2 CBSD incidence assessment

Five districts were targeted with three to four cassava growing divisions from each district for sampling. The data determined during sampling included CBSD incidence, prevalence, severity and the varieties grown by farmers. CBSD incidence was determined as a proportion of visibly diseased plants expressed as a percentage of the total number of plants observed. A total of 30 plants were randomly assessed along diagonals in each of 10 farmers' fields per division. The prevalence was recorded as the proportion in percentage of production units (farmer fields) in which the disease symptoms were observed. Severity of shoot symptoms was determined following a scale of 1 to 5 (Hillocks *et al.*, 1996) where; 1- no apparent symptoms, 2- slight foliar mosaic, no stem lesions, 3- foliar mosaic, mild stem lesions no die back, 4- foliar mosaic and pronounced stem lesions no die back and 5- defoliation with stem lesions and pronounced die back.

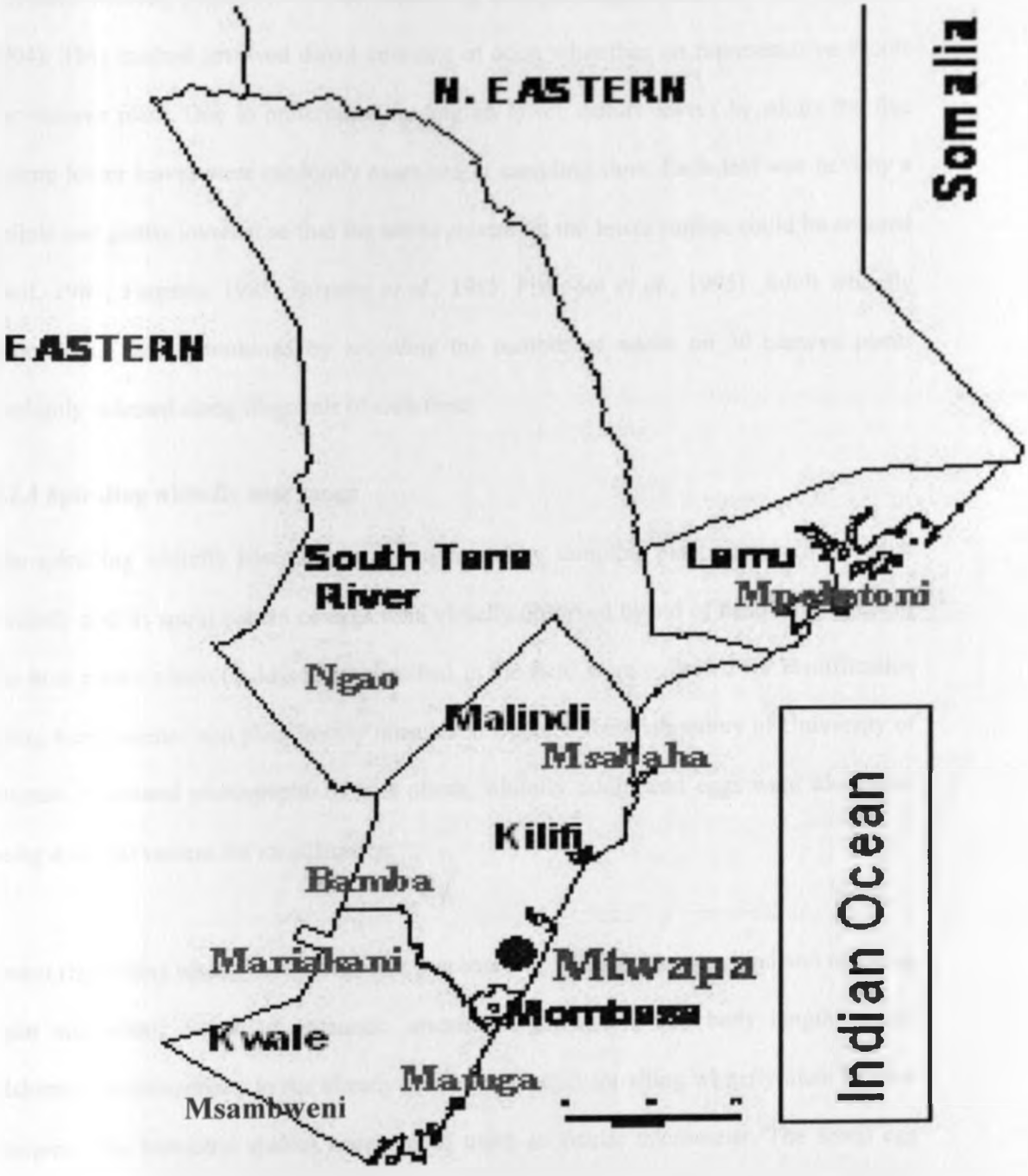

Fig 2: Coastal region map showing locations (Msabaha, Mtwapa, Msambweni, Matuga and Mariakani) surveyed for spiralling whitefly population, distribution, and host range in association with CBSD incidence.

4.2.3 Spiraling whitefly population assessment

The adult whitefly population was determined by the direct count method (Sseruwagi *et al.*, 2004). This method involved direct counting of adult whiteflies on representative shoots per cassava plant. Due to preferential feeding on lower mature leaves by adults the five mature lower leaves were randomly examined at sampling time. Each leaf was held by a petiole and gently inverted so that the adults present on the lower surface could be counted (Seif, 1981; Fargette, 1985; Fargette *et al.*, 1985; Fishpool *et al.*, 1995). Adult whitefly populations were determined by recording the number of adults on 30 cassava plants randomly selected along diagonals of each field.

4.2.4 Spiraling whitefly host range

The spiralling whitefly host range was determined by sampling plants in which the adult whitefly and its spiral pattern of eggs were visually observed by aid of hand lens. Some of the host plants which could not be identified in the field were collected for identification using weed science and plant botany manuals in Upper Kabete laboratory of University of Nairobi. Coloured photographs of host plants, whitefly adults and eggs were also taken using a digital camera for identification.

Insect (Spiralling whitefly) identification was based on its adult biology; hind and forewing span and width, length of antennae, antennae segmentation and body length (Head-abdomen) in comparison to the already documented adult spiralling whitefly identification features. The biometric studies were carried using an ocular micrometer. The spiral egg laying pattern specific to this whitefly was also a significant identification attribute used in this study. Finally the already documented host range was used to identify the host list and further helped to identify the whitefly.

Data analysis

The data collected included CBSD incidence and Spiralling whitefly adult population which were subjected to analysis of variance and mean comparison by least significant differences (LSD). The resulting variances were correlated to determine the association between CBSD incidence and spiralling whitefly population.

4.3 Results

4.3.1 Spiraling whitefly host range

Spiraling whitefly occurrence, biology and host range

The morphometric parameters of different adult spiraling whitefly structures showed males (known to be longer than females) to be larger than females and had elongate claspers at the distal end of the abdomen. The adult males were 2.49 ± 0.04 mm long and 1.24 ± 0.01 mm wide and the females were 1.85 ± 0.05 mm long and 1.19 ± 0.03 mm wide (Table 5). This spp was present in all cassava growing districts sampled and was found to be highly polyphagous with a wide host range (Table 6).

Spiraling whitefly *A. dispersus* was found to infest 56 host plants including different plant families (Table 6). Out of these, 11 families were represented by single species each. Five families; Cariceae, Rutaceae, Convolvulaceae, Aresaceae, and Anarcdiaceae were represented by two species each. Asteraceae and Malvaceae were represented by five species each whereas Cucurbitaceae and Euphorbiaceae by four and three species each respectively. There were eight and ten species of host plants, which came under Solanaceae and Fabaceae respectively. Euphorbiaceae was represented by one crop host (cassava) and the family that included the highest number of host plants of spiraling whitefly was in Fabaceae with 10 species.

The host range included trees, weedy spp, ornamentals, fruit trees, plantains, root-tuber crops and vegetables. These were common in all the locations sampled and were infested by the whitefly in all the districts except in farms visited in Mbuguni location of Kwale district and Kirumbi location of Kaloleni division.

Table 5: Morphometric parameters of adult male and female spiraling whitefly collected from cassava field in coastal Kenya (KARI-Mtwapa 2008).

Morphometric parameter (Male)	**Length (mm)**	**Morphometric parameter (Female)**	**Length (mm)**
Fore wing span	2.5±0.02	Fore wing span	2.05±0.01
Hind wing span	2.0±0.03	Hind wing span	1.9±0.05
Fore wing width	1.2±0.05	Fore wing width	1.0±0.06
Hind wing width	0.9±0.03	Hind wing width	0.8±0.04
Length of antennae	0.95±0.01	Length of antennae	0.75±0.01
Body width	1.24 ± 0.01	Body width	1.19 ± 0.03
Body length	2.49±0.04	Body length	1.85 ± 0.05

Morphometric parameters taken on 1000 (500 female and 500 males) adult spiraling whitefly using ocular micrometer (under compound microscope)

Table 6: A list of hosts of spiraling whitefly, their family, common and scientific names as identified in coastal Kenya

Family	Common Name	Scientific name
Amaranthaceae	Devils horse whip	*Achyranthesis aspera L.*
Anarcadiaceae	Mangoes	*Mangifera spp*
Anarcardiaceae	Cashew nut	*Anacardium occidentale*
Arecaceae	Coconut tree	*Cocos nucifera*
Arecaceae	Wild coconut tree	*Coco loco*
Asteraceae	Billy goat weed	*Ageratum conyzoides Linn*
Asteraceae	Chrysanthemum	*Chrysanthemum indica L.*
Asteraceae	Little ironweed	*Vernonia cinerea (Linn.) Less.*
Asteraceae	Nod weed/ starwort	*Synedrella nodiflora Gaertn.*
Asteraceae	Sow thistle	*Sochus asper Linn. Hill*
Bixaceae	Bixa plant	*Bixa orellana*
Caesalpiniaceae	Coffee senna	*Cassia occidentalis Linn*
Caricaceae	Papaya	*Carica papaya*
Convolvulaceae	Annual twinner	*Ipomoea eriopcarpa R. Br.*
Convolvulaceae	Morning glory weed	*Ipomoea involucrata P. Beauv.*
Convolvulaceae	Sweet potato	*Ipomea batata*
Cucurbitaceae	Cucumber	*Cucumis sativa*
Cucurbitaceae	Gourd plant	*Lagenaria siceraria*
Cucurbitaceae	Loofah/loofah gourd	*Luffa aegyptiaca Mill*
Cucurbiticeae	Watermelon	*Citrullus lanatus*
Elaeis	Palm tree	*Elaeis guineensis*

Family	Common name	Scientific name
Euphorbiaceae	Cassava	*Manihot esculenta*
Euphorbiaceae	Castor plant	*Ricinus communis*
Euphorbiaceae	Spurge weed/wild poinsettia	*Euphorbia heterophylla Linn*
Fabaceae	Acacia sp	*Acacia farnesiana*
Fabaceae	Cow pea	*Vigna unguiculata*
Fabaceae	Crotalaria like annual shrub	*Tephrosia bracteolata Guill. and Perr.*
Fabaceae	Green gams	*Vigna radiata*
Fabaceae	Mac donald eye weed	
Fabaceae	Mucuna cover crop	*Mucuna bracteata*
Fabaceae	Pigeon pea	*Cajanus cajan*
Fabaceae	Rattle box	*Crotalaria retusa Linn*
Fabaceae	Thorny shrub-Kilifi	*Acacia sp*
Lauraceae	Avocados	*Persia americana*
Malvaceae	Broom weed	*Sida acuta Burm.f.*
Malvaceae	Broom weed like	*Sida corymbosa R. E. Fries*
Malvaceae	Cotton	*Gossypium herbaceum*
Malvaceae	False mallow	*Malvastrum coromandelianum (Linn.) Garcke*
Malvaceae	Okra	*Abelmosc hus esculentus/(Hibiscus esculentus* L)
Marantaceae	Arrow roots	*Maranta arundnacea*
Moraceae	Jack fruit tree	*Artocarpus heterophyllus*
Musaceae	Bananas	*Musa acuminata*
Myrtaceae	Quavas	*Psidium quajava*
Nyctaginaceae	Bouginvillea	*Bouginvillea spectabilis*

Family	Common name	Scientific name
Rosaceae	Black rasp berry	*Rubus occidentalis*
Rutaceae	Lemon	*Citrus limon*
Rutaceae	Oranges	*Citrus sinensis*
Solanaceae	Black night shade	*Solanun nigrum*
Solanaceae	Capsicum	*Capsicum frutescens*
Solanaceae	Double thorn	*Oxygonum sp*
Solanaceae	Egg plant	*Solanum melongena*
Solanaceae	Hot pepper	*Capsicum annuum*
Solanaceae	Sodom apple	*Solanum incanum*
Solanaceae	Tomatoes	*Lycopersicum esculentum*
Solanaceae	wild cape goose berry	*Physalis angulata Linn*
Verbenaceae	Bastard vervain, Brazilian tea	*Stachytarpheta indica (Linn.) Vahl*

Note: The host plants were identified using weed science hand book: College of Agriculture and Veterinary Sciences Library; Univesrsity of Nairobi. (Author: Michieka, 1976)

The characteristic egg laying spiral pattern was observed in the entire host range, however adult stage was not present in all the hosts such as castor plant, certain ornamentals, and weed spp (*Crotalaria* spp). The adults were seen to have a migratory flight during early hours of the morning (6-7am). Egg laying was predominantly on the underside of young apical leaves (Plate 7) although occasionally the egg spirals were observed on the upper surface of the leaves (Plate 8). Egg laying was also seen to occur on fruits such as Papaya (Plate 9). Damage seemed to be mainly caused by the sap-sucking immature and adult whiteflies (Plate 10) that fed on the underside of the foliage. Preferential feeding was observed to be mainly on lower mature leaves with preferential oviposition on immature leafy apical shoots by the adults. Heavily infested plants had a black sooty appearance from mould growing on the sugary secretions that the whitefly immature excretes.

There were arthropod-pests that might have been natural enemies of *Aleurodicus dispersus* one of which morphologically appeared roundish and characteristically had attached spiral eggs round its body. This made it appear like a moving ball of spiral eggs under cassava leaves (Plate 11). This particular pest was also observed on other host plants (capsicum) infested by the spiraling whitefly.

Plate 7: Spiral egg pattern on upper surface of cassava leaves.

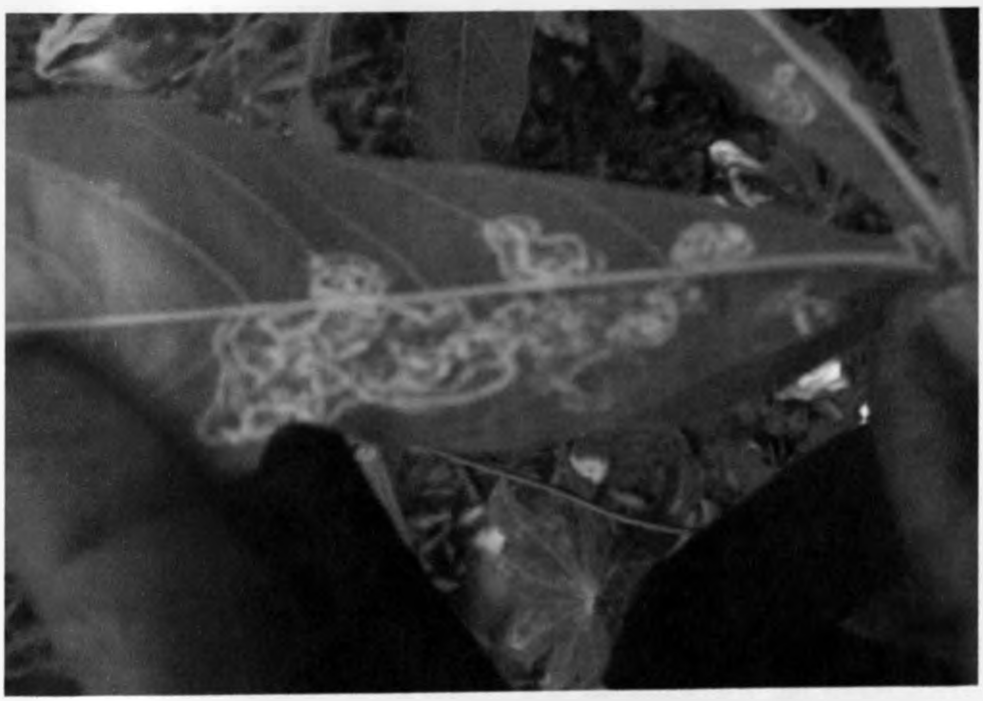

Plate 8: Spiral egg pattern on the under surface of cassava leaves.

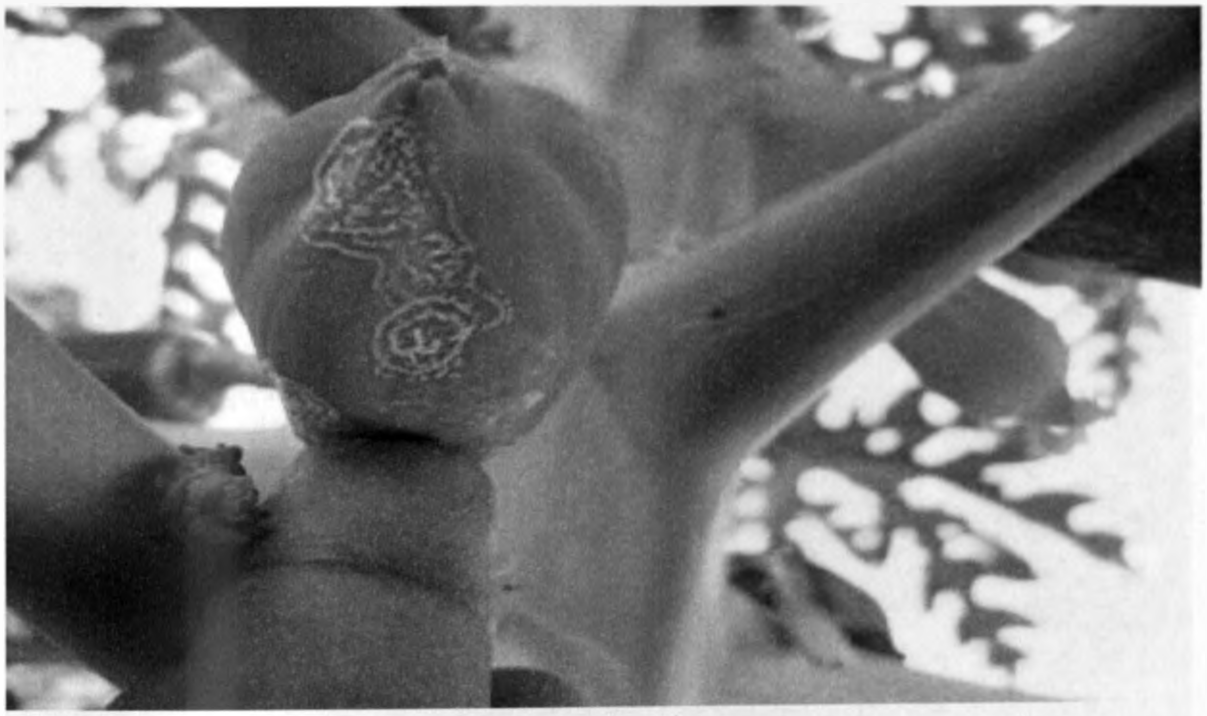

Plate 9: Spiral whitefly eggs laid on papaya fruit in Msambweni district

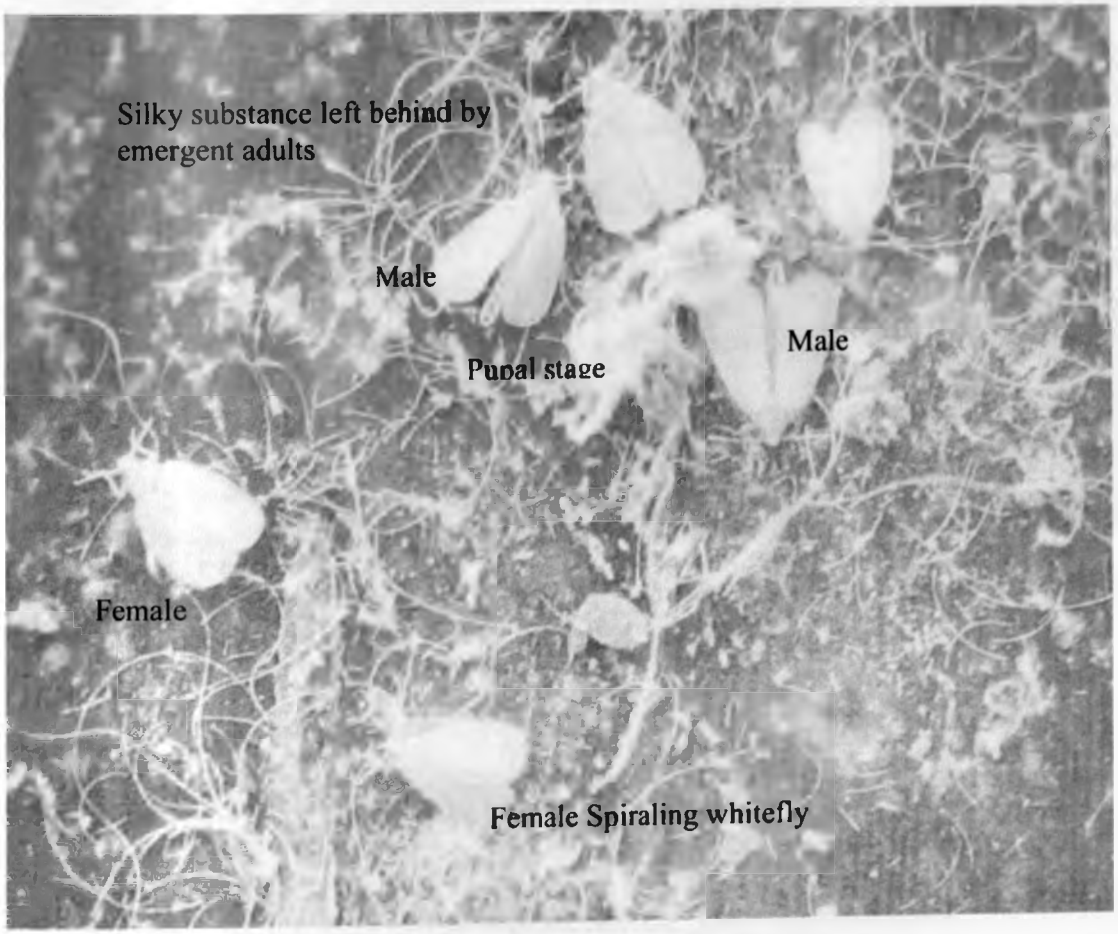

Plate 10: Adult Spiraling whitefly on coconut leaf in Coastal Kenya (KARI-Mtwapa)

Plate 11: Natural enemy like pest on the under surface of cassava feeding on spiraling whitefly eggs

4.3.2 CBSD incidence in popular cassava cultivars in coastal Kenya

Several cassava varieties were encountered in farmers' fields that include Kibandameno, Nguzo, Mtwapa 1, Agriculture, Kaleso, Sagalato, Korokoto and Ambari (Table 7). Of these cultivars Kibandameno was present in all the locations sampled. Different locations had certain common cultivars only known in those particular areas for example Sagalato (Mbuguni-Kwale), Korokoto (Kaloleni) and Ambari of Msambweni district. The cultivars showed varied sensitivity to both cassava mosaic disease and CBSD. Nguzo showed a considerable CMD tolerance and very low severity of CBSD through visual observation (Table 7). Similarly Sagalato had no apparent CBSD symptoms in Mbuguni location where the farmers there praised it as sweet, high yielding and early maturing. Kibandameno was the most susceptible with CBSD incidences of 80-100% wherever field it was sampled. Greatest tolerance to CBSD was observed in Sagalato and Ambari cultivars which are local land races.

4.3.3 CBSD Incidence in relation to spiraling whitefly population

The spiraling whitefly was present in all (100% prevalence) the coastal districts surveyed. There was a general trend showing low whitefly population in locations far away from the coast line. These included Chonyi (Kilifi), Kayafungo and Kirumbi (Kaloleni), Mbuguni (Kwale) and certain parts of Kubo division-Shimba hills (Kwale) (Table 8). The number of adult whitefly/plant differed significantly ($P>0.05$) between various districts. Whitefly (mean) populations were highest in Msambweni (23.69) and Kilifi (14.097) per each of the 5 lower most leaves per plant and lowest in Kwale (4.128) and Kaloleni (1.92) (Table 8) with an overall mean of 10.9 per leaf.

Table 7: CBSD incidences on different locally adopted cassava cultivars grown by farmers in coastal Kenya

Main cassava varieties	No of farms	No of samples	CBSD incidence (%)
Kibandameno	10	635	90 (3)
Agriculture	10	100	75(2)
Ambari branched	10	53	17 (1)
Ambari non branched	10	85	10.6 (1)
Nguzo	10	339	45 (2)
Korokoto	10	20	25 (2)
Sagalato	10	30	10 (2)
Kaleso	10	25	100 (3)

Note: 10 plants of each cultivar were sampled in selected field. Figures in parenthesis are severity scores.

In south coast the average whitefly population was at 13.909 per leaf in 2 districts (Msambweni and Kwale) surveyed. Low whitefly population was recorded in young cassava plants (2-3 months old) unlike mature (>5 months) plants a similar case with young and older leaves per plant. High whitefly population was recorded in districts situated nearer coast than those off the coastal belt by the time of survey (Kilifi and Msambweni). However there was significantly low whitefly population in Shimba hills (Kubo division) whereas no whitefly presence was recorded in four farms sampled in Mbuguni location of Matuga division in Kwale during this survey. This shows that the whitefly is spreading and had not reached certain locations in the coast region as at the time of this study. In general there was low whitefly population in Kaloleni district than any other district surveyed in coast region.

CBSD was prevalent in all the districts surveyed with the highest incidence being recorded in Msambweni (79.4%) with Kilifi (64.0%), Kwale at 68.3 and lowest in Kaloleni at 42.8 (Table 8). CBSD incidence also followed a general trend of decline away from coastal belt. For example Mtwapa (86.0%), Mavueni, Msambweni (86.4) and Mlalani (76.0%) which had higher CBSD incidences are closer to ocean than Mbuguni (42.0%), Chonyi (35.1%) and Kayafungo (45.0%). Nevertheless there were certain locations which did not fall in this trend such as Goloni (80.0%). There was a significant and positive correlation ($r=+0.5189$, $p<0.001$) between number of adult whitefly, *Aleurodicus dispersus*, and CBSD incidence (Fig 3). Similarly there was a significant and positive correlation ($p<0.001$) between number of adult whitefly vector, *Aleurodicus dispersus*, and CBSD severity. CBSD severity was high in Msambweni, Mtwapa and Mlalani locations. The varying severities were cultivar dependent with the greatest severity level of CBSD witnessed on Kibandameno. Wherever this cultivar was found there was presence of CBSD.

Table 8: CBSD Incidence (%) and mean whitefly (*Aleurodicus dispersus*) counts in selected cassava growing locations in coastal Kenya

District	Location	No of farms	*Aleurodicus dispersus* counts	Mean CBSD incidence
Kaloleni	Kayafungo	10	0.2±4.3	45.0±8.6
Kaloleni	Rabai	10	3.6±4.3	42.5±8.6
Kaloleni	Ruruma	10	1.7±12.1	35.0±17.1
Kilifi	Mavueni	10	7.3±5.0	68.3±9.9
Kilifi	Mtwapa	10	26.5±3.5	86.0±7.0
Kilifi	Mitangoni	10	0.6±8.6	20.0±17.1
Kilifi	Chonyi	10	2.5±4.6	35.1±9.2
kwale	Goloni	10	0.5±8.6	80.0±17.1
Kwale	Mbuguni	10	0.0±5.4	42.0±10.8
Kwale	Shimba Hills	10	4.7±6.1	57.5±12.1
Kwale	Tsima	10	7.8±4.6	90.0±9.2
Msambweni	Lungalunga	10	5.0±6.1	73.3±12.1
Msambweni	Mivumoni	10	27.8±5.0	80.0±9.9
Msambweni	Mlalani	10	13.9±5.4	76.0±10.8
Msambweni	Msambweni	10	40.2±5.0	86.4±5.0

Note: *Aleurodicus dispersus* is the spiraling whitefly. The figures are means for CBSD incidence and whitefly population for all farmer fields in each location.

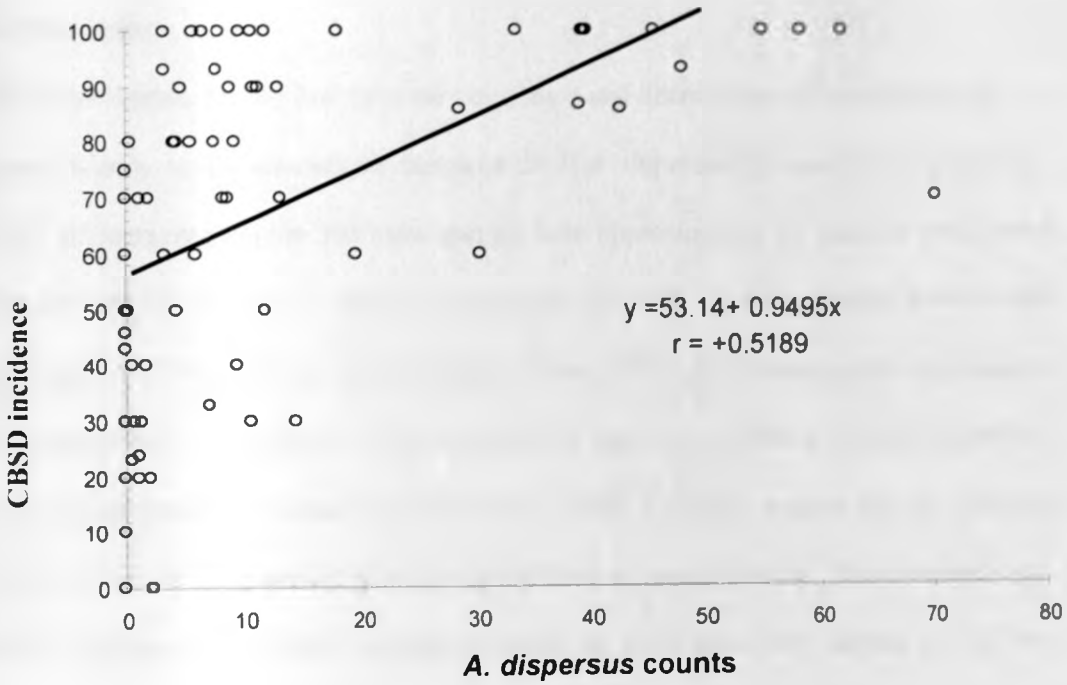

Figure 3: Correlation between CBSD incidence and *A. dispersus* counts

4.4 Discussion

This work reports for the first time the occurrence and distribution of spiraling whitefly in coastal Kenya. It also presents the first host list of *A. dispersus* in coastal Kenya, although fewer as compared to over 300 plant species from approximately 77 families which have been recorded as hosts of *A. dispersus* worldwide. Of these, the most detailed host list was published in 1994 by Wen *et al.* in Taiwan (Trevor, 1999). Its extensive host range covers 481 host plants belonging to 295 genera and 90 families, including several vegetables, fruits, ornamentals and avenue trees (Srinivasa, 2000). This may suggest that the whitefly has not exhaustively colonized all the available hosts in coastal Kenya. Plants that had egg spirals and flocculence only were also recorded as hosts since they support part of the pest's life cycle.

Several of these hosts recorded in Kenya are similar to the ones already identified as hosts of spiraling whitefly from various parts of Caribean region and other parts of the world. Russell (1965) recorded 44 plant species as hosts of spiraling whitefly from Florida, Central and South America and Caribbean Islands whereas it was found to infest 99 host plants including fruit trees, vegetable crops, ornamentals, shade and forest trees in Kanataka India. The 99 host plants belonged to 38 families (Kwaiswariaya *et al.*, 2007).

The whitefly is not native to African countries and therefore it must have been introduced or spread from a neighboring country where it had been introduced. It is not known how and when this pest was introduced in Kenya. However, spiraling whitefly was observed for the first time in Unguja Island (Tanzania) in late 2002 (Pallangyo, 2005). During a survey that was conducted in the Eastern Zone of Tanzania mainland in August 2004, the pest was found in Tanga Coast and Morogoro regions of Tanzania whereby twelve crops including

banana, cassava, cashew nut and a number of vegetables and fruit crops were found infested (Pallangyo, 2005), hosts similar to the ones reported in this study. This may suggest that possibly the whitefly spread from Tanzania along the coastal belt shared with Kenya. It is not surprising that the whitefly occurs mainly along the coastal region with the highest count being registered in Msambweni which borders coastal Tanzania. However, this may not rule out possible introduction through planting materials and inadvertently through fruits and vegetable imports since it was observed that this whitefly also oviposit on both foliage and fruits.

The results of the survey show that *A. dispersus* is prevalent and spreading in Coastal Kenya. This species had not been reported previously in Kenya hence this is the first direct evidence of the occurrence and distribution of spiraling whitefly in coastal Kenya. From the wide host range, the whitefly seems highly polyphagous attacking crops, weeds, trees and ornamentals presenting a very unique situation in pest management in Kenya. Besides the waxy nature, several reasons like wide host range, damage potential and rapid dispersal (migratory behavior) can enable the whitefly to maintain its status as a pest on a wide variety of plants. Hence, for a successful management strategy to be developed there will be need for further biology and host range studies to find out the vulnerable period during the lifecycle and also the perennial sources of infestation. The whitefly population on the non crop host plants serves are source of infestation that can switch on to cultivated plants.

The positive correlation between the disease incidence and the number of adult whiteflies indicates a considerable contribution of the whiteflies in the spread of the virus. It had been observed in Tanzania that considerable spread takes place between plants through vector transmission (Robertson, 1987). All the districts and locations where spiraling whitefly was

present are geographically located at low altitudes. This coincides with the general delimited distribution of CBSD along the coastal lowlands where high disease incidence occurs at altitudes below 300 m, less common between 300 m to 700 m and rare at altitudes above 700 m (Hillocks *et al*, 1999; Hillocks *et al*., 2002; Gondme *et al*., 2002). For instance in Msambweni there was the highest whitefly number (23) coinciding with the highest CBSD incidence (Table 8).

The cassava cultivar (Kibandameno) that was most preferred by farmers in coastal Kenya was seriously affected by CBSD. This calls for a management strategy which should include breeding for resistance focusing on both diseases without neglecting the need to incorporate the preferred attributes of Kibandameno into the new resistant varieties. However, the problem requires an immediate attention to curb the spread and distribution of the disease. It is imperative therefore that alternative varieties tolerant to CBSD be supplied to farmers whereas immediate effort to stop multiplication, supply and distribution of the varieties vulnerable to CBSV need to be implemented.

Extensive coverage surveys for spiraling whitefly need to be carried out in the regions not covered since not all cassava growing districts were sampled. Despite the evidence that the pest was not present in certain locations at the time of this survey it is necessary for complete coverage survey to be done in all the districts where cassava farming is done to ascertain pest status in these regions.

The information on CBSD incidence in each of the cultivar provides an indication of their susceptibility. It is unclear whether the least affected cultivars are resistant to infection or if they have simply escaped infection. It is important therefore to carry out molecular

characterization of these varieties and other necessary tests to determine the source of this resistance. This would help in identifying those locally adapted cultivars with low sensitivity to the disease such as low sensitivity to root necrosis. Such cultivars can be cleaned of virus and distributed to high risk areas. Further more such specific resistance alleles can be selected and used in transformation of the farmer preferred but susceptible cultivars such as Kibandameno. The data showed a positive correlation between whitefly population and CBSD incidence. In this respect a study to determine whether the whitefly (*Aleurodicus dispersus*) do transmit CBSV is important, as such information will have a direct bearing on the understanding of CBSV vector transmission hence its epidemiology.

5.0 CHAPTER FIVE

TRANSMISSION OF CASSAVA BROWN STREAK VIRUS IN KENYA

5.1 Introduction

Cassava brown streak disease (CBSD) described in Tanzania (Storey, 1936) attacks cassava leading to losses in root weight of up to 70% in susceptible cultivars (Hillocks *et al*., 2001). It is the second most significant biotic constraint to cassava production in Kenya after cassava mosaic disease. Unlike cassava mosaic disease (CMD) with which it is often associated, it attacks leaves, stem and roots, causing yellow/ brown, corky necrosis in the starch-bearing tissue, making the most severely affected roots unfit for consumption. It has spread along coastal cassava-growing areas from Southern Kenya, through Tanzania to the Zambezi River in Mozambique, and also occurs, in some inland areas of Malawi and Uganda, up to an altitude of 1000 m above sea level (Hillocks and Jennings, 2003).

The disease is caused by CBSV, an Ipomovirus in the family Potyviridae (Monger *et al.*, 2001b) a virus that is graft-transmissible from cassava to cassava (Storey, 1936) and mechanically transmitted from cassava to a number of herbaceous hosts (Lister, 1959). An earlier suggestion pointed out that CBSV was insect-transmitted and that the most probable vector was the whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) (Storey, 1939; Bock, 1994). Moreover work done by Maruthi *et al* (2004) reported *B. tabaci* exhibiting low transmission rates of 20-22% without a confirmatory study and second direct evidence supporting it to date. The efficiency of *B. tabaci* in CBSV transmission has not been determined. It has further been observed that *B. afer* (Priesner & Hosny), although less abundant than *B. tabaci,* reaches high population densities in some areas where CBSD incidence is high (Robertson, 1987; Munthali, 1992). This led to the proposal that *B. afer* was the most probable vector (Bock, 1994). This was similarly not confirmed and had no

direct evidence. The very low rates of natural spread of CBSD (Storey, 1939; Bock, 1994 and Maruthi *et al.*, 2004) are inconsistent with the high incidences of CBSD observed in the field surveys of up to 64% (Alicai *et al.*, 2007).

Although successful, virus transmission by *B. tabaci* has been reported, this does not preclude the possibility that under suitable conditions, *B. afer* whose population has been directly correlated to increase with the incidence of the disease (Robertson, 1987) and *A. dispersus* newly introduced and infesting cassava in coastal Kenya may also transmit CBSV. *A. dispersus* has been sighted in high population on lower mature CBSD symptomatic leaves during a whitefly collection survey in Kilifi, Malindi, Lunga lunga and Msambwueni within coastal cassava growing regions (Chapter 3). Its population was higher than that of *B. tabaci* with the latter found on young top-most leaves of the plant with the former mostly found on lower mature leaves. This observation coupled with the fact that *A. dispersus* ' high population seemed to coincide with resurgence of CBSD at the Coast together with the fact that transmission of this CBSV had not been clearly studied with the range of vectors remaining unknown promoted these transmission trials using the two whitefly spp.

The coat protein of CBSV has been sequenced and showed high sequence identity (27.3%) with the coat protein of mite transmitted tritimoviruses (Wheat streak mosaic virus and Brome streak virus) indicating possible role of the mites in its transmission (Monger *et al.*, 2001). Several pests (cassava green mites, red spider mites and cassava mealy bugs) that infest cassava might transmit CBSV during their mechanical feeding action. It became necessary to investigate whether cassava green mites; *Mononychellus tanajoa* (Bondar) and cassava red mites; *Tranychus urticae* can acquire and transmit the virus. However, the low

transmission rates (20-22%) and inconsistent results may have been due to technical difficulties in the transmission protocols and because the conditions that determine efficient vector transmission have not been fully understood and adopted (Maruthi *et al.*, 2004). In the light of this back ground a study was designed and implemented to determine the ability of *B. tabaci, A. dispersus* and other cassava infesting pests to transmit CBSV. Similarly their efficiency in CBSV transmission was investigated.

5.2 Materials and Methods

5.2.1 Collection of whitefly spp and other cassava pests

Populations of whiteflies (*B. tabaci*) were collected using an aspirator (Plate 12) from cassava plants during a CBSD survey in Western Kenya. The whitefly adults were transported under confinement in a whitefly tight meshed container and maintained on sweet potato and CBSV free cassava plants in insect-proof cages at Kenya Agricultural Research Institute-Kabete Centre in Nairobi. CBSV susceptible cassava cultivars MM96/5280 and MM96/4466 were identified during a CBSD diagnostic survey in Western Kenya. The cuttings of the cultivars were collected from KARI Kakamega established within green house and to ensure the absence of the Ipomovirus CBSV, all plant materials were subjected to RT-PCR test for CBSV infection as described by Monger *et al.* (2001) and then kept in whitefly exclusion cages (0.3mm mesh) prior to the start of the transmission experiments. For KARI- Mtwapa experiments same recipients' plants were used whereas the whitefly spp were collected from cassava plants and used directly for transmission experiments as time constraint could not allow for colony rearing procedure. Some populations were given 48-h AAP before transfer to target plants for inoculation access feeding period of 48 hours.

Plate 12: Collection of whitefly from cassava plants using an aspirator and a plastic jar.

5.2.2 CBSV Transmission Tests

Protocols for the whitefly transmission test were adopted from Maruthi *et al* (2004.), with modifications. Adult pests were given 48 hours access acquisition feeding time enmass (Field situation) and under no choice confinement (Falcon cages (Plate 13)) on diseased cassava plants and access inoculation feeding period of 48 hours on recipient plants within 0.3mm mesh cages under green house conditions (26°C ±2). In respect of the enmass feeding approximately 600 *B. tabaci* were allowed to acquire the cassava brown streak virus from CBSD infected cassava plants in whitefly-exclusion cages for 48 hours. The whiteflies were then collected by aspiration (Plate 12), transferred onto the test plants, and allowed inoculation access period on CBSV-free (MM96/5280 and MM96/4466) cassava plants in whitefly-exclusion cages for at least 48 hours.

The inoculation with CBSV was repeated four times at 7-day intervals. The whiteflies were then eliminated by spraying with the Brigade insecticide and symptom development monitored for 2-3 months. The experimental design involved 6 recipient plants 3 from each cultivar replicated four times summing up to 24 recipient plants per experiment. A control cage was set up with 6 (3 from each cultivar) CBSV free cassava plants infested with 'non viruliferous' (reared on sweet potato) *B. tabaci.* In each cage 6 diseased cassava plants were included to act as a continuous source of the virus (simulating field condition). The experiments were repeated three times.

Similar transmission experiments with slight variation were conducted using *B. tabaci* and *A. dispersus* at KARI-Mtwapa in coastal province of Kenya. These trials were done at the coast due to difficulty in raising enough colonies of *Spiraling whitefly* (*A. dispersus*) and *B. afer* at KARI/NARL in Nairobi. The whitefly populations were collected from cassava

plants within the KARI station and farmer plots around the station. The first transmission trial involved an adult whitefly population collected on CBSD infected cassava leaves (not given 48-h AAP) in the field and directly transferred to 9 recipient plants for 48-h IAP within 1x1x1.5 m whitefly tight cages under green house conditions (28°C ±2) . Three cages (9 recipient plants per age) were set up for each sp in first and second experiments (27 recipient plants in each experiment). In the second trial whiteflies were given 48-h AAP before transferring on to target plants for 48-h IAP. A modified falcon tube and 0.3 mesh cages were used to confine CBSD leaf petioles and infected cassava and respectively in cassava plots within the KARI centre (Plates 13 and 14 respectively).

In these cages the colonies of the whitefly spp collected were given 48 hours acquisition access feeding period then transferred onto 9 recipient plants of cultivar MM96/5280 for an inoculation access feeding period (IAP) of at least 48 hours within modified clip cages of water bottles and falcon tubes (Plate 13). The set up had 9 target plants replicated three times (27 recipient plants) for each whitefly spp. 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 inoculation with CBSV was repeated four times at 7-day intervals and each time using approximately 30 whitefly adults per plant. The 4 repeat inoculations were sequenced to start from first emergent leaves progressively upwards to the upper leaves. The whiteflies were then eliminated by spraying with Brigade insecticide and symptom development monitored specifically on inoculated leaves and entire recipient plant for 26-60 days.

Plate 13: Spiraling whitefly feeding on CBSD cassava leaves within a falcon tube cage

Plate 14: A 0.3 mesh cage containing recipient plants used for acquisition access feeding

A control cage for each whitefly spp was set up with 3 CBSV free cassava plants not infested with whitefly since the population collected were from already diseased plants in the field. In fact it was hard to find asymptomatic cassava plants during the adult whitefly spp collection in coastal Kenya. The experiments were repeated three times including a first trial experiment and 2 repeat trials for each of the 2 whitefly spp.

Fifty to a hundred adults of each of the other insect spp (cassava green mites, mealy bugs and red mites) reared on CBSV infected plants were transferred on to CBSV free recipient cassava plants in separate cages (Plates 16 and 17). Each cage contained 6 young CBSV virus-free plants with four cages being maintained for each insect species. These experiments were repeated three times. For each insect sp two control cages comprising 6 virus free plants were set up. Symptom development was monitored daily through visual observations followed by testing by RT-PCR using virus specific primers (Monger *et al.*, 2001) for the presence of CBSV. Percentage transmission was determined by the number of infected target plants over the total number of plants tested and the results were analyzed using probability estimates.

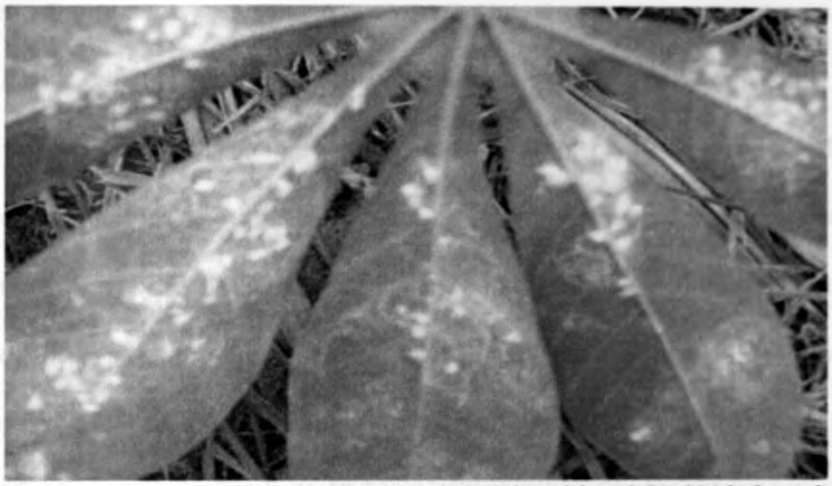

Plate 15: Spiraling whitefly adults feeding on the under side of CBSV infected cassava leaf

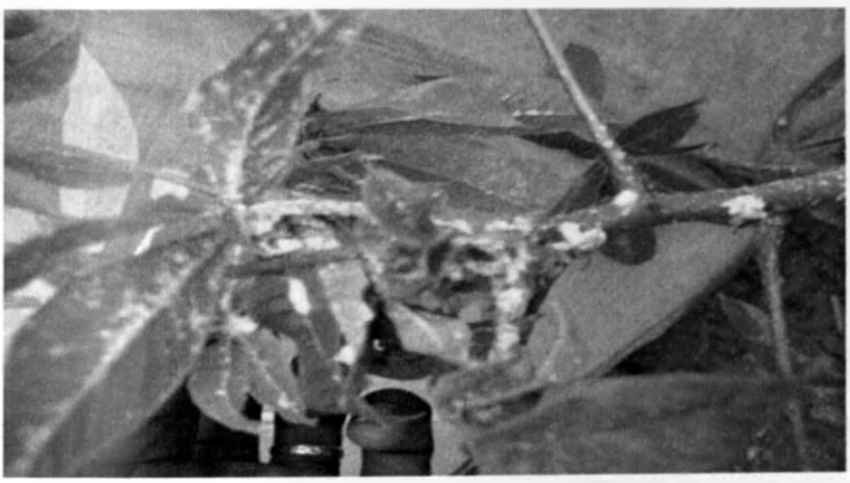

Plate 16: Mealy bugs fed on CBSD symptomatic cassava plant for AAP

Plate 17: Symptomatic leaf with mealy bugs introduced onto recipient plant for IAP

5.2.3 Transmission efficiency of cassava brown streak virus by whiteflies

To determine the transmission efficiency of the 2 whitefly spp a viruliferous population of each was introduced to feed on leaves of several target plants. Together, the effect of whitefly numbers on transmission efficiency was also assessed using 1, 5, 15 and 30 adult whiteflies of each sp on target plants. Parallel plant to plant transmissions were performed for each spp while varying the number of whitefly adults as indicated above. The colonies of the two whitefly spp were given 48 hours acquisition feeding period on CBSD symptomatic leaves then transferred onto 4 recipient plants of cultivar MM96/5280 for an inoculation feeding period of at least 48 hours within modified clip cages of water bottles and falcon tubes.

The 1, 5, 15, 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 inoculation with CBSV was repeated four times at 7-day intervals. The 4 repeat inoculations were sequenced to start from first emergent leaves progressively upwards to the top leaves. In this case 4 recipient plants of cultivar MM96/5280 were used per population for each whitefly spp. Four cages were set up for the (1, 5, 15, 30) different whitefly populations of each whitefly spp. A control cage for each whitefly spp was set up with 3 CBSV free cassava plants with out whiteflies. The whiteflies were eliminated by spraying with the brigade insecticide after every inoculation. Symptom development monitored specifically on inoculated leaves and entire recipient plant. Virus transmission efficiency was calculated as the percentage of the total number of plants infested with viruliferous whitefly sp that become infected. The transmission efficiency data were transformed as an arcsine square root prior to analysis, although the untransformed data are also presented in figures. Comparisons of virus transmission efficiency were made using

probability estimates of the probability of transmission by a single whitefly (Gibbs & Gower, 1960; Ng & Perry, 1999).

5. 3 Detection of cassava brown streak virus by RT-PCR

5.3.1 RNA Extraction

The presence of cassava brown streak virus in recipient plant tissues was assayed following the methods of Monger *et al.* (2001) with modifications. Cassava leaf (0.3 g) was ground to a powder with liquid nitrogen in a sterile pestle and mortar. One millilitre of grinding buffer was added (2% CTAB, 2% polyvinylpolypyrrolidone-40, 100 mm Tris pH 8'0, 20 mm EDTA, 1'4 m NaCl and 20 mm DTT added fresh). The suspension (800 mL) was transferred to a microfuge tube and incubated at 65°C for 15 minutes. After incubation 600 mL of chloroform: IAA (24: 1) was added and mixed by inverting the tubes. The phases were separated by microfuge at maximum speed of 13,000rpm for 10 minutes. The upper aqueous phase (300 mL) was removed and the chloroform extraction repeated. Ethanol precipitation of the nucleic acid was performed with 150mL of 5 m NaCl and 600mL of ice cold ethanol at -20°C for 30 min. The nucleic acid was collected by centrifugation at 6500 rpm speed for 10 min and re suspended in 150 mL of 2 m LiCl. The nucleic acid was left in the LiCl overnight at 4°C. The RNA was pelleted in a microfuge at 13,000 rpm speed for 30 min at room temperature. The LiCl was removed, the pellet washed with 70% molecular grade ethanol, dried and resuspended in 50mL of TE buffer.

5.3.2 cDNA Synthesis and RT-PCR

The single stranded RNA was synthesized into a complementary DNA (cDNA) following manufacturer's instruction using Moloney murine leukemia virus reverse transcriptase

enzyme from Invitrogen and gene specific primers CBSV (F) and CBSV 11(R) (Monger *et al.*, 2001).

The complementary DNA was amplified in a 25-mL PCR reaction volume containing 19.05 µl RNase-Free Water, 2.5µl of 10x PCR buffer, 0.75 µl of 50 mm $MgCl_2$, 0.5µl dNTPs Premix , 0.5µl of each primer (CBSV 10 (F), CBSV 11 (R)), 1mL of cDNA sample and 0.2µ Taq polymerase (Promega UK Ltd, Southampton, UK).The PCR program was set at 94°C initial denaturation for 2 min followed by one loop of 3 segments; 94°C for 1 min (denaturation), 57°C for 1 min (annealing) and 72°C for 1 min (Extension), repeated for 30 cycles then held at 72°C for 10 min (Final extension). Products were separated by 1% agarose gel electrophoresis containing10mg/ml ethidium bromide in TAE buffer at 80 volts for 45 minutes and viewed under UV light against a 1kb DNA ladder (Promega). The cDNA bands appearing at 231 base pair were positive for CBSV presence.

5.4 Results

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

In the first transmission experiment 3 of 12 (25%) cassava plants (MM96/5280) inoculated with 600 adult whitefly population of viruliferous (48-h AAP) *B. tabaci* produced typical CBSD symptoms. In this case the whiteflies were introduced in mass onto CBSV infected plants (48-h AAP) before being transferred through aspiration to recipient plants enclosed within meshed cages under green house conditions. High/mass mortality of the whiteflies was observed during hot conditions (>30°C). This was a great challenge since AAP required 48 hours similar to IAP whereas the whiteflies would die prior to transfer (within two days) to the recipient plants. Nevertheless this trial proceeded when water was applied on the green house floor and also by opening of the vents. The number of days that elapsed from inoculation to symptom appearance was 60 days in Nairobi and 26 days in Mtwapa. Symptoms in vector-inoculated plants were similar to those seen in plants obtained from infected cuttings (Plate 18, 19, 20, 21 and 22).

Initial symptoms appeared as yellowing of tertiary veins, which later extended into secondary and primary veins (Plates 18, 19 and 21). This was followed by leaf chlorosis in a characteristic pattern of feathering and stem necrosis. The RT-PCR test on the leaf samples showed the presence of the virus (Fig 4 (Lanes D-G and J)).

Plate 18: Initial symptoms of CBSV infected recipient cassava leaf (Apparent slight foliar mosaic; MM96/5280)

Plate 19: Prominent foliar mosaic of CBSV infected recipient cassava leaf (MM96/5280)

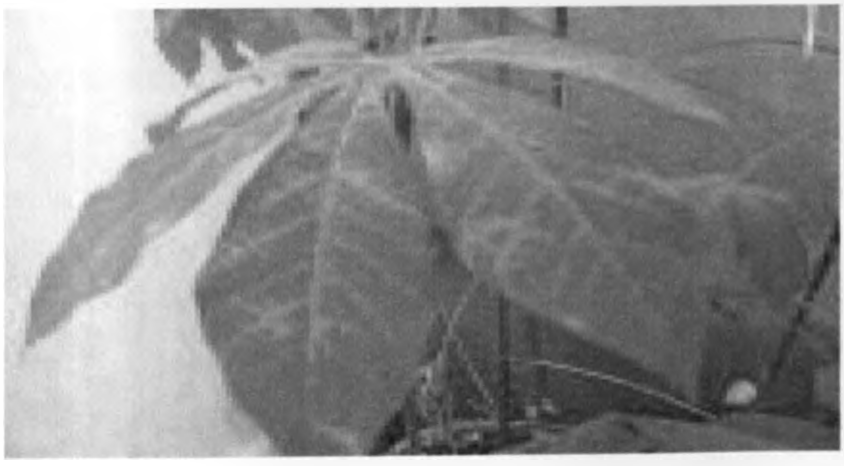

Plate 20: Clear foliar mosaic with characteristic feathering pattern on an infected recipient cassava leaf (MM96/5280)

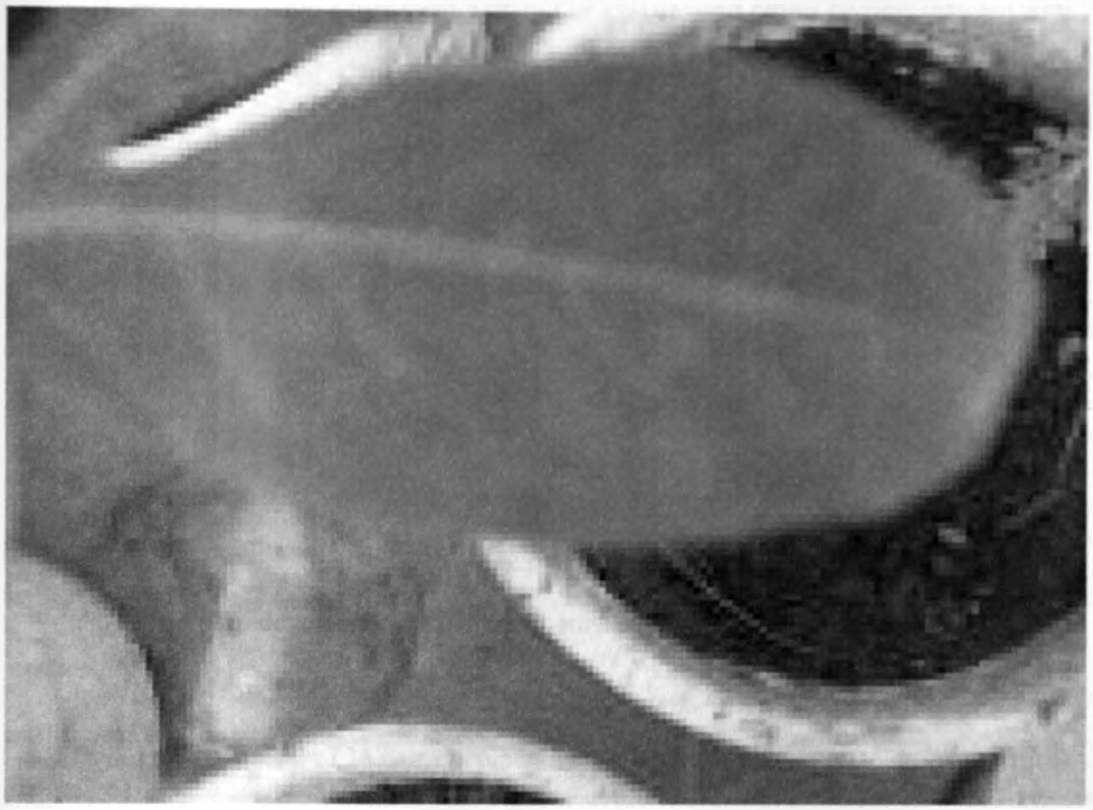

Plate 21: Initial symptoms on CBSV infected recipient cassava leaf of cultivar MM99/4466.

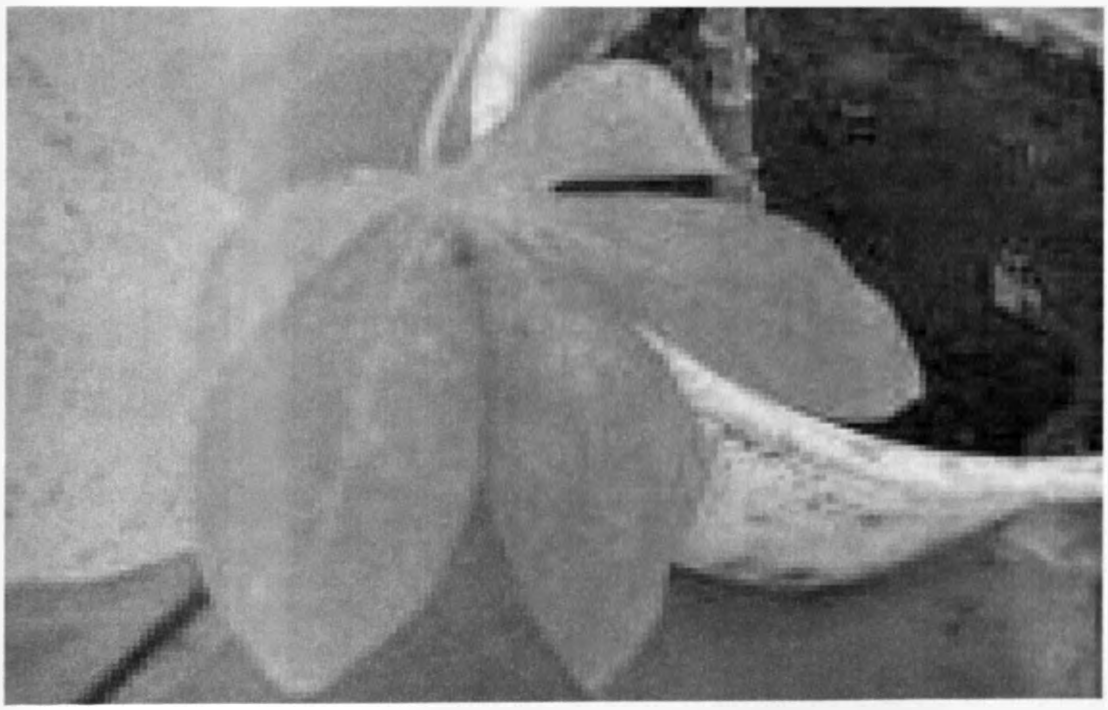

Plate 22: Advanced leaf chlorosis and mosaic on an infected recipient cassava leaf of cultivar MM96/4466

In the second transmission experiment 7 of 24 (29.2%) cassava plants became infected with CBSV. The infection of CBSV was confirmed by RT-PCR. It was determined in subsequent experiments with Cassava green mites (*M. tanajoa*), Red mites (*Tetranychus urticae*) and Cassava mealy bugs (*Phanococcus manihoti* that each of these spp does not transmit CBSV (Fig 4 (Lanes H-I)). This was confirmed when leaf samples of recipient plants from each of these trials were tested by RT-PCR. CBSV was not detected at all in the three trials.

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 cage) had 17 of 72 test plants (23.6%) developing CBSD symptoms. A total of 18 cassava plants var. MM96/5280 exposed to *A. dispersus* (7) and *B. tabaci* (11) collected in the field in KARI-Mtwapa from cassava showing symptoms of CBSD, developed symptoms. This was confirmed by RT-PCR tests (Fig 4 and 5).

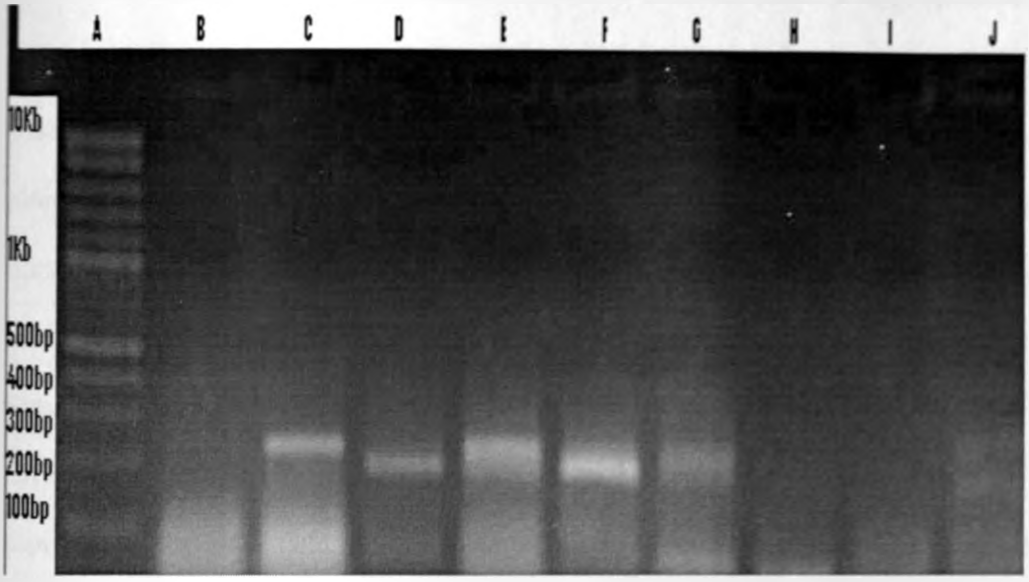

Fig 4: Photograph of PCR electrophoresis gel showing CBSV diagnostic bands (D,E,F.G and J transmission by *B. tabaci*) (231 base pairs), (A is 10Kb DNA ladder whereas C is a positive control, 10 positive control and Lanes H-I were samples from transmission trials by cassava green mites and cassava mealy bugs respectively.

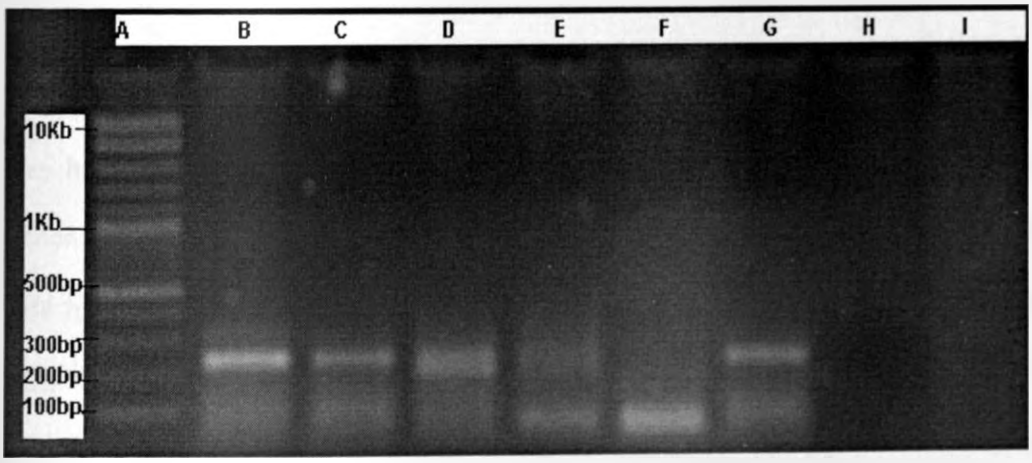

Fig 5: Photograph of PCR electrophoresis gel showing CBSV diagnostic bands (231 base pairs) A 10kb DNA ladder whereas (H) is a negative control, (I) water. (D) Positive control.

5.4.2 Field collected whitefly population versus Viruliferous (48-H AAP) population

It was determined that each of the whitely spp transmit CBSV independent of the other. CBSV was transmitted more efficiently when adult whiteflies were fed on infected cassava plants for 48 hours before allowing 48-h IAP on the recipient plants. For instance, transmission rate greater than 35% was achieved by *B. tabaci* population given 48-h AAP than non viruliferous population which gave a lower than 20% transmission rate (Table 9). Similarly *A. dispersus* was more efficient when given a 48-h AAP than when not (Table 9). Effectiveness of *B. tabaci* over *A. dispersus* as a vector was shown by high rate of transmission by both field collected and viruliferous population than those of spiraling whitefly. Transmission of the Ipomovirus by whiteflies fed on infected symptomatic leaves in over all rating was higher with *B. tabaci* at 40.7% when given 48-h AAP (no choice) whereas spiraling whitefly had an overall of 25.9%. Symptom development in plants required long incubation period (26-60 days) and none of the recipient plants showed symptoms until after 26 days.

The symptoms varied among the two cultivars used in these experiments (Plate 10). There was high humidity (>80%) within the falcon cages and high temperatures above 30°C which led to high mortality of the adult whiteflies during the experiments in KARI-Mtwapa. The net green houses where this work was conducted most of the time experienced high temperatures of 29-35°C. This affected this work adversely although *B. tabaci* appeared stronger withstanding the higher temperatures and humidity. *A. dispersus* was most vulnerable to the extent that in the event of high humidity within the falcon tube due to rainfall all the adults would die immediately.

Table 9: CBSV transmission rates by adult whitefly species allowed 48 AAP and those not allowed 48 h AAP.

Field collected adult whiteflies without 48-h AAP									
	Bemisia tabaci				*Aleurodicus dispersus*				
		No Infected					No Infected		
Rep	Recipient plants	Expt 1	Expt 2	Exp 3	Replicate	Recipient plants	Exp 1	Exp 2	Exp 3
1	9	2 (22.2)	0	3(33.3)	1	9	1 (11.1)	0	2(22.2)
2	9	1(11.1)	2 (22.2)	0	2	9	0	1(11.1)	0
3	9	1(11.1)	0	2(22.2)	3	9	3 (33.3)	0	0
		(14.8)	(7.4)	(18.5)			(14.8)	(3.7)	(7.4)
Probability									
Field collected adult whiteflies given 48-h AAP on CBSV infected plants									
	Bemisia tabaci				*Aleurodicus dispersus*				
Replicate	Recipient plants	No infected				Recipient plants	No infected		
		Exp 1	Exp 2	Exp 3	Replicate		Exp 1	Exp 2	Exp 3
1	9	3 (33.3)	6 (66.6)	3(33.3)	1	9	2 (22.2)	0	3(33.3)
2	9	2 (22.2)	2 (22.2)	5(55.5)	2	9	1 (11.2)	1(11.1)	4(44.4)
3	9	5 (55.5)	3 (33.3)	4(44.4)	3	9	3 (33.3)	4 (44.4)	5 (55.5)
		(37.0)	(40.7)	(44.4)			(22.2)	(18.5)	(37.0)
Probability									

Figures in parenthesis are percentage infection per replicate and each experiment in bold

5.4.3 Transmission efficiency

Efficiency of transmission differed among the two whitefly species examined in these studies. The most efficient transmission was observed with *B. tabaci* (Table 9), following a standard 48-h AAP on CBSV-infected cassava plants and a 48-h IAP. CBSV was transmitted at low levels by individual whiteflies of *Bemisia 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.

Even 15 whiteflies per plant resulted in greater than 50% transmission by *B. tabaci*. On the other hand *A. dispersus*, which is a recent invasive pest of cassava in coastal Kenya, was less efficient at transmitting CBSV (Fisher, $P < 0.0001$) (Table 10). However, unlike *B. tabaci*, transmission was not observed with individual spiraling whiteflies over the course of four experiments due to adverse conditions that lead to mortality. Non choice feeding on CBSD symptomatic leaves within falcon cages lead to greater efficiency of transmission by both spp.

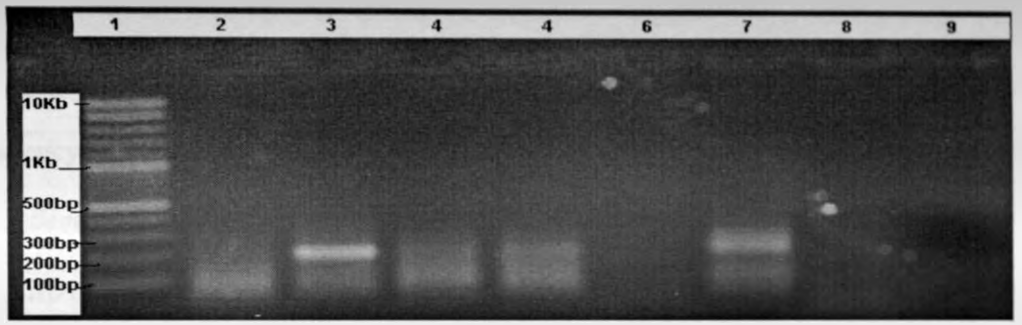

Fig 6: Photograph of PCR products showing CBSV diagnostic bands (231 base pairs) A 10kb DNA ladder whereas (i) is a negative control.

Table 10: Adult whitefly numbers used in CBSV transmission and transmission percentage per species

Field collected adult whiteflies without 48-h AAP

EXPERIMENT		*Bemisia tabaci*				*Aleurodicus dispersus*			
		Probability					Probability		
Replicate	Recipient plants	Expt 1	Expt 2	Exp 3	Replicate	Recipient plants	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)**

Field collected adult whiteflies given 48-h AAP on CBSV infected plants

EXPERIMENT		*Bemisia tabaci*				*Aleurodicus dispersus*			
	Recipient	Probability				Recipient	Probability		
Replicate	plants	Exp 1	Exp 2	Exp 3	Replicate	plants	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)**

Table 11: Probability of CBSV transmission by a single adult whitefly when allowed 48h AAP and when not given 48h AAP.

Adult whitefly sp efficiency of CBSV transmission										
EXPERIMENT	*Bemisia tabaci*					*Aleurodicus dispersus*				
	No of adults per plant					*No of adults per plant*				
	Recipient					Recipient				
Replicate	plants	1	5	15	30	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)
Probability		0.125								

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. Adult whiteflies were thirty per plant.

5.5 Discussion

CBSV was transmitted by *B. tabaci* but no transmission was observed when cassava green mite, cassava mealy bug and cassava red mites were used. Transmission of CBSV by *B tabaci* concurs with the whitefly transmission of other ipomoviruses (Hollings and Stone, 1976; Mansour and Al-Musa, 1993). Transmission rates of CBSV achieved here are near those of Cucumber vein yellowing virus (CVYV) (55%) (Mansour and Al-Musa, 1993), a new member of the genus Ipomovirus (Lecoq *et al.*, 2000), and but slightly lower relative to the high incidences of CBSD observed in some fields (Hillocks and Jennings, 2003). This suggests that CBSV may be transmitted poorly by *B. tabaci* and that the high incidences of CBSD seen in farmer fields may not only be due to cutting-borne infections (Maruthi *et al.*, 2004) but also other suspected vectors such as *B. afer* and *A. dispersus*.

The low transmission rate reported here may also be due to technical difficulties in the transmission protocols such as high temperatures within confined cages (mass mortality) and extreme humidity levels in falcon tubes. Environmental conditions may significantly affect transmission adversely (Maruthi *et al.*, 2004). For instance, high humidity within the clip cages lead to mass mortality of the spiraling whitefly although *B. tabaci* was able to survive humid condition but not absolutely.

The feeding behavior 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.*, 2004) whereas the most obvious CBSD symptoms and presumably higher virus titre develop in the lower leaves. The transmission mechanism employed here tried to over come this challenge by allowing *B. tabaci* to feed on the most symptomatic leaves of field-grown cassava 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.

Transmission of CBSV by field collected population of both the two whitefly spp. 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 have 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 infected cassava and also from non choice feeding then immediately transferred on to the recipient plants. In both cases transmission occurred meaning those adults that had acquired the virus did not loose the ability to transmit it during the transfer and also point to the fact that both the two vectors do not require a latent period before it can transmit the virus to the next host. Hence can be categorized as non-persistent or semi persistent mode of transmission. 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 and Fuchs, 2005). Further work need therefore to focus on categorizing the mode of CBSV transmission by the vectors involved.

Transmission of a 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. Similar results were achieved from this experiment when CBSV was transmitted by a single adult *Bemisia tabaci*.

One of the would be remarkable biological property of CBSV reported for the first time is its ability to be transmitted by two different whitefly vectors in two genera (*Bemisia* and *Aleurodicus* spp). This is not very unusual for a whitefly-transmitted virus. Earlier studies have demonstrated that Tomato chlorosis virus (ToCV) can be transmitted with equal efficiency by both *Trialeurodes abutilonea* and *Bemisia 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). More over both *Bemisia 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 (Winter mantel and Wisler, 2006).

The ability of *B. tabaci* to transmit CBSV seemed to be affected by the inoculation and acquisition feeding period. Percent transmission increased as the acquisition access feeding period of 48-h was allowed.

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 area in coastal Kenya. High population of whitefly in the field, which may be composed of *B. tabaci* and *A. dispersus* may be correlated and explain the high disease incidence observed under field

conditions. The results of the investigations on ability of the insects collected from the infected cassava field to acquire and inoculate the virus help us in understanding the role of the whitefly species in the development and spread of the CBSD in field conditions. The implication of this important finding is that host-plant resistance to whitefly may be considered as a possible method of dual control for CBSD (Hillocks *et al.*, 2005).

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 General discussion and Conclusions

CBSD from the findings is nolonger restricted in distribution along the coastal lowlands and has become wide spread in major cassava growing regions of Kenya. The study on spiraling whitefly presents the first host list (56) of *A. dispersus* in coastal Kenya, although narrower as compared to the extensive host range of 481 plants already reported belonging to 295 genera from 90 families (Srinivasa, 2000). The list already points to the polyphagous nature of the spiraling whitefly.

CBSV a causal agent of the disease is spread by *Bemisia tabaci* as found out in the transmission studies. The findings report for the first time the ability of spiraling whitefly (*Aleurodicus dispersus*) to transmit CBSV and may explain its contribution in the spread of CBSD in cassava growing districts in coastal Kenya. The results of the investigations on ability of the insects collected from the infected cassava field to acquire and inoculate the virus help us in understanding the role of the whitefly species in the development and spread of the CBSD in field conditions.

6.2 Recommendations

- Farmers lack accurate information on the symptoms, cause, spread and management of the disease. This information regarding CBSD symptoms, identification and management practices need to be availed to farmers to avoid further losses inflicted by this disease.

- CBSD problem requires an immediate attention to curb the spread of the disease in Kenya. It is imperative therefore that alternative varieties tolerant to CBSD be supplied to

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6.0 APPENDICES

Appendix 1: Analysis of variance tables

CBSD Incidence (Western, Eastern, Nyanza and Central Kenya)

Source	df	Sum of Squares	Mean Square	F value	Sig.
rep	12	2747.32	228.9	0.3	0.98
Between districts	8	90096.07	11262.0	16.5	0.001***
Experimental Error	74	50577.27	683.5		
Total	94	158048.48			

Bemisia tabaci **counts**

Source	df	Sum of Squares	Mean Square	F value	p value
rep	12	56.394	4.7	1.6	0.111
Between districts	8	267.947	33.5	11.4	0.001***
Experimental Error	74	217.831	2.9		
Total	94	657.200			

Pearson's correlation of CBSD incidence and *B. tabaci* counts

		CBSD Incidence	*B. tabaci* counts
CBSD Incidence	Pearson Correlation	1	+0.698**
	p-value	.	0.000
	Number of observations	95	95
B. tabaci counts	Pearson Correlation	+0.698**	1
	p-value	0.000	.
	Number of observations	95	95

**. Correlation is significant at the 0.01 level

CBSD severity

District	Average severity
Bondo	3
Bungoma	2
Busia	2
Embu	1
Kirinyaga	1
Machakos	1
Siaya	1
Teso	1
Thika	1

The severity of the disease followed the flowing trend. Bondo having the highest level of severity, then Bungoma and Busia having similar severity.

Appendix 2

6.4 The viruses of cassava, (Calvert and Thresh 2002)

Africa
Cassava mosaic geminiviruses (*Geminiviridae*: *Begomovirus*)
Cassava brown streak virus (*Potyviridae*: *Ipomovirus*)
Cassava Ivorian Bacilliform virus* (unassigned)
Cassava Kumi viruses A and B*
Cassava "Q" virus*
*Cassava common mosaic virus** (*Potexvirus*)

South/Central America
Cassava common mosaic virus (Potexvirus)
*Cassava virus X (Potexvirus)**
Cassava vein mosaic virus (Caulimoviridae)
*Cassava Colombian symptomless virus (Potexvirus)**
*Cassava American latent virus (Comoviridae: Nepovirus)**
Cassava frogskin "virus"

Asia/Pacific
*Cassava common mosaic virus** *(Potexvirus)*
Indian cassava mosaic virus (Geminiviridae: Begomovirus)
Sri Lankan cassava mosaic virus (Geminiviridae: Begomovirus)
*Cassava green mottle virus** *(Comoviridae: Nepovirus)*

Note
Viruses with names in italics are recognized species.
* Viruses with localized distributions and not of economic significance.

Appendix 3

FIELD SURVEY QUESTUIONARE

Date.....

Farmer's name;	
Acreage	
Location	
Division	
District	
Altitude	
Rainfall (average)	
Av. temperature	
Ecological zone	

What varieties of cassava do you grow on this farm?

1. .....
2. .....
3. .....
4. .....

Which variety is preferred by farmers and why not the others?

1. .....
2. .....

What are the diseases and pests of cassava you have observed in your farm?

1. .....
2. .....
3. .....
4. .....

Which is the most susceptible variety to CBSD?

1. Very susceptible.....
2. Tolerant .....
3. Resistant .....

Colored photo of disease symptom taken

4. Foliar.....
5. Root and stem lesion.....

What is the CBSD incidence?

Farm/plot	Total No of plants	Visibly diseased	Incidence (%)	Whitefly population

What is the severity of the disease?

Above ground severity; Table 3

1- No apparent symptoms, 2- Slight foliar mosaic, no stem lesions, 3- Foliar mosaic, mild stem lesions no die back, 4- Foliar mosaic and pronounced stem lesions no die back and 5- Defoliation with stem lesions and pronounced die back.

	Severity scale						
Farm/plot	1	2	3	4	5	Total	Remarks

Roots severity Table 4

2- No apparent root necrosis, 2- Less than 5% of the root necrotic, 3- 5 – 10% of the root necrotic, 4- 10 – 25% of the root necrotic, mild constriction and 5- 25% of the root necrotic, severe root constriction.

	Severity scale						
Plant	1	2	3	4	5	Total no of roots	Remarks
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							

Where do you obtain the planting materials?

Is there any presence of whitefly?

What are the challenges to cassava production in this area?

What is your response in dealing with the mentioned problems?

What is the relationship between the insect population and the incidence and severity levels of the disease?

What is the relationship between the shoot symptoms and the root symptoms?

Appendix 5:Table of Cassava Production by Province in Kenya

YEAR	Central		Eastern		Rift Valley		Coast		Nyanza		Western	
	Area	Production (MT)	Area	Production (MT)	Area	Production (MT)	Area	Production (MT)	Area	Production (MT)	Area	Production (MT)
1997	308	2,663	7,089	63,021	914	8,138	9,560	102,081	22,054	243,548	14,665	98,818
1998	230	1,934	5,997	42,298	1,885	20,858	6,463	58,604	31,053	203,231	11,466	47,788
1999	763	5,548	11,985	105,022	2,253	29,340	14,416	144,167	28,628	344,692	8,502	49,889
2000	346	1,892	10,864	56,054	-	-	14,307	143,091	25,211	165,880	9,545	51,704
2001	249	2,104	7,445	57,528	1,117	7,073	14,598	125,438	44,402	365,375	10,521	50,975
2002	340	1,893	14,659	83,654	2,037	19,263	9,371	93,724	45,877	340,915	11,628	81,216
2003	627	5,008	7,490	53,882	1,645	19,233	19,738	85,271	18,498	199,475	5,637	60,926
2004			8,378	37,052	2,598	32,981	11,303	115,297	27,894	184,290	5,587	120,572
2005	1041	6,122	-	-	2,559	27,423	10,891	73,123	-	-	107,247	60,561
2006	590	4928	10419	59,452	1934.9	21,302	11778	106911	19650	216,150	24130	247,790

Source: Ministry of Agriculture, 2007

Appendix 6: CBSD incidence and prevalence in various agro-ecological zones of Kenya

Survey region	District	CBSD incidence	Whitefly counts	Temperature (°C)	Rainfall (mm)	Altitude
Western	Busia	60[b]	1.7	21-23	1690	1220
	Bungoma	6[c]	2.3	18-21	2100	1211
	Teso	48[b]	2.92	21-23	1428	1220
Nyanza	Bondo	93[a]	6.77	21-22	864	1256
	Siaya	38[b]	2.2	21-22	996	1200
	Kisii	0[c]	0	17-21	1500	1420
Eastern	Embu	2[c]	0.7	18-22	1395	1478
	Machakos	0[c]	0.5	22-23	834	1097
	Mbere	0[c]	0	21-23	820	1220
Central	Kirinyaga	2[c]	0.5	20	1180	1312
	Thika	0[c]	0.7	20-21	879	1477

Appendix 7: Weather data for 2006-2007 at KARI-Mtwapa centre;

2007

	Jan.	Feb.	March	April	May	June	July	Aug.	Sep	Oct.	Nov.	Dec
Min.	32.2	23.3	24.8	24.7	23.8	20.4	22	27.1	22.2	22.8	22.7	23
Max.	31.3	31.15	32.8	31.8	29.7	26.2	29.1	26.2	28.8	28.6	30.7	31
R.H	69.04	67.3	71.5	79.9	82.5	68	79	78.9	81	77.7	74.5	71.1
Total Rainfall	3.2	1.4	21.4	228.8	802.7	57.7	116.4	133.1	147.3	57.8	141.3	83

2006

	Jan.	Feb.	March	April	May	June	July	Aug.	Sep	Oct	Nov.	Dec
Min.	24	24.8	24.9	24.6	23.6	22.5	22	22.2	22.1	23	23.5	23.8
Max.	32	32.6	32.2	30.6	29.8	28.2	27.9	28.8	28.2	29.7	30.2	31.6
R.H	67	68.5	64.6	81	83.4	79.5	78.5	78.5	77	81	80.9	76.5
Total Rainfall	1	1.6	49.7	321.2	305.9	158.6	103	88.3	143.1	282.6	365.1	49.7

Source: Courtesy of Metrological Department- Mtwapa, 2008.