OCCURRENCE AND INCIDENCE OF BANANA BUNCHY TOP DISEASE IN MAJOR BANANA GROWING REGIONS OF ZAMBIA

by

EMELIN MWENDA

A Dessertation submitted to the University of Zambia in partial fulfillment of the requirement for the degree of Master of Science in Agronomy.

University of Zambia

Lusaka

July, 2010.

DECLARATION

I, **EMELIN MWENDA**, do hereby declare that this research report is my original work and that this thesis has not previously been submitted to this or any other university for any degree or examination. Where other people's work has been referred to, I have acknowledged them and the details provided in the references section.

Signed:.....

EMELIN MWENDA

Date:.....

APPROVAL

This dissertation of **EMELIN MWENDA** is approved as fulfilling part of the requirements for the award of the degree of Master of Science in Agronomy by the University of Zambia.

NAME AND SIGNATURE **DATE**

1. **NAME**..... R. G. KAPOORIA (Prof.) .....
   SIGNATURE..... ..... 23-7-10 .....

2. **NAME**..... D. M. Lungu (Dr) .....
   SIGNATURE..... ..... 23/07/2010 .....

3. **NAME**..... E. M. Cakwiwa (Dr) .....
   SIGNATURE..... ..... 23/07/2010 .....

ABSTRACT

This study was conducted to determine the prevalence and incidence of Banana Bunchy Top Disease (BBTD) in major banana growing regions of Zambia. Banana Bunchy Top Disease is caused by Banana Bunchy Top Virus (BBTV) and is considered the most serious disease of banana (*Musa spp.*) worldwide. It can cause total crop loss if it is not diagnosed early.

A survey was conducted in Copperbelt, Eastern, Luapula, Lusaka and Southern Provinces of Zambia between May and November, 2009. A total of 15 districts were surveyed and involved 75 farmers. Farmers were interviewed regarding their knowledge on banana production and banana bunchy top disease using a questionnaire.

Based on visual symptoms, leaf samples were collected from symptomatic and asymptomatic banana plants in the field using FTA® cards. Serological tests were done using Polymerase Chain Reaction technique. Results of work done in the laboratory confirmed the presence of BBTV in all symptomatic plant samples analysed while only one asymptomatic sample tested positive for BBTV. Of the common banana cultivars grown in Zambia none showed resistance, although the level of susceptibility varied among them. Landraces showed low levels of susceptibility in comparison to the improved cultivars. Disease incidences were significantly different at $P \leq 0.05$ with average means ranging from 1 – 31.8%. The Presence of the Banana Bunchy Top Virus vector (*Pentalonia nigronervosa* Coq) was also confirmed in the field.

DEDICATION

My parents, Jonathan Zikanika Mwenda and Celia Mukosha Chintu, 'till we meet again.

ACKNOWLEDGEMENTS

I am greatly indebted to Prof. R.G. Kapooria, my research supervisor, for his valuable advice and guidance which made this study a success. My sincere gratitude also goes to Dr. L. Kumar of the International Institute of Tropical Agriculture (**IITA**), Ibadan, Nigeria for his input in the serological analysis of samples. My deepest gratitude goes to Mr P. Chikoti, R. Mulenga, M. Tembo and Mr G. Kaula for their guidance and allowing me access to the virology laboratory facilities at Mount Makulu Research Station.

I am also grateful to Dr. M. Mataa and Dr. P. Okori (Makerere University-Uganda) for their material support in form of books, critique and shaping this work at proposal level.

I owe a special thanks to the Zambian government and RUFORUM for financing this study through ZARI and UNZA- School of Agricultural Sciences respectively. Particularly, I would like to acknowledge the efforts of Dr. W. Mwale (Director-ZARI), Mr. D. Simumba (PARO-Training), Dr. M. Mwala, Dr. D.M.Lungu and Mrs. S.H.Ngandu for ensuring that financial and material support was made available on time.

Thanks to Kafula Chisanga and Mary Sikasote for a job well done during the survey and subsequent analysis of the data.

Finally, special thanks go to my husband Aubrey Chiselwa Sengebwila and daughter Isabella for their patience and love during my two (2) year study period at the University of Zambia.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

CHAPTER ONE

1.0. INTRODUCTION

Banana (*Musa spp.)* is one of the most important fruit crops in Zambia. Its production contributes significantly to food security and employment creation in both rural and urban areas. Banana cultivation thus contributes to the fight against poverty. The average yield of bananas for most small scale farmers in Zambia between 1993 and 2003 was reported at 3,182Kg/ha (FAO, 2004), with no report on commercial banana production. However, Zambia has potential to produce as much as 31,700 to 61,200 Kg/ha of bananas annually.

Present day bananas are a cross between *Musa acuminata* Colla and *Musa balbisiana* Colla species, which are believed to have originated in Malaysia and India respectively.

The best altitude for banana production is between sea level and 1800 metres (Ngeze and Gathumbi, 2004). Fertile, well drained soils with rainfall of 1000 mm to 1800 mm per annum are needed to achieve best yields. The optimum temperature for banana plants is between 25.5° C and 30.5° C. Being a tropical/ subtropical crop, temperatures below 16.5° C result in stunted growth.

The banana plant is a monocotyledonous, perennial-herbaceous plant belonging to the family *Musaceae.* It is composed of an above ground and underground stem. The above ground stem which is succulent and juicy bears leaves and inflorescence, while the underground stem, also known as a corm, bears roots. The banana inflorescence is covered with bracts which produce rows of female flowers and undeveloped male flowers. The fruits usually develop without fertilization, a term referred to as parthenocarpy. Propagation is vegetative by use of suckers or multiplication of disease free planting material through tissue culture. A fruit bunch is

harvested every nine (9) months, although this is dependent on the variety and management. The Pseudostem is always destroyed after the bunch is harvested. Commonly cultivated bananas are triploid in nature and are usually represented by AAB and ABB and have 2n = 3x = 33 chromosomes.

Bananas are available throughout the year in Zambia and are mainly taken as a dessert fruit belonging to the AAA triploid group. When ripe, a banana fruit contains 20% sugar, 3% protein, and small amounts of vitamins A, B, C, D and E. The fruit is also rich in potassium (K), Calcium (Ca), Iron (Fe), Sodium (Na) and Phosphorus (Chandler, 1995).

Bananas contain three natural sugars in the form of Sucrose, Glucose and Fructose. These simple sugars form carbohydrates which are a source of energy for the human body (Wardlaw and Kessel, 2002).

The banana fruit being a source of plant protein entails that it is important for tissue growth in human beings. When protein intake is inadequate but total caloric intake is sufficient, a condition referred to as kwashiorkor may occur (Balch and Balch, 1997).

Vitamins are chemical compounds that are required for normal growth and metabolism. Vitamin A (Beta- Carotene) is very important as it affects bones, eyes, hair, immune system, skin, soft tissues and teeth. Deficiency leads to allergies, loss of appetite, blindness, colds and insomnia among other things. Vitamin B is very important for body cells, eyes, gastrointestinal tract, hair, liver, mouth and nervous system. Deficiency symptoms include anemia, appetite loss, bad breath, depression, fatigue and hypertension. Vitamin C affects the adrenal glands, blood, bones, capillary walls, cells, heart, nervous system and teeth. Vitamin

C deficiency leads to anemia, bleeding gums, breath shortness, low infection resistance, stress and capillary wall ruptures (Harper, 1999; Balch and Balch, 1997).

Vitamin D affects the bones, heart, kidneys, nervous system, skin, teeth and thyroid gland. Inadequate intake of vitamin D leads to brittle and fragile bones, burning in the mouth and throat, diarrhoea, insomnia, irregular heartbeat, low blood calcium, rickets, soft bones and sensitivity to pain (Balch and Balch, 1997).

Vitamin E affects the arteries, blood vessels, heart, lungs, nerves, pituitary glands and skin. Its deficiency leads to enlarged prostate gland, A gastrointestinal disease, dry or falling of hair, impotence, muscle weakness and slow tissue healing (Balch and Balch, 1997).

In terms of minerals Calcium in bananas is very important for the maintenance of electrolyte balance in the body and also essential for bones. It is an essential mineral for overall health. Potassium on the other hand is important for blood, endocrine/digestive and nervous systems, heart, kidneys and muscles. Deficiency of potassium leads to constipation, continuous thirst, decreased blood pressure, dry skin, insomnia and hair problems (Harper, 1999).

Iron is essential for blood, bones, metabolic system, muscles, nails, skin and teeth. Deficiency symptoms include breathing difficulties, brittle nails, dizziness, anemia and sore or inflamed tongue (Morrison and Hark, 1999).

Sodium affects the lymphatic system, blood, muscles and nerves. Its deficiency leads to appetite loss, cramps, decreased resistance to infections, eye disturbances, fatigue, intestinal gas and vomiting (Morrison and Hark, 1999).

Finally, on the minerals, Phosphorus affects the bones, brain cells, circulatory and digestive system, eyes, liver, muscles, nerves and teeth. Its deficiency leads to bone pain, fatigue,

irregular breathing, nervous disorders, tooth problems, appetite loss, heart and kidney problems (Subar, 1998).

Despite all this importance attached to the banana fruit, its production in Zambia has declined over the years thereby failing to meet the increasing demand. This decline is partly due to pests and diseases, *inter alia.* Diseases caused by fungi are among the most important diseases of banana. Viral diseases rank as the second most serious problem which limits banana cultivation (Hadidi *et al.*, 1998). However, unlike fungal diseases, where chemical methods of disease control are successful, control of viral diseases is much more problematic. Viral infections of banana can generally cause losses of up to 30%, and occasionally up to 50 – 80% (Fraser, 1990).

There are four significant diseases of bananas that are caused by viruses. Banana Bunchy Top Disease (BBTD) is the most devastating and can cause total crop loss if early diagnosis and stringent sanitation is not practised. Other virus diseases include Banana Mosaic Virus (BMV), Banana Streak Virus (BSV), and Cucumber Mosaic Virus (CMV), (Jones, 2002; Ploetze *et al.*, 2003).

The presence of Banana Bunchy Top Disease has been suspected in banana growing areas of Southern, Copperbelt, Eastern, Luapula and Lusaka provinces of Zambia. Most farmers in Southern, Copperbelt and Lusaka provinces grow bananas on a commercial basis. In the other parts of the country, bananas are grown mostly on small family farms or traditional gardens located in wetlands or along streams.

The literature reviewed so far indicates that no research work has been done on the geographical distribution, prevalence and incidence of the virus causing Banana Bunchy Top

Disease in Zambia. Knowledge of the geographical distribution and prevalence of the disease can assist in the development of control measures like introduction of quarantine practices which can prevent the spread of the disease in Zambia. Disease incidence results can help plant inspectors to identify severely affected areas and prevent the movement of germplasm from such areas to non-affected areas in the country.

The overall objective of the study was to determine the prevalence and geographical distribution of Banana Bunchy Top Disease (BBTD) in major banana producing areas of Zambia. The specific objectives were to:

i. To assess the incidence of banana bunchy top disease in major banana growing areas of Zambia

ii. To confirm presence of the BBTV vector *Pentalonia nigronervosa* Coq in sampled fields.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. History and Geographical Distribution

The origin of Banana Bunchy Top Disease (BBTD) is not known with certainty. However, there is evidence to show its existence in Fiji in 1889 (Magnaye and Valmayor, 1995). Many Fiji plantations were abandoned by 1894 because of the severity of the infection, leading to the Island's banana export industry being threatened (Wardlaw, 1972). Exports fell from 788,000 bunches in 1892 to just 114,000 bunches in 1895 (Magee, 1953). The disease was subsequently reported in 1900 from Taiwan (Sun, 1961) and in 1901 from Egypt (Magee, 1953). This disease has also been reported from Sri Lanka and Australia around 1913 (Gowen, 1995). It is believed that Banana Bunchy Top Disease was introduced into Australia and Sri Lanka through infected banana suckers brought in from Fiji (Magee, 1927 and Wardlaw, 1972). By 1927, Magee reported that the disease incidence in Australia was in the range of 5 – 90%, although it has since been controlled through the implementation of strict phytosanitary control measures and government legislation (Harding *et al.*, 2001). In 1940, the disease was reported from India where it may have been introduced into Southern India from Sri Lanka (Magee, 1953). Interestingly, a very similar disease, abaca bunchy top disease in abaca (*Musa textilis*) was recorded in the Philippines in 1910 (Ocfemia, 1926). However, banana bunchy top disease was not recorded in the Philippines until 1960 (Castillo and Martinez, 1961). Vietnam was also affected by BBTD in 1968 (Vakili, 1969). Doon, (1995) later reported of the spread of the disease to Sarawak in Borneo of Malaysia. The disease has also been reported from Pakistan where damage recorded in some districts was more than 60% and production declined by 90% (Soomro *et al.*, 1992). According to Hu *et al.*, (1993)

and Ranasingh (2007) this disease has also been reported from China, Hawaii and Myanmar. Thomas and Iskra-Caruana (2000) and Pillay (2005) reported the occurrence of this disease from Angola, Burundi, Central African Republic, Congo, Democratic Republic of Congo (DRC), Gabon, Malawi and Rwanda (Thomas and Iskra-Caruana, 2000).

2.2. Symptomatology

The symptoms of Banana Bunchy Top Disease (BBTD) are quite distinctive and not likely to be confused with any other disease (Wardlaw, 1972). Symptoms may become apparent at any stage of plant growth. In severely infected banana plants, the leaves are typically bunched together at the apex (Figure 3), forming a congested rosette and hence the name "bunchy top" disease (Gowen, 1995).

Initial symptoms consist of dark green streaks in the veins of lower portions of the leaf midrid, petiole base and the pseudostem (Harding *et al.,* 1993). This symptom is also referred to as "Morse Code Streaking". This is due to the irregular streaks which resemble a series of breaking "dots" and "dashes". Infected leaves become stunted and chlorotic at the margins (Gowen, 1995). The leaves of infected plants become brittle in texture and the petioles are incompletely elongated. Severely infected plants do not bear fruit, and if fruits do develop, they are severely distorted, stunted and sometimes twisted or deformed and of little commercial value (Ranasingh, 2007). The lower hands of the bunch in infected plants often die off (PANS, 1977). Suckers that develop from infected "Mother" plants are also infected, indicating that the virus becomes widely distributed in the growing tissues (Figure 4). Magee (1953) noted that there are differences in disease symptoms shown by daughter suckers and plants infected from an outside source.

2.3. Economic Impact

Despite Banana Bunchy Top Disease being widespread in Asia and the South Pacific regions, there are no accurate estimates of the international economic impact. In most cases, the incidence of the disease is not well documented (Dale and Harding, 1998). However, Fiji, Australia, Pakistan and the Philippines can be used as examples where this disease has been well studied and has caused serious economic losses.

In the Islands of Fiji, the banana export industry began in 1877 with shipments of 3,100 bunches. Production rose to 359,000 bunches in the course of ten years; a peak being reached in 1892 with exports reaching 788,000 bunches. But with the outbreak of Banana Bunchy Top Disease three years later, banana export declined to 147,000 bunches (Wardlaw, 1972).

Banana Bunchy Top Disease was first recognized in Australia in 1913 and spread rapidly in the plantations on the border between the states of Queensland and New South Wales (Dale and Harding, 1998). By 1926, 90% of the original banana plantations in New South Wales were out of production (Wardlaw, 1972). Output fell from 460,000 cases of bananas in 1922 to 140,000 cases in 1925 (Dale and Harding, 1998). In the Currumbin district of South East Queensland, the number of banana plantations fell from 100 in 1922 to 4 in 1925 and production fell from 4,400 tonnes to 110 tonnes in the same period (Dale, 1987). In describing the devastating effect of the disease at the time, Magee (1927) reported of how difficult it was for someone, who had not visited the devastated areas, to visualize the completeness of destruction wrought in such a short time. Dale and Harding (1998) reports that the drastic impact on production was most certainly due to banana bunchy top disease alone. However, It is interesting to note that currently, due to a scheme of control based on

2.3. Economic Impact

Despite Banana Bunchy Top Disease being widespread in Asia and the South Pacific regions, there are no accurate estimates of the international economic impact. In most cases, the incidence of the disease is not well documented (Dale and Harding, 1998). However, Fiji, Australia, Pakistan and the Philippines can be used as examples where this disease has been well studied and has caused serious economic losses.

In the Islands of Fiji, the banana export industry began in 1877 with shipments of 3,100 bunches. Production rose to 359,000 bunches in the course of ten years; a peak being reached in 1892 with exports reaching 788,000 bunches. But with the outbreak of Banana Bunchy Top Disease three years later, banana export declined to 147,000 bunches (Wardlaw, 1972).

Banana Bunchy Top Disease was first recognized in Australia in 1913 and spread rapidly in the plantations on the border between the states of Queensland and New South Wales (Dale and Harding, 1998). By 1926, 90% of the original banana plantations in New South Wales were out of production (Wardlaw, 1972). Output fell from 460,000 cases of bananas in 1922 to 140,000 cases in 1925 (Dale and Harding, 1998). In the Currumbin district of South East Queensland, the number of banana plantations fell from 100 in 1922 to 4 in 1925 and production fell from 4,400 tonnes to 110 tonnes in the same period (Dale, 1987). In describing the devastating effect of the disease at the time, Magee (1927) reported of how difficult it was for someone, who had not visited the devastated areas, to visualize the completeness of destruction wrought in such a short time. Dale and Harding (1998) reports that the drastic impact on production was most certainly due to banana bunchy top disease alone. However, It is interesting to note that currently, due to a scheme of control based on

inspection, eradication and replanting with disease free material, this disease has been reduced to a minor one (Magee, 1953 and Wardlaw, 1972).

Similar losses due to Banana Bunchy Top Disease have been recorded in Pakistan where it was first recorded in 1988 in Sindh Province (Khalid *et al.,* 1993). Approximately, 60% reduction in production occurred. From 23,500 hactarage under banana production in 1988 it fell to 8,000 hactares in 1992 (Dale and Harding, 1998). This reduction was attributed to Banana Bunchy Top Disease (Khalid and Soomro, 1993).

In the Philippines and Vietnam the disease was so widespread that disease incidence in small holdings rose considerably to about 50% (Dale and Harding, 1998).

2.4. Aetiology and Epidemiology

Difficulties have been experienced in establishing the aetiology of Bunchy Top Disease (Gowen, 1995). This is because Banana Bunchy Top Virus was initially thought to be a *Luteovirus* (Mathews, 1982). This was based on the fact that the disease showed many characteristics of luteovirus infections such as not being sap transmissible, the persistent manner of transmission by aphids and induced phloem damage in infected plants (Dale and Harding, 1998). Subsequent evidence supported this theory. Dale *et al.,* (1986) successfully extracted dsRNA from Banana Bunchy Top Disease infected plants that was not present in non diseased plants and the electrophoretic pattern of this dsRNA was similar to the electrophoretic patterns of dsRNA extracted from plants infected with known luteoviruses. Iskra *et al.,* (1989) purified 28 nm isometric virus-like particles from plants infected with Banana Bunchy Top Disease. In contrast, Wu and Su (1990) purified 20-22 nm isometric virus like particles from infected plants and reported that these particles contained ssRNA of

about 6.0 kb. They described these particles as those of a small luteovirus and generated monoclonal antibodies for the detection of these particles (Dale and Harding, 1998).

However, it is now known that Banana Bunchy Top Virus is an isometric virus which is phloem limited and has a genome comprising of at least six different components of circular single stranded DNA (BBTV DNA -1 to -6) ranging in size from 1018 to 1111 nucleotides (Wu and Su,1990; Harding *et al.,* 2001; Burns *et al.,* 1994). This was proved when Harding *et al.*, (1991) and Thomas and Dietzgen (1991) purified 18- 20 nm isometric virus like particles from infected plants using modifications of the method of Wu and Su (1990) and Dale and Harding (1998).

Banana Bunchy Top Virus belongs to the *Nanovirus* group and is a member of the genus *Babuvirus* of the *Nanoviridae* family (Amin *et al.*, 2008). It has 18-22 nm isometric virions, a multicomponent ssDNA genome with a relative molecular mass of 2.0 x 106 and a coat protein sub unit with relative molecular mass of 20,000 (Thomas *et al.*, 2001 and Ranasingh, 2007).

Harding *et al.,* (1993) reported the sequence of the first component of the Banana Bunchy Top Virus genome. The component was shown to be circular ssDNA and 1.111kb (Dale and Harding, 1998). It contained a potential stem-loop structure, the loop sequence of which was almost identical to the invariant loop sequence of geminiviruses (Lazarowitz, 1992 and Dale and Harding, 1998).

The Bunchy Top Virus of bananas is disseminated in vegetative propagules through the movement of infected plants from one place to another (Gowen, 1995). But most importantly, it is spread from plant to plant by an aphid "*Pentalonia nigronervosa* Coq" which feeds on

infected plants and later transmits the virus to healthy banana plants (Magee, 1927, 1940, 1953 and Thomas *et al.*, 1994). Aphids are usually involved in short distance movement while diseased planting material is responsible for both short and long distance movement of the disease (Dale and Harding,1998).

Most of the epidemiological studies on Banana Bunchy Top Disease have been done in Australia. Allen (1978) reported that from an initial focus of infection the mean distance of new infections, presumably resulting from aphid infections, was 17.2 metres with 70% of new infections within 20 metres and 99% of new infections within 86 metres. Allen and Barnier (1977) found that when a new disease free plantation is set up, there is a chance of up to 88% getting the disease if it is located adjacent to a disease infested plantation. However, this dropped to a 27% probability if the nearest infected plantation was 50 to 1000 metres away (Dale and Harding, 1998).

The only confirmed hosts of the Banana Bunchy Top Disease are species within the genus *Musa* (*M. acuminata, M. balbisiana* and their hybrids, Fei bananas) and *Ensete ventricosum,* all members of *Musaceae* (Magee, 1927 and Dale and Harding, 1998). It is important to note that there is no known commercial cultivar of banana and plantain which has shown to be immune or highly resistant to Banana Bunchy Top Disease. However, variations do exist between cultivars in the rate of infection and the severity of symptoms (Magee, 1927, 1948 and Jose, 1981). The Dwarf Cavendish variety is very susceptible, much more so than some of the taller varieties (Wardlaw, 1972). *Musa ensete,* an African variety, is also susceptible to Banana Bunchy Top Disease (Magee, 1953).

infected plants and later transmits the virus to healthy banana plants (Magee, 1927, 1940, 1953 and Thomas *et al.*, 1994). Aphids are usually involved in short distance movement while diseased planting material is responsible for both short and long distance movement of the disease (Dale and Harding,1998).

Most of the epidemiological studies on Banana Bunchy Top Disease have been done in Australia. Allen (1978) reported that from an initial focus of infection the mean distance of new infections, presumably resulting from aphid infections, was 17.2 metres with 70% of new infections within 20 metres and 99% of new infections within 86 metres. Allen and Barnier (1977) found that when a new disease free plantation is set up, there is a chance of up to 88% getting the disease if it is located adjacent to a disease infested plantation. However, this dropped to a 27% probability if the nearest infected plantation was 50 to 1000 metres away (Dale and Harding, 1998).

The only confirmed hosts of the Banana Bunchy Top Disease are species within the genus *Musa* (*M. acuminata, M. balbisiana* and their hybrids, Fei bananas) and *Ensete ventricosum,* all members of *Musaceae* (Magee, 1927 and Dale and Harding, 1998). It is important to note that there is no known commercial cultivar of banana and plantain which has shown to be immune or highly resistant to Banana Bunchy Top Disease. However, variations do exist between cultivars in the rate of infection and the severity of symptoms (Magee, 1927, 1948 and Jose, 1981). The Dwarf Cavendish variety is very susceptible, much more so than some of the taller varieties (Wardlaw, 1972). *Musa ensete,* an African variety, is also susceptible to Banana Bunchy Top Disease (Magee, 1953).

2.5. The Aphid Vector

No other aphid, apart from the "*Pentalonia nigronervosa* Coq", is known to transmit Banana Bunchy Top Virus (Magee, 1927). Hafner *et al.,* (1995) reported that the Banana Bunchy Top Virus does not replicate inside the aphid vector.

The banana aphid is present worldwide where bananas (*Musa spp.*) are grown. Besides bananas, the banana aphid infests many tropical and subtropical plants which include *Alpina purpurata* (floral red and pink ginger), *Xanthosoma* (ape), *Cardamon, Heliconia* and tomatoes (Waterhouse, 1987 and Zimmerman, 1948). However, Xie *et al.,* (1996) reported that ginger is not a host of BBTV.

Like most aphids, the banana aphid is a phloem feeder that uses its long stylets to pierce plant tissue to suck the sap directly (Wardlaw, 1972).

Reproduction in the banana aphid is entirely parthenogenetic (without mating). The life cycle (nymph to adult) is completed in 9 to 16 days. The nymphs are more efficient vectors than adults (Magee, 1930).The virus is retained for at least 13 days after aphids have fed on an infected source and persists through the moult (Wardlaw, 1972). There is no egg stage, meaning that young ones are born live (Waterhouse, 1987).

Aphids may be spread throughout the plantation or a banana district by contact transfer from plant to plant in the same or adjacent rows; by movement of aphids over the soil; by the flight of winged individuals; by movement of aphid infected suckers; by translocation of aphid infected soil on the tools and on clothes of workers during cultural and harvesting operations (Magee, 1927 and Wardlaw, 1972).

The aphid colonies occur in the heart (crown) of the banana plant and between the pseudostem and the leaves, the conditions there being ideal for feeding and protection (Wardlaw, 1972). Throughout the greater part of the year, colonies consist of wingless females but later on develop wings and migration begins. This information helps to understand how new outbreaks may occur in isolated and protected areas planted with uninfected material (Wardlaw, 1972).

2.6. Ecological Relationship

Bunchy Top Disease is known to occur under tropical, subtropical and temperate conditions indicating that climatic factors have little influence on its incidence and development. However, seasonal fluctuations have a marked effect (Wardlaw, 1972). In summary, the most favourable conditions for the development of Bunchy Top Disease are periods of rain during the summer months.

2.7. Disease and Virus strains

The available evidence shows that there are different strains of Banana Bunchy Top Disease. For instance, Abaca Bunchy Top Disease in abaca (*Musa textilis*) was recorded in the Philippines in 1910.This disease was very similar to Banana Bunchy Top Disease in terms of biological properties, symptoms and transmission (Dale and Harding, 1998). However, Banana Bunchy Top Disease was not recorded in the Philippines until 1960. Ocfemia and Buhay (1934) could not transmit Abaca Bunchy Top Disease to bananas whereas Magee (1927) successfully transmitted Banana Bunchy Top Disease to abaca.

Karan *et al.*, (1994) worked on Banana Bunchy Top Virus isolates from 10 countries by sequencing and comparing their DNA component 1. It was found that there were two groups

of isolates, the Asian group (Taiwan, Philippines and Vietnam) and the South Pacific group (Australia, Burundi Egypt, Fiji, India, Tonga and Western Samoa). Two groups of isolates were also confirmed on Banana Bunchy Top Virus DNA component 2 to 6 (Karan, 1995). Dale and Harding (1998) believe these two groups represent the two groups of strains of Banana Bunchy Top Virus.

2.8. Control of Banana Bunchy Top Disease

Even though there are a number of control measures for Banana Bunchy Top Disease, it still continues to be a major limitation to banana production in some regions (Dale and Harding, 1998). Some of the control measures include:-

2.8.1. Prevention

Though Banana Bunchy Top Disease is widespread, it has not been reported from the Americas. This is the reason why the International Plant Genetic Resources Institute (IPGRI) and the Food and Agriculture Organization of the United Nations (FAO) have developed extensive recommendations on the movement of banana germplasm and planting material between regions (Frison and Putter, 1989). Where possible, the primary recommendation is that *Musa* germplasm be moved only as *in vitro* plantlets. However, Drew *et al.*, (1989) demonstrated that Banana Bunchy Top Disease could still be transmitted through plantlets *in vitro* when these plantlets are derived from diseased plants although, symptoms at this stage may not be evident.

Therefore, it is important that accurate and intensive diagnostic methods are employed to test *in vitro* material besides using visual symptoms before plantlets are released into non-infected areas (Dale and Harding, 1998). Wu and Su (1990) generated a range of monoclonal

antibodies that can be used in ELISA, while Thomas and Dietzgen (1991) developed both monoclonal and polyclonal antisera for the detection of banana bunchy top virus using ELISA. Harding *et al.*, (1991) also developed a DNA probe for the detection of Banana Bunchy Top Virus DNA component 1 using either dot blot or southern blot analysis. Karan *et al.*, (1994) developed a range of oligonucleotide primers for the amplification of all known sequence variants of Banana Bunchy Top Virus DNA components (Burns *et al.*, 1995; Karan, 1995 and Dale and Harding, 1998). Preventative measures such as stringent quarantine procedures, when adopted and carefully practised, have potential to reduce the risk of further dissemination of bunchy top virus (Gowen, 1995).

2.8.2. Virus free planting material

It is possible to provide large quantities of virus tested banana germplasm, with the advent of *in vitro* propagation and the availability of a range of sensitive and specific Banana Bunchy Top Virus diagnostic methods (Dale and Harding, 1998). Another method is through official inspection and certification schemes where disease-free plantings are identified as suitable sources of propagation material (Gowen, 1995). The only challenge with both approaches is to ensure the production of the required large quantities.

2.8.3. Resistant Cultivars

There have been reports of resistance in some varieties though they are not completely immune to the disease. Jose (1981) tested a range of banana cultivars and found that *Kanchikela* and *Venattukunnan* belonging to the *balbisiana* type (BB and BBB) were most resistant. Under experimental conditions neither plants were immune, but fewer plants became infected with Banana Bunchy Top Virus compared to the Gros Michel and many

other varieties of the *acuminata* group with AA or AAA genomes (Dale and Harding, 1998). This means that there is a possibility of selecting cultivars that can potentially slow the progression or reduce the incidence of the disease.

The differences in susceptibility to infection provide a scope for breeding a Banana Bunchy Top Virus resistant or tolerant variety. However, this is only possible when a suitable source of resistance is identified in a cultivar (Gowen, 1995 and Dale and Harding, 1998). However, work has been slow in this area and so far no immune variety has been identified. Dale (1987) also considers the possibility of use of biotechnology techniques to develop genetically engineered forms of resistance which can eventually play an important role in the control of the disease.

2.8.4. Vector control

Applications of insecticide to decrease the aphid population can be used to restrict the spread of Bunchy Top Virus (Gowen, 1995). However, other problems accompany the use of insecticides such as toxicity to consumers and operators, expense and harmful environmental effects.

2.8.5. Inspection and Roguing

Regular inspection of plantings and early eradication of diseased plants is an effective and important strategy for control of Banana Bunchy Top Disease (Dale and Harding, 1998). However, this requires knowledge and ability to diagnose the disease from early symptoms.

2.8.6. Transgenic Resistance

Transgenic virus resistance, based on virus- derived transgenes, has been widely demonstrated to be an effective strategy for the control of plant viruses (Dale and Harding, 1998). This strategy can specifically work for bananas as there is no known variety that is immune to Banana Bunchy Top Disease and conventional banana breeding programs have yet to prove effective (Dale, 1990). May *et al.,* (1995) and Sagi *et al.,* (1995) have reported of the regeneration of genetically transformed bananas. May *et al.*, (1995) transformed the variety Grand Nain using *Agrobacterium* - mediated transformation while on the other hand Sagi *et al.*, (1995) were able to transform the cultivar Bluggoe with the use of microprojectile bombardment. Six ssDNA components have been sequenced (Burns *et al.*, 1995; Harding *et al.,* 1993 and Xie and Hu, 1995). This work should lead to the Banana Bunchy Top Virus derived resistance genes being incorporated into bananas which can hopefully result in Banana Bunchy Top Virus resistant transgenic bananas (Dale and Harding, 1998).

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. General features of study area

A survey was conducted in Zambia's major banana growing regions during the period of May to October, 2009. A total of 15 districts were sampled which are spread out over five provinces of Zambia and different agro-ecological regions (Figure 1). The selected provinces were Copperbelt, Eastern, Luapula, Lusaka and Southern Provinces (Figure 2). On average, more than 40% of a province was sampled bearing in mind the status of banana production in a particular district of a chosen province.

Kitwe, Masaiti, and Ndola districts were covered in Copperbelt Province while Nyimba, Katete and Chipata distrcts were picked in Eastern Province. Luapula Province had Mansa, Mwense and Kawambwa. In addition to this, Lusaka and Kafue districts of Lusaka Province were surveyed, with Chiawa farmers being grouped together with Kafue farmers due to proximity reasons. The last province tackled was Southern province with Choma, Mazabuka, Monze and Siavonga districts. The selection of individual provinces was done with the help of the Department of Agriculture and the Zambia Agriculture Research Institute of the Ministry of Agriculture and Cooperatives. Provincial Agricultural Officers later on assisted with the selection of major banana growing districts in a province. Individual Farms in each district were then randomly selected.

3.1.1. Zambia's agro-ecological regions

Zambia has three different agro-ecological regions namely regions I, II and III (Figure 1).

3.1.1.1. Region I

Chiawa, Nyimba and Siavonga districts are found in region I. This region is characterized by major valleys such as Gwembe, Lunsemfwa and the Luangwa valleys, which lie between 300 and 900 metres above sea level. Other areas of region I include the Southern parts of Western and Southern Provinces with elevation between 900 and 1200 metres.

Region I has the shortest growing season with low mean annual rainfall which does not exceed 800 mm and more prone to droughts in comparison to other parts of Zambia.

Relatively high temperatures are characteristic of region I, with mean daily temperatures that may vary from 20^0 C to 25^0 C and be sometimes as high as 38^0 C in July. However, winters can be very cold with severe frosts expected in the southern parts of Western and Southern Provinces.

Region I soils are slightly acidic to alkaline with minor fertility limitations.

3.1.1.2. Region II

Lusaka, Choma, Mazabuka, Monze, Kafue, Chipata and Katete districts are in region II. This region is characterized by the Kalahari sand plateau with general elevation of 900 to 1300 metres above sea level.

The amount of rainfall received in this region is between 800 and 1000mm which is generally well distributed.

Mean daily temperatures range from 23^0 C to 25^0 C with maximum temperatures reaching 32^0 C in October. Low temperatures of about 10^0 C are experienced in this region in July. Soils of region II have slight to severe chemical and physical limitations to crop production.

3.1.1.3. Region III

Of the surveyed area, Mansa, Mwense, Kawambwa, Masaiti, Ndola and Kitwe fall under agro-ecological region III. This region is characterized by altitudes ranging from 1100 to 1700 metres above sea level. Region III receives mean annual rainfall exceeding 1000mm. Mean monthly temperatures vary from 16^0 C, while mean temperatures in the cold season averages about 18^0 C with the exception of a few places which experience frost problems averaging 17 days per year.

The soils in this region are generally highly weathered, leached and characterized by low pH of less than 4.5, which are often toxic to plant growth.

3.2. CONDUCT OF STUDY

Selection of Provinces and Districts for the survey was based on information obtained from Department of Agriculture and the Zambia Agriculture Research Institute of the Ministry of Agriculture and Cooperatives pertaining to banana production. Areas chosen had both local and improved cultivars of bananas grown by both small holder and commercial farmers. In the sampled areas, all the districts were accessible by road. Individual farms/homesteads were randomly selected within the district and the distances varied from province to province and ranged from 10 – 70 Km apart.

Global Positioning System (GPS) data was used to determine the altitude, latitude and longitude data and this information was used to produce a map showing the whole surveyed area as shown in Figure 2.

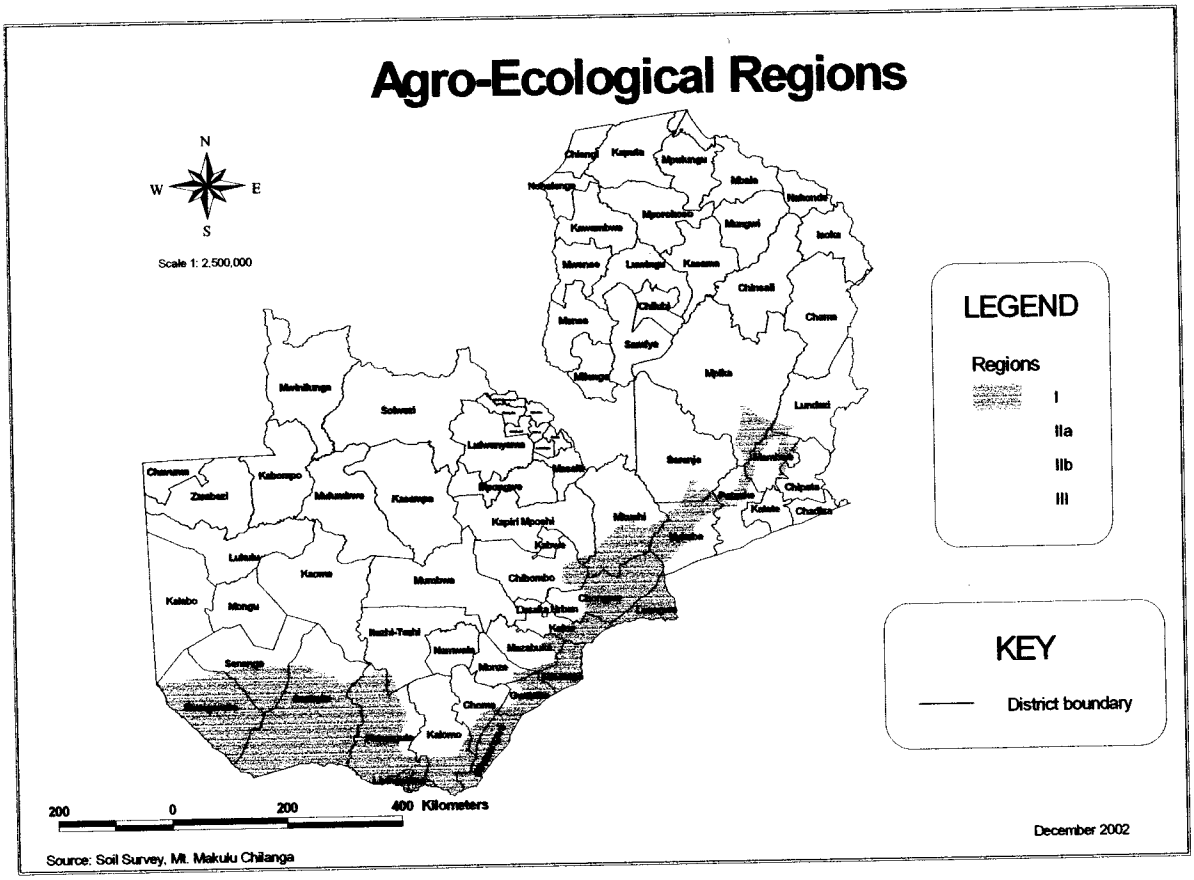

Figure 1. Map of Zambia showing Agro-Ecological Regions

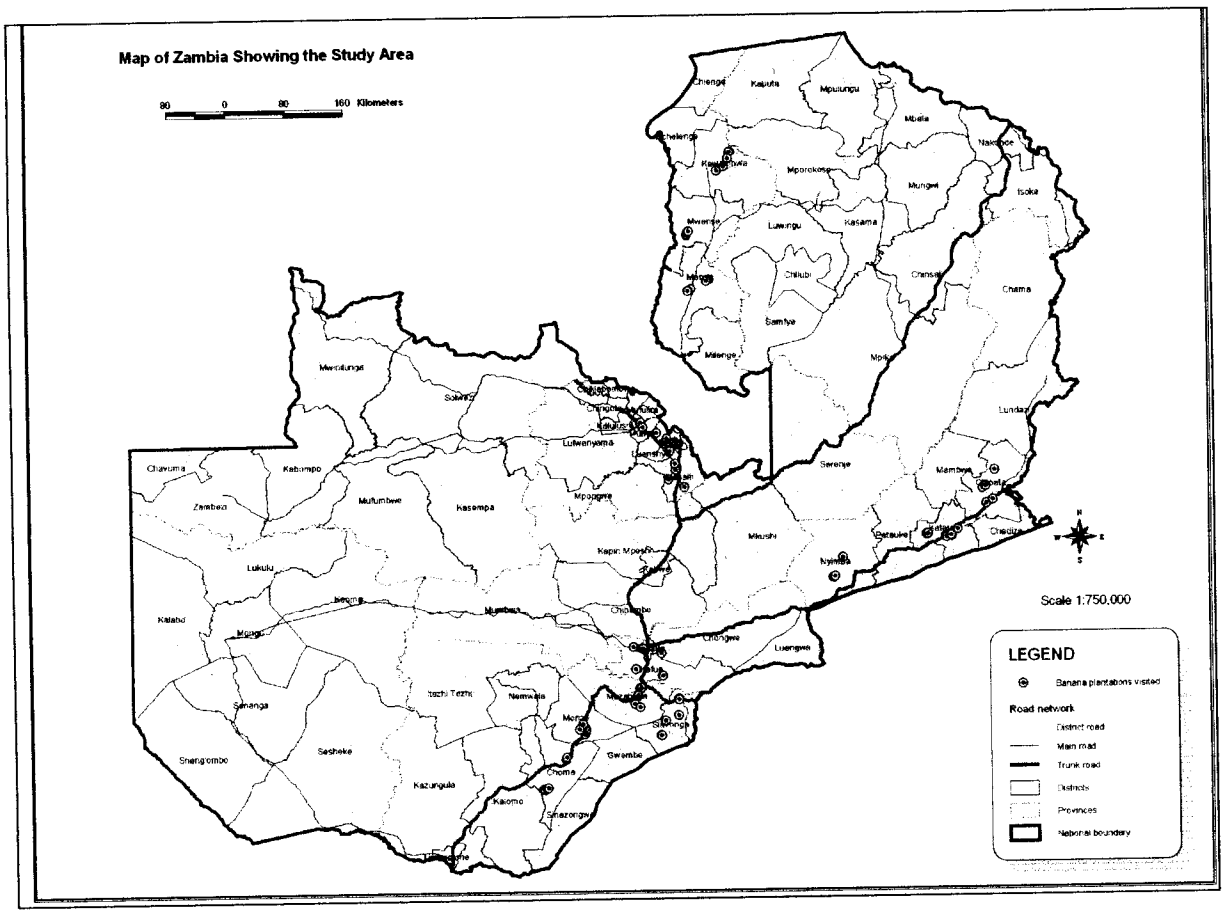

Figure 2. Map of Zambia showing study area of Banana Bunchy Top Disease Survey.

3.3. DATA COLLECTION AND ANALYSIS

3.3.1. Interviews

Seventy five farmers, both small scale and commercial farmers, were interviewed using a questionnaire (Appendix 1) in the fifteen districts which were surveyed. Responses from farmers were analysed using the Statistical Program for Social Sciences (SPSS) program version 16.

The respondents were randomly selected and there was no bias towards age, gender, farming system, farming experience, varieties grown or wealth. Each farmer was expected to give information pertaining to general banana production with specific emphasis on agronomic/ management practices, importance of bananas in comparison to other crops grown, knowledge of banana pests/diseases and finally if they knew anything about Banana Bunchy Top Disease and its vector *Pentalonia nigronervosa* Coq.

During the interview, the farmer was not told that the focus of the research was Banana Bunchy Top Disease so as to get as much information as possible on the farmer's knowledge on the disease and its vector.

3.3.2. Disease Incidence

For the purpose of recording disease incidence, twenty five (25) plants per block of banana field were randomly ear marked diagonally (Rao *et al.*, 2002). Infected plants were identified and marked based on typical Banana Bunchy Top Disease visual symptoms. These symptoms included short, erect leaves and severe rossetting with a bunchy top appearance (Figure 3).In addition to this, there were dark green dot-dash spots and streaks on leaf laminae and

pseudostem (Figure 5) with leaves showing excessive brittleness. These are classical symptoms of banana bunchy top disease (Magee, 1939).

The disease incidence for each farm was calculated using the formula by Gibbs (1983) and Allen *et al.*,(1983) as shown below.

Disease Incidence (%) = (Number of infected plants/Number of plants selected) **X** 100.

Disease Incidence data obtained was tested for homogeneity of variance before analysis. The data was later on transformed to arcsine values as described by Gomez and Gomez (1984). The transformed data was then subjected to analysis of variance (ANOVA) using Genstat Statistical Programme 11th Edition (Payne *et al.*, 2008). The experimental design used was completely randomized design (CRD) with districts being treatments while individual farms were used as observations (Gomez and Gomez, 1984). The number of farms selected per district was not equal because some districts had more banana growers than others.

Samples of the BBTV vector, *Pentalonia nigronervosa* Coq were collected from some fields and photographs taken as shown in Figure 6. Later on laboratory analysis and identification was done at Mount Makulu Research Station.

Figure 3. Banana plant showing symptoms of bunchy top disease (bunchy leaves).

Figure 4. Sprouting infected suckers .

OCCURRENCE AND INCIDENCE OF BANANA BUNCHY TOP DISEASE IN MAJOR BANANA GROWING REGIONS OF ZAMBIA

by

EMELIN MWENDA

A Dessertation submitted to the University of Zambia in partial fulfillment of the requirement for the degree of Master of Science in Agronomy.

University of Zambia

Lusaka

July, 2010.

DECLARATION

I, **EMELIN MWENDA**, do hereby declare that this research report is my original work and that this thesis has not previously been submitted to this or any other university for any degree or examination. Where other people's work has been referred to, I have acknowledged them and the details provided in the references section.

Signed:.....

EMELIN MWENDA

Date:.....

APPROVAL

This dissertation of **EMELIN MWENDA** is approved as fulfilling part of the requirements for the award of the degree of Master of Science in Agronomy by the University of Zambia.

NAME AND SIGNATURE **DATE**

1. **NAME**.....R. G. KAPOORIA (Prof.).....
   SIGNATURE..... ..... 23-7-10.....

2. **NAME**.....D. M. Lungu (Dr).....
   SIGNATURE..... ..... 23/07/2010.....

3. **NAME**.....E. M. Cakwiwa (Dr).....
   SIGNATURE..... ..... 23/07/2010.....

ABSTRACT

This study was conducted to determine the prevalence and incidence of Banana Bunchy Top Disease (BBTD) in major banana growing regions of Zambia. Banana Bunchy Top Disease is caused by Banana Bunchy Top Virus (BBTV) and is considered the most serious disease of banana (*Musa spp.*) worldwide. It can cause total crop loss if it is not diagnosed early.

A survey was conducted in Copperbelt, Eastern, Luapula, Lusaka and Southern Provinces of Zambia between May and November, 2009. A total of 15 districts were surveyed and involved 75 farmers. Farmers were interviewed regarding their knowledge on banana production and banana bunchy top disease using a questionnaire.

Based on visual symptoms, leaf samples were collected from symptomatic and asymptomatic banana plants in the field using FTA® cards. Serological tests were done using Polymerase Chain Reaction technique. Results of work done in the laboratory confirmed the presence of BBTV in all symptomatic plant samples analysed while only one asymptomatic sample tested positive for BBTV. Of the common banana cultivars grown in Zambia none showed resistance, although the level of susceptibility varied among them. Landraces showed low levels of susceptibility in comparison to the improved cultivars. Disease incidences were significantly different at $P \leq 0.05$ with average means ranging from 1 – 31.8%. The Presence of the Banana Bunchy Top Virus vector (*Pentalonia nigronervosa* Coq) was also confirmed in the field.

DEDICATION

My parents, Jonathan Zikanika Mwenda and Celia Mukosha Chintu, 'till we meet again.

ACKNOWLEDGEMENTS

I am greatly indebted to Prof. R.G. Kapooria, my research supervisor, for his valuable advice and guidance which made this study a success. My sincere gratitude also goes to Dr. L. Kumar of the International Institute of Tropical Agriculture (**IITA**), Ibadan, Nigeria for his input in the serological analysis of samples. My deepest gratitude goes to Mr P. Chikoti, R. Mulenga, M. Tembo and Mr G. Kaula for their guidance and allowing me access to the virology laboratory facilities at Mount Makulu Research Station.

I am also grateful to Dr. M. Mataa and Dr. P. Okori (Makerere University-Uganda) for their material support in form of books, critique and shaping this work at proposal level.

I owe a special thanks to the Zambian government and RUFORUM for financing this study through ZARI and UNZA- School of Agricultural Sciences respectively. Particularly, I would like to acknowledge the efforts of Dr. W. Mwale (Director-ZARI), Mr. D. Simumba (PARO-Training), Dr. M. Mwala, Dr. D.M.Lungu and Mrs. S.H.Ngandu for ensuring that financial and material support was made available on time.

Thanks to Kafula Chisanga and Mary Sikasote for a job well done during the survey and subsequent analysis of the data.

Finally, special thanks go to my husband Aubrey Chiselwa Sengebwila and daughter Isabella for their patience and love during my two (2) year study period at the University of Zambia.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

CHAPTER ONE

1.0. INTRODUCTION

Banana (*Musa spp.)* is one of the most important fruit crops in Zambia. Its production contributes significantly to food security and employment creation in both rural and urban areas. Banana cultivation thus contributes to the fight against poverty. The average yield of bananas for most small scale farmers in Zambia between 1993 and 2003 was reported at 3,182Kg/ha (FAO, 2004), with no report on commercial banana production. However, Zambia has potential to produce as much as 31,700 to 61,200 Kg/ha of bananas annually.

Present day bananas are a cross between *Musa acuminata* Colla and *Musa balbisiana* Colla species, which are believed to have originated in Malaysia and India respectively.

The best altitude for banana production is between sea level and 1800 metres (Ngeze and Gathumbi, 2004). Fertile, well drained soils with rainfall of 1000 mm to 1800 mm per annum are needed to achieve best yields. The optimum temperature for banana plants is between 25.5° C and 30.5° C. Being a tropical/ subtropical crop, temperatures below 16.5° C result in stunted growth.

The banana plant is a monocotyledonous, perennial-herbaceous plant belonging to the family *Musaceae.* It is composed of an above ground and underground stem. The above ground stem which is succulent and juicy bears leaves and inflorescence, while the underground stem, also known as a corm, bears roots. The banana inflorescence is covered with bracts which produce rows of female flowers and undeveloped male flowers. The fruits usually develop without fertilization, a term referred to as parthenocarpy. Propagation is vegetative by use of suckers or multiplication of disease free planting material through tissue culture. A fruit bunch is

harvested every nine (9) months, although this is dependent on the variety and management. The Pseudostem is always destroyed after the bunch is harvested. Commonly cultivated bananas are triploid in nature and are usually represented by AAB and ABB and have $2n = 3x = 33$ chromosomes.

Bananas are available throughout the year in Zambia and are mainly taken as a dessert fruit belonging to the AAA triploid group. When ripe, a banana fruit contains 20% sugar, 3% protein, and small amounts of vitamins A, B, C, D and E. The fruit is also rich in potassium (K), Calcium (Ca), Iron (Fe), Sodium (Na) and Phosphorus (Chandler, 1995).

Bananas contain three natural sugars in the form of Sucrose, Glucose and Fructose. These simple sugars form carbohydrates which are a source of energy for the human body (Wardlaw and Kessel, 2002).

The banana fruit being a source of plant protein entails that it is important for tissue growth in human beings. When protein intake is inadequate but total caloric intake is sufficient, a condition referred to as kwashiorkor may occur (Balch and Balch, 1997).

Vitamins are chemical compounds that are required for normal growth and metabolism. Vitamin A (Beta- Carotene) is very important as it affects bones, eyes, hair, immune system, skin, soft tissues and teeth. Deficiency leads to allergies, loss of appetite, blindness, colds and insomnia among other things. Vitamin B is very important for body cells, eyes, gastrointestinal tract, hair, liver, mouth and nervous system. Deficiency symptoms include anemia, appetite loss, bad breath, depression, fatigue and hypertension. Vitamin C affects the adrenal glands, blood, bones, capillary walls, cells, heart, nervous system and teeth. Vitamin

C deficiency leads to anemia, bleeding gums, breath shortness, low infection resistance, stress and capillary wall ruptures (Harper, 1999; Balch and Balch, 1997).

Vitamin D affects the bones, heart, kidneys, nervous system, skin, teeth and thyroid gland. Inadequate intake of vitamin D leads to brittle and fragile bones, burning in the mouth and throat, diarrhoea, insomnia, irregular heartbeat, low blood calcium, rickets, soft bones and sensitivity to pain (Balch and Balch, 1997).

Vitamin E affects the arteries, blood vessels, heart, lungs, nerves, pituitary glands and skin. Its deficiency leads to enlarged prostate gland, A gastrointestinal disease, dry or falling of hair, impotence, muscle weakness and slow tissue healing (Balch and Balch, 1997).

In terms of minerals Calcium in bananas is very important for the maintenance of electrolyte balance in the body and also essential for bones. It is an essential mineral for overall health. Potassium on the other hand is important for blood, endocrine/digestive and nervous systems, heart, kidneys and muscles. Deficiency of potassium leads to constipation, continuous thirst, decreased blood pressure, dry skin, insomnia and hair problems (Harper, 1999).

Iron is essential for blood, bones, metabolic system, muscles, nails, skin and teeth. Deficiency symptoms include breathing difficulties, brittle nails, dizziness, anemia and sore or inflamed tongue (Morrison and Hark, 1999).

Sodium affects the lymphatic system, blood, muscles and nerves. Its deficiency leads to appetite loss, cramps, decreased resistance to infections, eye disturbances, fatigue, intestinal gas and vomiting (Morrison and Hark, 1999).

Finally, on the minerals, Phosphorus affects the bones, brain cells, circulatory and digestive system, eyes, liver, muscles, nerves and teeth. Its deficiency leads to bone pain, fatigue,

irregular breathing, nervous disorders, tooth problems, appetite loss, heart and kidney problems (Subar, 1998).

Despite all this importance attached to the banana fruit, its production in Zambia has declined over the years thereby failing to meet the increasing demand. This decline is partly due to pests and diseases, *inter alia.* Diseases caused by fungi are among the most important diseases of banana. Viral diseases rank as the second most serious problem which limits banana cultivation (Hadidi *et al.*, 1998). However, unlike fungal diseases, where chemical methods of disease control are successful, control of viral diseases is much more problematic. Viral infections of banana can generally cause losses of up to 30%, and occasionally up to 50 – 80% (Fraser, 1990).

There are four significant diseases of bananas that are caused by viruses. Banana Bunchy Top Disease (BBTD) is the most devastating and can cause total crop loss if early diagnosis and stringent sanitation is not practised. Other virus diseases include Banana Mosaic Virus (BMV), Banana Streak Virus (BSV), and Cucumber Mosaic Virus (CMV), (Jones, 2002; Ploetze *et al.*, 2003).

The presence of Banana Bunchy Top Disease has been suspected in banana growing areas of Southern, Copperbelt, Eastern, Luapula and Lusaka provinces of Zambia. Most farmers in Southern, Copperbelt and Lusaka provinces grow bananas on a commercial basis. In the other parts of the country, bananas are grown mostly on small family farms or traditional gardens located in wetlands or along streams.

The literature reviewed so far indicates that no research work has been done on the geographical distribution, prevalence and incidence of the virus causing Banana Bunchy Top

Disease in Zambia. Knowledge of the geographical distribution and prevalence of the disease can assist in the development of control measures like introduction of quarantine practices which can prevent the spread of the disease in Zambia. Disease incidence results can help plant inspectors to identify severely affected areas and prevent the movement of germplasm from such areas to non-affected areas in the country.

The overall objective of the study was to determine the prevalence and geographical distribution of Banana Bunchy Top Disease (BBTD) in major banana producing areas of Zambia. The specific objectives were to:

i. To assess the incidence of banana bunchy top disease in major banana growing areas of Zambia

ii. To confirm presence of the BBTV vector *Pentalonia nigronervosa* Coq in sampled fields.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. History and Geographical Distribution

The origin of Banana Bunchy Top Disease (BBTD) is not known with certainty. However, there is evidence to show its existence in Fiji in 1889 (Magnaye and Valmayor, 1995). Many Fiji plantations were abandoned by 1894 because of the severity of the infection, leading to the Island's banana export industry being threatened (Wardlaw, 1972). Exports fell from 788,000 bunches in 1892 to just 114,000 bunches in 1895 (Magee, 1953). The disease was subsequently reported in 1900 from Taiwan (Sun, 1961) and in 1901 from Egypt (Magee, 1953). This disease has also been reported from Sri Lanka and Australia around 1913 (Gowen, 1995). It is believed that Banana Bunchy Top Disease was introduced into Australia and Sri Lanka through infected banana suckers brought in from Fiji (Magee, 1927 and Wardlaw, 1972). By 1927, Magee reported that the disease incidence in Australia was in the range of 5 – 90%, although it has since been controlled through the implementation of strict phytosanitary control measures and government legislation (Harding *et al.*, 2001). In 1940, the disease was reported from India where it may have been introduced into Southern India from Sri Lanka (Magee, 1953). Interestingly, a very similar disease, abaca bunchy top disease in abaca (*Musa textilis*) was recorded in the Philippines in 1910 (Ocfemia, 1926). However, banana bunchy top disease was not recorded in the Philippines until 1960 (Castillo and Martinez, 1961). Vietnam was also affected by BBTD in 1968 (Vakili, 1969). Doon, (1995) later reported of the spread of the disease to Sarawak in Borneo of Malaysia. The disease has also been reported from Pakistan where damage recorded in some districts was more than 60% and production declined by 90% (Soomro *et al.*, 1992). According to Hu *et al.*, (1993)

and Ranasingh (2007) this disease has also been reported from China, Hawaii and Myanmar. Thomas and Iskra-Caruana (2000) and Pillay (2005) reported the occurrence of this disease from Angola, Burundi, Central African Republic, Congo, Democratic Republic of Congo (DRC), Gabon, Malawi and Rwanda (Thomas and Iskra-Caruana, 2000).

2.2. Symptomatology

The symptoms of Banana Bunchy Top Disease (BBTD) are quite distinctive and not likely to be confused with any other disease (Wardlaw, 1972). Symptoms may become apparent at any stage of plant growth. In severely infected banana plants, the leaves are typically bunched together at the apex (Figure 3), forming a congested rosette and hence the name "bunchy top" disease (Gowen, 1995).

Initial symptoms consist of dark green streaks in the veins of lower portions of the leaf midrid, petiole base and the pseudostem (Harding *et al.,* 1993). This symptom is also referred to as "Morse Code Streaking". This is due to the irregular streaks which resemble a series of breaking "dots" and "dashes". Infected leaves become stunted and chlorotic at the margins (Gowen, 1995). The leaves of infected plants become brittle in texture and the petioles are incompletely elongated. Severely infected plants do not bear fruit, and if fruits do develop, they are severely distorted, stunted and sometimes twisted or deformed and of little commercial value (Ranasingh, 2007). The lower hands of the bunch in infected plants often die off (PANS, 1977). Suckers that develop from infected "Mother" plants are also infected, indicating that the virus becomes widely distributed in the growing tissues (Figure 4). Magee (1953) noted that there are differences in disease symptoms shown by daughter suckers and plants infected from an outside source.

2.3. Economic Impact

Despite Banana Bunchy Top Disease being widespread in Asia and the South Pacific regions, there are no accurate estimates of the international economic impact. In most cases, the incidence of the disease is not well documented (Dale and Harding, 1998). However, Fiji, Australia, Pakistan and the Philippines can be used as examples where this disease has been well studied and has caused serious economic losses.

In the Islands of Fiji, the banana export industry began in 1877 with shipments of 3,100 bunches. Production rose to 359,000 bunches in the course of ten years; a peak being reached in 1892 with exports reaching 788,000 bunches. But with the outbreak of Banana Bunchy Top Disease three years later, banana export declined to 147,000 bunches (Wardlaw, 1972).

Banana Bunchy Top Disease was first recognized in Australia in 1913 and spread rapidly in the plantations on the border between the states of Queensland and New South Wales (Dale and Harding, 1998). By 1926, 90% of the original banana plantations in New South Wales were out of production (Wardlaw, 1972). Output fell from 460,000 cases of bananas in 1922 to 140,000 cases in 1925 (Dale and Harding, 1998). In the Currumbin district of South East Queensland, the number of banana plantations fell from 100 in 1922 to 4 in 1925 and production fell from 4,400 tonnes to 110 tonnes in the same period (Dale, 1987). In describing the devastating effect of the disease at the time, Magee (1927) reported of how difficult it was for someone, who had not visited the devastated areas, to visualize the completeness of destruction wrought in such a short time. Dale and Harding (1998) reports that the drastic impact on production was most certainly due to banana bunchy top disease alone. However, It is interesting to note that currently, due to a scheme of control based on

inspection, eradication and replanting with disease free material, this disease has been reduced to a minor one (Magee, 1953 and Wardlaw, 1972).

Similar losses due to Banana Bunchy Top Disease have been recorded in Pakistan where it was first recorded in 1988 in Sindh Province (Khalid *et al.,* 1993). Approximately, 60% reduction in production occurred. From 23,500 hactarage under banana production in 1988 it fell to 8,000 hactares in 1992 (Dale and Harding, 1998). This reduction was attributed to Banana Bunchy Top Disease (Khalid and Soomro, 1993).

In the Philippines and Vietnam the disease was so widespread that disease incidence in small holdings rose considerably to about 50% (Dale and Harding, 1998).

2.4. Aetiology and Epidemiology

Difficulties have been experienced in establishing the aetiology of Bunchy Top Disease (Gowen, 1995). This is because Banana Bunchy Top Virus was initially thought to be a *Luteovirus* (Mathews, 1982). This was based on the fact that the disease showed many characteristics of luteovirus infections such as not being sap transmissible, the persistent manner of transmission by aphids and induced phloem damage in infected plants (Dale and Harding, 1998). Subsequent evidence supported this theory. Dale *et al.,* (1986) successfully extracted dsRNA from Banana Bunchy Top Disease infected plants that was not present in non diseased plants and the electrophoretic pattern of this dsRNA was similar to the electrophoretic patterns of dsRNA extracted from plants infected with known luteoviruses. Iskra *et al.,* (1989) purified 28 nm isometric virus-like particles from plants infected with Banana Bunchy Top Disease. In contrast, Wu and Su (1990) purified 20-22 nm isometric virus like particles from infected plants and reported that these particles contained ssRNA of

about 6.0 kb. They described these particles as those of a small luteovirus and generated monoclonal antibodies for the detection of these particles (Dale and Harding, 1998).

However, it is now known that Banana Bunchy Top Virus is an isometric virus which is phloem limited and has a genome comprising of at least six different components of circular single stranded DNA (BBTV DNA -1 to -6) ranging in size from 1018 to 1111 nucleotides (Wu and Su,1990; Harding *et al.,* 2001; Burns *et al.,* 1994). This was proved when Harding *et al.*, (1991) and Thomas and Dietzgen (1991) purified 18- 20 nm isometric virus like particles from infected plants using modifications of the method of Wu and Su (1990) and Dale and Harding (1998).

Banana Bunchy Top Virus belongs to the *Nanovirus* group and is a member of the genus *Babuvirus* of the *Nanoviridae* family (Amin *et al.*, 2008). It has 18-22 nm isometric virions, a multicomponent ssDNA genome with a relative molecular mass of 2.0 x 106 and a coat protein sub unit with relative molecular mass of 20,000 (Thomas *et al.*, 2001 and Ranasingh, 2007).

Harding *et al.,* (1993) reported the sequence of the first component of the Banana Bunchy Top Virus genome. The component was shown to be circular ssDNA and 1.111kb (Dale and Harding, 1998). It contained a potential stem-loop structure, the loop sequence of which was almost identical to the invariant loop sequence of geminiviruses (Lazarowitz, 1992 and Dale and Harding, 1998).

The Bunchy Top Virus of bananas is disseminated in vegetative propagules through the movement of infected plants from one place to another (Gowen, 1995). But most importantly, it is spread from plant to plant by an aphid "*Pentalonia nigronervosa* Coq" which feeds on

infected plants and later transmits the virus to healthy banana plants (Magee, 1927, 1940, 1953 and Thomas *et al.*, 1994). Aphids are usually involved in short distance movement while diseased planting material is responsible for both short and long distance movement of the disease (Dale and Harding,1998).

Most of the epidemiological studies on Banana Bunchy Top Disease have been done in Australia. Allen (1978) reported that from an initial focus of infection the mean distance of new infections, presumably resulting from aphid infections, was 17.2 metres with 70% of new infections within 20 metres and 99% of new infections within 86 metres. Allen and Barnier (1977) found that when a new disease free plantation is set up, there is a chance of up to 88% getting the disease if it is located adjacent to a disease infested plantation. However, this dropped to a 27% probability if the nearest infected plantation was 50 to 1000 metres away (Dale and Harding, 1998).

The only confirmed hosts of the Banana Bunchy Top Disease are species within the genus *Musa* (*M. acuminata, M. balbisiana* and their hybrids, Fei bananas) and *Ensete ventricosum,* all members of *Musaceae* (Magee, 1927 and Dale and Harding, 1998). It is important to note that there is no known commercial cultivar of banana and plantain which has shown to be immune or highly resistant to Banana Bunchy Top Disease. However, variations do exist between cultivars in the rate of infection and the severity of symptoms (Magee, 1927, 1948 and Jose, 1981). The Dwarf Cavendish variety is very susceptible, much more so than some of the taller varieties (Wardlaw, 1972). *Musa ensete,* an African variety, is also susceptible to Banana Bunchy Top Disease (Magee, 1953).

2.5. The Aphid Vector

No other aphid, apart from the "*Pentalonia nigronervosa* Coq", is known to transmit Banana Bunchy Top Virus (Magee, 1927). Hafner *et al.,* (1995) reported that the Banana Bunchy Top Virus does not replicate inside the aphid vector.

The banana aphid is present worldwide where bananas (*Musa spp.*) are grown. Besides bananas, the banana aphid infests many tropical and subtropical plants which include *Alpina purpurata* (floral red and pink ginger), *Xanthosoma* (ape), *Cardamon, Heliconia* and tomatoes (Waterhouse, 1987 and Zimmerman, 1948). However, Xie *et al.,* (1996) reported that ginger is not a host of BBTV.

Like most aphids, the banana aphid is a phloem feeder that uses its long stylets to pierce plant tissue to suck the sap directly (Wardlaw, 1972).

Reproduction in the banana aphid is entirely parthenogenetic (without mating). The life cycle (nymph to adult) is completed in 9 to 16 days. The nymphs are more efficient vectors than adults (Magee, 1930).The virus is retained for at least 13 days after aphids have fed on an infected source and persists through the moult (Wardlaw, 1972). There is no egg stage, meaning that young ones are born live (Waterhouse, 1987).

Aphids may be spread throughout the plantation or a banana district by contact transfer from plant to plant in the same or adjacent rows; by movement of aphids over the soil; by the flight of winged individuals; by movement of aphid infected suckers; by translocation of aphid infected soil on the tools and on clothes of workers during cultural and harvesting operations (Magee, 1927 and Wardlaw, 1972).

The aphid colonies occur in the heart (crown) of the banana plant and between the pseudostem and the leaves, the conditions there being ideal for feeding and protection (Wardlaw, 1972). Throughout the greater part of the year, colonies consist of wingless females but later on develop wings and migration begins. This information helps to understand how new outbreaks may occur in isolated and protected areas planted with uninfected material (Wardlaw, 1972).

2.6. Ecological Relationship

Bunchy Top Disease is known to occur under tropical, subtropical and temperate conditions indicating that climatic factors have little influence on its incidence and development. However, seasonal fluctuations have a marked effect (Wardlaw, 1972). In summary, the most favourable conditions for the development of Bunchy Top Disease are periods of rain during the summer months.

2.7. Disease and Virus strains

The available evidence shows that there are different strains of Banana Bunchy Top Disease. For instance, Abaca Bunchy Top Disease in abaca (*Musa textilis*) was recorded in the Philippines in 1910.This disease was very similar to Banana Bunchy Top Disease in terms of biological properties, symptoms and transmission (Dale and Harding, 1998). However, Banana Bunchy Top Disease was not recorded in the Philippines until 1960. Ocfemia and Buhay (1934) could not transmit Abaca Bunchy Top Disease to bananas whereas Magee (1927) successfully transmitted Banana Bunchy Top Disease to abaca.

Karan *et al.,* (1994) worked on Banana Bunchy Top Virus isolates from 10 countries by sequencing and comparing their DNA component 1. It was found that there were two groups

of isolates, the Asian group (Taiwan, Philippines and Vietnam) and the South Pacific group (Australia, Burundi Egypt, Fiji, India, Tonga and Western Samoa). Two groups of isolates were also confirmed on Banana Bunchy Top Virus DNA component 2 to 6 (Karan, 1995). Dale and Harding (1998) believe these two groups represent the two groups of strains of Banana Bunchy Top Virus.

2.8. Control of Banana Bunchy Top Disease

Even though there are a number of control measures for Banana Bunchy Top Disease, it still continues to be a major limitation to banana production in some regions (Dale and Harding, 1998). Some of the control measures include:-

2.8.1. Prevention

Though Banana Bunchy Top Disease is widespread, it has not been reported from the Americas. This is the reason why the International Plant Genetic Resources Institute (IPGRI) and the Food and Agriculture Organization of the United Nations (FAO) have developed extensive recommendations on the movement of banana germplasm and planting material between regions (Frison and Putter, 1989). Where possible, the primary recommendation is that *Musa* germplasm be moved only as *in vitro* plantlets. However, Drew *et al.,* (1989) demonstrated that Banana Bunchy Top Disease could still be transmitted through plantlets *in vitro* when these plantlets are derived from diseased plants although, symptoms at this stage may not be evident.

Therefore, it is important that accurate and intensive diagnostic methods are employed to test *in vitro* material besides using visual symptoms before plantlets are released into non-infected areas (Dale and Harding, 1998). Wu and Su (1990) generated a range of monoclonal

antibodies that can be used in ELISA, while Thomas and Dietzgen (1991) developed both monoclonal and polyclonal antisera for the detection of banana bunchy top virus using ELISA. Harding *et al.,* (1991) also developed a DNA probe for the detection of Banana Bunchy Top Virus DNA component 1 using either dot blot or southern blot analysis. Karan *et al.,* (1994) developed a range of oligonucleotide primers for the amplification of all known sequence variants of Banana Bunchy Top Virus DNA components (Burns *et al.,* 1995; Karan, 1995 and Dale and Harding, 1998). Preventative measures such as stringent quarantine procedures, when adopted and carefully practised, have potential to reduce the risk of further dissemination of bunchy top virus (Gowen, 1995).

2.8.2. Virus free planting material

It is possible to provide large quantities of virus tested banana germplasm, with the advent of *in vitro* propagation and the availability of a range of sensitive and specific Banana Bunchy Top Virus diagnostic methods (Dale and Harding, 1998). Another method is through official inspection and certification schemes where disease-free plantings are identified as suitable sources of propagation material (Gowen, 1995). The only challenge with both approaches is to ensure the production of the required large quantities.

2.8.3. Resistant Cultivars

There have been reports of resistance in some varieties though they are not completely immune to the disease. Jose (1981) tested a range of banana cultivars and found that *Kanchikela* and *Venattukunnan* belonging to the *balbisiana* type (BB and BBB) were most resistant. Under experimental conditions neither plants were immune, but fewer plants became infected with Banana Bunchy Top Virus compared to the Gros Michel and many

other varieties of the *acuminata* group with AA or AAA genomes (Dale and Harding, 1998). This means that there is a possibility of selecting cultivars that can potentially slow the progression or reduce the incidence of the disease.

The differences in susceptibility to infection provide a scope for breeding a Banana Bunchy Top Virus resistant or tolerant variety. However, this is only possible when a suitable source of resistance is identified in a cultivar (Gowen, 1995 and Dale and Harding, 1998). However, work has been slow in this area and so far no immune variety has been identified. Dale (1987) also considers the possibility of use of biotechnology techniques to develop genetically engineered forms of resistance which can eventually play an important role in the control of the disease.

2.8.4. Vector control

Applications of insecticide to decrease the aphid population can be used to restrict the spread of Bunchy Top Virus (Gowen, 1995). However, other problems accompany the use of insecticides such as toxicity to consumers and operators, expense and harmful environmental effects.

2.8.5. Inspection and Roguing

Regular inspection of plantings and early eradication of diseased plants is an effective and important strategy for control of Banana Bunchy Top Disease (Dale and Harding, 1998). However, this requires knowledge and ability to diagnose the disease from early symptoms.

2.8.6. Transgenic Resistance

Transgenic virus resistance, based on virus- derived transgenes, has been widely demonstrated to be an effective strategy for the control of plant viruses (Dale and Harding, 1998). This strategy can specifically work for bananas as there is no known variety that is immune to Banana Bunchy Top Disease and conventional banana breeding programs have yet to prove effective (Dale, 1990). May *et al.,* (1995) and Sagi *et al.,* (1995) have reported of the regeneration of genetically transformed bananas. May *et al.,* (1995) transformed the variety Grand Nain using *Agrobacterium* - mediated transformation while on the other hand Sagi *et al.,* (1995) were able to transform the cultivar Bluggoe with the use of microprojectile bombardment. Six ssDNA components have been sequenced (Burns *et al.,* 1995; Harding *et al.,* 1993 and Xie and Hu, 1995). This work should lead to the Banana Bunchy Top Virus derived resistance genes being incorporated into bananas which can hopefully result in Banana Bunchy Top Virus resistant transgenic bananas (Dale and Harding, 1998).

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. General features of study area

A survey was conducted in Zambia's major banana growing regions during the period of May to October, 2009. A total of 15 districts were sampled which are spread out over five provinces of Zambia and different agro-ecological regions (Figure 1). The selected provinces were Copperbelt, Eastern, Luapula, Lusaka and Southern Provinces (Figure 2). On average, more than 40% of a province was sampled bearing in mind the status of banana production in a particular district of a chosen province.

Kitwe, Masaiti, and Ndola districts were covered in Copperbelt Province while Nyimba, Katete and Chipata distrcts were picked in Eastern Province. Luapula Province had Mansa, Mwense and Kawambwa. In addition to this, Lusaka and Kafue districts of Lusaka Province were surveyed, with Chiawa farmers being grouped together with Kafue farmers due to proximity reasons. The last province tackled was Southern province with Choma, Mazabuka, Monze and Siavonga districts. The selection of individual provinces was done with the help of the Department of Agriculture and the Zambia Agriculture Research Institute of the Ministry of Agriculture and Cooperatives. Provincial Agricultural Officers later on assisted with the selection of major banana growing districts in a province. Individual Farms in each district were then randomly selected.

3.1.1. Zambia's agro-ecological regions

Zambia has three different agro-ecological regions namely regions I, II and III (Figure 1).

3.1.1.1. Region I

Chiawa, Nyimba and Siavonga districts are found in region I. This region is characterized by major valleys such as Gwembe, Lunsemfwa and the Luangwa valleys, which lie between 300 and 900 metres above sea level. Other areas of region I include the Southern parts of Western and Southern Provinces with elevation between 900 and 1200 metres.

Region I has the shortest growing season with low mean annual rainfall which does not exceed 800 mm and more prone to droughts in comparison to other parts of Zambia.

Relatively high temperatures are characteristic of region I, with mean daily temperatures that may vary from 20^0 C to 25^0 C and be sometimes as high as 38^0 C in July. However, winters can be very cold with severe frosts expected in the southern parts of Western and Southern Provinces.

Region I soils are slightly acidic to alkaline with minor fertility limitations.

3.1.1.2. Region II

Lusaka, Choma, Mazabuka, Monze, Kafue, Chipata and Katete districts are in region II. This region is characterized by the Kalahari sand plateau with general elevation of 900 to 1300 metres above sea level.

The amount of rainfall received in this region is between 800 and 1000mm which is generally well distributed.

Mean daily temperatures range from 23^0 C to 25^0 C with maximum temperatures reaching 32^0 C in October. Low temperatures of about 10^0 C are experienced in this region in July. Soils of region II have slight to severe chemical and physical limitations to crop production.

3.1.1.3. Region III

Of the surveyed area, Mansa, Mwense, Kawambwa, Masaiti, Ndola and Kitwe fall under agro-ecological region III. This region is characterized by altitudes ranging from 1100 to 1700 metres above sea level. Region III receives mean annual rainfall exceeding 1000mm. Mean monthly temperatures vary from 16^0 C, while mean temperatures in the cold season averages about 18^0 C with the exception of a few places which experience frost problems averaging 17 days per year.

The soils in this region are generally highly weathered, leached and characterized by low pH of less than 4.5, which are often toxic to plant growth.

3.2. CONDUCT OF STUDY

Selection of Provinces and Districts for the survey was based on information obtained from Department of Agriculture and the Zambia Agriculture Research Institute of the Ministry of Agriculture and Cooperatives pertaining to banana production. Areas chosen had both local and improved cultivars of bananas grown by both small holder and commercial farmers. In the sampled areas, all the districts were accessible by road. Individual farms/homesteads were randomly selected within the district and the distances varied from province to province and ranged from 10 – 70 Km apart.

Global Positioning System (GPS) data was used to determine the altitude, latitude and longitude data and this information was used to produce a map showing the whole surveyed area as shown in Figure 2.

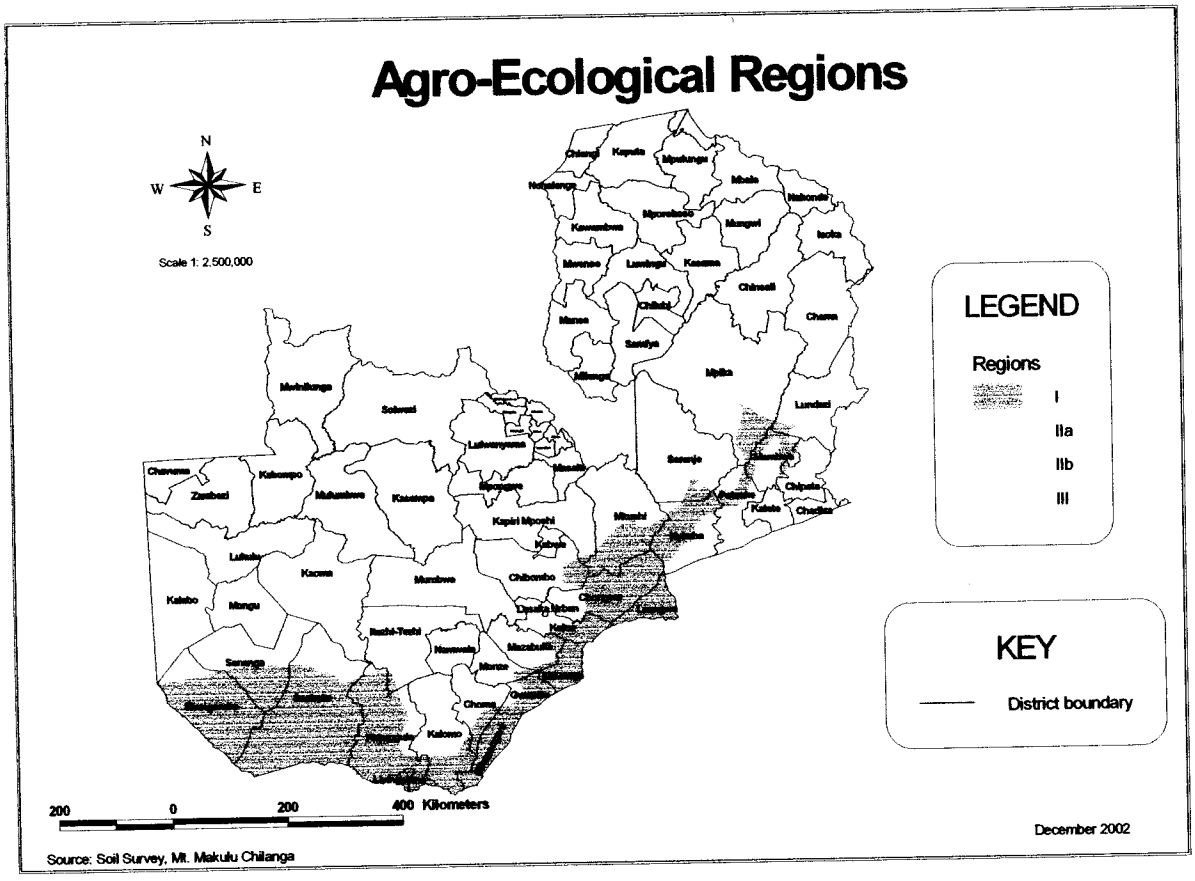

Figure 1. Map of Zambia showing Agro-Ecological Regions

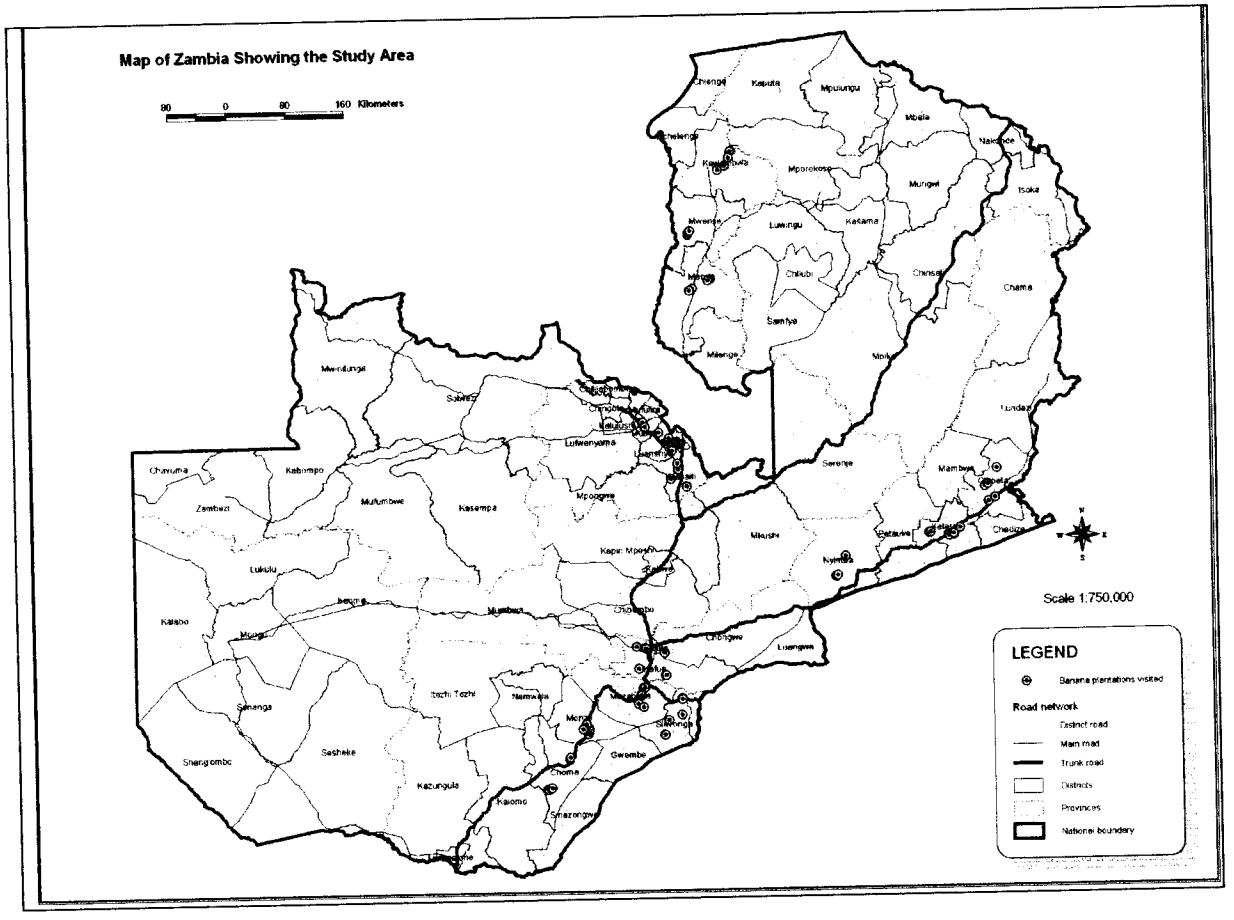

Figure 2. Map of Zambia showing study area of Banana Bunchy Top Disease Survey.

3.3. DATA COLLECTION AND ANALYSIS

3.3.1. Interviews

Seventy five farmers, both small scale and commercial farmers, were interviewed using a questionnaire (Appendix 1) in the fifteen districts which were surveyed. Responses from farmers were analysed using the Statistical Program for Social Sciences (SPSS) program version 16.

The respondents were randomly selected and there was no bias towards age, gender, farming system, farming experience, varieties grown or wealth. Each farmer was expected to give information pertaining to general banana production with specific emphasis on agronomic/ management practices, importance of bananas in comparison to other crops grown, knowledge of banana pests/diseases and finally if they knew anything about Banana Bunchy Top Disease and its vector *Pentalonia nigronervosa* Coq.

During the interview, the farmer was not told that the focus of the research was Banana Bunchy Top Disease so as to get as much information as possible on the farmer's knowledge on the disease and its vector.

3.3.2. Disease Incidence

For the purpose of recording disease incidence, twenty five (25) plants per block of banana field were randomly ear marked diagonally (Rao *et al.*, 2002). Infected plants were identified and marked based on typical Banana Bunchy Top Disease visual symptoms. These symptoms included short, erect leaves and severe rossetting with a bunchy top appearance (Figure 3).In addition to this, there were dark green dot-dash spots and streaks on leaf laminae and

pseudostem (Figure 5) with leaves showing excessive brittleness. These are classical symptoms of banana bunchy top disease (Magee, 1939).

The disease incidence for each farm was calculated using the formula by Gibbs (1983) and Allen *et al.*,(1983) as shown below.

Disease Incidence (%) = (Number of infected plants/Number of plants selected) **X** 100.

Disease Incidence data obtained was tested for homogeneity of variance before analysis. The data was later on transformed to arcsine values as described by Gomez and Gomez (1984). The transformed data was then subjected to analysis of variance (ANOVA) using Genstat Statistical Programme 11th Edition (Payne *et al.*, 2008). The experimental design used was completely randomized design (CRD) with districts being treatments while individual farms were used as observations (Gomez and Gomez, 1984). The number of farms selected per district was not equal because some districts had more banana growers than others.

Samples of the BBTV vector, *Pentalonia nigronervosa* Coq were collected from some fields and photographs taken as shown in Figure 6. Later on laboratory analysis and identification was done at Mount Makulu Research Station.

Figure 3. Banana plant showing symptoms of bunchy top disease (bunchy leaves).

Figure 4. Sprouting infected suckers .

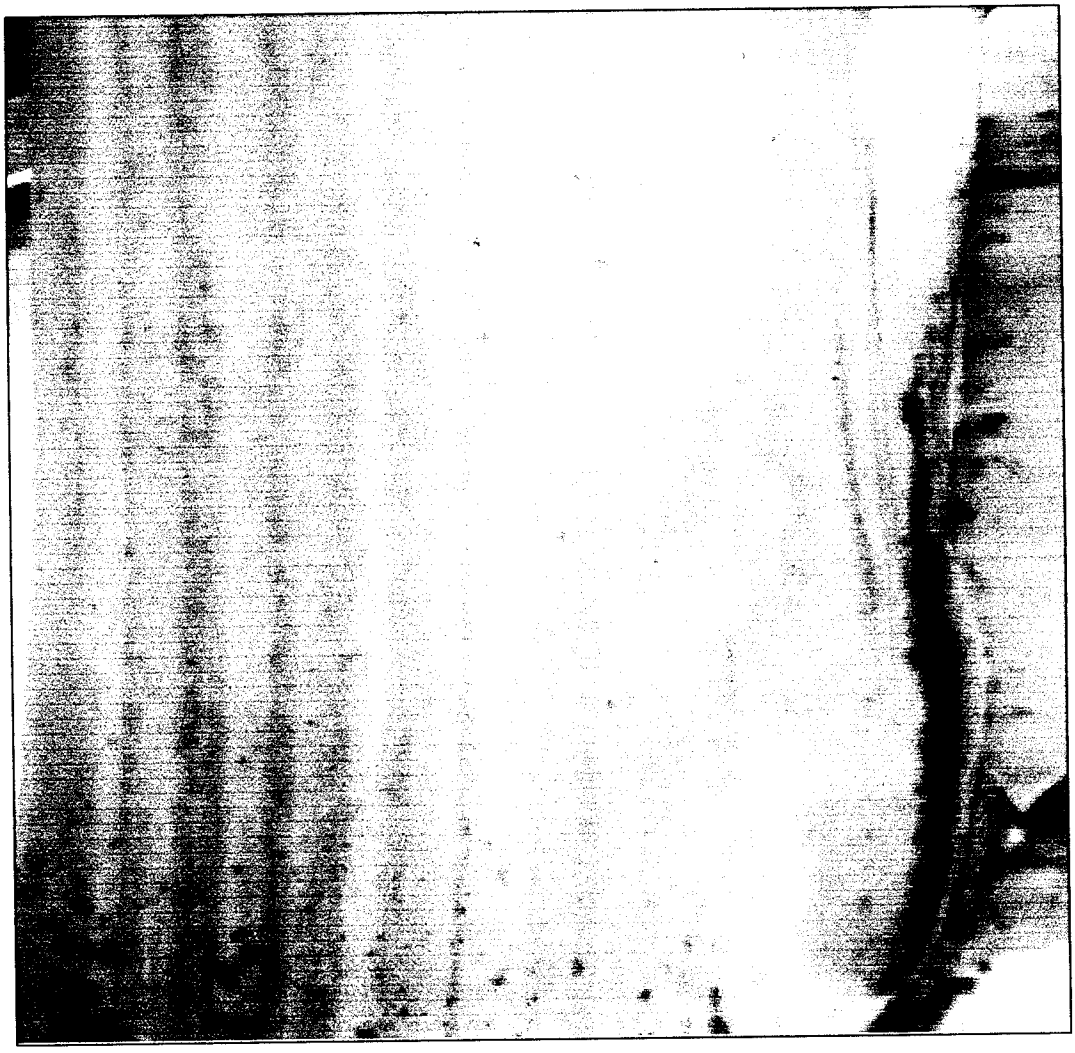

Figure 5. Shows the dark green streaks on a banana pdeudostem.

Figure 6. Colony of banana aphid (*Pentalonia nigronervorsa* Coq) on the base of leaf lamina

3.3.3. Collection of samples

A total of twenty eight (28) young leaf samples from both symptomatic and asymptomatic plants were collected. Asymptomatic plants were those that were growing three (3) to five (5) metres close to plants that showed Banana Bunchy Top Disease symptoms. A third leaf from the top of a banana plant was picked from selected plants. The third leaf was picked because it is the best tissue in terms of high viral titre (Ranasingh, 2007). Leaf tissue samples were collected using FTA® Classic Cards. This was done by placing a $16cm^2$ piece of parafilm over the plant tissue while the rounded end of a pestle was used to apply moderate downward pressure and slightly twisting until sap penetrated the reverse side of the FTA paper.

FTA® Classic cards were later dried at room temperature (around 25° C) overnight and stored in a paper bag for a period of 1 to 5 months before they were processed.

3.3.4. Processing of FTA for Polymerase Chain Reaction

Two (2) FTA measuring 1.2 mm each were punched from the FTA discs using 1.2 mm Harris Micro Punch (Whatman, Inc. U.S.A) and placed in a 1.5 ml Eppendorf containing 50 µl of FTA wash solution. This was left at room temperature for 15 minutes with regular shaking. The solution was later discarded with a pipette and the discs washed two times with 50µl FTA purification reagent. The FTA discs were rinsed first with 100 µl of TE^{-1} buffer (10 mM Tris- HCL, 0.1 mM EDTA, pH 8.0), followed by another rinse with 50 µl absolute ethanol for 5 minutes each. The FTA discs were then dried in an oven for 15 minutes at 56°C and were ready for PCR amplification.

3.3.5. Polymerase Chain Reaction Analysis

Polymerase Chain Reaction was performed at the International Institute for Tropical Agriculture (IITA) Virology Laboratory, Ibadan, Nigeria between 20th and 31st October, 2009 to detect Banana Bunchy Top Virus (BBTV) in the samples collected from Zambia's major banana growing regions. A pair of BBTV primers P_1 5'-GCA TAC CTT GTC AAA CCT TCT CCT C- 3' and P_2 5'- GCG TGA AAC GCA CAA AAG GCC- 3', which were able to amplify a region of the size 239 bp, were used. An extract of a healthy banana leaf sample served as a control for BBTV infected banana plant samples.

The PCR amplification was achieved in a final volume of 12.5 µl containing 2.5 µl of the 5 x Green buffer, 2.4 µl 25mM$MgCL_2$, 0.25 µl of 10 mM dNTP, 0.25 µl of 10 mM P_1 and P_2, 0.06 µl of the Go Taq Polymerase, 6.79 µl double distilled water and two FTA discs (Table 1.).

Polymerase Chain Reaction thermal conditions included a first step 94°C at 5 minutes followed by 35 cycles comprising denaturation at 94° C for 45 seconds. Annealing was done at 53° C for 30 seconds and elongation at 72° C for 30 seconds. At the end of the reaction, a final elongation step was achieved at 72° C for 5 seconds.

Polymerase Chain Reaction products were separated by electrophoresis in a 1.5% agarose gel containing ethidium bromide (1g/ 10ml) under a constant current of 100 mA. This migration was followed by visualization of the amplified bands under ultra violet (UV) light as shown in Figure 17.

Table 1. Reagents used as PCR reaction mix.

No.	Reagent	Volume
1.	5X Green Buffer*	2.5 µl
2.	25 mM $MgCl_2$*	2.4 µl
3.	10mM dNTPs	0.25 µl
4.	10mM BBTV- 1 Primer	0.25 µl
5.	10mM BBTV- 2 Primer	0.25 µl
6.	Polymerase	0.06 µl
7.	Templated DNA	2 µl
8.	Sterile double distilled water	4.79 µl

CHAPTER FOUR

4.0. RESULTS

4.1. SOCIAL AND ECONOMICAL ISSUES OF THE STUDY AREAS

The average farm size in the area under study ranged from 1 hactare to 2000 hactares. However land allocated to banana production varies from 0.25 hactares to 80 hactares as shown in Figure 7.

A total of 75 farmers randomly chosen were interviewed using questionnaires accompanied by physical inspections to their holdings. Twelve (12) farmers were from Kawambwa, Mwense and Mansa districts in Luapula province, Fifteen (15) farmers came from Lusaka province specifically picked from Lusaka and Kafue districts. Nineteen (19) and sixteen (16) farmers were chosen from Southern and Copperbelt provinces respectively. The districts targeted in Southern Province were Mazauka, Monze, Choma and Siavonga while Masaiti, Kitwe and Ndola were picked from the Copperbelt Province. Finally, 13 farmers were picked from Katete, Nyimba and Chipata districts of Eastern Province. The districts picked were those prominent in banana production and their numbers varied from province to province due to differences in size of provinces. The number of farmers chosen was also not uniform because some districts have more banana growers and higher population of people compared to others.

Of the farmers interviewed, 49% said that their main source of labor is casual/hired labour followed by 36% accounting for family labour. However, some farmers said that they used both hired and family labour as shown in Figure 8.

Most districts under study were accessible by road although distances from one farm to another varied in different districts, with some being 70 kilometres apart and hence impossible to sample more than 4 farms in a day per district.

4.2. Production Systems

The main livelihood for most respondents was mixed farming which included crop husbandry, poultry, livestock and fish farming. Eighty eight percent (88%) of the respondents across the whole study area grew other crops in addition to banana production while only 12% practiced monoculture where they were only involved in banana production. This picture was the same even in individual districts sampled and this is illustrated in Table 2.

The respondents that engaged in mixed agriculture mentioned maize, cassava, vegetables, groundnuts, soybeans, wheat, sugarcane, citrus, sweet potatoes, pineapples, palm oil, cotton and sunflower among other crops they grew in addition to banana production. A few farmers on the Copperbelt province said they were also involved in *Aloe vera* production.

Figure 9 shows that 70% of the farmers grew bananas as a source of income while 29% said that bananas were grown for both consumption and income. Only 1% of growers were engaged in banana production for research purposes.

Banana fruit processing was not widely practised among most of the respondents. Only about 11% of the respondents processed their banana fruits into products such as wine, relish and porridge, while 89% only consumed bananas as a fruit.

Figure 7. Land allocated to banana production across sampled districts.

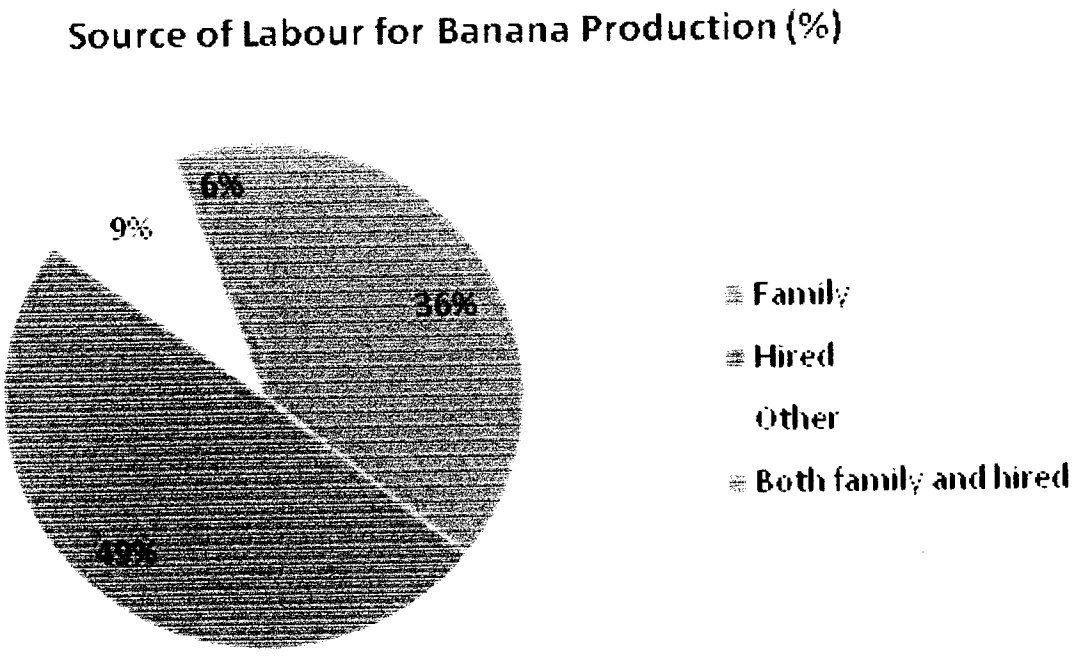

Figure 8. Source of labour for farmers across districts.

Table 2. Banana growers' production systems (%).

Districts	Banana only	Banana and other crops
Chipata	0	100
Katete	0	100
Nyimba	0	100
Kawambwa	0	100
Mansa	0	100
Mwense	0	100
Kitwe	16.7	83.7
Masaiti	0	100
Ndola	33.3	66.7
Choma	0	100
Mazabuka	0	100
Monze	0	100
Siavonga	40	60
Kafue	50	50
Lusaka	11.1	88.9

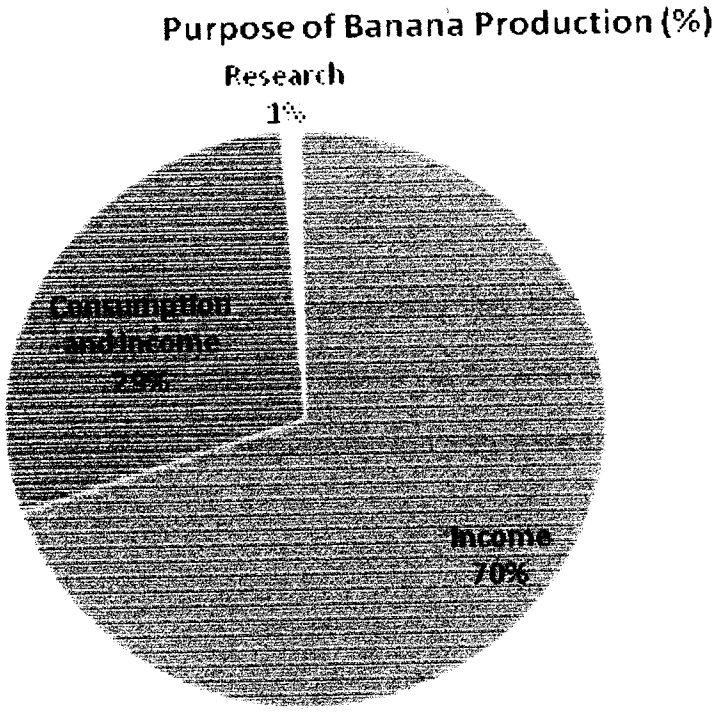

Figure 9. Purpose of banana production in different districts.

Once the banana plants reached fruition, the frequency of harvesting ranged from weekly to twice in a year among growers as shown in Table 3. However, 12% of the respondents had not yet started harvesting their crop as their plantations had just been set up at the time of the interview.

Most commercial farmers were able to provide estimates on the quantity of bananas which they produced per year with some farmers giving average estimates of 40 tonnes/hectare/year. Most small scale farmers on the other hand were not able to provide this information.

Figure 10 shows that more than half of the respondents sold their banana produce at the nearest town market while 24% and 22% sold their bananas at the local village market and farm gate respectively. It was only 2% of the farmers who had not yet started harvesting their fields.

4.3. Management practices

Banana plantations that were randomly selected had been set up from less than a year to 50 years before the study. Most plantations with local cultivars were being managed by smallholder farmers and had been set up as back as in the 1960s. The local cultivars would be called by different names in the different locations visited even though they referred to the same cultivar. Accordingly, 26.7% of the farmers set up their plantations more than 20 years ago, 13.3% being 10 years old, while, 37.3% and 22.7% were for plantations that were 5 and less than 3 years old respectively.

Source of planting material was very critical in this study. The results show 61.3% of the growers sourcing banana suckers from fellow farmers. The second highest group was that of

29.3% of respondents who bought their suckers from agro based companies and only 6.7% having had just inherited their planting materials from their parents and so said it was their own seed. A few farmers (2.7%) who belonged to local cooperatives sourced the planting material from their respective cooperatives (Figure 11). However, it is important to note that source of planting material varied from district to district.

The most common banana cultivars grown by farmers in the study area were William, Grandnain, Dwarf Cavendish and local varieties. Some farmers grew a combination of about two to three cultivars in one field. There were also differences in cultivars grown in different districts.

The majority of respondents followed the recommended spacing and plant density per station which is 1-3 suckers. Fifteen percent (15%) and eleven percent (11%) of the farmers had 10 and 5 plants per station respectively. Eight percent (8%) had 6-9 plants per station (Figure 12).

The general management of banana fields by small scale farmers was very poor with most of them not weeding or desuckering at all. A few farmers would remove excess suckers either monthly, twice or once per year. Most of the excess suckers removed were either replanted by farmers or sold to their neighbours. A few farmers were using the suckers as organic manure.

Table 3. Frequency of harvesting by banana growers (%).

District	Not yet	Weekly	Monthly	Quarterly	Twice per year
Chipata	20	0	20	20	40
Katete	0	0	25	50	25
Nyimba	0	0	0	25	75
Kawambwa	0	0	25	75	0
Mansa	25	0	0	25	50
Mwense	25	0	0	75	0
Kitwe	33.3	0	50	16.7	0
Masaiti	0	0	50	25	25
Ndola	16.7	0	50	33.3	0
Choma	0	0	50	50	0
Mazabuka	20	0	20	40	20
Monze	25	0	25	50	0
Siavonga	0	0	100	0	0
Kafue	0	16.7	83.3	0	0
Lusaka	11.1	0	66.7	22.2	0

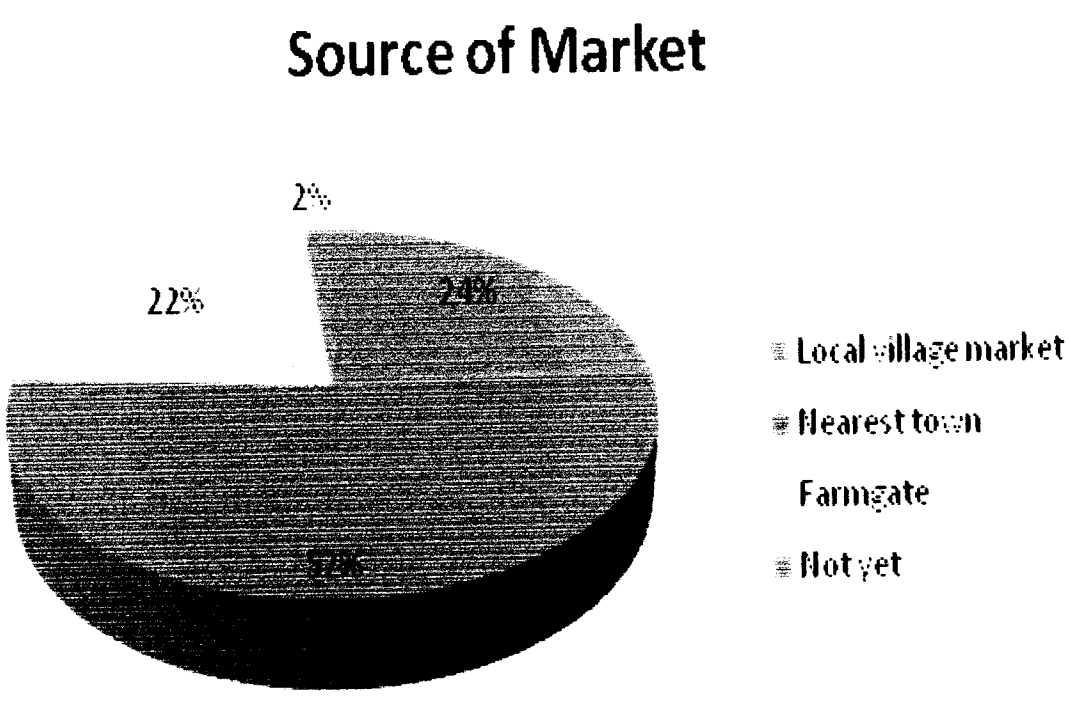

Figure 10. Source of market for bananas.

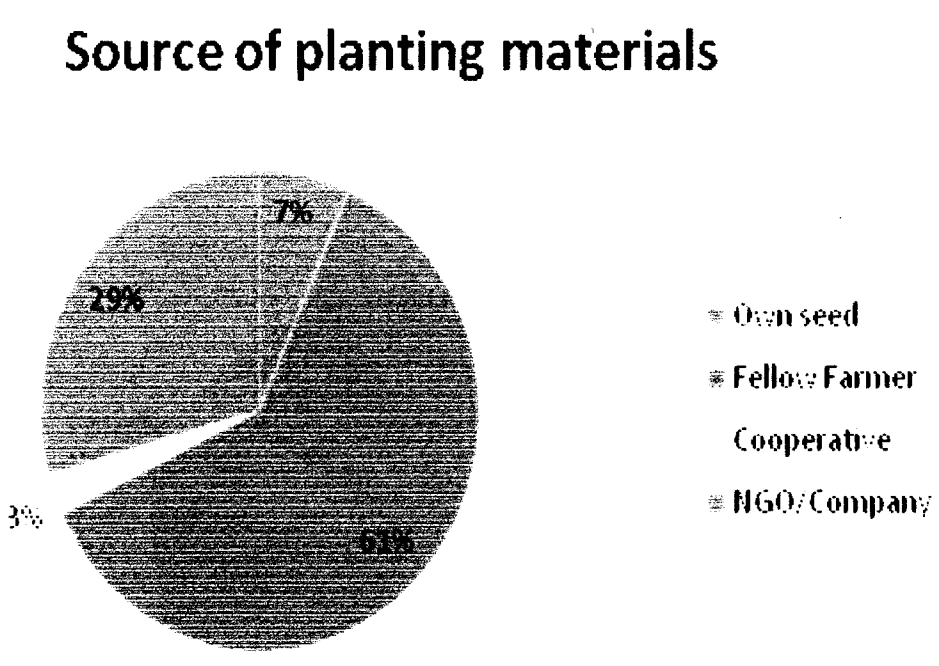

Figure 11. Source of planting material (%).

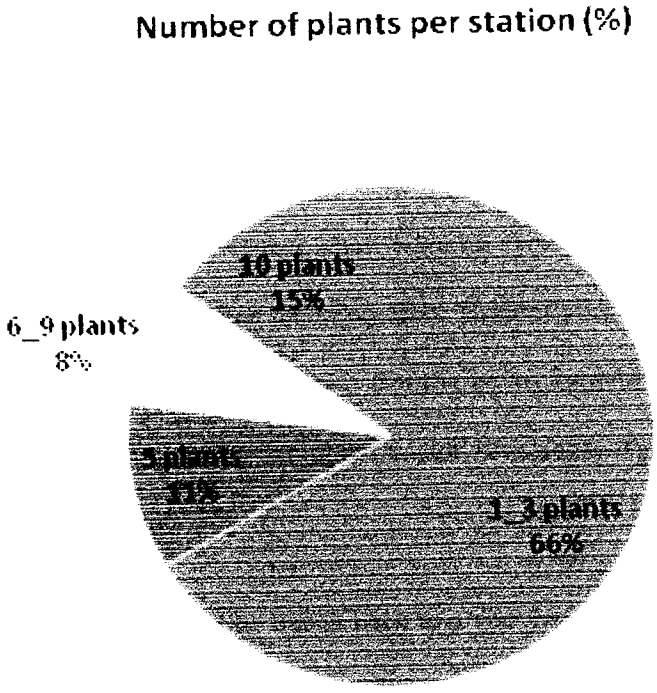

Figure 12. Number of plants per station.

Most of the banana growers interviewed could not afford inputs such as inorganic fertilizers and pesticides. Figure 13 shows that 81% of the respondents could not afford to purchase pesticides and only 19% could afford them. The most commonly used pesticides were Monocrotophos and Dimethoate.

Just over sixty percent (61.3%) of the respondents said they irrigated their fields either using micro jet or furrow irrigation. The majority of respondents who irrigated their fields did it weekly and a few, every fortnight.

4.4. Pest/Disease Prevalence

There were variations in terms of farmer knowledge on Banana Bunchy Top Disease from one district to another. Some districts recorded more farmers with knowledge while slightly more than half of the districts had farmers with little or no knowledge of disease. Most of these farmers could not even identify the aphid vector *Pentalonia nigronervosa* Coq. This is illustrated in Figure 14 and 15.

The disease in most districts could be traced back to a year before the study was undertaken. However, some farmers had first noticed the disease symptoms as way back as 5-10 years before. Chipata was the only district that had first reported the presence of Banana Bunchy Top Disease 10 years before.

Fifty eight percent (58%) of respondents uprooted the diseased banana plants even though they had scanty information on the disease, while 38% did not do anything about the problem in their fields. It was 4% of the respondents who used chemicals to try to eradicate the disease as shown in Figure 16.

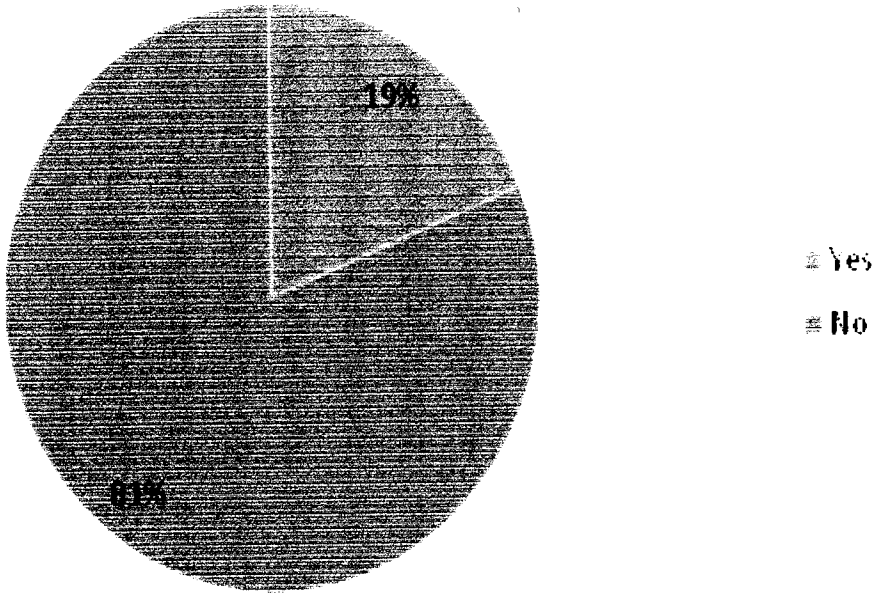

Figure 13. Farmer use of Pesticides (%).

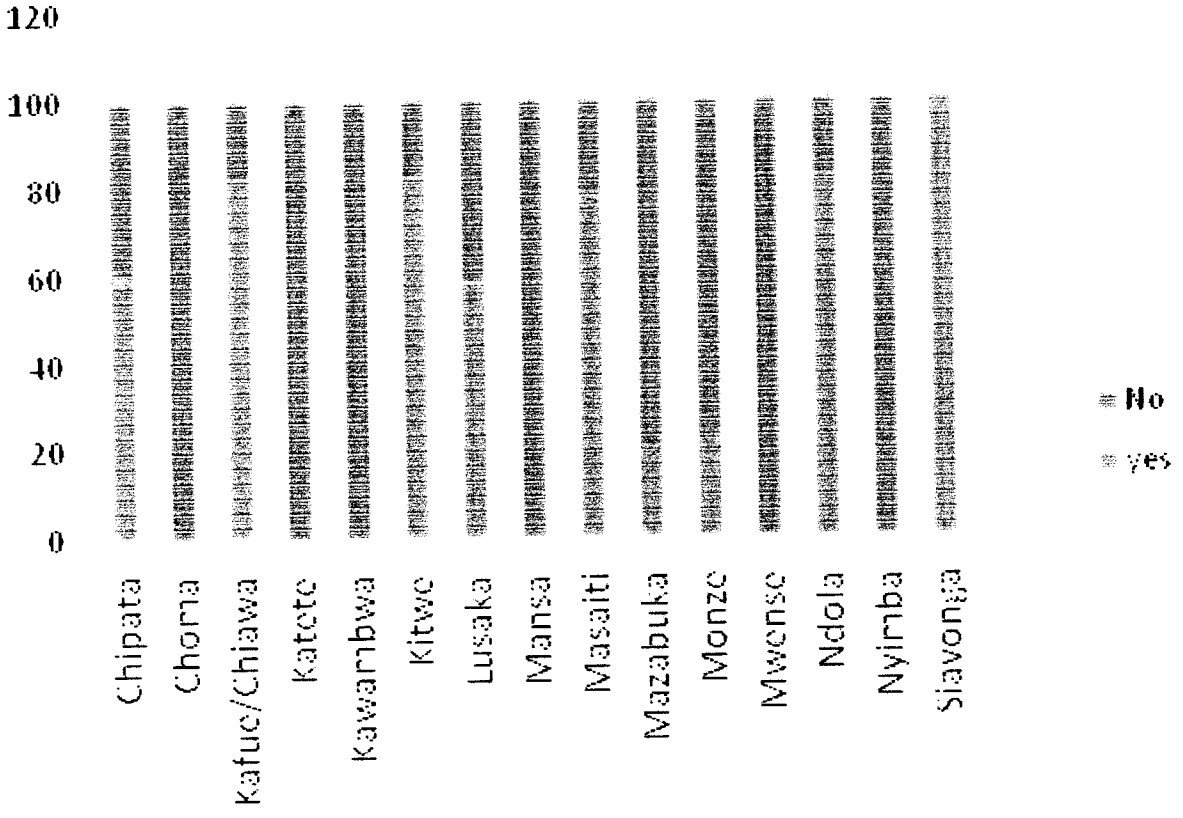

Figure 14. Farmer Knowledge of disease by district (%).

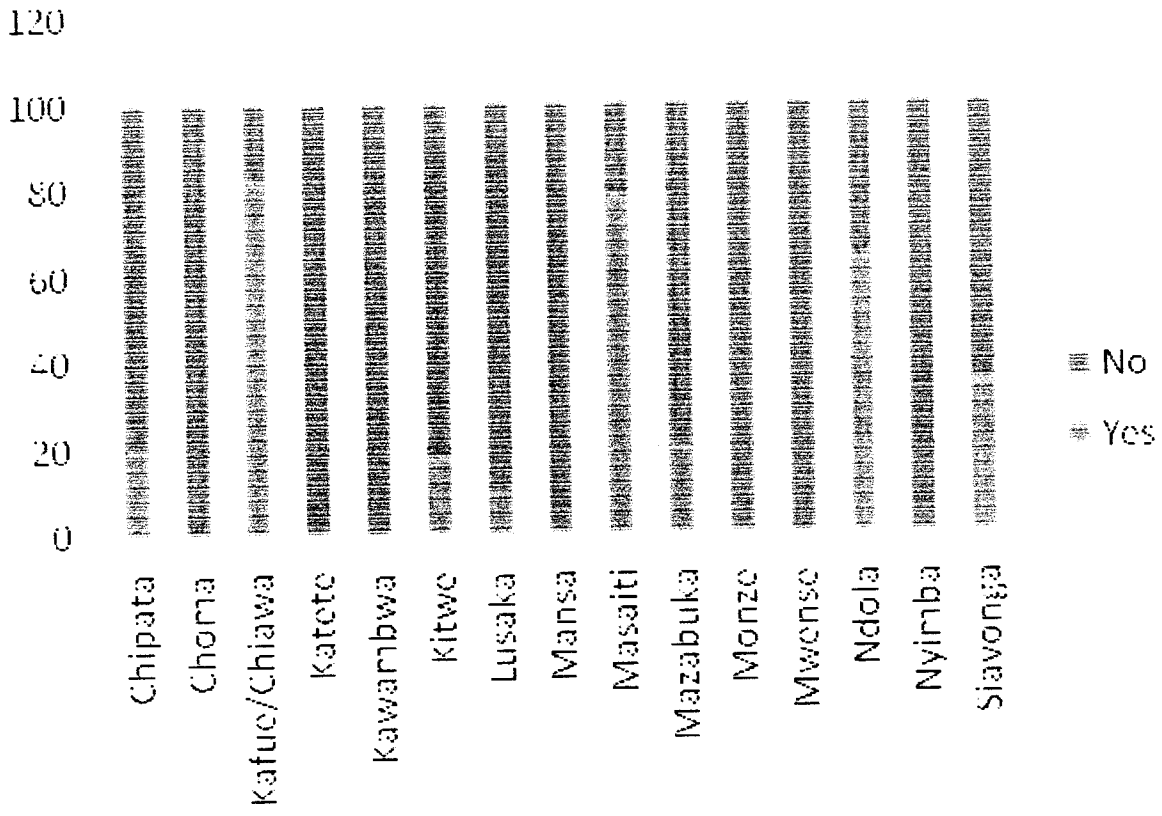

Figure 15. Farmer vector Identification in different districts (%).

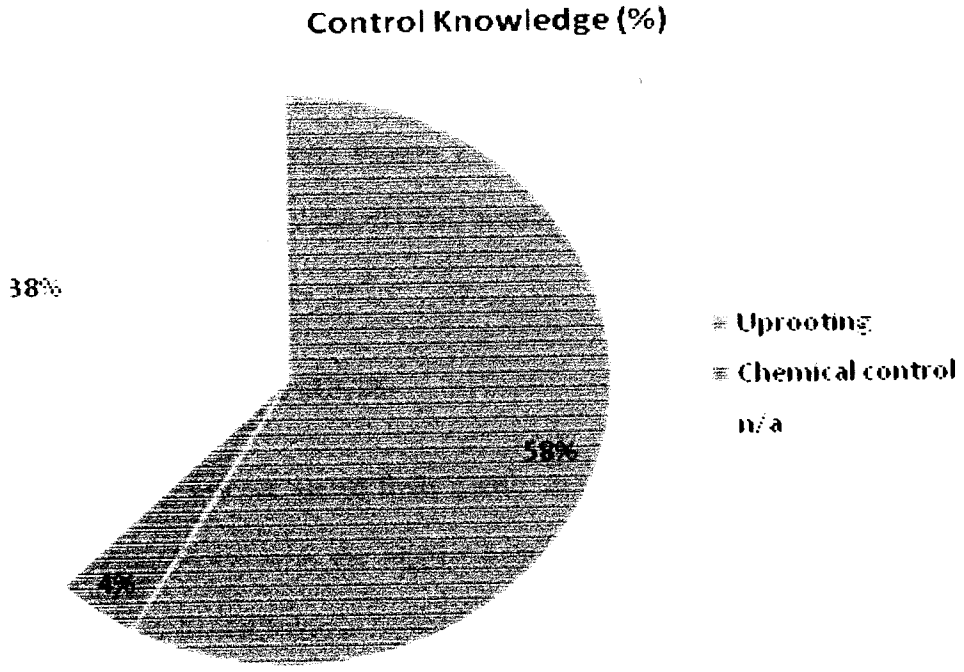

Figure 16. Control measure of disease by farmers across districts.

Upon physically inspecting the respondents' fields, 55% of the fields had plants that showed symptoms of Banana Bunchy Top Disease. It was from some of these fields that samples were collected for further analysis in the laboratory. There was no record of diseased plants in 45% of the farms surveyed.

4.5. PCR ANALYSIS RESULTS

Both symptomatic and asymptomatic banana leaf samples collected were tested for the presence of Banana Bunchy Top Virus via Polymerase Chain Reaction. The results are in Table 4 and Figure 17.

4.6. DISEASE INCIDENCE RESULTS

The disease incidence in percent varied from district to district as shown in Table 5. The average incidence was 16.4% with the highest being in Siavonga (31.8%), followed by Chipata (31.5%) and the lowest being in Nyimba, Mwense, Mazabuka and Monze (1%). The differences among districts were highly significant.

Table 4: BBTV detection in different cultivars in 15 districts via PCR

Province	Banana cv	Symptoms.	Tested samples	PCR detection No.	PCR detection%
Lusaka	Williams	Infected	.4	4	100
		Symptomless	2	0	0
Lusaka	D/Cavendish	Infected	0	0	0
		Symptomless	1	0	0
Lusaka	Grandnain	Infected	1	1	100
		Symptomless	0	0	0
Eastern	Williams	Infected	2	2	100
		Symptomless	0	0	0
Eastern	Landrace	Infected	1	1	100
		Symptomless	2	0	0
Luapula	Williams	Infected	2	2	100
		Symptomless	0	0	0
Luapula	D/Cavendish	Infected	1	1	100
		Symptomless	1	0	0
Copperbelt	Williams	Infected	5	5	100
		Symptomless	0	0	0
Southern	Grandnain	Infected	1	1	100
		Symptomless	1	1	100
Southern	Williams	Infected	0	0	0
		Symptoms	1	0	0
Southern	Landrace	Infected	1	1	100
		Symptomless	1	0	0
Southern	D/Cavendish	Infected	0	0	0
		Symptomless	1	0	0
Total PCR detection (%)			Infected	100	
			Symptomless	10	

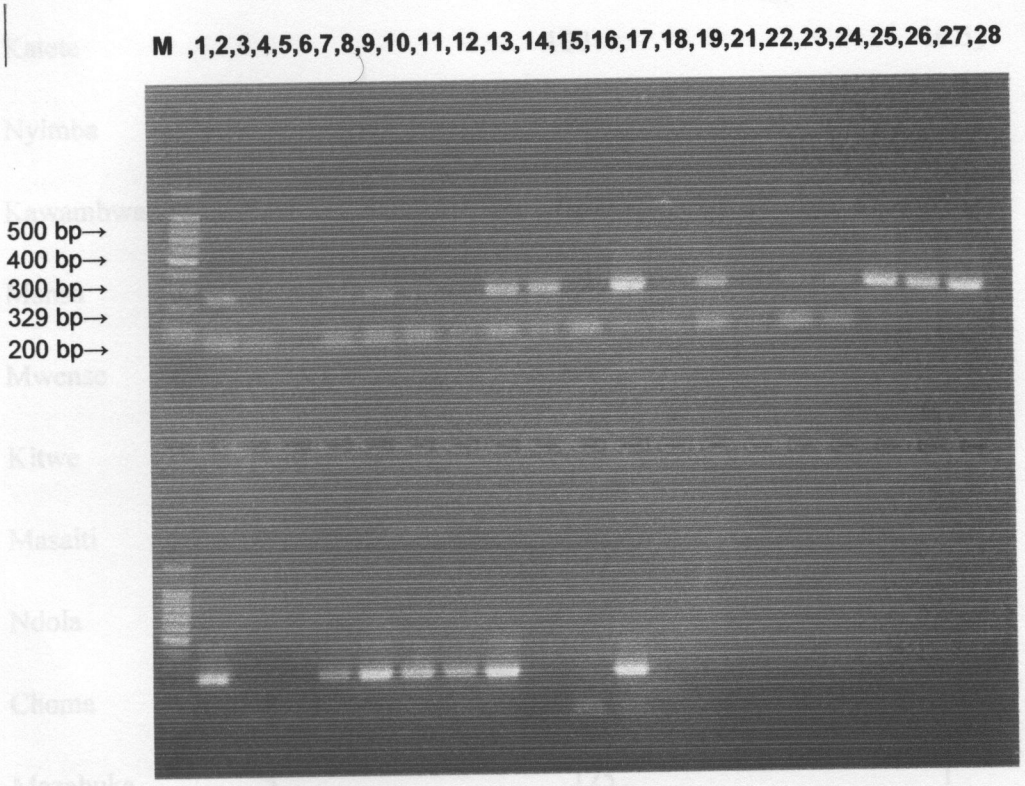

Figure 17. 1.5% Agarose gel shows PCR detection of BBTV in Symptomless and naturally virus infected banana samples. **M**: Marker.

Table 5: Shows mean BBTV infection in 15 districts in Zambia.

District	No. of farmers	Banana plants sampled	BBTV Incidence %
Chipata	5	125	31.5
Katete	4	100	10.5
Nyimba	4	100	1
Kawambwa	4	100	12.2
Mansa	4	100	4.9
Mwense	4	100	1
Kitwe	6	150	28.1
Masaiti	4	100	12.6
Ndola	6	150	17.4
Choma	4	100	18.6
Mazabuka	5	125	1
Monze	4	100	1
Siavonga	6	150	31.8
Kafue	6	150	20.8
Lusaka	9	225	25.8
Mean			16.4
LSD (0.05)			15.71
CV (%)			79.7

Disease incidence is angular transformed data.

CHAPTER FIVE

5.0. DISCUSSION

Banana Bunchy Top Disease (BBTD) incidence significantly varied from district to district and the range was from 1% to 31.8%. Siavonga showed the highest disease incidence (31.8%) followed by Chipata (31.5%) and Kitwe (28.1%) respectively. The lowest incidence among districts was 1%, which was recorded in Mazabuka, Monze, Mwense and Nyimba where most sampled fields showed little or no disease. The disease incidence data showed highly significant differences at $P \leq .05$ in the various districts sampled.

The presence of *Pentalonia nigronervosa* Coq in surveyed banana growing areas was confirmed. The aphid collections made during the course of this study were identified and confirmed at the Entomology Laboratory of Mount Makulu Research Station by entomologists. The aphid is still not widespread and was found to occur in only a few fields. Its presence in three of the 75 farms visited was found to be quite high. It is not easy to see the aphid on banana plants because it is usually concealed beneath the leaf bracts, a fact also reported by Khalid and Soomro (1993).

Results of banana leaf samples collected on FTA® cards were analysed using Polymerase Chain Reaction (PCR). The results confirmed the presence of Banana Bunchy Top Virus in Zambia's major banana growing areas (Table 3). Polymerase Chain Reaction was used because it can detect Banana Bunchy Top Virus DNA in very low concentrations. Hafner *et al.*, (1995) retested banana samples which were previously tested negative for Banana Bunchy Top Virus (BBTV) as being positive using the PCR technique. PCR technology is

very efficient even when virus titer is very low and can detect the virus even in symptomless plants (Soweha, 2005).

Of a total of twenty eight (28) banana samples tested, all eighteen (18) symptomatic samples were found to be positive where as only one out of the ten (10) asymptomatic samples tested positive with the other nine (9) producing negative results. Symptomatic plants showed the presence of dark green streaks and dot-dash symptoms which are typical symptoms of Banana Bunchy Top Disease as described by Thomas and Caruana (2000). One asymptomatic plant sample that tested positive to BBTV proved that some plants will not show symptoms but may carry the infection in latent stage.

These results also indicate that detection of Banana Bunchy Top Virus using visual symptoms alone can generally be relied upon by farmers especially if they are equipped with the knowledge of the virus. The other important point to note is that it is rare that asymptomatic plants will carry the virus. These results are similar to what Soweha (2005), found when, using the PCR technique he successfully tested positive all symptomatic banana samples but asymptomatic banana cultivars of Baradica, Grandnain and Maguraby showed 37.5%, 33.3% and 28.5% BBTV positive results respectively.

Polymerase Chain Reaction technology is very useful particularly in detecting the Banana Bunchy Top Virus even in asymptomatic plants especially during the early stages of infection. Early detection of the virus in banana plants using this technology can help to prevent the spread of the disease via diseased banana planting material.

Banana Bunchy Top Disease is likely to have been present in the surveyed areas of Zambia for quite some time, but remained unnoticed until now. This virus is known to remain latent

in symptomless plants as reported by Wardlaw (1972). Since, the disease has now reached alarming levels in major banana growing areas of Zambia, its spread should be prevented so that other areas do not get affected by it.

Of all the common banana cultivars grown in Zambia none showed resistance to Banana Bunchy Top Virus. The common banana cultivars grown in Zambia are Williams, Dwarf Cavendish, Grandnain and local varieties, and were all found to be infected with Banana Bunchy Top Virus. Smith *et al.*, (1998) and Dale and Harding (1998) reported that there was no known banana variety which was resistant to Banana Bunchy Top Virus, although some cooking bananas were tolerant and expressed symptoms more slowly. This study has also confirmed that the level of susceptibility to Banana Bunchy Top Virus differs markedly among banana cultivars. Local varieties generally showed low levels of susceptibility in comparison to the banana hybrids.

One other way in which Banana Bunchy Top Virus spreads from one place to another is via the use of infected planting material (Gowen, 1995 and Magee, 1927). Interestingly, the results of this study reveal low disease incidence in Mazabuka, Monze, Mwense and Nyimba where the majority of farmers exchange planting materials among themselves. Most farmers in these areas were small holder farmers and had little or no knowledge of Banana Bunchy Top Virus/Disease and its aphid vector "*Pentalonia nigronervosa*". These districts were expected to have high disease incidence, but this was not the case. This might be due to the fact that the disease has not been introduced into these districts and the majority of farmers grow only local varieties that possess low level of susceptibility to the disease.

Most farmers in high disease incidence districts such as Chipata, Kitwe, Lusaka and Siavonga had fair knowledge of the disease and its aphid vector *Pentalonia nigronervosa* Coq. These

farmers are mostly commercial farmers who import planting material directly or use planting material sourced from agro based companies and use banana cultivars which are highly susceptible to Banana Bunchy Top Virus infection. Chipata district which ranked second in disease incidence (31.5%), had farmers who became aware of the existence of the disease 10 years ago. This disease may have come from neighbouring Malawi (Kenyon *et al.*, (1997).

Chipata is a border town, where farmers introduce banana suckers privately and usually without quarantine inspections. These suckers are propagated and further distributed among farmers. The majority of farmers interviewed in Chipata actually indicated that they sourced their planting material from fellow farmers and used material passed on from generation to generation which they referred to as "own seed". This suggests that the distribution of diseased suckers combined with aphid activity and wind dispersal may have played an important role in the spread of the disease in Chipata.

Another possibility for the rise in disease incidence in districts with high disease incidence is that, as banana has been grown successfully for years without any major disease problem in Zambia, farmers were not familiar with the disease and its vector. By the time the disease was noticed, it was already established and had spread throughout the area via infected suckers and/or vector, causing heavy losses. In fact, some farms in Kitwe showed up to 90% disease incidence and in some cases total loss of entire fields. These results are in conformity with those of Rao *et al.*, (2002), Elangovan *et al.*, (1990), Allen *et al.*, (1983), Thresh, (1983) and Jose, (1981) who reported that Banana bunchy Top Disease can cause total crop loss in a plantation.

The few farmers that had some knowledge of the disease were generally commercial farmers who had access to the internet, reading books or had been visited by officers from the

Ministry of Agriculture. Some of these respondents even knew that the only remedy for Banana Bunchy Top Disease was to up-root diseased plants.

The survey also revealed that most small to medium scale farmers do not care about banana as a crop even though they wanted to reap its benefits. This was evidenced by most respondents' poor banana field management practices as they rarely weeded or desuckered their fields and their knowledge of the pest was also very poor. These farmers concentrated on annual crops like Maize and Vegetables compared to bananas. Some of them were even ignorant of the fact that bananas also needed to be irrigated. The general attitude was that bananas would survive even with minimal care and management. Inspite of this attitude the majority of farmers acknowledged the importance of banana as a crop on which they would depend for food and as a source of income when field crops fail.

Some of the poorly managed fields in Chiawa, Chipata, Kitwe, Lusaka and Siavonga districts were among the fields that showed high incidence of disease. The owners of these farms did not practise healthy crop husbandry and their fields were heavily infested with weeds. Some weeds have been reported to be alternative hosts of the banana aphid "*Pentalonia nigronervosa*". Suresh and Regupathy (1987) reported that the host range of the Banana Bunchy Top Virus (BBTV) vector is broad. Su (1993) confirmed this observation and named white ginger (*Hydechium coronarium*) and Canna (*Canna indica*) as suitable hosts of Banana Bunchy Top Virus.

Dead leaves were not removed from plants in most small holder fields, and only few used inorganic fertilizers which are vital for the best performance of most hybrid varieties. In addition to this, the spacing used by the farmers was not the recommended one.

The majority of farmers grew other crops in addition to banana, while others practised a monoculture production system with a banana crop only. The monoculture system was mainly practised by commercial farmers in Chiawa, Kafue, Kitwe, Lusaka, Masaiti, Ndola and Siavonga districts. These farmers grew banana hybrid cultivars and experienced high disease incidence. The high disease incidence could be attributed to the high level of susceptibility of banana hybrid cultivars mostly grown by these farmers and purchase of already infected suckers.

The second category comprised of small scale farmers who intercropped bananas with maize, sugarcane and vegetables. These farmers were found in some parts of Chipata, Choma, Katete, Mazabuka, Monze, Mwense and Nyimba districts. It is important to note that, crops that were grown side by side with bananas did not have any impact in terms of spread of the virus. This is because crops such as *Zea mays* (*Graminaea)* and *Solanum tuberosum* (*Solanaceae*) are not known to be alternative hosts of Banana Bunchy Top Virus and its aphid vector, (Magee, 1940).

CHAPTER SIX

6.0. CONCLUSION

All the eighteen (18) symptomatic banana leaf samples collected from the field tested positive to banana bunchy top virus which causes banana bunchy top disease, while only one of the 10 asymptomatic samples was positive. This shows that visual symptoms of banana bunchy top disease can be an effective and reliable diagnostic method which farmers can use to detect the presence of the disease in their field. The results also confirmed the presence of banana bunchy top disease in major banana growing areas of Zambia. The differences in disease incidence were highly significant among districts with average means varying from 1% to 31.8%.

The banana aphid *Pentalonia nigronervosa* Coq, which transmits Banana Bunchy Top Virus, was present in some fields and the collected samples showed that the identified aphid was *Pentalonia nigronervsa* Coq.

There was very little or no knowledge on Banana Bunchy Top Disease among the farmers in most districts surveyed. In order to correct this, there is need for the relevant authorities to facilitate the sensitization of farmers so that they are equipped with the knowledge and technical skills on how to prevent the spread of Banana Bunchy Top Virus.

There is less importance attached to good husbandry (Management) in banana production in most parts of Zambia. This was evidenced by poor management of banana fields which translated into very low yields especially among small holder farmers.

From the foregoing, it is recommended that the following measures be taken to control the spread of Banana Bunchy Top Disease in Zambia:

i). Regular field inspections for the diagnosis of Banana Bunchy Top infected plants.

ii). Spraying banana plants infested with the banana aphid *Pentalonia nigronervosa* Coq with Dimethoate or Kerosine oil.

iii). Cutting and burning of infected banana plants.

iv). Quarantine laws be strictly enforced on the importation of new suckers and movement of banana germplasm within the country.

vi). Development of new banana varieties with genetic resistance to banana bunchy top disease is desirable so as to prevent complete disappearance of banana plantations in the country.

In the absence of adhering to these recommendations, it is possible that Banana Bunchy Top Disease will continue to spread in the country and seriously affect the production of bananas in Zambia.

CHAPTER SEVEN

7.0. REFERENCES

Abdel- Hamid, I. A., Wagih, E. E., Mahfouz, H. T. and Sadik, A. S. 2003. Non radioactive detection of banana bunchy top nanovirus. *Arab Journal Biotechnology.*, 6, 327-336.

Allen, R. N. and Barnier, N. C. 1977. The spread of banana bunchy top disease between banana plantations in the Tweed River District during *1925-76. N.S.W. Plant Dis.Journal.* 46, 27-28.

Allen, R. N. 1978. Epidemiological factors influencing the success of rouging for the control of bunchy top disease of bananas in New South Wales, *Aust. J. Agric. Res.* 29, 535-544.

Allen, R. N., Plumb R. T. and Thresh J. M. 1983. Spread of banana bunchy top and other plant virus diseases in time and space.In: Plant Virus epidemiology.In: Plumb and Thresh J.M. (Eds), pr., 51-59.

Amin, I., Qazi, J., Mansoor, S., Ilyas, M. and Briddon, R.W. 2008. Molecular characterization of banana bunchy top virus from Pakistan. *Virus genes* 36(1), 191-198.

Balch, J. and Balch, P. 1997. Prescription for Nutritional Healing. http://www.something-fishy.org/dangers/vitamins.php.

Bunyolo, A., Chirwa, B. and Muchindu, M. 1995. *Agro-Ecological Zones of Zambia*: In *Zambia Seed Technology Handbook of the Ministry of Agriculture report.* Sweden.

Burns, T. M., Harding, R. M. and Dale, J. L. 1994. Evidence that banana bunchy top virus has a multiple component genome . *Arch. Virol.* 137, 371-380.

Burns, T. M., Harding, R. M. and Dale, J. L. 1995. The genome organization of banana bunchy top virus: analysis of six ssDNA components. *Journal of General Virology*. 76, 1471-82.

CABI, 2005. *Crop Protection Compedium*, 2005 edition. Wallingford, UK: CAB International.www.cabicompedium.org/cpc

Castillo, B. S. and Martinez, A. L. 1961. *Occurrence of bunchy top disease of bananas in the Philippines*. FAO Plant Protection Bull. 9, 74-75

Chandler, S. 1995. *The Nutritive Value of Bananas.* In: Bananas and Plantain (Gowen, S, Ed.). Chapman and Hall, UK. pp 456

Dale, J. L., Phillips, D. A. and Parry, J. N. 1986. Double Stranded RNA in banana plants with bunchy top disease. *Journal .General. Virology*. 67, 371-375

Dale, J. L. 1987. An economically important tropical plant virus disease. *Advances in Virus Research* 33, 301-325.

Dale, J. L. 1990. *Banana and Plantain.* In "Agricultural Biotechnology: "Opportunities for International Development ". G.J.Persley.ed. CAB International, UK.

Dale, J. L. and Harding, R.M. 1998. Banana Bunchy Top Disease: Current and Future Strategies for Control. *American Phytopathological Society*. 659 pp.

Dietzgen, R. G. and Thomas, J.E. 1991. Properties of virus like particles associated with banana bunchy top disease contain single stranded *DNA. Journal of General Virology* 72, 225-230.

Doon, Y. B. 1995. Personal Communication

Drew, R. A., Moisander, J. A. and Smith, M. K. 1989. The transmission of banana bunchy top virus in micropropagated bananas. *Plant Cell, Tissue Organ Culture*. 16, 187-193.

Dutta, A.C. 1970. *Botany for Degree Students*. Oxford University Press. Faraday House, Calcutta.

Elangovan, R., Mohan, J., Arumagan, R. and Jeyesrajan, R. 1990. A survey report on the incidence of major diseases of bananas in Tamil Nadu. *South India Hort.* 38, 339-340.

FAO. 2004. *Food and Agriculture Organization of the United Nations statistical YearBook and Selected Indicators of Food and Agriculture*. Rome, FAO.

Fraser, R. S. S. 1990. The genetics of resistance to plant viruses. *Annual Review of Phytopathology*.28, 179.

Frison, E. A. and Putter, C. A. 1989. *FAO/IBPGR Technical guidelines for the safe movement of musa germplasm.* Food and Agriculture Organization of the United Nations, Rome/ International Board of Plant Genetic Resources, Rome, 13.

Gibbs, A. 1983. *A* simple Convolution method for describing or comparing the distributions of virus affected plants in a plant community. In: *Plant Virus Epidemiology*. In: The Spread and Control of Insect-Borne Virus. Plumb, R.T and Thresh J. M (Eds), pp:39-50.

Gomez, K. A. and Gomez, A. A. 1984. *Satistical Procedures for Agricultural Research*, 2nd ed, Newyork: Wiley.

Gowen, S. 1995. *Bananas and Plantains*. Natural Resources Institute, Chatham, Uk. Chapman and Hall. London.

Hadidi, A., Khetarpul, R. K. and Koganezawa, H. 1998. Plant Virus Disease Control, APS Press, Minnesota.

Hafner, G., Harding, R. M. and Dale, J. L. 1995. Movement and Transmission of banana bunchy top DNA component one in banana. *Journal of General Virology.* 76, 2279-2285.

Harding, R. M., Burns T. M. and Dale J. L. 1991. Virus like particles associated with banana bunchy top disease contain single stranded DNA. *Journal of General* Virology 72, 225-230.

Harding, R. M, Burns, T. M., Hafner, G. J., Dietzgen, R. G. and Dale, J. L. 1993. Nucleotide sequence of one component of the BBTV genome contains putative replicase. *Journal of General Virlogy,*74, 323-328.

Harding, R. M., Dugdale, B., Hafner, G. J., Horser, C. L, Wanitchakorn, R. and Dale, J. L. 2001. The Molecular Biology of BBTV. *INFOMUSA,* 10, 1.

Harper, A, 1999. *Defining the essentiality of nutrients.* Cambridge.

http://bananas.bioversityinternational.org/- 10/01/2009.

Hu, J. S., Xu, M. Q., Wu, Z. C. and Wang, M. 1993. Detection of banana bunchy top virus in Hawaii. *Plant Disease.* 77, 952.

Imran, A., Qazi, J., Mansoor., Iyas, I. M. and Briddon, R. W. 2008. Molecular characterisation of Banana bunchy top virus (BBTV) from Pakistan. *Virus Genes,* Springer Netherlands, Volume 36, 1

Iskra, M. L., Garnier, M. and Bove, J. M. 1989. Purification of banana bunchy top virus: *Fruits*:2, 63-66.

Jones, D. R. 2002. *Diseases of Bananas, Abaca and Enset.* CABI publishing. Wallingford, Oxon, Uk. 544 pp.

Jose, P. C. 1981. Reaction of different varieties of bananas against bunchy top disease. *Agri. Res. Journal,* Kerala. 19, 108-110.

Karan, M., Harding, R. M. and Dale, J. L. 1994. Evidence for two groups of banana bunchy top virus isolates. *Journal of General Virology*. 75, 3541-3546.

Karan, M. 1995. Sequence diversity of DNA components associated with banana bunchy top virus. Ph.D thesis. Queensland University of Technology.

Kenyon, L., Brown, M. and Khonje, P. 1997. First Report Of Banana Bunchy Top Virus in Malawi. *Plant Disease* .81, 1096.

Khalid, S., Soomro, M. H. and Stover, R. H. 1993. First report of banana bunchy top virus in Pakistan. *Plant Dis*. 77, 101.

Khalid, S. and Soomro, M. H. 1993. Banana Bunchy Top Disease in Pakistan. *Pant Pathology*. 42, 923-926

Lazarowitz, S. G. 1992. Germniviruses: genome structure and gene function. *Crit. Rev. Plant Sci*. 11, 327-349.

Magee, C. J. P. 1927. Banana Bunchy Top Diseases of the Banana. *Council for Scientific and Industrial Research Bulletin No. 30.* Australia.

Magee, C. J. P. 1930. Transmission Studies on the banana bunchy top virus. *J. Aust.Inst. Agric. Scie*. 6, 109-110.

Magee, C. J. P. 1940. Transmission Studies on the banana bunchy top virus. *Journal Australian Industry Agricultural Sciences*. 6, 109-110.

Magee, C. J. P. 1948. Transmission of bunchy top to banana cultivars. *J.Aust. Inst. Agric.* Scie. 14, 18-24.

Magee, C. J. 1953. Some aspects of the Bunchy Top Disease of Bananas and other Musa spp. *J.proc.R.*500 N.S.W., 87, 1-18

Mathews, R. E. F. 1982. Classification and Nomenclature of viruses, *Intervirology* 17, 1-200.

May, G. M., Afza, R., Mason, H. A., Wiecko, A., Novak, F. J. and Arntzen, C. J. 1995. Generation of transgenic banana (*Musa acuminata*) plants via Agrobacterium –mediated transformation. *Biotechnology.* 13, 486-492.

Magnaye, L. V. and Valmayor, R. V. 1995. BBTV, CMV and other Viruses affecting banana in Asia and the Pacific.Bureau of plant industry, Dawao, Philippines, INIBAP Asia.

Morrison, G. and Hark, L. 1999. *Medical Nutrition and Disease*, 2nd ed. Cambridge.

Namaganda, M. J. and Ddunga, J. C. M. 1983. *Present production, potential and research needs of banana in Uganda.* In : R.A. Kirkby and Ngendahayo. D (eds). Banana Production and Research in Eastern and Central Africa. Proceedings of a regional workshop in Bujumbura, Burundi, 14-17 December, 1983.

Ngeze, P. and Gathumbi, M. 2004. *Learn how to grow and market bananas.* Technical Centre for Agricultural and Rural Cooperation ACP-EU. Netherlands.

Ocfemia, G. O. 1926. Progress report on bunchy top of abaca or Manilla hemp. *Phytopathology.* 16, 894.

Ocfemia, G. O. and Buhay, G. G. 1934. Bunchy Top of Abaca or Manilla hemp: II further studies on the transmission of the disease and a trial planting of abaca seedlings in a bunchy top devasted field. *Philipp. Agric.* 22, 567-581.

PANS Banana Manual No. 1. 1977. Pest Control, 3rd edition. London.

Payne, R. W., Murray, D. A ., Harding, S. A., Baird, D. B. and Soutar, D. M. 2008. Genstat for Windows (11th Ed.) Introduction. VSN International, Hempstead.

Pillay, M. 2005. Presence of banana bunchy top virus in Angola. *INFOMUSA*. 14 (1), 44

Ploetz, R. C., Thomas J. E. and Slaubaugh, J. 2003. Disease of banana and plantain. Pp 73-134 In: Diseases of Tropical Fruits Crops (R.c. Ploetz ed.). CABI Publishing, Wallingford, Oxon, UK.

Ploetz, R. 2004. Diseases and Pest: A review of their importance and management. *INFOMUSA*. 13, 2.

Ranasingh, N. 2007. Field Diagnosis and Management of Banana Bunchy Top Disease.Orissa Review. 78-80.

Rao, S. A., Abdul, L. K., Muhammed, A. K., Muhammed, A. R and Khalid, I. R. 2002. Occurrence and incidence of banana bunchy top disease in southern part of Sindh. Pakistan *Journal of Plant pathology*. 1, 2-4, 74 -75.

Renovat, B. 1983. *Banana Production Research in Burundi.* In: R.A Kirkby and Ngendahayo. D (eds). Banana Production and Research in Eastern and Central Africa. Proceedings of a regional workshop in Bujumbura, Burundi, 14-17 December, 1983.

Sagi, L., Panis, B., Remy, S., Schoofs, H., Desmet, K., Swennen, R. and Cammue, B. P. A. 1995. Genetic transformation of banana and plantain(Musa spp) via particle and bombardment. *Bio/Technology* 13, 481-485.

Soomro, M. H., Khalid, S. and Aslam, M. 1992. Outbreak of banana bunchy top virus in Sindh, Pakistan, FAO Plant Protect. Bull. 40, 95-99.

Stephen, A., Ferreira, E., Trujillo, E. and Desmond, Y. O. 1997. Banana Bunchy Top Virus. http//www2.cta hr.hawaii.edu/oc/freepubs/pdf/pD

Stover, R. H. 1972. Banana Plantation and Abaca Disease. Commonwealth Mycological Institute. London , 316 pp.

Smith, M. C., Holt, J., Kenyon, L. and Foot, C. 1998. Quantitative epidemiology of Banana Bunchy Top Virus Disease and its control. *Plant Pathology*. 47, 177-187.

Soweha, H. E. 2005. Detection of Banana Bunchy Top Nanovirus using Polymerase Chain Reaction in different Egyptian banana cultivars. *International Journal of Agriculture* and *Biology*, Vol 5, 698-700.

Su, H. J. 1993. Characterization and pathological nature of a novel DNA virus causing banana bunchy top disease. 6th International Congress of Plant Pathology. Montreal, Canada.

Su, H. J., Tsao, L. Y., Wu M. L. and Hung, T. H. 2003. Biological and molecular categorization of strains of banana bunchy top virus. *Journal of phytopathology* 151, 290-296.

Subar, A.F. 1998. Dietary Sources of Nutrients. *United States Diet Journal.* 98,537.

Sun, S. K. 1961. Studies on the bunchy top disease of bananas. *Spec. public.* College Agric. Taiwan Univ. 10,82-109.

Suresh, S. and Regupathy, A. 1987. *Banana bunchy top virus and its aphid vector.* An annotated bibliography. India: Keerthi Publishing House, PVT Ltd.

Swennen, R. 1984. A physiological study of the suckering behavior in Plantain (Musa cv. AAB). PhD Thesis Dissertationes de Agricultura no.132, Faculty of Agriculture, Katholieke Universiteit Leuven. 180pp.

Thomas, J. E. and Dietzgen, R. G. 1991. Purification, Characterization and Serological Detection of Virus like particles associated with banana bunchy top disease in Australia, *Journal of General Virology*. 72, 217-224.

Thomas, J. E., Iskra- Caruana, M. L. and Jones, D. R. 1994. Banana Bunchy Top Disease. *Musa disease factsheet* No. 4. INIBAP, Montpellier, France.

Thomas, J. E. and Iskra-Caruana, M. L. 2000. *Diseases caused by viruses*. Pp 241-253: In Diseases of Banana, Abaca and Enset (D.R. Jones, Ed.). CAB International, Wallingford, Oxon,Uk.

Thomas, J. E., Gambley, C. F., Geering, A. D. W., McMichael, L. A., Pay, J. N. and Sharma, M. 2001. Viruses and Musa germplasm. *INFOMUSA* : 10(1) in *PROMUSA VIII.*

Thresh, J. M. 1983. Plant virus epidemiology and control: Current trends and future prospects, In: Plant virus *epidemiology*, In: The spread and control of insect- borne viruses, R.T. Plumb and J.M. Thresh, (Eds), 349- 360.

Vakili, N. G. 1969. Control and eradication of bunchy top disease of bananas in South Vietnam. *Tech.Bull.No.1.* USAID/Vietnam.

Venten, H. J., Chu, P. W. G., Dale, J. L., Harding, R., Hu J., Katul, L., Kojima, M., Randles, J. W., Sano, Y. and Thomas, J. E. 2005. Nanoviridae. In : Faquet C.M, Mayo M.A, Maniloff .J, Desselberger .U, Ball LA, eds. *Virus Taxonomy*, VIIIth Report of the ICTV. London, UK: Elsevier/ Academic Press, 343- 354.

Wardlaw, C. W. 1972. *Banana Diseases.* 2nd edition , Longman, London.

Wardlaw, G. M. and Kessel, M. 2002. *Perspectives in Nutrition,* 5th ed. Boston

Waterhouse, D. F. 1987. *Pentalonia nigronervosa* Coquerel In: Biological Control: Pacific prospects. Waterhouse, D.F and Norris, K.R, Ed. Inkata Press. Melbourne 454 pp.

Wu, R. Y. and Su, H. J. 1990. Purification and Characterization of banana bunchy top virus. *Journal of Phytopathology* 128, 153 -160.

Wu, R. Y. and Su, H. J. 1990. Transmission of banana bunchy top virus by aphids to banana plantlets from tissue culture. *Botanical Bulletin of Academia* Sinka. 31, 7-10.

Xie, W. S., Hu, J. S and Sether, D. 1994. Molecular characterization and detection of banana bunchy top in Hawaii. *Journal of Phytopathol.* 84, 1105.

Xie, W. S. and Hu, J. S. 1995. Molecular Cloning, Sequence analysis and detection of banana bunchy top virus in Hawaii, *Phytopathology.* 85, 339-347.

Xie, W., Leonhardt, K. W., Wang, M., Sether, D. and Hu, J. S. 1996. Use of Polymerase Chain Reaction to study transmission of banana bunchy top virus by banana aphid (pentalonia nigronervosa Coq). *Ann. Appl. Biol.* Pp 128, 55-64.

Zimmerman, E.C. 1948. Insects of Hawaii, Vol 5. University of Hawaii Press, Honolulu: 464 pp.

APPENDICES

Appendix I: Questionnaire

BANANA BUNCHY TOP VIRUS SURVEY

A. HOUSEHOLD INFORMATION

Date.....Name of the Interviewer.....

A1. Farmer's Name.....

A2. District.....

A3. What is the size of your arable fields.....(ha)

A4. How much land is allocated to banana growing (a) 0-1ha (b) 2-5ha (c)>5ha

B BACKGROUND INFORMATION.

B1. Profession .....

B2. Highest level of education (a) Tertiary (b) Secondary (c) Primary (d) Never been

C AGRONOMIC /MANAGEMENT PRACTICES

C1. When was the banana plantation set up? (a) 20 years ago (b) 10 years ago (c) 5 years ago (d) < 3 years ago

C2. Where did you source planting materials from? (a) Own seed (b) Fellow farmer (c) Cooperative (d) NGO/Company

C3. Varieties being grown (a) Local/landrace (b) Dwarf Cavendish (c) Williams (d) Grandnain

C4. Number of plants per station (a) 1-3 (b) 5 (c) 6-9 (d) >10

C5.What is your desuckering interval? (a) Monthly (b) 6 months (c) Yearly (d) Not done

C6. Where are the excess suckers taken (a) Sold to local market (b) Replanted (c) Used as organic manure

C7. Any use of herbicides/pesticides (a) Yes (b) No (c) n/a

C8. If yes, which ones.....
.....

C9. Do you irrigate your field? (a) Yes (b) No (c) n/a

C10. What is the frequency? (a) Weekly (b) Two weeks (c) Not done, wait for rains

C11. Do you fertilize your bananas? (a) Yes (b) No (c) n/a

C12. If yes what fertilizer do you use? (a) Inorganic (b) organic (c) n/a

C13. Frequency of weeding (a) Once per month (b) once per year (c) Twice per year

C14. Source of labour (a) Family labour (b) Hired (c) Other

D IMPORTANCE

D1. Do you grow other crops besides banana (a) Yes (b) No (c) n/a

D2. If yes, which one (i).....(ii).....(iii).....(iv)...
.....

D3. Are bananas grown for subsistence or commercial.....

D4. Frequency of harvesting (a) Monthly (b) after 3 months (c) After 6 months

D5. Do you process any banana fruits, if so how?.....

D6. Quantity of bananas produced per year.... Eaten..... or Sold.....

D7. Market for your bananas (a) Local village market (b) Nearest town market (c) Farm gate

E PEST/DISEASE PREVALENCE

E1. Any knowledge on Banana Bunchy Top Virus/disease and its aphid vector (a) Yes (b) No (c) n/a

E2. Have you ever seen the banana aphid in your field? (a) Yes (b) No (c) n/a

E3 If yes, when did you first notice the disease symptoms or aphid? (a) A year ago (b) 3 years ago (c) 5 years ago

E4. Have you noticed any reduction in yield after noticing the Banana Bunchy Top disease? (a) Yes (b) No (c) n/a

E5. What control measures do you employ (a) Uprooting (b) Leave bananas (c) Spray with chemicals (d) Other

E6. Any other information on Banana production and Bunchy Top Disease and indicate whether disease was observed or not.

.....

Appendix II: Aphid collection form

Sample # . .13109.

INSECT PEST IDENTIFICATION FORM

Plant Protection Laboratory, Mount Makulu Central Research Station. Private Bag 7, CIIILANGA

SAMPLE RECEIVED BY..... NTHENGA ISAiAH DATE 12/05/2009

SUBMITTED BY.. EMELIN MWENDA Address . . .Mt. Makulu

CROP .BANANA VARIETY.. .WILLIAMS NECTARAGE. . .4 PLANTING DATE...! /20..,.

Last crop(s) grown Before last crop

PLANT PART AFFECTEDIDAMAGED

ROOTS: LI Apparently normal U Poor growth U Damaged roots E] Discoloured rotted

STEMS: U Apparently normal CJTunneled internally []'Darnaged externally U Top dieback LI Stalkrot TRUNK/BRANCH: [hi Apparently normal [1 Abnormal growth UTunneled internally U Damaged

Externally

LEAVES: LI Apparently normal U Perforated LI Damaged on the edges U Spotted yellow and wrinkled U Windowing

GRAIN: U Apparently Normal LI Abnormal U Tunneled internally U Holes (on the grain)

FRUIT/TUBERISEEDS: U Apparently normal U Abnormal growth U Tunneled internally U Shriveled U Holes (on the grain)

INSECT SPECIMEN: SUBMITTED LI NOT SUBMITTED

CHEMICAL APPLIED INSECTICIDE HERBICIDE FUNGICIDE

DIAGNOSIS AND RECOMMENDATIONS

The insect pests were diagnosed and identified as Banana Aphids, Peiztalonia nigronervosa Control: Chemical treatments are generally effective only if accompanied by careful eradication of infested plants.

Pesticides are cypermethrin, dicrotophos and parathion which should be sprayed at the plant crown and pseudostem bases below soil level between the outer leaf sheath and stem, and over the surrounding soil following recommendations by the ranufacturer on the label.

CHECKED AND SIGNED BY

Stage 1. Sylvia Tembo ,,

Stage 2. Nthenga Isaiah . . h.V

Stage 3. Mathews Matimel :i

Appendix III: ANOVA for disease incidence in 15 districts in Zambia

SOURCE	DF	SS	V.R	F Pr
District	14	653.7	3.83	<0.001
Residual	60	170.9		
Total	74			

CV: 79.7%