OCCURENCE OF BANANA *XANTHOMONAS* WILT IN KENYA AND POTENTIAL APPROACHES TO REHABILITATION OF INFECTED ORCHARDS

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DECLARATION

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

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DEDICATION

To my father the late Kilion Kwach Omolo and mother the late Rose Ogola Kwach for instilling in me a vision for the thirst of education at an early age. My brother John Awino Kwach who laid the foundation for my education.

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LIST OF ABBREVIATIONS

AEZ	-	Agro Ecological Zone
ANOVA	-	Analysis Of Variance
bp	-	base pair
BXW	-	Banana *Xanthomonas* wilt
EAHB	-	East African Highland banana
ELISA	-	Enzyme–Linked Immunosorbent Assay
FAO	-	Food and Agriculture Organisation
FAOSTAT	-	Food and Agriculture Organisation Statistic Data Base
FTA card	-	Flinders Technology Associates card
GLM	-	General Linear Model
ICIPE	-	International Centre for Insect Physiology and Ecology
IITA	-	International Institute of Tropical Agriculture
ISHS	-	International Society for Horticultural Science
KALRO	-	Kenya Agricultural and Livestock Research Organization
KARI	-	Kenya Agricultural Research Institute
M	-	Ladder
masl	-	Metres Above Sea Level
OD	-	Optical Density
PCR	-	Polymerase Chain Reaction
UV	-	Ultra Violet
Xcm	-	*Xanthomonas campestris* p.v. *musacearum*

GENERAL ABSTRACT

Banana *Xanthomonas* wilt (BXW) caused by *Xanthomonas campestris* p.v. *musacearum* (Xcm) affects all banana cultivars and causes up to 100% crop loss. The disease was first reported in western Kenya in 2006, spreads very fast and has no single effective control measure available, no natural resistance among the banana cultivars. This study was carried out to establish the spread of BXW in banana producing areas and develop integrated management options.

Two minute dipstick and FTA Whatman cards DNA capture techniques in polymerase chain reaction (PCR) diagnosis of the disease was evaluated using 258 samples of BXW from Burundi, D. R. Congo, Kenya, Rwanda, Tanzania and Uganda. The captured DNA by field kits were subjected to PCR and results compared with directly DNA issolated from fresh samples analysed by PCR and Enzyme–Linked Immunosorbent Assay (ELISA). The occurrence of BXW in banana growing regions of Kenya was determined through a survey. Diversity of insects associated with BXW in disease epidemic areas of western Kenya was determined by collecting insects visiting banana flowers and fruits in BXW affected and healthy farms. The effectiveness of single stem rouging and replanting in managing BXW was evaluated under field experiments in banana orchards with over 80% BXW incidence. Single stem rouging options evaluated included cutting at the base, uprooting of infected plants, injection with herbicide and total removal of whole mats. Efficacy of replanting was tested by total removal by uprooting all the plants in the affected orchard and replanting after three, four and six months. Susceptibility of the locally available banana germplasm was evaluated by inoculating 46 banana cultivars from eight genome groups with Xcm under screen house conditions.

The Two minutes dipstick DNA capture was most efficient in capturing Xcm DNA under field conditions and was comparable with PCR and ELISA on fresh samples. Out of 258 field samples of Xcm, 25.9% were positively diagnosed using 2 minutes DNA extraction capture field kit and only 1.5% with FTA cards. The survey confirmed presence of BXW only in Busia, Kakamega, Siaya and Kisumu . Stingless bees (*Apis* sp) were the most abundant insects found associated with banana as potential vectors for BXW. Injection with herbicide and uprooting of the infected plants were the most effective single stem rouging option in reducing BXW. The disease was significantly controlled after six months and bunch yield recovered after one year. Replanting banana on the affected fields three to four months after destruction of the BXW affected orchards reduced disease incidence to less than 2% for the five banana cultivars used. All the forty six banana cultivars evaluated were susceptible to BXW. Cultivars Mokoyo, Namukhila and Horn Plantain were most susceptible while cultivar Mysore, an apple dessert banana of genome AAB was least susceptible.

Two minutes dipstick was efficient in capturing BXW pathogen DNA for PCR diagnosis at field level. Banana *Xanthomonas* wilt was confirmed in Western and Nyanza regions of Kenya. Use of infected suckers, pruning implements and stingless bees contribute to BXW spread. Banana *Xanthomonas* wilt incidence was reduced by over 80% and 70% yields recovery within one year by rouging only the BXW symptomatic plants instead of destroying entire orchard.

CHAPTER ONE: GENERAL INTRODUCTION

1. 1. Background information

Banana is a major food security crop in East and Central African countries and an important source of nutrition. It is estimated to supply 75 % of the carbohydrates needed by more than 20 million people in these countries (Tripathi *et al.,* 2005; 2008 and 2009; Ocimati *et al.,* 2013a; Tripathi, 2014).

In Kenya banana production is threatened by the presence of the devastating bacterial pathogen *Xanthomonas campestris* p.v. *musacearum*. The disease affects all banana cultivars and can cause yield loss of 80-100% of affected plants (Tripathi *et al.,* 2011). There is no single effective control measure available for the disease in the region (Tushemereirwe *et al.,* 2004; Tripathi *et al*., 2009).

A combination of several integrated disease management methods such as timely removal of male bud flowers, routine disinfection of garden tools using sodium hypochlorite or heating farm tools can check the disease (Biruma *et al.,* 2007; Green, 2006; Jones *et al.,* 2007; Karamura *et al.,* 2008; FAO. 2009; Tinzaara *et al*., 2009; Tripathi *et al.,* 2009). Rouging options and cutting down and burying/drying the infected plant/mats with disease and herbicides application to kill the affected banana plants,kills the host there by kills the pathogen (Ssekiwoko *et al*., 2006a; Blomme *et al*., 2008; ProMusa, 2014; Kubiriba *et al.,* 2014). Use of clean planting materials will avoid the diasese spread. All the mentioned cultural practices may check the disease spread within the farm and from farm to farm (Ndungo *et al.,* 2003; Onyango, 2009; Mbaka *et al.,* 2008; Tripathi *et al.,* 2009; Tripathi *et al*., 2010; Ocimati *et al.,* 2013a). Leaving land fallow for six months probably needed longer periods before replanting, removal of affected material to far place which were more labour demanding than just cutting and leaving to dry/rot on the same spot. Uprooting the whole

plants in the orchard instead of rouging specific affected plants. The study aimed at reducing the crop for losses convinient of the farmers with affected banana plantations

1. 2. Problem statement

Banana *Xanthomonas* wilt (BXW) has spread to East and Central Africa region from Ethiopia (Yirgou and Bradbury, 1968). In Kenya BXW morbidity is very high and was first reported in 2006 in Teso, Bungoma and Busia areas and a year later in Bondo, Siaya, Mumias, Butere and Mt Elgon (Tripathi *et al*., 2007; Bioversity International, 2009; Mbaka *et al*., 2009). Since then the disease spread very fast and has reached Kisumu- Maseno 130 kms by 2009 (Onyango *et al.,* 2012). It has advanced to new areas in Busia county - Busia, Bumala, Amagoro and Matayos, Kakamega county - Bukura and Malakisi, Siaya county - Yala, Ukwala, Ugenya and Ugunja, Kisumu county - Masen (Mbaka *et al*., 2009; Tripathi *et al.,* 2010; Kwach *et al.,* 2012; Onyango *et al.,* 2012). In Western Kenya where BXW had been confirmed, Mbaka *et al.* (2008) estimated yield losses of 70% to 90 %. Tripathi *et al.* (2009) estimated production loss of about 53% and overall economic losses of US$2 billion to US$8 billion over a decade (Bioversity International, 2009). The impact of BXW devastation in East and Central Africa has been estimated to the extent of US$500 million (Mwanga *et al.* 2007; Biruma *et al.,* 2007; FAO, 2007; Bioversity International 2009; Ochola *et al.,* 2014). Kayobyo *et al.* (2005) predicted a decline in per capita consumption of bananas by 42% and total household incomes loss by about 32% in the BXW affected areas. Yusto *et al.* (2013) reported in their study in Kagera Tanzania that up to 100% banana plantation loss if no BXW management and control measures are employed and a 84% of banana production within one year in Kagera that increased banana prices by 38.3% after the outbreak of BXW acoompnied by 61.7% of the farmers' income annual loss. Abele and Pillay. (2007) reported that accumulated significant reductions in production of banana crop leads to increase in

price and that all these losses consumers bear the major share of the losses due to reduced supply and increasing prices of bananas, whereas producers benefit from higher prices and tend to lose less than consumers. Therefore, immediate intervention measures were undertaken in Kenya to reduce the effects of the disease and its spread to the communities. The aim was to reduce the spread of BXW which might have been attributed by the pathogen acquiring new traits, environmental changes, mutation or human activities such as cross border trade, exchange of planting materials, insects among others.

1. 3. Justification

Pathogenecity of BXW disease is still not yet understood (Aritua *et al.* 2007, Tushemereirwe *et al.,* 2003 and 2006). The disease affects all types of bananas including cooking, dessert and plantains (Tinzaara *et al.,* 2001; Ndungo *et al.,* 2005) and can cause yield loss of 80-100% (Biruma *et al.,* 2007; FAO, 2007; Boiversity International, 2009; Jogo *et al.,* 2013). Banana *Xanthomonas* wilt, a quarantined disease since it was report in 2006 in epidemic areas. The disease is highly transmissible and preads very fast through insects vectors, infected planting materials, farm pruning/harvesting garden tools.The disease is a threat to to other major banana producing regions in Kisii, Eastern, Central and Coast therefore, the need to curb its spread. Ability of the disease to assume epidemic proportion in a matter of months poses a special problem that needs to be addressed to save the crop. No robust host plant resistance among the cultivated banana varieties has been reported (Tripathi *et al.,* 2008; Ocimati *et al.,* 2013b; Tripathi *et al.,* 2013).

The broad objective of the study was to establish the occurence, mechanism of spread and develop integrated strategies for managing banana *Xanthomonas* wilt in affected banana fields in Kenya.

The specific objectives were to:

1. Evaluate the effectiveness of different DNA capture field kits in diagnosis of banana *Xanthomonas* wilt.
2. Determine the distribution of banana *Xanthomonas* wilt in major banana producing reions in Kenya.
3. Evaluate the effectiveness of rouging and orchard replanting in management of banana *Xanthomonas* wilt.
4. Evaluate banana cultivars for tolerance to banana *Xanthomonas* wilt.

CHAPTER TWO: LITERATURE REVIEW

2. 1. Banana production and its importance

Banana is a major food security crop in developing countries and is an important source of nutrition. It is estimated to supply 75 % of the carbohydrates needed by more than 20 million people in these countries (Tripathi *et al.,* 2005; Karamura *et al.,* 2008; Tripathi and Tripathi, 2009). Bananas and plantains are the worlds' fourth most important food crop after rice, wheat and maize (Kwach *et al.,* 2000; Tripathi *et al.,* 2005 and 2009; Viljoen, 2010; Yako *et al.,* 2011; Tripathi, 2013). Sub- Saharan Africa produces 35% of the world banana and plantains (Tripathi *et al.,* 2008). In East and Central Africa, the great lakes region covering Uganda, Tanzania, Rwanda, Kenya, Burundi and Congo, produces 15 million tons per year (Bioversity International, 2009; FAO, 2009; Odipio *et al.,* 2009; Jogo *et al.,* 2011; Kubiriba *et al.,* 2012; 2014). The region has the highest per capita consumption of bananas of 200 – 250 kg annually (Tushemereirwe *et al.,* 2002; Jones *et al.,* 2007; Biruma *et al.,* 2007; Nguthi, 2008; Tripathi and Tripathi, 2009; Tripathi, 2013).

In Kenya banana is a major crop (Table 2.1) covering 74,000 hectares equivalent to 2% of arable land with production of over 510,000 tons per year (MoA, 2008; Yako *et al.,* 2011). Bananas are grown in a wide range of altitudes from 0 – to slightly over 1800 masl. Cultivation of banana is largely carried out under rain fed conditions by small scale farmers with an average banana land holding of 0.3 hectares in Kenya. The production and yields per hectares vary within the regions of Kenya.

Table 2.1: Area under banana and production levels in different regions of Kenya

Production region	Area (ha)	Production (t)	Yield (t/ha)	Value in Ksh (million)
Central	1,5540	217,175	14	2,016
Coast	5,715	65,558	11	817
Eastern	12,834	8,0232	6	892
Nairobi	42	253	6	5
North Eastern	476	5,663	12	45
Nyanza	32,396	505,258	16	3,772
Rift Valley	2,870	47,613	17	386
Western	12,645	136,266	11	1,362
Total	82,518	1,058,018	12	9,295

Source: Yako *et al.,* 2011

Cooking and dessert banana cultivars are grown in different regions of Kenya (Yako *et al.,* 2011). The distribution of banana cultivars is influenced by factors such as local taste, eating habits and marketing conditions (Rice *et al.,* 1990; Nguthi, 2008; Bioversity International, 2009). Western and Nyanza regions of Kenya produce 64% of the total national production and predominantly the East African highland cooking banana (AAA), the Apple banana (AAB) and the Cavendish (AAA) (Kwach *et al.,* 2000; MoA, 2008; Nguthi, 2008; Mbaka *et al*., 2009; Onyango *et al.,* 2011). The presence of the devastating banana *Xanthomonas* wilt (BXW) disease in Kenya causes an alarm since the disease can cause absolute yield loss of 80-100% (FAO, 2007;Ocimati *et al.*, 2013a; Kubiriba *et al.*, 2014).

2. 2. Etiology of banana *Xanthomonas* wilt

Banana *Xanthomonas* wilt is caused by a bacterial pathogen, *Xanthomonas campestris* p.v. *musacearum,* a gram-negative, single straight rod about 0.4-0.7 x 0.7-1.6µm, motile by a single polar flagellum and xylem inhabiting bacterium (Aritua *et al.,* 2008; Smith *et al.,* 2008). The bacterium is Chemo-organotrophic and obligately aerobic, without special

structures or accumulations of poly-β-hydroxybutyrate or other storage products (Kidist, 2003; Aritua *et al.,* 2008; Smith *et al.,* 2008; Tripathi *et al*., 2009; Ocimati *et al.,* 2013b).

Xanthomonas campestris p.v. *musacearum* taxonomical identity is Bacteria; Proteobacteria; Gammaproteobacteria; Xanthomonadales; that belongs to the family Xanthomonadaceae (Aritua *et al.,* 2007). Other synonyms of the disease include; banana bacterial wilt, BXW, ensete wilt and *Xanthomonas* wilt of ensete. Local names used by farmers are "Adeka K'laboro" (AIDS of banana) in Teso community in Kenya and "Kiwotoka" in Uganda (Tushemereirwe *et al.,* 2006; Muhangi *et al.,* 2006 Odipio *et al.,* 2009; Onyango, 2009; Jogo *et al.,* 2013; Kubiriba *et al.,* 2014). Banana *Xanthomonas* wilt is the common name recommended by the Committee of Common Names of Plant Diseases, International Society for Plant Pathology (Aritua *et al.,* 2006; Jones *et al*., 2007; Smith *et al*., 2008).

Work based primarily on DNA sequence and fatty acid showed that strains of *Xcm* have very close homology to strains of *Xanthomonas vasicola* and most likely belong to this species(Aritua *et al*., 2008). Researchers proposed the name *Xanthomonas vasicola* for *Xanthomonas campestris* p.v. *musacearum* although this has not been formally approved as a new combination of names (Tindall *et al*., 2006; Aritua *et al*., 2008; Smith *et al*., 2008). The species *Xanthomonas vasicola* is currently of single pathovar membership (p.v. *holcicola*), comprising strains pathogenic to sorghum. However, studies have reassigned *Xanthomonas vasicola,* at the species level, a member of *X. axonopodis* p.v. *vasculorum* strains pathogenic to maize and sugarcane that were previously erroneously classified (Aritua *et al*., 2008; Smith *et al*., 2008). Whereas the species name *Xanthomonas vasicola* is accepted for the maize and sugarcane strains, the pathovar designation, p.v. *vasculorum*, proposed has not been approved (Young *et al*., 1991;Vauterin *et al*., 2000; Smith *et al*., 2008). Thus, no formally accepted pathovar status is recognised for strains of *Xanthomonas vasicola* from maize and sugarcane.

The limited pathogenicity studies undertaken to date have provided evidence for strains from ensete and banana to be designated as *Xanthomonas campestris* p.v. *musacearum*. A strain from banana was reported to elicit a pathogenic reaction on banana and maize while single strains of *Xanthomonas vasicola* of sorghum and maize were only able to elicit disease on maize (Aritua *et al*., 2007; Smith *et al*., 2008).

2. 3. Distribution of banana *Xanthomonas* wilt

Banana *Xanthomonas* wilt was first described on Ensete (*Ensete ventricosum*, *musaceae*) in 1968 (Yirgou and Bradbury, 1968; Kidist, 2003) and later on bananas (*Musa* species) in 1974 in the highlands of Ethiopia (Yirgou and Bradbury, 1974; Ssekiwoko *et al*., 2010). However, spread outside Ethiopia remained unreported for about four decades (Tripathi, 2013). In early 2001, an epidemic of the disease was detected for the first time outside Ethiopia on banana in Mukono and Kayunga Districts in Uganda (Tushemereirwe *et al.,* 2003; FAO, 2009; Tripathi, 2013; Ocimati *et al.,* 2013a; Kumakech *et al.,* 2013; ProMusa, 2014). The disease has been confirmed to affect all banana and plantains varieties in the Great Lakes region of eastern and central Africa including, Rwanda, north-eastern Democratic Republic of Congo, Uganda, Tanzania and Western Kenya (Ndungo *et al.,* 2005; Tripathi *et al*., 2007 and 2008; FAO, 2009; Ocimati *et al.,* 2012a and 2013b).

In Kenya the disease was first reported in 2006 in Teso, Bungoma, and Busia , and a year later in Bondo, Siaya, Mumias, Butere, Mt Elgon. The disease has advanced to more parts in Emuhaya, Gem, Kakamega, Ugenya, Ugunja, Kisumu and Vihiga (Mbaka *et al.,* 2009; Tripathi *et al.,* 2009; Onyango *et al.,* 2012; Jogo *et al.,* 2013; Kwach *et al.,* 2013; Kubiriba *et al.,* 2014). In the epidemic areas of western Kenya the disease severity within a farm with no control measures can be up to 100% killing all the plants (Onyango *et al.,* 2012).

2. 4. Symptoms of banana *Xanthomonas* wilt

The first visible symptoms expressed on banana infected by BXW include progressive yellowing and complete wilting of entire plant, premature and uneven yellowing, ripening and rotting of the bunch and eventually death of plants (Kidist, 2003: Jones *et al.,* 2007; EFSA, 2008; Tripathi *et al*., 2009; Ssekiwoko *et al.,* 2010).

When the bacteria enters through floral parts of banana, there is wilting of male bud bracts, followed by drying of the rachis leading to premature fruit ripening and drying and eventually death of the entire infected plant (Kubiriba *et al.,* 2014; ProMusa, 2014). Infected flowered plants show leaf wilting and eventual death of the plant. Infected banana fruit are characterized by brown internal colorations (Tripathi *et al*., 2005; 2013). When excised, pockets of pale yellow bacterial ooze appears within five to 15 minutes from cut banana tissue. There are also yellow or brown vascular streaks throughout the interior of infected plants. Internally, cross-sections of pseudostems show yellow bacterial ooze, while the cross sections of the fruits show rusty brown stains (Tushemereirwe *et al.,* 2004; Ssekiwoko *et al.,* 2010; Tripathi *et al*., 2013; ProMusa, 2014). The disease is devastating, completely kills the plant and affects both the flowered and the non flowered plants.

2. 5. Infection and colonization of banana *Xanthomonas* wilt

Xanthomonas campestris p.v. *musacearum* does not appear to have the ability to gain entry into the plant unless there is injury (Mwangi and Bandyopadhyay, 2006; Tripathi *et al.,* 2009; Tripathi *et al.,* 2013). Bacteria cannot enter plants via intact cuticles and entry is either through wounds or natural openings such as hydathodes and stomata (Kidist, 2003; Manners, 1993). Successful infection of a host plant by a bacterium involves the movement of the bacterium towards the host, contact between the two and penetration of the host by the

bacterium and proliferation of the bacterium inside the host immediately following ingress (Gnanamanickam *et al.,* 1999). Once the bacteria enters into the plant, they multiply in the intercellular spaces and move through the tissues. Cell death of the plant may follow due to toxins or pectolytic enzymes produced by the bacteria (Gnanamanickam *et al.*, 1999; Agrios, 2005).

The rate of spread of the disease within the host depends on the rate of multiplication of the pathogen, its motility, ability to produce pectolytic enzymes and the structure of the host, environmental conditions such as temperature and on the extent to which the host produces stimulants or inhibitors for bacterial growth and activity (Manners, 1993; Agrios, 2005).

2. 6. Factors favouring banana *Xanthomonas* wilt

Banana is a perennial crop that, whilst having seasonal peaks in productivity requires, continuous husbandry (Smith *et al.,* 2008). It is probable that opportunity for infection and spread occur throughout the year, but vary according to the season. The seasonality of airborne vectors, likely insects, coinsides with parallel patterns of rainfall to the peak periods of banana growth and flowering (Tripathi *et al.,* 2009). The risk of inflorescence infection will logically increase at these times (Ndungo *et al.,* 2005).

Symptoms progress faster during the wet season than the dry season. The time taken to reach different stages of symptom expression may differ with cultivar and environmental conditions such as temperature.

Plants show symptoms within three weeks of infection (Tripathi *et al.,* 2009). Symptoms develop rapidly under favourable conditions and are typically evident within three to four weeks under field conditions (Mwangi *et al.,* 2007b; Tripathi *et al.,* 2008). Altitude and land topography affect local environmental conditions, notably temperature and rainfall,

which influences suitability for growth of banana depending on the cultivar and airborne vector populations (Mwangi *et al.,* 2007b; Tripathi *et al.,* 2009).

Male bud infection, which are mediated by airborne vectors, has not been observed in Ethiopia at altitudes over 1700 masl (Addis *et al.,* 2004: Ndungo *et al.,* 2005: Smith *et al.,* 2008; Kumakech *et al.,* 2013). Infection through the banana inflorescence is not common above 1700 masl but is common around the lower elevations of Lake Victoria at altitude of 1135masl (Ndungo *et al.,* 2005). This suggests that climatic factors affect spread of BXW. The agro-ecology of the region under study lies between 800 to 1,500 masl, which are more suitable for insect vectors of the pathogen (Mwangi and Bandyopadhyay, 2006; Tripathi *et al.,* 2009; Tripathi and Tripathi, 2009; Tripathi *et al*., 2013).

2. 7. Spread and survival of banana *Xanthomonas campestris* p.v. *musacearum*

Banana *Xanthomonas* wilt is mainly spread by insects which pick up the bacteria externally on body parts or internally within organs when insects visit the banana inflorescence. The most common insects are stingless bees (*Plebeina denoiti*- Family *Apidae*), fruit flies (Family *Drosophilidae*) and grass flies (Family *Chloropidae*) and honeybees (*Apis mellifera*) (Gold and Bandyopadhyay, 2005; Gold *et al;* 2006; Tinzaara *et al.,* 2006; Bioversity international, 2009; Tripathi and Tripathi, 2009). Nectar-sucking birds and bats are suspected of transmitting the bacterium (Tinzaara *et al.,* 2006; Smith *et al.,* 2008; Tripathi *et al*., 2013).

Use of contaminated husbandry tools during banana orchrd operations spreads Xcm. The bacteria can be viable on metallic tools for upto three days if not sterilized (Mwangi and Muthoni, 2008). The commonly used tools include the knives (machetes) used in the removal of old age leaves and suckers to retain the required pseudostems per mat. Similarly, harvesting of banana fruits and leaves is undertaken by use of a knife or ‘panga (Tinzaara *et al.,* 2013; Tripathi *et al*., 2013). Spread of disease by contaminated knives and

‘pangas’(machete) is, therefore, a key mechanism for BXW spread (Eden-Green, 2004; Mwangi and Muthoni, 2008; Ssekiwoko *et al.*, 2010; ProMusa, 2010; Tinzaara *et al.*, 2013).

Spread of BXW has been associated with the movement of infected banana planting materials such as movement of banana suckers for planting new plantation or replanting (Thwaites *et al.,* 2000; Mwebaze *et al.,* 2006; Mwangi *et al.,* 2007a; Ocimati *et al.,* 2013a). Being a vascular wilt disease, Xcm is thought to be systemic, suckers obtained from severely infected mats with latent symptoms grew without showing BXW symptoms up to two years/bunch harvest (Ssekiwoko *et al.,* 2006a; Ocimati *et al.,* 2013b). As a result latent symptoms banana suckers have been transported from one ponit to the next leading to importation of diseased plant material from point/place to the next,thus spread of BXW (Smith *et al.,* 2008; Tripathi *et al.,* 2008). Fresh banana leaves and or dry leaves infected with the BXW when used as cushion for packing harvested banana bunches to market places can be a source of importation of the disease from one point such as from home to marketing place. The leaves debris may be disposed at a place they may be in contact with clean banana plant and a point of pathogen entry may be condusive, can be a possible way of disease spread. Infected male buds when used as bungs for water containers, when disposed in banana plantations maybe a source of transmission if the pathogen could find an entry point onto healthy banana plant (Onyango *et al.,* 2013; Tripathi *et al.,* 2013).

Bacteria could also penetrate into plants through roots, especially if injured by soil borne pests, namely, nematodes and weevils (Mwangi *et al.*, 2007a; Tripathi *et al.,* 2009). Different infection rates were observed when infested soils were placed on the rhizomes of various cultivars of AAA genome, some had less incidence of the disease (Smith *et al.,* 2008; Tripathi *et al.,* 2008; FAO, 2009).

Persistence of banana Xcm in soils is not well understood and may be between three to six months. The bacteria can survive in non sterile soils for 35 days, while in sterile soils

for up to 90 days. When the pathogen is associated with the plant debris it can survive only for 21days when buried or on the soil surface. *Xanthomonas campestris* p.v. *musacearum* can survive for over 90 days when stored in refrigerator. When the host debris and residues are present it can survive more than four month. (Mwebaze *et al.,* 2006; Ssekiwoko *et al.,* 2006b; Mwangi *et al.,* 2007b; Smith *et al.* 2008; Welde-Michael *et al.,* 2008, Tripathi *et al.,* 2010). The pathogen is known to survive for up to three months in the soil in the absence of a host (Mwebaze *et al.,* 2006; Tumushabe *et al.,* 2006: Tripathi *et al.,* 2007; Ocimati *et al.,* 2013b).

2. 8. Banana cultivars tolerant to banana *Xanthomonas* wilt

Previous studies have not found a natural tolerance in cultivated *Musa* spp. against BXW (Tripathi *et al.,* 2008). However some cultivars posses characteristics such as lack of male bud or persistent bracts that make it difficult for the pathogen to infect under natural conditions (Ssekiwoko *et al.,* 2006b; 2006c; Muchunguzi *et al.,* 2007; Ssekiwoko *et al.,* 2009; Lewis *et al.,* 2010; ProMusa, 2010; 2014). Prospects of developing cultivars with resistance to BXW through conventional breeding are limited because so far no source of cultivated banana germplasm exhibiting resistance against Xcm has been identified (Tripathi *et al.,* 2008; and 2010; Ocimati *et al.,* 2013b).

2. 9. Diagnosis of banana *Xanthomonas* wilt

The emergence of BXW disease has threatened the livelihoods of tens of millions of East-African banana farmers (Tushemereirwe *et al.,* 2004; Biruma *et al.,* 2007). The disease may be diagonesed in several ways depending on the aim and resources, some methods are less costly while others like polymerase chain reaction (PCR) need specialised facilities.

Field observations are used for visible symptoms expressed on banana already affected by BXW such as yellowing of leaves wilting of male bud bracts, followed by drying

of the rachis, premature and uneven ripening of fruit characterized by brown internal colourations, cross section of the infected pseudostem shows pockets of pale yellow bacterial ooze that appears within five to 15 minutes (Yirgou and Bradbury, 1968; Yirgou and Bradbury, 1974; Ssekiwoko *et al.,* 2010; Tripathi *et al.,* 2013; Kubiriba *et al.,* 2014; ProMusa, 2014). A part from visual symptoms BXW can be diagonised by several scientific apprved technologies these include capturing Xcm DNA using field capture kits such as Flinders Technology Associates (FTA) Whatman card field kit and 2 minute DNA dipsticks field kit which are later subjected to PCR analysis and eclectophoresis process taking a gep image then read in ultra violet lights (UV). Enzyme –Linked Immunosorbent Assay (ELISA) for testing specificity and sensitivity are some of the methods (Mahuku, 2004; Ssekiwoko *et al.,* 2006c.; Kumakech *et al.,* 2013).

Xanthomonas campestris p.v. *musacearum* can be diagonised and confirmed by several other biochemical tests such as Anaerobic growth, Aesculine hydrolysis, Carbohydrate utilization, Urease production, Catalase production, Starch hydrolysis andH_2S production from organic sulphur compounds (Mahuku, 2004; Kumakech *et al.,* 2013*).*

2. 10. Management of banana *Xanthomonas* wilt

Banana *Xanthomonas* wilt spread to other areas in East and Central Africa is a threat to banana commodity that requires some control measures thus calls for specific research to generate data on its management and control. The measures may include integrated disease management methods.

Routine disinfection of garden tools after use on each mat using sodium hypochlorite assists in killing the bacteria and thus reduces the spread rate from one plant to the next (Green, 2006; Jones *et al.,* 2007). Alternatively heating garden tools, such as hoes, machete, knives and slashers over a fire until the metal is too hot to touch is also effective in killing the

bacteria (Biruma *et al.,* 2007; Karamura *et al.,* 2008). Where contaminated tools are the main means of pathogen dissemination, disease spreads in a systematic manner starting from the infected mat and expanding outward thus disinfection to have clean farm operation tools is vital (Karamura *et al.,* 2008; Tripathi *et al*., 2010; Ocimati *et al.,* 2013a). The fields can also be kept clean by spot checking any BXW symptoms and can be reinforced by having a suspended mechanical weeding/operations for at least three months to observe the latent symptoms during the period weeding can be done by herbicides (Karamura *et al.,* 2008; Okurut *et al.,* 2006).

Use of clean planting banana material like tissue culture plantlets and or conventional rapid field macro propagated banana suckers in disease free areas checks BXW spread from farm to farm (Ndungo *et al.,* 2003; Onyango, 2009; Mbaka *et al.,* 2008; Tripathi *et al.,* 2009; Tripathi *et al*., 2010; Tripathi, 2013; ProMusa, 2014). Entire infected mats which have not flowered can be removed to ensure that no infected suckers reach flowering, avoiding sources of inoculum for insect vectors (Muchunguzi *et al.,* 2007).

Timely removal of male buds with a forked stick is important in where insect vector that visit the diseased flower parts carry the pathogen mechanically from one flower to the next (Tinzaara *et al*., 2009; FAO, 2009; Tinzaara *et al*., 2013). The disease can be contained in fields where de-budding is effectively practiced (Green, 2006; Tinzaara *et al*., 2009; Tripathi, 2013; Ocimati *et al.,* 2013a; Ocimati *et al.,* 2013b; Kubiriba *et al.,* 2014). The practise should be carried out just after the female flowers have been formed three to four weeks after flowering.

Rehabilitation of previously affected banana orchards by removal of the infected plants within a mat reduces the disease spread from one plant to other plants mostly for the infection by insects through flower buds at initial stages before the disease has advanced to the soil (Tripathi *et al.,* 2009; Kubiriba *et al.,* 2014). Cutting down and burying whole mats

with disease symptoms kills the pathogen as they cannot survive without banana. The use of herbicides to kill the affected banana plants has also been effective (Ssekiwoko *et al*., 2006a; Blomme *et al*., 2008; Addis *et al.,* 2010). Once BXW occurs in a field, the recommended remedy is to rougue out all infected plants, completely dig out the rhizomes and leave the field fallow for a period thereby killing the pathogen,which eventualy reduces the pathogen build up (Karamura *et al*., 2008; Blomme *et al*., 2008; Tripathi *et al.*, 2009; Kwach *et al.*, 2012; Kubiriba *et al.*, 2014).

CHAPTER THREE: DAIGNOSIS OF BANANA *XANTHOMONAS* WILT BY DNA AND SEROLOGICAL ANALYSIS

3. 1. Abstract

Symptoms of banana *Xanthomonas* wilt (BXW) are confused with *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *cubense*. Therefore field diagnostic tools for BXW that are easy to use, fast, precise and effective are important in detecting the disease at early stages. The objective of the study was to evaluate the effectiveness of DNA capture field kits in polymerase chain reaction (PCR) diagnosis of BXW. Two-hundred and fifty eight samples suspected to be BXW were collected from East and Cetral African countries using the Two-minute DNA capture field kit and Flinders Technology Associates (FTA) Whatman card field kits. Another set of of 36 fresh samples from the field in Kifu Uganda were collected and these were analysed by two-minute DNA capture field kit, FTA card field kits, ELISA and direct extraction of DNA for comparison. The captured DNA was subjected to PCR analysis for presence of *Xanthomonas campestris* p.v. *musacearum* (Xcm). Two-minute dipstick DNA capture field kit was found to be most efficient in capturing Xcm DNA under field conditions and it gave comparable results with Enzyme –Linked Immunosorbent Assay (ELISA) and direct extraction of DNA from fresh samples. Out of 258 field samples, 25.9% were positively diagnosed using two-minute dipstick DNA capture field kit and only 1.5% with FTA cards while out of 36 fresh samples 38.8% were positive with two-minute dipstick DNA capture field kit, 25% FTA cards, 38.8% direct for DNA extraction and 38.8% with ELISA methods. Two-minute dipstick DNA capture field kit was efficient in capture of *Xcm* DNA from both symptomatic and asymptomatic but infected banana plants. The two-minute dipstick DNA capture field kit enables sampling in remote areas without the need for complicated equipment.

Keywords: *Xanthomonas campestris* p.v. *musacearum*, DNA capture field kits, PCR, ELISA

3. 2. Introduction

Banana *Xanthomonas* wilt (BXW) visual field symptoms plants include wilting and/or drying of male buds, premature ripening of bunches, yellowing of leaves, vascular discoloration and production of yellow ooze on cut vascular tissues (Yirgou and Bradbury, 1968; Tushemereirwe *et al.,* 2004;). These symptoms easily confused with those due to a fungal disease *Fusarium oxysporum* f.sp. *cubense* that causes wilting/or nutritional disorders (Aritua *et al.,* 2008(b); Studholme *et al.,* 2010; Carter *et al.,* 2010; Adikini *et al.,* 2011). Therefore diagnostic tools that are easy to use, fast, precise and effective in capturing Xcm are vital to help field workers and farmers for better BXW management (López *et al.,* 2009; Ssekiwoko *et al.,* 2010; Jogo *et al,* 2011; Kubiriba *et al.,* 2014).

Some methods are expensive comparatively, while others may be simple and can capture the pathogen in the field and later be diagnosed in the laboratory. Some of the methods of capturing the DNA can store the information for long, Mbogori *et al.* (2006) reported FTA cards can maintain DNA integrity for over 14 years, under good storage conditions for successful polymerase chain reaction (PCR) amplification. Further more, some of these methods like the two-minute dipstick DNA capture field kit and FTA Whatman card field kits are applicable for samples that can be carried from one country to the other to avoid spread of the disease across border (Ndungo *et al.,* 2006; Tomlinson *et al.,* 2007; Mwang *et al.,* 2007; Aritua *et al.,* 2008(a); Biruma *et al.,* 2008; Carter *et al.,* 2009; Ramathani and Beed, 2013). *Xanthomonas campestris* p.v. *musacearum* has been reported to have latent expression upto two years/from planting of banana to flowering which gives chances for the disease to spread when planting materials are carried without visual field symptoms (Biruma *et al.,* 2008; Ocimati *et al.,* 2013a). The longer latent period of BXW might attribute to elusiveness and re-appearance of BXW in the epidemic areas where the BXW had been

controlled (Kuburiba *et al.,* 2012; Ocimati *et al.*, 2013b; Tinzaara *et al.,* 2013). The study objective was to evaluate effectiveness of DNA field capture kits in diagnosing Xcm.

3. 3. Materials and methods

3. 3. 1. Experimental materials

Two hundred fifty eight (258) samples were collected from suspected BXW infected banana crop;. Rwanda (60), Tanzania (60), Kenya(44), Uganda (44), Burundi (30), and twenty from Democratic Republic of Congo (Table 3.1). The samples by the the two-minute dipstick DNA capture field kit and FTA Whatman cards from BXW symptomatic and healthy looking banana plants. Another set of thirty six fresh samples from ten plants in a field which had been infected with BXW (Table 3.2) five non-symptomatic and five symptomatic plants were randomly picked for BXW diagnosis.

Table 3.1: Suspected banana *Xanthomonas* wilt samples collected from East and Central African countries for diagnosis of *Xanthomonas campestris* p.v. *musacearum*

Country	Total number of samples	Condition of the sample		
		Diseased	Suspected	Healthy
Burundi	30	3	22	5
DR Congo	20	20	0	0
Kenya	44	22	17	5
Rwanda	60	30	0	30
Tanzania	60	20	30	10
Uganda	44	22	14	8
Total	258	117	83	58

Table. 3.2. Fresh samples collected at Kifu, Uganda for diagnosis of banana *Xanthomonas* wilt

Sample code	Plant part	Condition of plant
1L	Leaf	Symptomatic
1P	Pseudostem	Symptomatic
1C	Corm	Symptomatic
1R	Rachis	Symptomatic
1F	Fruit	Symptomatic
2L	Leaf	Symptomatic
2P	Pseudostem	Symptomatic
2C	Corm	Symptomatic
2R	Rachis	Symptomatic
2F	Fruit	Symptomatic
3L	Leaf	Symptomatic
3P	Pseudostem	Symptomatic
3C	Corm	Symptomatic
4L	Leaf	Symptomatic
4P	Pseudostem	Symptomatic
4C	Corm	Symptomatic
5L	Leaf	Symptomatic
5P	Pseudostem	Symptomatic
5C	Corm	Symptomatic
5R	Rachis	Symptomatic
5F	Fruit	Symptomatic
6L	Leaf	Symptomatic
6P	Pseudostem	Symptomatic
6C	Corm	Symptomatic
7L	Leaf	Symptomatic
7P	Pseudostem	Symptomatic
7C	Corm	Symptomatic
8R	Rachis	Symptomatic
8P	Pseudostem	Symptomatic
8C	Corm	Symptomatic
9L	Leaf	Non-symptomatic
9P	Pseudostem	Non-symptomatic
9C	Corm	Non-symptomatic
10L	Leaf	Non-symptomatic
10P	Pseudostem	Non-symptomatic
10C	Corm	Non-symptomatic

3. 3. 2. Capture of *Xanthomonas campestris* p.v. *musacearum* DNA by two-minute dipstick field kit

The *Xanthomonas campestris* p.v. *musacearum* DNA extraction involved addition of macerated sample to the two-minute dipstick DNA capture field kit which comes with its bottle containing extraction buffer (Tris-Cl, NaCl, EDTA and n-lauroylsarcosine). Once the sample is put inside the bottle, then the lid closed and the bottle containing sample was shaken for 30 seconds. Four dipsticks with the glass fibre were inserted to be in contact with the buffer were allowed to run for approximately two minutes in the buffer then were removed. The dipsticks were placed on a clean paper towel and allowed to air-dry without exposing to direct sunlight. Paper towels were then discarded. The captured DNA a single punch 2 mm^2 disc was taken and processed for PCR analysis.

3. 3. 3. Capture of *Xanthomonas campestris* p.v. *musacearum* DNA by Whatman FTA cards

Samples of symptomatic plant parts were crushed and extracted smeared on to the Whatman FTA card to capture the Xcm DNA. The cards were left to dry overnight and then kept in a desiccator. From the above sample, the DNA of Xcm was eluted onto Whatman FTA card a single punch 2 mm^2 disc was taken for PCR analysis.

3. 3. 4. Direct extraction of *Xanthomonas campestris* p.v. *musacearum* DNA from fresh samples

Fresh symptomatic and non symptomatic plant samples were collect in the field at Kifu, in Uganda (Table 3.2). Each sample was cut into small 3mm^2 pieces and surface sterilized in 70% ethanol for two to three minutes. Then a rinse was thoroughly done in

sterile distilled water blotted dry. A pproximately 3g of surface sterilized plant tissue was put into to the mortar and 3mL of distilled water was added then crushed using a pestle. A loopful of the extract was streaked onto 20ml nurtrient agar and incubated at 25 ^{o}C for 24 hours. A single bacterial colony was sub cultured onto fresh nutrient agar.

3. 3. 5. Polymerase Chain Reaction of *Xanthomonas campestris* p.v. *musacearum*

Polymerase chain reaction analysis was performed following the method described by Adikini *et al.* (2011) and Ramathani and Beed (2013) using primers Xcm-38F (5′CCGCCGG TCGCAA TGTGGGTAAT3′) and Xcm-38R (5′CAGCGGCGCCGGTGT ATTGAGTG3′) primer pairs. A 20 ml reaction mix containing 1× reaction buffer (50 mM KCl, 10 mM Tris-HCl pH 8.3), 1.5 mM MgCl, dNTPs (Promega) at 0.25 mM, 0.5 pmol of each primer and 1.0 U of Taq DNA polymerase was placed in a thermocycler with the DNA template. Thermocycler were set at an initial denaturation of 94°C for 5 minutes, followed by 40 cycles of 94°C for 20 seconds, 60°C for 20 seconds and 72°C for 1 minute, with a final extension step of 72°C for 10 minutes. The PCR products were separated by electrophoresis in a 2.0% agarose gel containing ethidium bromide (l g/10 ml or 5 ml of ethidium/100 ml of TAE (Tris-acetate-EDTA buffer) under a constant current of 100 V, running in 1× TAE buffer. The amplified DNA fragments were visualized under UV light using a UV Transilluminator and gel photo were read that was corresponding to the Xcm primer DNA bands 650bp of the Xcm positve control.

3. 3. 6. Enzyme –Linked Immunosorbent Assay (ELISA) for the detection of *Xanthomonas campestris* p.v. *musacearum*

Direct Antigen Coated ELISA (DAC-ELISA) method using polyclonal antibodies (PCAs) as described by Clark and Bar-Joseph (1984), Hobbs *et al.* (1987), Cerovska *et al.* (2006), Ssekiwoko *et al.* (2006), Vemana and Jain (2010) and Zia-Ul-Hussnain *et al.* (2013) was used. Banana plant tissues were ground fresh in carbonate coating buffer (1:1 w/v); containing 2% polyvinyl pyrollidone; pH 9.8) at a rate of 100 mg/ml. About 100µl of the extract was dispensed into each well of a new ELISA plate and incubated at 4^{o}C overnight. The coated plates were washed thrice with Phosphate Buffer Saline Tween (PBS-T) containing 0.05% Tween-20, allowing three minutes for each wash. The BXW polyclonal antiserum was diluted to 1:10000 in Phosphate Buffer Saline-Tween, polyvinyl pyrollidone ova albumin (PBS-TPO) containing 2% polyvinyl pyrollidone and 0.2% egg ova albumin and was dispensed onto 100µl into each well and incubated at 37^{o}C for one hour. This was washed again with PBS-T allowing three minutes for each wash. Another dilution of Goat anti-rabbit Alkaline phosphatase (ALP) conjugate to 1:10,000 in PBS-TPO was dispensed onto 100µl into each well and incubated in plates at 37^{o}C for one hour, thereafter washed again with PBS-T. A fresh 100µl of p-Nitro phenyl Phosphate (pNPP) substrate buffer containing 10% diethanolamine and *p*-nitro phenyl phosphate at 0.5mg/ml, pH 9.8) was added to each well and the plates incubated in the dark at room temperature of 24^{0}C. The plates were observed for any colour changes and absorbance measured at 405nm in an ELISA plate reader after one and two hours.

3. 3. 7. Data analysis

Data was analyzed using SPSS statistical package version 11.0. (Sabine and Brian 2004) and percentages expressed.

3. 4. Results

Out of 258 samples from East and Central African countries, the percentage of positive amplified by the 2minutes dipstick method were higher than FTA cards tested using primers Xcm-38F and Xcm-38R that produced bands at 650 bp size with 100bp ladder. (Figures 3. 1, 3. 2, 3. 3 and Table 3.3). While from the fresh samples of 36 using the same primer Xcm-38F and Xcm-38R were also positively amplified with the same primer (Figure 3. 4 and Table 3. 4). Two minutes DNA dipstickfield kit was singficanlty better than FTA but were comparable with direct DNA extraction and ELISA.

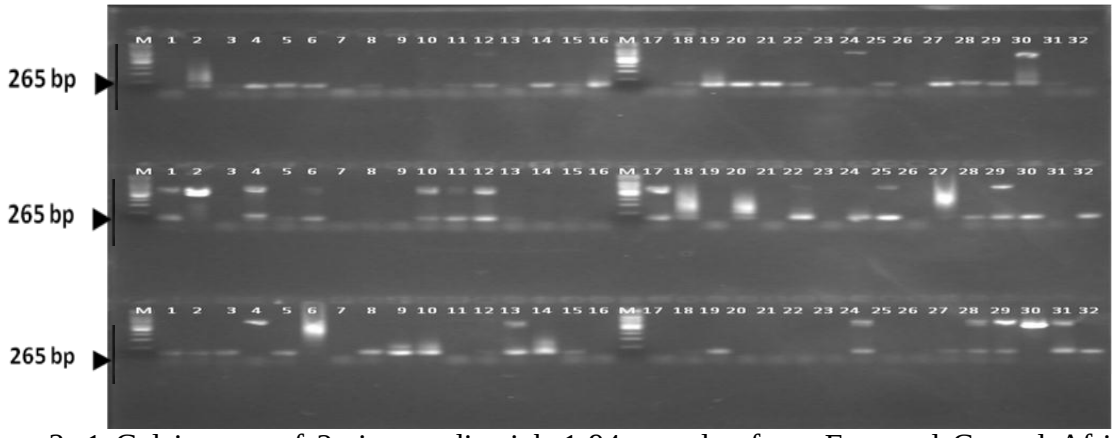

Figure 3. 1 Gel images of 2minutes dipstick 1-94 samples from East and Central African countries.
Key: First row 1-30 from Burundi, 31-32 DR Congo, Second row 1-19 from DR Congo, 20-32 from Kenya Third row from 1.-30 from Kenya, bp= Primer base pair, M=Ladder

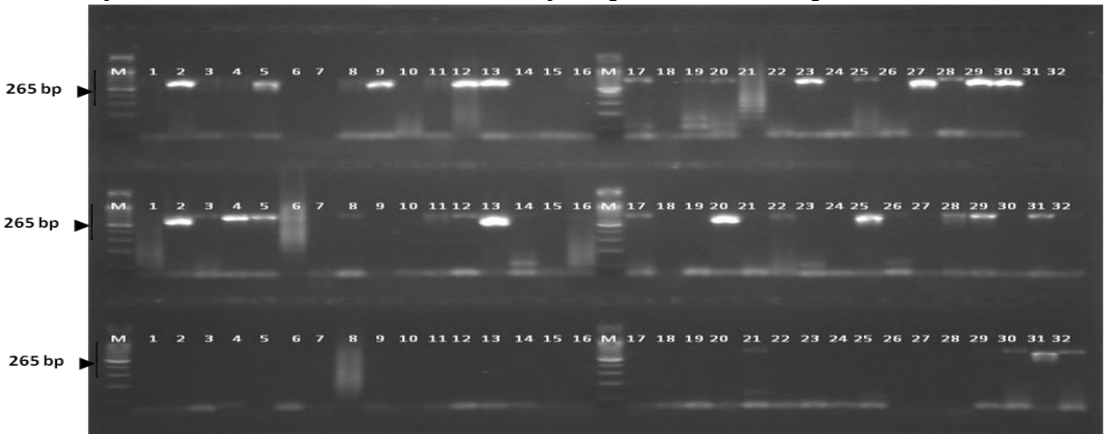

Figure 3. 2 Gel images of 2minutes dipstick 95-188 samples from East and Central African countries.
Key: First row 1-2 from Kenya, 3-32 from Rwanda, Second row 1-30 from Rwanda, 31.-32 from Tanzania , Third row 1-28 Tanzania, 29-32 from Uganda, bp= Primer base pair, M=Ladder

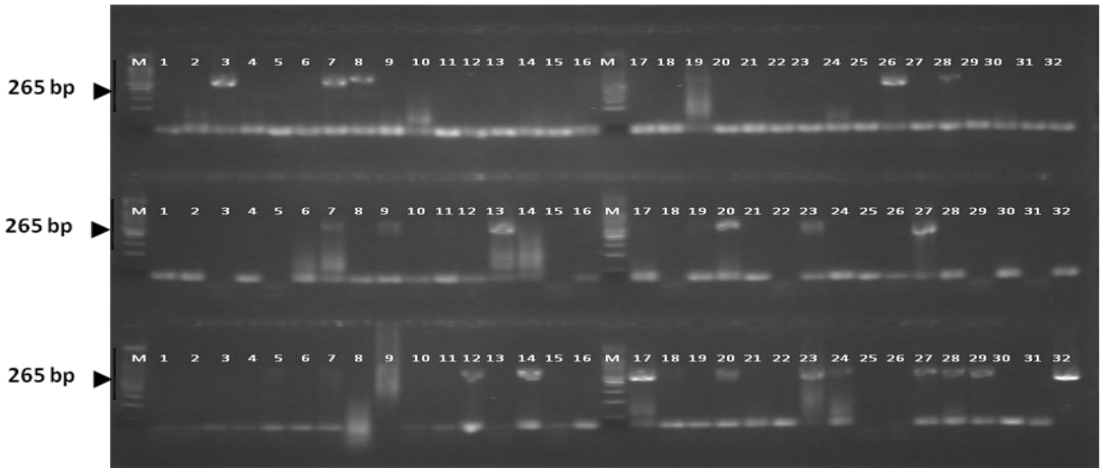

Figure 3. 3 Gel images of 2 minutes dipstick 189-258 samples from East and Central African countries.
First row 1-32 from Uganda, Second 1-8 from Uganda, bp= Primer base pair, M=Ladder

The ELISA test was negative (Table 3.3 and Figure 3.4) the average optical density (OD) reading from duplicate sample wells was less than twice the average OD of that in the negative sample control. For the positive controls are all above 1.0 OD after 60 minutes incubation were greater than twice.

Banana *Xantomonas* wilt was more prevalent in Rwanda and D.R. Congo (Table 3.3). with above 40% positive results with two minutes dipstick while the least was in Tanzania with less than 10% of the samples analysed. FTA had the highest positive results in Burundi however they were lower than 7%

Table 3.3: Percent samples from East and Central African positive for *Xanthomonas campestris* p.v. *musacearum* DNA captured by 2 minute dipstick and FTA Whatman card field kits

Country	Total number of samples	Percent positive samples	
		Two-minute	FTA
Burundi	30	10.0	6.7
DR Congo	20	40.0	0.0
Kenya	44	22.7	2.2
Rwanda	60	55.0	1.7
Tanzania	60	8.3	0.0
Uganda	44	18.2	0.0
Total	258	26.0	1.5

Out of the total number of leaf samples 50% were negative, of which 25% from symptomatic and 25% from non-symptomatic samples. 70% positive from pseudostem symptomatic samples. 10% were negative symptomatic while 20% were negative from non-symptomatic samples. All the fruit/rachis were positive from symptomatic samples only pseudostem had the highest positive for two mines dipstick, FTA and PCR while ELISA was only high for rachis/fruit. FTA had lower positive results than the other methods used.

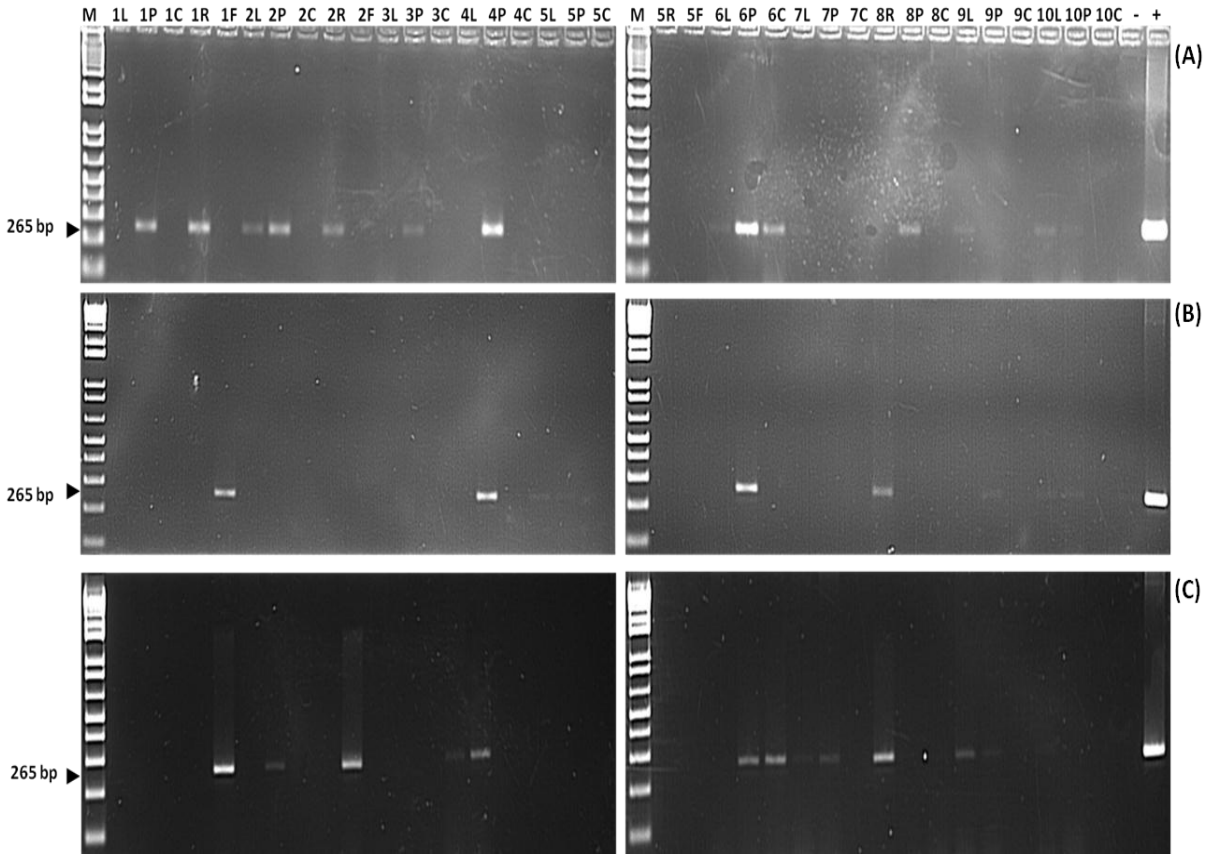

Figure. 3. 4. Gel images for *Xanthomonas campestris* p.v. *musacearum* field fresh samples from Kifu

Key: (A) 2 minute DNA capture kit, (B) FTA cards and (C) Direct DNA extraction, bp= base pair, M=Ladder, L=Leaf sample, P=Pseudostem, C=corm, R=Rachis, F=Fruit, - Negative control and + Positive control

Table 3.4 Percent samples positive for *Xanthomonas campestris* p.v. *musacearum* determined by DNA two-minute dipstick and FTA Whatman card field capture kits, direct extraction of PCR and DAC-ELISA methods

Plant part	Total No.of samples	Field DNA capture kits		Fresh samples	
		Two-minute	FTA card	PCR	DAC-ELISA
Leaf	8	50	25	50	50
Pseudostem	10	70	50	60	30
Corm	10	20	0	19	20
Rachis/Fruit	8	15	26	26	55
TOTAL	36	38.8	25	38.8	38.8

3. 5. Discussion

The two-minute dipstick DNA capture field kit, direct DNA extraction method and ELISA gave higher number of positive results compared to FTA Whatman card. Ramathani and Beed (2013) reported that two-minute dipstick DNA capture field kit method had higher results than FTA DNA capture kits and were more efficient, simple to use in the field and also provided a rapid means of diagnosis using crude DNA. The field DNA capture kits do not require purification of DNA before PCR amplification as direct DNA extraction protocols, no liquid nitrogen for grinding plant tissue (Mbogori *et al.,* 2006).

The samples from Uganda, D.R.Congo, Burundi, Rwanda, Tanzania and Kenya tested positive for Xcm. The presence of the disease in these countries is in agreement with Ramathani and Beed (2013) who tested for banana bunchy top and BXW also confirmed the disease in all the countries used the two minute and FTA DNA field kits. The current study was able to detect and captured Xcm pathogen DNA by the field capture kits among diseased, suspected samples as well as among those considered to be healthy, an indication that its an effective method that can help in detection at field level to detect latent stages of BXW (Ramathani and Beed, 2013).

ELISA method gave similar results to the two-minute dipstick DNA capture field kit, however ELISA cannot be used alone for accurate conclusion the result might be more sensitive due to reaction of the Xcm with the plants antigens (Hobbs *et al.,* 1987; Ngobich *et al.,* 2003 and Rajasulochana *et al*., 2008). Use of the field kits in combination with PCR offers quick results with the greatest level of accuracy and requires minimum resources, as a single fragment of DNA can provide evidence of a pathogen (Rajasulochana *et al*., 2008).

Polymerase chain reaction gives a clear-cut deduction of the causal agent, and most especially when using gene-specific primers that target the presence of specific DNA fragments thus acurate and give definate diagnosis (Chandler and Jarrell, 2005). Two-minute

dipstick DNA capture field kit containing DNA can be stored easily and amplified at any time provided the samples are kept at room temperature (Ramathani and Beed, 2013). The two-minute dipstick DNA capture field kit was simple to use in the field and also provided a rapid means of diagnosis using crude DNA, contrary to direct DNA extraction and ELISA which require purification of DNA before PCR amplification (Ramathani and Beed, 2013). Further more, liquid nitrogen for grinding plant tissue is not required (Ramathani and Beed, 2013). The Xcm bacterial DNA was captured in the field from the diseased, suspected or asymptomatic and the result obtained was sufficient and efficient with greater sensitivity as compared to ELISA method and direct DNA extraction that requires samples to be transported to laboratory.

The two-minute dipstick DNA capture field kit simplified the steps of DNA collection, reduced the cost and time required to process DNA to the final step of purification (Mbogori *et al.,* 2006; Ramathani and Beed, 2013). Two-minute dipstick DNA capture field kit sticks are impregnated with chemicals that allow for the breakdown of the cell wall, and thus immobilize the DNA within the matrix without interfering with Xcm integrity (Mbogori *et al.,* 2006). The study showed that two-minute dipstick DNA capture field kit was significantly better than FTA cards, as 26% was positve with Xcm for two-minute dipstick DNA capture field kit as compared to 1.5% for FTA cards. This means the two-minute dipstick DNA capture field kit sampling kits demonstrated their usefulness in capturing crude Xcm for diagnosis and can be applied in epidemiological studies in the disease endemic areas and cross border sampling to different countries without carrying live pathogen (Ramathani and Beed, 2013). The two-minute dipstick DNA capture field kit was able to detect the Xcm and confirmed its presece in all the East and Central African countries.

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CHAPTER FOUR: OCCURENCE OF BANANA *XANTHOMONAS* WILT IN KENYA

4. 1. Abstract

Banana *Xanthomonas* wilt (BXW) caused by *Xanthomonas campestris* p.v. *musacearum* (Xcm) was first reported in western Kenya in 2006. The disease is devasitating, spreads very fast and affects all banana cultivars. The objective of the study was to determine the occurrence and transmission of BXW in banana growing areas of Kenya. A survey was carried in major banana growing regions of Nyanza, Western, Eastern, Central and Coast. In addition, the diversity of insects associated with BXW infected and healthy banana orchards were colleted was determined from banana flowers and fruits in BXW endemic areas of western Kenya. Presence of BXW was confirmed in Busia, Kakamega, Bungoma, Siaya, Kisumu and Bondo areas. The disease was significantly higher in Western than Nyanza regions. All other banana producing areas in Kenya were free from BXW. Insects found associated with banana as potential vectors of BXW in epidemic areas were stingless bees (*Apis* sp), grass flies (*Drosophila* sp), banana fruit flies (*Tephritida Ceratitis*), beetles (*Neomyia ruissima* sp), and black ants (*Plectroctena* sp). Stingless bees were most abundant followed by fruit flies on flowers and fruits of banana plants. Insects are potential vectors of BXW. Banana *Xanthomonas* wilt was present in Kenya and spread in Western and Nyanza regions only.

Key words: Banana *Xanthomonas* wilt, status, transmission, Kenya

4. 2. Introduction

Banana *Xanthomonas* wilt was first reported in Kenya in 2006 in Teso, Bungoma and Busia and a year later in Bondo, Siaya, Mumias, Butere, Mt Elgon (Mbaka *et al.,* 2009; Tripathi *et al.,* 2009). The disease had advanced to more parts in Emuhaya, Gem, Kakamega, Ugenya, Ugunja, Kisumu and Vihiga (Onyango *et al.,* 2012; Jogo *et al.,* 2013; Kwach *et al.,* 2012; Kubiriba *et al.,* 2014). Bananas *Xanthomonas* wilt spreads very fast mainly by insect (Tinzaara *et al.,* 2006; Tripathi *et al*., 2013; Kubiriba *et al.,* 2014). Insect vector transmission in banana occurs when the insects are foraging giving a possibility of picking up the bacteria from the wounds of infected inflorescences during feeding and or collecting nectar and later as they visit the next inflorescence (Addis *et al.,* 2010; Ekesi and Muchugu, 2007; Shimelash *et al.,* 2008; Bioversity, 2011; ProMusa, 2014). The common insects that may play the role of transmission include stingless bees, fruit flies and grass flies, black ants (Gold and Bandyopadhyay, 2005; Gold *et al.,* 2006; Tinzaara *et al.,* 2006; Bioversity international, 2009; Tripathi and Tripathi, 2009).

Use of of infected planting materials and movement from one point to the next for replanting or establishing new orchard(s) has contributed to the spread of BXW (Thwaites *et al.,* 2000; Mwebaze *et al.,* 2006; Mwangi *et al.,* 2007a; Ocimati *et al.,* 2013a). Mainly suckers obtained from infected mats with latent symptoms carry the pathogen and can grow in the new field/site up to two years or up to bunch harvest is when BXW symptoms wii be expressed (Ssekiwoko *et al.,* 2006; Mwangi *et al.,* 2007b; Ssekiwoko *et al.,* 2010a; Ocimati *et al.,* 2013b). Infected planting materials has played a major role in importation and introduction of BXW especially from far distances (Smith *et al.,* 2008; Tripathi *et al.,* 2008).

Contaminated garden tools spreads BXW and the commonly used tools are the knives and machetes for pruning, at harvesting of banana fruits and leaves (Tinzaara *et al.,* 2013;

Tripathi, 2013). Tools used during weeding can spread BXW when an infected mat is cut by the hoe that may carry the pathogen. It is also possible that borrowing of farm tools or communal harvesting of banana bunches from one farm to the next using the same tools can spread BXW. The bacteria can be viable on metallic tools for upto three days if not sterilized (Eden-Green, 2004; Mwangi and Muthoni, 2008; Ssekiwoko *et al.,* 2010; ProMusa, 2010; Tinzaara *et al.,* 2013). The objective of the study was to establish the occurrence and insect diversity associated with banana *Xanthomonas* wilt in banana producing ares of Kenya.

4. 3. Materials and methods

4. 3. 1. Determination of occurence of banana *Xanthomonas* wilt in banana producing areas of Kenya

Stratified random sampling was used to identify the banana growing households for interview using a questionnaire (Appendix I) administered between February and April, 2010. A total of 500 households were interviewed, 233 from Nyanza, 197 from Western, 32 from Eastern, 20 from Central and 18 from Coast regions (Table 4.1) which were the five leading banana producing regions. Two from each area, two divisions per county, two villages per division and fifteen farmers per village were targeted. From one village to the next average 15 kilometers apart in the direction where the BXW was advancing. From one farmer interviewed to the next was at least two kilometers and must own a banana plantation with at least ten stools.

Table 4.1. Specific sites and number of farms sampled during the survey of banana *Xanthomonas* wilt in banana producing areas of Kenya.

Agro ecological Zones	Regions	Specific (site) Division	No. Farms sampled Total (n)*= 500 farms
UM_1**	Central	Muranga	8
UM_3	Central	Kaguini	2
UM_3	Central	Kirinyaga	10
CL_3	Coast	Kilifi	7
LM_4	Coast	Taveta	11
LM_3	Eastern	Imenti South	12
UM_1	Eastern	Imenti South	17
UM_2	Eastern	Imenti South	3
LH_1	Nyanza	Nyamira	25
LH_1	Nyanza	Kisii	31
LH_2	Nyanza	Kisii	3
LM_2	Nyanza	Siaya	18
LM_2	Nyanza	Kisumu West	33
LM_2	Nyanza	Gem	16
LM_2	Nyanza	Ugenya	10
LH_2	Nyanza	Kisii	28
UM_2	Nyanza	Nyamira	2
UM_1	Nyanza	Nyamira	10
UM_1	Nyanza	Kisii	6
UM_1	Nyanza	Gucha	35
UM_1	Nyanza	Marani	16
LM_1	Western	Busia	37
LM_1	Western	Kakamega	32
LM_1	Western	Samia	17
LM_1	Western	Bungoma	1
LM_2	Western	Bungoma	29
LM_3	Western	Teso	25
LM_3	Western	Bungoma	5
UM_1	Western	Mount Elgon	26
UM_1	Western	Hamisi	7
UM_2	Western	Hamisi	1
UM_3	Western	Bungoma	14
UM_3	Western	Teso	3

Key: (n)*= 233 Nyanza, 197 Western, 32 Eastern, 20 Central, 18 Coast, UM_1**-Agroecological zone Upper Midland 1, CL_3-Coastal Lowland 3, LH_1-Low Highland 1, LM_4-Low Midland 4

Data was collected on the percentage of banana mats infected per orchard in a farm, cultivars grown, cropping system within the orchard, types of insects visiting male buds, removal of male buds and disposal of BXW infected plant debris.

4. 3. 2. Determination of insects associated with Banana *Xanthomonas* wilt

A survey was conducted in April 2011 in BXW epidemic areas of Western and Nyanza regions of Kenya. The specific sites were Butula, Butere, Gem, Ugunja and Ugenya (Table 4.2). A total of 250 farms were surveyed and in each farm ten mats were randomly sampled for the insects present on banana plant. All types of insects visiting floral parts of banana were collected from the male inflorescences of healthy bananas and BXW infected plants using a cotton net trapping bag. The insect net trapping bag was put around the flower taking care not to disturb the banana flower to trap the insects. After trapping the insect with a net, a cotton wool wetted with chloroform was dropped at the bottom of the insects net so that the fume/ vapour of chloroform could knock out the insects in the net within one minute to ease the handling of the traped insects. The insects were then carefully emptied from the insect net, put in a bottle of alcohol and labelled for further identification in the laboratory. Insects were identified using Stereomicroscope (10-250X), according their mode of feeding as per their mouth parts, either sucking, chewing, nectors collectors/pollinators. The stucture of their wings whether flying or crowling. Other symbiosis relationship with banana such as feeding on banana plant/parts, trapers of other insects for their feed of othat insects that visit banana plant. These features were used to link whether they could be potential vectors of BXW.

Table 4.2. Specific sites and number of farms sampled for insect diversity in banana *Xanthomonas* wilt epidemic areas of western Kenya

Agroecological zones	Region	Area	Total farms sampled	Healthy farms	BXW infected farms
LM_2*	Western	Butula	20	17	3
LM_1	Western	Butere	10	6	4
LM_2	Nyanza	Gem	50	38	7
LM_1	Nyanza	Ugunja	60	38	11
LM_2	Nyanza	Ugenya	110	53	21
Total			250	152	46

Key:*LM_1- Agroecological zone Low Midland 1, LM_2 Low Midland 2

4. 3. 3. Data analysis

Data was analyzed using SPSS statistical package version 11.0. (Sabine and Brian 2004) that determined the areas affected by BXW, the diversity and abundance of insects associated with banana.

4. 4. Results

4. 4. 1. Banana cropping system in Kenya

The cropping system of banana was mainly pure and intercrops. Between 44 to 79% of the Kenya farmers intercrop banana plantations while 21 to 53% do pure stand depeding on the region (Table 4.3). Most of the farmers intercrop to reduce the initial establishment costs. In farmrs where employees alone were present and were incharge of that farm especially if the owner of the farm were far, the employees were reluctant to give full information of the farm or did not know.

Table 4.3: Percentage of banana farmers practicing various banana cropping system in Kenya

Cropping system	Western	Nyanza	Central	Eastern	Coast
Pure banana crop	21.2	30.5	52.1	35.0	53.3
Intercrop	60.8	78.6	43.8	61.7	46.8
Both pure stand and intercrop	1.4	0.4	0.0	0.0	0.0
Don't know*	33.8	40.5	4.2	3.4	0.0

*not willing to disclose information majority were farm employee

4. 4. 2. Ocurrence of banana *Xanthomonas* wilt in Kenya

The disease was present in Western and Nyanza regions only and in these two regions 20% of the mats had BXW. Within a farm between 14 to 40% mats affected and three percent of the farmers had their farms with 100% disease infection. Banana production regions of Central, Eastren and Coast were free from the disease (Table 4.4). The disease was observed to be on increase to new areas between 22 to 28% in Western and Nyanza regions. Between three to eight percent the disease had been completely controlled. Fouty five percent of farmers in disease free regions of Central, Eastren and Coast were not aware of BXW (Table 4.5). Farmers knowledge of BXW symptoms was only observed in Western and Nyanza regions. In the epidemic regions higher number of farmers were aware of the wilting of the leaves, premature ripening of banana fruits and rotting of male bud than yellow ooze and pulp discouration (Figure 4.1).

Table 4.4: Percentage of mats affected by banana *Xanthomonas* wilt in Kenya

Percent mats affected	Western	Nyanza	Central	Eastern	Coast
None	65.7	51.5	100.0	100.0	100.0
<10	21.9	19.3	0.0	0.0	0.0
11-40	6.8	6.5	0.0	0.0	0.0
41-70	2.5	1.4	0.0	0.0	0.0
71-100	3.0	2.5	0.0	0.0	0.0

Table: 4.5: Percentage of farmers indicated the trend of banana *Xanthomonas* wilt status in Kenya

BXW trend	Western	Nyanza	Central	Eastern	Coast
Increasing	27.9	21.8	0.0	0.0	0.0
Constant	16.3	5.7	0.0	0.0	0.0
Decreasing,	5.2	7.0	0.0	0.0	0.0
Complete control	8.2	2.7	0.0	0.0	0.0
Don’t know	45.3	47.7	100.0	100.0	100.0

Farmers knew of BXW spread mechnisms (Figure 4.2) thirty to fourty percent in Western region, four to seventeen percent in Nyanza region inluding insect as a vector, infected planting material and contarminated farm tools.

In epidemic areas between 45% to 15% of the farmers knew the control options of destroying infected plants and between 16 % to 36 % of farmers debud to control BXW. Use of clean planting material as a control measure was low and the least known option by the farmers was quarantine. In BXW free regions the farmers were not aware of these control options.

Recommended control practices for BXW only carried out in Nyanza and Western regions with removal of infected mats being the most practiced and the least practiced being disnfection of farm tools by using sodium hypochlorite or heating. In Coast, Central and Eastern regions farmers practise debudding to controll cigar end rot and earlier fruit ripening (Table 4.6).

Table: 4.6 Percentage of farmers practising the recommended banana *Xanthomonas* wilt control practices in Kenya

Control options	Western	Nyanza	Central	Eastern	Coast
De-budding early*	15.0	17.0	28.0	24.0	33.0
Disinfecting tools	7.0	4.0	0.0	0.0	0.0
Clearing all infected field	8.0	16.0	0.0	0.0	0.0
Removing only infected mats	39.0	27.0	0.0	0.0	0.0
Using forked stick or hand	36.0	20.0	0.0	0.0	0.0

*In Central, Eastern and Coast practiced for control of banana cigar end rot and early fruit ripening

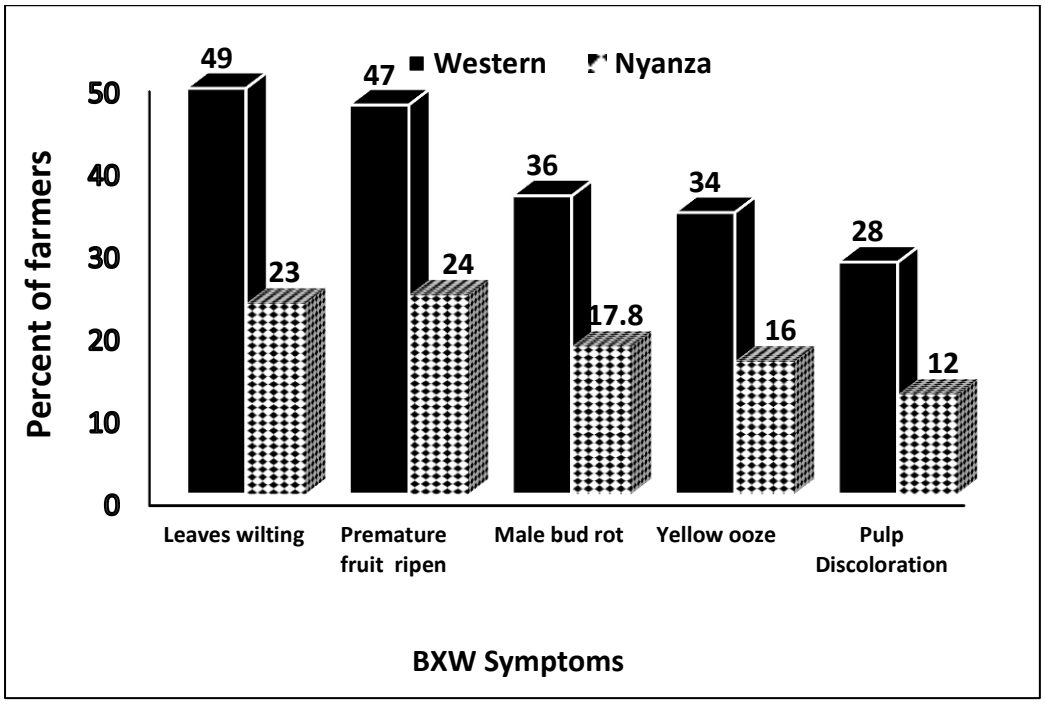

Figure 4.1: Percent farmers' who are aware of banana *Xanthomonas* wilt symptoms in Western and Nyanza regions.

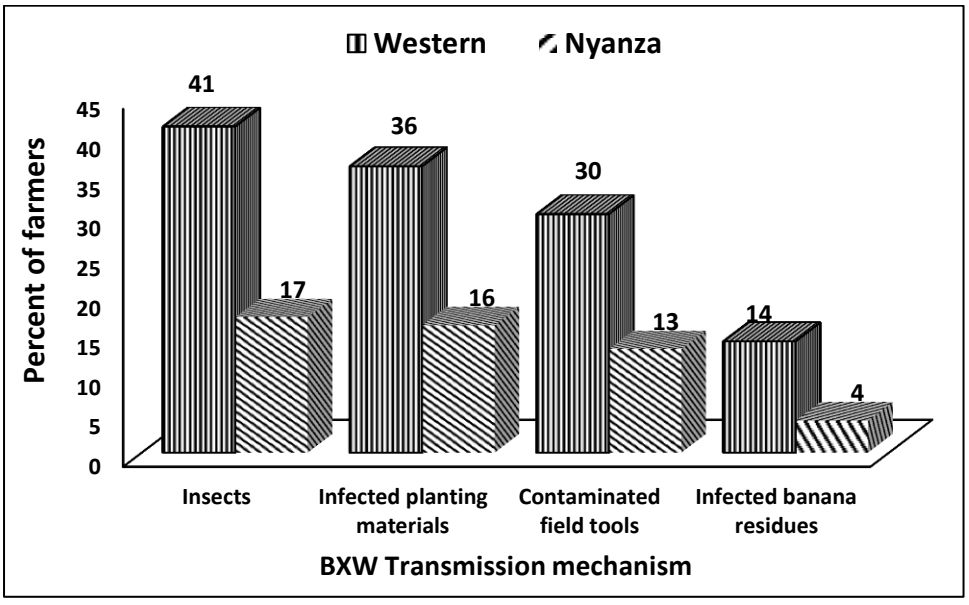

Figure: 4.2: Percent farmers who are aware of banana *Xanthomonas* wilt modes of transmission in Western and Nyanza regions

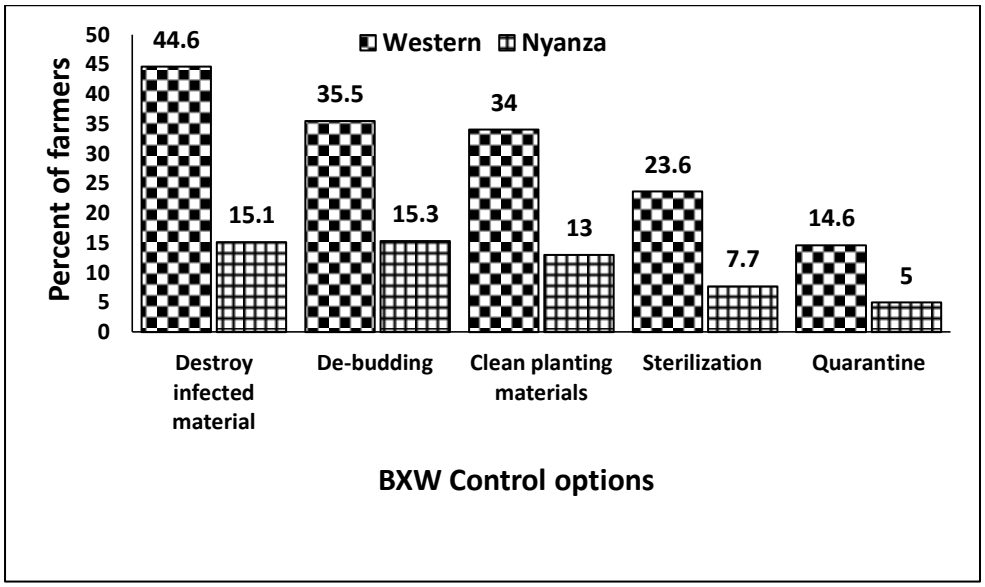

Figure 4.3: Percent farmers' who are aware of banana *Xanthomonas* wilt control options in Western and Nyanza regions

4. 4. 3. Diversity of insects associated with banana *Xanthomonas* wilt in epidemic areas in Kenya

A total of 3615 insects were collected with stingless bees (*Apis* sp), grass flies (*Drosophila* sp), banana fruit flies (*Tephritida Ceratitis*), beetles (*Neomyia ruissima* sp), and black ants (*Plectroctena* sp), beetles (*Neomyia ruissima* sp) being the most abundant (Table 4.7). Stingless bees were the most common insects observed followed by banana fruit flies, grass files, beetles and black ants on banana plant. More than three times insects were obsevred in low altitudes of 1224-1282 masl than medium to high altitude areas. There was an increasing order from Busia, Kakamega and Siaya, dessert types of banana had significantly more insects than cooking types. Insects observed on fruits were similar to those on flowers. There were similar insects on healthy plants as on those diseased (Tables 4.7). Stingless bees were more abundant in dessert banana cultivars as compared to cooking cultivars. There were moderate banana fruit flies populations observed on FHIA17, Valery and Mysore. Other insects such as beetles and black ants were few (Tables 4.8).

Table 4.7. Percent and insects associated with banana plants in banana *Xanthomonas* wilt epidemic areas

	Total insects collected	Stingless bees	Fruit fly	Grass Fly	Beetles	Black ants
Altitude (masl)*						
1224-1282	2180	42	29	15	6	5
1283-1340	658	46	37	7	1	6
1399-1455	777	66	16	8	2	7
County						
Siaya	3250	46	28	14	4	6
Busia	260	69	20	4	1	1
Kakamega	105	7	2	0	1	0
Type of banana usage						
Dessert	3139	45	29	13	1	0
Cooking	476	66	18	7	1	6
Part of the banana where insect collected						
Fruit	1743	51	25	11	6	6
Flower	1578	48	30	12	2	4
Status of banana plant						
Healthy looking	2600	49	30	12	1	6
Diseased looking	1015	45	24	12	13	5
Disease suspected						
BXW	532	40	20	11	21	4
Yellow sigatoka	446	50	26	14	25	6

*masl-metres above sea level at 1341-1398 disease free areas no data

Table 4.8: Percent and insects on banana cultivars associated with banana in banana *Xanthomonas* wilt epidemic areas

Cultivar	Total insects collected	Stingless bees	Fruit fly	Grass Fly	Black ants
FHIA 17	2044	46	32	13	6
Garisa short	224	56	14	7	15
Garisa tall	61	100	0	0	0
Giant sweet	158	37	24	21	13
Gold finger	89	74	16	0	8
Kayinja	269	30	18	7	0
Muraru	44	41	50	9	0
Mysore	127	31	38	24	0
Ngombe	70	80	13	0	0
Sirio	106	73	15	12	0
Uganda green	239	51	28	7	13
Valery	184	50	27	17	0

4. 6. Discussion

Over 40% of the farmers in the study area intercrop banana plantations with other low cover crops like beans or vegetables in the first year of establishment. This concurs with C3Project, (2007) and Onyango *et al.* (2012) reports that intercopping banana is a common practice within East and Central African region to reduce the intial cost of establishment.

The study confirmed presence of BXW in Western and Nyanza regions and confirmed farmers are familiar with the disease unlike in Central, Eastern and Coast region (Kwach *et*

al., 2012; Onyango *et al.,* 2012). The study also cofirmed BXW had spread fast since it was reported in 2006 in Teso and Busia only (Mbaka *et al.,* 2009) to new areas within Busia county including Busia, Bumala, Amagoro and Matayos; in Kakamega county it was confimed in Bukura, Butere and Emuhaya; in Bungoma county in Malakisi, while in Siaya county BXW had spread in Yala, Ukwala, Ugenya and Ugunja; and in Kisumu county in Maseno area. The disease had spread to Bondo and Mt Elgon as new areas.

In the study insects, planting materials and contarminated farm tools played a major role within a farm concuring with earlier reports in Uganda, Rwanda, Burundi and Kenya (Tripathi *et al*., 2007; Ndungo *et al*., 2008; Mbaka *et al*., 2009; Tripathi *et al.,* 2010). Farmers' in Western and Nyanza areas of Kenya recognised wilting of leaves and premature ripening of fruits as the symptoms of BXW. In epidemic areas in Uganda these signs have been recognised and used to develop control measures for the disease (Tushemereirwe *et al.,* 2004; Jogo *et al.,* 2012), in Ugunja Kenya farmers had used the same symptoms to effectively identify and control the disease (Onyango *et al.,* 2012; Jogo *et al.,* 2012). The study confirmed that farmers destroy infected plant material, use clean planting material and debud to control in BXW epidemic areas of Kenya.

The types of insects associated with banana plant in BXW epidemic areas were stingless bee, grass fly, beetles, black ants and banana fruit fly. Stingless bees were the highest 48% followed by banana fruit flies 27% and grass fly 12%. These observations concurs with earlier reports in Ethiopia and Uganda (Gold *et al.,* 2006; Tinzaara *et al.,* 2006; Shimelash *et al.,* 2008). The study confirmed more insects were observed in low altitude areas of 1224 masl of 60% of the total insects populations observed than in high altitudes areas in agrrement with Ndungo *et al.* (2005) who reported more insects population around the lower elevations of Lake Victoria at altitude 1135masl than at 1700

masl. This suggests that male bud removal wuold check the disease spread in low altitude areas where insect activities are high and abundance.

The study confimed that there were more insects on the flowers and fruits. The results agree with others who reported that the insects collect nector (Tinzaara *et al.,* 2006; Billah *et al.,* 2007; Shimelash *et al.,* 2008; Tripathi *et al.,* 2008). Significantly more insects were observed in the banana dessert cultivars namely Gros Michel, Valery, FHIA 17, Garisa and Gold finger cultivars than the cooking type Uganda green this concurs with studies in Ethiopia where Shimelash *et al.* (2008) showed that Dwarf Cavendish hosted the highest diverstity of insect families. Tinzaara *et al.* (2006) reported that dessert banana had more nector attacting more insects than the cooking type because the dessert banana type has higher sugar content as compared to the cooking. The dessert banana type also produce more pollen than the cooking type therefore attracts more insects.

Stingless bees, fruit flies and grass flies were abundant and may be associated with banana inflorescence as they forage for nectar or other symbiotic relationships. The forage behavior makes the insects associated with banana plants/flowers is hypothezised to provide opprtunity for picking Xcm from infected plants and transmitting to the non infected plants. Tinzaara *et al.* (2006) in their study on the role of insect in the transmission of BXW were able to isolate Xcm cells from stingless bees, fruit flies and grass flies that were collected from male flowers of both symptomatic and non symptomatic plants. They further confirmed that amount of Xcm cells isolated from stingless bees were twice as much as other insect groups thus confirming their role in the transmission of BXW. Stingless bees and flies were identified as important vectors in Moko disease of banana (Buddenhagen and Elsasser, 1962). These insects were causing infection through the moist cushions or scars of recently dehisced male flowers and floral bracts thus had a role in transmission (Buddenhagen and Elsasser 1962; Gold *et al.,* 2006; Tinzaara *et al.,* 2006; Tinzaara *et al.,* 2009). Shimelash *et al.* (2008)

while evaluating insects as potential vectors of Xcm were successful in isolating Xcm from bees, grass flies fruit flies and wasps that were visiting banana infected male inflorescences in Kaffa Ethiopia. In Hondurus bees were found to carry *Ralstonia solanacearum* in Bluggoue cultivar plantations (Buddenhagen and Elsasser, 1962).

In our study observed stingless bees, fruit flies and grass flies may play a major role as vectors for transmission of Xcm while black ants and beetles may also be agents of transmission of Xcm Shimelash *et al.* (2008) Tinzaara *et al.,* (2009) and Gold *et al.* (2006) had all reported that these insects are vectors of BXW. Although insects like bees collect nectar from male flowers of banana they have no pollination role for the formation of the edible banana fruits. Banana crop has its edible fruits formed parthenocarpically, suggesting the fruits are formed without pollination and thus do not affect production of the edible fruits (Rice and Tindal, 1990). This suggests that insects or pollinators are not required in banana plantations for banana fruits formation/production. Therefore removing off the male buds (Kubiriba *et al.,* 2014), after the edible fruits are formed was recommended to reduce bacterial entry sites through male buds (Tinzaara *et al.*, 2009) and minimize Xcm spread.

Timely debudding, reduces chance of transmission of BXW in banana farms infected with BXW because the banana floral part which attracts insects activities are eliminated. Understanding the diversity of these insects associated with banana will likely assist banana farmers in the management of BXW in epidemic areas. Banana *Xanthomonas* wilt disease is present in Kenya and was spread in Western and Nyanza regions only. No report of BXW disease in other parts of the country.

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CHAPTER FIVE: EFFECTIVENESS OF ROUGING AND ORCHARD REPLANTING IN MANAGEMENT OF BANANA *XANTHOMONAS* WILT

5. 1. Abstract

Xanthomonas campestris p.v. *musacearum* affects all banana cultivars and causes up to 100% loss. The disease has no single effective control measure. The study objective was to evaluate the effectiveness of rouging options and the optimum replanting period for rehabilitation of banana *Xanthomonas* wilt (BXW) affected orchards. Field experiments were conducted on an orchard with 80% BXW incidence in Bumala, Busia between February 2010 to January 2011. The rouging options were evaluated included cutting infeced plants at the base, uprooting of infected plants, injection of infected plants with herbicide and total removal of whole mats. Effect of time of replanting was tested by uprooting all the plants in the affected orchard and replanting at three, four and six months after removal. Data was collected monthly on number of plants infected by BXW, dead plants and the bunch yields. All the single stem rouging options significantly reduced BXW incidence from 80% to less than 1.4%. Injection with Glyphosate herbicide (Roundup®) and uprooting of the infected plants were the most effective methods. Bunch yield was recovered up to 70% after one year with the highest bunch yields of 24.5 tons ha^{-1} while control option in the infected orchard yielded 3.5 tons ha^{-1}. Replanting three and four months after destruction reduced BXW incidence to less than 2% in five banana cultivars tested. The study confirmed that single stem rouging of BXW infected orchards and replanting in three months was very effective in management of BXW.

Keywords: Glyphosate, rouging, replanting, rehabilitation *Musa*, *Xanthomonas*

5. 2. Introduction

Banana crop is under serious threat from the banana *Xanthomonas* wilt (BXW), its spread to other areas in East and Central Africa has been fast and affects all banana cultivars (Turyagyenda *et al.,* 2008; Mwangi 2007; Reeder *et al.,* 2007; Carter *et al.,* 2009; Tripathi 2013; Kubiriba *et al.,* 2014). The disease requires some interventions and measures for its control (Addis *et al.,* 2004; Ndungo *et al.,* 2005; Mwebeze *et al.,* 2006; Karamura *et al.,* 2008; Karamura *et al.,* 2008; Tripathi *et al.,* 2009; Carter *et al.,* 2009; Addis *et al.,* 2010). The measures include integrated disease management methods such as rouging diseased plants within a mat and replanting at the approprate time to control the disease (Addis *et al.,* 2010; Ssekiwoko *et al*., 2006; Tinzaara *et al*., 2009; Viljoen, 2010; Tripathi, 2013; Jogo *et al*., 2013). By incorparating several integrated measures for BXW control, disease incidence decreases and the spread can be contained through reduction of the inoculum's sources (Smith *et al.,* 2008). Once BXW occurs in a field, probably the best remedy is to cut down all infected plants, completely dig out the rhizomes, and place the field under fallow (Karamura *et al.,* 2008; Tripathi *et al.,* 2008; Tinzaara *et al*., 2009; Tripathi 2013; Kubiriba *et al.,* 2014).

The study was carried out to evaluate the effectiveness of rouging and replanting time in managemen of banana *Xanthomons* wilt affected orhards.

5. 3. Materials and methods

5. 3. 1. Evaluation of single stem rouging options for mat rehabilitation of banana *Xanthomonas* wilt affected orchards

Field experiment was conducted on a farmers banana orchard with 80% BXW incidence between February 2010 and January 2011 in Bumala, Busia. The farm is at an altitude of 1311m asl at latitude N00 27 64 7^0 and longitude of E 034 17 5 70^0 in a Lower Midland 2 (LM_2) Agroecological Zone. Five single stem rouging options were implemented

namely, (i) injection of Glyphosate herbicide (Roundup®) at 20 ml per plant at the pseudostem base at the soil level using a syringe, (ii) cutting the diseased stems at the base in the mat using a machete, (iii) uprooting the diseased stems of the affected mat using hoe, (vi) cut all the plants in the affected mat using machete, (v) control which consisted of whole infected mats being left intact. The experiment was laid out as randomised complete block design, with each rouging option having six mats and replicated four times. Farm implements used in uprooting or cutting the plants were sterilized by dipping into sodium hypochlorite : water solution (1:5 v/v). All male buds were removed except in the control plots. Weeds were controlled twice during the experiment period of one year using Glyphosate herbicide (Roundup®).

Data was collected on the incidence of BXW by observing the wilted plants, dead plants and severity score. Clean fresh banana bunch weight were taken for every rouging option and expressed in yield tons ha^{-1}. Disease severity was rated on a scale of 1-5 (1= 0% no symptoms on plant/no damage, 2 = 1 to 25%: mild symptoms on the leaves of the suckers, 3 = 26 to 50%: mild symptoms on suckers, some stunting and burnt, 4 = 51 to 75%: mild symptoms on suckers, stunting and wilting of suckers, 5 = 76 to 100% most suckers stunted and dying.

5. 3. 2. Evaluation of effective replanting time in management of banana *Xanthomonas* wilt affected orchards

Banana orchard with over 80% BXW infection was destroyed by uprooting all the mats. The uprooted materials were removed from the field and the field was left fallow for three months. Replanting was done from March, 2010 at three, four and six months regimes after destruction of the infected orchard. The objective of the study was to evaluate the optimum replanting period for management of BXW affected orchards. Clean BXW planting

material of corm suckers from five banana cultivars were used (Table 5.1). A spacing of 3 x 2 metres for 15 plant of each cultivar in a gross plot of 6 metres by 8 metres was laid out in a randomised complete block design (RCBD) replicated three times. At replanting 120 gms DAP fertiliser was applied per hole of every sucker and later after six months topdressed with 100gms CAN fertiliser. Data was collected on the number of wilted plants, severity of BXW, number of dead plants every two weeks. Weeds were controlled by Glyphosate herbicide (Roundup®).

Table 5. 1 Banana cultivars evaluated for replanting time of banana *Xanthomonas* wilt infected orchard

Cultivars	**Genome**	**Source**	**Description**
Ng'ombe	AAA	KALRO Kisii	East African Highland cooking banana very tall heavy bunch weight
Nusu Ng'ombe	AAA	KALRO Kisii	East African Highland cooking banana medium tall moderate bunch weight
Exera	AAA	KALRO Kisii	Dessert banana moderate height moderate bunch weight
Gros Michel	AAA	KALRO Kisii	Dessert banana very tall heavy bunch weight
Gold finger	AAAB	KALRO Kisii	Dessert banana hybrid medium height heavy bunch weight

5. 3. 3. Data analysis

Data was analysed using the Statistical Analysis package General Linear Model for the analysis of variance and mean separation using LSD ($P \leq 0.05$) was applied to determine the differences among the treatment and interaction means.

5. 4. Results

5. 4. 1. Effectiveness of single stem rouging in managing banana *Xanthomonas* wilt

After application of treatments BXW incidence reduced significantly ($P \leq 0.05$) between first one to three months in all the rouging options (Table 5. 1 and Figure 5. 2). Infection of infected stems with Glyphosate herbicide (Roundup®) was most effective. There was significantly reduction in disease severity from start up to six months. Between six to twelve months the disease was significantly ($P \leq 0.05$) controlled by the rouging options except the control which had the pathogen inoculum.

Table 5.2: Rouging options on banana *Xanthomonas* wilt incidence, severity and yield from 0 to 12 months

Rouging options	Percent		BXW severity score	Clean bunch yields (tons ha^{-1})
	Wilted plants	Dead plants		
Zero month				
Herbicide	28.1	7.5	3.1	-
Cut at base	24.2	3.6	3.8	-
Uproot	27.6	1.5	3.7	-
Cut all	24.8	0.8	3.4	-
Control	31.6	1.9	3.9	-
Mean	27.3	3.1	3.6	-
LSD (P≤0.05)	12.2	10.3	1.2	-
CV%	28.9	20.2	21.6	-
Three months				
Herbicide	9.2	0.5	3.8	-
Cut at base	3.9	0.0	2.1	-
Uproot	3.0	0.0	1.6	-
Cut all	5.4	1.5	2.3	-
Control	11.5	1.5	3.0	-
Mean	6.6	0.7	2.6	-
LSD (P≤0.05)	6.0	2.0	1.4	-
CV%	18.2	16.9	15.3	-
Six months				
Herbicide	4.0	0.8	3.7	0.7
Cut at base	4.4	0.2	2.8	0.0
Uproot	4.8	0.0	2.7	0.4
Cut all	1.8	0.0	1.7	0.7
Control	7.6	1.3	3.2	0.0
Mean	4.5	0.5	2.8	0.2
LSD (P≤0.05)	4.9	1.1	1.8	1.4
CV%	12.4	19.1	16.9	13.3
Nine months				
Herbicide	0.9	0.0	1.0	1.1
Cut at base	1.1	0.0	0.7	4.0
Uproot	0.8	0.0	0.4	2.3
Cut all	1.0	0.3	1.0	2.5
Control	11.3	1.1	3.1	3.1
Mean	3.0	0.3	1.2	2.5
LSD (P≤0.05)	4.8	1.0	1.4	6.0
CV%	14.1	18.1	13.6	28.9
Twelve months				
Herbicide	0.5	0.0	0.3	14.0
Cut at base	1.1	0.0	0.8	6.4
Uproot	1.4	0.0	0.8	24.5
Cut all	0.4	0.0	0.4	12.5
Control	3.9	0.4	2.0	3.5
Mean	1.5	0.1	0.9	12.5
LSD (P≤0.05)	2.8	0.6	1.5	18.0
CV%	24.1	14.5	21.1	17.6

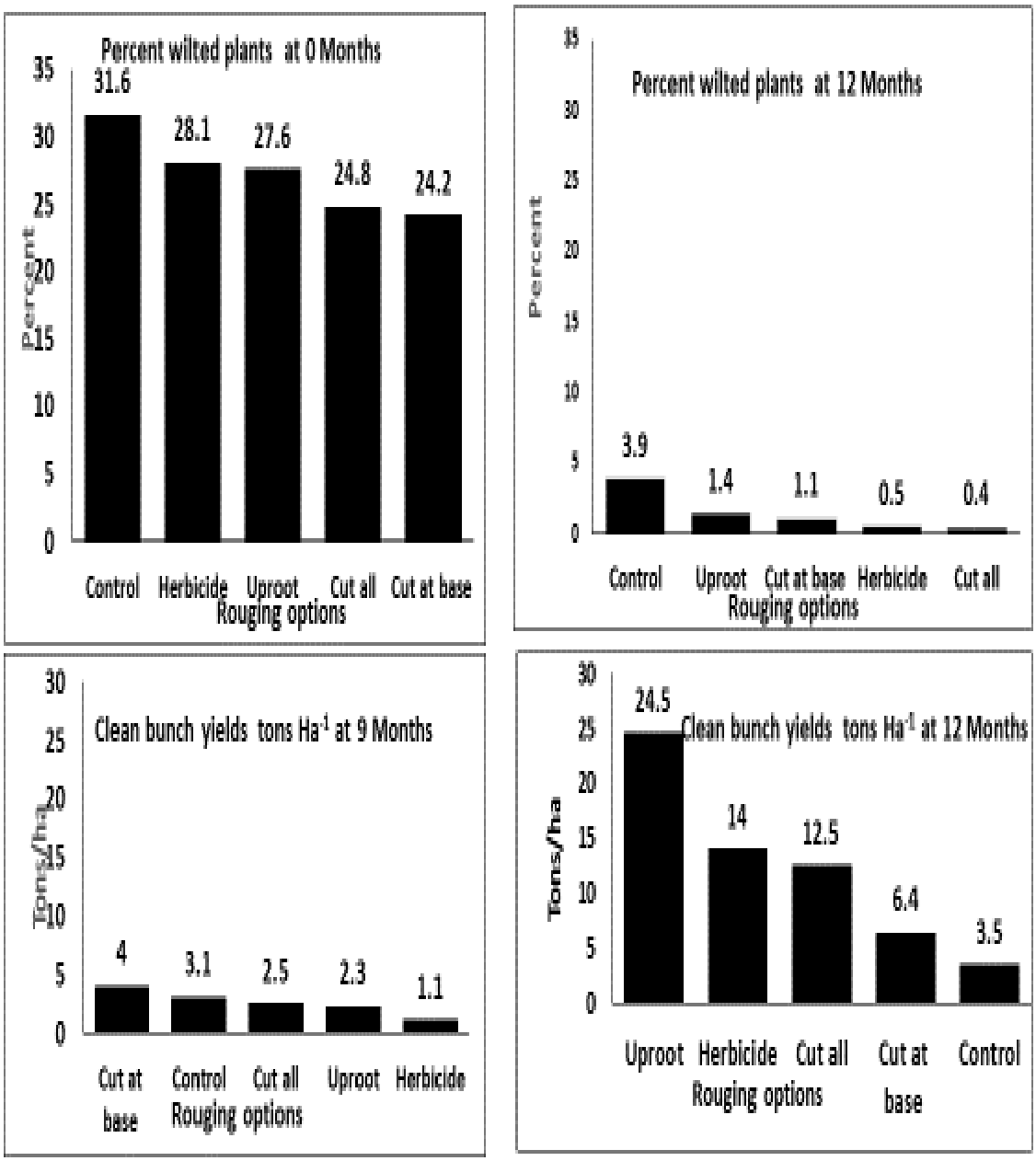

Figure 5.1 Percent banana plants wilted at 0, 12 months and clean bunch yields (tons ha^{-1}) at 9 and 12 months of various rouging options for management of banana *Xanthomonas* wilt.

5. 4. 2. Optimum replanting period for banana *Xanthomonas* wilt management

There were significant difference in BXW incidence among and within the banana cultivars used. All the cultivars used were affected by BXW (Table 5. 3 and Table 5. 4). The cultivar Ngombe had the highest incidence of BXW attack. Cultivars Ngombe, Nusu Ngombe, Gold Finger and Gros Michel were affected for the three months replanting period, however the infection reduced as the period advanced and by sixth month there were no further incidences of BXW observed. Three months replanting period had higher infection compared to four while six months replanting had no incidence of BXW.

Table 5.3: Percent affected plants by banana *Xanthomonas* wilt after replanting at 3, 4 and 6 months

Cultivar	**After one month**			**After four months**		
	3	4	6	3	4	6
Ngombe	2.0	0.0	0.0	0.0	0.0	0.0
Nusu Ngombe	2.0	0.3	0.0	2.0	0.0	0.0
Gros Michel	2.0	0.3	0.0	2.0	0.0	0.0
Gold Finger	1.0	0.0	0.0	0.6	2.0	0.0
Exera	0.0	0.0	0.0	0.0	0.0	0.0
Mean	1.4	0.1	0.0	0.9	0.4	0.0
LSD (P≤0.05)	1.9	0.7	0.0	1.6	0.6	0.0
CV%	17.8	29.5	0.0	25.0	23.4	0.0

Table 5.4: Percent dead plants due to banana *Xanthomonas* wilt after replanting at 3, 4 and 6 months

Cultivar	**After one month**			**After four months**		
	3	4	6	3	4	6
Ngombe	2.0	0.0	0.0	0.0	0.0	0.0
Nusu Ngombe	2.0	0.7	0.7	2.0	0.0	0.0
Gros Michel	2.0	2.0	0.0	0.7	0.0	0.0
Gold Finger	1.0	0.7	0.7	0.0	2.0	2.0
Exera	0.0	0.0	2.0	0.0	0.0	0.0
Mean	1.4	0.7	0.7	0.5	0.4	0.4
LSD (P≤0.05)	7.0	7.0	6.0	7.0	7.0	6.0
CV%	17.8	18.1	27.2	21.0	23.4	23.2

5. 5. Discussion

5. 5. 1. Effectiveness of single stem rouging options for banana *Xanthomonas* wilt management

All the single stem rouging methods had BXW incidence significantly reduced at $p \leq 0.05$, with Glyphosate herbicide (Roundup®) injection and uprooting of the infected plants being the most effective. The disease was significantly controlled by the third month for all the the rouging options. Kubiriba *et al.* (2014) reported a reduction of BXW incidence by the seventh month from over 25% to less than 3%. Complete uprooting of affected plants and removing plant debris on ridges, leaving the debris to rot/dry was an effective measure for managing *Xanthomonas* wilt (Mwangi, 2007; Turyagyenda *et al*., 2008; Kubiriba *et al.*, 2014).

After the apllication of rouging options there was signifcant reduction $p \leq 0.05$ of dead plants to zero by the third month. The infection which from the upper parts of the banana plant by insects vectors or cutting using machetes when harvesting/deleafing or pruning was eliminated by rouging that intercepted the disease transmission. Therefore is was possoble to chieved control before the disease could move downwards (Ssekiwoko *et al.,* 2006, Kubiriba *et al.,* 2014). Single infected stems are rouged leaving other clean plants on the mat rather than rouging the whole mat.

The number of dead plants were high in the first three months for Glyphosate herbicide (Roundup®) and by the sixth month the disease was controlled. Okurut *et al.* (2006) reported that all plants injected with 2,4-D herbicide snapped and died within 30-60 days while plants injected with Glyphosate herbicide (Roundup®) wilted and dried up within the first month and by six month the disease was controlled. Mwangi, (2007) reported 2, 4 D-Amine herbicide as the most effective chemical causing stems to topple over quite rapidly while Glyphosate acts gradually causing plants to wither and wilt after several weeks.

Injecting infected plants with herbicide was effective and comparable to removal by uprooting or cutting the plants at the base. The results also concurs with Blomme *et al.* (2008) and Okurut *et al* (2006) that Glyphosate was effective in control of BXW infected banana mats. Kubiriba *et al.* (2014) also reported that single stem injection with Glyphosate herbicide (Roundup®) of diseased banana plants killed and controlled the BXW within six months. The significant reduction of Xcm by the sixth month in the herbicide treatments might be attribued by the death of the plant material that led to the death of the Xcm, as the pathogen has no live host to make it survive.

However herbicide application may have some related enviromental hazards. The effectiveness of the herbicide would to some extent depend on the expertise of the person applying. Herbicide can be cost-effective in terms of labour as compared to physical uprooting of mats or cutting at the base only. Smith *et al.* (2008) also reported eradication of large acreage can be done in a relatively short time. Herbicide reduce soil disturbance and hence minimise risks of erosion on farms located on the hillsides. Herbicide also minimizes the use of cutting tools, which can easily spread the disease in areas of high disease incidence (Blomme *et al.,* 2008). Glyphosate was effective however the related knowledge of application, initial resources to purchase the herbicide and its related application equipments might be high for an average low resource farmer (Smith *et al.,* 2008). The study confirmed that Glyphosate herbicide (Roundup®) was very effective and controlled BXW within the first six month.

In addition, unlike single stem removal by uprooting or cutting at the base, farmers may not be happy to see their infected plantations being destroyed at once, due to fear of loss of income instantly and food (Bagamba *et al.,* 2006). The single stem rouging was an effective and low-labour intensive strategy of managing BXW without instant loss of yield as the unaffected plants would come to early bunch bearing.

The reduction of the disease incidence might have contributed to the significant increment at $p \leq 0.05$ on bunch production from the treatments from nine to twelve months. Bunch yield was recovered after one year. Highest bunch yields of up to 24.5 tons ha^{-1} was obtained in plots where affected plants were uprooted compared to 3.5 tons ha^{-1} in control plots. Rouging option of herbicide application to the BXW affected plant, uproot the affected plants and cut all the affected plantd were able to give yields from 12.5 to 24.5 tons ha^{-1} by the end of the first twelve months which was above the national farmer average banana bunch yield of 12 tons ha^{-1} (Yako *et al.,* 2011) while control yielded 3.5 tons ha^{-1} confirming that BXW causes significant loss to banana crop as also been reported earlier (Tripathi and Tripathi, 2009, Ocimati *et al.,* 2013a; Kubiriba *et al.,* 2014). The rouging of only the infected plant from the mat and leaving the clean plants in the same mat reduced the chances of diseased plants, thus would not allow the plant to flower thereby reduced the chances of transmission of the disease by the insects through male bud flower. There was also reduced cost of the uprooting the whole mat, that led to the significantly early yields within twelve months probably from the clean plants in the mat in agreement with other researchers (Blomme *et al.,* 2008). Removal of all affected mats might be high labour demand, less immediate reward for effort invested, no immediate alternative source of income and food security challenging (Mwangi, 2007; Ndongo *et al.,* 2008). The study confirmed the possibility to reduce BXW incidence by over 80% in one year, resulting in yields recovery by up to 70% within one year by removing only the BXW symptomatic plants, compared to destroying the whole orchard. Three months replanting time was effective in control of BXW.

5. 5. 2. Optimum replanting period for banana *Xanthomonas* wilt mat rehabilitation

Replanting banana on BXW affected fields three to four months after destruction of the BXW affected orchards reduced disease incidence from a field with over 80% infection to less than 2% for the five banana cultivars Ngombe, Nusu Ngombe, Exera, Gold Finger and Gros Michel. Turyagyenda *et al*. (2008) reported that banana suckers replanted after a one-month fallow period had a 25% re-infection rate, while all suckers replanted after seven and eight months of fallowing survived. They further reported that incidence of re-infection was highest with treatments of re-sprouting suckers and lowest in treatments of completely uprooting. Mwebaze *et al.* (2006) reported that Xcm survives in the soil for less than three months under laboratory conditions. Welde-Michael *et al.* (2008) and Mwebaze (2006) reported that Xcm cannot survive in soil in the absence of enset plant material for more than 90 days. Welde-Michael *et al.* (2008) and Kubiriba *et al.* (2014) reported that removal of affected banana plants from a field, cut into small pieces and heaped/buried/composted killed the pathogen in banana orchard. Suckers of four cultivars Ngombe, Nusu Ngombe, Gold Finger and Gros Michel had reduced infection within the first three months of replanting period, however the infection significantly reduced at $p \leq 0.05$ as the period increases.

The systemicity of Xcm was able to be alive within the mat for some period allowing the disease to appear within the first three months. Ocimati *et al*. (2012) reported that Xcm due to its systemic nature was able to be alive within a mat for long periods between 5–16 months without expressing visual BXW symptom. The period before expression may vary from fourteen days to two years (Ocimati *et al.,* 2013b). Being a vascular bundle disease, Xcm must colonise the corm and spread to others parts of banana plant (Ocimati *et al.*, 2013a). Most bacteria populations decline rapidly after their introduction into the soil as a result of biological, chemical and physical factors that affect their survival. The above phenominon was able to explain why we observed a very significant control after six months

the Xcm might have died without a host. The study confirmed that replanting period of three months can rehabilitate infected BXW orchard and restore yield within one year.

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CHAPTER SIX: TOLERANCE OF BANANA CULTIVARS TO BANANA *XANTHOMONAS* WILT

6. 1. Abstract

Banana *Xanthomonas* Wilt (BXW) caused by *Xanthomonas campestris* p.v. *musacearum* (Xcm) attacks all banana cultivars. The infected banana plants are killed leading to total yield loss and no natural resistance among the banana cultivars has been reported. The objective of the study was to evaluate the tolerance of locally available banana cultivars grown in Kenya to BXW. Forty six banana cultivars representing eight genomic groups were evaluated for tolerance to BXW. Seedlings of each banana cultivar were inoculated by injecting one millitre of the *Xanthomonas campestris* p.v. *musacearum* suspension equvalent to 10^8cfu ml^{-1} of Xcm bacterial. into the base of pseudostem. The experiment was arranged in completely randomised design, replicated three times, each treatment had 10 banana plants per cultivar. Data collected included days to first symptoms, number of plants with BXW symptoms, number of wilted leaves per plant and BXW severity. All the forty six banana cultivars evaluated were susceptible to BXW. Cultivars Mokoyo, Namukhila and Horn Plantain were most susceptible showing BXW symptoms in 14 days after inoculation while cultivar Mysore, an apple dessert banana of genome AAAB was the least susceptible expressing symptoms 26 days after inoculation. The study confimed that though all the banana cultivars were susceptible to BXW.

Key words: Susceptibility, genome, musa, cultivars, bacterial wilt, net house.

6. 2. Introduction

The BXW disease is very destructive, affecting all banana cultivars, including both East African Highland bananas and exotic types of banana (Kidist, 2003; Tushemereirwe *et al.,* 2003; Ssekiwoko *et al.,* 2006c;. Reeder *et al.,* 2007; Carter *et al.,* 2009; Ocimati *et al.,*

2013b). Banana *Xanthomonas* wilt is very devastating to banana industry since the disease is known to cause an annual loss of over US$500 million through yield loss of 80-100% (Biruma *et al.,* 2007; Boiversity International, 2009; FAO, 2007).

Symptoms expressed on infected banana are progressive yellowing of leaves and complete wilting of entire plant, premature and uneven yellowing, ripening and rotting of the bunch and eventually death of plants (Yirgou and Bradbury, 1968 and 1974; Tushemereirwe *et al.,* 2004). Previous studies have not found a natural tolerant cultivated *Musa* spp. against the disease (Tripathi *et al.,* 2008; Turyagyenda *et al.,* 2009; Viljoen, 2010; Onyango *et al*., 2011; Onyango *et al*., 2012). Some cultivars posses, characteristics such as lack of male bud, persistent bracts that makes the cultivars difficult for insects to transmit the pathogen naturally (Ssekiwoko *et al.,* 2006b; Ssekiwoko *et al.,* 2006c; Muchunguzi *et al.,* 2007; Ssekiwoko *et al.,* 2009; Lewis *et al.,* 2010; ProMusa, 2010). Prospects of developing banana cultivars resistant to BXW through conventional breeding are limited because no cultivated banana exhibits resistance to Xcm (Tripathi *et al.,* 2008; Tripathi *et al.,* 2010).

In Kenya various banana cultivars prefered and grown include East African Highland cooking banana subgroup (AAA genome), beer brewing cultivar (ABB genome), the dessert cultivars (AAB genome) and the Cavendish subgroup (AAA genome), plantains (AAB genome) and tetraploid hybrid cultivars (AAAA genome) (Nguthi, 1996; Nguthi, 2008; Onyango *et al*., 2011).

The objective of the study was to evaluate locally available banana cultivars for tolerance to BXW as a management strategy.

6. 3. Materials and methods

6. 3. 1. Experimental materials

Forty six banana cultivars representing eight genomic groups (Table 6.1) from ex-ICIPE banana project collection between 1989 to 1994 (ICIPE, 1997) which were transferred to KALRO Kisii banana germplasm collection (KARI, 2005). These cultivars were multiplied through tissue culture and planted in potted subtrate of soil, sand, and farm yard manure at a ratio of 1: 7: 2 parts dry weight steam sterilized as described by World Agroforestry Centre (2012). The tissue culture suckers were hardened for five months for evaluation in a screen house in western Kenya at Alupe, Busia, N 00 28.28' .6" and E 034.07.28' .8". In a completely randomised design experiment, ten banana suckers of each cultivars were replicated three times and were inoculated with Xcm. Another ten plants of each treatment were used as control and were inoculated with distilled water. The experiment was implemented from September 2012 to November 2012.

Table 6. 1 Banana cultivars evaluated for tolerance to *Xanthomonas campestris* p.v. *musacearum* according to genomes

Genome	Genetic group	Banana cultivars	Source
AAA	East African Highland cooking bananas	Uganda Green, Ng'ombe, Kiganda Lusumbi, Nakabululu, White Nakabululu, Jamaga, GN giant, Kikuyu-I, Sialamule, Libukusu, Litambi, Ishighame, Ntobe, Turbo and Namakhila	KALRO Kisii (Ex ICIPE)
AAA	East African Highland beer bananas	Mokoyo, Musera, Mtagato GN, Mukubu-2, Mpologoma	KALRO Kisii (Ex ICIPE)
ABB	Asian cooking bananas	Bokoko, Ngonja and Matumbo	KALRO Kisii (Ex ICIPE)
ABB	Beer/juice bananas	Blugoye -10 and Kayinja	KALRO Kisii (Ex ICIPE)
AAB	Plantains/roasting bananas	Gonja I, Horn plantain and Spambia	KALRO Kisii (Ex ICIPE)
AAB AAAB,	Apple dessert bananas	Mysore and Geraldine tucker	KALRO Kisii (Ex ICIPE)
AAA, AAAA, AAAB	Dessert bananas	Chinese Cavendish, Valery, Grand naine, Dwarf Cavendish, FHIA-17, FHIA-25, X-Oyugis, Kampala, Pelipita, Gold finger, Gros Michel, Bogoya MD	KALRO Kisii (Ex ICIPE)
AA	Diploid Muraru dessert bananas	Mraru mshare, Njuru, Mraru mlalu, Kamunyilya	KALRO Kisii (Ex ICIPE)

6. 3. 2. Isolation, culturing and inoculation of *Xanthomonas campestris* p.v. *musacearum*.

Freshly harvested sample of banana (pseudostem) infected with BXW from the field in western Kenya was surface sterilised with sodium hypochlorite : water solution (1:5 v/v) then a piece was cut, then crushed in small amount of distilled water to let Xcm bacteria ooze

from the banana tissue. The suspension was streaked on nurtrient agar and incubated at 25 ^{o}C for 24 hours. The bacterial colony was purified on nutrient agar and multiplied enough to inoculate the total number of plants for the experiment. For inoculation harvesting of the bacteria was done by flooding in sterilized distilled water and standardized based on serial dilution into a suspension on pricipal of bacteria suspension: water 1:9 ratio equivalent to 10^{8}cfu ml^{-1} Xcm bacterial suspension. Each banana seedling was inoculated by injecting one ml of the suspension into the base of pseudostem by use of hypodermic syringe. The inoculated plants were covered with transparent polythene for 12 hours to maintain humid conditions for infection. The experiment was arranged in a completely randomised design (CRD) with each cultivar had ten plants inoculated with the bacteria and replicated three times. An equivalent number of plants were inoculated with sterilized distilled water as control. The experiment was conducted over two cropping cycles. Data collected included days to first symptoms, number of plants with BXW, number of wilted leaves per plant and BXW severity.

6. 3. 3. Banana *Xanthomonas* wilt assesment

Banana *Xanthomonas* wilt severity was assessed on individual plant as described by (Michael *et al.,* 2006; Tripathi *et al.,* 2008 and Tripathi *et al.,* 2009) using a scale of 1= 1-20% with chlorosis symptoms on leaves, 2 = 21 – 40% leaves with chlorosis and necrosis, 3 = 41-60% leaves with yellowing, 4= 61-80% leaves with wilt and curled, 5= 81-100% all leaves wilted and plants dead. The cultivars were rated for susceptibility based on percentage number of plants with BXW symptoms as as described by (Tripathi *et al.,* 2008 and Tripathi *et al.,* 2009), as highly susceptible (HS) 90-100%, plants affected, moderately susceptible (S) 50-89%, plants affected, tolerant (T) 0-49%, plants affected.

6. 3. 5. Data analysis

Data was analysed using the Statistical Analysis for Scientists (SAS) statistical package. General Linear Model, analysis of variance and mean separation by LSD ($p \leq 0.05$) was applied to determine the significant differences among treatments.

6. 4. Results

All the banana genomic groups evaluated were suscptible to BXW. There were significant difference at $p \leq 0.05$ for days to first disease appearance, number of wilted leaves per plant and BXW severity among the cultivars. Within a genomic group the East Africa Highland (EAH) cooking banana, EAH beer banana, Asian cooking, beer/juice, plantains/roasting, apple dessert, dessert and muraru dessert were moderately susceptible to highly susceptible to Xcm. *Xanthomonas campestris* p.v. *musacearum,* being a xylem inhabiting bacterium there was 100% recovery after nine weeks from inoculation to all the fouty six cultivars evaluated. All the cultivars evaluated had high BXW disease incidence above 70% percent across the genome. All the plants inoculated had reaction of yellowing, necrosis, chlorosis, and wilt symptoms on leaves. The BXW symptoms were shown on newly opened leaves from 20-80% for all the cultivars evaluated. The severity of the disease was between 40-80% for all the cultivars evaluated. The earliest yellowing symptoms were observed from cultivar Mokoyo, Namukhlia and Horn Plantain fourteen days after inoculating while the latest was cultivar Mysore twenty six days after inoculation.

The East African Highland cooking banana genome AAA (Table 6.2) showed significance at $p \leq 0.05$ for disease symptoms to appear days after inoculation (dai) from the earliest observed 14dai for cultivar Namukhila. Severity score on leaves were high with disease incidence between 70-95%. Within this genome 100% Xcm was recovered from all

the cultivars nine weeks after inoculation the genome showed a very high suscptibility 70% of the cultivars.

Table 6. 2. Reaction of AAA East African Highland cooking banana cultivars to *Xanthomonas campestris* p.v. *musacearum*

Cultivar	Days to first symptoms	Percent		Severity score	Recovery of Xcm	Disease rating
		Plants affected	Wilted leaves			
Turbo	18.0cd	71.0c	62.5ab	2.0bc	Positive	S
Sialamule	25.0a	79.0abc	29.4bc	3.0abc	Positive	S
Libukusu	16.0ef	82.0ab	25.6bc	3.0abc	Positive	S
Nakabululu	19.0c	84.0ab	30.3bc	3.0abc	Positive	S
G.N. Giant	17.0de	85.0ab	22.7bc	3.0abc	Positive	S
Litambi	18.0cd	91.0ba	43.4bc	4.0ab	Positive	HS
Ishigame	24.0ab	92.0ab	27.0bc	2.0bc	Positive	HS
White Nakabulu	18.0cd	93.0ab	45.4ab	3.0abc	Positive	HS
Ntobe	15.0fg	94.0ab	29.4ab	3.0abc	Positive	HS
Kiganda Lusumba	15.0fg	94.0ab	41.6ab	3.0abc	Positive	HS
Kikuyu-I	16.0ef	94.0ab	77.0ab	3.0abc	Positive	HS
Ng'ombe	23.0b	95.0ab	80.0ab	3.0abc	Positive	HS
Namukhila	14.0h	95.0a	80.0ab	3.0abc	Positive	HS
Uganda Green	24.0ab	96.0a	71.4ab	3.0abc	Positive	HS
Jamaga	18.0cd	97.0a	64.5ab	4.0ab	Positive	HS
Mean	19.0	89.0	48.6	3.0		
LSD (P≤0.05)	1.0	17.6	38.8	1.2		
CV%	10.0	11.0	14.0	15.0		

Means with the same letter are not significantly different within each column, S- Susceptible, HS- Highly susceptible

The ABB East African Highland beer banana (Table 6.3) showed significance at p≤ 0.05 for disease symptoms to appear dai from the earliest observed 15dai for cultivar Mpologoma. Severity score on leaves were very high with disease incidence ranged from 90-95% and 100% Xcm was recovered from all the cultivars nine weeks after inoculation. All the cultivars within this genome were highly suscptibile.

The ABB Asian cooking banana (Table 6.3) there was significant at p≤ 0.05 for disease symptoms to appear dai the earliest was cultivar Bokoboko 17 dai while Cultivars Gonja and Matumbo 18 dai. Cultivar Gonja had severity significantly scored high while the other two cultivars Matumbo and Bokoboko were moderate. The number of plants affected was 87 percent for cultivar Matumbo the lowest while cultivars Bokoboko had 90 and Gonja 93 percent respectively. There was a 100% Xcm recovered from all the cultivars, cultivars Gonja and Matumbo were higly susceptible.

The ABB Beer/juice bananas (Table 6.3) there was significant at p≤ 0.05 for disease symptoms to appear dai. The cultivar Kayinja showed symptoms 15 dai while 18 dai for cultivar Bluggoe-10. Severity score on leaves were very high with disease incidence of 90% for Kayinja and 95% for cultivar Bluggoe-10. Within this genome 100% Xcm was recovered from the two cultivars and were all highly suscptibile.

The ABB Plantains/roasting banana (Table 6.4) there was significant differences at p≤ 0.05 for disease symptoms dai. The cultivar Horn Plantain showed symptoms in two weeks while cultivar Gonja-I showed after three weeks after inocutation. The cultivar Spambia had the lowest serverity score. Within this genome 100% Xcm was recovered from the two cultivars. Cultivars Spambia and Gonja-I were highly suscptibile while Horn Plantain suscptibile.

Table 6. 3. Reaction of AAA East African Highland beer banana, Asian cooking and Beer/juice ABB banana cultivars to *Xanthomonas campestris* p.v. *musacearum*

Cultivar	Days to first symptoms	Percent		Severity score	Recovery of Xcm	Disease rating
		Plants affected	Wilted leaves			
East African Highland beer banana cultivars AAA						
Mpologoma	15.0b	90.0a	95.2a	2.0c	Positive	HS
Mukubu-2	18.0a	91.0a	47.6bc	3.0b	Positive	HS
Mtagato GN	18.0a	93.0a	68.9b	3.0b	Positive	HS
Musera	18.0a	95.0a	35.7c	3.0b	Positive	HS
Mokoyo	14.0b	95.0a	35.0c	4.0a	Positive	HS
Mean	16.0	93.0	56.5	2.8		
LSD (P≤0.05)	2.0	19.3	11.9	0.4		
CV%	12.0	8.0	18.0	18.0		
Asian cooking bananas cultivars ABB						
Matumbo	18.0a	87.0a	38.4b	2.0a	Positive	S
Bokoboko	17.0b	90.0a	33.3b	2.0a	Positive	HS
Gonja	18.0a	93.0a	74.0a	3.0b	Positive	HS
Mean	17.3	90.0	48.5	2.8		
LSD (P≤0.05)	1.0	18.0	35.0	0.6		
CV%	9.0	9.0	14.0	17.0		
Beer/juice bananas cultivars ABB						
Kayinja	15.0b	90.0a	35.7b	3.0a	Positive	HS
Bluggoe-10	18.0a	95.0a	80.0a	3.0a	Positive	HS
Mean	16.0	92.0	57.8	3.0		
LSD (P≤0.05)	1.0	23.2	43.0	0.4		
CV%	6.0	7.0	16.0	13.0		

Means with the same letter are not significantly different within each column , S- Susceptible, HS- Highly susceptible

The AAB and AAAB dessert banana (Table 6.4) there was significant difference at p≤ 0.05 for disease symptoms to appearance dai with cultivars Geraldine Tucker 18 dai and 26 dai for Mysore. There was 100% Xcm was recovered and were all highly suscptibile.

Table 6. 4. Reaction of Plantains/roasting genome AAB, Apple dessert genome AAB and AAAB to *Xanthomonas campestris* p.v. *musacearum*

Cultivar	Days to first symptoms	Percent		Severity score	Recovery of Xcm	Disease rating
		Plants affected	Wilted leaves			
Plantains/roasting banana cultivars AAB						
Horn Plantain	14.0c	88.0a	80.0a	2.0b	Positive	S
Spambia	16.0b	91.0a	38.4b	2.0b	Positive	HS
Gonja-I	23.0a	97.0a	74.0a	3.0a	Positive	HS
Mean	18.0	92.0	64.0	3.0		
LSD (P≤0.05)	1.0	17.4	35.5	0.3		
CV%	14.0	9.0	13.0	12.0		
Apple dessert banana cultivars AAB and AAAB						
G.T*AAAB	18.0b	93.0a	74.0a	3.0a	Positive	HS
Mysore AAB	26.0a	97.0a	83.8a	3.0a	Positive	HS
Mean	20.0	95.0	78.9	3.0		
LSD (P≤0.05)	2.0	17.5	40.0	0.5		
C V%	16.0	8.0	18.0	14.0		

Means with the same letter are not significantly different within each column, GT*- Geraldine Tucker AAB , S- Susceptible, HS- Highly susceptible

The AAA, AAAA and AAAB dessert banana (Table 6.5) there was significant difference at p≤ 0.05 for disease symptoms to appear dai the earliest observed on cultivars X-Oyugis, Grand Naine and Chinese Cavendish 17 dai while the latest was cultivar Kampala 23 dai. Severity score on leaves were high with disease incidence between 75-95% for cultivar Valery. Cultivar Bogoya had low BXW serverity while all the ramaing cultivars in the genome had higher severity. Within this genome 100% Xcm was recovered, 67% of the cultivars were suscptibility.

The AA Muraru dessert banana (Table 6.5) significant at p≤ 0.05 for disease symptoms the latest was cultivar Kamunyilya 20 dai. Severity score on leaves were very high with disease incidence between 90-95% for the cultivars. Within this genome 100% Xcm was recovered from all the cultivars, the cultivars in this genome were higly suscptible

Table 6. 5. Reaction of Dessert banana genomes AAA, AAAA, AAAB and Muraru AA Diploid banana cultivars to *Xanthomonas campestris* p.v. *musacearum*

Cultivar	Days to first symptoms	Percent		Severity score	Recovery of Xcm	Disease rating
		Plants affected	wilted leaves			
Dessert banana cultivars genome AAA, AAAA and AAAB						
X-Oyugis AAA	17.0ef	75.0b	28.5b	3.0a	Positive	S
Bogoya MD AAA	22.0ab	80.0ab	21.7b	3.0a	Positive	S
Dwarf Cavendish AAA	18.0def	80.0ab	37.7a	3.0a	Positive	S
FHIA 17 AAAA	18.0def	85.0ab	37.0b	3.0a	Positive	S
Kampala AAA	23.0a	85.0ab	25.6b	3.0a	Positive	S
FHIA-25AAAA	20.0cd	86.0ab	47.6ab	3.0a	Positive	S
Gold FingerAAAB	19.0def	87.0ab	33.3b	3.0a	Positive	S
Gros Michel AAA	18.0def	89.0ab	30.3b	3.0a	Positive	S
Valery AAA	18.0def	91.0ab	21.7b	2.0b	Positive	HS
Grand Naine AAA	17.0ef	91.0ab	55.5a	3.0a	Positive	HS
C. C*AAA	17.0ef	92.0ab	43.4a	3.0a	Positive	HS
Pelipita AAA	21.0bc	97.0a	52.6a	3.0a	Positive	HS
Mean	19.0	86.0	36.4	3.0		
LSD (P≤0.05)	2.0	20.6	33.2	0.4		
CV%	19.0	14.0	20.0	13.0		
Muraru dessert banana cultivars genome AA Diploid						
Njuru	16.0b	90.0a	45.4b	3.0a	Positive	HS
Kamunyilya	20.0a	92.0a	27.0a	3.0a	Positive	HS
Mraru Mlalu	16.0b	94.0a	66.6b	3.0a	Positive	HS
Mraru Mshare	16.0b	96.0a	62.5b	3.0a	Positive	HS
Mean	17.0	93.0	50.3	3.0		
LSD (P≤0.05)	0.8	23.1	39.5	0.5		
CV%	8.0	12.0	15.0	14.0		

Means with the same letter are not significantly different within each column, C. C*-Chinese Cavendish, S-Susceptible, HS- Highly susceptible

6. 5. Discussion

All the plants that were inoculated with Xcm expressed BXW symptom of yellowing, wilting and necrosis on the leaves similar to young plants with the BXW symptoms under natural infection. In Ethiopia forty local and exotic banana cultivars were evaluated for resistance to BXW raised from suckers planted in field trial. All the inoculated cultivars developed BXW symptoms within forty five to one hundred and twenty days and by 75 days

94% had shown the BXW symptoms. Cultivars Dwarf Cavendish, Valery and Matoke and East African Highland Banana were susceptible (Welde-Michael *et al*.,. 2006).

Tripathi and Tripathi (2009) in Uganda screened two sets of ten cultvars of banana for Relative susceptibility to BXW one set were generated from apical shoot tips through micropropagation which were 3-4 leaves in an in vitro screening, the second set were raised from tissue culture which were ten weeks old. The ten cultivars were all susceptible among them were cultivars Dwarf Cavendish, Giant Cavendish FHIA17 and Kayinja which were highly susceptible, Nakitembe was least susceptible the others were susceptible except *Musa balbisiana* was resistant, a fertile deploid parent.

This study further confirmed that most of the cultivars evaluated were highly susceptible. This was further confirmed when the inoculated banana cultivars sampled were reisolated and cultured in nutrient agar grown at 25^0C and in 24 to 72 hours a growth of yellow, dome shaped, mucoid, circular and shinny colonies was observed for all the cultivars. Cultivars Mokoyo, Namukhila and Horn Plantain were the most susceptible showing BXW symptoms in fourteen days after inoculation while cultivar Mysore, an apple dessert banana of genome AAB was the least susceptible expressing symptoms twenty six days after inoculation. A recovery of Xcm in all plants inoculated meaning the Xcm survived in banana plants during the experimental period. The study confirmed that all the forty six banana cultivars evaluated were susceptible to BXW thus the need to breed resistant banana cultivars.

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CHAPTER SEVEN: GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7. 1. General discussion

Two minute dipstick DNA field capture kit was effective in diagnosis of banana *Xanthomonas* wilt than Whatman FTA cards. Ramathani and Beed (2013) reported that the kit was efficient in daigonis of BXW. The kit was able to detect the Xcm from diseased, suspected and as well as among those considered to be healthy an indication that its an effective method that can help in detection at field level even the latent stages of BXW (Ndungo *et al.,* 2005; Mbaka *et al.,* 2008; Ramathani and Beed, 2013). The two-minute dipstick DNA capture field kit was simple to use in the field and also provided a rapid means of diagnosis using crude DNA, without purification of DNA before PCR amplification (Ramathani and Beed, 2013). Farmers can use this kit to collect samples and send to laboratory for confirmation of diseased, suspected or asymptomatic banana plants. The result of information obtained would be sufficient and efficient with greater sensitivity as compared with ELISA method and direct DNA extraction that requires samples to be transported to laboratory. The study showed that two-minute dipstick DNA capture field kit was significantly better than FTA cards as had been reported by Ramathani and Beed (2013).

In Kenya the study confirmed BXW in Western and Nyanza areas and farmers were familiar with the disease but not in Central, Eastern and Coast areas. The study has also cofirmed BXW has spread fast since it was reported in 2006 to new areas of Busia, Bungoma Siaya and Kakamega, Kisumu. (Kwach *et al.,* 2012; Onyango *et al.,* 2012; Kubiriba *et al.,* 2014). The study has cofirmed that there are management options are available to curb BXW. In BXW epidemic area the leading mode of transmission of BXW was insects, planting materials and contarminated farm tools. In Uganda, Rwanda and Burundi the same mode of

transmission were reported (Tripathi *et al*., 2007; Ndungo *et al*., 2008; Mbaka *et al*., 2009; Tripathi *et al.,* 2010).

Insects found to be associated with banana plant in BXW epidemic areas were stingless bee, grass fly, banana fruit fly and black ants. Stingless bees were the most abundant followed by banana fruit flies and grass fly. In Ethiopia and Uganda stingless bee were confirmed to transmit Xcm on banana as the pathogen was isolated from those insects that visited infected plants (Gold *et al.,* 2005; Tinzaara *et al.,* 2006; Shimelash *et al.,* 2008). Significantly more insects were observed in the dessert banana cultivars namely Gros Michel, Valery, FHIA 17, Garisa and Gold finger cultivars than the cooking type Uganda green. This concurs with studies in Ethiopia where Shimelash *et al.* (2008) showed that dwarf Cavendish hosted the highest number of insect families. Tinzaara *et al.,* (2006) reported dessert banana had more nector thus attacts insects more than the cooking type.

Single stem rouging methods were effective in reducing BXW incidence, with the injection with herbicide and uprooting of the infected plants being the most effective. The disease was significantly controlled after six months and in the treatments where only the BXW affected plants were removed. Bunch yield was recovered after one year. Turyagyenda *et al*.(2008) reported that banana suckers replanted after a one-month fallow period had a 25% reinfection rate, while all suckers replanted after seven and eight months of fallow survived. The study confirmed single stem injection with Glyphosate herbicide (Roundup®) to BXW infected plants killed them and controlled the BXW disease within six months. The study confirmed that after three months the Xcm load was low and by sixth month total control was achieved. Mwebaze *et al.* (2006) reported that Xcm survives in the soil for less than three months under laboratory conditions. Welde-Michael *et al.* (2008) reported that Xcm cannot survive in soil in the absence of enset plant material for more than 90 days. Welde-Michael *et al.* (2008) and Kubiriba *et al.,* (2014) reported that removal of affected

banana plants from a field, cut into small pieces and heaped/buried/composted killed the pathogen in banana orchard. This might be why the study was able to control the disease in six months because the pathogen survival after three months is low without the host plant. The application can be used by farmers for management of BXW in the first three months.

In the study complete uprooting was an effective option and reduced the BXW. Turyagyenda *et al* (2008) indicated that complete uprooting of affected plants and removing plant debris on ridges, leaving the debris to rot/dry was an effective measure for managing BXW (Mwangi, 2007; Kubiriba *et al.,* 2014). The single stem rouging was an effective and less labour intensive strategy of managing BXW without complete loss of yield. Glyphosate herbicide (Roundup®) was effective however the related costs, technical knowledge of application, initial resources to purchase the herbicide and its related application equipments might be high for an average low resource farmers. The study confirmed that it was possible to reduce BXW incidence by over 80% in one year, resulting in yield recovery by up to 70% within one year by removing only the BXW symptomatic plants compared to destroying the orchard followed by replanting period after three months. This offers options of BXW management depending on stakeholders ability.

The 46 banana cultivars screened for tolerance to BXW were found to be susceptible. The study confirmed cultivars Mokoyo, Namukhila and Horn Plantain were the most susceptible showing BXW symptoms in fourteen days after inoculation while cultivar Mysore, an apple dessert banana was the least susceptible expressing symptoms twenty six days after inoculation. *Xanthomonas campestris* p.v. *musacearum* was able to survive in the plants as 100% recovery was observed from all inoculated plants. Studies in Uganda reported by Tinzaara *et al.* (2013), Tushemereirwe *et al.* (2004) and Ndungo *et al* (2006) in Democratic Republic of Congo reported that all banana cultivars were susceptible to Xcm (Ndungo *et al.,* 2008). In Ethiopia forty local and exotic banana cultivars evaluated for

resistance to BXW were all susceptible including Dwarf Cavendish, Valery and Matoke and East African Highland Banana (Welde-Michael *et al.,* 2006).

7. 2. Conclusions

Banana *Xanthomonas* wilt disease is present in Kenya and had spread in Busia, Bungoma Siaya and Kakamege, Kisumu of Western and Nyanza regions only. Other parts of the country are still free from BXW. Two minute dipstick DNA capture kit was effective in diagnosis of BXW. Rehabilitation of severely BXW affected banana orchards was achieved by carrying out single stem rouging options, such as removal by uprooting, cutting at the base, injecting with Glyphosate herbicide (Roundup®). For orchards with above 80% infection, uprooting the whole mat followed by replanting after three months is effective. The rehabilitation program was able to recoverd yields by 70% after one year. All banana cultivars screened for BXW were susceptible.

7. 3. Recommendations

1. Quarantine enforcement by Government agencies to prevent spread of BXW to non infected areas.
2. Awareness creation among banana farmers in BXW endemic areas on management of BXW by use of cultural control methods, sterilization of farm implements in infected orchards, rougingof infected banana plants and removal of male banana flower buds to be encoraged by banana farmers, Agricultural extension agents, other trained stakeholders on BXW control, use of mass media, posters, during Agricultural shows and field days.
3. Policy on use of clean planting materials and regular inspection/screening for the approved banana nurseries to dispense BXW disease free materials.

4. Production of free BXW such as banana tissue culture, train and encourage more banana nurseries producers/tissue culture hardening nurseries to avail clean banana planting materials.

5. Further studies on breeding for banana cultivars with resistance to BXW.

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Appendix I Survey Questionnaire: Occurrence of banana *Xanthomonas* wilt in Kenya

Questionnaire number ___ Date of interview ____ Name by _____

1. DETAILS OF RESPONDENTS

A, Region ___ **B**, District: ____ **C**, Division: _____**D**, Location: ___ **E**, Sub-location_____ **F,** Village: _____**G**, Name of farmer: _____**H**.Tel. number____, **I** Occupation _____ **J**. 1.Full time farmer, **2**. Part time farmer, **K**, If not full time farmer, main occupation **1**. Formal employment, **2**. Casual employment, **3**. Self employed, **4**. Other __

2. PLANTATION STATUS AND PLANTING MATERIALS

A) GPS readings: Altitude_Latitude__Longitude_

B) Area under bananas (acres) (**estimate**): **_**or mats number _

C) Banana varieties grown

i) Name banana cultivars grown, ii) Brief description(s) of eah cultivar named, iii) Indicate type/use; 1. dessert, 2. cooking, 3 other (specify) per cultivar

iv) Types of planting material per cultivar named; 1=Suckers, 2=Tissue culture, 3=corm

iv) What source of planting material used per cultivar for establishing plantation; 1=Own 2=Neighbors 3=MoA, 4=Researchers 5=Others (specify) e.g. NGO's

v) Who plants banana? __, Who owns the bananas?__, Who sells banana __; 1. Husband, 2. Wife, 3. Child, 4. Husband and wife, 5. All 1, 2, 3&4, 6. Farm worker

vi) What cropping system do you practice 1. Pure banana crop 2. Intercrop and why for each practice?, ii) If intercrop, state crops used.

3. BXW STATUS AND TRANSMISSION MECHANISM

A) Current status of BXW on farms 1=Not present, 2=Present

i) If not present, has BXW been on this farm before?1=Yes, 2=No, ii) If Yes, the first time disease seen on farm? 1=≤ a month, 2=≤ three months, 3= ≤ six months, 4=≥ a year

iii) What % of mats is infected by BXW; 1=0, 2=<10, 3= 11-40, 4=41-70, 5= 71-100%

vi) Is the BXW status over the past year; 1=Increasing, 2=Constant, 3= Decreasing, 4=Complete control.

B) Farmer knowledge on means of spread and control of BXW

i) Do you know BXW? (1 = Yes, knowledgeable 2= No not knowledgeable)

ii) How do you recognize the disease?, iii) Where did you obtained the knowledge for BXW symptoms; discoloration of finger, section pulp, flower, including fruits, wilting leaves, premature ripening of bunch, rotting of male bud and bunch, others (1=neighbor, 2=Extension, 3= Researcher, 4= Radio/TV, 5= other (specify)

iii) How does BXW spread? and how do you control it?

iv) Where did you obtain the knowledge for its control