INCIDENCE, SEVERITY AND SPREAD OF CASSAVA MOSAIC DISEASE IN WESTERN KENYA

By

BY CHARLES LWANGALWOLE

A THESIS SUBMITTED IN PARTIAL FULFILMENT FOR THE DEGREE OF MASTER OF SCIENCE IN PLANT PATHOLOGY AT KENYATTA UNIVERSITY

Declaration by the candidate

This thesis is my original work and has not been presented for a degree in any other University or for any other award.

Charles Lwanga Lwole

Signed Date

Declaration by the Supervisors

This thesis is submitted with our approval as University supervisors

Dr. Ethel O. Monda
Department of Botany,
Kenyatta University,
Nairobi, Kenya.

Signed Date

Dr. G. W. Otim-Nape
National Agricultural Research Organisation,
Entebbe, Uganda.

Signed Date
DEDICATION

This work is dedicated to the African farmer who needs the status of cassava to be elevated from a subsistence commodity to a highly valuable commercial crop through research.
ACKNOWLEDGEMENTS

I would like to acknowledge the financial support provided by the Rockefeller Foundation, Nairobi without which this research would not have realised the light of the day.

I would like to give special thanks to Dr. Moses Onim of Lagrotech Consultants in Kisumu for linking me to Rockefeller Foundation Nairobi. Without his desire to help I would never have known that funds existed for this kind of research in that organisation.

I would like to acknowledge the untiring efforts of my supervisors Dr. Monda and Dr. Otim-Nape who constantly gave me invaluable suggestions and criticisms and ensured that the work was finished on time.

Special thanks go to Dr. James P. Legg for training me in PCR diagnostics and allowing access to the PCR laboratory analysis facilities of IITA-ESARC in Uganda, the only centre in the region that is doing routine PCR analysis for plant viruses. I would like to remember very fondly the patience of Robert Obonyo and Geoffrey Okao-Okuja the PCR specialists at IITA-ESARC who assisted me to understand the procedure and interpret of the results.

Prof. Mike Thresh has been more than a fatherly figure to me. He went out of his way to ensure that everything was running smoothly and there are no better words for thanking him.

I would like to remember KARI-RRC for using their centre as a base and provided free access to all their facilities. Special thanks to Christopher Mburu, Maurice A. Mudeheri and Caleb Wituka for helping with statistical analysis and computer operations.

Finally I would like to thank my uncle Mr. F.B. M. Khalumi for giving his car for my work even when he needed it more than I did and my wife Elizabeth N. Mukolwe for her patience, commitment, encouragement, material and spiritual support. All those who contributed to my work in all ways I sincerely thank them.
The impact and spread of cassava mosaic disease was studied through a survey, by harvesting of diseased and healthy plants in farmer's fields to determine current yields and the effects of cassava mosaic disease on yield, cassava mosaic disease spread experiments, yield of spread experiments, determination of virus type by polymerase chain reaction and determination of cassava mosaic disease incidence in ratoons of cassava variety SS-4. The survey of incidence and severity of cassava mosaic disease and whitefly populations was carried out in October to November 1999 in Mumias/Butere, Kakamega, Busia and Teso in Western Province and Siaya and Suba in Nyanza Province. Many varieties of cassava were encountered, indicating a rich genetic diversity. Individual districts showed high cassava mosaic disease incidence and the overall cassava mosaic disease severity for all varieties was 3.33 (p<0.01) on the 1-5 scale of increasing severity. Abundant whitefly populations associated with cassava mosaic disease were encountered in Suba district only, indicating that the spread of the pandemic is still active. The overall cassava mosaic disease incidence in the region had dropped to 62.3% and was attributed to an infusion of improved cassava varieties by farmers of Busia and Teso districts from Uganda. A survey of yield from farmer's fields revealed that fresh tuberous weight per plant ranged from 0.3-4.4 (±0.86) kg in local and improved varieties. Cassava mosaic disease accounted for 28% of the total fresh tuber yield variability in the districts, and cassava mosaic disease infections accounted for 53% poor harvests encountered in all the district, thus indicating that cassava mosaic disease reduces overall yield in western Kenya.
considerable spread between April and October 1999, at all sites. There was little spread at the Siaya site between, August 1999 and March 2000 and no spread at Kakamega site at the same period. Most spread occurred at Siaya and it was significantly greater than at other sites. The local variety Serere used as a control showed severe infection towards the end of experiment of April 1999 plantings, whereas the improved varieties TMS 30337, TMS 30572 showed apparent decline in disease incidences towards the end of the experiment. Cassava mosaic disease incidences at Kakamega were higher than at Bungoma in plantings of April 1999. Mean cassava mosaic disease severity was not significantly different between Siaya (mean = 2.1, p = 0.05) and Kakamega (mean = 2.0, p = 0.05) but their severity was significantly different from that at Bungoma (mean = 1.6, p = 0.05). The variety SS-4 had only one plant infected at Kakamega in plantings of April 1999. Numbers of adult whiteflies differed significantly between sites, and between varieties in all experiments.

Evaluation of yield from the four cassava varieties showed that the improved varieties yielded more than the local variety Serere. Combined yield for all varieties in all sites ranged from <0.1 – 5.0 (±1.0) kg per plant. The yield of improved varieties varied from the lowest 11.1 t/ha of SS-4 at Bungoma to 27.7 t/ha of SS-4 at Kakamega, the local variety Serere had a production of 11.24 t/ha at Bungoma to 18.43 t/ha at Kakamega. Adult whiteflies were not encountered on the plants at harvest 10 months after planting. Cassava mosaic disease was caused by the newly discovered Uganda Variant/East African cassava mosaic virus-Uganda Variant that predominated in the region. SS-4 was highly resistant to cassava mosaic disease and its ratoons showed low disease incidence whereas its cuttings showed no disease and can be used for propagation.
# TABLE OF CONTENTS:

Title

Declaration ................................................................. I

Dedication ........................................................................ II

Acknowledgements ......................................................... III

Abstract ................................................................. IV

**CHAPTER ONE** .......................................................... 1

1.0 General Introduction .................................................. 1
1.2 History of cassava mosaic disease (CMD) in Kenya 1970s to the present: Pandemic Emergence .................................................. 5
1.2.1 Estimated cassava production losses due to CMD .................. 7
1.2.2 Strategy for combating CMD ........................................ 8
1.3 Hypothesis .................................................................. 12
1.4 Objectives .................................................................. 12

**CHAPTER TWO** .......................................................... 13

2.0 Literature review ......................................................... 13
2.1 History, cause and distribution of CMD ......................... 13
2.2 CMD Symptoms Expression ............................................. 15
2.3 Effect of mode and stage of infection on yield .................. 17
2.4 Management of CMD ................................................... 18
2.5 Factors influencing the spread of CMD .......................... 21
2.5.1 Sensitivity of the varieties grown ................................. 22
2.5.2 Infection “pressure” ................................................... 23
2.5.3 Systemic nature of CMD in infected plants
   The concept of “reversion and recovery” ............................. 24
2.6 Detection and differentiation of CMGs ............................. 24
2.7 Epidemiology of CMGs ................................................ 25
2.7.1 Mode of spread ....................................................... 25
2.8 *Bemisia tabaci*: The whitefly vector of CMGs .................. 26
2.8.1 Biology of the whitefly *Bemisia tabaci* ......................... 27
2.8.2 *B. tabaci* and its biotypes ........................................ 27
2.8.3 Climatic effect on population size and activity of *B. tabaci* ...... 28
2.8.4 *B. tabaci* and cassava ............................................. 31
2.8.5 Sampling techniques for populations of *B. tabaci* ............ 32
<table>
<thead>
<tr>
<th>Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.8.6</td>
</tr>
<tr>
<td>2.8.7</td>
</tr>
<tr>
<td>2.9</td>
</tr>
<tr>
<td>2.9.1</td>
</tr>
<tr>
<td>2.9.2</td>
</tr>
<tr>
<td>2.9.3</td>
</tr>
<tr>
<td>2.10</td>
</tr>
<tr>
<td>2.10.1</td>
</tr>
<tr>
<td>2.10.1.1</td>
</tr>
<tr>
<td>2.10.2</td>
</tr>
<tr>
<td>2.10.2.1</td>
</tr>
<tr>
<td>2.10.2.2</td>
</tr>
<tr>
<td>2.10.2.3</td>
</tr>
<tr>
<td>2.10.2.4</td>
</tr>
<tr>
<td>2.11</td>
</tr>
<tr>
<td>2.12</td>
</tr>
<tr>
<td>2.13</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chapter Three</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0</td>
</tr>
<tr>
<td>3.1</td>
</tr>
<tr>
<td>3.1.1</td>
</tr>
<tr>
<td>3.1.2</td>
</tr>
<tr>
<td>3.1.3</td>
</tr>
<tr>
<td>3.2</td>
</tr>
<tr>
<td>3.2.1</td>
</tr>
<tr>
<td>3.2.2</td>
</tr>
<tr>
<td>3.2.3</td>
</tr>
<tr>
<td>3.2.4</td>
</tr>
<tr>
<td>3.2.5</td>
</tr>
<tr>
<td>3.3</td>
</tr>
<tr>
<td>3.4</td>
</tr>
<tr>
<td>3.4.1</td>
</tr>
<tr>
<td>3.4.2</td>
</tr>
<tr>
<td>3.4.3</td>
</tr>
<tr>
<td>3.4.4</td>
</tr>
<tr>
<td>3.4.5</td>
</tr>
<tr>
<td>3.5</td>
</tr>
<tr>
<td>3.6</td>
</tr>
</tbody>
</table>
3.6.1 Sample collection ........................................................................54
3.6.2 Extraction of viral DNA from CMD-affected cassava leaves ...55
3.6.3 Sample DNA amplification and testing for the presence of ACMV, EACMV and UgV ..............................................................55
3.6.4 Testing for the presence of ACMV and UgV by primers of ACMV and UgV ...........................................................................57
3.6.5 Testing for the presence of EACMV and UgV by primers of EACMV and UgV ...........................................................................57
3.6.6 Agarose gel preparation ................................................................57
3.6.7 Sample loading .............................................................................58
3.6.8 Separation, amplification and precipitation of PCR product ....58
3.6.9 Restriction digestion ....................................................................59
3.7 Incidence of CMD in ratoons SS-4 variety ...................................59

CHAPTER FOUR ...............................................................................61

4.0 Results ..........................................................................................61
4.1 Vector populations, incidence and severity of CMD in western Kenya ...61
4.1.1 Common cassava varieties ..........................................................61
4.1.2 CMD incidence in Western Kenya ...............................................61
4.1.3 Severity of shoot symptoms ........................................................62
4.1.4 Adult whitefly populations ..........................................................62
4.1.5 Mapping ......................................................................................63
4.2 Survey of yield in ‘relic’ cassava in post-pandemic zone in western Kenya .................................................................68
4.2.1 Varieties encountered ..................................................................68
4.2.2 Correlation of growth and yield parameters ..............................70
4.2.3 Correlation of whitefly population, CMD symptoms severity score and growth and yield of cassava ........................................71
4.3 Spread of CMD in four cassava cultivars in western Kenya ..........72
4.3.1 Disease progress .................................................................72
4.3.2 Area under disease progress curve(AUDPC) .............................82
4.3.3 CMD symptom severity .........................................................82
4.3.4 Recovery ...............................................................................84
4.3.5 Adult whitefly populations ......................................................85
4.4 Growth and yield of varieties under trials ..................................86
4.4.2 Adult whitefly populations ......................................................87
4.4.3 CMD severity .................................................................87
4.4.4 Correlation of growth and yield parameters ..............................88
4.5 Virus types in CMD affected plants in Western Kenya .................92
4.5.1 Identification of viruses by PCR ..............................................92
4.5.2 Identification of viruses by RFLPs .........................................92
4.6 CMD incidence in SS-4 cassava ratoons ......................................93
LIST OF TABLES

Table 1  Scale for scoring symptom severity ................................. 49
Table 2  Disease reaction of four cassava cultivars to CMD ............. 52
Table 3  Primers used for PCR. ...................................................... 56
Table 4  Common cassava varieties ................................................. 61
Table 5  Mean number of adult whitefly population counts per shoot in six different districts........................... 63
Table 6  Separated mean values of yield characteristics of health and diseased plants in all districts....................... 69
Table 7  Mean plant weight and harvest index of cassava in Mumias/Butere, Teso and Siaya ..................................... 70
Table 8  Pearsons correlation coefficients matrix for cassava growth, yield parameters, CMD symptom scores and adult whitefly population ........................................... 71
Table 9  Mean value of plant characteristics and percentage of total $R^2$ due to variability in districts .................... 72
Table 10  Diseases incidence (%) by site for plantings in April 1999 to August 1999 ....................................................... 73
Table 11a  Disease incidence (%) in each cultivars at 7 MAP in April – October 1999 ......................................................... 75
Table 11b  Disease incidence in all varieties combined in relation to stage of growth ......................................................... 75
Table 12  Interaction values for CMD incidence ............................... 75
Table 13  Maximum mean disease incidence in each variety............. 76
Table 14  Areas under the disease progress curves (AUDPC) for CMD in different cassava varieties in on-farm trials at three sites in district of western Kenya April – October 1999 and at two sites in August 1999 – March 2000 .... 82
Table 14a  Disease severity in each cultivars (April – October 1999) at 7MAP ................................................................. 83
Table 14b  Combined disease severity in each cultivars (April – October 1999) at 7MAP ......................................................... 83
Table 15  Interaction of CMD severity .............................................. 84
Table 16  Pearsons correlation coefficients matrix for cassava growth, yield parameters, CMD symptom scores and adults whitefly population ........................................... 88
Table 17  Percentage yield loss due to CMD .................................... 90
Table 18  Disease incidence in ratoons of 55-4 ............................... 93
LIST OF FIGURES BAR CHARTS, MAPS AND PLATES

Plate 1 Whitefly-infected cassava showing symptoms restricted to youngest leaves ................................................. 29
Plate 2 Cutting-infected cassava with earliest leaves expressing symptoms ....................................................... 29
Plate 3 Healthy cassava plant .......................................................................................................................... 30
Plate 4a Samples with light band showing positive reaction for UgV ............................................................... 94
Plate 4b Sample showing positive reaction for ACMV ...................................................................................... 94
Plate 5a Sample indicated by arrow showing positive reaction with EcoRV ................................................. 95
Plate 5b Sample showing positive reaction for both UgV and EACMV ......................................................... 95
Plate 6a Samples showing positive reaction for UgV only ............................................................................. 96
Plate 6b Samples showing UgV only, EACMV only and a mixture of UgV and EACMV ............................................. 96
Map 1 Survey of CMD incidences in Western Kenya in October-November 1999. Plant age of fields surveyed was 7-12 months ................................................................. 65
Map 2 Survey of CMD severity in Western Kenya in October-November 1999. Plant age of fields surveyed was 7-12 months ........................................................................... 66
Map 3 Distribution of adult whitefly population in Western Kenya in October-November 1999. Plant age of fields surveyed was 7-12 months ......................................................................... 67
Figure 1 Cassava production in tonnes in Western Kenya ................................................................................. 8
Figure 2 Cumulative incidences and incidence in MIUs graphs ........................................................................... 77-78
Figure 3 Cumulative and actual incidence ........................................................................................................ 79-80
Figure 4 Whitefly population counts graphs ................................................................................................ 81
Figure 5 Yield in all varieties at all sites .............................................................................................................. 91
CHAPTER ONE

1.0 GENERAL INTRODUCTION

Cassava (Manihot esculenta Crantz) is an edible root crop found in the tropics. Its origin is South America, from where it was widely distributed to other tropical continents, especially Africa and Asia. In S. E. Asia, it is grown on a large scale and is sold mainly to Europe for animal feed (FAO, 1997).

In Africa, cassava is a major food crop that is used as human food, source of income on a small scale and as animal feed. As food, it is used in diverse ways. Fresh tuberous roots are cooked and eaten, fresh leaves are cooked and eaten as a vegetable contributing up to 20% of the protein in the diet (Banea, 1993). Dried chips are ground into flour, which may be mixed with flour from millet and sorghum to make many traditional dishes. The supplementary uses of cassava as a livestock feed have been reviewed (Iyayi and Tewe, 1994).

Cassava grows in diverse environmental conditions. It is grown in a wide range of rainfall from 400 mm to > 3000 mm per annum, and its tolerance to drought and low soil fertility make it an ideal food reserve for marginal areas. The crop is also tolerant to highly acidic soil conditions. It can be grown in a wide range of altitudes from sea level to 2000 metres above sea level, and is less sensitive to climatic changes than most other crops (Porto, 1995).

The main limit of the cassava growing area in Africa lies within the latitude
30°N and 30°S. The belt has a human population of 427 million people. Cassava distribution is largely tropical and excludes most of northern and southern Africa (Akoroda, 1997). Presently the tropical areas of sub-Saharan Africa (SSA) form the main production region of cassava.

Cassava comes second in importance as a staple food after maize, but in terms of total fresh weight production, cassava is first in Africa with a production of 86 million tonnes (FAO, 1999). It provides on average over 200 calories per day per person (IITA, 1988). Almost 200 million people (40% of the population) in the SSA region depend on cassava. In the Republic of Congo, Gabon, Mozambique and the Democratic Republic of Congo, the population consumes about 1000 calories per day per person from cassava out of the daily requirement of 2500 calories. Root yields from farms average 9-10 t/ha, although yields of over 70 t/ha have been achieved under experimental conditions. However, 15-20 t/ha is attainable with improved production methods but without fertilizer use (Akoroda, 1997). Cassava can yield up to 20 t/ha of fresh leaves, which contain 20-30% protein on a dry weight basis. A 100 g sample of leaves contains: 300 mg vitamin A, 0.25 mg thiamine, 300 mg calcium, 7.6 mg iron, 0.60 mg riboflavin, 2.4 mg niacin, and 310 mg ascorbic acid (Ojeba et al., 1996).

The main pests of cassava are nematodes, mites, and mealybugs (IITA, 1985). Nematodes cause root-knot, which is prevalent in the tropical and sub-tropical regions where cassava is grown. The causal pests responsible are *Meloidogyne* spp., which include *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla*. 
Symptoms appear as galls or knots, occurring singly but which often coalesce into a string of knots. Cassava yield losses due to root-knot nematodes are assessed to be 17-50%. Control measures employed involve crop rotation and use of nematode resistant cultivars where available (IITA, 1985).

Several species of mites are associated with cassava. The major ones are the cassava green mites (CGM) *Mononcychellus tanajoa* and *M. progresivus*. These occur throughout the cassava belt in Africa and the neotropics (Bellotti *et al.*, 1994). Attacks result in 20-80% loss in tuberous root yield. Biological control and host plant resistance are used to contain these mites (IITA, 1985; IITA, 1990;). The release of the phytoseid might, *Typhlodromalus aripo* has been reported to provide effective biological control of CGM in many countries of sub-Saharan Africa. However *T. aripo* has reportedly failed to establish in some regions, including Zambia (Sakala *et. al.*, 1998).

Four species of cassava red mite occur in the cassava belt in Africa, namely *Oligonychus gossypii*, *Tetranychus telarius*, *T. neocaledonicus* and *T. cinnabrinus*. They cause most damage to the plant at the beginning of the dry season. No control measures are known (IITA, 1985).

Cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero (Homoptera: Pseudococcidae), which was accidentally introduced from South America in the 1970s is now found in all cassava growing areas of Africa (Neuenschwander, 1994a; IITA, 1985; Anonymous, 1999). Continent-wide release of the parasitic wasp *Apoanagyrus lopezi*, introduced from South
America has provided effective control of *P. manihoti* in cassava growing regions of Africa. More recently there has been successful establishment of *A. lopezi* as a natural enemy of *P. manihoti* in Zambia. Recent surveys have confirmed reduced populations of the cassava mealybug and reduced damage to cassava (Sakala *et al.*, 1998).

The whitefly *Bemisia tabaci* (Gennadius 1889)(Homoptera: Aleyrodidae) is a vector of the viruses causing Cassava Mosaic Disease (CMD) (IITA, 1985; Fishpool and Burban, 1994).

The major cassava diseases are CMD caused by cassava mosaic geminiviruses (CMGs) (Family: *Geminiviridae*; Genus: *Begomovirus*) (Rybicki, 1994), cassava brown streak disease (CBSD) caused by *Cassava brown streak virus* (Family: *Potyviridae*, Genus: *Ipomovirus*), cassava bacterial blight (CBB) caused by *Xanthomonas campestris* pv *manihoti*, cassava anthracnose disease (CAD) caused by *Colletotrichum gleosporoides* f. sp. *manihotis*, cassava brown leaf spot caused by *Cercosporidium henningsii* Allesch. and cassava white leaf spot caused by *Cercospora caribaea* Cif. (IITA, 1985).

CMD is generally regarded as the most important disease of cassava and was ranked as the most important vector-borne disease of any food crop in Africa (Geddès, 1990). The disease occurs in Africa, southern India and Sri Lanka. The CMGs causing CMD have particles averaging 20-30nm, are also disseminated through cuttings used for planting. Few quantitative data exist on which to base definitive estimates of the losses caused by CMD. However,
various estimates have been made, most recently by Thresh et al., (1997) and Otim-Nape and Thresh (1998) who suggest overall losses of 15-24% in Africa. The most comprehensive study was done in Uganda between 1990 and 1992 where overall incidence of CMD was 54% in the whole country (Otim-Nape, 1993; Otim-Nape et al., 1998a). High disease incidences of CMD have also been reported in Benin, Cameroon, Ghana and Nigeria (Wydra and Msikita, 1998). Since 1988, a series of serious epidemics of CMD have been reported in Uganda where large areas of the cassava crop have been destroyed, leading to severe food shortages and famine (Otim-Nape, 1993; Otim-Nape et al., 1997b, Otim-Nape et al., 2000; Thresh et al., 1994b, Thresh et al., 1997; Thresh et al., 1998a). One of the management strategies in Uganda is through the introduction of improved germplasms (e.g., TMS 60142, TMS 30572 and TMS 30337) selected originally at the International Institute of Tropical Agriculture (IITA) Ibadan in Nigeria. These germplasm have good levels of resistance to CMD.

1.2 History of CMD in Kenya 1970s-to the present: pandemic emergence

In Kenya the earliest record of work on CMD was from a plant virology research project done from the 1970s through to the mid-1980s, at a time the incidence of CMD incidence was low. This work has been reviewed by Bock (1983, 1994).

Assessment of CMD at 13 locations in Western and north Nyanza provinces in 1993 (Legg et al., 1999b) revealed an overall CMD incidence of 20% and there was little evidence of active transmission by whiteflies. Symptoms were mainly
moderate to mild. This situation reflected low infection pressure consistent with earlier findings (Bock, 1983; Bock, 1994). However, the later 1995 survey (Gibson, 1996) provided the first evidence for a change in disease incidence and severity in western Kenya, most notably in the area along the Uganda-Kenya border between Malaba and Busia. In 1997 a severe form of CMD was noticed (Legg et al., 1999b; Legg 1999b). This change in disease status was similar to that reported earlier in Uganda in 1988.

CMD incidence in fields sampled in the area to the north and west of Kakamega was as high as or exceeded 80%. *B. tabaci* abundance was similar to that associated with the CMD pandemic in Uganda. The affected area along the Kenya-Uganda border was characterized by a high disease incidence from whitefly infection. Disease severity was moderate to severe and as in Uganda farmers’ responses to the disease have commonly been either to abandon or drastically reduce cultivation of cassava (Otim-Nape et al., 1997c, 2000; Otim-Nape and Thresh, 1998; Legg et al., 1999b).

Recent surveys have highlighted the rapid spread of the pandemic through all parts of Western province and north Nyanza in Kenya between 1995 and 1998. Spread into south Nyanza is considered to be slow, probably due to natural barriers, such as the Winam Gulf and the Nyando Plain where little or no cassava is grown (Anonymous, 1999). The disease incidence in South Nyanza was only 5% and symptoms were largely mild (Legg and Okao-Okuja, 1999). Indications are that CMD is still spreading rapidly through south Nyanza and further surveys are required to confirm this. Also information is lacking on the
effect of CMD in parts of Western province and north Nyanza, which have already been swept by the pandemic.

1.2.1 Estimated cassava production losses due to CMD

The production and financial losses associated with the CMD pandemic in western Kenya have been crudely estimated based on a series of plausible assumptions (Legg et al., 1999b). Assuming a 'normal' production figure for cassava in the pandemic area of western Kenya of 430,000 tonnes, which is half of total Kenyan production (FAO, 1997). If CMD incidence were 84%, yield losses attributable to severe CMD of 40%, of total losses would be estimated to exceed 140,000 tonnes (fig 1.). Assuming a convertible value of 100 US dollars per tonne of fresh roots, total financial losses would exceed 14 million USD. Comparable calculations from Uganda have estimated annual losses to CMD (Otim-Nape et al., 1997b; Thresh and Otim-Nape 1994) in the pandemic affected areas to be USD 60 million. Farmers in the areas affected by the pandemic still retain "relic" mainly diseased cassava. There is need to determine how much yield is obtained from such stands which should contribute to better understanding of current national production.
1.2.2 Strategy for combating CMD

The approach taken to address the Kenyan condition is similar to that applied in Uganda, mainly introduction of improved varieties resistant to CMD. Initially open quarantine was allowed for the emergency importation from Serere, Uganda of two CMD-resistant cultivars {Tropical Manihot Series (TMS) 30572 and a Ugandan variety SS-4} as stem cuttings, and after evaluation at Alupe open quarantine site they have been released for rapid multiplication. In addition to the two CMD-resistant varieties a further 500 clones were imported from Serere, Uganda to Alupe open quarantine for evaluation before selection and release for on-farm testing.
Thresh et al., (1994d) noted that only few, and mainly small, unguarded experiments on yield loss caused by CMD have been performed with improved CMD-resistant types now being promoted in many African countries. Information is limited or entirely lacking on several of the TMS varieties that are being released in quantity to farmers or for use as parents in national breeding programmes. It is not known whether the reaction of these varieties is similar in all agroecological zones, which led to the research done and reported in this thesis.

Several studies on the spread of CMD to cassava genotypes in multi-locational trials in Tanzania, Kenya, Côte d'Ivoire, Cameroon and Uganda have revealed big differences between genotypes and locations (Storey and Nichols, 1938; Bock and Guthrie, 1978; Fauquet et al., 1988a; Hahn et al., 1989; Fondong et al., 1997; Otim-Nape, 1993; Otim-Nape et al., 1998a). Other studies have also been done on the symptomatology and epidemiology of CMD, detection, diagnosis and properties of the viruses involved, transmission by whiteflies, ecology and biology of whitefly vectors, yield loss by and control of CMD. Recent studies in Benin on the incidence and severity of cassava anthracnose disease (CAD) on ratoons of different cassava varieties have shown significantly increased or lowered incidence or severity of CAD when different cassava varieties are ratooned at the start or end of the rainy season respectively, (Msikita, 1998). Such inconsistencies in the cassava crop response to disease indicate that even if the virus is introduced by the whitefly into resistant varieties such as the Ugandan cassava variety SS-4 it is bound to
cause different reactions and raise questions such as: Is the virus fully translocated with photosynthates into the tuberous roots? Do the stems of SS4 retain the virus when the crop is mature? Do SS-4 ratoons and stems cut from the SS-4 variety express CMD symptoms during growth? Such questions necessitate more studies in varied agro-ecological zones in Africa. These studies are needed because CMD still threatens food security in many African countries and continues to pose problems in cassava growing areas (Thresh et al., 1994a).

Epidemiological information obtained in Côte d'Ivoire, Nigeria, Togo, Kenya and Uganda (Thresh et al., 1994c) represents only some of the very diverse agro-ecologies in which cassava is grown. It is now clear that CMD spreads more rapidly in some areas than in others. This implies that additional information is required on rates of spread in other agro-ecologies in order to develop effective control measures. Information is needed especially on the rates of spread in Kenya under current pandemic conditions.

Detection and diagnosis of CMD usually involves visual inspection (Otim-Nape, 1993; Fargette et al., 1987b; Guthrie, 1991; Thresh et al., 1994b) followed by laboratory analysis, using serology or polymerase chain reaction (PCR) to analyse leaf samples collected from the field (Harrison et al., 1997a). Otim-Nape (1993) and Thresh et al., (1994b) indicate that visual detection of symptoms which involves physical examination of plants for disease tend to underestimate the proportion of virus-infected plants because cassava plants only express disease symptoms some weeks after inoculation (Fauquet and
Moreover, some infected plants may become symptomless or leafless after damage or drought. In the recent past laboratory diagnosis has relied on tests using triple antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA)(Sequiera and Harrison, 1982; Fargette et al., 1987b; Thomas et al., 1986; Otim-Nape, 1993). This method was used to distinguish *African cassava mosaic virus* (ACMV), *East African cassava mosaic virus* (EACMV) and *Indian cassava mosaic virus* (ICMV). A problem with TAS-ELISA is that it does not distinguish between ACMV and the new Uganda Variant/ East African cassava mosaic virus (UgV/EACMV- UgV)(Deng et al 1997 and Zhou et al 1997) because they both have a closely related coat protein (Harrison et al., 1997b). The distinction was later made by PCR studies of CMG's DNA. Similarly greater reliance is being placed upon analysis of respective virus DNA by nucleic acid hybridisation techniques. To determine which viruses is/are causing severe infection of cassava in Kenya it is necessary to complement visual inspection of disease with laboratory analysis of the infected samples by PCR.

In epidemiological studies, information is lacking on detailed records taken on individually numbered plants on successive occasions throughout the period of growth. Such data can be used to produce disease progress curves based on the *actual* and *cumulative* proportion of plants that expressed symptoms at any one or more times. A complete assessment of the behaviour of varieties and the tendency to recover and become symptomless can be obtained by comparing the *cumulative* and *actual* curves of disease progress (Thresh et al., 1998c; Colon, 1984).
To address the gaps identified above, the following hypotheses were developed:

1.3 HYPOTHESIS:

1. CMD incidence and severity are high in nearly all parts of western Kenya.
2. Different cassava varieties react differently to CMD under conditions of similar disease pressure.
3. CMD in western Kenya is caused by a new strain of CMD virus found recently in Uganda.

1.4 OBJECTIVES

1. To survey the spread of CMD in western Kenya.
2. To determine the response of four cassava varieties to CMD under field conditions in western Kenya.
3. To determine CMD infection in ratoons of cassava variety SS-4.
4. To determine the virus types in cassava at representative sites using diagnostics based on the Polymerase Chain Reaction Assay (PCR) and Restricted Fragment Length Polymorphism (RFLP).
CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History, cause and distribution of CMD

Cassava is routinely propagated vegetatively by cuttings obtained from its woody stem. These cuttings are planted as "seed" by farmers. Many crops that are propagated vegetatively are notoriously prone to virus infection and cassava is no exception. Eighteen different viruses or putative viruses have been described from cassava, of which eight are known to occur in Africa (Thresh et al., 1994b). The most important of these are the whitefly-borne geminiviruses (Family: Geminiviridae; Genus: Begomovirus)(Rybicki 1994), which cause cassava mosaic disease.

CMD was first observed and reported by Warburg (1894) as "Krauselkrankheit" in the present Tanzania, but it is now known in all the main cassava growing regions of Africa (Golding, 1936; Storey and Nichols, 1938; Guthrie, 1987; Thresh et al., 1994b; Thresh et al., 1998a; Bock et al., 1978) and the Indian sub-continent. It was soon shown that the disease is transmissible by grafts and whiteflies, and as there was no visible pathogen it was attributed to virus infection (Zimmermann, 1906). Martin (1928) termed the disease "mosaic". There has since been reference to African Cassava Mosaic Disease (ACMD), East African Cassava Mosaic Disease (EACMD) and Indian Cassava Mosaic Disease (ICMD) (Harrison et al., 1996). The distinction between Indian cassava mosaic virus and the two African cassava
mosaic viruses has led to difficulties as to what should be the most appropriate designation of the diseases they cause. A recent publication has attempted to distinguish between African and East African cassava mosaic diseases (Chikaunga et al., 1996). Thresh et al., (1998) and Otim-Nape and Thresh (1997) have asserted, however, that the disease should be referred to as cassava mosaic disease (CMD) to avoid confusion and to distinguish the African/Indian Cassava Mosaic Diseases from the Cassava Common Mosaic Disease of the Neo-tropics. The latter in south/central America is caused by a completely different virus of the genus Potexvirus (Costa and Kitajima, 1972). No virus particles were detected in CMD-infected plants until 1975 when sap inoculations from cassava to Nicotiana clevelandii Gray succeeded (Bock, 1975). The geminivirus isolated from these inoculations to N. benthamiana was later characterised and caused typical CMD symptoms when returned to cassava, fulfilling Koch's postulates (Bock and Woods, 1983). A related virus which also causes CMD has been reported in India and Sri Lanka (Abraham, 1956).

Recent PCR diagnostic studies have shown that the severe form of CMD observed in local cultivars in Uganda is a result of a new and extremely virulent virus strain. This has been shown to be a recombinant hybrid of the two previously recognised CMGs from Africa, namely: African cassava mosaic virus (ACMV) and East African cassava mosaic virus (EACMV) and is referred to as either the Uganda variant (UgV)(Zhou et al., 1997) or as a distinctive strain of EACMV (EACMV-UgV) (Deng et al., 1997) until there is agreement on the appropriate designation. The variant has been referred to as
UgV/EACMV-UgV (Legg, 1999b). Moreover symptoms are most severe if UgV/EACMV-UgV occurs together with ACMV in a mixed infection (Harrison et al., 1997b; Fondong et al., 2000). No local cultivars have shown resistance to this severe form of CMD. In South Africa a new cassava mosaic geminivirus has been reported and is being referred to as *South African cassava mosaic virus* (SACMV) (Rey and Thompson, 1998; Berrie et al., 1998), and in Cameroon a variant of EACMV called EACMV/Ca has been reported (Fondong et al., 2000).

### 2.2 CMD Symptoms Expression

The symptoms of CMD, were described by Storey and Nichols (1938). They occur as characteristic leaf mosaic patterns that affect discrete areas and are determined at an early stage of leaf development (Acland, 1971; Fargette et al., 1987d; Hill and Waller, 1988; Otim-Nape, 1993; Thresh et al., 1998a). The chlorotic areas fail to expand fully so that stresses set up by unequal expansion of the leaf or leaflets cause malformation and distortion. The leaves are reduced in size, are misshapen and twisted, with yellow areas separated by areas of normal green colour. Severely affected plants are stunted and the young leaves absciss (Storey and Nichols, 1938; Cours, 1951). It has been noted that some leaves situated between affected ones may seem normal and give the appearance of having recovered or reverted.

These recovery and reversion characteristics are manifestations of plant resistance and are influenced by ambient temperature (Deighton, 1935; Storey and Nichols, 1938; Rossel et al., 1987; Gibson, 1994). Furthermore,
symptoms may recur on a recovered plant when environmental conditions again favour symptom expression. The first few leaves produced by an infected cutting are sometimes symptomless and may be followed by severely affected leaves. There is a tendency for symptoms to diminish as the plant ages, especially in resistant varieties (Storey and Nichols, 1938; Rossel et al., 1987). Cuttings obtained from the bases of mature stems of resistant varieties are more likely to contain virus than the upper stem portion, which means that the virus concentrates at the base of the stem (Jennings, 1960; Gibson and Otim-Nape, 1997).

Physiological and histological examinations reveal that infected leaves have palisade cells that are either quite undifferentiated or shorter than those of the spongy mesophyll tissues (Beck and Chant, 1958; 1959). Leaves of CMD-infected plants show marked reductions and distortions of the chloroplasts, increased respiration and peroxidase activity, and decreased total carbohydrate and rates of photosynthesis (Chant et al., 1971).

The leaf chlorosis may be pale yellow or nearly white or just discernibly paler than the normal green. The chlorotic areas are usually clearly demarcated and vary in size from that of a whole leaflet to small flecks or spots. Leaflets may show a uniform mosaic pattern or the mosaic pattern is localised to a few areas, which are often at the base of leaflets. Distortion, reduction in leaflet size and general stunting appear to be secondary effects associated with symptom severity (Storey and Nichols, 1938; Massala, 1987; Hill and Waller, 1988). Symptoms vary from leaf to leaf, shoot to shoot, and plant to plant, even with
the same variety and virus strain in the same locality (Storey and Nichols, 1938; Massala; 1987; Fargette et al., 1987b).

2.3 Effect of mode and stage of infection on yield

Thresh et al., (1994a) summarise various reports on yield loss caused by CMD from different parts of Africa. It is indicated that plants raised from infected cuttings lose much more yield than plants infected during growth by whiteflies. Fargette et al., (1988) indicate that plants grown from infected cuttings sustain greater losses (55%-77%) than those infected by whiteflies (35%-60%). This concurs with earlier findings in Zanzibar, where yield of plants grown from infected cuttings was only 5%-44% of equivalent healthy controls (Tidbury, 1937). In Kenya Bock and Guthrie, (1978) and Robertson (1987), reported 14%-56% loss from infected cuttings, and in other trials 25%-76% loss was reported (Seif, 1982). Corresponding studies in Côte d'Ivoire, Nigeria and more recently Uganda are consistent with these findings (Fargette et al., 1988; Terry and Hahn, 1980; Anonymous, 1981; Anonymous, 1991; Hahn et al., 1989; Otim-Nape et al., 1994; Otim-Nape et al., 1997a). The effect of CMD on yield is most severe in plants where all the branches and leaves are totally affected and express conspicuous symptoms. But in plants with only one branch infected, such infection does not decrease yield (Golding, 1936). Higher yield losses, are expressed by those plants that are infected, by the whitefly vector, in the early stages of growth. Infection that occurred 4 months or more after planting had no significant effect on yield in a study in Côte d'Ivoire (Fargette et al., 1988). CMD decreases other components of yield such as root number, root size and harvest index (Otim-Nape et al., 1994). Further losses by
CMD are realised through use of diseased planting material because planting infected cuttings contributes to maximum loss and rapid spread of CMD from such crops (Robertson, 1987).

2.4 Management of CMD

Many attempts have been made to control the disease by use of virus-resistant varieties or through sanitation, which involves the use of CMD-free cuttings and roguing (Bock, 1982; Thresh and Otim-Nape 1994). Early workers on CMD in East Africa bred for resistance to CMD in cassava by introducing foreign varieties, which were crossed with local varieties. They also introduced resistance from other species including *Manihot glaziovii* Muell.-Arg (Nichols, 1947). Removal of infected cassava from plantings to maintain the health of a crop has been recommended by many workers (Guthrie, 1990; Thresh and Otim-Nape, 1994). Despite these efforts, CMD is still prevalent in many cassava growing countries, and causes serious losses in yield (Thresh *et al.*, 1998c). The principal control measures used to manage CMD are host plant resistance and phytosanitation.

2.4.1 Phytosanitation measures

Phytosanitation is a term which implies in a general way the various means used to improve the health status of cassava planting material and ways in which sources of inoculum for the whitefly are decreased or eliminated in an area. It has been widely advocated that CMD control requires the selection and use of virus-free planting material, crop hygiene and roguing of any further sources of inoculum for whiteflies (Guthrie, 1990; Thresh and Otim-Nape, 1994; Thresh
et al., 1998b). The effects of phytosanitation were studied in Kenya by Bock (1994) and he reported a widespread and indiscriminate use of CMD-infected planting materials in areas where infection is prevalent.

In Kenya, for example, popular local varieties are very highly contaminated with CMD. The benefits of planting CMD-free cuttings are considerable as healthy cuttings establish more readily and grow faster than infected ones. Yields are also substantially higher, even if plants are infected later by the whitefly (Fargette et al., 1988). Widespread adoption of sanitation is complicated by unavailability of sufficient CMD-free planting materials for farmers and even when available these may become infected and require frequent introduction of CMD-free materials (Thresh and Otim-Nape, 1994).

It is now known that farmers are usually reluctant to remove diseased plants that still contribute a little to yield and it is difficult to ensure that roguing is done efficiently and with the diligence required to control CMD. Moreover few farmers who rogue will not reap its full benefits if all farmers in nearby localities do not adopt it. Such limitations add to the difficulty of developing a simple and acceptable set of measures for use by farmers. However, roguing could be used with full benefits in production of CMD-free planting material in large commercial farms (Thresh and Otim-Nape, 1994).

2.4.2 Control of CMD by use of resistant material

CMD became a serious problem in the late 1920s and early 1930s, though it had been known as early as 1894. Agriculturists soon became aware of the
need for CMD-resistant varieties. In instances where there is considerable spread of the disease by vectors resistant varieties offer an obvious advantage. Decrease of yield losses caused by viruses due to resistance has formed a high priority in cassava breeding programmes in Africa (Nichols, 1947; Jennings, 1976; 1994; Hahn et al., 1980). Initial studies were in Tanzania in the 1930s and 1940s which were followed by others in Ghana, Madagascar and Nigeria. Since 1971 the most influential resistance breeding activities have been located at IITA, Ibadan, Nigeria (Jennings, 1994; Mahungu et al., 1994).

Only a few of the improved varieties released by IITA are highly resistant to CMD. Others are described as 'resistant' 'moderately resistant' or 'moderately susceptible' and their resistance to CMD is manifest in different ways (Rossel et al., 1992). Some of the improved varieties are more difficult to infect than unimproved ones, but when infected they develop conspicuous symptoms that occur throughout the plant. This means that they are not immune to infection by CMD, furthermore disease incidence depends on the prevailing inoculum pressure but is consistently less in resistant than in susceptible varieties of the same age exposed to similar amounts of inoculum (Thresh et al., 1994a; 1998c).

An obvious advantage of these resistant varieties is that they sustain little damage or yield loss due to CMD, even under high inoculum pressure. This is to be expected since the proportion of infected plants is low and therefore they are unable to contribute to much further spread. In the resistant varieties the viruses are often restricted to certain parts of the shoot and branches, which
consequently restricts the symptoms. Such restricted systemic distributions means that the subsequent shoots are not affected and become symptomless i.e. 'recover'. Moreover they are free of virus and grow into uninfected plants if used for further propagation.

For these reasons the incidence of infection is unlikely to increase progressively in successive cycles of propagation, as might otherwise be expected with a vector-borne disease of a vegetatively-propagated crop (Fargette et al., 1994a; Thresh et al., 1998b).

2.5 Factors influencing the spread of Cassava Mosaic Disease

The rate of spread of CMD can be measured locally or regionally and can be expressed as 'infection pressure', which may be high or low. Infection pressure is a measure of the number and infectivity of the vectors encountered and the extent to which the inherent susceptibility of the varieties exposed is influenced by environmental factors. Moreover, infection pressure is influenced in part by the susceptibility of the clones exposed to inoculum, and it is also evaluation of the degree of varietal susceptibility in response to infection pressure which determines whether control by adopting CMD-free planting material of any specific clone is feasible (Bock, 1994).

It has been determined that the severe form of CMD observed in local cultivars in Uganda is as a result of a new extremely virulent virus strain (Zhou et al., 1997; Deng et al., 1997). No local cultivars have shown resistance to this severe attack.
How rapidly stocks of clonally propagated cassava will degenerate when exposed to CMG infection is dependent on both abiotic and biotic factors influencing spread by vectors and the effectiveness with possible control measures adopted. The principal biotic elements are vector, virus and cassava clone, and environmental factors affecting and interacting with them such as rain, wind and sunshine.

2.5.1 Sensitivity of the varieties grown

Some varieties are more readily infected by CMGs than others when exposed to similar amounts of inoculum (Anonymous, 1981, Nichols, 1947). There are differences among varieties in their response to infection (Cours, 1951). Susceptible varieties when infected are so severely affected and grow so poorly that fewer cuttings can be obtained from them for further propagation. Fargette et al., (1994b) notes that an important consequence of this effect is that infected cuttings are underrepresented when stems are collected at random from a mixed stand of healthy and infected plants. This underrepresentation is increased when farmers select cuttings, from vigorously growing healthy source plants and not from weak diseased ones. Furthermore infected cuttings tend to sprout later and grow less vigorously than uninfected ones (Pandey, 1986; Raffaillac and Nedelec, 1988). Finally in unfavourable conditions, high sensitivity to CMD leads to death of infected cuttings and they are less likely to withstand weeding operations or competition from neighbouring cassava or intercrops (Otim-Nape, 1993).
2.5.2 Infection "Pressure"

Infection pressure is assessed empirically for any given site and season from the proportion of plants of a susceptible cultivar that are infected when exposed to natural infection by whitefly vectors. Differences in infection pressure between sites or seasons can be determined by exposing plants of the same variety, or preferably a range of different varieties, in standard plots of similar size, shape, spacing and configuration.

The advantage of using a standard range of varieties of different degrees of susceptibility is that infection pressure can be assessed whatever the situation; it can also be compared between sites, seasons or planting dates (Bock, 1994). To detect infection pressure where it is low, susceptible varieties are required, whereas resistant varieties are needed where it is high, since susceptible ones would soon be totally infected and much multiple infection would occur (sensu Gregory, 1948). Infection pressure has been assessed in Côte d'Ivoire (Fauquet et al., 1988a;) and in coastal and western Kenya (Bock, 1983) where it was found to be low. (Bock, 1994). However big differences in infection pressure have also been demonstrated recently in Uganda and Cameroon (Otim-Nape, 1993; Otim-Nape et al., 1998; Legg et al., 1997; Fondong et al., 1997).

2.5.3 Systemic nature of CMD in infected plants.

The concept of "reversion" and "recovery"

Some cuttings taken from CMD-infected plants grow into uninfected plants (Storey and Nichols, 1938), and it is especially so for resistant cultivars (Jennings, 1960) such progeny are probably virus-free (Pacumbaba, 1985).
This could be because cuttings were obtained from a plant that had recently been infected and before CMD is fully systemic. This indicates the ability of some cassava varieties to localise the virus distribution. This condition is called 'recovery' if an initially diseased plant produces symptomless shoots. 'Reversion' is used to describe the phenomenon by which symptomless plants arise from cuttings derived from diseased plants (Gibson and Otim-Nape, 1997; Alicai et al., 1999).

The reversion phenomenon is of great epidemiological importance because it has a self-cleansing effect and influences the dynamics of disease progress and the losses sustained. Reversion is important in preventing the gradual build-up of infection in cassava stocks which otherwise can occur in crops maintained by repeated cycles of vegetative propagation (Fargette et al., 1994; Fargette and Vié, 1995). Moreover, plants that develop from uninfected cuttings and are infected by whiteflies are not severely damaged compared with plants infected from the outset as cuttings (Thresh et al., 1994a; Thresh et al., 1998b).

2.6 Detection and differentiation of CMGs

The four cassava geminiviruses; ACMV, EACMV, UgV/EACMV-UgV and ICMV can be detected and differentiated by either serological or DNA-based methods. TAS-ELISA has been used reliably to detect ACMV, EACMV and ICMV (Harrison et al., 1997a), but does not distinguish ACMV from UgV/EACMV-UgV. The most sensitive detection of CMGs is achieved when samples for test are taken from young leaves (Fargette et al., 1987b).
In the recent past increasing attention has been paid to DNA-based tests (Harrison et al., 1997a). The increased accuracy, specificity and sensitivity achieved through nucleic acid hybridisation tests with radioactive or non-radioactive probes makes PCR the preferred test in most instances (Harrison et al., 1997a). Two approaches based on PCR are applied. First approach relies on selecting primers based on nucleotide sequences that do not occur in other whitefly-transmitted geminiviruses; so that only the target virus is detected. The second approach uses degenerate primers based on sequences occurring in several whitefly-transmitted geminiviruses and the target viruses are distinguished by the pattern of fragments obtained by restriction endonuclease treatment of the amplified DNA (Rojas et al., 1993; Deng et al., 1994).

2.7 Epidemiology of Cassava Mosaic Geminiviruses (CMGs)

2.7.1 Mode of Spread

CMGs are disseminated through planting infected cuttings and they are transmitted by the feeding activities of the whitefly B. tabaci (Storey, 1936; Storey and Nichols, 1938; Chant, 1958; Bock, 1983; Bock et al., 1978; Dubern, 1994; Fargette et al., 1985). The relative importance of the two modes of transmission seems to differ from place to place and resultant infection pressure depends on the number of whiteflies and sources of infection (Bock, 1983; Otim-Nape, 1993; Byabakama et al 1997; Legg et al., 1997). Results obtained from the ODA-funded project of the 1970s/1980s in Kenya showed that dissemination of diseased propagules was the most important epidemiological factor in coastal and western areas (Bock, 1983). This
situation has changed drastically recently in western Kenya and other parts of East Africa where the increased CMD incidence is a consequence of whitefly transmission (Otim-Nape, 1993; Gibson et al., 1996; Legg, 1995; Legg and Ogwal; 1998; Thresh et al., 1998a). A plant that is infected and develops symptoms may later appear healthy on inspection but still retains CMGs in portions of its stem and these usually grow as infected cuttings when planted. Uninformed farmers will transport these as planting material for considerable distances, thereby inadvertently spreading CMGs, sometimes into areas where spread by whitefly is low (Chant, 1958; 1959; Bock, 1983; Bock and Woods, 1983; Fargette et al., 1985; Fargette et al., 1988; Fauquet and Beachy, 1989; Otim-Nape, 1993).

From the two modes of spread it can be shown in the field that CMD-infection is of two distinct types, that from cuttings and that due to whitefly. Infection from cuttings is distinguished by symptom expression on the first leaves that emerge after planting. Symptoms progress upwards and may later disappear (recovery). This contrasts with current season whitefly infection in which symptoms appear on the topmost leaves and not on the lowest first-formed leaves (see Plates 1, 2 and 3)(Otim-Nape, 1993; Gibson et al., 1996).

2.8 Bemisia tabaci: The whitefly vector of CMGs

B. tabaci is a polyphagous pest of crops in many different parts of the world mainly the tropics and sub-tropics (Greathead, 1986). Bemisia species are thought to have originated in Asia. B. tabaci is the most economically important species of the genus Bemisia (Mound, 1963). It can affect plants,
firstly by feeding on the leaves, which may develop chlorosis. Heavy feeding can lead to reduced plant vigour (Byrne et al., 1990). Most importantly, however, *B. tabaci* is a major vector of plant pathogens, especially viruses (Ohnesorge, 1986; Duffus, 1987; Cohen, 1990; Fishpool and Burban, 1994). Many of the pathogens transmitted are viruses (Muniyappa, 1980; Otim-Nape et al., 1995).

### 2.8.1 Biology of the whitefly *Bemisia tabaci*

Adult whiteflies feed on the undersides of young cassava leaves, where they lay eggs and the immature stages develop. The developmental cycle from eggs to adult ranges from 19-30 days depending on temperature (Gerling et al., 1986). Legg (1995) found the development period of *B. tabaci* on cassava in central Uganda ranged from 20-28 days, with a mean of 23.6 days. The higher the temperature the faster the rate of development. Optimum temperatures are 30°-33°C. Generation times on cassava are up to 23 days in the dry season and up to 30 days in the relatively cool wet season (Fishpool and Burban, 1994). Longevity of up to 48 days have been recorded on cassava in Uganda (Legg, 1996). Reproduction is arrhenotokous whereby unmated females produce males and mated females produce both males and females, also fecundity is highly variable depending on environmental conditions (Gerling et al., 1986). Fecundity is 28-43 eggs per female, and 6-8 eggs are produced per day. (Fishpool et al., 1989; Fishpool and Burban, 1994).

### 2.8.2 *B. tabaci* and its biotypes

The taxonomy of whiteflies has traditionally been based on differences in
morphology of the final stage of the fourth instar nymph (or pupa). Other forms of classification have emphasised crop hosts, localities and more recently genetic heterogeneity (Mound, 1963; Cohen et al., 1992). Many of these species have been named B. tabaci (Russel, 1957). Isoenzyme analyses (Perring et al., 1991) and random amplified polymorphic DNA obtained via PCR have indicated important biochemical differences between whitefly species (Perring et al., 1993).

Work done in Côte d'Ivoire has indicated two host-restricted B. tabaci strains that infest cassava, other cultivated crops and weeds. One strain only colonised cassava, whereas the other colonised a wide range of other cultivated crops and weeds but not cassava (Burban et al., 1992). In Uganda, there is evidence of both geographical and host-associated strains of B. tabaci (Legg et al., 1994). Recent evidence from Côte d'Ivoire and Uganda suggests that cassava is the only significant host of the cassava strain of B. tabaci (Legg et al., 1994). Two other whitefly species, B. afer Priesner and Hosny and Aleurodicus dispersus Russel (Homoptera, Aleurodidae) occur in Africa. The former has been considered to be a vector of Cassava brown streak virus. A. dispersus causes direct feeding damage (Storey, 1936; Thresh et al., 1994; Robertson, 1985; Neuenschwander, 1994b).

2.8.3 Climatic effect on population size and activity of B. tabaci

Populations of B. tabaci seem to be governed mainly by abiotic factors. In Kenya, Seif (1981a) found seasonal fluctuations of the whitefly populations on cassava could be correlated with temperature and relative humidity, but rainfall
Plate 1 White fly - inoculated cassava plant showing CMD symptoms restricted to youngest leaves

Plate 2. Cutting infected cassava plant with earliest leaves expressing symptoms
Plate 3 Healthy cassava plant.
had no direct effect on the whiteflies. In the Sudan, heavy rains were usually followed by a drop in population levels (Khalifa and El-Khidir, 1964; Gameel, 1970), but high temperatures tended to favour their increase. Optimum conditions for population increase are high temperature, low rainfall and low humidity.

2.8.4 *B. tabaci* and cassava

The immigration, establishment and migration of *B. tabaci* populations into and out of cassava plantings is influenced by planting date, cassava variety and climatic conditions (Fishpool *et al.*, 1988; Fishpool *et al.*, 1989; Fishpool *et al.*, 1994). New cassava fields are usually colonised by whiteflies which immigrate from nearby older cassava fields. Population gradients have shown decreasing abundance from the windward to the centre of the crop and a slight increase towards the leeward side of a new field (Fishpool *et al.*, 1995; Legg, 1995). Such gradients resemble CMD gradients of a crop that was initially CMD-free and is CMD infected (Fargette *et al.*, 1990; Otim-Nape 1993). Results of studies of colonisation of cassava by *B. tabaci*, obtained from different parts of Africa are similar (Fishpool *et al.*, 1988, 1995; Robertson, 1987; Legg, 1995; Otim-Nape, 1993). The adult insects occur on cassava throughout the growing period and populations are related to the stage of crop growth. The adult whitefly slowly moves into and establishes within the crop as soon as the plants have grown sufficiently to be colonised by the whitefly (Fishpool and Burban, 1994). Reproduction starts thereafter and within the first three weeks new adults appear. Rapid population build-up follows until 3-4 months after
planting, which is the time when the cassava foliage is able to support increased whitefly population. The population is maintained, though with fluctuations, for a short time. It then declines rapidly to a low level which is maintained in the remaining growth period (Fishpool and Burban, 1994; Fishpool et al., 1988, 1995; Otim-Nape, 1993). Some cassava varieties are more prone to infestation by B. tabaci than others (Fishpool and Burban, 1994). It is thought that this is due to optimum nutritional quality of the food in the plant leaves in the early stages of growth. This quality deteriorates 3-5 months after planting when the leaves age and root tuberization begins, thereby whiteflies migrate to other younger plants. Moreover parasites, parasitoids and pathogens of B. tabaci are in low numbers on the young plants (Fishpool and Burban, 1994; Otim-Nape et al., 1995;). Localized movement leads to dissemination of CMGs within plantings, whereas spread between fields is ascribed to flights over long distances (Fauquet and Fargette, 1990).

2.8.5 Sampling techniques for populations of B. tabaci

The three main approaches used in sampling whiteflies are: attractive or non-attractive traps that sample flying adults, direct counts or vacuum collection of adults in the crop and those for enumerating nymphs.

To monitor B. tabaci in cassava direct counts are best done early in the morning when conditions are cooler and the adults are less active than later in the day (Mound, 1965; Gerling and Horowitz, 1984; Fargette et al., 1985; Otim-Nape, 1993;). The counts involve carefully turning over the youngest four or five leaves at the apex and recording the adults present. Although
Butler *et al.*, (1986) says that in sampling the whitefly on cotton the technique can give inconsistent results because adults fly away or drop off the leaves, it has been found satisfactory for doing counts on cassava plants (Seif, 1981a; Fargette, 1985; Fargette *et al.*, 1985; Otim-Nape, 1993; Legg, 1995). Though Fargette *et al.*, (1985) stressed that counts should be done in the morning when temperatures are cool and whiteflies are slow, recent studies indicate insignificant differences between morning and afternoon counts of adults on cassava (Legg, 1995). This is because relatively few adult whiteflies are found on cassava and these are usually localized within the top five leaves (Legg, 1995).

2.8.6 Adaptation of *B. tabaci* within new cassava plantings

Whiteflies move within the foliar canopy of cassava early in the morning in search of suitable feeding or oviposition sites. In adverse conditions the insects are swept up the plant by wind and are eventually blown out of the canopy (Fishpool *et al.*, 1988). Average flight speeds of an adult whitefly within the plant canopy are c. 0.2ms\(^{-1}\) (12 m per min) in wind speeds of c.0.1-0.4ms\(^{-1}\). The insect cannot control its flight in high winds (Yao *et al.*, 1987).

2.8.7 Transmission of CMGs by *Bemisia tabaci*

Preliminary studies by Bondar (1924), China (1930), Hedin (1931), Kufferath and Ghesquire (1932) showed that CMD is transmitted by whitefly. The mode of transmission was studied by Storey and Nichols (1938), Chant (1958) and Seif (1981b) who considered transmission by adults, and Dubern (1994) who demonstrated transtadial transmission (i.e. from one instar to the next in
nymphal stages) but not transovarial transmission.

The viruses are transmitted in a persistent manner following acquisition access periods of 3 to 5 hours. Successful inoculation takes 10 minutes and latent periods for successful transmission are 3-6 hours (Dubern, 1994). The virus is retained by the insect for at least 9 days and persists during moulting (Dubern, 1994). The adult whitefly transmits the virus only to the younger leaves of the cassava plant. About 95% of adult whiteflies occur on the younger leaves of cassava plants, usually the top 10 from the apex (Fauquet and Fargette, 1990).

A factor of significant epidemiological importance is that whiteflies are viruliferous for at least 9 days, and can therefore easily spread CMGs over long distances when carried by strong winds. It has been shown that short distance movements (<10 km) of viruliferous whiteflies is an important factor in the expansion of the pandemic of severe CMD in East Africa (Legg and Ogwal, 1998; Otim-Nape et al., 1997).

2.9 Vector Control

2.9.1 Biological control/Natural enemies

Natural enemies of the whitefly affect populations by causing mortality. Predators and parasitoids of *B. tabaci* have been reviewed by Gerling (1986, 1990). Predacious mites are of the genera *Typhlodromus* and *Amblyseius*. They are considered to be potential biological control agents. Mites have been recorded as predators on *B. tabaci* in coastal Kenya and on cassava in Côte d'Ivoire (Robertson, 1985; Otim-Nape et al., 1995).
Over 30 species of parasitoids of the family Aphelinidae are known to attack *B. tabaci*. They lay eggs in the fourth instar nymphs and on hatching the larvae penetrate their hosts (Fishpool and Burban, 1994). Not much information is available on the effectiveness of biological control, as a means of controlling CMD. Greater emphasis has been placed on the development and deployment of virus-free and resistant clones (Fauquet and Fargette, 1990; Fishpool and Burban, 1994).

2.9.2 Insecticides

One method of controlling insect-borne viruses is by use of pesticides. This approach has seldom been adopted to control CMD in Africa due to toxicological, economic and environmental considerations. Other reasons include the limited effectiveness of insecticides in controlling insect-borne viruses such as CMGs whose main spread is into and not within crops. Insecticides are unlikely to kill incoming viruliferous whiteflies before they have had an opportunity to introduce CMD, since transmission of CMGs by *B. tabaci* requires only short feeding periods (Dubern, 1994). Inoculum is introduced into new plantings from external sources (Fargette et al., 1990). The occurrence of virulent readily transmitted virus isolates and efficient and fecund whitefly vectors may mean that relatively small whitefly populations can be very effective in spreading CMGs. Since cassava remains in the field for up to 12 months or even longer and so is exposed to infection for a long period, while continuos spraying will be uneconomical. Cassava is grown as a low value subsistence crop in most of sub-Saharan Africa. *B. tabaci* may be indigenous to Africa, and has an established fauna of natural enemies (Legg,
1999a). Subsistence farmers may not be able to purchase insecticides or spray machines (Thresh and Otim-Nape, 1994).

2.9.3 Vector-Resistant Varieties

*B. tabaci* adult populations tend to be small on cassava and it is considered a less favourable host than cotton and some other crops (Fargette *et al*., 1987d). By breeding varieties that are even less susceptible to *B. tabaci* than those being grown it should be possible to check the whiteflies population and the spread of CMD but this possibility has received little attention (Leuschner, 1977). However recent studies have identified possible genotypes somewhat resistant to whiteflies and shown that resistance to the vector and to CMD are independent traits that can be combined to give a favourable combination of properties (Fauquet *et al*., 1988a).

2.10 Progress of CMD within space and time

Virus-infected cassava represents the main host and source of the spread of CMD. It is from this source that viruliferous whiteflies move to healthy crops, where they cause primary spread, and later lead to secondary dissemination within the crop (Thresh, 1987; Alaux and Fauquet, 1990). Spread of CMD is positively correlated to the activity, infectivity and size of the vector population about one month earlier (Fargette *et al*., 1985), which corresponds to the latent period between inoculation and CMD symptom development (Fargette *et al*., 1990; 1994a) and by the magnitude, proximity, distribution and potency of sources of infection, especially cassava plantings located upwind and planted nearby. These and similar studies in Uganda (Otim-Nape, 1993) show that
mean numbers of adult whiteflies correspond to increases in incidence of CMD until the crop is 3-5 months old. Succeeding increases in populations to maximum at crop maturity were not related to whitefly populations as insect numbers declined with crop age. In most cases, rapid CMD increases occurred in periods of rapid cassava growth, and at the beginning or soon after the end of the dry season (Bock, 1987; Robertson, 1987; Fargette et al., 1994a; Otim-Nape et al., 1995; Fishpool et al., 1995).

In Uganda, sites with much CMD spread usually had corresponding high population densities of whiteflies (Otim-Nape et al., 1995). Fauquet et al., (1988a) found large differences in rates of CMD spread between sites in lowland coastal areas of Côte d'Ivoire and attributed these to differences in the potency, prevalence, and distribution of nearby sources of infection rather than to differences in whitefly populations. Much spread occurred even in isolated fields several kilometres from the nearest infected cassava field, which suggested spread over considerable distances (Fauquet et al., 1988a). In Kenya, however, effective spread of CMD leading to comparatively high incidence of disease in susceptible crops was variable and restricted to short distances of tens of metres (Bock, 1987). Differences in conditions for cassava growth, the intensity of cassava cultivation, abundance of infected plants and whitefly populations were considered the main causes behind the variation (Robertson, 1987). Such discrepancies in results call for more studies on dispersal by B. tabaci and spread of CMD (Otim-Nape et al., 1995).
2.10.1 Factors governing spatial distribution of CMD

2.10.1.1 Wind direction and edge effects

Whiteflies are usually more abundant at the margins of crops, especially along the windward and to lesser extent leeward sides. Smaller numbers occur within fields, irrespective of field size or whitefly population density (Fargette et al., 1985). The crop canopy acts as a windbreak and becomes a barrier that creates turbulence at crop boundaries and within plots (Yao et al., 1987). Otim-Nape (1993) and Legg (1995) reported similar findings in Uganda, where CMD incidence was higher at the edge of crops in which spread took place. The results are consistent with findings that viruliferous vectors are carried by the prevailing wind from infected sources and are deposited at the upwind edges of fields (Yao et al., 1987; Pedgley, 1982).

2.10.2 Whitefly populations and spread of CMD in relation to cropping practices

2.10.2.1 Planting date

Planting date, spacing/crop density, crop deployment, and intercropping can have considerable influence on vector populations and virus spread (Thresh, 1982; Fargette et al., 1985; Fargette et al., 1987d; Thresh, 1987; Fauquet et al., 1988a, Otim-Nape, 1986; Otim-Nape, 1993; Otim-Nape et al., 1995).

Change of planting date can avoid plants being exposed to infection when the vector population is high and the young crop is most vulnerable (Thresh, 1987). Reports from West Africa (Fargette et al., 1985), Kenya (Bock, 1983) and recently Uganda (Otim-Nape, 1993; Byabakama et al., 1997) indicate seasonal variations in vector numbers and spread of CMD. In some areas, it is
known that there are likely to be advantages in planting later in the year provided that crop establishment and growth are not impaired, this is because spread is greatest from March to July and least from August to November (Thresh, 1987; Byabakama et al., 1997; Muimba-Kankolongo et al., 1987).

2.10.2.2 Plant spacing and crop density

CMD incidence expressed as a percentage of the total stand decreased as plant density increased in trials in Uganda (Otirn-Nape and Ingoot, 1986) and Côte d'Ivoire (Fargette et al., 1990). This implies that adoption of uniform and dense plant stands while avoiding breaks in colony and patches of bare ground is a justifiable means for reducing CMD incidence.

2.10.2.3 Crop deployment

Studies from Côte d'Ivoire indicate that the number of whiteflies and the incidence of CMD are higher at the periphery of plantings, notably those that are orientated perpendicular to the direction of the prevailing wind (Alaux and Fauquet, 1990). This means that cassava plantings could be deployed in such a way as to decrease the incidence of CMD (Thresh and Otim-Nape, 1994). Planting large, compact blocks and orientating elongated plots along rather than across the prevailing wind direction, will decrease the proportion of most vulnerable peripheral areas (Thresh, 1987; Thresh, and Otim-Nape, 1994). According to Alaux and Fauquet (1990) it could suffice to plant upwind, use windbreaks, or grow rows of another crop to serve as a barrier to intercept the vectors, and reduce the spread of the virus, but these possibilities have seldom been considered (Thresh, 1987; Fargette et al., 1985; Thresh and Otim-Nape,
2.10.2.4 Intercropping

Although cassava in Africa is largely grown as an intercrop, usually with other crops such as maize, sorghum, groundnut, beans or sweet potato, nearly all experiments on whitefly vector populations and spread of CMD have been on monocultures of cassava (Otim-Nape \textit{et al.}, 1995). Only one experiment done in Côte d'Ivoire (Fargette and Fauquet, 1988) considered the effects of maize introduced at two spacing and three planting dates on whitefly populations and spread of CMD. The effects were complex and difficult to interpret, but whitefly populations and virus spread were consistently more in plots with maize at low density than in high density plots or in the cassava monoculture (Fargette and Fauquet, 1988; Otim-Nape \textit{et al.}, 1995). Results suggested that intercropping may have an adverse effect on cassava growth, although further research is required to evaluate the effects of the main intercrops used by African farmers.

2.11 Exploitation of genetic variability for CMD resistance

Thresh \textit{et al.}, (1997) indicate that extreme caution should be applied when generalizations are made regarding the economic importance of CMD in Africa. On the limited evidence available they distinguish at least three types of situations that influence the need to exploit genetic variability. These three states are referred to as "\textit{benign}, "\textit{endemic}" and "\textit{epidemic}".

The "\textit{benign}" state is common in vast areas of Africa where the incidence of
infection is generally low. The symptoms are usually mild and affected plants do not suffer obvious yield losses (Thresh et al., 1994a; Thresh et al., 1997). This state occurs in Mozambique and at altitudes exceeding 1200m in Cameroon, Malawi and Burundi and until recently in many parts of Tanzania, (Thresh et al., 1994a).

The **endemic situation** is encountered in areas like coastal Kenya, Côte d'Ivoire and Ghana, where CMD is prevalent but is largely due to the planting of infected cuttings. Symptoms are somewhat more severe than is the case in the benign state (Thresh et al., 1997). Production is stable and satisfactory, though yields are impaired (Fargette et al., 1988). CMD is not regarded as serious by farmers and agricultural extensionists. However, yields could be improved by adopting control methods.

In the **epidemic condition**, CMD spreads rapidly and the symptoms are very severe. It is associated with high populations of whiteflies (Otim-Nape, 1993; Legg, 1995; Legg and Ogwal, 1998, Thresh et al., 1997). Cassava yield losses are unacceptably high, threatening food security, and hunger has been reported in affected areas (Otim-Nape et al., 1997c). A concerted effort to combat the disease is needed on all fronts by introduction of other crops, promotion of resistant cassava varieties, farmer education and research (Thresh et al., 1997). This situation was first encountered in Madagascar in the 1930s-1940s (Cours, 1951; Cours et al., 1998), more recently in Uganda (Otim-Nape et al., 1997c) and now is seen in all parts of western Kenya and the Kagera region of NW Tanzania (Thresh et al., 1998a; Legg et al., 1999b; Legg, 1999b). The
dramatic shift from the benign to the epidemic state associated with the CMD pandemic in East Africa calls for the adoption of resistant varieties to prevent continuing crop failure (Otim-Nape et al., 1997; Cours et al., 1998).

The importance of exploiting the genetic variability of the genus Manihot in breeding for resistance to CMD has been stressed by many researchers (Hahn et al., 1979; Charrier and Lefevre 1987; Jennings and Hersey, 1985). Breeding and selection of cassava for resistance to CMD began independently in East Africa in 1938 (Nichols, 1947) and in Madagascar in 1935 (Cours, 1951; Cours-Darne, 1968; Cours et al., 1998). Attempts to identify CMD-resistant varieties from *M. esculenta* germplasm existing at the time were unsuccessful as the level of resistance available was inadequate. Greater success was achieved through inter-specific crosses of *M. glaziovii* Muell.-Arg. with cassava (Cours-Darne, 1968; Jennings, 1994; Cours et al., 1998) to produce hybrids. In both programmes a satisfactory starch content and high level of resistance to CMD was obtained by back-crossing hybrids of *M. glaziovii* x *M. esculenta* to cassava. The breeding programme in East Africa produced germplasm which has provided the main source of resistance used in the international breeding programme at IITA in Nigeria and other national breeding programmes (Jennings, 1976; 1994; Guthrie, 1991; Hahn et al., 1989; Mahungu et al., 1994; Ssemakula et al., 1997).

Field resistance if expressed as the percentage of CMD-affected plants is a good measure of cassava resistance (Fauquet et al., 1987a). In breeding work in Tanzania, resistance assessments were initially based on the number of
months that plants remained free of CMD symptoms, severity of symptoms produced and the incidence (Jennings, 1994). Subsequently three levels of resistance were defined: susceptible, intermediate and high.

2.12 CMD elimination strategy

Many workers have recognized the importance of reducing losses from CMD through the use of resistant varieties (Jennings, 1976; 1994; Cours, 1951; Cours et al., 1998; Otim-Nape et al., 1997c; Thresh et al., 1998b). However, achievements have been limited up to the present day because highly resistant varieties are not widely available and therefore make up only a small fraction of the c. 9.5 million hectares in Africa (FAO, 1997). Nweke et al., (1994) revealed several socio-economic and other factors that influence cassava production expansion and choice of cultivar. Over 1200 differently named varieties were recorded and about 92 and 176 different clusters of varieties based on a limited number of botanical features, were encountered in West and East Africa, respectively (Nweke, 1998; Nweke et al., 1994; Thresh et al., 1998). Although information regarding CMD resistance was not sought, it is widely admitted that farmers consider insensitivity to CMD, other diseases and pests as a key factor among others including root yield, early bulking, good cooking properties, ease of processing, low cyanide content and ability to suppress weeds (Otim-Nape et al., 1994; Nweke et al., 1994; Nweke, 1998; Thresh et al., 1998c). These multiple-requirements and deliberate consumption of CMD-infected leaves by some groups indicate that CMD is one of many items on a list that influences the farmers choice of a genotype. The level of technical "know how" will influence a farmers adoption of a cultivar such that
it may be deliberate or inadvertent and unintentional (Otim-Nape et al., 1994).

Combined use of phytosanitation and resistance has received little promotion since there are currently no clear guidelines on their use together. It is difficult to apply the practice of roguing to resistant varieties, since they "recover" and become symptomless, subsequently making it impossible to rogue effectively and unnecessary to do so (Thresh et al., 1998b; Thresh et al., 1998c). Moreover, most farmers do not have access to the resistant varieties and few recognise the potential benefit of this measure in situations where incidence is low or symptoms are mild. In places where resistant varieties are grown there is little incentive to rogue since incidence tends to be low and the yield penalty incurred by diseased plants is small. Where local varieties are grown and CMD incidence is low or symptoms are mild as is typical in the 'benign' state, the justification for phytosanitation is unclear. The paucity of information on the merits of adopting this measure necessitates further study before recommending it to farmers.

The ability of healthy plants to compensate for the impaired growth of their diseased neighbours has hardly been considered in experiments which have demonstrated the substantial yield losses incurred by some resistant varieties (Bock and Guthrie, 1978; Anonymous., 1980; 1981; Terry and Hahn, 1980; Seif, 1982). Unless the incidence of infected plants exceeds a certain critical level in a stand of a resistant variety their yield may remain unaffected (Otim-Nape, 1993; Otim-Nape et al., 1997; Sserubombwe, M.Sc 1998). It follows that roguing may only be useful for obtaining 'clean' disease-free planting material and only in those circumstances where rates of infection are low
(Otim-Nape et al., 1997). This indicates that CMD will have little effect on the yield of stands of resistant varieties, which is consistent with modeling predictions made by Fargette and Vié (1995), which suggest that the use of resistant varieties alone could effectively control CMD (Thresh et al., 1998c).

2.13 Integrated Control of CMD

It is only in recent years that serious progress has been made on control of CMD in West and East Africa, even though the disease has been studied since 1894 and much information is available on possible control measures (Thresh et al., 1994c). One problem is that cassava is a neglected crop, despite its dietary importance and potential for export as animal feeds or fuel. Limited resources have been allocated to cassava for CMD research and extension activities in Africa. Moreover, much effort has gone into combating cassava green mites, mealybugs and bacterial blight. These now cause in some countries more obvious damage than the insidious effect of CMD on growth and yield of cassava (Thresh and Otim-Nape, 1994).

Cassava suffers a generally low status in Africa because it is grown mainly for consumption by subsistence farmers and receives little or no attention compared to crops grown for export or on large commercial farms by influential farmers. Hence the difficulties extension services encounter in implementing CMD control research findings in competition with research findings of other crops. Other problems include limited personnel for extension and advisory services, shortage of finance, poor research facilities and unavailability of varieties resistant not only to CMD but also to other pests and
diseases of cassava (Thresh and Otim-Nape, 1994).

Limited progress has been made in developing an integrated approach to the control of cassava pests and diseases. One approach has been to rely on resistant varieties and routine screening tests against pests and diseases of cassava are adopted in breeding programmes mainly at IITA and in many other countries national programs. Biological control of green mites and mealybugs has been given extensive attention but no attempt has been made to co-ordinate control by the use of resistant varieties and natural enemies (Thresh and Otim-Nape, 1994).

One major difficulty that will be encountered when introducing integrated control measures will be the very diverse ecological conditions under which cassava is grown in Africa. It is grown in a wide range of altitudes and climates, as a major and minor crop and in areas having very different growing seasons and patterns of crop production. The result is there are likely differences in whitefly populations, virus strains and virus rates of spread between regions and therefore control measures or varieties that are appropriate in one place may be ineffective elsewhere. This is clear from contrasting epidemiological evidence obtained from Kenya, Côte d'Ivoire and Uganda. This is a serious limitation in devising an appropriate control strategy for each region to integrate within an overall crop protection strategy (Thresh and Otim-Nape, 1994).
3.0 MATERIALS AND METHODS

3.1 Vector Populations, Incidence and Severity of CMD in western Kenya

3.1.1 Selection of survey area: The survey was carried out between 20th and 5th November 1999 to assess vector populations, incidence and severity of CMD in western Kenya. The importance of cassava to the area was an important criterion used to select the study area. Administrative Western and Nyanza Provinces of Kenya were selected for the study. In Western Province the survey was done in the districts of Mumias-Butere, Kakamega, Busia and Teso while in Nyanza Province, Siaya and Suba were covered (Maps 1, 2 and 3). These are districts where a majority of farmers grow cassava.

3.1.2 Sample size: At least 30 plants were assessed in each of the 10-14 different farmers fields sampled per district. The fields sampled varied in size from 0.2 ha to 0.3 ha. The fields were selected with the aid of agricultural extension officers who were familiar with the area.

3.1.3 Sampling procedure: In all districts of Western Province and in Siaya district of Nyanza Province the fields were sampled along the main roads at intervals of 5 Km and alongside paths within villages where fields were randomly sampled. While in Suba district in south Nyanza sampling was done along the
shores of both Rusinga and Mfangano Islands in Lake Victoria, because cassava is grown only along the shores of the lake. Plants were selected along two diagonals across each field assessed, 15 consecutive plants were taken along the first diagonal and this was repeated with the second diagonal. Information about cassava varieties and age of the crop was obtained from the farmer. Disease severity, incidence and whitefly population were assessed as explained in sections 3.2.1 - 3.2.4.

3.2 Parameters Measured

3.2.1 Disease incidence: This refers to the number of plant shoots that were visibly diseased (Krantz, 1988), relative to the total number of plants assessed.

\[
\text{CMD incidence (\%)} = \frac{\text{Number of plants with symptoms}}{\text{Total number of plants assessed}} \times 100\%
\]

3.2.2 Disease severity: Disease severity is the area of plant tissue diseased (Krantz, 1988), relative to the total area. Severity is estimated or measured and expressed as either total area or proportion or percentage of plant tissue with symptoms of the disease. It is common to express severity on an arbitrary scale indicating the extent of symptom development. The scale of 1 to 5 (Fargette, 1985; Otim-Nape et al., 1998) commonly used in Uganda and Kenya was used in this study. The scale is as indicated in Table 1.
Table 1: Scale for scoring CMD symptom severity (Farrette, 1985 Otim-Nape et al., 1998a)

<table>
<thead>
<tr>
<th>Scale</th>
<th>Severity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Apparently healthy shoots, no symptoms</td>
</tr>
<tr>
<td>2.</td>
<td>Mild chlorotic patterns affecting most leaves; mild distortions at the base of most leaves while the remaining parts of the leaves and leaflets appear green and normal.</td>
</tr>
<tr>
<td>3.</td>
<td>Pronounced mosaic pattern on most leaves; narrowing and distortion of the lower one-third of the leaflets.</td>
</tr>
<tr>
<td>4.</td>
<td>Severe mosaic distortion of two thirds of most of the leaves and general reduction of leaf size; some stunting of shoots.</td>
</tr>
<tr>
<td>5.</td>
<td>Very severe mosaic symptoms on all leaves, distortion, twisting, malformation and severe leaf reduction of most leaves accompanied by severe stunting of plants.</td>
</tr>
</tbody>
</table>

3.2.3 Type of Infection: CMD was categorised as due to use of infected cuttings ('C'), or infection by the whitefly vector *B. tabaci* ('W') where cutting infection is distinguished by the appearance of disease symptoms on both the lowest and either all or some of the upper leaves of a diseased plant and whitefly-borne infection was characterised by the appearance of disease symptoms only on the uppermost leaves of a plant (Sseruwagi et al., 1999)(see plates 1, 2 and 3 on pp 26). This enabled me to tell the predominant mode of CMD spread at the time of sampling. The type of infection was expressed as a percentage of the total sample collected per field.

3.2.4 Adult whitefly population: The population of adult whiteflies was assessed on each plant sampled. At sampling time four top-most apical leaves of a representative shoot where most adults are found were randomly selected for examination. Each leaf was held by the petiole and gently turned upside down to count the total number of adults present. The average number of
whiteflies per plant shoot was calculated and then grouped into categories of 0-1, 1-3 and >3 to represent the whitefly abundance at each locality sampled.

3.2.5 **Mapping:** For map analysis co-ordinates for each of the sampling sites were estimated by interpolating between points or towns for which co-ordinates were known. The co-ordinates were fed into Acer View GIS 3.0 computer mapping system for visual representation of the incidence and severity of CMD and its whitefly population.

3.3 **Yield of 'relic' cassava in the post-pandemic zone of western Kenya**

A detailed survey of cassava yields in farmers’ fields was carried out in January 2000 in Mumias/Butere, Teso, and Siaya districts of western Kenya. The fields assessed for yield were different from the fields used in section 3.1. Assessment was made of growth, yield, CMD symptom severity and adult whitefly populations on 70 cassava plants from Teso district, 30 cassava plants from Mumias/Butere district and 30 plants from Siaya district. In Teso district most farmers were willing to have their crop harvested to facilitate yield assessment. All crop stands sampled were 7-12 months old. Plants were randomly selected and harvested in each field. The CMD symptom severity scoring system of 1-5 (Table 1) was used. Adult whitefly were counted on the top-most four expanded leaves of one shoot per plant, before the plant was harvested. Records of total plant weight, tuberous root weight and tuberous root numbers were weighed then other parameters were taken as explained in sections 3.2.1 - 3.2.4. Harvest index (HI) was calculated as in Cock *et. al.*, (1979).
HI = $\frac{\text{Weight of tuberous root}}{\text{weight of above ground parts} + \text{weight of tuberous roots}}$

One-way analysis of variance was used to assess differences among districts in CMD symptom severity, cassava growth and yield characteristics. Correlation analysis was carried out to check the interrelationships among observed variables.

3.4 Resistance to CMD of four cassava varieties under field conditions in western Kenya

The trials were conducted in farmers' fields in three different agro-ecological zones namely the Upper Midland 1 (UM$_1$) at Kakamega, which has a bimodal rainfall 2000-2200 mm p.a. with very good yield potential and temperatures between 18-21° C, Lower Midland 2 (LM$_2$) at Bungoma which has good yield potential, temperatures between 21-24° C, rainfall 1100-1300 mm p.a. and the Lower Midland 3 (LM$_3$) at Siaya which has fair yield potential, temperatures between 21-24° C and rainfall 900-1400mm p.a. (Jeatzold, 1983). Plantings were established during the long rains season from April to July 1999 and also during the short rain season from August to October 1999.

3.4.1 Selection of cuttings and planting.

Mature stem cuttings, 10-20cm long were obtained from symptomless plants, being grown at KARI-Kibos and at Sondu, Nyakach District. The cuttings were buried in furrows 10 cm deep. The cuttings were taken from plant clones with known reaction to CMD (Table 2). Recommended cultural practices were
adopted, neither fertilizers nor pesticides were applied.

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Disease Reaction</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>30572 (Migyera)</td>
<td>Resistant (R)</td>
<td>IITA-Nigeria</td>
</tr>
<tr>
<td>SS-4 (Nase-4)</td>
<td>Resistant (R)</td>
<td>NARO- Uganda</td>
</tr>
<tr>
<td>TMS 30337 (Nase-2)</td>
<td>Moderately Resistant (MR)</td>
<td>IITA-Nigeria</td>
</tr>
<tr>
<td>Serere (local)</td>
<td>Susceptible (S)</td>
<td>Kenya</td>
</tr>
</tbody>
</table>

### 3.4.2 Test Varieties, Locations and Experimental Designs

In each experiment at Bungoma, Kakamega and Siaya districts, three cassava clones TMS 30572 (Migyera), TMS 30337 (Nase-2), SS-4, recently introduced from NARO, Uganda and one local variety Serere were evaluated. Cuttings were planted in plots of 10m x 10m at a spacing of 1m x 1m in a randomized complete block design (RCBD) with four replications at Siaya, at first planting in April 1999. For the Kakamega and Bungoma trials due to shortage of land four farms were selected randomly per district and each field formed a replicate. During the second planting of August 1999 Kakamega and Siaya had four replicates each in a RCBD design. All plants that had primary infection at emergence were removed and replaced with healthy ones of the same age from a reserve site.

### 3.4.3 Monthly Climatic Records

Wherever possible, rainfall (mm), and ambient maximum and minimum temperatures were obtained from meteorological services to determine the relationship with whitefly population density and CMD infection and spread. (Appendix 1)
3.4.4 Disease Assessment.

Data was taken at each location on disease incidence, severity of symptom expression on shoots of infected plants (symptom score) and adult whitefly population count on the four top-most expanded leaves of representative shoots. Records were made starting from 1 to 6 months after planting (MAP). A scale of 1-5 was used for recording symptom severity. Disease incidence was indicated by presence or absence of leaf symptoms. Other symptoms caused by cassava green mite, cassava anthracnose disease, cassava bacterial blight and leaf spots were scored using a scale of 1-5, where 1 indicates no apparent symptoms observed and 5 severe symptoms. Plants that had recovered ('R') (loss of symptoms) from CMD on each assessment were recorded by noting the absence of symptoms on the newest growing shoot/leaves and the presence of symptoms on the lower leaves of the same plant. Actual CMD incidences were transformed for multiple infection (sensu Gregory, 1948). Data for changes in mean incidence and adult whitefly populations for each variety were plotted on graphs for each location and experiment.

3.4.5 Statistical Analyses

Homogeneity of variance was used to detect any anomalies in the data before analysis of variance was carried out on arcsine-transformed values of incidence, on logarithm-transformed values of adult whitefly populations and on actual values for symptom severity (Gomez and Gomez, 1984). Tukeys test for separation of means was used. Regression analysis was used to investigate the relationship between whitefly population, disease incidence and symptom severity. Area under disease progress curves were calculated for each variety.
3.5 Effect of CMD on yield of four cassava cultivars in three agro-ecological regions

Yield data were obtained from plantings of April 1999 at 10 MAP when both healthy and diseased plants were harvested at each site. Assessment was made on 20 healthy plants and 20 diseased plants of each cultivar at each site except for SS-4 which had only one plant infected in all the trials. All plants were harvested at 10 MAP. The characteristics taken into account were yield, CMD symptom severity and adult whitefly populations. The plants were picked randomly from the four plots in each locality. The plant selected was harvested and the total number of tuberous roots, fresh tuberous root yields, average individual tuberous weight and total fresh plant weight were recorded. The harvest index was calculated as described in section 3.3. Homogeneity of variance was used to detect any anomalies in the data before analysis of variance was carried out to assess differences between sites, and to determine the effect of CMD on growth and yield. Correlation and regression analysis were used to study interrelationships between CMD symptom severity, cassava growth and other yield parameters. The Statistical Analysis System (SAS, Institute, Cary, NC, USA) was used to analyze the data.

3.6 Virus types in CMD-affected plants in western Kenya

3.6.1 Sample collection

Young cassava leaves were collected from the uppermost shoot of each
diseased plant sampled at experimental sites in Kakamega and Siaya. Eleven samples were collected from Kakamega and ten samples from Siaya. The severity score of the sampled plant was taken on a scale of 1-5.

3.6.2 Extraction of viral DNA from CMD-affected cassava plant leaves

One punched leaf sample of diameter 1 cm was ground with kontes pestles in a micro-fuge tube containing 500μl of extraction buffer (Dellaporta et al., 1983), this buffer is composed of 100mM Trizma Base 1.21g, 8.5mM EDTA 0.316g, 500mM NaCl 2.922g and 10mM β-mercaptoethanol 78μl all mixed in 100ml of distilled water and adjusted to pH 8.0. In each tube was added 33μl of 20% Lauryl sulphate and mixed thoroughly and incubated in a waterbath at 65°C for 10 minutes. To each tube was added 160μl of 5M Potassium acetate and mixed thoroughly. The tube was kept in an icebox for 10 minutes and then centrifuged (Sanyo, Micro Centaur) for 10 minutes at 13000 rpm. From each tube 450μl of the supernatant was removed and mixed with 450μl of cold Isopropanol. The tube was spun at 13000 rpm for 10 minutes to precipitate the DNA. To each tube was added 500μl of 70% ethanol and spun at 13000 rpm for 5 minutes. The supernatant was removed to leave behind DNA. To every tube containing the DNA precipitate, 500μl of distilled water was added to resuspend it. This DNA was stored in the fridge at 4°C for further analysis.

3.6.3 Sample DNA amplification and testing for the presence of ACMV, EACMV and UgV

An equal amount, 5μl of diluted DNA was prepared from each sample and put into one Eppendorf tube for each sample. Amplification was performed in
18.7 μl, volume consisting of 0.2 μl of 1 unit/μl Taq polymerase, 1.0 μl of 10X dNTPs, 1.5 μl of 25mM MgCl2, 2.5 μl of 5% Tween-20, 2.5 μl of 10X Thermo buffer and 10.6 μl distilled water. To all the 21 samples in Eppendorf tubes was added a mixture of 0.2 μl of Universal primer 1 and 0.2 μl of Universal primer 2 (Table 3). Each tube was layered with 30 μl of mineral oil. These tubes were then loaded into a thermocycler (Hybaid Omn-E and Techne Progene PCR machines) for amplification which was programmed as follows: **Step 1**: Denaturing or breaking the polypeptide bonds, 1 cycle at 94°C for 2 minutes, 54°C for 1 minute 30 seconds and 72°C for 2 minutes; **Step 2**: Annealing or building nucleotides, 35 cycles at 94°C for 1 minute, 54°C for 1 minute 30 seconds and 72°C for 2 minutes and **Step 3**: Extension or elongating the polymers, 1 cycle at 94°C for 1 minute, 54°C for 1 minute 30 seconds and 72°C for 10 minutes. After completion of the cycles the samples were maintained at 4°C in the refrigerator (Zhou *et al.*, 1997) until required.

### Table 3 Primers used for PCR

<table>
<thead>
<tr>
<th>Primer pair designation*</th>
<th>Primer sequence (5' to 3')#</th>
<th>To Detect</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV-AL1/F1</td>
<td>TGTCTTTCTGGGACTTGTGTG</td>
<td>UgV/EACMV</td>
</tr>
<tr>
<td>UV-AL1/R1</td>
<td>AACCTATCCCCCGATGCTCAT</td>
<td>UgV/EACMV</td>
</tr>
<tr>
<td>ACMV-AL1/F</td>
<td>GCGGAATCCCTAACATTTATC</td>
<td>ACMV</td>
</tr>
<tr>
<td>ACMV-CP/R3</td>
<td>TGCCCTCCTGATGATTATGTC</td>
<td>ACMV/UgV</td>
</tr>
<tr>
<td>EACMV/CP/Ra</td>
<td>ACTCTATGAGTAATACCCGA</td>
<td>EACMV/UgV</td>
</tr>
<tr>
<td>EACMV/CP/Rb</td>
<td>ACTCTATGGGTAATGCGCTGA</td>
<td>EACMV/UgV</td>
</tr>
<tr>
<td>Universal 1</td>
<td>TAATATTACCKGWKGRCCSC</td>
<td>E/A/UgV</td>
</tr>
<tr>
<td>Universal 2</td>
<td>TGGACYTTRCAWGGWCCTTCACA</td>
<td>E/A/UgV</td>
</tr>
</tbody>
</table>

*Designations containing /F denote forward primers and those with /R are reverse primers
#Y represents A or C; R represents A, T, or G; W represents C, T or A; S represents A, G or C; K represents C, T, G, or A.
3.6.4 Testing for the presence of ACMV and UgV by primers of ACMV and UgV

In separate trial run, each sample of 5μl of diluted DNA from each site was put into two separate tubes designated ‘A’ for ACMV and “U” for UgV, to enable testing for the presence of both ACMV and UgV viruses in each sample. Amplification was performed in 18.5μl volume consisting of 10.6 μl of distilled water, 2.5μl of 10X thermo buffer, 2.5μl of 5% Tween-20, 1.5μl of 25mM Magnesium chloride, 1.0 μl of 10X dNTPs, and 0.2μl of 1 unit/μl Taq polymerase. To the first set of ‘A’ tubes was added 0.2 μl of ACMV mixed primer pairs and 0.2 μl UgV primer pairs was added to the second set of “U” tubes. Each tube was layered with 30μl of mineral oil. The samples were then loaded into a thermocycler that was set to PCR conditions as in section 3.6.3.

3.6.5 Testing for the presence of EACMV and UgV by primer pairs of EACMV and UgV

To test for the presence of EACMV and UgV the amplification procedure followed steps similar to those in section 3.6.4 except to each tube designated ‘E’ 0.2 μl EACMV mixed primer pairs were added and to each tube designated ‘U’ 0.2 μl UgV mixed primer pairs were added. Each tube was layered with 30μl of mineral oil. The samples were then loaded into a thermocycler, which was set to PCR conditions as in section 3.6.3.

3.6.6 Agarose gel preparation

Agarose gel (1.2%) was prepared by dissolving 1.2 g agarose in 100ml of Tris
acetate EDTA (TAE) buffer. The mixture was heated to boiling to dissolve the agarose. Ethidium bromide was added to the solution and allowed to cool to 38°C. The molten solution was poured into an electrophoresis tank that had been pre-fitted with two combs. The mixture was allowed to solidify and the combs were removed. The gel was immersed into an electrophoresis tank (Electrophoretic Gel System, Savant HG 340) containing Tris-acetate buffer of 1090ml.

3.6.7 Sample loading
About 2μl of loading dye was put into the wells of the omni-plate wells corresponding to the number of samples prepared. Ten micro-litres of amplified DNA sample was put onto the dye in each omni-plate well and mixed thoroughly. Each mixture of the sample was then put into one well in the gel using a predetermined loading order. 10μl of standard DNA marker [(Sigma PCR marker 50-2000bp, USA) Appendix 3a II] was put into the last well of each half of the gel. The samples were run at 100 volts for one hour.

3.6.8 Separation amplification and precipitation of PCR product
Ten micro-litres of the PCR product sample were loaded per sample per well in 1.2% ethidium bromide stained agarose gel. The gel was run at 100 volts for 1 hour and was visualized under ultra-violet light source and photographs were taken. Samples giving positive results with the universal primers were precipitated for RFLP. To precipitate the PCR product for digestion, the product was carefully removed into a new tube by leaving behind the mineral oil. An equal volume of Isopropanol was added to the PCR product. This
mixture was centrifuged at 13000 rpm for 10 minutes. The supernatant was removed and the DNA pellets were washed with 100 ml of 70% ethanol. This solution was further centrifuged at 13000 rpm for 10 minutes. The supernatant was removed and the pellets were left in air to dry. The pellets were then resuspended in distilled water for further use.

3.6.9 Restriction Digestion

Restriction enzymes EcoRV and MluI were used. Two sets of 21 tubes each, were prepared for testing with each enzyme. The restriction digestion reaction consisted of 10 μl of diluted DNA sample mixed with 7.5 μl of distilled water, 2.0 μl 5X of react buffer and 0.5 μl of 1 unit/μl the respective enzyme. These were pulsed in a centrifuge to ensure thorough mixing of the contents. The samples were incubated at 37°C for 1-2 hours. Twenty micro-litres of the sample mixed with loading dye was loaded into the 1.2% agarose gel stained with ethidium bromide, a standard DNA marker was put in the last well and set running at 100 volts for 45 minutes. The gels were photographed under uv-light on a Polaroid film. The results were interpreted by using patterns obtained from RFLP score sheet (Appendix 3).

3.7 Incidence of CMD in ratoons of SS-4 variety

The cassava variety SS-4 was grown from healthy stem cuttings planted in April 1999 at the Siaya, Bungoma and Kakamega districts as described in section 3.4.2. Ratooning was done in February 2000 and the mature stems were cut 5 cm above the ground. At Siaya 60 plants were cut whereas in Kakamega 80 plants were cut and in Bungoma 100 plants were cut. The plants
were left to grow to maturity at 10 MAP during which data on adult whitefly population, CMD severity and CMD incidence were collected monthly for six months as described in section 3.2.1 - 3.2.4. Each stem from the ratooned plant was divided into three equal parts namely bottom, middle and top. Each of the bottom, middle and top parts were cut into 10 cm long pieces. All cuttings were marked to correspond to the parent plant. The cuttings were then buried 10 cm deep in the soil and were left to sprout into young shoots. The cut back parent plants were also left to develop young ratoon shoots. After three weeks, observations were made on the new ratoon shoots and on each set of three cuttings. Data were collected for CMD incidence in both ratoons and new plants from stem cuttings. The CMD severity in the ratoons was recorded on a scale of 1-5 (Table 2). T-test was calculated by the method of paired-comparisons. Statistical analysis system package (SAS, Cary, NC, USA) was used to analyse the data.
CHAPTER FOUR

4.0 RESULTS

4.1 Vector Populations, Incidence and Severity of CMD in western Kenya

4.1.1 Common cassava varieties

The most frequently encountered varieties at each locality are shown in table 4. Varieties Serere and Ebwanateraka were among the most widely cultivated varieties in all six localities.

Table 4 Common cassava varieties in each district

<table>
<thead>
<tr>
<th>Locality</th>
<th>Main varieties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Teso</td>
<td>TMS 30572, Ebwanateraka,</td>
</tr>
<tr>
<td>Busia</td>
<td>Ebwanateraka, TMS 30337, TMS 30572</td>
</tr>
<tr>
<td>Suba</td>
<td>Nyaluo, Nduruma, Nyasigulu.</td>
</tr>
<tr>
<td>Siaya</td>
<td>Adhiambolera.</td>
</tr>
<tr>
<td>Kakamega</td>
<td>Serere, Ebwanateraka.</td>
</tr>
<tr>
<td>Mumias/Butere</td>
<td>Wetoto, Serere, Bii</td>
</tr>
</tbody>
</table>

4.1.2 CMD incidence in western Kenya

CMD occurred in all the 82 fields assessed. The overall mean incidence of CMD was 62.3% for the six districts surveyed. Incidences for individual districts were 83% and 82% for Siaya and Mumias/Butere respectively, which were significantly ($p<0.05$) higher than the other four district where mean CMD incidences ranged from 50% in Kakamega to 60% in Busia in which incidence was not significantly different.

All the varieties recorded including TMS 30572 (also known as Nigeria in Teso and Busia) and TMS 30337 found mainly in Teso and Busia districts expressed CMD symptoms. The mean incidence in the six most common varieties namely
Ebwanateraka, Nyasigulu, Nduruma, Adhiambolera, Serere and Wetoto was 76%. For individual varieties the CMD incidence ranged from 24% in the TMS 30572 to 100% in Ebwanateraka and Bii.

4.1.3 Severity of shoot symptoms

The overall mean CMD severity score for all the varieties was 3.33. The mean severities differed significantly between districts. The shoot symptom severity of 5 was highest in Mumias/Butere and Siaya districts, and was not significantly different between the two, whereas it was significantly different from the severity in the other four districts namely Teso, Busia, Suba and Kakamega. The later were not significantly different in severity. The lowest shoot symptom severities were observed in Teso and Busia. This was not surprising as farmers in these districts had through their own initiative introduced the resistant varieties (TMS 30572 and TMS 30337) from Uganda. There was a highly significant correlation ($r = 0.88, p < 0.01$) between disease incidence and disease symptom severity, which was an indication that in this particular case high severity scores are associated with high disease incidence.

4.1.4 Adult whitefly populations

The overall mean for adult whitefly population was 0.43 whiteflies per shoot. Large numbers of adult whiteflies were encountered in Suba district, on the offshore island of Rusinga in Lake Victoria (Map 3). There were very few whiteflies found on another island Mfangano 10 Km to the SW in the same district. The mean adult whitefly population on logarithm-transformed values as in Gomez and Gomez (1984) for Suba district was 1.22 ($p = 0.05$) and was
significantly different from the whitefly population means obtained in other
districts. All the other districts means were not significantly different (Table 5).

Table 5 Mean number of adult whitefly population
counts per shoot in six different districts

<table>
<thead>
<tr>
<th>Locality</th>
<th>Mean adult whitefly number per shoot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Teso</td>
<td>0.25b</td>
</tr>
<tr>
<td>Busia</td>
<td>0.18b</td>
</tr>
<tr>
<td>Suba</td>
<td>1.22a</td>
</tr>
<tr>
<td>Siaya</td>
<td>0.06b</td>
</tr>
<tr>
<td>Kakamega</td>
<td>0.20b</td>
</tr>
<tr>
<td>Mumias/Butere</td>
<td>0.15b</td>
</tr>
</tbody>
</table>

Mean with different letters are separated by Tukey statistic at the \( p < 0.05 \) level.

Mapping

After surveying and collection of data the sites were then categorised according
to their CMD incidence as described by Legg (1999b). For the CMD incidence
map (Map 1) the five categories were:

<table>
<thead>
<tr>
<th>category</th>
<th>incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0-20%</td>
</tr>
<tr>
<td>2</td>
<td>20-40%</td>
</tr>
<tr>
<td>3</td>
<td>40-60%</td>
</tr>
<tr>
<td>4</td>
<td>60-80%</td>
</tr>
<tr>
<td>5</td>
<td>80-100%</td>
</tr>
</tbody>
</table>

For CMD severity (Map 2) the three categories were:

<table>
<thead>
<tr>
<th>category</th>
<th>severity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>2</td>
</tr>
<tr>
<td>Moderate</td>
<td>2-3</td>
</tr>
<tr>
<td>Most Severe</td>
<td>4-5</td>
</tr>
</tbody>
</table>
For whitefly abundance (Map 3) means of numbers of adult per plant shoot were used and the categories were:

0-1 per plant shoot,

1-3 per plant shoot

and >3 per plant shoot.
Map 1

Survey of CMD incidences in Western Kenya in October - November 1999. Plant age of fields surveyed was 7 - 12 months.
Plant age of fields surveyed was 7 – 12 months.
Map 3
Distribution of adult whitefly population in Western Kenya October – November 1999. Plant age of fields surveyed was 7 – 12 months.
4.2 Survey of yield of 'relic' cassava in post-pandemic zone in western Kenya

4.2.1 Varieties encountered

In the areas surveyed, nine main cassava cultivars were grown widely. The data presented in Table 6 was obtained from the separated data for healthy and diseased plants. The varieties Adhiambo lera and Serere were common in Mumias/Butere and Siaya, but not in Teso where Serena and Kelezenzia predominated. Some varieties like Tamisi occurred as single plants in Mumias/Butere, whereas Jolejo and Otugo diep occurred in Siaya. All varieties were susceptible to CMD except Otugo diep that was grown in Siaya. This was said to be toxic possibly indicating a high cyanogenic potential. This variety could be resistant, as the few plants encountered did not show any disease symptoms.

Mean plant height varied from 0.9 to 1.9 m and total fresh plant weight ranged from 0.5 to 4.0 kg. No plant of any variety exceeded a height of 2 m. The weight of the improved variety SS-4 in Teso exceeded 4 kg. The means of total numbers of tuberous roots, mean individual tuberous root weight, fresh tuberous root weight and harvest index were 0 - 4.4 (± 2.81), 0 -3.8 (± 0.37) kg, 0 - 4.4 (± 0.86) kg and 0 - 5.0 (±1.34) kg, respectively. SS-4 gave fresh tuberous root weights equivalent to 23.6 t/ha compared to 7.2t/ha of Serere and 3t/ha of Kelezenzia. These were the local varieties which showed the highest yield during the survey.
Table 6. Separated mean values of yield characteristics of healthy and diseased plants in all districts

<table>
<thead>
<tr>
<th>Variety</th>
<th>plant height (m)</th>
<th>Plant weight (kg)</th>
<th>Number of tuberous roots per plant</th>
<th>Fresh tuberous root weight per plant(Kg)</th>
<th>Mean individual tuberous root weight(g)</th>
<th>Harvest index</th>
<th>CMD score</th>
<th>Number of whiteflies per plant shoot$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D</td>
<td>H</td>
<td>D</td>
<td>H</td>
<td>D</td>
<td>D</td>
<td>H</td>
<td></td>
</tr>
<tr>
<td>Bumba</td>
<td>1.4(0.09)</td>
<td>-</td>
<td>1.5(0.35)</td>
<td>-</td>
<td>3.0(0.63)</td>
<td>0.7(0.2)</td>
<td>-</td>
<td>25(0.7)</td>
</tr>
<tr>
<td>Adhiambolera</td>
<td>1.4(0.16)</td>
<td>-</td>
<td>3.0(0.51)</td>
<td>-</td>
<td>2.8(0.8)</td>
<td>0.6(0.2)</td>
<td>-</td>
<td>9.2(3.1)</td>
</tr>
<tr>
<td>Bk 8</td>
<td>-</td>
<td>2.0</td>
<td>-</td>
<td>3.0</td>
<td>-</td>
<td>0.7(0.1)</td>
<td>-</td>
<td>13(3.0)</td>
</tr>
<tr>
<td>Serere</td>
<td>2.0(0.12)</td>
<td>-</td>
<td>2.0(0.3)</td>
<td>-</td>
<td>7.8(0.0)</td>
<td>0.9(0.1)</td>
<td>-</td>
<td>10(0.6)</td>
</tr>
<tr>
<td>Tamisi</td>
<td>1.2(-)</td>
<td>-</td>
<td>0.6(-)</td>
<td>-</td>
<td>0.8(-)</td>
<td>0.2(-)</td>
<td>-</td>
<td>17(5.0)</td>
</tr>
<tr>
<td>Salanyingi</td>
<td>2.0(0.2)</td>
<td>-</td>
<td>4.0(2.0)</td>
<td>-</td>
<td>8.0(3.0)</td>
<td>1.4(0.8)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SS-4</td>
<td>-</td>
<td>1.6</td>
<td>-</td>
<td>4.2</td>
<td>-</td>
<td>8.0(3.0)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Serena</td>
<td>1.5(0.07)</td>
<td>-</td>
<td>1.6(0.1)</td>
<td>-</td>
<td>4.0(0.3)</td>
<td>0.6(0.1)</td>
<td>-</td>
<td>14(2.1)</td>
</tr>
<tr>
<td>Kelesenza</td>
<td>2.0(0.04)</td>
<td>-</td>
<td>2.0(0.3)</td>
<td>-</td>
<td>4.1(0.4)</td>
<td>0.7(0.1)</td>
<td>-</td>
<td>17(2.0)</td>
</tr>
<tr>
<td>Bk11</td>
<td>-</td>
<td>1.0</td>
<td>-</td>
<td>2.0</td>
<td>-</td>
<td>0.3(0.0)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Jolejo</td>
<td>-</td>
<td>1.1</td>
<td>-</td>
<td>3.0</td>
<td>-</td>
<td>0.3(0.0)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nyaboro</td>
<td>1.0(-)</td>
<td>-</td>
<td>1.0(-)</td>
<td>-</td>
<td>2.0(-)</td>
<td>0.3(-)</td>
<td>-</td>
<td>15(-)</td>
</tr>
<tr>
<td>Otugo diep</td>
<td>-</td>
<td>2.0</td>
<td>-</td>
<td>2.3</td>
<td>-</td>
<td>5(0.0)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

CMD symptoms score 1 = no symptoms 2-5 symptoms of increasing severity

$^2$ Mean whitefly population per plant on four top-most-leaves

Values in parentheses are SEM.

H = Healthy
D = Diseased
Table 7 Mean plant weight and harvest index of cassava in Mumias/Butere, Teso and Siaya districts.

<table>
<thead>
<tr>
<th>District</th>
<th>No of plants</th>
<th>Mean plant Weight (kg)</th>
<th>Harvest Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mumias/Butere</td>
<td>30</td>
<td>0.90a</td>
<td>0.42a</td>
</tr>
<tr>
<td>Teso</td>
<td>70</td>
<td>0.89a</td>
<td>0.35a</td>
</tr>
<tr>
<td>Siaya</td>
<td>30</td>
<td>0.86a</td>
<td>0.43a</td>
</tr>
</tbody>
</table>

Figures followed by same letter in column one and two are not significant at 1% level. Means calculated for all diseased and healthy plants assessed. Tukeys test used. Different varieties sampled in one site.

Mean plant weight, mean plant height, fresh tuberous root weight, mean individual tuberous root weights were not significantly different between the three districts. CMD severity score was not significantly different between Teso and Siaya ($p < 0.05$). Also, the mean adult whitefly population was not significant between Teso and Siaya, Mumias/Butere and Siaya but differed significantly between Teso and Mumias/Butere ($p > 0.05$). The harvest index was not significantly different in all the districts (Table 7).

4.2.2 Correlation of growth and yield parameters

Data from the three districts were used to construct Pearson’s correlation matrix for growth and yield parameters for CMD symptom severity scores and adult whitefly populations (Table 8).

There were significant positive correlations between total plant weight and four of the other five growth parameters. There was a positive and highly significant correlation between plant weight and harvest index. Fresh tuberous root weight was positively correlated with four of the five growth parameters (Table 8).
Table 8. Pearson Correlation Coefficients matrix for cassava growth, yield parameters, CMD symptom scores and adult whitefly populations (Combined data for all districts; n = 115)

<table>
<thead>
<tr>
<th></th>
<th>PHt(m)</th>
<th>PWt</th>
<th>TNO</th>
<th>TWt</th>
<th>ITWt</th>
<th>HI</th>
<th>CMD:S</th>
<th>WF</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHt(m)</td>
<td>1.00000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PWt</td>
<td>0.40786</td>
<td>1.00000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNO</td>
<td>0.52177</td>
<td>0.45854</td>
<td>1.00000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TWt</td>
<td>0.44975</td>
<td>0.80196</td>
<td>0.65580</td>
<td>1.00000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ITWt</td>
<td>0.10859</td>
<td>0.40811</td>
<td>0.01401</td>
<td>0.55939</td>
<td>1.00000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HI</td>
<td>-0.42684</td>
<td>0.10621*</td>
<td>-0.43097</td>
<td>-0.20379</td>
<td>-0.15021*</td>
<td>1.00000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMD:S</td>
<td>-0.12269*</td>
<td>-0.44111†</td>
<td>-0.03649*</td>
<td>-0.33528†</td>
<td>-0.15312*</td>
<td>-0.56533†</td>
<td>1.00000</td>
<td></td>
</tr>
<tr>
<td>WF</td>
<td>-0.17999 ns</td>
<td>-0.08936 ns</td>
<td>0.00000</td>
<td>-0.03162 ns</td>
<td>-0.03156 ns</td>
<td>0.17069 ns</td>
<td>-0.05273 ns</td>
<td></td>
</tr>
</tbody>
</table>

* Significant at the 5% level, † Significant at the 1% level. PHt(m) = Plant height, PWt = Plant weight, TNO = Tuberous root numbers per plant, TWt = Tuberous root weight, ITWt = Individual tuberous root weight, HI = harvest index, CMD:S = CMD severity, WF = Whitefly population.

4.2.3 Correlation of whitefly populations, CMD symptom severity score and growth and yield of cassava

There was no significant correlation between whitefly numbers and any of the cassava growth or yield parameters (Table 8), and the correlations were all negative. The relationship between CMD severity score and the different growth and yield characteristics were all negative and, highly significant (p < 0.01), except for tuberous root per plant that was negative and not significant.
Table 9 Mean values of plant characteristics and percentage of total $R^2$ due to variability in districts (n = 115 in all districts)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean</th>
<th>$R^2$</th>
<th>F values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Tuberous root weight (kg)</td>
<td>0.79</td>
<td>28</td>
<td>21.8*</td>
</tr>
<tr>
<td>Plant height (m)</td>
<td>1.58</td>
<td>4</td>
<td>2.4*</td>
</tr>
<tr>
<td>Plant weight (kg)</td>
<td>2.19</td>
<td>21</td>
<td>14.8*</td>
</tr>
<tr>
<td>Number of tubers per plant</td>
<td>3.96</td>
<td>12</td>
<td>7.5*</td>
</tr>
<tr>
<td>Harvest index</td>
<td>0.86</td>
<td>53</td>
<td>63.4*</td>
</tr>
<tr>
<td>Mean Individual Tuber weight (kg)</td>
<td>0.21</td>
<td>1</td>
<td>6.81*</td>
</tr>
</tbody>
</table>

* F values significant at the 0.01% level

The effect of CMD on growth attributes was highly significant in all districts ($p < 0.01$). CMD infection accounted for 28% of the total fresh tuber yield variability in the districts. The effect of CMD infection on harvest index was highly significant ($p < 0.01$) and infection accounted for 53% of the poor harvest encountered in all the districts (Table 9).

4.3 Spread of CMD in four cassava cultivars in western Kenya

4.3.1 Disease Progress

There was no spread at Kakamega in experiment 2 planted in August 1999, whereas at least some spread occurred in all other experiments. More spread of the disease occurred in one multi-locational experiment in Siaya ($\text{mean} = 22 \ p = 0.05$) than in two on-farm trials in Kakamega ($\text{mean} = 14 \ p = 0.05$) and
Bungoma (mean = 12 p =0.05) (Table 10).

Table 10 Disease incidence (%) by site for plantings in April 1999 and August 1999

<table>
<thead>
<tr>
<th>Site</th>
<th>Disease Incidence</th>
<th>Disease Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>April 1999</td>
<td>August 1999</td>
</tr>
<tr>
<td>Siaya</td>
<td>22a</td>
<td>4.1a</td>
</tr>
<tr>
<td>Kakamega</td>
<td>14b</td>
<td>0.0b</td>
</tr>
<tr>
<td>Bungoma</td>
<td>12c</td>
<td>-</td>
</tr>
</tbody>
</table>

Means followed by a letter are significantly different at 5% level

Disease progress curves are presented for each of the four sites where spread occurred. In the plantings of April 1999, CMD spread rapidly in the local cultivar Serere and less rapidly in cv TMS 30337 in Siaya, than in the other improved genotypes, and this was more so at Kakamega than in Bungoma (Fig. 2a, 2c 2e, 2g ). The data are also presented after transformation for multiple infection where much spread occurred (Fig 2b, 2d, 2e, 2h). Most of the plants of Serere were infected by the end of the growing season in Siaya, whereas fewer plants were infected in Kakamega and Bungoma.

Apart from Serere, which showed conspicuous disease symptoms at all stages of growth, disease incidence in cvs TMS 30337, TMS 30572 and SS-4, was usually low at 7 MAP and during the final observation at harvesting of the multi-locational trial and the OFTs planted in April 1999.

In the OFTs, the mean incidence was significantly higher in Kakamega than in
Bungoma, in April 1999 plantings. The spread of CMD within different genotypes differed between sites, and at different stages of growth (see Fig. 2). The maximum disease incidence reached in each cultivar at 7 MAP is shown in Table 11a. There were significant differences in the mean disease incidence 1, 2 and 3 months after planting (MAP), and no significant differences in mean CMD incidence 4, 5 and 6 MAP in plantings made in April 1999 (Table 11b) was observed. There were significant interactions for CMD incidence between site and month, site and cultivar and month and cultivar, where month represented crop age (Table 12).

The disease incidence was significantly greater in Serere (overall mean = 39, p=0.05) than in cv TMS 30337 (overall mean = 16, p = 0.05), TMS 30572 (overall mean = 8, p = 0.05) and SS-4 (overall mean = 0.3, p = 0.05) at all locations where spread occurred. The maximum mean disease incidence reached in all varieties compared across locations, within locations and in different seasons is shown in Table 13. All three TMS genotypes showed an apparent decline in disease incidence after reaching maxima 4-6 months after planting (MAP) (see Fig. 2a-h).

Cumulative disease incidence graphs indicate that area under disease progress curves (AUDPC) in the local variety Serere did not decline and did not recover, whereas in the improved varieties tended to decline as they recovered (see fig.3a-i). Transformation of actual disease incidence to multiple infection units confirmed that in terms of CMD incidence, Serere still consistently performed poorly in comparison to the improved varieties (fig. 2a-h). SS-4 was less
infected than TMS 30572 and TMS 30337, while TMS 30572 performed better than TMS 30337 (see MIU graphs in fig 2).

**Table 11a** Disease incidence in each cultivar at 7MAP in April-Oct 1999 (N = 24 for each variety)

<table>
<thead>
<tr>
<th>Variety</th>
<th>Site</th>
<th>Kakamega</th>
<th>Siaya</th>
<th>Bungona</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serere</td>
<td>7.0(6)</td>
<td>32.0(15)</td>
<td>51.0(27)</td>
<td>34.0(15)</td>
</tr>
<tr>
<td>TMS 30572</td>
<td>13.0(7)</td>
<td>7.0(6)</td>
<td>47.0(27)</td>
<td>5.0(7)</td>
</tr>
<tr>
<td>TMS 30337</td>
<td>24.0(12)</td>
<td>17.0(11)</td>
<td>24.0(12)</td>
<td>9.0 (7)</td>
</tr>
<tr>
<td>SS-4</td>
<td>0.8(2)</td>
<td>0.0(0)</td>
<td>0.0(0)</td>
<td>0.0(0)</td>
</tr>
</tbody>
</table>

Values in parenthesis = SD

**Table 11b** Disease incidence in all varieties combined in relation to stage of growth

<table>
<thead>
<tr>
<th>Growth Stage (or MAP)</th>
<th>Mean Disease Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3a</td>
</tr>
<tr>
<td>2</td>
<td>11b</td>
</tr>
<tr>
<td>3</td>
<td>18c</td>
</tr>
<tr>
<td>4</td>
<td>21d</td>
</tr>
<tr>
<td>5</td>
<td>21.5d</td>
</tr>
<tr>
<td>6</td>
<td>21.6d</td>
</tr>
</tbody>
</table>

Mean followed by a different letter are significantly different at 5% level

**Table 12** Interaction values for CMD incidence

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &lt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site*Month</td>
<td>10</td>
<td>106</td>
<td>3.29</td>
<td>0.01</td>
</tr>
<tr>
<td>Site*Cultivar</td>
<td>6</td>
<td>588</td>
<td>18</td>
<td>0.01</td>
</tr>
<tr>
<td>Month*Cultivar</td>
<td>15</td>
<td>651</td>
<td>20</td>
<td>0.01</td>
</tr>
</tbody>
</table>

CV = 36, R² = 0.92, Mean CMD incidence = 16
Table 13 Maximum (%) mean disease incidence in each variety

<table>
<thead>
<tr>
<th>Variety</th>
<th>April 1999</th>
<th>August 1999</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Siaya</td>
<td>Kakamega</td>
</tr>
<tr>
<td>Serere</td>
<td>51(24)</td>
<td>32(24)</td>
</tr>
<tr>
<td>TMS 30337</td>
<td>24(24)</td>
<td>17(24)</td>
</tr>
<tr>
<td>TMS 30572</td>
<td>13(24)</td>
<td>7 (24)</td>
</tr>
<tr>
<td>SS-4</td>
<td>0 (24)</td>
<td>1 (24)</td>
</tr>
<tr>
<td>Overall</td>
<td>22(96)</td>
<td>14(96)</td>
</tr>
</tbody>
</table>

Values in parentheses = number of plants assessed
Fig 2a. Cumulative CMD incidence (%) at Bungoma April-Oct 1999

Fig 2b. CMD incidence in multiple infection units (MIUs) at Bungoma April-Oct 1999. Legend: L = local cassava, M = Migyera, N = Nase-2 and S = SS-4.

Fig 2c. Cumulative CMD incidence (%) at Kakamega April-Oct 1999

Fig 2d. CMD incidence in MIUs at Kakamega April-Oct 1999. Legend: L = Local cassava, M = Migyera, N = Nase-2, S = SS-4.
Fig. 2e. Cumulative CMD incidence (%) at Siaya April-Oct 1999

Fig. 2g. Cumulative CMD incidence(%) at Siaya August 1999 - Mar 2000

Fig. 2f. CMD incidence in MIUs at Siaya April-Oct 1999.
Legend: L = local cassava, M = Migyera, N = Nase-2, S = SS-4

Fig. 2h. CMD incidence in MIUs at Siaya August 1999 - March 2000.
Legend: L = Local cassava, M = Migyera, N = Nase-2, S = SS-4.
Fig. 3a Cumulative and actual CMD incidence at Bungoma April - October 1999

Fig. 3b Cumulative and actual CMD incidence at Bungoma April - October 1999

Fig. 3c Cumulative and actual CMD incidence at Bungoma April - October 1999

Fig. 3d Cumulative and actual CMD incidence at Bungoma April - October 1999

Fig. 3e Cumulative and actual CMD incidence at Kakamega April - October 1999

Fig. 3f Cumulative and actual CMD incidence at Kakamega April - October 1999
Fig. 3g. Cumulative and actual CMD incidence at Siaya April - October 1999

Fig. 3h Cumulative and actual CMD incidence at Siaya April - October 1999

Fig. 3i Cumulative and actual CMD incidence at Siaya April - October 1999
Fig. 4a. Whitefly population at Siaya April-October 1999

Fig. 4b. Whitefly population at Siaya August 1999-March 2000

Fig. 4c. Whitefly population at Kakamega

Fig. 4d. Whitefly population at Kakamega

Fig. 4e. Whitefly population at Bungoma
4.3.2 Area under disease progress curve (AUDPC)

There was a significantly larger AUDPC at Siaya than all other sites in plantings of April 1999. There was no significant difference in AUDPC between Kakamega and Bungoma. In Siaya where disease incidence was also recorded for a second planting, there was a significantly larger AUDPC of 28.39 (p = 0.05) for April 1999 plantings than 1.79 (p = 0.05) for August 1999 plantings. The improved varieties TMS 30572, TMS 30337 and SS-4 had consistently small AUDPCs at all times in all localities. Serere showed the largest AUDPC in all experiments and at all sites (Table 14).

Table 14 Areas under the disease progress curves (AUDPCs) for CMD in different cassava varieties in on-farm trials and multi-locational trials at three sites in districts of western Kenya April-Oct 1999 and at two sites in Aug 1999-Mar 2000

<table>
<thead>
<tr>
<th>Variety</th>
<th>Kakamega 1999</th>
<th>Siaya 1999</th>
<th>Bungoma 1999</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serere</td>
<td>205</td>
<td>0</td>
<td>645</td>
</tr>
<tr>
<td>TMS 30572</td>
<td>12</td>
<td>0</td>
<td>37</td>
</tr>
<tr>
<td>TMS 30337</td>
<td>61</td>
<td>0</td>
<td>114</td>
</tr>
<tr>
<td>SS-4</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mean</td>
<td>70</td>
<td>0</td>
<td>199</td>
</tr>
</tbody>
</table>

Lsd (p = 0.05) for site differences = 16 in 1999 and 18 in 1999-2000
Lsd (p = 0.05) for cultivar differences =25, ns = not significant

4.3.3 CMD symptom severity

The mean CMD symptom severity score were not significantly different for Siaya (mean = 3.1, p = 0.05) and Kakamega (mean = 3.0 p = 0.05) sites but were different for Bungoma (mean =2.6 p = 0.05) site in plantings of April 1999. In plantings of August 1999, the mean severity score at Siaya was 2.1 ( p = 0.05 ) and was significantly different from that at Kakamega ( mean = 0, p = 0.05 ). The disease severity in each cultivar is shown in Table 14a. The combined mean CMD severity score of all the varieties differed significantly
Table 14a Disease severity in each cultivar (April -Oct 1999) at 7 MAP

<table>
<thead>
<tr>
<th>Variety</th>
<th>Site</th>
<th>Kakamega</th>
<th>Siaya</th>
<th>Bungoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serere</td>
<td></td>
<td>3.06 (0.78)</td>
<td>4.20 (1.26)</td>
<td>3.15 (0.85)</td>
</tr>
<tr>
<td>TMS 30572</td>
<td></td>
<td>2.04 (0.42)</td>
<td>2.09 (0.10)</td>
<td>2.04 (0.07)</td>
</tr>
<tr>
<td>TMS 30337</td>
<td></td>
<td>2.19 (0.21)</td>
<td>2.26 (0.21)</td>
<td>2.08 (0.11)</td>
</tr>
<tr>
<td>SS-4</td>
<td></td>
<td>2.00 (0.01)</td>
<td>1.00 (0.00)</td>
<td>1.00 (0.00)</td>
</tr>
</tbody>
</table>

Values in parenthesis = SD

Table 14b Combined disease severity in each cultivar (April -Oct 1999) at 7 MAP

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>(N)</th>
<th>Mean Severity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serere</td>
<td>(72)</td>
<td>4.0 a</td>
</tr>
<tr>
<td>Nase-2</td>
<td>(72)</td>
<td>2.3 b</td>
</tr>
<tr>
<td>TMS30572</td>
<td>(70)</td>
<td>1.5 c</td>
</tr>
<tr>
<td>SS-4</td>
<td>(72)</td>
<td>0.1 d</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td>2.0</td>
</tr>
</tbody>
</table>

CV = 56, R² = 0.6  * Means significant at p = 0.05

Symptoms were usually more severe on Serere than on TMS 30337, while TMS 30337 developed more conspicuous symptoms than TMS 30572 and SS-4. SS-4 did not show symptoms at Siaya and Bungoma, while at Kakamega only one plant developed conspicuous symptoms in plantings of April 1999. The cv TMS 30572 and TMS 30337 had lower CMD severity scores at all sites in all experiments and OFTs. Significant differences existed among varieties at different stages of growth, especially at 1-3 MAP, but no clear trends were observed in the final stages of growth at 4-6 MAP.
Significant interactions for CMD symptom severity were observed between site and month, site and cultivar and also between month and cultivar (Table 15). CMD symptoms were evident in Serere 2-3 MAP and reached maximum mean severity score of 4 (p =0.05) at the final record 6 MAP when symptoms were barely noticeable on TMS 30572 and SS-4. In SS-4 the symptoms reached a maximum score of 2 and the one plant infected at Kakamega had recovered at harvesting, 10 MAP. The trends in symptom severity were consistent in all sites, in plantings of April 1999 but in August 1999 plantings at Siaya, the overall maximum mean severity score of 2.6 ( p = 0.05) was reached at 6 MAP. In plantings of August 1999 CMD severity in Serere reached a significant maximum mean severity of 2 (p = 0.05) compared to TMS 30572 and TMS 30337 which reached maximum mean severity of 2.2 ( p= 0.05) for both. SS-4 was not infected.

Table 15 Interaction between CMD severity and site, month and cultivar

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Mean square</th>
<th>F-value</th>
<th>Pr&lt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site*Month</td>
<td>10</td>
<td>0.2</td>
<td>3.5</td>
<td>0.01</td>
</tr>
<tr>
<td>Site*Cultivar</td>
<td>6</td>
<td>2.2</td>
<td>40</td>
<td>0.01</td>
</tr>
<tr>
<td>Month*Cultivar</td>
<td>15</td>
<td>2.4</td>
<td>43</td>
<td>0.01</td>
</tr>
</tbody>
</table>

CV = 17, R² = 0.93, Mean CMD severity = 1.4

4.3.4 Recovery

The TMS clones recovered and all were symptomless towards the end of the experiment (3a-h). There was a significant relationship (P < 0.05) between all the sites in the number of plants recovered at each site. There was a significantly (p<0.05) greater recovery in the period between 4th and 5th MAP
than 1st and 4th MAP but there was no significant recovery during the 5th and 6th month (0.09, p < 0.05). There was significantly more recovery in the variety TMS 30337 than in any of the other clones studied. There was no significant difference in recovery between SS-4 and Serere (difference between means = 0.01, p = 0.05). This was because SS-4 was least infected due to its good levels of resistance, whereas no plant recovered in Serere, which was highly susceptible to CMD at the time of the experiment (fig. 3a-i).

4.3.5 Adult whitefly populations

Numbers of adult whiteflies increased to maxima 1-3 and 3-5 MAP in plantings of April 1999 and in plantings of August 1999, respectively (Figure 4a-e). Numbers were greatest 1 and 2 MAP in all sites in plantings of April 1999 and declined from 3-6 MAP. Early significant interactions (p < 0.01) between genotypes and stage of growth for whitefly numbers were evident at 1,2 and 3 MAP for cassava grown in April 1999, while there were more pronounced significant interactions in plantings of August 1999 at 3-6 MAP.

Maximum adult whitefly populations were significantly different at all locations in both sets of trials. Numbers were significantly larger at Siaya (mean of all observations = 0.94, p = 0.05), than at Kakamega (mean of all observations = 0.81, p = 0.05) and at Bungoma (mean of all observations = 0.65, p = 0.05) in the plantings of April 1999. In plantings of Aug 1999 the numbers were significantly larger at Siaya (mean of all observations = 2, p = 0.05) than at Kakamega (mean of all observations = 1, p = 0.05).
In plantings of April 1999 the populations of adult whiteflies were significantly different among all genotypes, TMS 30337 had the highest mean of all observations 1.09 (p = 0.05) while the lowest population mean of all observations 0.64 (p = 0.05) was obtained in SS-4. In plantings of Aug 1999, the highest mean of all observations was 2.07 (p = 0.05) for SS-4 and in Serere the lowest mean of all observations was 1.13 (p = 0.05).

4.4 Growth and yield of varieties under trials

The mean plant height calculated for all plants of each variety varied from 0.6 m in Nase-2 to 2.6 m in Serere and total plant weight from 0.3 kg in Serere to 9.0 kg in Nase-2. Mean total fresh tuberous root weight, total number of tuberous roots per plant, harvest index and average weight per individual tuberous root were <0.1-5.0(±1.0) kg; 1-16(±0.12); <0.18-0.98(±0.14) and <0.05-1.5(±0.19) kg, respectively. Fresh tuberous root weights were equivalent to 27.75 t/ha for SS-4 at Kakamega, 17.36 t/ha at Siaya and 11.1 t/ha at Bungoma; TMS 30572 yielded 24.20 t/ha at Kakamega, 21.00 t/ha at Siaya and 18.21 t/ha at Bungoma; TMS 30337 yielded 22.43 t/ha at Kakamega, 11.70 t/ha at Siaya and 15.90 t/ha at Bungoma; Serere yielded 18.43 t/ha at Kakamega, 13.18 t/ha at Siaya and 11.24 t/ha at Bungoma (Fig.5).

The highest yield was obtained from the variety SS-4 at the OFT Kakamega whereas it gave the lowest yield in the OFT trial at Bungoma. Plant height differed significantly between Kakamega and Bungoma, Siaya and Kakamega, but there was no significant difference in plant height between Siaya and Bungoma. Kakamega and Siaya were significantly different in plant weight,
but Kakamega and Bungoma, Siaya and Bungoma did not differ significantly in plant weight. The total number of tuberous roots per plant differed significantly across all sites. Fresh tuberous root weight did not differ significantly between Siaya and Bungoma, but was significantly different between Kakamega and Siaya and also between Kakamega and Bungoma. The mean individual tuberous root weight was not significantly different between Kakamega and Siaya, but was significantly different between Siaya and Bungoma and between Bungoma and Kakamega. The harvest index was significantly different between Bungoma and Kakamega only.

4.4.2 Adult Whitefly Populations

Adult whiteflies were not seen on any of the plants at harvest 10 MAP. This is because at this stage, whiteflies have migrated to younger cassava plants in new fields.

4.4.3 Cassava Mosaic Disease severity

The CMD severity scores at harvest were highly significant between all cultivars (p = 0.01). There was a significant difference in the mean severity scores between plants at Siaya and those at Bungoma (p = 0.05), and was also between Kakamega and Bungoma, but not between Siaya and Kakamega. The mean severity score was 2.73 (±2.32) across all sites. The highest mean severity score was shown by the cultivar Serere to be 4.22 (±2.77), and the lowest in TMS 30572 was 2.20 (±1.47). An intermediate CMD score of 2.47(±1.68) was obtained in TMS 30337. Plants harvested, of the variety SS-4 did not express CMD symptoms. The highest significant yield loss due to CMD
was experienced in Serere at Bungoma 0.8 kg and in TMS 30337 at Siaya 0.8 kg.

### 4.4.4 Correlation of growth and yield parameters

Correlation analysis of data from all sites was used to construct a Pearson's (Table 16) correlation matrix for growth and yield parameters.

<table>
<thead>
<tr>
<th></th>
<th>PHt</th>
<th>PWt</th>
<th>TNO</th>
<th>TWt</th>
<th>ITWt</th>
<th>HI</th>
<th>CMD:S</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHt</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PWt</td>
<td>-0.2*</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNO</td>
<td>-0.7*</td>
<td>0.6*</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TWt</td>
<td>-0.3*</td>
<td>0.9*</td>
<td>0.6*</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ITWt</td>
<td>-0.2*</td>
<td>0.4*</td>
<td>-0.3*</td>
<td>0.5*</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HI</td>
<td>-0.3*</td>
<td>0.02*</td>
<td>0.02*</td>
<td>0.3*</td>
<td>0.4*</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>CMD:S</td>
<td>0.8*</td>
<td>-0.3*</td>
<td>-0.2*</td>
<td>-0.4*</td>
<td>-0.2*</td>
<td>-0.3*</td>
<td>1.0</td>
</tr>
</tbody>
</table>

* significant at the 5% level, ‡ significant at the 1% level. PHt = Plant height, PWt = Plant weight, TNO = Tuberous root numbers per plant, TWt = Fresh tuberous root weight, ITWt = Mean individual tuberous root weight, HI = harvest index, CMD:S = severity.

There were significant and negative correlations (p<0.01) between health status and all six of the growth and yield parameters. Total plant weight was significantly correlated with four of the five other growth and yield characteristics. Total number of tuberous root, fresh tuberous root weight and mean individual tuberous root weight were correlated with the harvest index. The mean individual tuberous root weight was negatively correlated to tuberous root number. The correlation between symptom score with plant
weight, tuberous root number fresh tuberous root weight and mean individual

tuberous root weight and harvest index were all negative and highly significant
(p<0.01). There was a slight positive correlation between CMD severity score

and plant height, this could be due to the infected plants having originated from
healthy material, such that CMD had only a slight deleterious effect on plant

height performance.

CMD significantly affected plant weight in all sites but the effect was less

pronounced in Bungoma, where it accounted for only 4% loss in total

variability. Total tuberous weight was decreased significantly in Kakamega and

Siaya where CMD accounted for 22% and 17%, respectively, while it

accounted for 9% decrease of total tuber weight decrease in Bungoma.

Tuberous root numbers, average individual tuberous root weight and harvest

index were significantly affected by CMD in all sites. However effect of CMD

on tuberous root numbers in Bungoma was less pronounced.

Regression models fitted to the untransformed data by site indicated that for
each unit increase in CMD severity score, fresh tuberous root weight and total

plant weight per plant decreased by 0.24 kg and 0.34 kg, respectively. 

Tuberous root number, average individual tuberous root weight and harvest

index decreased by 0.42 tubers, 0.03 kg and 0.36, respectively.

The yield data plotted in (Fig.5) show a general trend in weight decrease in
plants that were diseased. Results are not available for SS-4, which had only
one diseased, plants showing symptoms during the growth period. TMS 30572
yielded averagely 2.0 kg tuberous roots per plant in all the sites. In Bungoma the diseased TMS 30572 yielded more than the healthy plants.

**Table 17** Percentage yield loss in 4 cassava genotypes at 3 different locations due to CMD

<table>
<thead>
<tr>
<th>Variety</th>
<th>Siaya</th>
<th>Bungoma</th>
<th>Kakamega</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS-4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TMS 30572</td>
<td>13</td>
<td>0</td>
<td>32</td>
</tr>
<tr>
<td>Serere</td>
<td>30</td>
<td>43</td>
<td>50</td>
</tr>
<tr>
<td>TMS 30337</td>
<td>50</td>
<td>41</td>
<td>25</td>
</tr>
</tbody>
</table>

LSD at 5% = 29, cv = 1%

At 5 % level the difference in yield between healthy and diseased plants harvested was significant for all areas. TMS 30572 showed a significant loss in yield between healthy and diseased plants in Kakamega only (Table 17).
Figure 5. Yield in all varieties at all sites

Siaya April 99-March 2000

**Key**
- H – Healthy
- D – Diseased
- N/A – Not available
4.5 Virus types in samples from CMD spread experiments in western Kenya

4.5.1 Identification of viruses by PCR

Amplification of the virus DNA by the universal primer identified that eighteen of twenty-one samples contained virus DNA. The primers UV-AL1/F1 and UV-AL1/R1 (Table 3) detected UgV in 9 of 11 samples from Kakamega site and UgV in 9 of 10 samples from Siaya. The primers ACMV-AL1/F and ACMV-CP/R3 detected ACMV in only one sample and this was from Siaya (Plate 4b). The primers EACMV-CP/Ra and EACMV-CP/Rb detected EACMV in all the eighteen samples in which UgV had been detected by UgV primers (Plate 4a).

4.5.2 Identification of viruses by RFLPs

Four samples from Kakamega and six samples from Siaya showed a positive reaction for both EACMV and UgV when digested with EcoRV, which does not distinguish between the two because these on digestion produces fragments of very close molecular weights i.e. EACMV has 2199 bp and 585 bp whereas UgV has 2197 bp and 585 (Plate 5a and 5b). However, following digestion by MluI it was established that the 3 samples from Kakamega had UgV only, whereas 4 of the six samples from Siaya had UgV only (Plate 6a) and the fifth had a co-infection of UgV and EACMV in the same plant; the sixth sample had EACMV only (Plate 6b). Digestion with MluI releases three fragments for EACMV, these being 1212 bp, 1057 bp and 515 bp, whereas it releases 4 fragments for UgV, these being 1212 bp, 670 bp, 515 bp and 385 bp.
4.6 CMD incidence in SS-4 cassava ratoons

At Siaya 60 symptomless plants of SS-4 cassava were ratooned. Of these two (3.6%) expressed CMD symptoms on the ratoon growth, whereas at Kakamega three (3.8 %) plants of 80 were infected as ratoons. At Bungoma none of the SS-4 cassava plants was affected by CMD. The three stem cuttings derived from each plant from all sites were not infected with CMD. All cuttings sprouted with healthy shoots. The CMD incidence in the ratoons at all sites was not significantly different (p = 0.05).. Results indicating the whitefly population and CMD incidence of the parent SS-4 crop have already been reported in section 4.3 of this thesis. The results also show that the virus moves down to the base of the plant on infection and is later transported upwards when new shoots develop from the infected plant.

Table 18 Disease incidence in ratoons of SS-4

<table>
<thead>
<tr>
<th>Site</th>
<th>Healthy Plants sampled</th>
<th>Infected plants sampled</th>
<th>Infected Cutting</th>
<th>Ratoon Growth (Diseased)</th>
<th>Disease Incidence*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Siaya</td>
<td>60</td>
<td>0</td>
<td>0</td>
<td>2/60</td>
<td>3.6a</td>
</tr>
<tr>
<td>Kakamega</td>
<td>79</td>
<td>1</td>
<td>0</td>
<td>3/80</td>
<td>3.8a</td>
</tr>
<tr>
<td>Bungoma</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0/100</td>
<td>0 a</td>
</tr>
</tbody>
</table>

*Means followed by the same letter are not significant at p = 0.05
Plate 4a. (i) Fragments obtained from 21 samples after amplification of viral DNA using UV-ALI/Fl and UV-ALI/RI primers and gel electrophoresis. Lane 23 = Positive reaction for UgV. Lane 24 = Wells. Lane 25 = Control for UgV. Lane 26 = Standard PCR marker. (ii) Amplification of viral DNA of the same 21 samples (picture not shown) using EACMV-CP/Ra and EACMV-CP/Rb and gel electrophoresis gave the same result as in above picture.

Plate 4b. Fragments obtained from 21 samples after amplification of viral DNA using ACMV-ALI/F and ACMV-CP/R3 primers and gel electrophoresis. Lane 23 = Wells. Lane 24 = Positive reaction for ACMV. Lane 25 = Control for ACMV. Lane 26 = Standard PCR marker.
Plate 5a. Fragments obtained after digestion of viral DNA with EcoRV and electrophoresis in 1.2% agarose gel. Lane 23 = Wells. Lane 24, 25 and 26 = positive reaction with EcoRV, indicating either UgV or EACMV. Lane 27 = Standard PCR marker. Samples from Kakamega.

Plate 5b. Fragments obtained after digestion of viral DNA with EcoRV and electrophoresis in 1.2% agarose gel. Lane 23 = Standard PCR marker. Lane 24 = positive reaction with EcoRV, indicating either UgV or EACMV. Samples from Siaya.
Plate 6a. Fragments obtained after digestion of viral DNA with Mlu1 and electrophoresis in 1.2% agarose gel. Lane 23 = Wells. Lane 24 and 25 = positive reaction for UgV only. Lane 26 = Standard PCR marker. Samples from Kakamega.

Plate 6b. Fragments obtained after digestion of viral DNA with Mlu1 and electrophoresis in 1.2% agarose gel. Lane 23 = Standard PCR marker. Lane 24 25 and 26 = positive reaction for UgV only. Lane 27 = Mixture of UgV + EACMV. Lane 28 = positive reaction for EACMV only. Lane 29 = Wells. Samples from Siaya.
5.1 Vector Populations, Incidence and Severity of CMD in western Kenya

5.1.1 Varieties grown, incidence, severity and spread of CMD by *B. tabaci*

Thirteen cassava varieties were found growing in the localities surveyed. The predominant variety Serere is mainly preferred because it is 'sweet' and has good boiling properties. Some of the other cassava varieties grown are mainly long-term and because they store well in the ground, they can be left for up to 2 years or longer which is one of the reasons cassava is regarded as a famine reserve crop (Otim-Nape *et al.*, 1998b).

Bock (1983) indicates that CMD probably causes higher production losses in cassava in Kenya than any other disease of any other crop. This is consistent with the overall CMD incidence of 62.3% and the yield loss data obtained in the farmers' fields in Mumias/Butere, Teso and Siaya. All the varieties examined were to some extent affected by CMD but the overall incidence of infection was less than the 89% incidence reported in Western and Nyanza provinces, in 1998 (Legg *et al.*, 1999b). This is explicable because in districts like Teso and Busia as a consequence of the epidemic the farmers had introduced TMS 30572 and TMS 30337 varieties that are resistant to CMD.

An informal small scale survey of Nyanza in July 1998 showed little evidence of the pandemic which suggested that Winam Gulf and Nyando plain had acted as barriers to the movement of the whitefly from areas to the North that had already been affected (Anonymous., 1999). The situation had changed markedly...
by the time of the latest survey, which detected active CMD in parts of Suba
district, which is beyond Winam Gulf on the southern side of Lake Victoria.
This showed that Nyando Plains and Winam Gulf could not act as barriers to
stop the spread. In Mfangano and Rusinga islands a high CMD incidence was
associated with whitefly infection as was evidenced by new whitefly infection
exhibited by plants sampled.

Legg and Ogwal (1998), Otim-Nape and Thresh (1997) and Otim-Nape et al.,
(2000) report a southward expansion of the CMD epidemic 'front' in Uganda at
c. 20 Km per year. Their observations like our results as at Suba (Table 5), show
that CMD spread, in the epidemic area, is associated with large populations of
B. tabaci. There are several possible explanations for their abundance at the
'front' and they include: genetic changes in B. tabaci populations or changes in
their dispersion behaviour, environment, new virus strain, synergistic
relationship between virus and whitefly (Colvin et. al., 1999) and a possibly
new strain of B. tabaci associated with the pandemic (J.T. Legg, pers. comm.).

The severe symptoms associated with the CMD epidemic in this study have
been reported in Uganda (Gibson et. al., 1996). Results on PCR assay in this
study confirm the occurrence of the UgV virulent strain of CMD. This strain is
also associated with the epidemic in Kenya. The structural and physiological
changes induced in the cassava plant by this virus render the plant more suitable
for colonisation and development by B. tabaci (Colvin et. al., 1999).
Differences, encountered in whitefly number and activity, as well as differences in infection pressure could be due to seasonal factors associated with localized climatic and agro-ecological conditions. Similar results have been obtained in previous multi-locational studies in Uganda and Cameroon (Otim-Nape et al., 1998a; Legg, 1995; Fondong et al., 1997).

5.2 Yield of 'relic' cassava in post-pandemic zone in western Kenya

There is considerable genetic diversity of cassava in the three districts surveyed Butere/Mumias, Teso and Siaya. This is evident from the numerous and different varieties encountered. This is due to different preferences of farmers, and is consistent with the findings of Otim-Nape and Zziwa (1990) and Otim-Nape et al., (1994) in Uganda where different varieties are grown to satisfy food requirements and tastes, and also provide some security against the risks of pests diseases and the effects of unfavourable environment.

All the varieties encountered in the relic cassava sampled have been grown in the districts for a long time except variety SS-4. This is an improved variety released by National Agricultural Research Organisation in Uganda and its superior performance (yield = 23.6t/ha) was to be expected from experience in Uganda. The other varieties such as Serere and Kelesenzia are highly susceptible to CMD and generally yielded poorly 7.2t/ha and 3 t/ha, respectively. Otim-Nape et al., (1994) in Uganda, Fauquet et al., (1988a) in Côte d'Ivoire and Terry and Hahn (1980) in Nigeria reported similar poor yields of local cassava varieties.
The low tuberous root yields were related to the few tuberous roots per plant, low mean individual tuberous root weight and low harvest index (Table 7), which is an indication of poor accumulation of, assimilates in the storage organs (Cock, et al., 1979). The significant correlations (Table 8) between fresh tuberous root weight and other yield characteristics stresses the reliance of yield to these attributes. Cock (1978) found that leaves, petioles, stems and storage organs are all important components that determine yield and any factors that interfere with them will also affect yield.

5.2.1 Whiteflies, CMD severity, plant growth and yield

Adult whiteflies were recorded on ten plants assessed in Siaya and Teso districts, and not elsewhere and were not correlated to CMD severity at the time of recording. Lack of correlation between CMD severity score and the number of whiteflies present (Table 8) is attributed to the period of approximately one month required for symptoms to be expressed in a plant after inoculation by *B. tabaci* (Fauquet and Fargette, 1990). Therefore no significant relationship is to be expected between the presence of the whitefly and CMD incidence at this time because symptoms observed at sampling time must be the result of an earlier infection. Moreover whiteflies are more associated with rates of CMD spread, than with symptom severity and incidence (Otim-Nape et al., 1994), which were the parameters recorded. Fauquet et al., (1988a) similarly found that rates of spread at sites in Côte d'Ivoire were correlated to adult whitefly numbers recorded one month earlier.
Lack of correlations between adult whitefly numbers and growth characteristics and yield was to be expected because whiteflies cause little damage to cassava, and are important mainly as vectors of CMD. The low CMD incidence and low disease severity in the variety SS-4 indicate that the use of improved varieties with such high levels of resistance could improve yields, where CMD is a problem. The potential benefits to be gained by adopting this variety are emphasised by the poor yields and moderate to severe symptoms encountered in the susceptible varieties being widely grown in the districts.

The fact that CMD largely accounts for the poor yields recorded as indicated by the harvest index means that CMD has a severe debilitating effect on growth which mainly influence yield and therefore CMD reduces overall yield in western Kenya. The findings on yield loss caused by CMD are similar to those of Otim-Nape et al., (1994) in Uganda, Bock and Guthrie (1978) and Seif (1982) in Kenya, Terry and Hahn (1980) in Nigeria and Fargette et al., (1988) in Côte d'Ivoire; who reported that reduction in yield is mainly through the effects of the disease on growth parameters.

Results from the study show that CMD effect on yield accounted for 28% of the yield variability in all the districts which is consistent with simulations by (Cock et al., 1979; Otim-Nape et al, 1994; 1998) indicating that a reduction in the rate of vegetative growth of only 10% is likely to decrease yield by 20%.

Considering that a large proportion of the plants sampled were infected as cuttings and so had greatly impaired yields, this could be related to the mode of infection
(cutting or vectors), whereby yields are severely reduced if the infection is by cutting, and less so by vector, depending on stage at which the plant is inoculated (Terry and Hahn, 1980; Fargette et al., 1988; Sserubombwe, 1998). For CMD to reduce yield significantly it could have disrupted the plants metabolic activities and photosynthetic processes. Chant et al., (1971) and Ayanru and Sharma (1982) have reported similar finding.

The findings in this study clearly show that CMD is a major constraint to cassava production in western Kenya, and the need to introduce CMD-resistant varieties cannot be over emphasised. The study stresses the continuing need to assess yields in relation to CMD infection encountered and initiate appropriate CMD control measures.

5.3 Resistance of four cassava varieties to CMD in western Kenya

It is evident from the data presented that the spread of CMD in western Kenya was high at the beginning of the study and decreased towards the end of the experiment (Table 13). These results are not inconsistent with studies done elsewhere. Surveys done in Uganda (Otim-Nape, 1993) and much more recently in Kenya (Legg, 1999) indicated that an initially high rate of spread is a feature of the early stages of the pandemic. The low rates of spread encountered in plantings made in August 1999 are typical of those recorded during the late stage of the pandemic when there is little or no inoculum due to severe losses that occurred earlier and led to a significant reduction in the amount of cassava grown in the area.
Genotypes performed differently at different sites (Fig 2). This is an indication of the influence of climatic and other factors on the rate of spread. It may also be due to seasonal fluctuations in infection pressure as was reported by Fargette et al., 1985. Unfortunately this factor was not investigated in the study and is an area requiring further investigation in Kenya in future. The lack of spread at Kakamega (Table 13) is consistent with the results obtained in Uganda (Legg and Ogwal, 1998), where the amount and proximity of sources of inoculum largely determines how much spread can occur. By the time the study was done the pandemic front had already passed through Kakamega and the amount of cassava grown was greatly reduced. Moreover, the KARI-Regional Research Centre where the trial was done is surrounded by an area where little or no cassava was found growing within the distances of 250 m considered by Legg and Ogwal, (1998) to be optimum for spread from without. Furthermore Kakamega is surrounded by large areas of forest land and sugar cane plantations. The reduced rates of spread in Siaya in plantings of August 1999 compared with high rates of spread in plantings of April 1999 could probably be due to seasonal influences.

Climatic factors have been known to greatly influence adult whitefly populations and the rate of spread of CMD (Seif, 1981a). In plantings of April 1999 the rate of CMD spread was higher at Siaya than at Kakamega and Bungoma, which correlates with the rainfall and temperatures conditions at the sites. Rainfall reduced the adult whitefly population numbers in plantings of April 1999 at Siaya at 5 MAP (Fig 3b). The number of whiteflies shows an increase 7 MAP (Fig. 3c) and this could be due to a slight dry spell that was experienced at the time thereby favouring whitefly population increase. This coincided with the period of the main ('long') rains. Similar results
have been obtained in other studies in Kenya (Robertson, 1987), Côte d'Ivoire (Fauquet et al., 1988) and in Uganda (Otim-Nape, et al., 1998a). As the crops aged, fewer adult whiteflies occurred which could be due to adult whitefly migration as the physiological and nutritional status of the plants change and photosynthates begin to be translocated to the tuberous roots for storage (Fishpool et al., 1995; Legg, 1995).

5.4 Yield evaluation of four cassava cultivars grown under epidemic conditions in western Kenya

The cultivar Serere, yielded more than the improved Ugandan variety SS-4 in Bungoma (Fig.5). This may be because the performance of SS-4 is influenced by agro-ecological conditions such that the variety is best suited to high potential zones. The TMS varieties 30572 and 30337 yielded consistently well at all sites, although TMS 30572 yielded more than TMS 30337. This finding is consistent with results from similar studies in Uganda under CMD pandemic conditions (Sserubombwe, 1998). The high yield of SS-4 in Kakamega could be in response to favourable climatic conditions experienced in UM1, which differ markedly from those in LM2 in Bungoma and LM3 in Siaya.

5.4.1 CMD severity, plant growth, yield and whitefly population

All varieties were affected to at least some extent by CMD which was most prevalent in cv Serere. With the exception of SS-4 all the other three genotypes experienced losses due to CMD. This implies that under severe CMD pressure conditions like those of the trials, Serere would experience more yield loss than TMS 30337 (Fargette et al., 1987c; Otim-Nape et al., 1998a; Thresh et al., 1994a; 1998c). The higher yield
losses sustained by TMS 30337 in Siaya and by Serere in Bungoma could be due to the low levels of resistance in these varieties compared to TMS 30572.

The findings in this study are similar to results in other detailed studies in Uganda (Otim-Nape et al., 1994) and to less detailed ones in which yield was the main parameter recorded and vegetative growth was not assessed (Bock and Guthrie, 1978; Terry and Hahn, 1980; Seif 1982; Fargette et al., 1988).

The mode of infection and the time of first symptom expression are important factors in determining the effects of CMD on the yield of cassava (Terry and Hahn, 1980; Fargette et al., 1988; Sserubombwe, 1998; Sserubombwe et al., 1999). Diseased plants of TMS 30572 yielded more than healthy plants at Bungoma thereby emphasizing that late infection of cassava plants by whiteflies has little or no effect on cassava yield. Infection as cuttings or early inoculation by whitefly has more deleterious effects. There were no whiteflies at the time of harvest, which is consistent with previous studies showing that whiteflies find the older plants unpalatable and therefore migrate to younger plants (Fishpool and Burban, 1994; Fishpool et al., 1995; Legg, 1995). Though whiteflies were absent it was clear the plants that became diseased had been inoculated earlier by the whitefly. There is no reason to expect a direct relationship between the incidence of CMD and the whitefly population sampled at the same time because symptoms are known to appear 3-4 weeks later, that time being the latent period between inoculation of virus by the whitefly and symptom expression (Fauquet and Fergette, 1990), but a correlation has been found between the whitefly population at 3-4 weeks earlier and symptom expression 3-4 weeks later (Fauquet et al., 1988a).
5.5 Detection of virus type in samples from CMD spread experiments in western Kenya by Polymerase Chain Reaction and Restriction Fragment Length Polymorphism (RFLP)

Results from the PCR experiments indicate that new CMG variant EACMV/UgV (Zhou et al., 1997; Deng et al., 1997) is involved in the severe infection encountered in the experiments conducted in western Kenya. The results also indicate that co-infection of UgV and EACMV took place as reported earlier in Uganda (Harrison et al., 1997b). It has been reported that co-infected plants sustain more severe effects of the disease than plants that have a single infection (Harrison et al., 1997b; Fondong et al., 2000). The apparent inability of the PCR procedure and the restriction enzyme EcoRV to distinguish EACMV from UgV could be due to their DNA-A molecules sharing 92% similarity in nucleotide sequence as reported by Zhou et al., (1997) and Harrison et al., (1997b) but the restriction enzyme Mlu1 was able to distinguish the two viruses. Based on serological assays of a limited number of samples it was earlier reported that ACMV predominated to the west of the Rift Valley while EACMV was mainly found on the coast of East Africa (Harrison et al., 1997a). It appears that there is not such a clear separation of the two because EACMV was identified in this study and in a recent survey (Ogbe et al., 1996), which revealed EACMV in parts of Nyanza province in Kenya. The presence of UgV in Kenya is consistent with the view that Ugandan pandemic has been able to move rapidly from adjacent parts of Uganda into W. Kenya. It could also be that there is a high concentration of the virus in UgV and EACMV affected plants, such that whitefly populations migrating from these...
plants would be highly viruliferous. Results from Cameroon indicate that co-infected plants have a high concentration of the virus (Fondong et al., 2000). Vector transmission of geminiviruses is controlled by their coat protein (Briddon et al., 1990; Azzam et al., 1994) and whitefly-transmitted geminiviruses with different coat proteins are differentially adapted for transmission by different biotypes of B. tabaci (McGrath and Harrison, 1995). Thus the close association of EACMV with UgV could be because they are adapted to a similar mode of transmission as the two viruses could be sharing similar epitopes used in the transmission of the viruses.

Whitefly-transmitted geminiviruses are widespread in crops and weeds in the tropics and sub-tropics worldwide (Harrison, 1985). The geminiviruses are efficiently transmitted from plant to plant by Bemisia spp. (Harrison, 1985; Bedford et al., 1994; Markham et al., 1994). Though few studies have been reported on the movement of geminiviruses into and within crop fields, primary spread between fields has been shown to be more important than secondary spread within fields for CMD (Fauquet et al., 1988a; Fauquet and Fargette, 1990; Otim-Nape, 1993; Byabakama et al., 1997).

In this study planting was with 'clean' cassava cuttings and any plants that emerged with shoots bearing symptoms were rogued, so all the infected plants from which samples were extracted were due to primary infection from viruliferous whiteflies from neighbouring infected field plots. This implies that such spread of the UgV is very important and that only use of resistant planting material can provide effective control.
5.6 CMD incidence in SS-4 cassava ratoons

Fauquet and Fargette (1990) state that because immune varieties are not available for use in Africa, and that because the vector, virus and host are prevalent in many areas, successive plantings of the same clonally propagated stock should degenerate progressively and ultimately show disease incidence of 100%. This did not occur with SS-4 ratoons in my experiment. The percentage disease incidence was quite low (Table 19), which implies that SS-4 is highly resistant. It appears that it restricts movement of the virus altogether, thereby preventing its multiplication or else the virus is fully translocated to the tuberous roots with the photosynthates. This could be the only reason explaining the absence of the virus in the shoots from the stems of the parent plants. Knowledge of reversion and recovery (Fargette et al., 1994b; Rossel et al., 1992; Fauquet et al., 1988b; Alicai et al., 1999) indicate that in improved varieties like SS-4, virus incidence tends to reach an equilibria as plants become infected and others recover and express reversion. Such low rates of spread in SS-4 could easily be counter balanced by reversion and recovery. Since SS-4 has shown tolerance to CMD, it implies that it can be a good source of cuttings for further propagation, as discussed by Fargette et al., (1994b). Yield losses that would be experienced by extensive roguing can easily be avoided by introducing this variety both for multiplication and farmer adoption. Results recorded in section 4.3 indicate that SS-4 did not succumb to the infection pressure encountered at the time of growth, whereas Serere a local variety was severely infected under the same conditions. The absence of CMD in cuttings collected from the parent plant, whose ratoons emerged with CMD infections could be indicating that there was little or no virus in the stems. Serological studies of virus concentration in resistant and susceptible varieties have shown lower concentrations in resistant varieties (Fargette et al., 1996c). This is an important
their adoption will decrease the amount of inoculum present in a locality (Thresh et al., 1998), thus restricting spread of CMD to susceptible varieties being grown. This topic has been generally discussed such that statements and inferences have not been supported by detailed references or data because this work has not been reported elsewhere. Moreover the study was done in one year and conclusive assertions cannot be reached from these results alone, more detailed studies are needed.

5.7 The impact of CMD on cassava productions and practical implications

To control CMD, the strategy of choice, which is widely recommended, is deployment of resistant varieties and phytosanitation. Their relevance and applicability have been extensively reviewed (Thresh et al., 1994c; Thresh and Otim-Nape, 1994; Thresh et al., 1997; Thresh et al., 1998b; Legg, 1999b; Legg et al., 1999b; Otim-Nape et al., 2000). It is important to consider control strategies in areas of western Kenya that have been severely devastated by the CMD epidemic, and where crop yields are severely impaired. Precedents are provided by the approach used in Uganda to control the CMD epidemic, which have been very successful (Otim-Nape et al., 2000). To address the problem in Kenya CMD-resistant varieties TMS 30572 and SS-4 are currently (May 2000) being multiplied for distribution to farmers. A further fifteen to twenty best clones from new material obtained from IITA-ESARC regional breeding programme based at Serere in Uganda are being evaluated for resistance to other pests and diseases in Kenya and will be sent for on-farm trials. These materials will soon be made available to farmers after monitoring in On-Farm Trials (Legg et al., 1999a). Selection of healthy planting material by farmers might be effective in zones of low inoculum pressure referred to as 'post-epidemic' (Anonymous., 1997), if the material is derived from uninfected 'clean'
crops of varieties like Serere when improved clones are neither available nor acceptable once CMD has been brought under control. Informal surveys in Teso and Busia indicated there has already been an improvement in the health status of hitherto severely diseased local varieties and this needs further investigation.

Farmer varietal preferences and utilisation of cassava influence control strategies. Unpalatable ‘bitter’ cultivars no matter how CMD-resistant or productive are unlikely to find ready acceptance among local farmers who traditionally cultivate ‘sweet’ varieties (Bock, 1994a). This view is consistent with informal discussions with farmers involved in the multi-locational and OFTs who reported that TMS 30572, TMS 30337 and SS-4 were ‘bitter’ when cooked. This could be one reason why farmers seem to retain the many diseased local genotypes despite the poor yields.

Further, informal discussions with farmers reveal that they have little or no knowledge of what CMD is, which means they rarely select ‘clean’ material when planting. Similar attitudes have been encountered in Uganda (Otim-Nape et. al., 1997c). In areas where serious losses have been sustained, abandonment of cassava and adoption of other crops like sweet potato may have led to the observed differences in disease incidence and severity between districts.
5.8 Conclusions and recommendations

In the study a high genetic diversity of cassava was encountered. This indicates a need to conserve the local germplasm, as it may be highly preferred to the improved varieties being introduced. More study is needed to reveal other underlying reasons for such a high genetic diversity. Possibilities of conserving desirable local germplasm through tissue culture methods should be exploited, as it has not been investigated in Kenya.

The cassava variety SS-4 appears to translocate all virus particles to the roots, although further studies are needed to confirm this. The devastation caused by CMD to local cassava did not significantly affect the improved varieties under test in the region, which means that introduction, and deployment of these new resistant varieties or the sources of resistance they contain will restore cassava production.

Emergence of healthy cassava plants in localities previously swept by the pandemic shows that it is essential to encourage selection of healthy planting material if farmers are already practising the technology, or if not efforts should be made to inform farmers on the importance of selecting healthy materials and roguing diseased plants. More studies on the merits and limitations of selection and roguing may have to be done before campaigns for and against the practice are done. There is need for
farmers to be persuaded to grow improved varieties and also be educated about spread of CMD through cuttings.

From the study it emerges that the new virus strain UgV/EACMV-UgV appears to be moving rapidly within the affected zone and no local cassava variety is resistant to it. This means that no planting material should be moved from the pandemic zone to elsewhere like coastal Kenya.

As this study reveals the effectiveness of deploying CMD-resistant varieties, it is essential that other viable CMD control measures like biological control for the whitefly be considered due to its potential sustainability and lasting impact.

Results from the study reveal a decrease in CMD incidence since the height of the epidemic in 1998, which means that continuous monitoring of CMD spread through surveys is crucial before money and personnel are engaged in CMD control.

Though SS-4 was revealed as a highly resistant variety to CMD in this experiment, there is need to investigate its yield potential in more and diversified agro-ecological zones including those of limited fertility.

There is need to investigate what other factors influence rapid transmission of UgV and EACMV, apart from relying on the protein coat and clarify the underlying cause.
REFERENCES


ANONYMOUS, (1999). In: *Emergency Programme to combat the cassava mosaic disease pandemic in East Africa; A system wide whitefly IPM affiliated project*. IITA Nov 1999 pp. 38


APPENDIX 1: RAINFALL AND TEMPERATURE

Rainfall at Kakamega 1999-2000

Rainfall (mm)
### APPENDIX 2

**SOIL TESTS**

Soil samples assayed to determine the levels of mineral nutrients in each locality.

<table>
<thead>
<tr>
<th>Site</th>
<th>N</th>
<th>P</th>
<th>C</th>
<th>Na</th>
<th>K</th>
<th>Ca</th>
<th>Hp</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bungoma</td>
<td>0.13</td>
<td>Trace</td>
<td>0.42</td>
<td>0.14</td>
<td>0.32</td>
<td>1.4</td>
<td>-</td>
<td>5.9</td>
</tr>
<tr>
<td>Kakamega</td>
<td>0.18</td>
<td>2</td>
<td>0.26</td>
<td>0.14</td>
<td>0.22</td>
<td>0.80</td>
<td>0.5</td>
<td>4.3</td>
</tr>
<tr>
<td>Siaya</td>
<td>0.14</td>
<td>Trace</td>
<td>0.16</td>
<td>0.11</td>
<td>0.18</td>
<td>1.20</td>
<td>0.2</td>
<td>4.7</td>
</tr>
</tbody>
</table>

Deficiencies underlined

Soil tests done at KARI-NARL, Nairobi, Kenya.
### APPENDIX 3

#### RFLP Patterns using restriction enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Restriction site sequence</th>
<th>ACMV-K</th>
<th>ACMV-N</th>
<th>ACMV-NOg</th>
<th>EACMV</th>
<th>UgV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dra I</td>
<td>5'-TTT/AAA -3'</td>
<td>2 fragments: 2 fragments: 3 fragments: 2 fragments:</td>
<td>1 - 1723 bp 1 - 1771 bp 1 - 1723 bp 1 - 2614 bp</td>
<td>2 fragments: 1 - 993 bp 3 fragments:</td>
<td>2 fragments: 2 fragments: 2 fragments: 2 fragments: 2 fragments:</td>
<td>1 - 2612 bp 2 - 170 bp 1 - 170 bp</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 - 1039 bp 2 - 991 bp 2 - 50 bp 2 - 170 bp</td>
<td>1 - 1723 bp</td>
<td>2 fragments:</td>
<td>1 - 2614 bp</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 - 1723 bp 2 fragments: 3 fragments: 2 fragments: 2 fragments:</td>
<td>2 fragments:</td>
<td>1 - 2614 bp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eco RI</td>
<td>5' - G/AATTC -3'</td>
<td>3 fragments: 1 fragment: 1 fragment: 1 fragment: 2 fragments:</td>
<td>1 - 1915 bp 2 fragments: 2 fragments: 2 fragments: 2 fragments:</td>
<td>2 fragments:</td>
<td>2 fragments: 2 fragments: 2 fragments:</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 - 769 bp 2 fragments: 2 fragments: 2 fragments: 2 fragments:</td>
<td>2764 bp 2764 bp 2784 bp</td>
<td>1 fragment:</td>
<td>1 - 1863 bp 1 - 919 bp</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 - 78 bp 1 - 2764 bp 1 - 2764 bp 1 - 2784 bp</td>
<td>1 fragment:</td>
<td>2 fragments:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eco RV</td>
<td>5'-GAT/ATC-3'</td>
<td>2 fragments: 2 fragments: 2 fragments: 2 fragments: 2 fragments:</td>
<td>1 - 1480 bp 2 fragments: 2 fragments: 2 fragments: 2 fragments:</td>
<td>2 fragments:</td>
<td>2 fragments: 2 fragments: 2 fragments: 2 fragments:</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 - 1282 bp 2 fragments: 2 fragments: 2 fragments: 2 fragments:</td>
<td>1 - 1478 bp 2 fragments: 2 fragments: 2 fragments: 2 fragments:</td>
<td>2 fragments:</td>
<td>1 - 2197 bp 2 - 585 bp 2 - 585 bp</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 - 1286 bp 2 fragments: 2 fragments: 2 fragments: 2 fragments:</td>
<td>2 fragments:</td>
<td>2 fragments:</td>
<td>1 - 2199 bp 2 - 585 bp 2 - 585 bp</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 fragments: 2 fragments: 2 fragments: 2 fragments: 2 fragments:</td>
<td>1 - 1284 bp 2 fragments: 2 fragments: 2 fragments: 2 fragments:</td>
<td>2 fragments:</td>
<td>1 - 2199 bp 2 - 585 bp 2 - 585 bp</td>
<td></td>
</tr>
<tr>
<td>Hinc II</td>
<td>5'-GT(T,C)/(A,G)AC-3'</td>
<td>4 fragments: 3 fragments: 4 fragments: 2 fragments: 2 fragments:</td>
<td>1 - 1269 bp 1 fragments: 2 fragments: 3 fragments:</td>
<td>2 fragments:</td>
<td>2 fragments: 2 fragments: 2 fragments: 2 fragments:</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 - 1269 bp 2 fragments: 3 fragments: 3 fragments: 4 fragments:</td>
<td>1 - 1650 bp 3 fragments: 2 fragments: 4 fragments:</td>
<td>2 fragments:</td>
<td>1 - 1661 bp 2 - 1123 bp</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 - 697 bp 3 fragments: 3 fragments: 4 fragments: 2 fragments:</td>
<td>2 - 695 bp 3 fragments: 4 fragments: 2 fragments:</td>
<td>2 fragments:</td>
<td>1 - 1661 bp 2 - 1123 bp</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 - 417 bp 4 fragments: 3 fragments: 4 fragments: 2 fragments:</td>
<td>3 - 417 bp 4 fragments: 3 fragments: 4 fragments:</td>
<td>2 fragments:</td>
<td>1 - 1661 bp 2 - 1123 bp</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 - 379 bp 3 fragments: 4 fragments: 3 fragments: 4 fragments:</td>
<td>4 - 379 bp 4 fragments: 3 fragments: 4 fragments:</td>
<td>2 fragments:</td>
<td>1 - 1661 bp 2 - 1123 bp</td>
<td></td>
</tr>
<tr>
<td>Mlu I</td>
<td>5' A/CGCCTT3'</td>
<td>2 fragments: 1 fragment: 1 fragment: 3 fragments: 4 fragments:</td>
<td>1 - 1550 bp 1 fragment: 1 fragment: 3 fragments:</td>
<td>2 fragments:</td>
<td>2 fragments: 2 fragments: 2 fragments: 2 fragments:</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 - 1212 bp 1 fragment: 1 fragment: 3 fragments: 4 fragments:</td>
<td>2764 bp 2764 bp</td>
<td>1 fragment:</td>
<td>1 - 1212 bp 1 - 1212 bp 1 - 1212 bp</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 fragment: 1 fragment: 3 fragments: 4 fragments: 2 fragments:</td>
<td>2764 bp 2764 bp</td>
<td>1 fragment:</td>
<td>1 - 1212 bp 1 - 1212 bp 1 - 1212 bp</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 fragments: 4 fragments: 2 fragments: 2 fragments: 2 fragments:</td>
<td>2 fragments:</td>
<td>2 fragments:</td>
<td>1 - 1212 bp 1 - 1212 bp 1 - 1212 bp</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 fragments: 4 fragments: 2 fragments: 2 fragments: 2 fragments:</td>
<td>3 fragments:</td>
<td>4 fragments:</td>
<td>1 - 1212 bp 1 - 1212 bp 1 - 1212 bp</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 fragments: 4 fragments: 2 fragments: 2 fragments: 2 fragments:</td>
<td>4 fragments:</td>
<td>4 fragments:</td>
<td>1 - 1212 bp 1 - 1212 bp 1 - 1212 bp</td>
<td></td>
</tr>
</tbody>
</table>
### APPENDIX 3a I POLYMERASE CHAIN REACTION EXPERIMENTS

#### Sources of Samples

<table>
<thead>
<tr>
<th>Kakamega</th>
<th>Universal Primer test</th>
<th>Viruses detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>U+E</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>U+E</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>U+E</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>U+E</td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td>U+E</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>U+E</td>
</tr>
<tr>
<td>11</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Siaya</th>
<th>Universal Primer test</th>
<th>Viruses detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>-</td>
<td>U+E</td>
</tr>
<tr>
<td>15</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>18</td>
<td>+</td>
<td>U+E</td>
</tr>
<tr>
<td>19</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>21</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

#### 3a II SIGMA PCR MARKER (50-2000 bp)

Fragment sizes: base pairs

| 50       |
| 150      |
| 300      |
| 500      |
| 750      |
| 1000     |
| 1500     |
| 2000     |