EFFECTS OF HARVESTING INTERVALS AND QUALITY OF PLANTING MATERIALS ON CASSAVA BROWN STREAK DISEASE AND YIELD OF CASSAVA

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CROP SCIENCE OF SOKOINE UNIVERSITY OF AGRICULTURE.

MOROGORO, TANZANIA.

2014

ABSTRACT

Investigations were conducted at Kibaha Sugarcane Research Institute, Coast region, Tanzania to identify cassava brown streak viruses (CBSVs) and their variants by Real-Time Polymerase Chain Reaction (RT-PCR) and to assess the effect of time of cassava harvesting on quality and yield loss associated with cassava brown streak disease (CBSD) infection. This study was prompted by the reported reduction in yield of cassava in Tanzania caused by CBSD. Two field experiments were conducted where the first sought to evaluate the effect of CBSD on above ground yield components and root yield of CBSD-infected and CBSD-free planting materials of Kiroba cassava variety. The second experiment assessed the effect of harvesting time on yield and quality traits for virus-free planting materials of Kiroba and Mwari varieties. The viruses (CBSV and UCBSV) were identified using RT-PCR assays from 220 cassava leaf samples of both varieties. Results indicated that plants affected by CBSVs may or may not express symptoms of CBSD but only molecular (PCR) diagnosis can be used to confirm the presence of either or both of the causal viruses. Use of CBSD-infected planting materials of Kiroba decreased root weight by 24%. However, the symptoms of CBSD were delayed for the plants whose planting materials were free from virus. The highest incidence (24.3%) of root necrosis for Mwari was recorded at 14 months after planting (MAP) and at the highest level of CBSD incidence (5.2%). Kiroba had the highest incidence (22 to 26.8%) of root necrosis from 12 to 16 MAP at the lowest (0%) to the highest CBSD incidence (14.5%). The highest yield (8.8 t ha^{-1}) for Mwari was recorded at 14 MAP, which dropped to 8.0 t ha^{-1} at 16 MAP. The highest yields (8.1 and 9.3 t ha^{-1}) for

Kiroba were recorded at 14 to 16 MAP. It was recommended that virus-free planting materials should be used in order to avoid spread of CBSVs and reduce the intensity of root necrosis. More studies should be conducted using similar planting materials in a non-isolated field and during the second growing season to ascertain the reproducibility of the findings of this study before the findings are recommended to farmers.

DECLARATION

I, MARY BISWALO YONGOLO, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within period of registration and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

5-11-2014

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The above declaration is confirmed by;

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Dr. P.J. Njau **Date**

(Supervisor I)

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ACKNOWLEDGEMENTS

All acclamations and appreciations are for Almighty GOD, Who is the Creator of the Universe, the Cherisher and Sustainer of the world, Lord of all things, Master of the Day of Judgment and Hath Power over all things (Most Gracious, Most Merciful). All respects are due to Holy Jesus Christ (Peace be upon him), the greatest social reformer and revolutioner, who is a symbol of guidance and fountain of knowledge (GOD's bless for us all).

I wish to thank my academic supervisors: Dr. P.J. Njau of the Department of Crop Science and Production, Sokoine University of Agriculture, Morogoro, Tanzania and Dr. J. P. Legg of the International Institute of Tropical Agriculture (IITA) Tanzania, for their helpful advice and assistance. Acknowledgements are made to the Ministry of Agriculture, Food Security and Co-operatives, Tanzania for the award of the fellowship.

I am highly indebted to the East African Agricultural Productivity Program (EAAPP) for their financial support. Special thanks to Root and Tuber National program in Tanzania and the staff of IITA's Virus Vector Ecology Group for their advice and assistance. Lastly but not least, I am greatly indebted to my family for their encouragement and support throughout the duration of my studies. They have been a constant source of love, concern, support and strength for all these years.

DEDICATION

This work is dedicated to my husband Charles Yongolo, my children Isaya, Rebeca and Paul and to my mother Lucia Mkome Biswalo for their patience, endurance, understanding and encouragement during the whole period of my study.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
CBSD	Cassava brown streak disease
CBSV	Cassava brown streak virus
$CBSV_S$	Cassava brown streak viruses
CGM	Cassava green mite
CMD	Cassava mosaic disease
CMG_S	Cassava mosaic geminiviruses
CMV	Cassava mosaic virus
Ct	Threshold cycle
CTAB	Cetyl trimethyl ammonium bromide
DAP	Days after planting
DNA	Deoxyribonucleic acid
DRC	Democratic Republic of Congo
EAAPP	East African Agricultural Productivity Program
EACMD	East African cassava mosaic disease
EACMV	East African cassava mosaic virus
EDTA	Ethylene -diamine-tetra-acetic acid.
ELISA	Enzyme linked immunosorbent assay
FAO	Food and Agriculture Organization of the United Nations
FAOSTAT	Food and Agriculture Organization of the United Nations Statistics
GDP	Gross domestic product
GLCI	Great Lakes Cassava Initiative

IITA	International Institute of Tropical Agriculture
M	Mole
MAFSC	Ministry of Agriculture, Food Security and Co-operatives
MAP	Months after planting
Masl	Metres above sea level
MiRNAs	Micro ribonucleic acids
mM	Millimolar
MT	Metric tons
NaCl	Sodium chloride
PCR	Polymerase chain reaction
PQS	Plant quarantine station

CHAPTER ONE

1.0 INTRODUCTION

1.1 Origin, Production, Distribution and Utilization of Cassava

Cassava (*Manihot esculenta* Crantz) is a perennial root crop. It originated in South America and has become one of the most widely grown food crops with ability to survive in diverse environmental conditions (Fermont *et al.*, 2009). Cassava is cultivated throughout the lowland tropics, typically between 30° N and 30° S of the equator, in areas where the annual mean temperature is greater than 18 °C. It provides efficient carbohydrate production of high cash value and with potential for value-added processing, while being tolerant of low soil fertility. It is the sixth major staple crop in the world after rice, wheat, maize, potato, and sweet potato with an annual production of 262 585 741 tons, and area of production was 20 385 206 ha (FAOSTAT, 2012).

Africa accounts for more than half (0.51) of the world production, whereas Asia and Latin America harvest 0.29 and 0.19, respectively. In Africa, cassava is the second most important food crop after maize; it provides more than half of dietary calories for over half of both the rural and urban populations in sub-Saharan Africa.

Cassava forms an important food and cash crop in Africa. Of all agricultural commodities produced in this continent, cassava is considered the second most important source of calories after maize and third most important dietary source of proteins after beans and maize (IITA, 1990). Cassava is grown in the majority of African countries, but most production is in Nigeria 54 000 000 MT, DRC 16 000

000 MT, Ghana 14 547 279 MT, Angola 10 636 400 MT, Mozambique 10 636 400 MT, Tanzania 5 462 454 MT, Uganda 4 924 560 MT, Malawi 4 692 202, MT Cameroon 4 200 000 MT, Sierra Leone 3 520 000 MT, Madagascar3500000 MT, Benin 3 295 785 MT, and Rwanda 2 716 421 MT (FAOSTAT, 2012). In Africa total area under cassava cultivation is estimated to be approximately 14 million hectares annually (FAOSTAT, 2013). The total annual production in Africa is 157 987 234.4 MT million metric tons. This accounts for 85% of the global production of cassava (FAOSTAT, 2013). In East Africa, Tanzania is the leading cassava producer at 5 400 000 metric tons , followed by Uganda and Kenya 2 228 000 MT and 1 112 420 MT per annum respectively (FAOSTAT, 2013). These authors also narrate that cassava is an important root crop in the medium and low potential areas of Tanzania. The crop plays a major role in smallholder farming system in Tanzania, henceforth improve social economic base of rural community.

Tanzania is an agricultural based country with her economy largely depending on agriculture which contributes about 50% of the GDP, about 75% of the foreign exchange earnings and employs about 70% of the active labour force of the population (Ministry of Agriculture and Food Security, (MAFS, 2004). About 80% of the population live in rural areas and is mainly composed of small- scale farmers who depend on agriculture for their livelihood. The population growth rate is still quite high and therefore the demand for food is always increasing

1.1.1 Socio - economic importance of cassava in Tanzania

Cassava is considered by the Ministry of Agriculture, Food Security and Cooperatives (MAFSC) to be a top priority among other food crops in Tanzania

(Fermont *et al.*, 2009). Area under cultivation in Tanzania is 954 509 hectares (FAOSTAT, 2012). The other merits of cassava are that it has the ability to do well in marginal and stressful environments, tolerance to low rainfall and poor soils; it is not labour intensive, ease of propagation through stem cuttings, and some varieties can be left *in situ* for appreciably long periods of time (2 years) without spoilage (Hillocks *et al.*, 2001). Thus farmers grow and reserve cassava for famine in drought prone areas. Cassava in Tanzania is cultivated in mono and mixed cropping with maize, sorghum and pigeon pea (Hillocks and Thresh, 2001). Cassava is credited for having a flexible harvesting time ranging from 8-24 months after planting (Fermont *et al.*, 2009), which allows for piecemeal harvesting.

1.1.2 Conventional field cassava production in Tanzania

Cassava is propagated using stem cuttings. Usually several cuttings are obtained from a single mother plant at the age of 8-18 months. In few occasions in Tanzania, farmers obtain clean planting materials from research centres and other sources, but it is a common practice for Tanzanian farmers to use stakes obtained from previous crops. However, this practiced way of propagation results into accumulation of viruses in cassava farms from one season to another.

According to Fermont *et al.* (2009), the total production of cassava (1.9 million tons) in Tanzania is higher than that of sweet potato (1.3 million tons) and Irish potato (0.7 million tons). Its yield per unit area (10 t ha^{-1}) is also higher than that of sweet potato (6.0 t ha^{-1}) but similar to that of Irish potato (9.2 t/ha).The yield of cassava on smallholders' fields, however, could be increased to 80.0 t ha^{-1} by adopting improved genotypes, applying appropriate fertilizers, improved crop establishment

and timely control of insect pests and diseases (Hillocks *et al.*, 2000). Thus, there is an appreciable yield gap between actual and maximum potential yield.

1.1.3 Constraints to cassava production in Tanzania

Cassava production in Tanzania is constrained by both abiotic and biotic factors, which are aggravated by sub-optimal management practices and availability of good quality planting materials (Hillocks *et al.*, 2001). The abiotic factors include inadequate rains and soil fertility. Diseases and insect pests constitute the biotic constraint of cassava in Tanzania. The insect pests and diseases of cassava include mealybugs (*Phenacoccus manihoti* Matile-Ferrero), cassava green mites (*Mononychellus tanajoa* Bondar), anthracnose (caused by a fungus *Colletotrichum gloeosporioides*f. sp. *manihotis* Penz.) and bacterial blight (*Xanthomonas axonopodis* pv. *manihotis* Berthet and Bondar) and virus diseases (Fermont *et al.*, 2009).

The most economically important cassava viral diseases in Tanzania are: cassava mosaic disease (CMD) and cassava brown streak disease (CBSD). Both have been recognized in the region since the 1930s but have become increasingly damaging in recent years. CMD is caused by viruses of the family Geminiviridae: genus Begomovirus, referred to collectively as Cassava mosaic geminiviruses (CMGs). Recent studies show that CBSD is caused by at least two distinct ipomoviruses viz. Cassava brown streak virus (CBSV) and Uganda Cassava brown streak virus (UCBSV). As CMV, both CBSV and UCBSV are transmitted by the whitefly, *Bemisia tabaci* (Gennodius).

Recent outbreaks of CBSD in the Lake Zone of Tanzania, and elsewhere in the Great Lakes region of East and Central Africa (Legg *et al.*, 2011), indicated that CBSD is no longer confined to low altitude (<1 000 meters above sea level) coastal areas of East Africa. Virus characterization studies have shown that a second virus species exists in East Africa called *Ugandan cassava brown streak virus* (UCBSV), the two species in some cases co-exist in infected cassava with CBSD (Mbanzibwa *et al.*, 2010). Both virus species, often in mixed infections, were found to be common in both the Coast and Lake Zones of Tanzania (Mbanzibwa *et al.*, 2010). Currently, CBSVs are the most important biotic constraints to cassava production in Tanzania. The CBSD associated viruses are gaining in severity, threatening food and livelihood securities for millions of farmers and cassava consumers in the region. In this study, the two viruses that cause CBSD are referred to collectively as Cassava brown streak viruses (CBSVs), whilst CBSV and UCBSV refer specifically to the two species. At present, most of the cassava varieties grown in Tanzania are susceptible to CBSD.

1.2 Problem Statement

The potential production of the cassava crop in Tanzania has been greatly reduced by CMD and CBSD (Mtunda *et al.*, 2002). CBSD is a major disease which reduces yield and quality of cassava in Tanzania. It has been reported to cause up to 70% yield loss by reducing the root sizes and causing pitting and constriction on roots (Hillocks *et al.*, 2001). CBSD causes a dry necrotic rot in the storage roots, which render them unpalatable and unmarketable (Nichols, 1950), hence destroying yield and causing significant reductions in quality. The damage done by this disease is

severe when cassava is grown under low soil fertility, drought conditions, short rainy season or in late planted crop, leading to total crop loss (Cuambe *et al.*, 2007). Higher losses caused by CBSD have been observed in farmer's fields due to planting infected planting materials but the levels and extent of losses have not yet been thoroughly investigated (Hillocks *et al.*, 2001). No single method is ideal in controlling CBSD, thus several control methods can be combined as an integrated approach to reduce the damage caused by this disease.

1.3 Justification

In Tanzania, cassava production is second to maize in importance as a stable food crop. However, current production of this crop is below the yield potential that can be achieved under good management practices. The current situation is one of cassava deficit due to CBSD as one of the major production constraints. This calls for the need to increase cassava yields per unit area in order to feed the ever-increasing population. The yield and quality of cassava in CBSD prone areas can be improved by proper choice of planting materials and time of harvesting. Control of CBSD using the quality of planting materials and time of harvest has not yet been fully explored. Harvesting cassava earlier may reduce the effects of CBSD on the crop as necrosis tends to affect roots of sensitive varieties from 5-6 months after planting and increases as harvesting is delayed. However, early harvesting to avoid the effects of the disease can still result in reduced yields because the crop is not in the field long enough to reach its full yield potential. This study sought to establish how delayed harvesting and quality of planting materials can be optimized to improve the yield and quality of cassava in CBSD prone areas.

1.4 Overall Objectives

The study aims at generating information needed to establish the optimum harvesting time of cassava in CBSD prevalent areas with a view to improving the yield and quality of cassava

1.4.1 Specific objectives

i. To identify CBSV and its variants by polymerase chain reaction (PCR).

ii. To determine the effect of CBSD on the above ground growth components of cassava.

iii. To determine the effect of quality of planting materials and time of harvesting on yield and quality losses associated with CBSD infection.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Description of Cassava

Cassava (*Manihot esculentum* L.) is a dicotyledonous plant belonging to the Euphorbiaceae family (Onwueme, 1978). It is a tropical and sub-tropical root crop that is mainly propagated by vegetative stems (Fregene *et al.*, 2003). The crop produces fruits that naturally split explosively (shuttering) after pollination and fertilization (Onwueme, 1978; Osiru *et al.*, 1996). *Manihot esulenta* produces starch - bearing tuberous roots that have led to its domestication (Chiwona-Karltun, 2001).

The plant of cassava grows as a shrub and is propagated mainly from the stem cuttings (IITA, 1990; Hallack, 2001). Onwueme (1978) and IITA (1990) reported cassava heights to be dictated by the varieties themselves or the environment where the plant is grown. The stems may reach up to four meters (Osiru, 1996) or attaining less than one meter for the dwarf varieties (Onwueme, 1978; IITA, 1990).

2.2 Cassava Production, Distribution and Importance

Cassava is grown principally for its swollen roots, though its leaves are also eaten as a vegetable in some parts of Africa. The roots are 25 to 35 % starch, but the leaves contain a significant amount of protein and other nutrients (Appendix 1).

As well as being the main source of dietary calories for over half the rural and urban populations in sub-Saharan Africa, cassava roots have industrial use in the

production of animal feed, starch, glucose, paper, and more recently in bio-fuel production (Nassar and Ortiz, 2007). Cassava is a popular crop amongst subsistence farmers due to the ease of propagation through stem cuttings, ability to grow in suboptimal conditions and the year round availability for harvest (Mtunda *et al.*, 2002).

About 95 % of the cassava produced in Africa is used for human consumption and five percent for industrial uses such as starch (Nweke and Haggblade, 2009). Also, cassava is increasingly being used as an animal feed and as a raw material for various industrial products such as ethanol (Doretto, 1993).

Cassava plants are cultivated mainly for their storage roots (Ravindran, 1993), and produce potentially more calories per unit area than any other crop in the world except sugar cane. However, besides the storage roots being rich in starch, the cassava plant canopy has gained importance because it might be a valuable source of proteins, vitamins and minerals for human and animal nutrition (Carvalho *et al.*, 1985; Carvalho *et al.*, 1994). In Tanzania, cassava is an integral component of most cropping systems and is among the most important staples in many zones. It plays an important role as a food security crop and provides useful opportunities for extending labour use and exploiting price peaks in the food market.

2.3 Incidence, Distribution and Importance of Cassava Brown Streak Disease in Tanzania

The CBSD is caused by two viruses of the genus *Ipomovirus*, which is one of the four genera comprising the *Potyviridae* (Monger *et al.*, 2001). However, its causal

viruses have only recently been confirmed as CBSV and UCBSV. CBSD was reported at mid altitude levels (above 1000 masl) in Democratic Republic in Congo (DRC) (Mahungu *et al.*, 2003), Uganda (Alicai *et al.*, 2007), western Kenya and the Lake zone areas of Tanzania (Legg and Jeremiah, 2008). The CBSD was reported in the coastal and lake shore areas of Malawi in eastern and southern Africa and at altitudes below 1 000 masl (Nichols, 1950; Hillocks *et al.*, 1999). The CBSD has shown to be highly damaging with 10–100% incidence that can result in up to 70 % decrease in root weight of infected plants compared to healthy plants (Hillocks *et al.*, 2001). Jennings (1960) regarded the disease as a serious problem in Tanzania and selection for resistance to CBSD and the other virus disease affecting the crop in the region, cassava mosaic disease (CMD), was part of the cassava improvement programme at Amani Research Station throughout the 1940s and 1950s. Recent outbreaks of CBSD in the Lake Zone of Tanzania and elsewhere in the Great Lakes region of East and Central Africa (Legg *et al.*, 2011) indicated that the disease is endemic in cassava growing areas in Tanzania (Fig. 1).

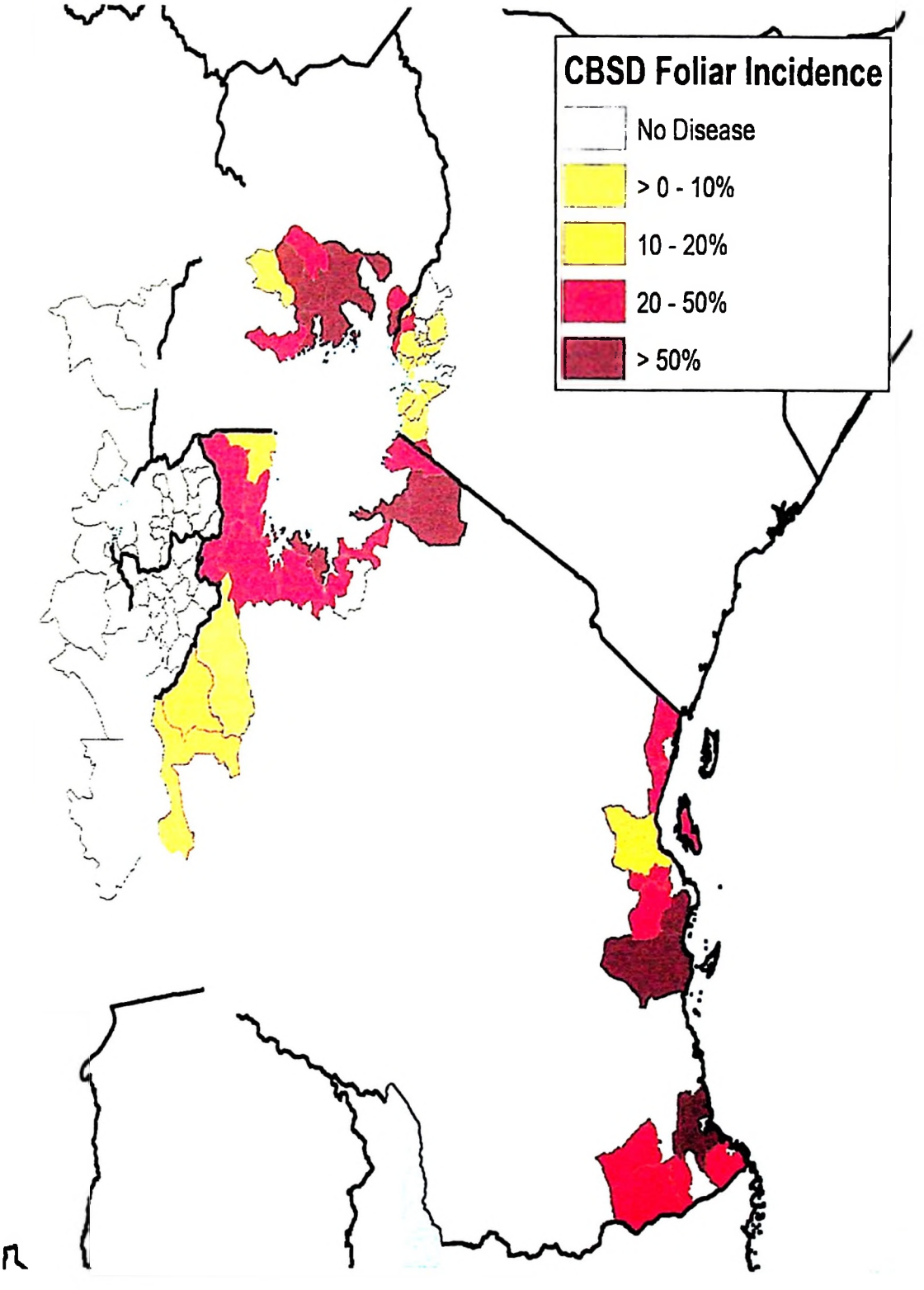

Figure 1: Distribution and importance of cassava brown streak disease in Tanzania

Source: IITA Image Library.GLCI maps (2010)

2.4 Symptoms of Cassava Brown Streak Disease

The name 'brown streak' was given to CBSD because of the brown elongate necrotic lesions that develop on the young green stem tissue of affected plants (Plate 1a).

The symptoms of CBSD are unusual in that they can affect a wide range of organs including leaves, stems, tuberous roots and fruits. The CBSD foliar symptoms (Plates 1b and 1c) vary greatly but are characterized mainly by leaf chlorosis in feathery patterns, appearing first along the margins of veins and later developing into chlorotic blotches (Nichols, 1950). However, CBSD symptoms are often masked in the field due to plants also being affected by cassava green mite (*Mononychellus tanajoa*), mould (growing on the honeydew excreted by whiteflies) and cassava mosaic disease (CMD). Symptoms also vary with the variety, crop age and environmental conditions (Hillocks and Raya, 1999) the tendency of cassava to shed older mature symptomatic leaves, especially during prolonged dry periods, further add to the complexity of disease identification. Characteristic CBSD foliar symptoms normally occur only on mature leaves, and the young expanding leaves are symptomless. The economically damaging symptom occurs on the tuberous roots as a yellow/brown, corky necrosis in the starch-bearing tissues (Plate 1d and 1e), and radial root constriction occurs in very severe infections.

The necrosis begins as discrete areas, but in fully susceptible cultivars, it may affect most of the root, rendering the roots unfit for human consumption. Serological and/or molecular techniques have been developed in order to provide more reliable

diagnosis without having to rely on variable disease symptoms. Moreover, the symptoms are very variable in type and severity and some varieties are affected much less than others and frequently express symptoms only during the early stages of growth (Nichols, 1950).

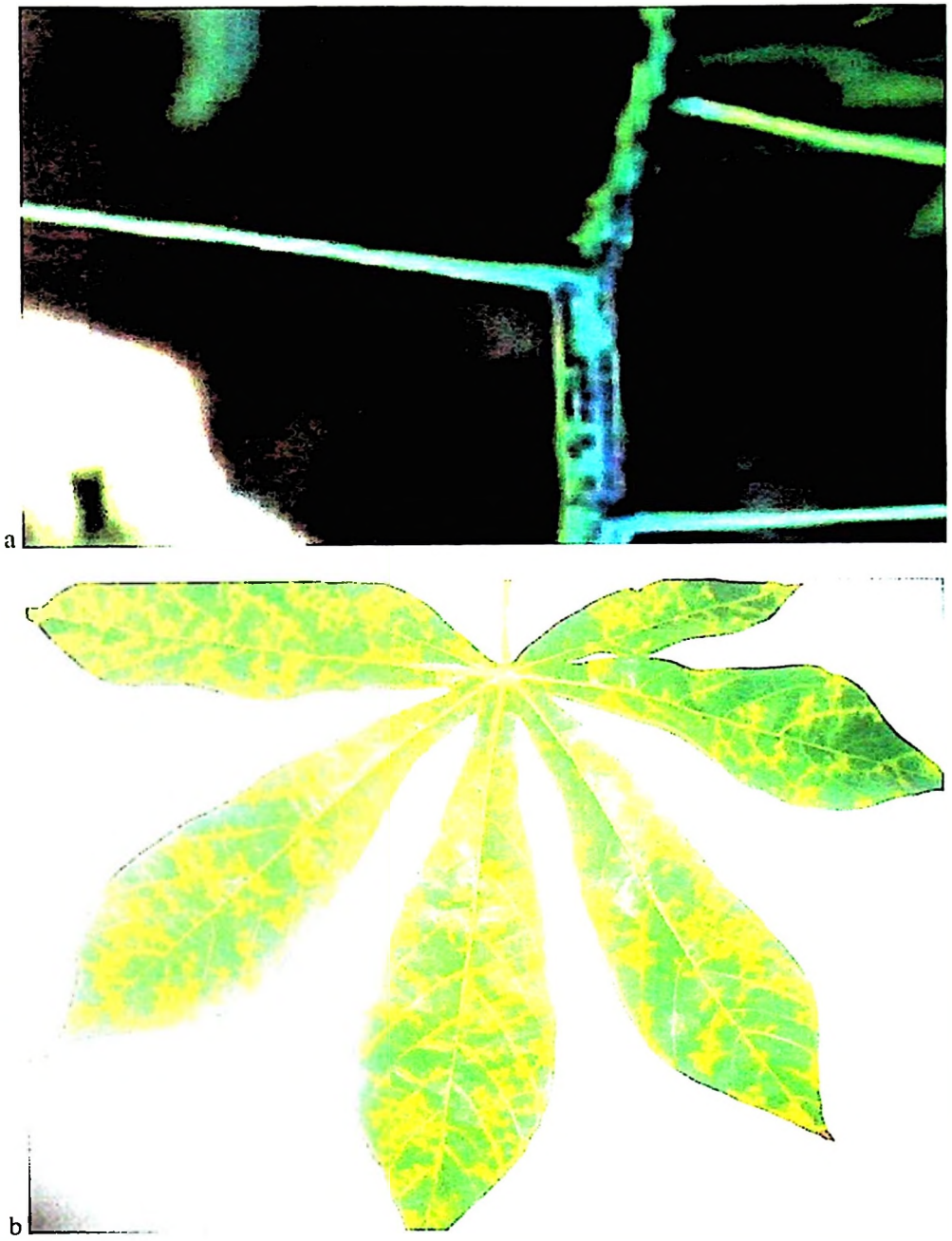

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Plate 1: Brown elongated necrotic lesions of CBSD on the young green cassava stem (a), CBSD foliar chlorosis symptoms (b) and yellow blotches on mature leaves (c) Cassava root in cross section showing yellow/brown necrosis in cross section (d) and CBSD longitudinal section showing corky necrosis in the starch-bearing tissues (e)

2.5 Identification of the Causal Agent

Precise identification of the causal agent is the first step in management of CBSD. Although accurate description of symptoms is necessary to describe the disease, virus diagnosis should not be based on symptoms alone, because several unrelated viruses cause similar symptoms and the same virus or its strains can result in different symptoms on the same host or on different host species. Several diagnostic methods are available for the identification of CBSV including Enzyme linked immunosorbent assay (ELISA) and Polymerase chain reaction (PCR) (Mbazimbwa

et al., 2011). Identification of CBSVs based on symptoms is reported as unreliable because the symptoms are inconsistently expressed in leaf, stem and root and are difficult to distinguish from mite damage and nutrient disorders (Hillocks and Jennings, 2003). Also, there is no evidence of any symptom differences between CBSV and UCBSV, making differentiation of CBSVs by visual symptoms impossible. For these reasons a diagnostic test is required to accurately identify the presence of viruses for research, policy (e.g. quarantine) and planting material multiplication purposes.

The first RT-PCR assay for the detection of CBSV was developed using only the small number of sequences available at the time (Monger *et al.*, 2001a), and as a result this assay has proven not to be comprehensive for all strains of CBSVs. Recently, gel-based conventional RT-PCR assays for the detection of both viruses (Mbanzibwa *et al.*, 2011) and a real-time RT-PCR assay for CBSV have been developed (Moreno *et al.*, 2011). Real-time PCR is widely recognized as providing greater sensitivity than both ELISA and conventional PCR. However, the most significant advantages of the technique are realized in routine testing of samples: the absence of a gel electrophoresis step enables larger sample numbers to be processed at a reduced cost and the closed tube system effectively eliminates post-PCR contamination and resultant false positive results (Appendix 3).

2.6 Transmission of Cassava Brown Streak Virus

The disease was first reported and distinguished from CMD in Tanzania during the 1930s (Storey, 1936). Soon after, the whitefly, *Bemisia tabaci* (Gennadius) (Plate 4)

was suspected as a possible vector (Bock, 1994). Successful transmission of CBSV by *B. tabaci* between cassava plants was achieved in 2004 (Maruthi *et al.*, 2005). Currently, *B. tabaci* has been identified as the vector and studies are being carried out on understanding CBSV and *B. tabaci* relationships in order to understand the epidemiology of this important disease. In Southern Tanzania where the main period for planting cassava is November–January, whitefly populations are greatest during February–May (sometimes to June), when there is usually vigorous fresh green cassava growth. Following the appearance of CBSD symptoms derived from planting infected cuttings; the main period of new (secondary) infections by vectors coincides with peak whitefly populations.

During the dry season between May and June and September, whitefly numbers on cassava decrease with the decline in young green shoots and new infection with CBSD is rare. Both CMD and CBSD can spread rapidly in the field from cutting-borne infection foci, and it has been observed that the seasons in which the spread of CBSD is greatest are also those in which whitefly populations are high (Hillocks *et al.*, 2001). In the cassava growing seasons of 2002 and 2003, when whitefly numbers were low in Tanzania, little spread of CBSD occurred. This contrasted with the 2004 season when whitefly numbers were high in February and March and rapid spread of CBSD occurred (Maruthi *et al.*, 2005).

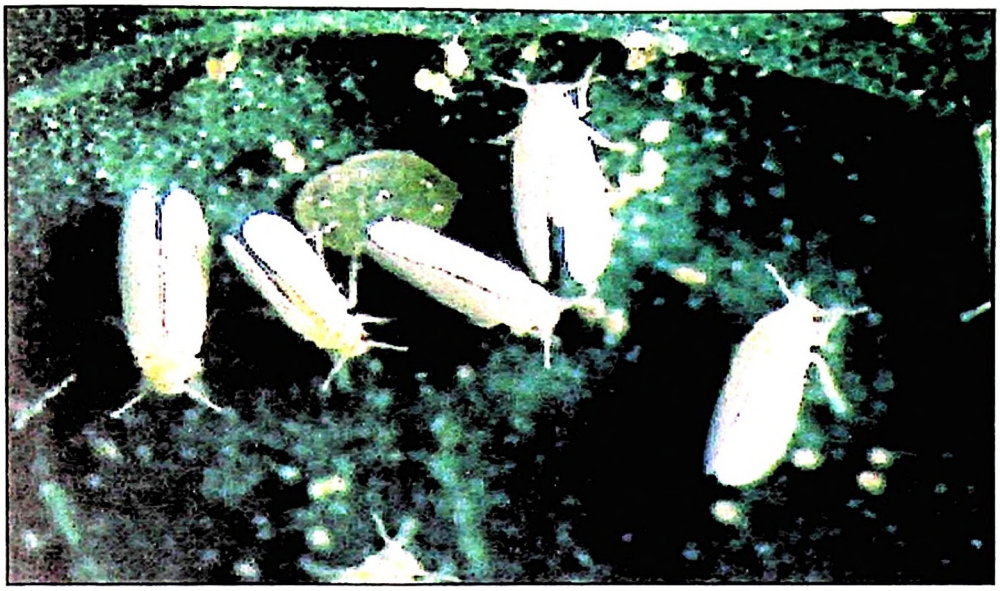

Plate 2: Whiteflies, *Bemisia tabaci*, vector of CBSV

2.7 CBSD Distribution and Effects on Cassava Yield

The CBSD is currently occurring in areas that were believed to be unsuitable for the disease such as high altitude areas away from the Indian Ocean coastal belt in Kenya, Tanzania and Mozambique (Rwegasira *et al*, 2011). The virus is spread short distances by whiteflies vectors and long distances through the exchange of cassava cuttings from diseased plants. Studies in Tanzania, however, have demonstrated that most (> 90%) plants of sensitive varieties sprouting from cuttings taken from diseased stems express leaf symptoms, and that many of the same plants (12–59 %, depending on variety) show root symptoms at harvest (Hillocks *et al*., 1999). It was also shown that sensitive varieties may lose up to 70% of fresh root yield, due principally to the effects of die-back. These losses are then compounded by the effects of necrosis on root quality, which prevent harvested roots from being marketed or encourage premature harvesting to avoid the most severe damage. For susceptible varieties, these additional losses ranged from 2 to 29%.

Symptoms of root necrosis and yield loss increase as the age of the crop increases (Nichols, 1950). The losses are particularly acute for local varieties in which root necrosis begins to increase from six months after planting, encouraging farmers to harvest prematurely (Alicai *et al.*, 2007). An important consequence is that such varieties cannot be relied on as a food reserve for use in times of drought. By contrast, other apparently tolerant varieties, such as Nanchinyaya in southern Tanzania, begin to show mild root necrosis only beyond 12 months after planting, and as such, incur virtually no yield loss and can be harvested at the optimal and most convenient time.

2.8 Control of CBSD

Strategies for CBSD management are mostly aimed at eradicating the source of infection to prevent it from reaching the crop, delaying the time of infection, minimizing the effects of infection once it has occurred, and interfering with the movement of vectors to prevent the spread of the disease, or ideally combinations of both strategies. The most cost effective and environmentally friendly means of controlling CBSD is through the use of resistant varieties. However, until now most cassava cultivars are not resistant to CBSVs.

Research on breeding for resistance initiated at the Amani Station in the 1930s considered both CMD and CBSD. Initially, a large collection of cassava varieties was made from many international and local sources, but none appeared to have adequate levels of resistance to either CMD or CBSD. Two Brazil-derived varieties, Aipin Valenca and Macaxeira, have limited levels of resistance to CBSD. Aipin

Valenca continues to be grown widely in Tanzania, but neither of these two varieties was considered sufficiently resistant to CBSD for large-scale promotion. Following the success of the interspecific crossing technique using *M. glaziovii* to introduce CMD resistance to cultivated cassava, a similar approach was explored for CBSD, although in this case, using the other wild relative *M. melanobasis* Muell. Arg. The first crosses were made in 1950, and over the subsequent decade, substantial progress was made in developing cassava that combined good levels of resistance to both CMD and CBSD.

The approach of selecting CBSD-free stems when replanting has been clearly demonstrated (Omongo, (2003), although advocating a phytosanitation programme to farmers has two major drawbacks. The first is the major educational and training input required and the second is the difficulty that farmers or even researchers can face in correctly identifying CBSD-free material. Farmers have been introduced to the control of virus diseases in cassava through phytosanitation in southern Tanzania on a small scale (Hillocks *et al.*, 2001).

However, the relative merits of such an approach compared to the promotion of tolerant varieties are yet to be evaluated. It seems that resistant and tolerant varieties and phytosanitation by uprooting diseased plants (roguing) and selection of disease-free stems for new planting may all have an important role to play in managing CBSD, but considerable research remains to be done on the conditions under which each is most appropriate and on how best to combine them into an integrated strategy. Phytosanitation has mainly been used within the framework of schemes for

the multiplication of planting materials. In future, there might be opportunities for the development of improved resistance through genetic transformation techniques.

Viruses of the family *Potyviridae* are particularly amenable to coat protein-mediated resistance approaches, and CBSV might therefore be an appropriate target for such a strategy. Another key facet of CBSD management given its apparently restricted distribution will be the prevention of movement between countries through the implementation of strict quarantine procedures. Some of Africa's major cassava producers, including Nigeria, Ghana, Bénin, and Ivory Coast appear to have favourable environments for CBSD. It is therefore critical that movements of germplasm in vegetative form should to be strictly controlled (through tissue culture) and that virus indexing laboratories that test tissue culture material prior to export are fully equipped to test for CBSVs.

Other methods used to control CMD and CBSD on the African continent include thermotherapy and meristem-tip culture. Heat therapy and tissue culture are frequently used at plant quarantine stations to free imported, vegetatively propagated plant materials of systemic pathogens such as the CMGs and potato viruses. At Muguga Plant Quarantine Station, tissue culture combined with thermotherapy was used to free five East Africa lines of CMGs and CBSVs.

CHAPTER THREE

3.0 MATERIAL AND METHODS

3.1 Experimental Site

The study was conducted at the Sugarcane Research Institute (SRI), Kibaha, Coast Region, situated at 06° 46′ S and 38° 55′ E at an elevation of 107 meters above sea level. The soils of the area are Loamy sandy texture well-drained and relatively fertile to support crop production. The rainfall data for 2011 to 2012 was 1196.7 mm. The rainfall data from September 2012 to August 2013 was 728 (URT, 2013). This amount of rainfall was below the minimum average rainfall of Kibaha per year. Kibaha has an average annual rainfall of 800 – 1 000 mm with an average temperature of about 24 to 28 °C (URT, 2013).

3.2 Identification of CBSV by Symptoms and RT-PCR

3.2.1 CBSV Symptoms

A total of 220 cassava leaf specimens (each weighing 10 g) for species speciation of CBSV were randomly collected from test plants in experiment 1 and 2. For each specimen collected, records were kept of the sample and plant number, symptoms, and variety. Plant Pathology laboratory at Kibaha and dried before they were tested for CBSV and UCBSV by RT-PCR (Mbazibwa *et al.*, 2010).

3.2.2 Real-time polymerase chain reaction (RT-PCR)

3.2.2.1 RNA extraction

The RNA extraction from cassava leaves was carried out using the CTAB method (Mbazibwa *et al.*, 2010) as described in Appendix 2.

3.2.2.2 RT-PCR amplification

PCR amplification technique was used to determine the species of the CBSV infecting the cassava genotypes at Kibaha. Two hundred and twenty (220) leaf specimens collected (section 3.2.1) were pooled in a group of five or three leaves per pool to form 46 pooled leaf samples. These pooled samples were tested for the presence of CBSV and UCBSV in PCR as described by Mbazibwa *et al.* (2010). A total of 13 pools from both experiment 1 and 2 showed a +ve reaction to CBSV whereas 14 pools were –ve for CBSV infection. To identify which specimens were infected with the CBSV or UCBSV species, individual leaf specimen from the RT-PCR positive pools were re-tested using PCR amplification technique and the CBSV and UCBSV specific primers. Accordingly, a total of 65 and 68 individual leaf specimens were tested for CBSV and UCBSV, respectively. The specific primers used were those developed by Zhou *et al.* (1997). The PCR amplification was conducted according to the cycle described by Deng *et al.* (1994) and Were *et al* (2004). The RT-PCR results obtained were compared with the recorded field symptoms of CBSD. The overview of the RT-PCR test used was as follows:

Procedural Overview

Prepare total RNA

↓

Perform reverse transcription Protocol

↓

65 min

Create and set up a plate document

↓

15 min

Prepare the PCR reaction plate

↓

15 min

Run the PCR reaction plate

↓

90 min

Analyze results

(2 min)

3.3 Assessment of the Effect of CBSD on the Above-ground Yield Components of Cassava

3.3.1 Treatment description and experimental design

The planting materials used in this experiment were the CBSD- free and CBSD-infected cassava cuttings of variety Kiroba. The CBSD- free Kiroba cuttings were

obtained from IITA experimental farm in Tanga while the CBSD- infected cuttings were obtained from SRI, Kibaha.

CBSD-infected plants were identified based on the typical symptoms of CBSD and by PCR as describe in section 3.1.2. The experimental design was a randomized complete block design (RCBD) with four replications. The experimental block size was 31 m x 12 m, whereas the plot size was 7 m x 6 m with 6 rows and 7 plants per row. This experiment was established in plots that were at least 100 m away from other cassava fields in order to minimize the chances of spread of CBSVs into the trial plot from neighbouring fields.

3.3.2 Crop establishment and management

The experimental plots were ploughed once before planting. Ploughing was done on 14th April, 2012. Planting of cassava was done on 16th April 2012 at a spacing 1 m x 1 m to give equivalent of 10 000 plants/ha (Plate 3). The CBSV infection and White flies infestation was natural. Weeding was done thrice to control weeds, as the rain was particularly high in the season. Watering during dry periods was done. The experimental plots were attacked by termites and cassava mealybug, but no chemical control measure was done. This aimed at avoiding interference with the whitefly population. Guarding was done from eight months after planting (8 MAP) till the end of the experiment to protect the trial against thieves and small animals.

Plate 3: Cassava plants in the experimental field at SRI, Kibaha

3.3.3 Variables measured

The plants were examined for distinct virus symptoms such as chlorotic and necrotic lesions, mosaic and other abnormalities every month starting from 2 months after planting (2MAP) to the end of the experiment. Measurements of the above-ground yield components such as plant height (cm), and shoot weight (kg) were also taken.

3.3.3.1 Percentage plant establishment

This was done at 30 days after planting (30DAP) by counting the number of sprouted plants and expressed as a percentage of the total planted plants. Total

number of planted CBSD- free cassava cuttings was 196; however, 192 (97.95%) cuttings sprouted while 185 (94.38%) out of 196 CBSV- infected Kiroba planted in the experiment sprouted. Gap filling was done 2 weeks after planting for both CBSV- infected Kiroba and CBSV- free Kiroba.

3.3.3.2 Foliar CBSD rating

Foliar CBSD severity rating was done every month starting from 2MAP for 12 months by using the IITA (2009) disease severity scale of 1-5, where: 1= no symptoms on leaves or stem, 2 = mild/slight vein yellowing or chlorotic blotches on leaves, no brown streak/lesions on green stem portions, 3 = mild/slight vein yellowing or chlorotic blotches on leaves, mild brown streak/lesions on green stem portions, 4 = severe/extensive vein yellowing or chlorotic blotches on leaves, severe brown streak/lesions on green stem portions, no defoliation, stem dieback or stunting, and 5 = severe/extensive vein yellowing or chlorotic blotches on leaves, severe stem dieback. Disease incidence was estimated by counting the number of symptomatic plants, expressed as a percentage of the total number of plants planted per plot.

3.3.3.3 Foliar CMD rating

The CMD severity rating was done every month starting from 2MAP for 12 months by using the IITA (2009) disease severity scale of 1-5, where 1 = no symptoms, 2= mild chlorotic mosaic on leaves, little distortion of leaf shape, 3 = moderate chlorotic mosaic on leaves, leaf shape with cupping, 4 = bright yellow chlorosis covering much of leaf area, severe distortion of leaf shape with reduced size, down-

turned petioles, and 5 = bright yellow chlorosis affecting much of leaf area, severe distortion of leaf shape with reduced size, down-turned petioles with leaf drop, plant stunted. Because of the existing interaction among CBSD, CMD and whiteflies, this information was taken to compare plants with both CMD and CBSD leaf symptoms and plants with CBSD leaf symptoms only. CMD incidence was estimated by counting the number of symptomatic plants and expressed as a percentage of the total number of plants planted per plot.

3.3.3.4 Foliar CGM rating

Severity for CGM was recorded by using a scale of 1-5 i.e 1=no damage, 2=<5%chlorotic, 3=>5%, <50% chlorotic, >50%chlorotic, 5=dead leaf, leaf drop. CGM incidence was estimated by counting the number of damaged plants, and expressed as a percentage of the total number of plants planted per plot.

3.3.3.5 Whiteflies count

The population of whiteflies was assessed weekly by counting the number of whiteflies on the five top most expanded leaves of a representative shoot on 10 plants per plot for 12 months. Whiteflies were found on the lower leaf surfaces.

3.3.3.6 Shoot weight (kg)

The vegetative part of the cassava plants was cut and measured by using Salter weighing balance with accuracy of 0.1 g.

3.3.3.7 Plant height (cm)

The height of each plant was measured in cm from the soil level to the tip of the top-most foliage using a tape measure at 3, 6, 8, 10, and 12 MAP. It is anticipated that healthy plants grow taller than diseased plants. All plants/plot were measured.

3.3.3.8 Rating of root symptoms of CBSD

Each cassava root was cut transversely into five pieces to observe and record the extent of root necrosis. For each root, necrosis severity was determined by using the IITA (2009) root necrosis severity scale (RNSS) of 1-5, where 1 = no visible symptoms; 2 = less than 5% of storage tissue necrotic; 3 = 5-10% of storage tissue necrotic; 4 = 10-25% of storage root necrotic, root surface mildly constricted; and 5 = more than 25% of storage tissue necrotic, root surface severely constricted.

3.3.4 Yield data

At harvesting, records were kept of weight of shoots (kg), the number of plants harvested/plot; the number of roots harvested/plant; the number and weight of storage roots (kg) for each treatment and the extent of root necrosis. The weight of roots and shoots were measured by using a weighing balance, Mettler Toledo, accuracy 0.1 g.

3.3.5 Statistical model and data analysis

The model used was: $Y_{ik} = \mu + \beta_i + \alpha_k + \varepsilon_{ik}$

Where: Y_{ik} is the response, μ = General mean for all treatments, βi = Effect of blocks/replications, α_k = Effect of treatments, i = Variety and k = General

experimental error. Data were subjected to analysis of variance (ANOVA) and Regression analysis to compare yield and severity data using SIGMAPLOT 11 statistical software (Levesque, 2007). Analysis of variance was a one-way in randomized complete block design.

3.4 Experiment 2

To assess the effect of quality of planting materials and time of harvesting on yield and quality losses associated with CBSD infection.

3.4.1 Source of planting materials and experimental design

Two cassava varieties viz. Kiroba (tolerant to CBSD) and Mwari (susceptible to CBSD) were used in this portion of the study. Both varieties were from virus-free planting materials obtained from isolated planting material at the IITA multiplication site in Tanga Region. The cassava cuttings, each 30cm long, were planted on 6th April 2013 at the SRI Kibaha. The experimental design was a split plot in RCBD with three replications. The main plot treatments were the two cassava cultivars while the subplot treatments were the six harvesting times, which were 6, 8, 10, 12, 14, and 16 MAP. The spacing used was 1 m x 1 m and the plot size was 2 m x 12 m for each variety in a sub-plot.

3.4.2 Data collection and field management

3.4.2.1 Percentage plant establishment

Plant establishment was determined at 30 DAP by counting all sprouting plants in each plot. A total of 382 (88.42%) out of 432 of planted Mwari cuttings and 410

(94.9%) out of the 432 planted Kiroba cuttings sprouted. Gap filling was done at 14 DAP. Plant establishment was estimated as a percentage number of plants established.

3.4.2.2 Rating of foliar symptoms of CBSD, CMD, and CGM

Disease severity for CBSD, CGM and CMD as well as disease incidences were recorded by using the IITA disease severity scales as described in experiment 1 for CBSD, CMD, and CGM disease severity scale. Disease incidence was estimated by counting the number of symptomatic plants, expressed as a percentage of the total number of plants planted per plot.

3.4.2.3 Plant height(cm)

Plant height was measured and recorded at 3 MAP, 6 MAP, then once every two months for 16 months as described in section.3.3.3.7.

3.4.2.4 Cassava green mite

Severity and incidence for CGM was also recorded by using a scale of 1-5 i.e 1= no damage, 2=<5% chlorotic, 3=>5%, <50% chlorotic, >50% chlorotic, 5=dead leaf, leaf drop.

3.4.2.5 Whitefly counts

More recently, the whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: *Aleyrodidae*) was shown to transmit CBSVs. Adult whitefly numbers on the lower surfaces of the

five top-most leaves of each test plant was counted to determine the effect of whitefly numbers on the rate of CBSV spread.

3.4.2.6 Yield at harvesting

The number of plants harvested and the number of roots/plot was recorded. The shoot weight (kg), the number and weight of storage roots for each treatment was taken and measured using a weighing balance. The extent of root necrosis was recorded at harvest by using the scale of 1-5 as described in section 3.3.3.8.

3.5 Statistical Model and Data Analysis

Data on yield was analyzed by using a split plot statistical model: $Y_{ijk} = \mu + \beta_i + \alpha_j + \omega_{ij} + \gamma_k + (\alpha\gamma)_{jk} + \varepsilon_{ijk}$, where by Y_{ijk}= is the response, μ= the overall mean, β_i = Replication/block effect, α_j = Main factor effect, ω_{ij} = Main plot random error effect, γ_k = Subplot factor effect, $(\alpha\gamma)_{jk}$ = Interaction effect, ε_{ijk} = Subplot random error effect, i = 1,2,3...i^{th} level of factor A=Main plot (Variety), j = 1,2,3...j^{th} level of factor B=Sub plot (Time of harvest), k = general experimental error.

Collected data was analyzed by using SIGMAPLOT 11 statistical software (Levesque, 2007). Analysis of variance was done by using a two way system in a randomized complete block design. Factors were variety and time. The values were CBSD severity, CBSD incidence, CMD severity, CMD incidence, CGM severity, CGM incidence, Plant height, number of plants harvested, number of roots, weight of roots, shoot weight, root necrosis severity, root necrosis incidence. Significant treatments means were compared using the LSD test at the 5% level of probability.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Identification of CBSV and its Variants

4.1.1 Cassava brown streak disease symptoms on the test plants

Symptoms of CBSD observed on the test plants for both experiment 1 and 2 were interveinal leaf chlorosis, yellow spots, and stem necrosis (Plate 4). Others were not easy to describe as they showed an overlap of different symptoms, and so were referred to as "mixed" (Table 1). Interveinal leaf chlorosis was the most prevalent symptoms and it occurred 1MAP initially on infected Kiroba and latter, 2MAP, on a few plants of uninfected Kiroba as faint chlorotic specs. The yellow blotches type symptoms occurred 4MAP on plants of Mwari as yellow spots which latter coalesced to form yellow blotches. The stem necrosis consisted of darkish brown symptoms on the stems and base of petioles, while the "mixed" type symptoms characterized by general leaf yellowing, mosaic, and interveinal chlorosis were observed on a few plants of Mwari and infected Kiroba but not on clean Kiroba. Generally, most samples collected for CBSV identification via symptoms and for comparison with the RT-PCR amplification results were symptomatic (Table 1).

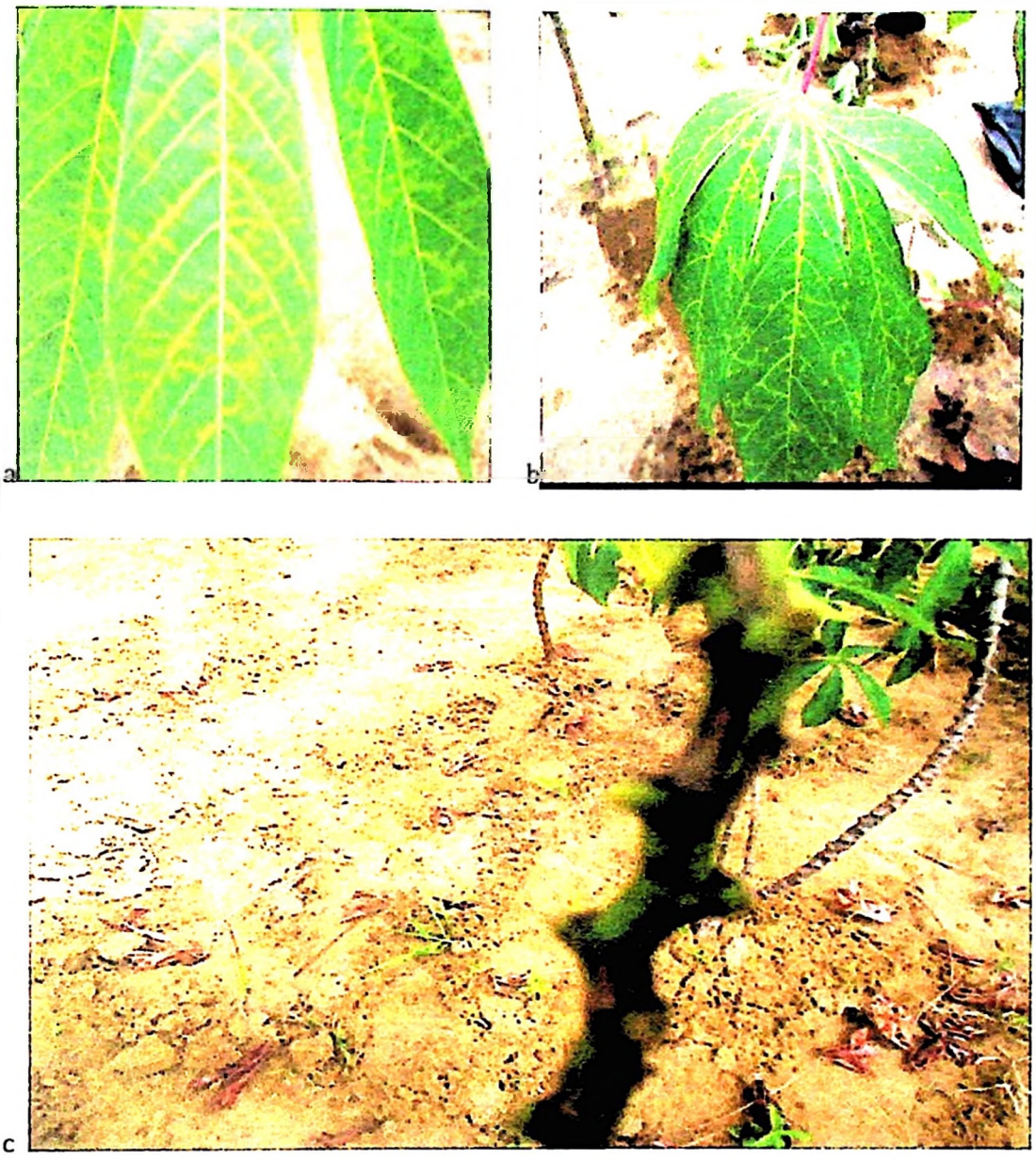

Plate 4: Leaf chlorosis in feathery patterns on cassava variety Kiroba (a); chlorotic blotches on cassava variety Mwari (b) and stem necrosis on cassava variety Kiroba (c)

Table 1: Disease symptoms on cassava leaf samples collected from plots of experiment 1 and 2

Sample code	Field symptoms Description	Sample code	Field symptoms Description	Sample code	Field symptoms Description
M180	Ivc , Chl	M5	Ys, ChlMo,Chl	M71	Chl
M181	Ivc , Chl	M6	ChlMo, Chl	M72	Chl
M182	Ivc , Chl	M7	Chl	M73	Ys, ChlMo,, Chl
M183	Ivc , Chl	M8	ChlMo, Chl	M74	Chl
M184	Ivc , Chl	M9	Chl	M95	ChlMo, Chl
M188	Ivc , Chl	M10	Chl	M96	Chl
M189	Ivc , Chl	M11	Chl	M97	Chl
M190	Ivc , Chl	M12	Chl	M98	ChlMo, Chl
M191	Ivc , Chl	M13	ChlMo ,Chl	M99	Chl
M192	Ivc , Chl	M14	Chl	M115	Chl
M196	Sl/ Chl	M15	A	M116	Chl
M197	Ivc , Chl	M16	Chl	M117	Chl
M198	Ivc , Chl	M17	Chl	M118	ChlMo, Chl
M199	Ivc , Chl	M18	A	M119	Chl
M200	Ivc , Chl	M19	Chl	M145	Chl
M204	Ivc , Chl	M20	A	M146	Chl
M205	Ivc , Chl	M21	ChlMo, Chl	M147	Chl
M206	Ivc , Chl	M22	Chl	M148	Chl
M207	Ivc , Chl	M23	Chl	M149	Chl
M208	Ivc , Chl	M24	Chl	M170	Chl
M212	Ivc , Chl	M25	ChlMo, Chl	M171	Chl
M213	Ivc , Chl	M31	ChlMo	M172	Chl
M214	Ivc , Chl	M32	Chl	M173	Chl
M215	Ivc , Chl	M33	ChlMo, Chl	M174	Chl
M216	Ivc , Chl	M34	Chl		
M217	Mxd	M35	Chl		
M218	A	M36	Chl		
M219	A	M37	Chl		
M1	Chl	M38	Chl		
M2	ChlMo, Chl,	M39	Chl		
M3	Ys,Chl, ChlMo	M40	Ys, Chl		
M4	Chl	M70	Chl		

Key

1. Symptom description is based on descriptions made by Brunt *et al.* (1990), 2. Sample codes were derived from plant number and name (e.g. M = Mary, 180 = Plant number 180), 3. Chl = chlorosis, Ivc = Interveinal leaf chlorosis, Ys = yellow spots, ChlMo = chlorotic mosaic, 4. A = symptoms absent, Sl = stem necrosis, Mxd = Mixed symptoms.

4.1.2 Verification of symptoms and identification of viruses

In order to confirm the fact that symptoms observed on cassava were certainly due to virus, PCR amplification tests were conducted. A total of 38 leaf samples from experiment 1 and 180 from experiment 2 were tested for CBSV and UCBSV species (Table 2 and Table 3). Twenty-three (23) and 25 of 38 leaf samples for experiment 1 tested positive for CBSV and UCBSV, respectively. In experiment 2, eight and 12 specimens of 180 were positive for UCBSV and CBSV, respectively. In spite of positive results reported above, seven (7) infected cassava samples were negative for both CBSV and UCBSV. However, some negative samples had yellow spots and leaf chlorosis. It could not be established as to why these samples were negative.

Identified viruses occurred in cassava singly, or in mixtures whereby both CBSV and UCBSV were found in the same plant (Table 2). The CBSV + UCBSV mixture was detected in 19 leaf samples. However, in five leaf samples, UCBSV was found alone. In addition, four of five leaf samples obtained from symptomatic plants, had UCBSV alone while 1 leaf sample had CBSV alone. Furthermore, the results indicated that one leaf sample obtained from asymptomatic plant was infected by UCBSV (Table 2).

The present observation confirms earlier reports by Mbazibwa *et al.* (2010) in which cassava leaves collected from Tanzania were found infected with both CBSV and UCBSV. The high rate of recombination of different viruses co-infecting cassava (Patil and Fauquet, 2009) can result in the formation of virulent strains, which reduce yield as observed in Uganda for the East African Cassava Mosaic virus

(EACMV) and the Ugandan variant (EACMV-Ug) (Pita *et al.*, 2001). Furthermore, the presence of both CBSV and UCBSV in Tanzania may be one of the contributing factors to low cassava yields in the country.

The present results also confirm the report by Arbashi *et al.* (2010) and Winter *et al.* (2009) that the symptoms of CBSD on Cassava are not specific to CBSV or to its variants. Samples that tested positive for CBSV and UCBSV either had leaf chlorosis, yellow blotches, "mixed", stem necrosis or asymptomatic. Consequently, one cannot rely fully on symptoms to specifically identify CBSV or its variants, even though some viruses are named according to symptoms (Brunt *et al.*, 1990). The inability to use symptoms to identify CBSVs has also been reported by Hillocks and Thresh (2000) who asserted that field symptoms of CBSD could be masked by those of senescence, other diseases and damage caused by mites. Since, most Tanzanian cassava varieties recommended for commercial and small-scale cultivation exhibit tolerance to CBSD, it is important that molecular testing for example RT-PCR be used to index planting materials prior to dissemination.

Even after successfully identifying CBSVs, there were samples with virus disease like leaf chlorosis and yellowing symptoms, which still tested negative to the PCR test used. According to Mbazimbwa *et al.* (2009), there could be viruses belonging to other families, which cause Cassava brown streak virus-like symptoms or another group of viruses caused the symptoms. As such, there is high possibility that unidentified viruses could belong to families reported elsewhere or may even be new viruses (Bock, 1982).

Table 2: Grouping of samples according to foliar symptoms and naturc of infection (single, mixed or negative) for experiment 1

CBSV single tests				UCBSV single tests				
Sample no.	Foliar symptoms	Virus sp	Ct (dRn)	Sample no.	Foliar symptoms	Virus sp	Ct (dRn)	Diagnosis
M180	Ivc , Chl	CBSV	16.36	M180	Ivc , Chl	UCBSV	21.08	C+U
M181	Ivc , Chl	CBSV	22.53	M181	Ivc , Chl	UCBSV	26.98	C+U
M182	Ivc , Chl	CBSV	21.07	M182	Ivc , Chl	UCBSV	25.67	C+U
M183	Ivc , Chl	CBSV	17.27	M183	Ivc , Chl	UCBSV	19.42	C+U
M184	Ivc , Chl	CBSV	No Ct	M184	Ivc , Chl	UCBSV	25.8	U
M188	Ivc , Chl	CBSV	No Ct	M188	Ivc , Chl	UCBSV	30.79	U
M189	Ivc , Chl	CBSV	19.4	M189	Ivc , Chl	UCBSV	20.59	C+U
M190	Ivc , Chl	CBSV	22.34	M190	Ivc , Chl	UCBSV	28.42	C+U
M191	Ivc , Chl	CBSV	19.1	M191	Ivc , Chl	UCBSV	22.74	C+U
M192	Ivc , Chl	CBSV	18.54	M192	Ivc , Chl	UCBSV	23.15	C+U
M196	Sl/ Chl	CBSV	17.7	M196	Sn/ Chl	UCBSV	24.51	C+U
M197	Ivc , Chl	CBSV	16.52	M197	Ivc , Chl	UCBSV	No Ct	
M198	Ivc , Chl	CBSV	16.95	M198	Ivc , Chl	UCBSV	23.57	C+U
M199	Ivc , Chl	CBSV	16.87	M199	Ivc , Chl	UCBSV	20.26	C+U
M200	Ivc , Chl	CBSV	16.91	M200	Ivc , Chl	UCBSV	25.46	C+U
M204	Ivc , Chl	CBSV	16.98	M204	Ivc , Chl	UCBSV	23.53	C+U
M205	Ivc , Chl	CBSV	18.47	M205	Ivc , Chl	UCBSV	22.72	C+U
M206	Ivc , Chl	CBSV	17.64	M206	Ivc , Chl	UCBSV	24.41	C+U
M207	Ivc , Chl	CBSV	16.74	M207	Ivc , Chl	UCBSV	19.99	C+U
M208	Ivc , Chl	CBSV	18.64	M208	Ivc , Chl	UCBSV	No Ct	
M212	Ivc , Chl	CBSV	17.36	M212	Ivc , Chl	UCBSV	23.05	C+U
M213	Ivc , Chl	CBSV	16.76	M213	Ivc , Chl	UCBSV	25.09	U
M214	Ivc , Chl	CBSV	19.44	M214	Ivc , Chl	UCBSV	28.77	C+U
M215	Ivc , Chl	CBSV	18.07	M215	Ivc , Chl	UCBSV	No Ct	
M216	Ivc , Chl	CBSV	17.89	M216	Ivc , Chl	UCBSV	23.72	C+U
				M217	Mxd	UCBSV	25.69	U
				M218	A	UCBSV	No Ct	
				M219	A	UCBSV	No Ct	

Key

1. Symptom description is based on virus symptom description and descriptions made by Brunt *et al.* (1990), 2. Sample codes were derived from plant number and name (e.g. M = Mary, 180 = Plant number 180), 3. Chl = chlorosis, Ivc = Interveinal leaf chlorosis, Ys = yellow spots, ChlMo = chlorotic mosaic, 4. A = symptoms absent, Sl = Stem lessions, Mxd = Mixed symptoms, C = CBSV; U = UCBSV; C+U = Mixed infection of CBSV and Uganda variant of CBSV; Ct = Number of PCR cycles to pass the threshold value; No Ct = Negative results

Table 3: Grouping of samples according to foliar symptoms and nature of infection (single, mixed or negative) for experiment 2

CBSV single test				UCBSV single test				
Sample no.	Foliar symptoms	Virus sp	Ct (dRn)	Sample no.	Foliar symptoms	Virus sp	Ct (dRn)	Diagnosis
M1	Chl	CBSV	No Ct	M1	Chl	UCBSV	No Ct	
M2	ChlMo, Chl,	CBSV	No Ct	M2	ChlMo, Chl,	UCBSV	No Ct	
M3	Ys,Chl, ChlMo	CBSV	No Ct	M3	Ys,Chl, ChlMo	UCBSV	31.92	U
M4	Chl	CBSV	26.29	M4	Chl	UCBSV	No Ct	C
M5	Ys, ChlMo,Chl	CBSV	No Ct	M5	Ys, ChlMo,Chl	UCBSV	No Ct	
M6	ChlMo, Chl	CBSV	No Ct	M6	ChlMo, Chl	UCBSV	No Ct	
M7	Chl	CBSV	No Ct	M7	Chl	UCBSV	33	U
M8	ChlMo ,Chl	CBSV	No Ct	M8	ChlMo ,Chl	UCBSV	No Ct	
M9	Chl	CBSV	No Ct	M9	Chl	UCBSV	24.24	U
M10	Chl	CBSV	30	M10	Chl	UCBSV	No Ct	
M11	Chl	CBSV	39.86	M11	Chl	UCBSV	No Ct	
M12	Chl	CBSV	28.19	M12	Chl	UCBSV	No Ct	
M13	ChlMo,Chl	CBSV	No Ct	M13	ChlMo,Chl	UCBSV	No Ct	
M14	Chl	CBSV	28.09	M14	Chl	UCBSV	No Ct	
M15	A	CBSV	28.79	M15	A	UCBSV	28.4	C+U
M16	Chl	CBSV	29.13	M21	ChlMo, Chl	UCBSV	No Ct	
M17	Chl	CBSV	No Ct	M22	Chl	UCBSV	No Ct	
M18	A	CBSV	No Ct	M23	Chl	UCBSV	No Ct	
M19	Chl	CBSV	No Ct	M24	Chl	UCBSV	No Ct	
M20	A	CBSV	28.66	M25	ChlMo, Chl	UCBSV	No Ct	
M21	ChlMo, Chl	CBSV	No Ct	M31	ChlMo	UCBSV	No Ct	
M22	Chl	CBSV	No Ct	M32	Chl	UCBSV	No Ct	
M23	Chl	CBSV	No Ct	M33	ChlMo, Chl	UCBSV	No Ct	
M24	Chl	CBSV	No Ct	M34	Chl	UCBSV	No Ct	
M25	ChlMo, Chl	CBSV	28.76	M35	Chl	UCBSV	21.23	U
M36	Chl	CBSV	No Ct	M95	ChlMo, Chl	UCBSV	No Ct	
M37	Chl	CBSV	No Ct	M96	Chl	UCBSV	No Ct	
M38	Chl	CBSV	30.53	M97	Chl	UCBSV	28.83	C+U
M39	Chl	CBSV	No Ct	M98	ChlMo, Chl	UCBSV	No Ct	
M40	Ys, Chl	CBSV	28.47	M99	Chl	UCBSV	No Ct	
M70	Chl	CBSV	No Ct	M145	Chl	UCBSV	No Ct	
M71	M71	CBSV	No Ct	M146	Chl	UCBSV	23.23	U
M72	M72	CBSV	No Ct	M147	Chl	UCBSV	No Ct	
M73	M73	CBSV	No Ct	M148	Chl	UCBSV	No Ct	
M74	M74	CBSV	No Ct	M149	Chl	UCBSV	No Ct	
M115	Chl	CBSV	No Ct	M170	Chl	UCBSV	No Ct	
M116	Chl	CBSV	No Ct	M171	Chl	UCBSV	No Ct	
M117	Chl	CBSV	No Ct	M172	Chl	UCBSV	27.29	U
M118	ChlMo, Chl	CBSV	28.89	M173	Chl	UCBSV	No Ct	
M119	Chl	CBSV	No Ct	M174	Chl	UCBSV	No Ct	

Key

1. Symptom description is based on descriptions made by Brunt *et al.* (1990), 2. Sample codes were derived from plant number and name (e.g. M = Mary, 180 = Plant number 180), 3. Chl = chlorosis, Ivc = Interveinal leaf chlorosis, Ys = yellow spots, ChlMo = chlorotic mosaic, 4. A = symptoms absent,Sn = stem lessions, Mxd = Mixed symptoms, C = CBSV; U = UCBSV; C+U = Mixed infection of CBSV and Uganda variant of CBSV; Ct = Number of PCR cycles to pass the threshold value; No Ct = Negative results

4.2 Assessment of the Effect of CBSD on the Above-ground Growth Components of Cassava

4.2.1 CBSD severity and incidence in CBSD- affected and CBSD- free Kiroba

The results in Table 4 showed that there was significance difference in severity and incidence between CBSD- affected and CBSD- free Kiroba. CBSD leaf symptoms started to be observed in May that is one month after planting for CBSD- infected Kiroba and in June that is two months after planting for clean Kiroba (Fig. 2). The symptoms started with a score of class 2 in both affected and CBSD- free Kiroba. In general CBSD leaf symptoms were initially more in diseased Kiroba than they were in initially healthy Kiroba, and there was no clear change in this pattern over the course of the trial. This indicates that the extent at which disease severity exists is relatively higher in CBSD- affected Kiroba than in CBSD- free Kiroba which could be related to the nature of the planting materials used. These observations are similar to the findings reported by Rwegasira and Rey (2012) that CBSV-infected susceptible cultivars obviously exhibit the foliar, stem and root symptoms while the resistant cultivars undergo active growth giving no time for the expression of CBSD symptoms.

In addition, the positive trend obtained for CBSD severity in these Kiroba types followed similar trend to that of disease incidence (Fig. 2). The rate of disease spread was generally higher in CBSD- affected Kiroba than in CBSD- free Kiroba where the initial stable value was 83.2%, which however, increased to 100% as from 2 MAP. It was also observed that disease incidence was not realized early in CBSD

free Kiroba. The CBSD incidence increased up to 46% (Table 4). This indicates that disease severity increased as general means of disease incidence also increased. The findings of the present study are in line with the findings of Seem (1984) and Cardoso *et al.* (2004) who reported that in some pathosystems the incidence and severity increased until most susceptible tissues became infected; thereafter, increase in disease could only result from increase in severity. Furthermore, studies conducted by Abaca *et al.* (2012) indicated that the local genotypes of cassava showed foliar CBSD symptoms with incidence ranging from 0-98% and severity from 1-3 whereas, ten of the elite genotypes did not show foliar symptoms. Munga (2008) indicated that host plant which is resistance is one option available to cassava breeders to reduce the detrimental effects of cassava brown streak disease. Mallowa *et al.* (2011) insisted that selecting CBSD symptom free cassava plants as planting materials as it is for CMD is another strategy to manage the spread of CBSD in the cassava fields.

Table 4: Incidence and severity of CBSD for CBSD-affected and CBSD-free Kiroba

Treatment	Mean CBSD severity	Mean CBSD incidence (%)
CBSD- affected Kiroba	2.40±0.07**	100.00±0.0***
CBSD- free Kiroba	2.10±0.07	46.90±5.0
P	0.005	<0.001

Key: ** = p ≤0.01; *** = p <0.001, P = Probability value, CBSD = Cassava brown streak disease

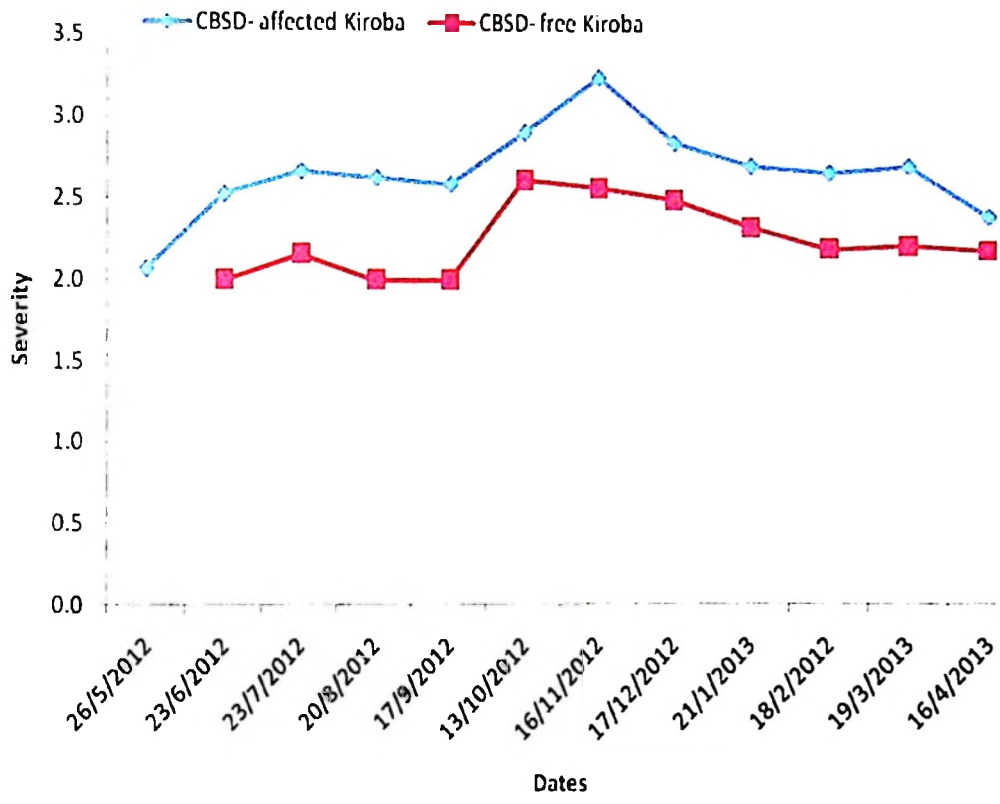

Figure 2: Trends of leaf symptoms of CBSD severity in CBSD-affected and CBSD-free Kiroba

4.2.2 General field weather, whitefly population and cassava brown streak virus disease trends

Cassava cuttings for experiment 1 were planted on 12 April 2012. During plant establishment it was the rainfall period; however decreased in May. The maximum temperature was 32°C (Fig.3).

In the experimental field, a positive relationship was observed between the number of whiteflies recorded and the cassava plants expressing CBSD symptoms (Table 5). At the same time, whitefly populations in the field went down as the cassava

crops grew (Fig.4). It was observed that these trends coincided with rainfall patterns. Thus, during the dry season, the whitefly population declined to a minimum average of 5 whiteflies counted per plant. In the wet season, nine months after planting, it went up to a maximum of 45 whiteflies per plant. At 9 MAP there was good growth of cassava, because of adequate rainfall and increasing temperature. The findings also suggested that as the temperature increases the whitefly population also increased and at maximum temperature of 33.8 ^{0}C the highest number of whitefly was recorded per cassava plant (Figs. 3 and 4).

The results indicated that there was no difference in whitefly number between clean and infected Kiroba (Fig 4). The whitefly abundance recorded was low to moderate and the highest average number was 41 whiteflies recorded in January (9 MAP). In addition, the results also indicated that the average total number declined to below 5 whiteflies per plant from September to November. Furthermore, there was fluctuation of whitefly abundance in February and mid-March in which there was a decrease and an increase in the number of whitefly population. However, the whitefly population increased to 35 whiteflies per plant between mid March to April (Fig.4).

During the months of November, December and late January there was heavy rains and the temperature was high with maximum temperature of 32.3, 33.5, 32.5 and these favoured increases in whitefly population. This could be associated with increased new tender leaves which made it easier for the whiteflies to acquire sap from the leaves. This also increased chances for the whitefly to reproduce and multiply in number.

The findings of this study showed that the CBSD-affected Kiroba expressed more severe symptoms of CBSD than the CBSD- free Kiroba. These findings suggest that CBSD is not rapidly expressed in clean Kiroba plants probably because they were health and had no disease initially. However, the CBSD expression in health Kiroba was slightly expressed as the plants aged and the relatively high expression was observed in April that is 12 MAP. These findings are in agreement with those of Maruthi *et al.* (2005) who reported that the highest increase in CBSD incidence at Kibaha occurred in April, coinciding with the peak adult whitefly populations. The findings of this study further showed that the number of whiteflies started to increase in February although it decreased slightly in March and increased again throughout April that is 12 months after planting (Fig.4). The findings of Maruthi *et al.* (2005) also showed similar observation that whitefly numbers declined almost to zero after July and this pre-empt further spread of CBSD to cassava plants.

Generally, the trend at which the CBSD incidence was increasing between the two types of Kiroba was almost similar although there were very insignificant variations (Table 5) According to Legg *et al.* (2011), the CBSD pandemic seems to be a 'new encounter' situation between host and pathogen and the disease outbreaks occur after whitefly population has increased. The results obtained for the numbers of whitefly caught in the field in every month in the present study demonstrated that the increased number of whitefly correspond to increased incidence of CBSD in the cassava fields with few exceptions, where the number of whitefly population gradually increased up to 40 and then decreased up to 30. The decline in whitefly population might be due to the rainfall, which affected their reproduction potentials

and increased the mortality. In addition, the inconsistent increase in whiteflies population might be related to the increased maturity of the cassava plants, whose leaves did not favour feeding of the whitefly. The relationship observed in this study was somewhat significant and showed a positive trend indicating that there is a direct effect of rainfall on the whitefly population build-up in the cassava field. The findings of this study are in agreement with those of Akanda and Rahman (1993) and Rahman *et al.* (2006) who stated that there was a positive correlation between whitefly population build-up and the relative humidity prevailing over the tomato field.

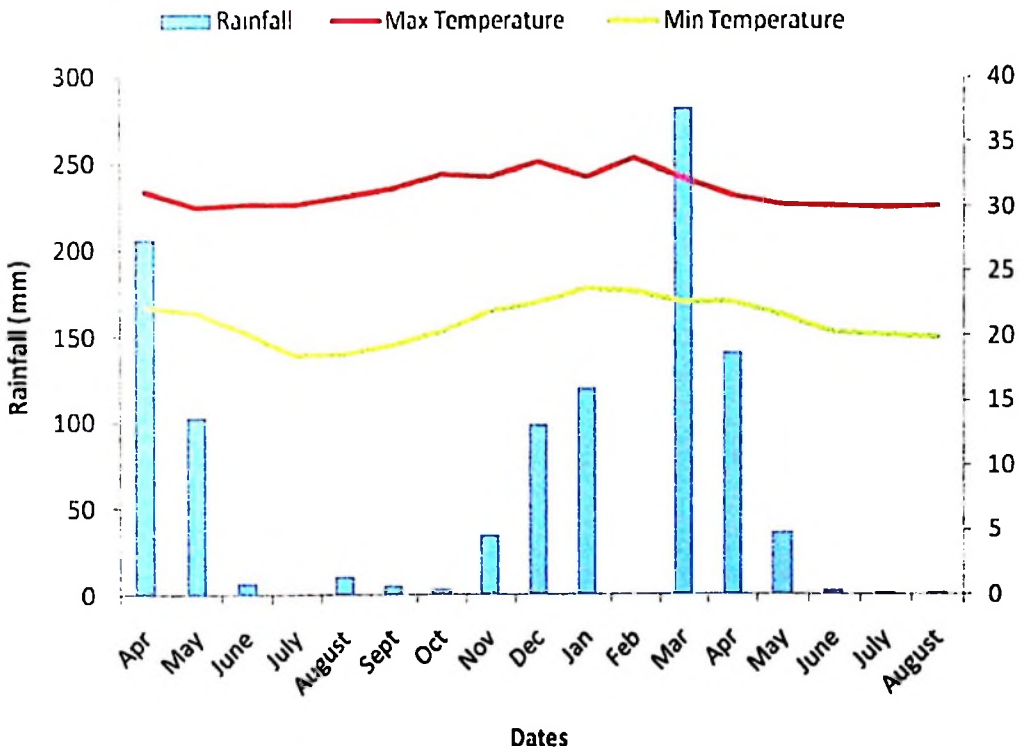

Figure 3: Mean monthly rainfall (mm) and temperature (°C) at the study area during the study period

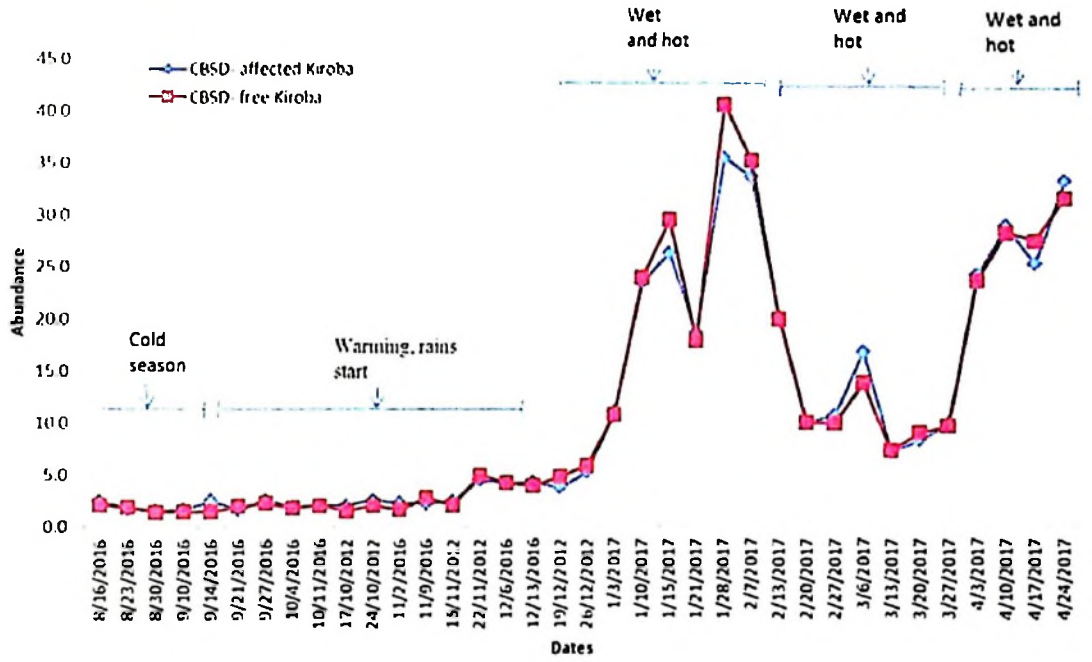

Figure 4: Whitefly abundance in CBSV-Affected and CBSV-free cassava variety Kiroba

Table 5: Mean whitefly counts per month and mean number of CBSD- affected plants, per plot in experiment 1

		CBSV- affected Kiroba			CBSV- free Kiroba		
Crop growth stage	**Dates**	**WF abundance**	**CBSV %incidence**	**CBSV severity**	**WF abundance**	**CBSV %incidence**	**CBSV severity**
Vegetative growth	26/5/2012	-	49.5	2.1	-	0	0
-/-	23/6/2012	-	100	2.5	-	0.2	2
-/-	23/7/2012	-	100	2.7	-	1.2	2.2
-/-	20/8/2012	1.9	100	2.6	1.8	2	2
-/-	17/9/2012	2.1	100	2.6	1.8	2	2
-/-	13/10/2012	2.1	100	2.9	1.8	3.2	2.6
-/-	16//11/2012	2.8	100	3.3	2.8	3.8	2.6
-/-	17/12/2012	4.3	100	2.9	4.7	4	2.5
-/-	21/1/2013	23	100	2.7	24.6	4.2	2.3
-/-	18/2/2013	18.6	100	2.7	18.8	5	2.2
-/-	19/3/2013	10.5	100	2.7	9.9	6.2	2.2
Harvesting	16/4/2013	28	100	2.4	27.8	18.2	2.2

Key: WF = Whiteflies, CBSV = Cassava brown streak virus, - Data not collected

4.2.3 CMD severity and incidence

The data in Table 6 showed that there was very little CMD infection in the trial; accordingly there was no significance difference with respect of CMD between CBSD- free and CBSD- affected Kiroba treatments (Table 6).

However, CMD was generally higher in affected Kiroba than was in CBSD-free Kiroba and this could be attributed to the nature of planting materials used and to the susceptibility to CMD of the CBSD- affected Kiroba compared to the clean (CBSD-free) Kiroba suggesting that the genetic resistance might be linked to the cultivar as well as general defence signalling mechanisms in cassava. Further to that, it is obvious that CMD existence is favoured by vegetative growth of the cassava plant. These findings concur with the results by Omongo (2003) that the preferential feeding on cassava by the virus vector, whiteflies, is related to the phloem content together with morphological and topographical differences in the leaf structure.

Table 6: Incidence and severity of CMD in CBSD-free and CBSD-affected Kiroba

Treatment	CMD severity	CMD incidence (%)
CBSD- affected Kiroba	2.5±1.0 NS	2.5±2.6 NS
CBSD- free Kiroba	1.9±1.0	2.0±2.4
P	0.418	0.78

Key: NS = p >0.05, CMD = Cassava mosaic disease, P = Probability value

4.2.4 Assessment of cassava green mite damage in cassava plants

There was no significance difference in CGM damage between CBSD- affected and CBSD- free Kiroba. The results of the assessed damage due to cassava green mites

in CBSD affected and CBSD- free Kiroba cassava plants are as presented in Fig. 5. The results showed that CGM was initially observed in the experiment at 2 months after planting. The CGM damage severity score for infected Kiroba was class 3 but the trend of damage fluctuated between class 2 and 3 for both CBSD-free and CBSD-affected Kiroba plants (Fig. 5). The results also indicated that there was a persistent decrease in the extent of infestation to class 2 at 11 and 12 months after planting in March 2013 and April 2013, respectively. The highest intensity of CGM damage recorded on both CBSD-free and CBSD- affected Kiroba was class 3 observed between 6 MAP and 10 MAP that is from October 2012 to February 2013.

These findings suggest that the highest severity of CGM was favored by dry spells after the short showers which occurred in the study area. The sudden increase in CGM damage could be attributed to the fact that after the little showers there was a vigorous formation of new tender cassava leaves, which provided sufficient nutrition to the whiteflies which enabled them to rapidly multiply in number hence the increased CGM severity.

There was no significance difference in CGM incidence between the CBSD-affected and CBSD- free Kiroba. However, CGM incidence was increasing at equal proportion in both CBSD- affected and CBSD- free Kiroba (Fig.6). The high CGM incidence for both CBSD-free andCBSD- affected Kiroba was observed to start at 8MAP and persistently increased throughout the period of experiment up to 12MAP, which was probably attributed to the dry spells in the study area except some showers in March and April (Fig. 6). These findings are similar to those of Opoku-

Asiama *et al.* (1999) who reported that the incidence, severity and population of the CGM are higher in the dry season or during the short dry spell in the rainy (wet) season.

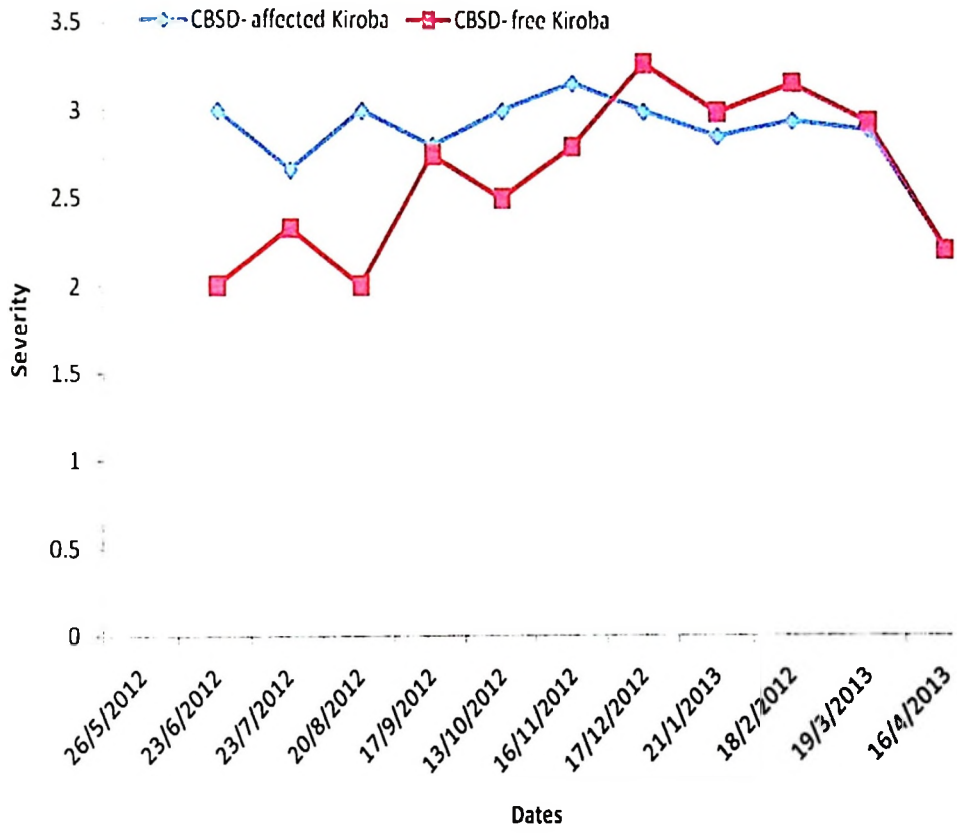

Figure 5: Severity of CGM damage in CBSD- free and CBSD- affected Kiroba

In addition, Abaca *et al.* (2014) found that the CGM effects are greater during the dry period than the wet period.

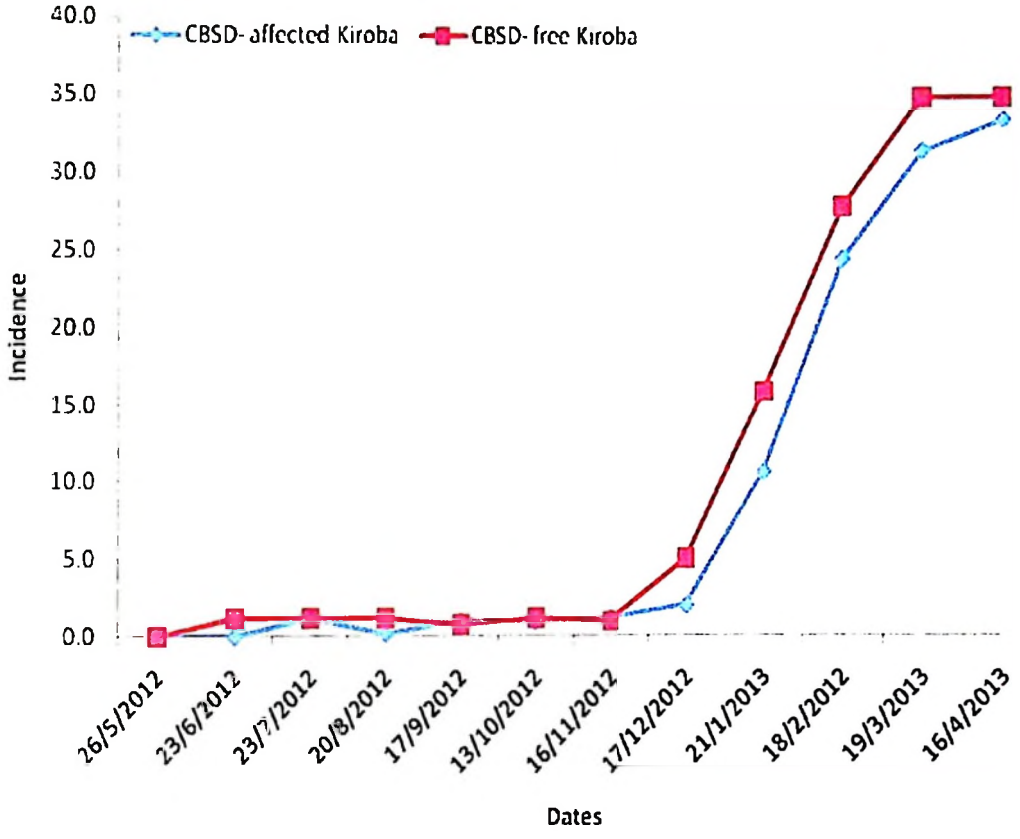

Figure 6: Incidence of CGM damage in CBSD- free and CBSD- affected Kiroba

4.2.5 Plant height

There was no significant ($p > 0.05$) difference in plant height between the infected and clean Kiroba (Fig. 7). However, the height of CBSD- free Kiroba was consistently taller than that of CBSD- affected Kiroba at every period of observation and plant age (Fig. 7). The lack of significant treatment difference in plant height for the CBSD-free and CBSD-affected Kiroba could be attributed to moisture stress because the experiment was established during off-season, which delayed plant establishment although irrigation was adopted later. According to Laban *et al.*

(2013), water stress has significant and devastating effects on vegetative and yield parameters of cassava. Also, El-Sharkawy (2007) reported that the varietal variability among cassava genotypes in response to water stress, with some genotypes having high levels of drought tolerance and others susceptible.

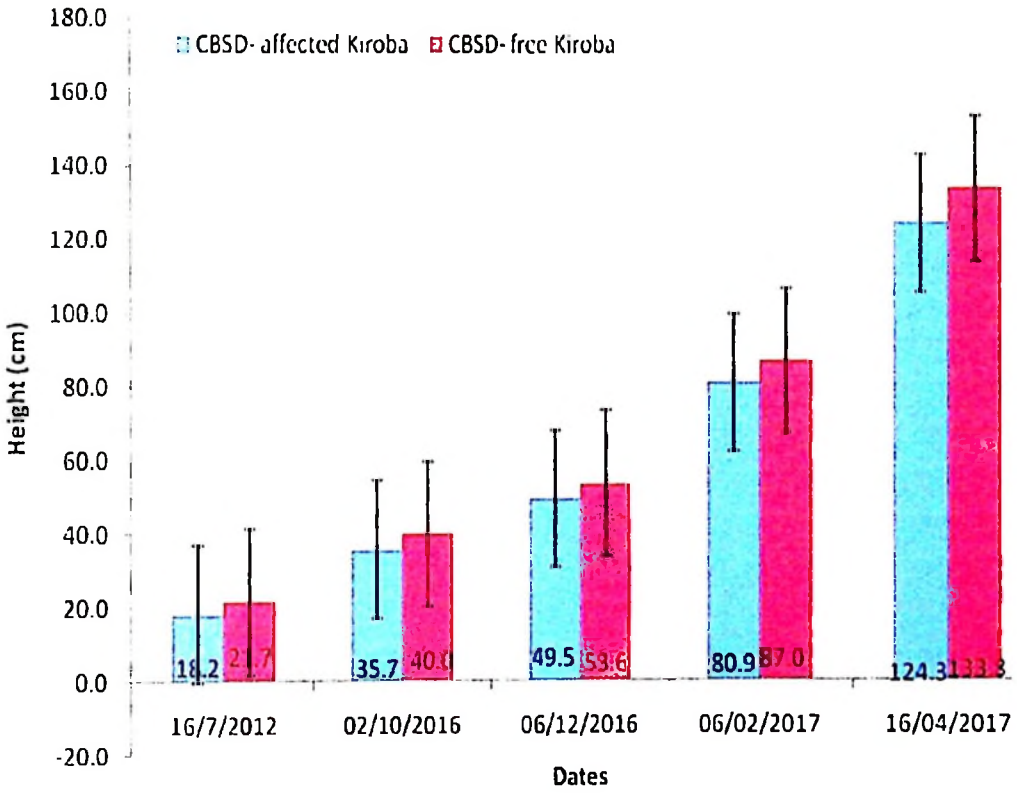

Figure 7: Difference in plant heights between the CBSD- free and CBSD-affected Kiroba plants

4.2.6 Relationship between yields of CBSD free and CBSD affected Kiroba

Results indicated that there was a significant ($p < 0.05$) difference between the weights of roots of CBSD- affected and CBSD-free Kiroba (Table 7). The results also indicated that there was no significant ($p > 0.05$) difference for the number of

plants harvested, number of roots/plant, root necrosis, and shoot weight. The number of roots harvested in CBSD-free Kiroba was almost similar to that of CBSD-affected Kiroba but weighed more than the latter. Similar observations were also observed by Adriko *et al.* (2011) who obtained few roots and lower average root weights in disease infected cassava plants compared to the un-infected, stressed that the vegetative and yield parameters of cassava depended on the variety and stage of disease infection.

4.2.7 Percentage reduction in yield of cassava due to CBSD

Generally the root yield of infected Kiroba was lower than that of clean Kiroba. Based on these results, there was a 24% loss in cassava yield as a result of CBSD infection as derived from the ratio of the difference between the yields obtained from clean Kiroba (7.0 t ha^{-1}) and that of infected Kiroba (5.3 t ha^{-1}) and the actual yield obtained from clean Kiroba, that is:

$$\text{Yield loss} = \frac{7.0 - 5.3}{7.0} \times 100 = 24\% \qquad \text{.. (1)}$$

The loss in yield observed could be attributed largely to the nature of planting materials used and the extent of CBSD infection as it was observed and discussed in section 4.2.1 of this study. Similar observation was observed by Hillocks *et al.* (2001) that losses in root weight of over 60% were recorded in plots containing plants grown from symptomless mother plants as compared with those grown from mother plants showing symptoms of CBSD.

The effect of CBSD on root weight reductions was as high as 70% in the most sensitive cultivars. The lower yield loss obtained in our study could be attributed to the low CBSD intensity on the test plant or because the trial was established during the off-season. The difference observed in yield loss between the CBSD- affected and CBSD- free Kiroba could be attributed to the length of time between the appearance of foliar symptoms and the development of root necrosis which is a varietal characteristic. Similar findings have also been reported by Hillocks *et al.* (2001) who observed that CBSD decreased root weight and patches of root necrosis made roots unmarketable but the difference in yield was determined by the characteristics of the planting materials.

Table 7: Effect of CBSD on yield and growth parameters of CBSD-free and CBSD-affected plants of cassava variety Kiroba

Treatments	Plt hrvst	No of rts	Wt of rtst/ha	Sh Wt t/ha	Rt Nec Sev	Rt Nec Inc
CBSD infected Kiroba	23±2.2	1.9±0.6	5.3±0.6	5.1±0.6	1.25±0.5	1.25±2.5
CBSD clean Kiroba	24.5±0.6	1.8±0.2	7.0±1.1*	4.8±0.7	1.5±0.6	2±2.3
t			2.6			
α						0.05: 0.05
P	0.23	0.851	0.039	0.575	0.537	0.6

Key:

N.S = p >0.05; * = p ≤0.05; ** = p ≤0.01; *** = p <0.001; 1= Infected Kiroba, 2= Clean Kiroba, Sev = Severity, Inc = Incidence, Plt hght = Plant height, No of rts = Number of roots, Wt= Weight, rts = Roots, Rt Nec = Root necrosis, Shwt =shoot weight, Plt hrvst = Plant harvested

4.2.8 Regression coefficient of CBSD severity against number of roots harvested, weight of roots, shoot weight, and root necrosis

The results of the linear regression models generated for the number of roots, shoot weight and root necrosis severity were, respectively, YnR = 1.729 + (0.0859*CBSD

sev), YwtS = 507.126 + (3.801*CBSD sev) and YrNS= 1.014 + (0.00141*CBSD sev) (Table 8). Results indicated that the powers of the performed tests with respect to the model were below the desired powers of 0.8 which indicated less confidence to detect the differences which existed between CBSD severity and number of roots, shoot weight and the severity of root necrosis. However, negative coefficient was observed for the weight of roots indicating that there is a likely decrease in the weight of roots with increase in CBSD severity. In addition, results indicated that the weight of roots was significant ($p < 0.05$) reduced by the CBSD severity which had a regression model of YwtR = 826.057 - (93.736*CBSD sev). These findings suggest that the number of roots does not necessarily determine the weight of roots, hence yield. This could be associated with many roots which are affected and their weight being reduced in turn.

Table 8: Regression coefficient of CBSD severity against number of roots harvested, weight of roots, shoot weight, and root necrosis

	No. of rts	Wt of rts	Sht wt	Rt Nec
CBSD Severity				
r	0.0567	0.19*	0.009	0.008
P	0.458	0.012	0.897	0.913
Regression model	YnR = 1.729 + (0.0859*CBSD sev)	YwtR = 826.057 – (93.736*CBSD sev)	YwtS = 507.126 + (3.801*CBSD sev)	YrNS= 1.014 + (0.00141*CBSD sev)

Key: * = p ≤0.05, Wt = Weight, CBSD = Cassava brown streak disease, No of rts = Number of roots, Wtrts = Weight of roots, Sht wt = Shoot weight, Rt Nec = Root necrosis.

4.3 Effect of Quality of Planting Materials and Time of Harvesting on Yield Losses Associated with CBSD Infection

4.3.1 Analysis of variety and time

The results of the analysis of variance for CBSD, CMD and CGM incidence, plant height, plant harvested, number of roots, incidence and severity of root necrosis, and weight of roots are shown in Table 9. The variety main effects had no influence on all the variables studied. The main effects of harvesting time were not significant for all characters examined except CBSD incidence, plant height and weight of shoots. Similarly, all characters examined varied among the genotypes. In general, all interactions were not significant for most of the tested characters except for CBSD incidence. Results also indicated that the variety had no influence except on CMD incidence, plant height, number of harvested plants, number of roots, severity of root necrosis, and the incidence and shoot weight.

The difference in the mean values among the different levels of variety was not great enough to exclude the possibility that the difference was just due to random sampling variability after allowing for the effects of differences in harvesting time. However, this observation was not statistically ($p > 0.05$) different.

Table 9: Analysis of variance for CBSD, CMD and CGM incidence, plant height, plants harvested number of roots, incidence and severity of root necrosis, and shoots weight of cassava cultivars harvested at different times, 2013 and 2014.

Treatments		CBSD Inc.	CMD Inc	CGM Inc	Plt hght	Plt hrvst	No of rts	Rt Nec sev	Rt Nec inc	Wtrts	Shwt
Mwari		2.39	8.51	44.71	77.06	17.60**	3.70**	2.68*	14.55	4.06	4.611***
Kiroba		2.94***	26.47**	60.77*	88.04***	19.33	3.24	2.23	19.59*	4.82***	3.917
	V	0.489	0.002	0.099	0.057	0.004	0.132	0.06	0.10	0.30	0.246
P	**T**	<0.001	0.357	0.01	<0.001	0.919	0.001	0.33	0.02	<0.001	<0.001
	V x T	<0.001	0.493	0.581	0.97	0.25	0.217	0.275	0.915	0.926	0.631

Key: N.S = p >0.05; * = p ≤0.05; ** = p ≤0.01; *** = p <0.001, V = Variety, T = Time, V x T = Interaction between Variety and Time, Plt hght = Plant height, Plt hrvst = Plant harvested, No of rts = Number of roots, Rt Nec sev = Root necrosis sev, Rt Nec inc = Root necrosis inc, Wtrts = Weight of roots, Shwt = shoot weight, CBSD Inc = Cassava brown streak incidence, CMD Inc = Cassava mosaic disease , incidence Cassava green mites incidence.

4.3.2 CBSD, CMD and CGM incidence, plant height, plants harvested number of roots, incidence and severity of root necrosis, and shoots weight of two cassava cultivars Kiroba and Mwari

The difference in the mean values of the parameters among the different levels of time was greater than would be expected by chance after allowing for effects of differences in variety. The latter observation indicated that the difference was statistically significant ($p < 0.05$). These differences were isolated by the use of a multiple comparison procedure of Holm-Sidak method (Aickin and Gensler, 1996) and the results are as indicated in Table 10. Results also indicated that the effect of different levels of variety does not depend on what level of time is present.

4.3.2.1 Severity and incidence of CBSD in Mwari and Kiroba cassava varieties

The results showed that the CBSD symptoms for cassava variety Mwari were not visible until 14MAP with severity of class 4 (Fig. 8). The results showed that CBSD symptoms for cassava variety Kiroba were not visible until 11MAP. At this stage of cassava plant growth mild symptoms were observed with disease score class 2 (Fig 8) and the disease incidence was 14% (Table 10). The disease incidence for variety Mwari and Kiroba was 4% and 14.3%, respectively, which was recorded during the 5th harvest that is 14MAP (Table 10).

Generally the number of affected plants by CBSD was very low for both varieties. The delayed CBSD expression for varieties Mwari and Kiroba was attributed probably to the nature of the planting materials which were clean (Fig. 8). The

findings of this study are in line with those of Hillocks *et al.* (2001) whose reported that the inherent characteristics of the susceptibility or resistance of the respective variety leads to varied response to the CBSV infection. According to Rwegasira and Rey (2012), most susceptible cultivars exhibit pronounced foliar and root symptoms, and the syndrome begins soon after sprouting in the cutting-derived infection. In addition, Hillocks and Thresh (2000) advocate that the symptoms of CBSD vary from leaf to leaf, shoot to shoot and plat to plant, even for the same variety and virus strain even in the same locality. This suggests that starting with clean planting materials delayed CBSD expression in the studied Kiroba and Mwari cassava varieties.

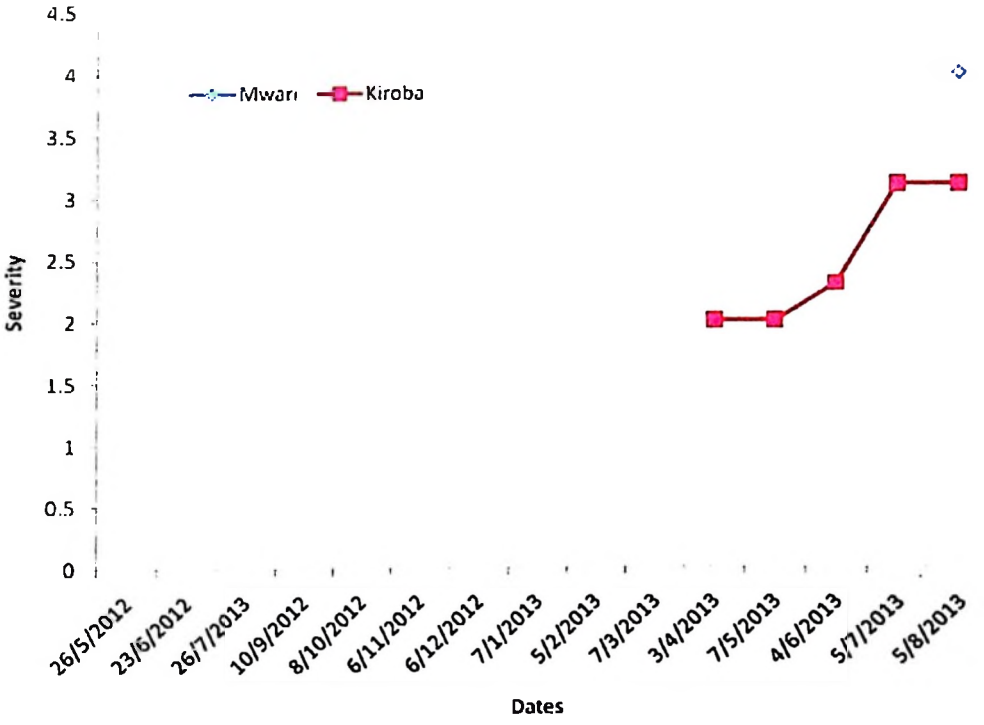

Figure 8: CBSD severity on Mwari and Kiroba cassava varieties

4.3.2.2 Severity and incidence of CMD in Mwari and Kiroba cassava varieties

The findings indicated that generally Kiroba variety had higher CMD severity than Mwari variety (Fig. 9). However, in August 2013 CMD severity generally increased for Kiroba and decreased for Mwari. In addition, the CMD incidence increased in both cassava varieties to July 2013 that is 15MAP at which it decreased in both varieties in August 2013 that is 16MAP (Table 10). The results also indicated that the highest CMD incidences of 15 and 46% were recorded during harvest 4 that is 12MAP for Mwari and Kiroba, respectively. The inconsistent observation made during maturity stage of the two cassava varieties could be related to the drop-out of old leaves which decreased the extent at which the disease was expressed. In addition, the level of expression of the disease during maturity in Mwari variety was higher than in Kiroba variety. This finding suggests that CMD is more expressed in Mwari variety as opposed to Kiroba.

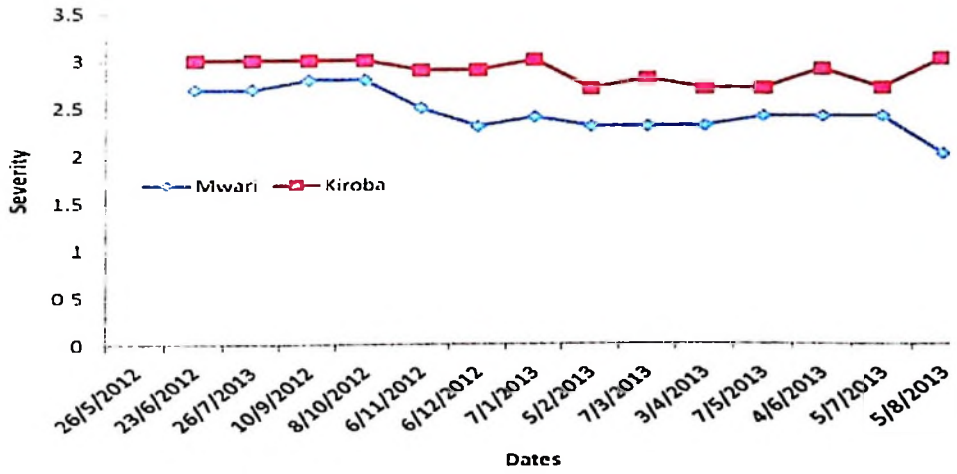

Figure 9: CMD severity for on Mwari and Kiroba cassava varieties

4.3.2.3 Severity and incidence of CGM damage in Mwari and Kiroba cassava varieties

The increase in cassava green mite's damage was due to high temperature and rainfall (Fig. 10). Results indicated that the highest CGM incidence for Mwari and Kiroba was recorded during 3 (73%), and, 4 (89%) and 5 (92%) harvests, respectively, that are 10MAP, 12 and 14MAP (Table 10). The latter variations in the CGM incidence between the two varieties and at different harvesting times were statistically at par. In addition, the increase in CGM incidence observed for Mwari and Kiroba cassava varieties from October to March that is 6 to 11MAP could be related to intermittent dry periods experienced in the experimental site. The findings, however, depicted that the extent of incidence of cassava green mite's damage was almost similar for both Mwari and Kiroba varieties (Table 10) throughout the growing periods. The findings of this study suggest that the relatively dry conditions favour the incidence of cassava green mites and their damage. These findings are consistent to those of Abaca *et al.* (2014) who observed that CGM effects were greater during the dry period than the wet period. Skovgård *et al.* (1993) also obtained similar findings with the cassava green mites in selected dry areas in Kenya.

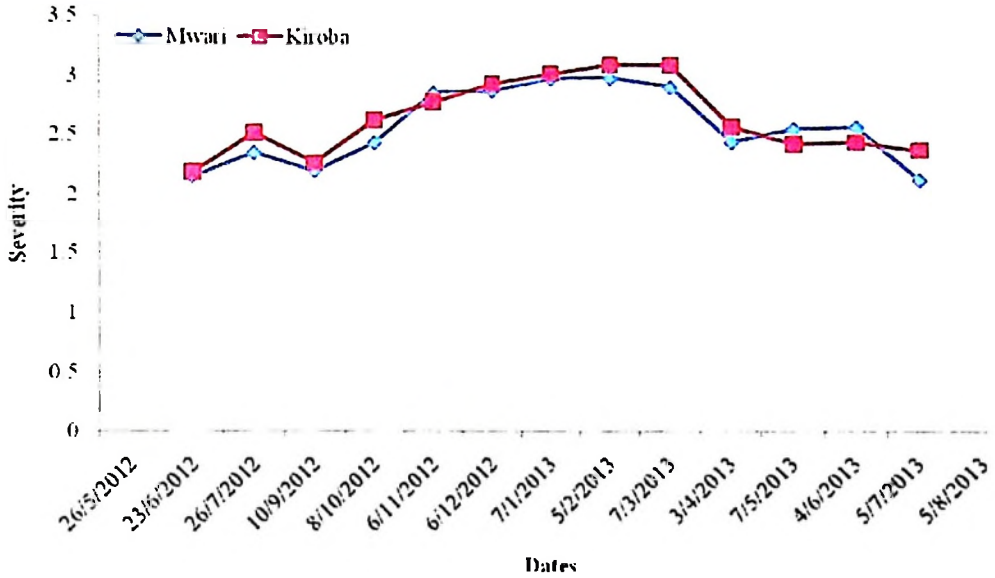

Figure 10: CGM severity for Mwari and Kiroba cassava varieties

4.3.2.4 Plant height

The results in Table 10 showed that there was no big difference in vegetative growth of the cassava plants regardless of the varieties but the variety Kiroba grew relatively faster than variety Mwari. Results indicated that the significant changes in increased plant height for Mwari variety was observed during 4 to 6 harvesting times but the highest height (123 cm) was recorded at harvest 5 that is 14MAP. On the other hand, the significant changes in increased height were for Kiroba variety was observed during 3 to 6 harvesting times but also the highest height (127 cm) was observed at harvest 5 as for Mwari variety. On the other hand, the lowest plant height recorded for Mwari was between 30 and 40 cm, and for Kiroba plants was 40 cm that is at 2MAP (Table 10). These findings suggest that Kiroba variety increased in height relatively faster than Mwari variety probably due to immediate adaptation and adjustment to the changing environmental conditions.

4.3.2.5 Effects of CBSD on the roots quality and yield loss

Results of the effects of CBSD on the quality and yield loss in Mwari and Kiroba varieties are presented in Table 10. The observations were deduced from the number of plants and the roots harvested, severity of root necrosis, weight of shoot and yield. Results indicated variation in the levels of root necrosis depending on the number of plants harvested. Generally, many roots per plant (3 to 6) were harvested from Mwari and Kiroba varieties during harvests 4 to 6 that are 12 to 16MAP.

The findings of this study could be related to those of previously conducted similar studied. Hillocks *et al.* (2001) reported that in Tanzania, studies have demonstrated that most (> 90%) plants of sensitive varieties express leaf symptoms, and that many of the same plants (12–59%, depending on variety) show root symptoms at harvest. Rwegasira and Rey (2012) reported that sensitive varieties may lose up to 70% of fresh root yield, due to the effects of die-back.

According to Hillocks *et al.* (2001), these losses are compounded by the effects of necrosis on root quality, which prevent harvested roots from being marketed or encourage premature harvesting to avoid the most severe damage. The findings of this study indicated mild symptoms of severity of root necrosis for variety Mwari which were relatively greater than a scale of 2. These symptoms were observed starting at time 1 of harvest that is 6 MAP. Similar symptoms were also observed for variety Kiroba in different harvesting times but harvests 1 and 3 that are 6MAP and 10MAP were relatively lower than the class of 2. The findings of this study suggest that early or delayed harvest of Kiroba and Mwari varieties does not necessarily help

in preventing yield loss through root necrosis but it is largely determined by the extent of CBSD infection. These findings are consistent with those of Nichols (1950) who depicted that the symptoms of root necrosis and yield loss does not only increase as the age of the crop increases but also depend on the level of diseases infection. However, Hillocks *et al.* (2001) observed that yield losses are particularly acute for local varieties in which root necrosis begins to increase from six months after planting, encouraging farmers to harvest prematurely.

Table 10: Means of CBSD, CMD and CGM incidence, plant height, plants harvested number of roots, incidence and severity of root necrosis, and shoots weight of cassava cultivars harvested at different times, 2013 and 2014

Variety	Time	CBSD inc	CMD inc	CGM inc	Plt hght	Plt hrvst	No of rts	Rt Nec sev	Rt Nec inc	Wtrts	Shwt
Mwari	1	0.00a	12.53a	14.20a	38.33a	18.00d	2.77a	3.33a	5.63a	1.27a	0.50a
	2	0.00a	8.60a	32.03a	50.43a	18.67f	3.33a	2.67a	13.97a	2.17a	1.53a
	3	0.00a	10.17a	72.77b	57.83a	18.33e	2.40a	2.00a	7.10a	1.10a	2.37a
	4	4.67b	1.43a	56.23b	75.53b	17.33c	3.87a	2.23a	18.40a	3.00a	4.03a
	5	5.2b	14.83a	49.90b	123.27b	16.33a	5.53b	3.17a	24.33a	8.83b	11.70b
	6	4.067b	3.47a	43.13a	116.97b	16.67b	4.23a	2.70a	17.87a	7.97b	7.533b
Kiroba	1	0.00a	11.43a	18.47a	50.40a	19.33h	2.53a	1.93a	10.33a	1.93a	0.50a
	2	0.00a	28.00a	57.57b	65.23a	19.00g	3.23a	2.50a	17.83a	3.13a	1.73a
	3	0.00a	18.87a	68.07b	73.47b	18.00d	2.67a	1.73a	13.30a	3.13a	2.73a
	4	0.00a	22.70a	88.63b	91.00b	19.67i	4.30a	2.67a	26.83a	3.33a	3.60a
	5	3.17b	46.17a	92.43b	127.43b	19.33h	4.00a	2.23a	22.60a	8.13b	9.10b
	6	14.47c	31.67a	39.43a	120.7.00b	20.67j	2.73a	2.33a	26.67a	9.27b	5.83b
LS Mean		1.48	9.07	16.20	9.516	0.97	0.49	0.38	5.08	1.26	1.01

The means bearing similar letter(s) along the same column did not differ significantly based on Holm-Sidak method (Aickin and Gensler, 1996). LS = Least significance, CBSD inc =Cassava brown streak disease incidence, CMD inc = Cassava mosaic disease incidence, CGM inc = Cassava green mites incidence

Plt hght = Plant height, Plt hrvst = Plant harvested, No of rts = Number of roots, Rt Nec sev = Root necrosis sev, Rt Nec inc = Root necrosis inc, Wtrts = Weight of roots, Shwt = shoot weight, MAP= months after planting, Time 1 = 6MAP, 2= 8MAP, 3= 10MAP, 4= 12MAP, 5= 14MAP, 6= 16MAP

4.4 Whitefly Population

The findings indicated that number of whiteflies in late October to mid December 2012 was higher in Kiroba than in Mwari cassava varieties but the whitefly population decreased between late December and late January 2013 (Fig. 11). In early February 2013, the number increased to 29 and 26 for the Kiroba and Mwari, respectively. This number decreased between February and early April 2013 which reached 35 whiteflies in each cassava variety in May 2013 but the number decreased far below this throughout the remained period of experiment.

These findings revealed that the whitefly population was low during the dry season with low temperatures (Fig. 11). The findings also suggested that as the temperature increases the whitefly population also increased and at maximum temperature of 33.8 ^{0}C the highest number of whitefly was recorded per cassava plant (Fig. 11). In addition, it was observed that during the rainy season or rather the presence of showers the whitefly population decreased (Fig. 11). The findings indicated that during the months of November, December and late January as the study area received rains, the temperatures were high and these favoured increase in whitefly population. This could be associated with increased new tender leaves on cassava plants which availed easiness of acquiring sap from the leaves. These also increased chances for the whitefly to reproduce and multiply in number but the extent of replication generally appeared to be higher in Kiroba than in Mwari.

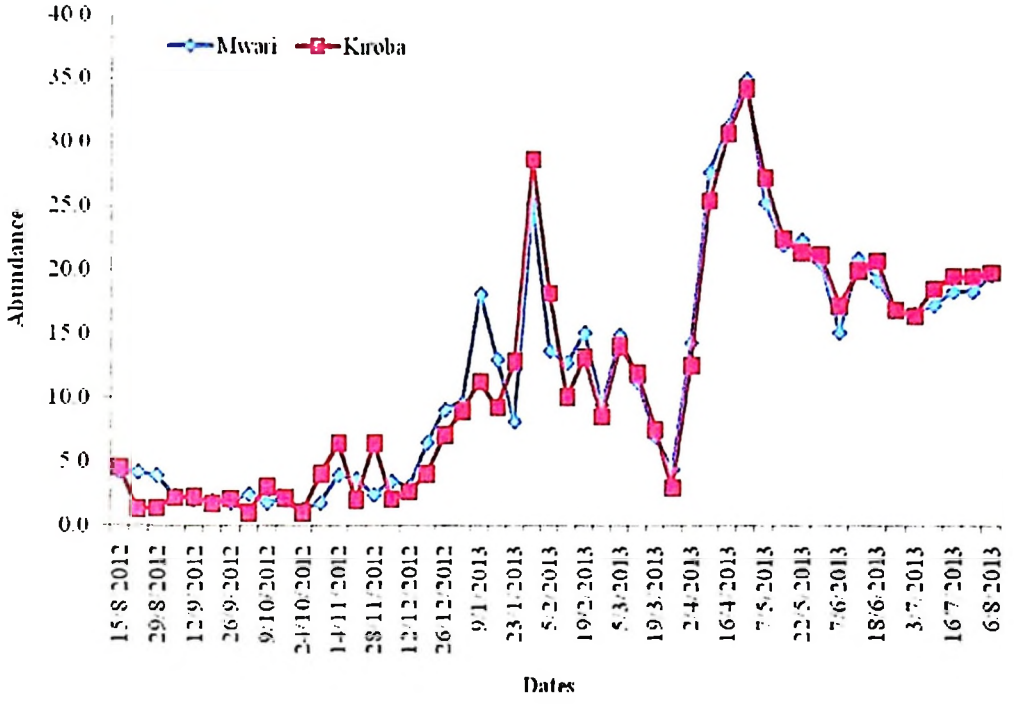

Figure 11: Whitefly abundance for Kiroba and Mwari cassava varieties

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusions

i. The detection and identification of CBSVs and their variants were done by using Real-Time Polymerase Chain Reaction (RT-PCR). It was found that the CBSD prevailing at Kibaha is caused by CBSV and UCBSV infection. Although plants affected by CBSD may or may not express symptoms, symptomatology alone cannot be used to unequivocally identify CBSVs, and there are no obvious differences in symptoms caused by the two species.

ii. CBSD-free planting materials of the Kiroba variety gave higher yields (7.0 ± 1.1 t ha^{-1}) than those obtained with CBSD-infected planting materials (5.3 ± 0.6 t ha^{-1}).

iii. It was found that the use of CBSD-infected planting materials of variety Kiroba resulted in a yield loss of 24%. Initially CBSD-free planting materials became infected towards the end of their growth cycle, since they were planted adjacent to plots of initially CBSD-infected materials. In spite of this late infection, yields of the initially CBSD-free material were much greater than those for the initially CBSD-infected material. These results suggest that in the absence of adjacent infected plots, the yields of the initially CBSD-free materials would have been even higher.

iv. The findings of this study showed that CBSV and UCBSV infection at Kibaha was higher than that by the cassava mosaic geminiviruses that cause CMD. This highlights the particular importance of CBSVs as key pathogens of cassava in coastal Tanzania.

v. There was no clear relationship between the incidence of CBSD root necrosis in varieties Mwari and Kiroba and time of harvesting. Part of the reason for this was the generally low incidence of root necrosis, which was due to the fact that the trial was planted during a season which was unfavorable for whitefly population increase and subsequent spread of CBSVs.

vi. The effects of CBSD on yield in varieties Mwari and Kiroba with harvesting times followed a similar trend to that of root necrosis. The highest yield (8.8 t ha^{-1}) for variety Mwari was recorded at harvest 14 MAP, which dropped to 8.0 t ha^{-1} at 16 MAP. The highest yields (8.1 and 9.3 t ha^{-1}) for Kiroba variety were observed at 14 MAP and 16 MAP respectively. It was concluded that early or delayed harvest of Kiroba and Mwari varieties does not necessarily prevent root necrosis and increase yield but it is largely determined by the extent of CBSD infection. The absence of any obvious effect of CBSD on yield is the fact that there was not enough CBSD.

5.2 Recommendations

Based on the findings of this study it is recommended that:

i. The absence of a clear relationship between CBSD symptoms and virus detection suggests that further research needs to be conducted to determine exactly how virus presence relates to symptom expression, during the course of growth of cassava plants. Additionally, it would be valuable to determine the effects of single and mixed infections of CBSVs and to measure changes of their concentrations over time, and relate these to symptom expression.

ii. CBSD-free planting materials of cassava should be used in order to avoid further spread of CBSD infection above-ground. This is expected to account for the yield loss of 24% caused by CBSD infection. Further to that, if seed multiplication is the aim, virus-free cuttings should be planted in an isolated field to reduce the extent of CBSD spread. There is currently an insufficient supply of virus-free planting materials for farmers in Tanzania.

iii. Based on yield data alone, the study has demonstrated that optimal harvesting dates for Mwari and Kiroba varieties are from 12-16 MAP. Further studies, conducted in high CBSD pressure seasons and locations, are required to develop more refined guidelines on harvesting periods. In addition, both varieties used in this study are relatively resistant to CBSD. Future related studies should use varieties that are popular with farmers but

which are highly susceptible to CBSD, such as Rasta or Kikombe. In order for this to be feasible, researchers will first need to establish virus-free blocks of these varieties.

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APPENDICES

Appendix 1: Nutrient contents of cassava

Nutrient	Unit	Value per 100 g	cup 206g	root 408g
Proximates				
Water	g	59.68	122.94	243.49
Energy	kcal	160	330	653
Protein	g	1.36	2.8	5.55
Total lipid (fat)	g	0.28	0.58	1.14
Carbohydrate, by difference	g	38.06	78.4	155.28
Fiber, total dietary	g	1.8	3.7	7.3
Sugars, total	g	1.7	3.5	6.94
Minerals				
Calcium, Ca	mg	16	33	65
Iron, Fe	mg	0.27	0.56	1.1
Magnesium, Mg	mg	21	43	86
Phosphorus, P	mg	27	56	110
Potassium, K	mg	271	558	1106
Sodium, Na	mg	14	29	57
Zinc, Zn	mg	0.34	0.7	1.39
Vitamins				
Vitamin C, total ascorbic acid	mg	20.6	42.4	84
Thiamin	mg	0.087	0.179	0.355
Riboflavin	mg	0.048	0.099	0.196
Niacin	mg	0.854	1.759	3.484
Vitamin B-6	mg	0.088	0.181	0.359

Folate, DFE	µg	27	56	110
Vitamin B-12	µg	0	0	0
Vitamin A, RAE	µg	1	2	4
Vitamin A, IU	IU	13	27	53
Vitamin E (alpha-tocopherol)	mg	0.19	0.39	0.78
Vitamin D (D2 + D3)	µg	0	0	0
Vitamin D	IU	0	0	0
Vitamin K (phylloquinone)	µg	1.9	3.9	7.8
Lipids				
Fatty acids, total saturated	g	0.074	0.152	0.302
Fatty acids, total monounsaturated	g	0.075	0.154	0.306
Fatty acids, total polyunsaturated	g	0.048	0.099	0.196
Cholesterol	mg	0	0	0
Other				
Caffeine	mg	0	0	0

Key: µl=miroliter, rpm=revolutions perminute, w/v = weight per volume, mg =milligram, iu=International Units, RAE= Retinal Activity Equivalents, DFE = Dietary folage equivalent, Kca=Kilocalorie

Source: National Nutrient Database for Standard Reference Release 26 Software v.1.3.1

Appendix 2: Protocol for DNA/RNA extraction part of materials and methods

Preparation of the extraction buffer (100 mL)

2.0% (w/v) CTAB 2.0g

2.0 M NaCl 11.688g

2.0% PVP 2.0g

25mM EDTA-5.0 ml of 0.5 M stock

100 mM Tris-HCL, pH 8.0 - 10.0 ml of 1M

0.2%β – mercaptoethanol (only add immediately before use)

Extraction protocol used was described below:

1. Measure approximately 100 mg of fresh leaf or 45 mg of dried leaf into a mortar or thick gauge plastic bag.

2. Add 1 ml CTAB extraction buffer and grind into a fine paste.

3. Transfer 750 µl of the extract into a 1.5 ml micro-centrifuge tube and incubate at 65 ° C for 15 min.

4. Mix the extracts with an equal volume (750µl) of chloroform: isoamyl alcohol (24:1); vortex briefly and centrifuge at 12 000 rpm (13 000g) for 10 min at 4 ° C.

5. Transfer the top aqueous solution (500µl) into a new micro-centrifuge tube.

6. Add 0.6 vol (300µl) cold isopropanol and incubate at – 20 for at least 10 min.

7. Centrifuge the samples at 13 000 rpm (15 600g) for 10 min at 4 ° C and discard the supernatant.

8. Wash the nucleic acid pellet in 700µl 70% ethanol by voltexing and incubating at – 20 ° C for at least 10 min and centrifuging for 5 min at 13 000 rpm.

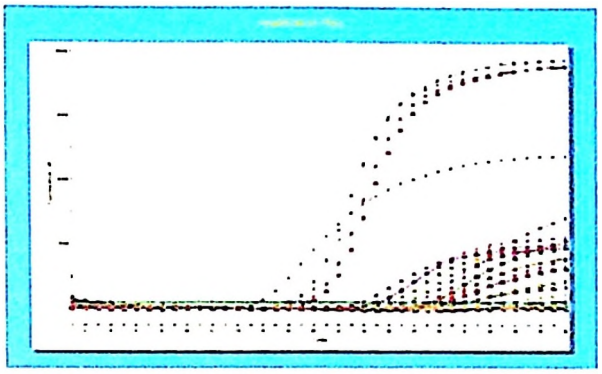
9. Remove the ethanol and dry the pellet. NB: *Ethanol should be completely dried up*

10. Resuspend the dried pellet in 100 µl sterile water (SDW) or low concentration TE on ice for about 30min. Store at – 20 o C (or -80 o C for long term storage)

Appendix 3: RT-PCR Amplification plots part of results

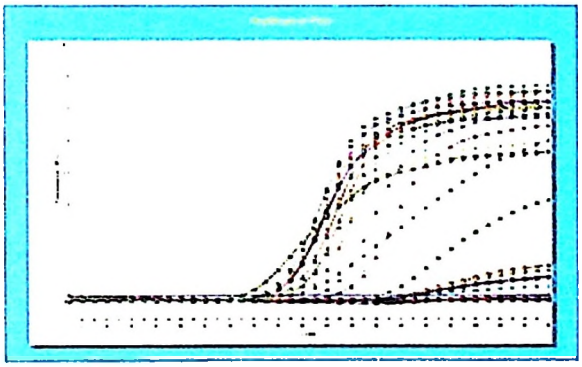
Amplification Plots

Mary y Tracie 02-11-2013 mqg



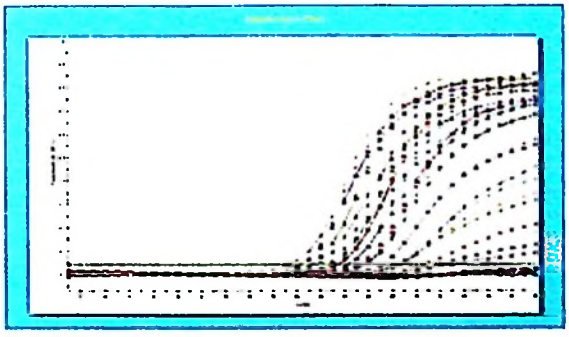
Amplification Plots

Mary y Tracie 02-11-2013 -CBSV simplex.mqg



Amplification Plots

Mary Tracie 02-11-2013 simplex-UCBSV.mqg



Appendix 4: General PCR practices to prevent contamination.

These include:

- Maintaining separate areas, dedicated equipment, and supplies for sample preparation, PCR setup, PCR amplification, and analysis of PCR products.
- Amplified PCR products into the PCR setup area were not allowed.
- Clean lab coat and gloves were used
- The reactions and components were capped as much as possible.
- The positive-displacement or aerosol-resistant pipette tips were used.
- The lab benches and equipment periodically with freshly diluted 10 % bleach solution.
- Gloves were changed whenever suspected that they are contaminated.
- All sample tubes and reaction plates were handled, opened and closed carefully.

Appendix 5: Ct value

The Ct value means the number of PCR cycles where the reporter dye signal is sufficiently high to cross an automatically or manually determined threshold value. The earlier the signal passes the threshold value, the stronger the PCR amplification of the target sequence. In a standard setup 40 cycles are run. The Ct "undetermined" (No ct) means that the signal stays under the threshold during all the 40 cycles, which practically means a negative result, or that the amplification is so low that it can't be detected. It is a relative measure of the concentration of target in the PCR reaction. The Ct (threshold cycle) is the intersection between an amplification curve and a threshold line. It is also the first cycle at which the instrument can distinguish the amplification generated fluorescence as being above the ambient background signal. This Ct value can be directly correlated to the starting target concentration for the sample. The greater the amount of initial DNA template in the sample, the earlier the Ct value for that sample.

SPE