OCCURRENCE OF SUGARCANE RATOON STUNTING DISEASE IN NYANDO SUGAR BELT AND ITS MANAGEMENT BY HOT WATER TREATMENT

BY

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DECLARATION

This thesis is my original work and has not been presented for a ward of a degree in any other University

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I dedicate this work to my husband Ginson M. Riungu and family members who always encouraged and motivated me to carry on with my education. Through their love and enormous support I was able to successfully complete this study.

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LISTS OF ABBREVIATIONS

AEZS Agro-ecological zones

CIRAD The Centre for International Cooperation in Agricultural Research for Development

HWT Hot water treatment

KESREF Kenya Sugar Research Foundation

KSB Kenya Sugar Board

LM Lower midland

NCB Nitrocellulose membrane

PCM Phase contrast microscope

RSD Ratoon stunting disease

SRI Sugar research foundation

TB-EIA Tissue blot enzyme immunoassay

TBS Tris buffered solution

ABSTRACT

Sugarcane yield in Kenya has been on decline due to many factors including pests and diseases. Ratoon stunting disease (RSD) caused by *Leifsonia xyli* subsp. *xyli* is considered to be the most serious disease of sugarcane worldwide because it can cause up to 50% yield loss. This study was carried out with the objective of determining the prevalence and incidence of the RSD in Nyando sugar belt. Survey for the occurrence of RSD and sugarcane production practices was conducted from November, 2014 to February 2015 in three agro-ecological zones (AEZs) of Nyando sugar belt in Kisumu County. Sugarcane stalks above the age of nine months were randomly selected from each sampled farm and the presence of RSD was detected by phase contrast microscope and Tissue Blot Enzyme Immunoassay. Information on sugarcane variety, source of seed cane, acreage, and major sugarcane disease and production practices was collected using a questionnaire.

Effect of hot water treatments in the management of RSD was determined on three sugarcane varieties (CO421, D8484, KEN 83737) at 45°C, 50°C, 52°C and 55°C for two hours. Control cane was treated with cold water at room temperature (25°C). The treated cane was planted in the field and in the greenhouse and data collected included germination, cane girth, cane height, number of millable stalks, number of internodes, cane weight and sucrose content.

Results of the survey showed that 55% of the farmers grow sugarcane on small land holdings of less than two acres and mainly use seed cane from neighbours and own farms. Most farmers (96%) grow old sugarcane varieties such as CO421, CO945, CO617, and N14. Awareness on ratoon stunting disease was low, only 35% of farmers had information on the disease. Ratoon stunting disease was found to be highly prevalent (67%) in the Nyando sugar belt with disease

incidence of up to 25%. All the three main varieties grown were found to have RSD incidences ranging from 10 to 35%. Hot water treatment at 45, 50 and 52°C significantly reduced RSD and increased cane germination, cane and sucrose yield. Hot water treatment at 55°C completely eliminated RSD but significantly reduced germination and cane yield. The cane setts which were treated with cold water at 25°C were stunted and had low overall cane yield.

The results showed widespread occurrence of RSD in Nyando sugar belt which could be attributed to the use of infected setts, preference of old commercial sugarcane varieties coupled with low awareness to ratoon stunting disease. Hot water treatment at 50°C for two hours was the most effective and produced the highest cane yield. However results showed that cane yield at temperature 50°C and 52°C was not significantly different. Therefore any of the two temperatures are hereby recommended for management of ratoon stunting disease of sugarcane.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Sugarcane (*Saccharum officinarum*) is grown in the tropics and subtropical regions of the world. It was estimated that sugarcane was cultivated in over 26 million hectares in more than 90 countries with worldwide harvest of 1.84 billion tones (FAO, 2012). In Kenya, it is one of the important crops alongside tea, coffee, horticulture and maize (KSB, 2010). In Kenya sugarcane farming is mainly done by small scale farmers who contribute about 90% of the production. The remaining (10%) coming from the large scale farmers and the factory nucleus estates.

The Kenya sugarcane industry plays a significant role in social-economic development of the country (KSB, 2008). The sector directly supports 200,000 small scale farmers who supply the cane milled by the sugar companies (Wawire *et al*., 2006; Odenya *et al*., 2007; KSB, 2008). An estimated six millions Kenyans derive their livelihood directly or indirectly from the sugar industry. The industry is estimated to employ 12,500 Kenyans in sugar plantations and sugar factories (KSB, 2008). In addition, the industry saves Kenya in excess of US$ 250 million in foreign exchange annually. Other benefits accruing from the industry are social amenities such as schools, roads and bridges, health facilities provided to the communities by the Sugar industry and out grower institutions. The sugar industry also provides raw materials to other industries such as bagasse for power co-generation and molasses for a wide range of industrial products including ethanol.

In Kenya, sugarcane is grown on fairly flat regions in Western, Nyanza and Coast. These include Kwale, South Nyanza, Mumias, Busia, Nyando (Chemelil, Muhoroni, and Kibos), Nzoia and West Kenya. About 85% of sugarcane supply is from the small-scale growers whilst the

remaining is from the nucleus estates owned by the sugar factories. Currently there are eleven active companies which support sugar processing six of which are privately owned (Transmara , Sukari, Butali, Kibos, Soin , Sukari mills and West Kenya) and four are still under majority Government ownership (Muhoroni, Chemelil, Nzoia, Sony, Mumias sugar company).

The national annual production of sugar ranges from 450,000 to 590,000 metric tonnes of sugar (KSB, 2013).This does not meet the annual demand of 760,000 metric tonnes, which leaves a deficit of up to 200,000 metric tonnes that is met by imports from other countries (KSB, 2013). Sugarcane yields in Kenya have been reported to have declined since 2009 from 85 metric tonnes per hectare to 69 tonnes per hectare in 2013 (Table 1).

Pests and diseases, low adoption to agricultural technology, soil infertility, poor road networks and use of poor seed cane (Wawire *et al*., 2006) are the major factors limiting sugarcane production in Kenya. Most important diseases that attack sugarcane are smut (*Sporisorium scitaminea), sugarcane mosaic virus*, Pineapple disease (*Ceratocystis paradoxa)* and ratoon stunting disease (*Leifsonia xyli* subsp *xyli*) (KESREF, 2013). Ratoon stunting is considered the most serious sugarcane disease worldwide (Davis and Bailey, 2000, McFarlane, 2001). The disease is of major concern in many sugar producing countries of the world, as its impact on cane production can be very severe. It has been reported that the disease reduces both cane and sucrose yield (Zvoutete, 2004; Johnson and Tyagi 2010; Yohannes *et al*., 2012).

Table1. Area under sugarcane cultivation and production in Kenya (2005-2013)

Year	Area under sugarcane	Yield(tonnes/ha)	Production(Mt)
2005	144,765	84.9	4,800,820
2006	147,730	90.3	4,932,839
2007	158,568	87.9	5,204,214
2008	169,421	93.9	5,112,000
2009	154,298	85.3	5,610,702
2010	165,800	83.1	5,709,586
2011	180,912	83.3	5,338,562
2012	207,483	65.5	5,822,633
2013	206,809	69.4	5,900,000

Source: Kenya Sugar Board, 2008, Food Agricultural Organization, 2013)

1.2. Problem Statement and Justification

In Kenya sugarcane production stands at 60 tonnes per hectare which is way below the potential yield of 100 tonnes per hectare under rain-fed conditions (KSB, 2014). Cane census conducted in 2014 by Kenya sugar industry showed that the average cane yields dropped from 65.5 tonnes per hectare recorded in previous cane census to 60.54tonnes as at the end of February 2014(KSB, 2014). This decline has been reported in all sugar growing areas of this country and it's attributed to many factors including pests and diseases (Wawire *et al*., 2006). Ratoon stunting disease caused by a bacterium *Leifsonia xyli* subsp *xyli* it's well known to be an economically important disease of sugarcane in the world (Bailey and Bechet, 1995, Davis and Bailey, 2000, McFarlane, 2001).

Depending on the susceptibility of the variety and weather condition, the impact of RSD has warranted serious concern as loss in yield can reach over 60%, especially in ratoon crop (Scarlett, 1980). The disease causes both quantitative and qualitative losses in sugarcane (Johnson and Tyagi, 2010). Direct yield loss results from production of thinner and shorter stalks. The disease also reduces the sucrose content of the cane (Zvoutete, 2004, Yohanes *et al*., 2012). The results of four different trials confirmed the presence of ratoon stunting disease in sugar growing areas of Kenya and estimated yield losses in the major variety CO 421 at between 17 and 21% in Mumias and about 4% in Ramisi, the percentage loss of available sucrose was 22.8 in Mumias (Early, 1973). However, the incidence and severity of this particular disease has not been documented in sugarcane growing areas of Kenya. Ratoon stunting is spread through infected cuttings or mechanically through farm implements. A survey conducted in Nyando found that farmers lack knowledge and awareness of the existence of clean and disease free planting materials (Odenya *et al*., 2009). It was reported that 68.9% of sugarcane farmers source their seed cane from others and 12.2% planted their own seed enhancing the spread of the diseases (Odenya *et al*., 2009). It is therefore important to determine the current prevalence, incidence and severity of RSD so that farmers could be enlightened on better management strategies.

The fact that over 50% of farmers use uncertified or untreated seed cane means the disease can easily spread over large areas within a short time (Riungu *et al*., 2012). There is a need to come up with management strategy to help sugarcane farmers obtain quality and healthy seed cane in order to prevent the introduction and spread of this disease in their field. Ratoon stunting disease has always been managed by heat therapy of planting material combined with well organized multiplication nurseries. Hot water treatment at 50°C for two to three hours has been the most

commonly used method of heat treatment (Johnson and Tyagi, 2010). However, conflicting results show that hot water treatment (HWT) may have either positive or negative effects on yields based on the variety, temperature and duration of the therapy (Johnson and Tyagi, 2010). It is therefore important to evaluate the effect of HWT in control of RSD and its effects on cane germination and yield .

1.3. Objectives

The broad objective was to contribute to enhanced sugarcane productivity through improved diagnosis and management of ratoon stunting disease by hot water treatment.

The specific objectives were:

1. To determine prevalence and incidence of ratoon stunting disease in farmer fields in Nyando sugar belt.
2. To determine the effectiveness of hot water treatment in management of ratoon stunting disease in different sugarcane varieties.

1.4. Hypothesis

1. The RSD prevalence and incidence is not different in the three agro-ecological zones of Nyando sugar belt.
2. Hot water treatment has no effect on reduction and control of RSD, cane growth, yield and quality.

CHAPTER TWO: LITERATURE REVIEW

2.1. Sugarcane production in Kenya

Sugarcane is an important agro- industrial crop grown in tropical and subtropical regions of the world. It is a perennial grass that forms lateral shoots at the base to produce multiple stems, typically three to four metres high and about five centimetres in diameter. The stems grow into a cane stalk which when mature constitutes approximately 75% of the entire cane. A mature stalk is typically composed of 11-16% fibre, 12-16% soluble sugars, 2-3% non -soluble sugars and 63-73% water. Sugarcane juice is extracted and used for making white sugars, brown sugars, jaggery and ethanol. The main by-products of sugar industry are bagasse and molasses.

Sugarcane is one of the most important crops in Kenya alongside tea, coffee, maize and horticultural crops. The crop is mainly grown in Nyando zone which covers Chemelil, Kibos, Muhoroni and Soin areas, South Nyanza zone covering Sony sugar, Sukari and Transmara and Western region which includes Mumias, Nzoia, West Kenya and Butali area at elevations ranging between 1,300m and 1,700m above sea level (KESREF, 2013). The major sugarcane varieties in Kenya are CO 617, CO 421, N 14 and CO 945 (KESREF, 2013). Varieties CO 617 and CO 421 are dominant in the Nyando and Nzoia sugar zones while N 14 and CO 945 dominate the Mumias and Sony sugar zones. In 2002, six "KEN" varieties such as KEN 82 – 216, KEN 82 – 219, KEN 82 – 247, KEN 82 – 401, KEN 82 – 808 and KEN 83 – 737 were released. Positive attributes of these varieties include early maturity at 15 – 19 months, high sugar and cane yields. So far reports from the field indicate that varieties KEN 82 – 216, KEN 82– 247, KEN 82 – 808 and KEN 83 – 737 are gaining popularity in the out grower zones in Nyando and Mumias. In 2007, KESREF released four improved varieties, D 8484, EAK 73-335,

KEN 82-62 and KEN 82-472 (KEPHIS, 2015). The variety KEN series are early maturing and high yielding and are expected to be adopted by farmers. The current commercial varieties for example varieties CO 421 and CO 945 are late maturing, have low sucrose content and are susceptible to the major diseases such as smut, mosaic and ratoon stunting disease.

Over 85% of sugarcane production is contributed by small scale farmers, the remaining 15% coming from large scale farmers and factory nucleus estates. Sugarcane industry supports directly or indirectly 6 million Kenyans and it's a source of livelihood for farmers in sugar growing areas in Kenya (Wawire *et al*., 2006; Odenya *et al*., 2007). The total area under cane is 206,809 hectares (KSB, 2013). Sugarcane production stands at 60 tonnes per hectare which is way below the potential yield of 100 tonnes per hectare under rain-fed conditions (KESREF, 2009).

2.2 Constraints to sugarcane production

The major constrains to sugarcane production in Kenya include low adoption to agricultural technology, soil infertility, poor road networks (Wawire *et.al*. 2006), use of poor seed cane, pests and diseases. The major diseases affecting sugarcane production in Kenya include sugarcane smut, ratoon stunting, sugarcane mosaic virus and pineapple disease (KESREF, 2013).

Smut caused by fungus *Sporisorium scitaminea* is considered to be one of the major diseases affecting sugarcane in Kenya. It was first reported in Kenya in 1958 in Nyanza and Coastal Provinces (Robinson, 1959). Presently sugarcane smut occurs in all sugarcane growing areas of Kenya (KESREF, 2002). Most recognised symptoms of smut disease are black soot, whip like stalks and grass like leaves. The emerged whips are composed of a central core of host tissue surrounded by a thin layer of black spores that is covered by a thin silver-white membrane. The

whips vary in size from only a few centimetres to large whips up to 1.5 m long extending high above the foliar canopy (Viswanathan, 2012). The infected stools may appear grassy with an abnormally high number of small diameter stalks with terminal whips. The whips reduce the yield and quality of sugarcane and jiggery (Nzioki and Jamoza, 2006). The disease can be controlled effectively through use of resistant varieties, rouging of the infected plants before whip open and burying or burning the plants and hot water treatment of setts at 50°C for 2hrs.

The sugarcane common rust is caused by fungi *Puccinia melanocephala.* Common rusts is characterized by pustules (uredinia) that occur mainly on the underside of the leaves (Ryan and Egan, 1989; KESREF, 2013).The pustules are 2-20 mm long by 1-3 mm wide and lie parallel to the vascular bundles. Severely infected leaves have large numbers of pustules that coalesce, causing large areas of leaves to become necrotic. The pustules are reddish brown to brown. The disease can be managed by planting resistant cultivar. Cultivar diversification is also recommended due to the possible presence of rust variants.

Pineapple disease (sett rot) of sugarcane caused by fungus *Ceratocystis paradoxa* causes rotting of sugarcane setts (Viswanathan, 2012). The disease causes considerable losses in sett germination in almost all countries where sugarcane is grown. The disease is severe in heavy textured soils and poorly drained fields and can reduce germination up to 47%. The infected setts have red to black internal discolouration and emit a smell resembling that of mature pineapple fruit (KESREF, 2013). Later the centre of the cuttings breaks down and turns black because of the dust-like chlamydospores which are distributed between the vascular bundles. Adopting field operations which result in better drainage and better tillage will help to control the disease. Use of fungicides and planting resistant varieties it's also recommended.

Sugarcane mosaic virus has been reported in sugarcane growing areas of Kenya (KESREF, 2013). Symptoms include appearance of pale yellow patches or blotches on the leaves and sometimes the whole plant may be stunted. The disease causes reduction of yield in infected plants and the loss depends on susceptibility of the cultivar, incidence of infection, environmental conditions and stage of growth. The disease can be controlled through use of healthy clean seed cane, use of resistant varieties and roguing and destruction of infected plants if the infection is high (Viswanathan, 2012).

2.3. Ratoon stunting disease

2.3.1. Occurrence and distribution

Ratoon stunting disease (RSD) of sugarcane caused by the bacterium *Leifsonia xyli* subsp. *xyli* is extremely common in most of the sugarcane growing countries in the world causing losses up to 30% per year (Hughes, 1974). The disease was first detected in 1944 in the ratoon crop of Q28, in Queensland (Steindl, 1961). To date the disease has been reported from Australia, USA, India, Brazil, Florida, China, Fiji, Philippine and Africa (Viswanathan, 2001; Dela *et al*., 2002; Johnson and Tyagi, 2010). The disease has been reported as being well established in most sugarcane growing regions of the world causing most yield loss worldwide (Davis and Bailey, 2000, McFarlane, 2001; Tiwari *et al*., 2010). The results of four trials confirmed the presence of ratoon stunting disease in sugar growing areas of Kenya (Early, 1973). Surveys based on estates or small scale grower productions have revealed that numbers of infected fields in Kenya are 60-90% (Bailey, 1999). In South Africa, approximately 9% of commercial cane fields contain some levels of RSD infection. Numbers of fields infected in other countries are 30% in Swaziland, Zambia 50%, Kenya, Malawi, Uganda, and Zimbabwe 60-70%, Tanzania and Mafambisse estate in Mozambique 100 % (Bailey, 1999).

2.3.2. Causal organism of ratoon stunting

Although ratoon stunting disease had been recognised as an important disease of sugarcane for over 40 years (Steindl, 1950), it was only in 1970s that Gillaspie *et al*., (1973) and Teakle *et al*., (1973) independently found the bacterium associated with the disease in Louisiana and Australia, respectively. These findings were confirmed in South Africa (Bailey, 1976), Taiwan (Chen *et al*., 1975), Mauritius (Ricaud *et al*., 1976), India (Rish and Nath, 1978), Brazil (Gillaspie *et al*., 1979) and Florida (Davis and Dean, 1984). The bacterium was identified as a corynebacterium and was taxonomically designated *Clavibacter xyli* subsp *xyli* based on phenotypic characteristics *(*Davis *et al*., 1984). Evtushenko *et al*., (2000) reclassified the bacterium along with *Clavibacter xyli* subsp *cynodontis* as *Leifsonia xyli* subsp *xyli* and *Leifsonia xyli* subsp *cynodontis*, respectively based on rRNA gene analysis.

Leifsonia xyli subsp *xyli* is a small gram positive, xylem inhabiting, coryneform bacterium that maybe detected in xylem sap extracts using phase-contrast or dark -field microscopy(x1000). The bacterium is unicellular, measures about 0.25-0.50 x 1-4µm in size. The bacterium colonizes xylem vessels and remains in vessels and adjoining tracheids, parenchyma and lacunae of the xylem. Pathogen populations vary among varieties and are greatest in the basal portion of the mature stalks during later part of the growing season.

The pathogen is extremely fastidious in its nutritional requirements and can only be grown in axenic culture on special media such as the synthetic complete (SC) medium (Davis *et al*., 1980). Due to slow growth, exacting nutritional requirements and lack of a selective medium for isolation, diagnosis based on isolation of the pathogen in culture is rarely used (Croft *et al*., 1994). Microscopy, serology and DNA based diagnostic techniques are the main method used to detect the pathogen (Fegan *et al*., 1998; Grisham *et al*., 2007). The pathogen has been found only

in sugarcane in nature and has no known insect vectors. It is transmitted mechanically through farm implements and spread through infected propagation materials (Young *et al*., 2006).

2.3.3 Symptoms of ratoon stunting disease

Ratoon stunting disease produces no reliable or distinct external symptoms (Steindl, 1961; Gillaspie and Teakle, 1989). The diseased clumps usually display stunted growth, reduced tillering, thin stalks with shortened internodes and yellowing leaves. In stubble or ratoon crops, diseased plants are slower to initiate growth, and death of individual plants of extremely susceptible varieties may occur. Ratoon crops usually suffer more severely than plant crops. Stunting in the field is not uniform from clump to clump and diseased fields show a characteristic up and down appearance, even if all plants are diseased. Yield losses caused by RSD is enhanced by stress particularly moisture stress (Gao *et al*., 2008; Comstock and Gilbert, 2009). The reduction in yield is due to the production of thinner and shorter stalks rather than a reduction in the total number of canes (Dean and Davis, 1990). Some highly susceptible varieties may show wilting under moisture stress and develop a necrosis of leaves at the tips and margins. The root system of diseased cane may be reduced in size, proportionally to the above young parts, but the roots appear to be normal.

The internal symptom caused by the bacterium includes vascular discoloration in fully differentiated nodes of relatively mature stalks or less commonly in young stalks (Gillaspie and Teakle, 1989, Viswanathan, 2012). The stalks appear as yellow to reddish –brown dots, commas, or short lines when viewed by slicing longitudinally through nodes (Gao *et al*., 2008). The discoloration does not extend into the internodes unlike similar symptoms due to other diseases (Gao *et al*., 2008). The presence of colour and the intensity of discolouration of the growing

points and vascular bundles may vary with the stage of growth of the stalks, the degree of infection and will differ among cultivars.

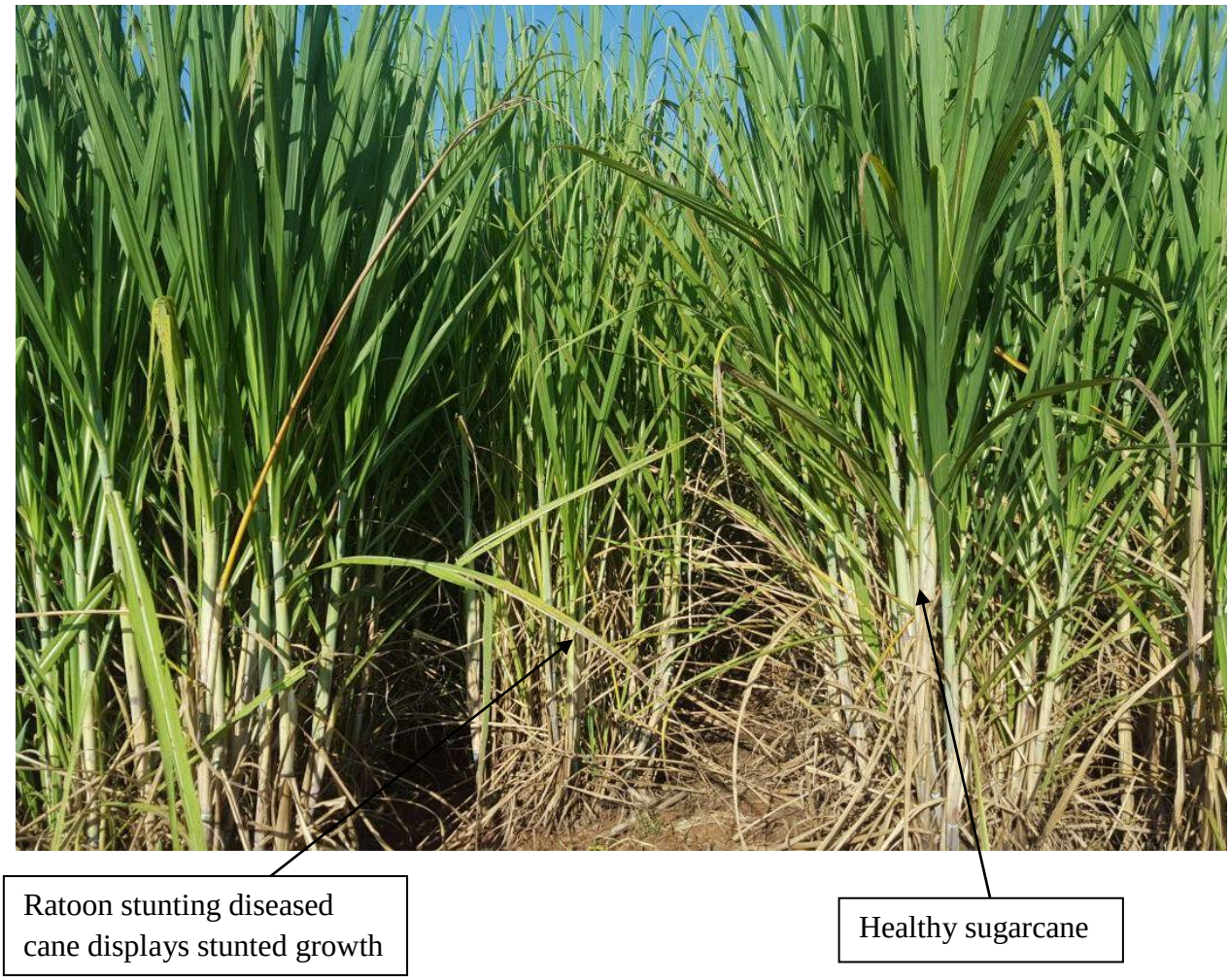

Figure 2.1.Ratoon stunting diseased sugarcane versus healthy sugarcane

2.3.4 Disease development, spread and pathogen survival

The pathogen has been found only in sugarcane in nature and has no known insect vector (Tiwari *et al*., 2012). Since the disease cannot be detected by external appearances, the bacterium is spread unknowingly from one area to another (Tiwari *et al.*, 2012). Systemic infection of the xylem takes place through wounds during farm operation. The life cycle of the bacterium is limited to xylem vessels of sugarcane plants. The bacterium produces cell wall -degrading

enzymes, pectinase and cellulase (Monteiro-Vitorello *et al*., 2004) which may be involved in extraction of nutrients from xylem vessels. Production of gelatinous material due to host defence response or by bacterium itself plug xylem vessels of infected plant impairing translocation of water and nutrients which may lead to poor growth of the infected plants (Kao and Damann, 1978). Monteiro-Vitorello et al., 2004 reported that the bacterium may be involved in the synthesis of abscisic acid hormone which is reported to be a plant growth inhibitor. The hormone could be the cause of stunting and poor tiller growth in sugarcane infected with *Leifsonia xyli* subsp *xyli.*

The disease is mechanically transmitted through cutting tools during field operations. Bailey and Tough (1992) and Comstock *et al*., (1996) showed that RSD could be transmitted when sap from infected stalks contaminates blades of harvesting equipment and is spread from plant to plant. Mechanical harvesters have also been known to spread the disease (Damann,1992; Hoy *et al*., 1999).The pathogen is also spread during propagation by planting seed cane from RSD infected crop (Comstock and Gilbert, (2012); Viswanathan, (2012). It is not seed transmitted. It may survive for several months on infected crop debris left on soils or soil itself contributing to the persistence of the disease in areas where the disease is common (Autrey *et al*., 1991; Bailey and Tough, 1992). However, the extent of infection by the pathogen surviving in the soil is not known (Comstock and Gilbert, 2009). Volunteer regrowth from former infected crops provides a common source of inoculum of new plantings. Disease expression (stunting) is enhanced by drought and subsequent water logging (Viswanathan, 2012). The rate of disease spread and extent of colonization of in infected plants are directly to varietal susceptibility

2.3.5 Effect of ratoon stunting disease on sugarcane

The bacteria reduces yield and sugar content of the cane (Johnson and Tyagi, 2011; Yohannes *et al*., 2012). Concentration of the bacteria in the vascular bundles causes blockage, interfering with uptake of water and nutrients that ultimately leads to poor and stunted growth of the sugarcane plant (Viswanathan, 2012). The diseased crop produces few tillers, thin stalks with shortened internodes which lead to reduction in cane yield (Viswanathan, 2012). Yield reductions are sometimes greater in successive ratoon crops, possibly due to increased disease incidence (Gao *et al*., 2008). Johnson and Tyagi, (2010) found the reduction in cane yield was more on ratoon crops than on plant crop. Field experiments have shown ratoon stunting disease can cause reductions in yield of 15-30% under good irrigated conditions and 20-40% under average rain-fed conditions in cultivars that are widely grown in Africa. Even greater losses can occur in some cultivars under drought conditions (Bailey, 1999; Johnson *et al*., 2010). In Fiji yield losses averaged at 29% (Jonson and Tyagi, 2010).

Field experiments in South Africa have shown an average 20-40% yield reduction due to ratoon stunting disease for rain- fed conditions and 21 to 32% yield loss under irrigated conditions depending on cultivar (Bailey and Bechet, 1997; Bailey and McFarlane, 1998). Stalk population and length were reduced due to the disease in both rain- fed and irrigated environments (Bailey and Bechet, 1997). In Louisiana, cane and sugar yield were reduced by 24% in the second-ratoon crop of L99-226 and by an average of 32% across the plant-cane, first-ratoon and second - ratoon crops of L99-233 (Grisham, *et al* 2009). In Florida, Comstock (2008) compared the cane and sugar yield of *Leifsonia xyli* subsp *xyli* infected plants and healthy plants and showed that plants infected with *Leifsonia xyli* subsp *xyli* had fewer stalks, reduced sugar and reduced cane yields compared with healthy plants.

2.3.6 Diagnosis of ratoon stunting disease

In the field it is very difficult to diagnose ratoon stunting disease because of the absence of visible symptoms (Viswanathan, 2012), its presence can only be positively determined by laboratory analysis of the sap samples. Studies on ratoon stunting disease infected plants have shown the bacterium to be in the xylem vessels frequently adjacent to the vessel wall (Kao and Damann, 1980). Microscopy, serology and DNA based diagnostic techniques are the main method used to detect the pathogen (Fegan *et al.*, 1998, Grisham *et al.*, 2007).

The characteristic stunting and thriftiness associated with RSD can be used to identify the disease in the field (Grisham *et al.*, 2007). Diseased stalks of some varieties may exhibit an internal discolouration of vascular bundles at the lower portion of nodes. They appear as yellow to reddish brown dots, commas or short lines when viewed by slicing longitudinally through nodes (Gao et al., 2008)**.**

Leifsonia xyli subsp *xyli* is a xylem -limited, coryneform bacterium. Xylem sap extracts can be examined by either phase -contrast (PCM) or dark field-microscopy at x1000 for the presence of pathogen (Gillapsie *et al.*, 1973), Davis& Dean, 1984) which is thin, rod shaped, straight or slightly curved. Culture of the bacterium is extremely difficult because of its fastidious nature and can only be grown in axenic culture on special media such as the synthetic complete (SC) medium (Davis *et al*., 1980).

The bacteria can be detected using various immunological tests. Two procedures that permit multiple samples to be analysed simultaneously are Tissue-blot enzyme immunoassay (TB-EIA) and Evaporative-Binding Enzyme Immunoassay (EB-EIA) (Croft *et al*, 1990, Croft *et al*., 1994). In both protocols, antibodies specific to the bacterium are used. Evaporative-Binding Enzyme

Immunoassay is a modified ELISA procedure for analysis of vascular sap extracts. Tissue- blot EIA enables detection and enumeration of colonized vascular bundles in stalk cross-section.

Polymerase chain reaction (PCR) assays based on detection of the 16S ribosomal RNA gene of the pathogen have been developed for detection and identification (Pan *et al*., 1998b; Fegan *et al.,* 1998). PCR provide greater sensitivity and specific detection than other molecular means

2.3.7 Management of ratoon stunting disease

The RSD pathogen is easily transmitted mechanically hence Sanitation is important in keeping healthy cane from becoming infected (Comstock *et al.,* 1996). Precaution should be taken to avoid transmission of pathogen. Cutting implements which have been used in diseased sugarcane fields should be disinfected with disinfectants like sodium hypochlorite before being used in another field. Sugarcane fields that are believed to be free from, or with a lower incidence of ratoon stunting disease can be harvested first each day (Gillaspie and Davis, 1992). Planting healthy cane can be used to control ratoon stunting disease. Seed cane can be monitored for freedom from the disease using appropriate diagnostic techniques.

Seed cane can be heat -treated to eliminate the pathogen (Gillaspie and Davis, 1992). Hot water, hot air, moist air and aerated steam treatments have been used. Hot water treatment at 50°C for 2-3 hours has been the most commonly used method and helps in establishment of pathogen free nurseries which supply planting materials for commercial fields (Johnson and Tyagi, 2010). Different cultivars differ in their tolerance to injury by heat. Seed cane from mature plants is usually less affected by heat and generally germinates better after hot-water treatment than that from immature plants. Precautions should be taken during hot-water treatment of the seed cane. Accurate control of temperature and time is essential for effective treatment. Seed cane should not be trashed cleanly, as a little trash around the nodes helps protect the buds from damage

(Viswanathan, 2012). Destruction of plant debris and old crop before fields is replanted is important as volunteer re-growth may be a source of infection (Bailey and McFarlane, 1999). Fields which are known to be infected requires longer breaks from cane for at least six months before fields are replanted (Bailey and McFarlane, 1999).

CHAPTER THREE

OCCURRENCE OF SUGARCANE RATOON STUNTING DISEASE IN NYANDO SUGAR BELT

3.1. Abstract

Ratoon stunting disease (RSD) of sugarcane caused by *Leifsonia xyli* subsp *xyli* is an economically important disease of sugarcane worldwide. The disease causes more yield loss than any other sugarcane disease. In Kenya, RSD could be one of the diseases causing reduction of cane yield but need to be scientifically verified. This study was carried out with the objective of determining the prevalence and incidence of the RSD in Nyando sugar belt. Survey on occurrence, prevalence, severity of RSD and sugarcane production practices was conducted from November 2014 to February 2015 in three agro-ecological zones (AEZs) of Nyando sugar belt in Kisumu County. Information on sugarcane variety, source of seed cane, acreage, and major sugarcane disease and production practices was collected using a questionnaire. Sugarcane stalks above the age of nine months old were randomly selected from each of the sampled farms and the presence of RSD was detected by phase contrast microscope and Tissue Blot Enzyme Immunoassay.

Results of the survey showed that 55% of the farmers grow sugarcane on small land holdings of less than two acres and mainly use seed cane from neighbours and their own farms. Most farmers (96%) grow old sugarcane varieties such as CO421, CO945, CO617, and N14. Awareness on ratoon stunting disease was low, only 35% of farmers had information on the disease. Ratoon stunting disease was found to be highly prevalent (67%) in the Nyando sugar

belt with disease incidence of up to 25%. All the three main varieties grown were found to have RSD incidences ranging from 10 to 35%. The results showed widespread occurrence of RSD in Nyando sugar belt which could be attributed to the use of infected setts, preference of old commercial sugarcane varieties coupled with low awareness to ratoon stunting disease. It is therefore important to sensitize farmers on RSD management strategies like planting of healthy seed cane and field sanitation.

Keywords: Ratoon stunting disease (RSD), Sugarcane, *Leifsonia xyli* subsp.*xyli*, disease diagnosis

3.2 Introduction

In Kenya, sugarcane industry plays a significant role in social -economic development of the country. It supports directly or indirectly over six millions Kenyans. It's a source of livelihood to about 200,000 farmers in Western Kenya (Wawire *et al*., 2006; Odenya *et al*., 2007). In Nyando, the crop is ranked as the most important cash crop followed by rice. However, the yield of cane has been on significant decline hence the need to determine the cause.

Ratoon stunting disease caused by *Leifsonia xyli* subsp.*xyli* may be one of the possible causes but needs to be verified. Ratoon stunting is the most economically important disease of sugarcane worldwide (Bailey and Bechet, 1995; Davis and Bailey, 2000). The disease is of great concern in many countries as loss in yield can reach over 60%, especially in ratoon crops (Scarlet, 1980; Davis and Bailey, 2000). Comstock and Lentini, (2005) proved that the pathogen can cause 5 to 15% yield loss without farmers realizing their farms were infected. The disease leads to poor sett germination, production of thin stalks with shortened internodes and reduction of sucrose content which all results to poor quality cane (McFarlane, 2002; Zvoutete, 2004; Yohanes *et al*., 2012).

Ratoon stunting disease does not produce reliable external symptoms making it very difficult to diagnose or identify in the field (Viswanathan, 2012) hence farmers can spread the disease unknowingly from one field to another though infected cuttings.

The presence of ratoon stunting disease was first confirmed in Kenya in 1973 (Early, 1973). There is however, no documentation of the actual spread and current incidence of the disease in farmer fields in different sugarcane growing zones in the country. Nyando is among the four zones that produces sugarcane in Kenya, therefore conducting the study in the area would play a great role in the documentation of the actual amounts of disease in farmer fields.

The objective of this study was to determine the prevalence, incidence and severity of ratoon stunting disease in different agro-ecological zones of Nyando sugar-belt.

3.3. Material and methods

3.3.1. Sampling of farms and sample collection

The study was conducted from November 2014 to February 2015 in three agro- ecological zones (AEZs) in which sugarcane is grown in the Nyando sugar belt. The AEZs considered were Lower Midland 1(LM1) known as sugarcane growing zone with average annual rainfall above 1600mm, Lower Midland 2 (LM2) marginal sugarcane zone with average annual rainfall of 1300-1600 and Lower Midland 3 (LM3) cotton growing zone with annual average rainfall ranging from 1100-1350mm. These three AEZs fall in the administrative areas of Muhoroni, Kisumu East and Soin sub counties respectively.

Ten farms that had cane above the age of nine months per AEZ were systematically sampled, the 50^{th} farm along a transect, in case the farm had no sugarcane at the right age the next farm was considered. Both large scale and small scale farms with maturing cane at 9months to 14 months

mostly ratoon one to four were considered. Information on sugarcane variety, source of seed cane, acreage on sugarcane, yield per unit area, major disease affecting sugarcane, and disease control methods was collected using a questionnaire with open-ended and closed-ended questions (Appendix1).

At a distance of 20m inside the field along the perimeter, ten sugarcane stalks were chosen at random in a diagonal transect. The stalks were cut as close to the ground as possible with a cutting knife and the knife sterilized using 10% sodium hypochlorite after cutting each sample. They were then bundled, tied and labelled with tags bearing the name of the farm and the variety. The collected samples were then taken to the Sugar Research Institute laboratory for analysis and isolation of the bacterium.

3.3.2. Microscopic examination for detection of *Leifsonia xyli* subsp *xyli*

Short lengths of cane were cut from the lower nodal region of the stalk, one node and internode length. One end of the stalk was cut at right angle and the other at 45^{o} angle. Air was forced through the cane using a 12 volt air pressure pump and xylem sap collected using a dropper. To avoid contamination, one dropper was used for each sample collected. The extracted xylem sap was placed on a microscope slide using a dropper, oil immersion was added onto the sap and observed under the phase contrast microscope (Carl Zeiss Imager D.2) at x1000 magnification (Gillaspie *et.al.,* 1973, Lemma *et al*., 2013,) for the presence of characteristic bent rod- shaped bacteria. Sap from a stalk known to be infected with RSD was used as a positive control (Hoy *et al*., 1999).

3.3.3. Detection of *Leifsonia xyli* subsp *xyli* by Tissue Blot Enzyme Immunoassay

The procedure for Tissue Blot Enzyme Immunoassay (TB-EIA) for diagnosis of *Leifsonia xyli* subsp. *xyli* used was one provided by J-H Daugrois, 2015 (CIRAD, Montpellier). The cane pieces collected in section 3.3.1 above were cut transversally with a sharp knife to have a clear cut section. The samples were then pressed onto a Nitrocellulose Membrane (NCM) (Whatman, Protan BA85) placed on absorbent paper sheet (Whatmann 3mm Cr) on a rigid laboratory bench. The cut section of the cane was pressed on the NCM as vertically as possible for 15 seconds to do the printing. After all printings were done, the absorbent paper sheet was taken off and the NCM was allowed to dry in cool dry atmosphere. The blotted NCM were immersed in 3% Bovine Serum Albumin (BSA) (6g BSA, 20ml of 10x TBS, 200ul of 50% Tween-20, 180ml water) in Tris Buffered Saline(TBS) (8g NaCl, 0.2g KCL, 3g Tris base, 800ml water) with the print side facing up for 30 minutes.

For the first antibody the Nitrocellulose membranes were incubated in goat Lxx polyclonal antibody 1Gg diluted 1:10000 in 1% BSA(6g BSA, 20ml of 10x TBS, 200ul of 50% Tween-20), 180ml water) in TBS for 1.5 hours at room temperature with the print side facing down. The nitrocellulose membranes were then washed with three rinses of five minutes each in TBS Tween (0.05% tween in TBS). For the second antibody, the rinsed membranes were then incubated in rabbit antigoat 1Gg alkaline phosphatase conjugate diluted 1:10000 in 1% BSA in TBS for one hour at room temperature with the print side facing down. The membranes were again washed with three rinses of five minutes each in TBS Tween. After the three rinses the membranes were then immersed in alkaline phosphates substrate (1 NBT tablet per 20-30ml) for 5 to 20 minutes at room temperature. The reaction was stopped by dipping the membranes in distilled water. After drying the prints were observed under the microscope where the colonized

vascular bundles appeared blue for positive sample. A positive sample from Sugar Research Institute laboratory was included as a standard.

3.3.4. Isolation of *Leifsonia xyli* subsp *xyli* from sugarcane stalk samples

Further confirmation of the bacterium from positive stalk samples obtained from TB-EIA procedure was done. Xylem sap from the stalks was extracted as described in 3.3.1. Using a wire loop the sap was streaked into petri dishes containing Yeast extract, dextrose, calcium carbonate (YDC) media (10g of yeast extract, 20g of dextrose, 20g of calcium carbonate, USP powder 15g of Agar) (Nancy *et al*., 2008). The cultures were incubated at 27°C for 14 days. The colonies were identified based on cultural characteristics like the size, shape and the colour.

3.3.5. Assessment of disease prevalence, incidence and severity

The disease prevalence, incidence, and severity over the agro-ecological zone were computed from the results obtained from TB-EIA analysis. The disease prevalence was assessed as the number of farms with infected cane over the total number of farms sampled per AEZ. Incidence of the disease was expressed as a percentage of infected stalks out of the total sampled stalks per farm. Disease severity was determined using a scale (0=no infected stalk, 1-2 infected stalks= slight infection, 3-4 infected stalks= moderate infection, 5-7 infected stalks =severe infection, >8 infected stalks= very severe infection) which was a modification of RSD rating scale developed by Bailey and Fox 1984.

3.4. Data analysis

The data on crop production practices, disease prevalence, incidence and severity was analysed using the Statistical Package for Social Sciences version 11 and Microsoft Excel, 2010 to

determine disease prevalence and incidence in different agro-ecological zones and how farmer's practices influence the disease incidence and spread.

3.5. Results

3.5.1 Sugarcane production practices in Nyando sugar-belt.

Majority of the sugarcane farmers are small scale with land under sugarcane production being less than two acres (Figure 3.1). Most sugarcane farmers surveyed sourced their seed cane from neighbours (45%) and own farms (26%). Those who sourced seed canes from Sugar Research Institute (SRI) (former KESREF) were less than 10% (Figure 3.1). Majority of farmers preferred and grew old commercial sugarcane varieties such as CO421 and CO617 as opposed to new improved varieties like D8484 (Table 3.2). Majority of farmers in Nyando were not aware of RSD. Among the farmers interviewed across the three AEZs only 35% had information on the disease.

3.5.2. The occurrence of ratoon stunting disease (RSD) in farmer fields

The average disease prevalence over the three AEZs was 67% (Table 3.1). Agro-ecological zones, LM1 and LM3 had farms with the highest prevalence of 70%. The lowest prevalence among the AEZs was 60% recorded in LM2 (Figure 3.2). The mean percentage disease incidence was 20%. The highest disease incidence was from LM2 (25%) and the lowest was from LM1 (15%) (Figure 3.2). The severity of RSD in the three AEZs ranged from slight to very severe (Figure 3.3). Majority of the farms had slight to moderate disease infection with only three farms in LM2 and LM3 with severe and very severe infection. Most of the varieties surveyed were infected with ratoon stunting disease (Table 3.2). Variety CO421 had the highest number of infected stalks across the three agro-ecological zones compared to other varieties (Table 3.2). Percentage disease incidence was higher in farms planted with own seed cane and farms planted

with seed cane obtained from neighbours (Figure 3.5). After isolation, the bacteria colonies in YDC media were creamy white, shinny and round in shape (Figure 3.4).

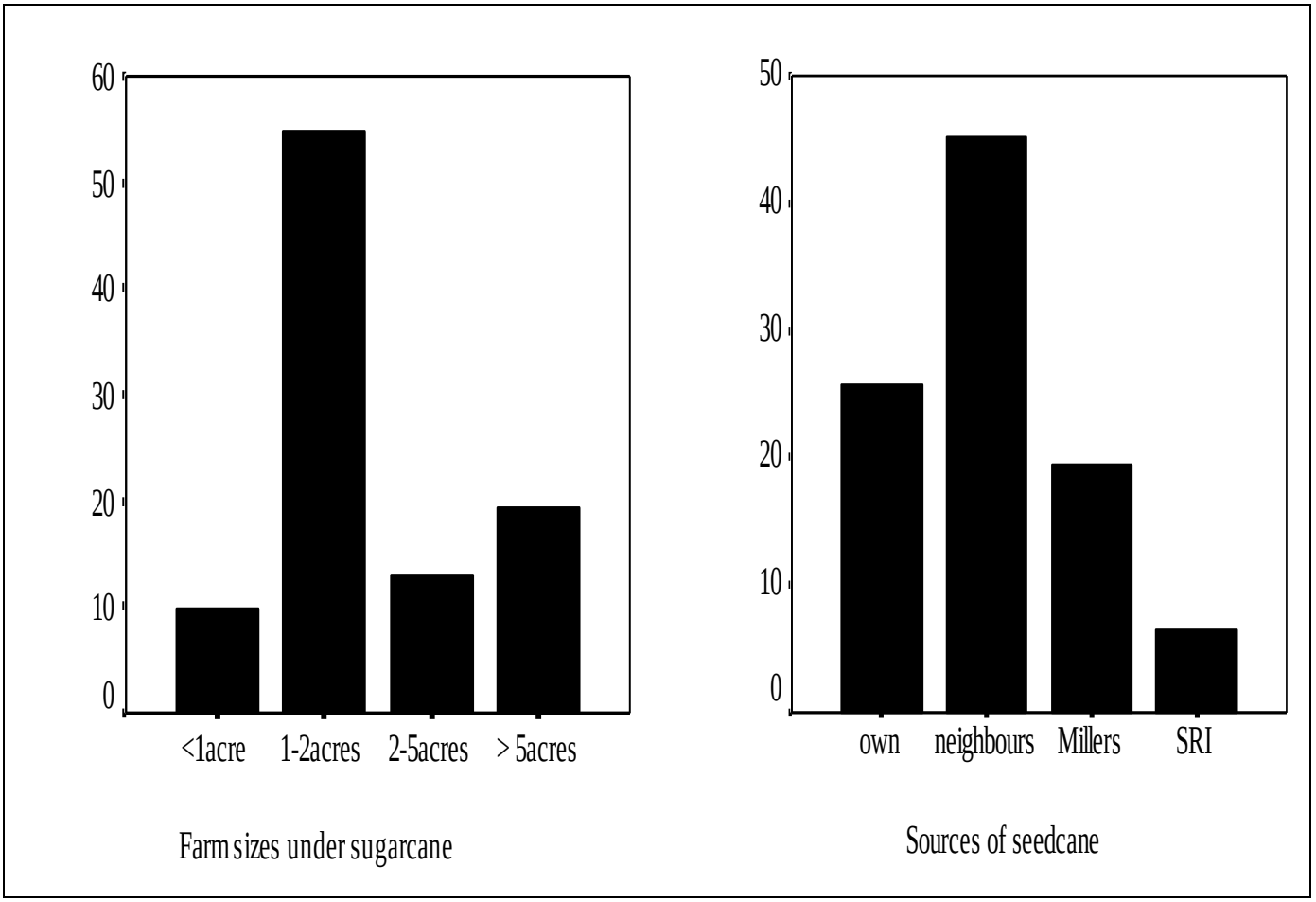

Figure .3.1. Farm sizes under sugarcane and farmers sources of seed cane in Nyando sugar-belt (SRI=Sugar Research Institute).

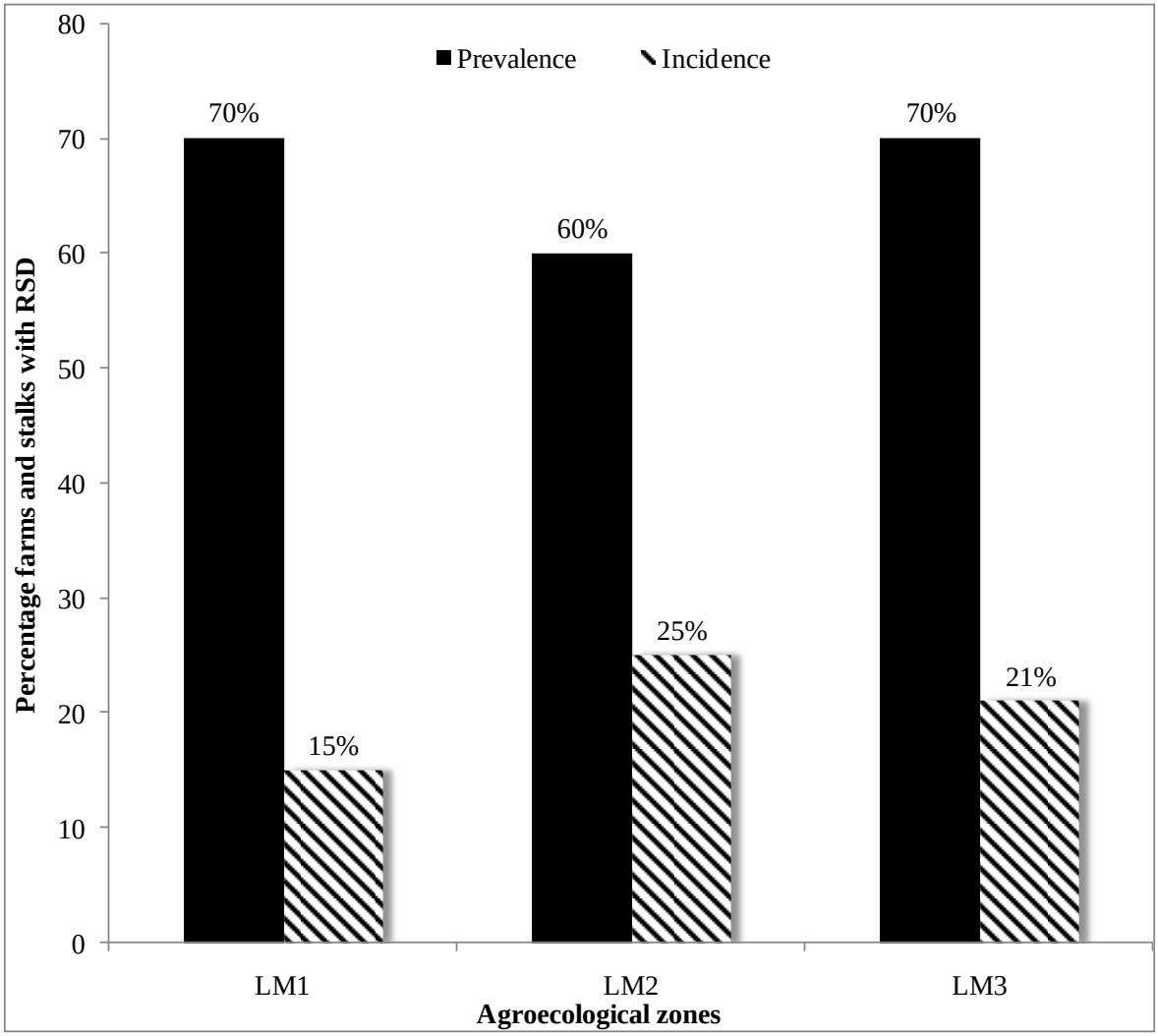

Figure 3.2. Percentage prevalence and incidence of ratoon stunting disease in different agro-ecological zones in Nyando (LM1=Lower Midland 1; LM2=Lower Midland 2; LM3= Lower Midland 3).

Table 3.1. Percentage positive number of farms for ratoon stunting disease based on Phase-contrast microscope (PCM) examination and immunoassay analysis (TB-EIA Tissue blot enzyme).

AEZs	No. of farms sampled	Percentage farms positive (PCM)	Percentage farms positive (TB-EIA)
LMI	10	40	70
LM2	10	50	60
LM3	10	30	70
Average	10	40	66.7

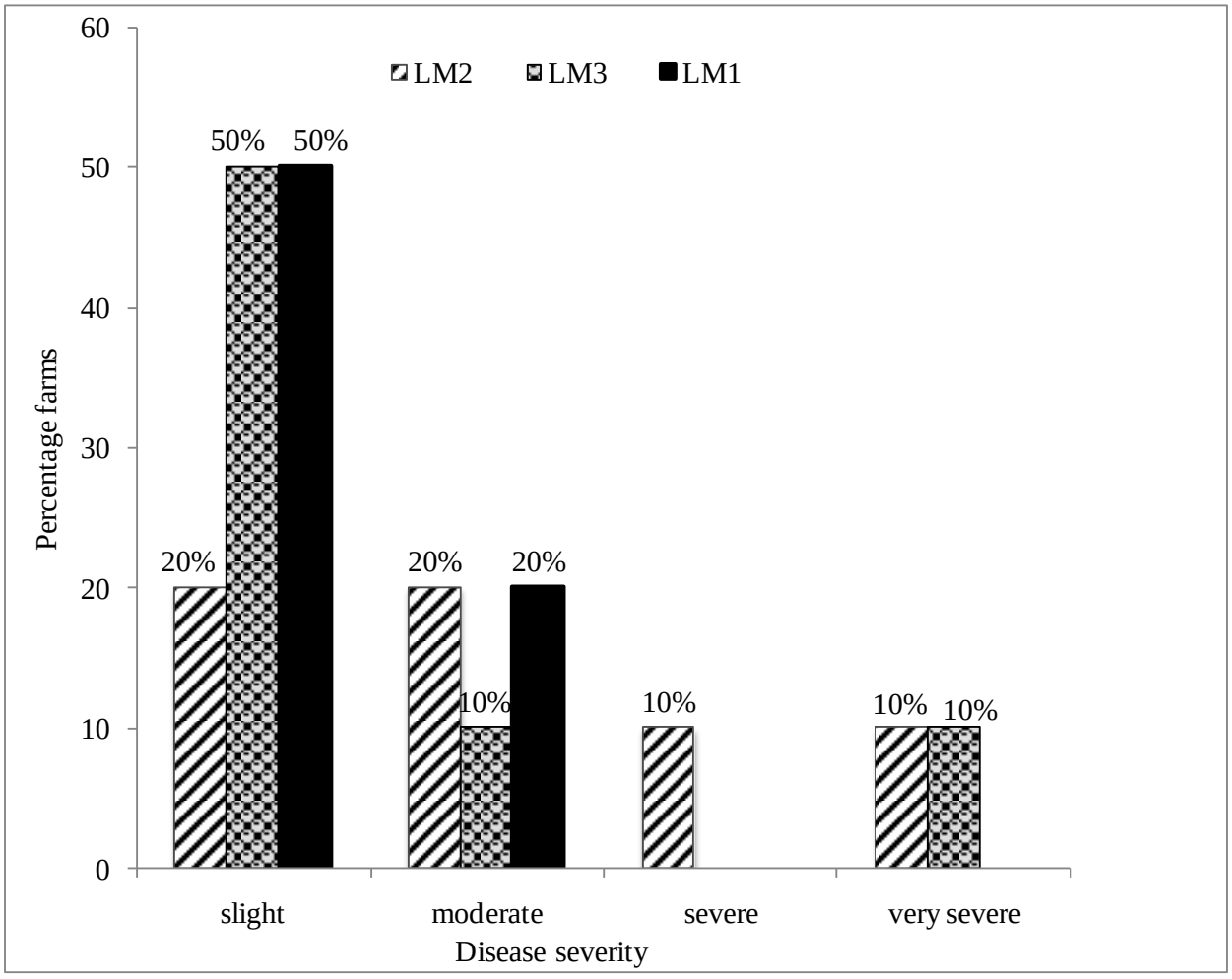

Figure 3.3.Severity of ratoon stunting disease in different agro-ecological zones in Nyando

Table.3.2. Percentage of Ratoon stunting disease infected farms and cane stalks of major commercial sugarcane varieties in different agro-ecological zones in Nyando Sugar belt.

Variety	Agro-ecological zone	% farms sampled	% farms infected	% infected stalks
CO421	LM1	80	75	16
	LM2	60	67	35
	LM3	40	75	33
CO617	LM1	0	0	0
	LM2	40	50	10
	LM3	20	50	10
CO945	LM1	20	50	10
	LM2	0	0	0
	LM3	10	100	20
N14	LM1	0	0	0
	LM2	0	0	0
	LM3	20	100	20
D8484	LM1	0	0	0
	LM2	0	0	0
	LM3	10	0	0

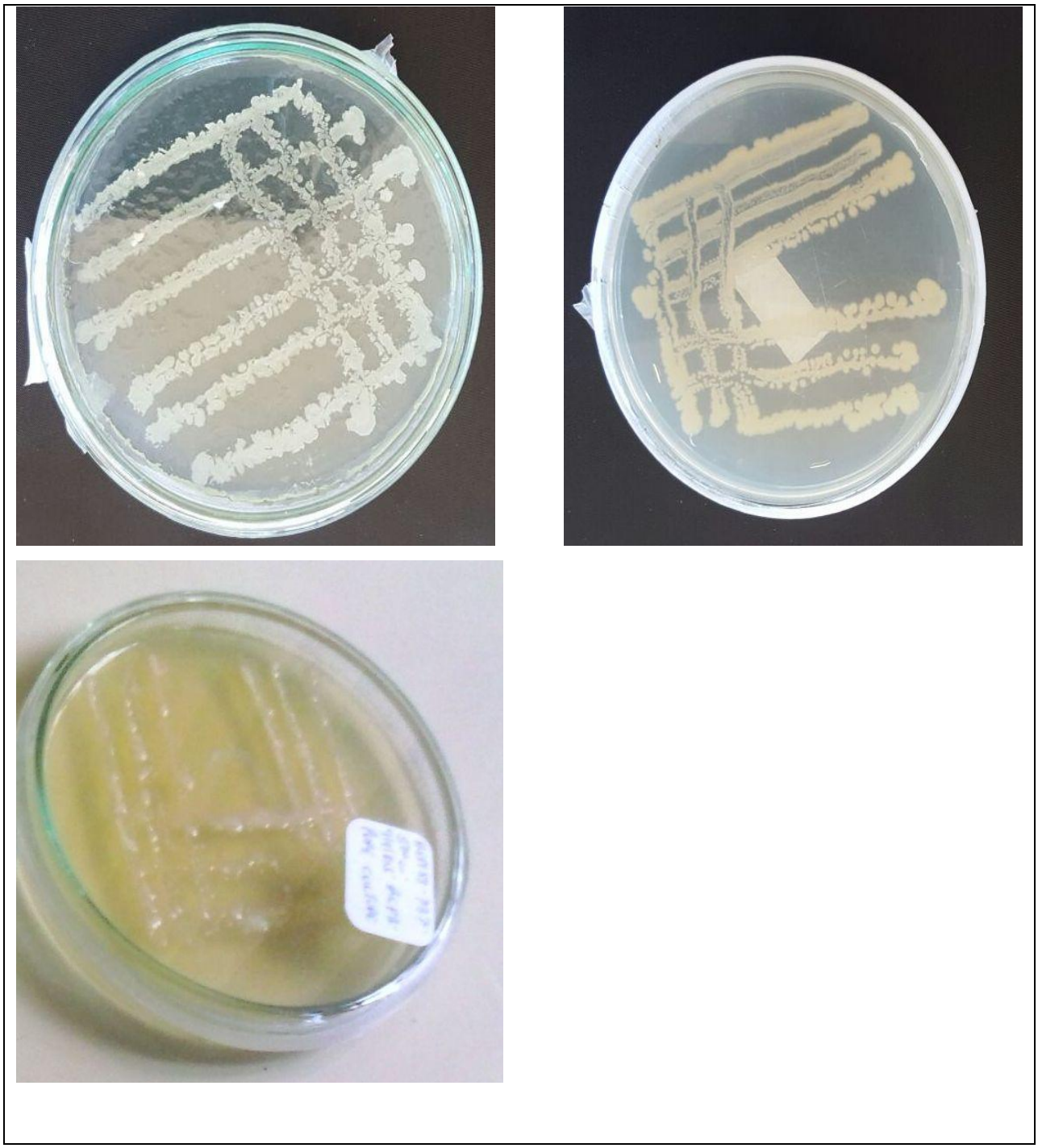

Figure 3.4.White cream colonies of ratoon stunting bacterium isolated from sugarcane stalk samples in yeast extract, dextrose, and calcium carbonate (YDC) media.

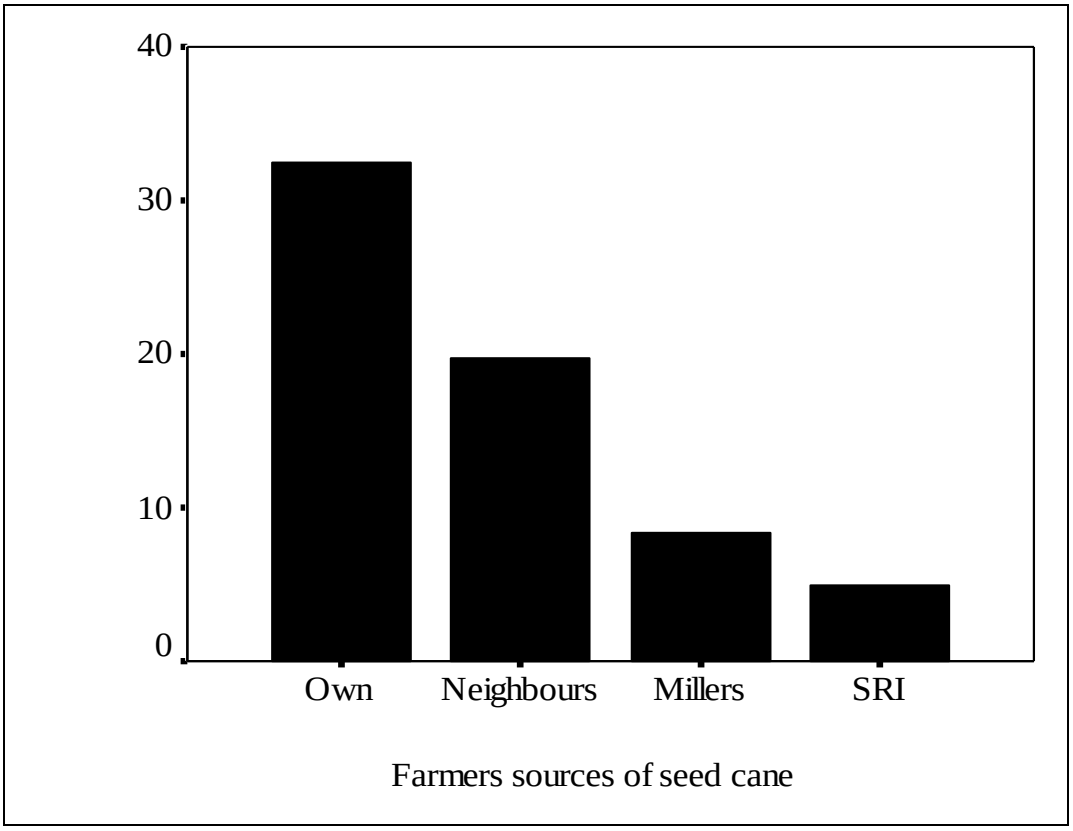

Figure.3.5. Percent ratoon stunting disease incidence in farms with different seed cane sources in Nyando sugar belt (SRI=Sugar Research Institute)

3.6. Discussion

The study found that most of the sugarcane farmers in Nyando sugar belt grew the crop on small land holdings of less than two acres and mainly use seed cane from neighbours and own farms. Most grew old sugarcane varieties and awareness on ratoon stunting disease was very low. These findings are in agreement with Wawire *et al*., 2007 and Odenya *et al*., 2008, who found that, land sizes under sugarcane in Nyando were relatively small due to redistribution and fragmentation as farm households increases. The results also concurs with those by Odenya *et al*., 2009 who found that farmers in Nyando mainly sourced seed cane from neighbours and own crop and

they preferred old commercial sugarcane varieties as opposed to new improved varieties due to their ratoonability and they are resistant to drought . Use of seed cane from neighbours and own crop that were not certified could have enhanced the spread of ratoon stunting disease. The study found that RSD incidence was higher in farms where farmers sourced seed cane from neighbours and own crop. This observation is also reported by Viswanathan (2012) who found that RSD cannot be detected by external appearances hence the bacterium is spread unknowingly through setts from diseased plants.

The preference of old commercial sugarcane varieties such as CO421, CO 617, CO 945, and N14 could be contributing to low sugarcane productivity in Nyando sugar belt. These old varieties are characterised by late maturity, low sucrose content and susceptibility to major sugarcane diseases such as smut, mosaic and ratoon stunting (KESREF, 2013, KEPHIS, 2015). Varietal screening has shown some resistance to RSD based on yield loss and level of vascular colonisation by the bacteria of some varieties. In South Africa, variety N27 was found to have some resistance to RSD based on the fact that it suffered little yield loss and showed low levels of colonization by *Leifsonia xyli* subsp *xyli* while variety N14 was found to be highly susceptible (McFarlane, 2002; Lemma *et al*.,2013).

The tradition of sourcing seed cane from neighbours and own crop could lead to spread of sugarcane diseases such as, red rot, smut, wilt, grassy shoot, leaf scald, yellow leaf mosaic virus and ratoon stunting (Viswanathan, 2012). This may be impacting on the productivity in the Nyando sugar belt. Viswanathan, (2012) reported that most of sugarcane diseases are transmitted through seed cane hence adequate care should be taken when selecting seed cane.

The study confirmed the presence of ratoon stunting disease in Nyando sugar belt with average prevalence of 67% and average disease incidence of 20%. This confirms earlier studies by Early, (1973) who showed the presence of ratoon stunting disease in the Kenya and estimated yield losses in major variety CO421 at between 17 and 21% at Mumias and about 4% at Ramisi. Surveys conducted worldwide have shown that ratoon stunting disease is extremely common in many sugar growing countries of the world ,causing great losses in cane yield (Damann,1990; Bailey and Mcfarlane,1998; Paulraj,2000; Daugrois,2006; Derrick *et al*., 2007; Lemma *et al*., 2013).

The use of seed cane from neighbours and own crop rather than from milling companies or Sugar Research Institute could be contributing to the spread of ratoon stunting disease in Nyando. Rott *et al*., (2000)., Comstock and Gilbert, (2012)., Viswanathan, (2012), reported that ratoon stunting disease is mainly spread by propagation with infected cuttings hence there is need to use of disease free seed cane to control the disease.

Low awareness on ratoon stunting disease means no direct sanitation measures are undertaken which explain high disease incidence in farmers' field. Comstock et al., (1996), Viswanathan, (2012) reported that sanitation is important in keeping healthy cane from becoming infected since the pathogen is easily transmitted mechanically through farm implements. In a study by Bailey and Tough (1992) found that use of infected cutting tools leads to rapid spread of ratoon stunting diseases from one stool to another.

The use of the old sugarcane varieties could be contributing to the spread and build up of ratoon stunting disease in Nyando. In a study by Bailey *et al*., (2000), Yohannes *et al*., 2012, Lemma *et al*., (2013) found that, some of these old varieties like N14and CO421 had high susceptibility to

ratoon stunting disease. Cultivation of older varieties for many years in a region results in build up of bacterial titre in the cane stalks and thereby loss of vigour due to ratoon stunting disease (Viswanathan, 2012). From the findings of this study it was recommended that farmers should be sensitized on ratoon stunting disease in order to create awareness of the disease and its management strategies like planting healthy seed cane and disinfection of farm implements.

CHAPTER FOUR

EFFECT OF HOTWATER TREATMENT ON SUGARCANE RATOON STUNTING DISEASE, CANE YIELD AND QUALITY

4.1. Abstract

Sugarcane ratoon stunting disease (RSD) caused by bacterium *Leifsonia xyli* subsp *xyli* causes huge losses to sugarcane ratoon crops. Hot water treatment (HWT) of sugarcane setts before planting is commonly used in many countries to control RSD but conflicting results show that it may have either positive or negative effects on yield based on variety, temperature and duration of therapy. The objective of this study was to determine the effectiveness of hot water treatment in the management of ratoon stunting disease. Three sugarcane varieties (CO421, D8484, KEN 83737) were treated with hot water at 45, 50, 52 and 55°C for two hours. Control cane was treated with cold water at room temperature (25°C). The treated cane was planted in the field and in the greenhouse and data was collected on germination, number of tillers, cane girth, cane height, number of millable stalks, and number of internodes, cane weight and sucrose content.

Hot water treatment significantly reduced ratoon stunting disease and had notable effect on germination and cane yield. Hot water treatment at 45, 50 and 52°C significantly reduced RSD and increased cane germination, cane and sucrose yield. Hot water treatment at 55°C completely eliminated RSD but significantly reduced germination and cane yield. The cane setts which were treated with cold water at 25°C were stunted and had low cane yield and sucrose content. Hot water treatments at 50°C was the most effective and produced the highest cane yield followed closely by treatment at 52°C. Therefore the two temperatures are hereby recommended for management of ratoon stunting disease of sugarcane. Treatment of sugarcane setts at high

temperatures of 55°C for two hours though effective in reducing RSD is harmful through reduction in germination of setts and thus total plant population and eventually low cane yield.

Key words: Ratoon stunting disease, hot water treatment, sugarcane, cane yield, production, *Leifsonia xyli* subsp *xyli.*

4.2 Introduction

Ratoon stunting disease caused by a bacterium *Leifsonia xyli* subsp *xyli* is considered as the most serious disease of sugarcane worldwide (Davis and Bailey, 2000, McFarlane, 2001,). It is well known to be an economically important disease in almost all countries where sugarcane is grown (Bailey and Bechet, 1995). Depending on the susceptibility of the variety and weather condition, loss in yield due to RSD can reach over 60%, especially in ratoon crop and this has warranted serious concern in many counties (Scarlett, 1980). The disease causes both quantitative and qualitative losses in sugarcane (Johnson and Tyagi, 2010). Direct yield loss results from production of thinner and shorter stalks. The disease also reduces the sucrose content of the cane (Zvoutete, 2004; Yohanes *et al*., 2012). The disease can cause about 5-15% loss in crop yield without the grower even knowing his fields have been infected (Comstock, 2002).

Minimizing the effects of ratoon stunting disease in sugarcane production should be a high priority in the sugar industries throughout the world. Ratoon stunting has always been managed through heat treatment of setts followed by careful management of the crop through proper disinfection of harvesting tools to keep healthy cane from being infected (Bailey and Tough, 1992; Gillaspie and Davis, 1992; Comstock *et al*., 1996; Viswanathan, 2012; Comstock and Gilbert, 2012. Various thermal treatments (hot air, steam and hot water) have been used to successfully eliminate the *Leifsonia xyli* subsp *xyli* from sugarcane setts (Davis and Bailey, 2000;

Tiwari *et al*., 2012). Hot water treatment at 50°C for two to three hours is the most commonly used method of heat treatment used to establish pathogen free seed cane (Steindl, 1961; Gillaspie and Davis, 1992; Johnson and Tyagi; 2010). Hot water treatment kills bacteria in xylem vessels and diminishes RSD infection hence is the primary control strategy for RSD. However, conflicting results show that hot water treatment may have either positive or negative effects on yield based on variety, temperature and duration of therapy (Johnson and Tyagi, 2010). Ongoma, (1992) showed that Variety EAK 70-153 had a marked positive response to hot water treatment with a 35% increase in germination of buds. There was an approximate 10% increase in germination in varieties EAK 69-40 and EAK 69-41. Hot water treatment had little effect on the germination of varieties CO421, EAK 69-47 and EAK 70/97. In case of variety CO331 germination was reduced by 13%. In a study by Johnson and Tyagi, (2010) found hot water treatment at 50°C for two hours gave higher average yield compared to hot water treatment at 50°C for three hours.

Ratoon stunting disease is mainly spread though infected planting material hence production and supply of healthy initial seed cane though hot water treatment will help in the control of the disease , increase cane productivity and farmers income. Therefore the objective of this study was to determine the effectiveness of hot water treatment in management of ratoon stunting disease for improved cane productivity hence increased income for sugarcane farmers in Kenya.

4.3 Material and Methods

4.3.1 Experimental materials and application of hot water treatment

Sugarcane varieties selected for this experiment were D8484 and KEN 83-737 which are high yielding new improved varieties and CO421 which is one of the oldest but most widely grown variety in Nyando sugar-belt. Twelve months old RSD diseased canes of these varieties were

obtained from the Sugar Research Institute breeding station. Presence of ratoon stunting disease in these diseased canes was confirmed through Tissue Blot Enzyme Immunoassay procedure as provided by J-H Daugrois (CIRAD, Montpellier).

The cane stalks were cut into three budded setts. For each variety the setts were split into five batches. The batches were put in gunny bags, placed on metallic baskets and immersed in a tank containing hot water. The batches were treated at different water temperatures of 45, 50, 52 and 55°C for two hours. Temperature was maintained constant using a digital control panel fitted with a thermostat. After two hours the cane setts were removed from the tank and cooled in distilled water containing fungicides (bayleton) for 30 minutes. For control, the setts were soaked in distilled water for two hours at a room temperature at 25°C.

4.3.2. Field and greenhouse evaluation of hot water treated sugar cane setts

In the green house, three setts were planted horizontally in a 20cm bucket and covered slightly with planting medium which consisted of forest soil plus manure at a 3: 1 ratio. Fertilizer DAP was applied at the rate of 5g per pot. Each of the five hot water treatment levels (25, 45, 50, 52 and 55°C) and three varieties (D8484, KEN 83-737, and CO421) were replicated thrice and experiment laid out in a Completely Randomized Design (CRD). The setts were irrigated once a day.

For the field trial, the experiment was set at Sugar Research Institute farm at Kibos two sites (upland with poor soils, low moisture content and in low land with deep fertile soils with good moisture holding capacity). The land was ploughed and harrowed evenly to a depth of 20cm. Plot size was four furrows of 2.5m length spaced at 1.2m. Treated setts were planted end to end at a rate of 25 setts per furrow making a total of 100 setts per treatment plot. DAP fertilizer was

applied at the rate of 200kg per hectare. Each of the five hot water treatment (25, 45, 50, 52 and 55°C) and three varieties (D8484, KEN 83-737 and CO421) were replicated thrice. The field experiment was under rain fed condition. Weeding was done thrice at 30 days, 60days and 90 days after planting. At four months the crop was top dressed with CAN at the rate of 350kg per hectare. The design of the experiment was 3×5 factorial laid out in a Randomized Complete Block Design (RCBD). Both experiments were carried out for only one crop cycle.

4.3.3. Assessment of sett germination and tillering

Assessment on sett germination was done at 30 and 45 days after planting while tiller count data was taken at three months. All buds which had germinated in the whole plot were counted while at three months the number of tillers was counted in each plot.

4.3.4. Determination of the effect of hot water treatment on RSD bacterium

At 11 months, ten stalks were harvested from each treatment plot starting from the controls. The cutting knives were disinfected at the end of each plot using 3% sodium hypochlorite. The bacterium was detected in the harvested stalks using Tissue blot enzyme immunoassay procedure as described in section 3.3.3.

4.3.5. Determination of effect of hot water treatment on sugar cane yield and quality.

At 11 months, millable stalks, cane weight, cane thickness (girth), cane height, the number of internodes and sucrose content were determined. In the field trial, the millable stalk count was taken from two middle rows in each plot. Then 10 stalks were cut randomly from each net plot to determine cane weight, cane girth, cane height and sucrose content. The cane height in centimetre was determined using a tape measure while cane weight in kilogram was determined

using a weighing balance. The weight obtained from the 10 stalks was converted to kilogram per hectare using the following formula

$$\text{Cane weight (kg/ha)} = \frac{\text{weight of 10 stalks} \times \text{No. of stalks per net plot x 10000}}{10 \times \text{area of net plot}}$$

The weight was then converted to cane yield per hectare (Mt/ha). In the greenhouse, all the stalks in the pot were harvested and weighed to determine weight per pot. The cane thickness (girth) was measured with vernier calliper. The sugar content was determined using a digital hand held refractometer (Bellingham +Stanley, E-line Refractometer). Using a plastic rod, juice from sampled cane was placed on the prism area of refractometer. Through the eyepiece, readings were taken from the border line light/dark demarcation line. The degree brix readings obtained gave the percentage sucrose content of the cane juice.

4.4. Data analysis

Data on sett germination, tiller count, cane yield and disease incidence from hot water treated cane was subjected to analysis of variance using ANOVA procedure of Genstat 13th Edition to determine the effect of hot water treatment on ratoon stunting disease, cane yield and quality and differences among treatment means were compared using the Fishers protected LSD test at 5% probability level to determine if there were significant differences between treatments.

4.5. Results

4.5.1 Effect of hot water treatment on sett germination and tillering

There was a significant difference in germination at different treatment temperatures ($p<0.001$) in both sites (Table 4.1). The setts planted in lowland had higher germination than those planted in upland. The germination of setts treated at 45, 50 and 52°C was higher than that of control

(25°C). There was a significant increase in germination to setts treated at these temperatures compared to the control (Figure 4.1). However, setts treated at 50°C had the highest germination compared to other temperatures in both sites. Germination of setts subjected to HWT at 55°C was the lowest. In lowland the varieties were not significantly different in germination. However, interaction of temperature and variety was highly significant ($p<0.001$). Variety CO421 germinated well at 52 and 55°C compared to D8484 and KEN 8373. In the upland site, there was a significant varietal effect ($p=0.006$) but the varietal –temperature effect was not significant (Table 4.1).

In the greenhouse, hot water treatment affected germination of setts and the treatment temperatures were significantly different ($P < 0.001$) (Table 4.2). Germination at both 30 and 45 days was found to be higher to those setts subjected to HWT at 50°C and lowest for setts subjected to HWT at 55°C (Table 4.2).Varieties were significantly different. Variety CO 421 responded well to all temperatures and recorded the highest average germination compared to others (D8484 and KEN83737). Interaction between temperature and variety was not significant.

Table.4.1.Number of buds germinated per plot for three sugarcane varieties treated with hot water at different temperatures and planted at the two sites

		Temperature° C					
Site	variety	25	45	50	52	55	Mean
Lowland site							
	D8484	103_{de}	193_{ab}	189_{ab}	121_{cde}	11_{g}	123.5_{ns}
	CO421	87_{ef}	146_{bcd}	185_{ab}	195_{a}	45_{fg}	131.7_{ns}
	KEN83737	160_{abc}	193_{ab}	187_{ab}	99_{de}	2_{g}	128.2_{ns}
	Mean	116_{b}	177_{a}	186_{a}	138_{b}	19_{c}	
	LSD $_{(p\leq 0.05):}$ Temp 27, Variety 21, temp*variety 47, C.V (%) = 22.3						
Upland site							Mean
	D8484	68_{ns}	81_{ns}	128_{ns}	109_{ns}	3_{ns}	78.2_{b}
	CO421	70_{ns}	95_{ns}	138_{ns}	120_{ns}	10_{ns}	86.9_{b}
	KEN83737	118_{ns}	133_{ns}	153_{ns}	108_{ns}	0.3^{ns}	102.8_{a}
	Mean	86_{c}	103_{bc}	140_{a}	113_{b}	5_{d}	
	LSD $_{(p\leq 0.05):}$ Temp 18, variety 14, temp*variety 32, C.V(%) = 21.7						

Values followed by the same letters within the rows and columns are not significantly different; LSD= Least significant difference, CV= Coefficient of variation, Temp=Temperature, ns=Not significant

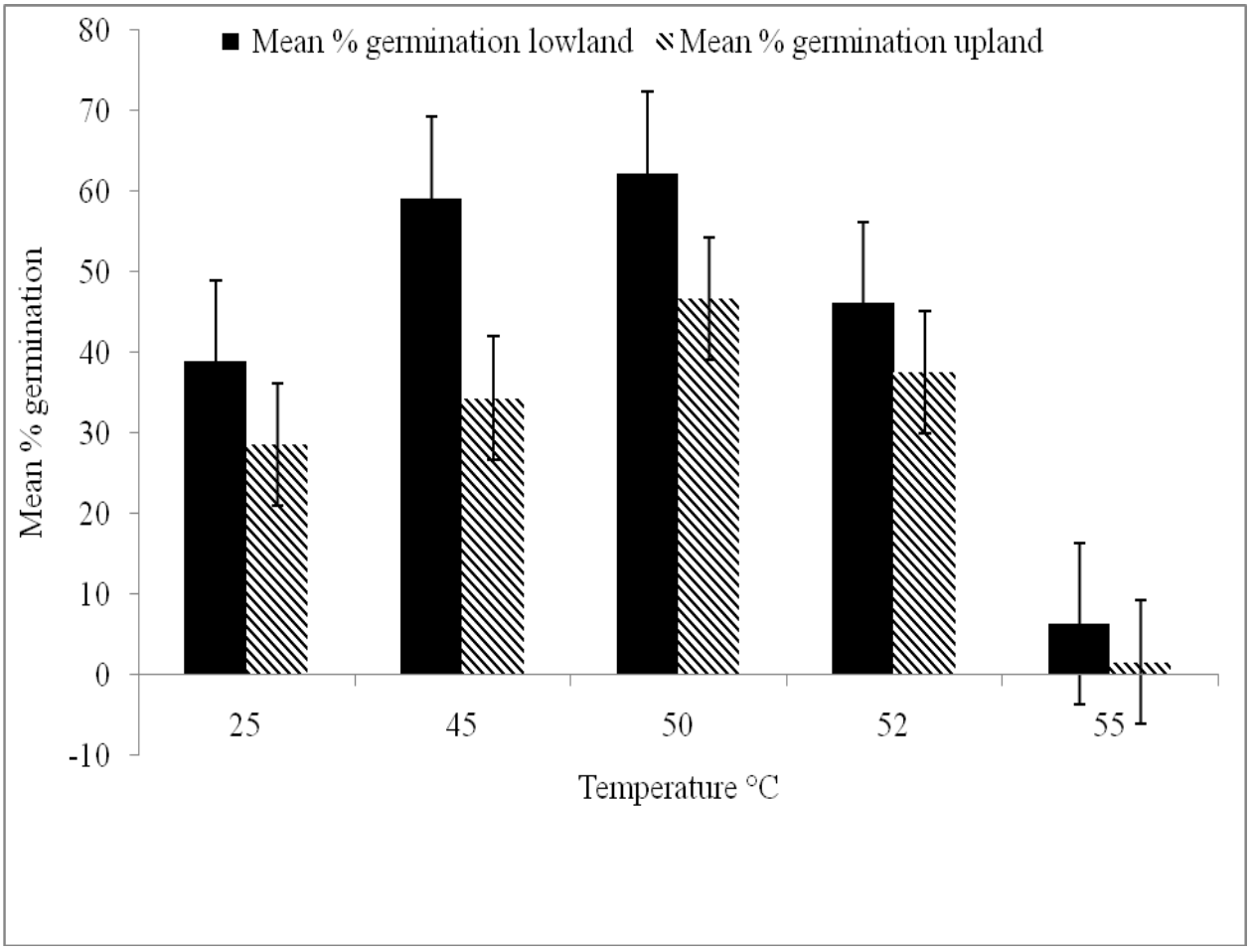

Figure 4.1. Percentage sett germination under field condition in two different sites.

Table 4.2. Number of buds germinated per pot for three sugarcane varieties treated with hot water at varying temperatures under greenhouse conditions

	Temperature° C					
variety	25	45	50	52	55	Mean
30 days after planting						
D8484	3.3_{ns}	3.3_{ns}	4.0_{ns}	2.7_{ns}	1.0_{ns}	2.9_{b}
CO421	4.0_{ns}	3.7_{ns}	4.7_{ns}	4.0_{ns}	2.7_{ns}	3.8_{a}
KEN83737	2.7_{ns}	3.7_{ns}	4.3_{ns}	3.7_{ns}	0.7_{ns}	3.0_{b}
Mean	3.3_{b}	3.6_{ab}	4.3_{a}	3.4_{ab}	1.4_{c}	
LSD $_{(p\leq 0.05)}$: Temp 1.0, variety 1.0, temp* variety 1.7. %CV=30.7						
45 days after planting						
D8484	6.0_{ns}	7.7_{ns}	9.0_{ns}	6.0_{ns}	4.3_{ns}	6.6_{b}
CO421	8.3_{ns}	8.7_{ns}	10.0_{ns}	7.7_{ns}	6.0_{ns}	8.1_{a}
KEN83737	6.0_{ns}	7.7_{ns}	8.7_{ns}	6.0_{ns}	4.0_{ns}	6.5_{b}
Mean	6.8_{b}	8.0_{ab}	9.2_{a}	6.6_{b}	4.8_{c}	
LSD $_{(p\leq 0.05)}$: Temp 1.7, variety 1.3, temp*variety 2.9. %CV=24.3						

Values followed by the same letters within the rows and columns are not significantly different
LSD= Least significant difference, CV= Coefficient of variation, Temp=Temperature, ns=Not significant

Hot water treatment had significant effect on tillering (Table 4.3) ($p<0.001$) in both sites. There was more tiller establishment in the lowland than in the upland site. In both sites, setts subjected to HWT at 45, 50 and 52 °C had more tillers than the control (25°C), however setts treated at 50°C produced more tillers compared to other temperatures. In the lowland, tillering at 50°C was not significantly different from 45°C which was not significantly different from temperature 52°C. In both sites setts treated at 55°C produced the lowest number of tillers. Varieties were significantly different in tillering ($p=0.003$ in the lowland and $p=0.019$ in the upland) with variety CO421 producing the highest number of tillers. Interaction between temperature and variety was significant only in the lowland. Varieties D8484 and KEN 83737 produced more tillers at 50°C while CO421 produced more tillers at 52 °C. In the greenhouse there was also a significant difference in tillering among treatment temperatures ($p< 0.001$). Tillering was highest on setts treated at 50°C while lowest number of tillers was found at 55°C. Varieties and interaction effects were not significant.

Table.4.3. Number of tillers for sugarcane varieties treated at different temperature levels under field and greenhouse conditions

		Temperature° C					
site	variety	25	45	50	52	55	Mean
Lowland site							
	D8484	176.3_{d}	255.3_{b}	261.3_{b}	189.7_{cd}	23.3_{f}	181.2_{b}
	CO421	183.0_{d}	252.0_{b}	282.3_{b}	342.7_{a}	81.7_{e}	228.3_{a}
	KEN83737	243.3_{bc}	274.3_{b}	295.3_{ab}	170.0_{d}	1.0_{f}	196.8_{b}
	Mean	200.9_{c}	260.6_{ab}	279.7_{a}	234.1_{b}	35.3_{d}	
	LSD $_{(p\leq 0.05):}$ Temp 33.0, variety 25.6, temp*variety 57.2, C.V (%) =16.9						
Upland site							
	D8484	117.0_{ns}	154.3_{ns}	211.0_{ns}	169.0_{ns}	7.0_{ns}	131.7_{b}
	CO421	150.0_{ns}	190.3_{ns}	266.7_{ns}	216.0_{ns}	23.3_{ns}	169.3_{a}
	KEN83737	201.3_{ns}	181.3_{ns}	212.0_{ns}	180.3_{ns}	0.3_{ns}	155.1_{ab}
	Mean	156.1_{b}	175.3_{b}	229.9_{a}	188.4_{b}	10.2_{c}	
	LSD $_{(p\leq 0.05):}$ Temp33.2, variety 25.7, temp*variety 57.4. C.V(%) = 22.6						
Greenhouse							
	D8484	8.7_{ns}	9.7_{ns}	10.7_{ns}	6.7_{ns}	4.7_{ns}	8.1_{ns}
	CO421	9.0_{ns}	9.0_{ns}	11.3_{ns}	7.7_{ns}	5.3_{ns}	8.5_{ns}
	KEN83737	5.3_{ns}	9.7_{ns}	10.0_{ns}	7.7_{ns}	5.0_{ns}	7.5_{ns}
	Mean	7.7_{b}	9.4_{bc}	10.7_{c}	7.3_{b}	5.0_{a}	
	LSD $_{(p\leq 0.05):}$ Temp 2.08, variety 1.61, temp*variety 3.59 C.V(%) =26.9						

Values followed by the same letters within the rows and columns are not significantly different.
LSD= Least significant difference, CV= Coefficient of variation, Temp=Temperature, ns=Not significant

4.5.2. Effect of hot water treatment on ratoon stunting bacterium

Hot water treatment reduced RSD bacteria and there were significant differences between temperatures (P<0.001) (Table 4.4). The percentage infected stalks were lower in the treated cane compared to the control (25°C) and reduced as temperature increased. Cane treated at 55°C had the lowest percent of infected stalks. There was no significant difference among the varieties at both sites. Interaction between variety and temperature at both sites was not significant.

Table 4.4. Percent stalks positive for RSD pathogen after hot water treatment

		Temperature° C					
	variety	25	45	50	52	55	Mean
Low land site							
	D8484	93.3_{ns}	63.3_{ns}	16.7_{ns}	10.0_{ns}	3.3_{ns}	37.3_{ns}
	CO421	93.3_{ns}	63.3_{ns}	30.0_{ns}	13.3_{ns}	0.0_{ns}	40.0_{ns}
	KEN83737	93.3_{ns}	60.0_{ns}	20.0_{ns}	10.0_{ns}	3.3_{ns}	37.3_{ns}
	Mean	93.3_{d}	62.2_{c}	22.2_{b}	11.1_{a}	2.2_{a}	
	LSD $_{(p\leq 0.05)}$: Temp 9.3, variety 7.2, temp*variety 16.1. CV(%)= 25.2						
Up land site							Mean
	D8484	96.7_{ns}	70.0_{ns}	16.7_{ns}	16.7_{ns}	6.7_{ns}	41.3_{ns}
	CO421	100.0_{ns}	60.0_{ns}	30.0_{ns}	23.3_{ns}	3.3_{ns}	43.3_{ns}
	KEN83737	96.7_{ns}	60.0_{ns}	16.7_{ns}	13.3_{ns}	0.0_{ns}	37.3_{ns}
	Mean	97.8_{d}	63.3_{c}	21.1_{b}	17.8_{b}	3.3_{a}	
	LSD $_{(p\leq 0.05)}$: Temp 10.8, variety 8.3, temp*variety 18.7. C.V (%)= 27.4						

Values followed by the same letters within the rows and columns are not significantly different.
LSD= Least significant difference, CV= Coefficient of variation, Temp=Temperature, ns=Not significant

4.5.3. Effect of hot water treatment on cane growth

Hot water treatment had effect on number of millable stalks, height, girth, cane internodes, and there were significant differences ($p<0.001$) between the treatment temperatures in both field and in the greenhouse (Table 4.5, 4.6, 4.7, 4.8,). The cane treated at 45, 50 and 52°C performed better in all yield parameters compared to control (25°C). For the number of millable stalks, there was no significant difference at 45, 50 and 52°C in the lowland while in the upland there was no significant difference in number of millable stalks between treatment at 45°C and 50°C and also between 50°C and 52°C. In the greenhouse, there was a significant difference in number of millable stalks between temperature 52°C and 45°C (Table 4.5). In both sites the highest number of millable stalks was produced at 50°C while the lowest was at temperature 55°C. There was a significant difference in the number of millable stalks among the varieties in both sites in the field ($p<0.001$) and also in the greenhouse ($p=0.007$) with variety KEN 83-737 and CO 421 producing the highest number in the upland and lowland sites while variety D8484 and CO421 were better in the greenhouse. The effect of interaction between temperature and variety on number of millable stalks was significant in both lowland and upland sites.

In field and greenhouse trial, canes treated at 45, 50 and 52°C were taller compared to the control (25°C) (Table 4.6). There was no significant difference in height at temperature of 50°C and 52° however, treatment at 50°C produced the tallest cane. Canes treated at 55°C were the shortest. There was a significant difference among the varieties in height ($p=0.002$ in lowland, $p<0.001$ upland and $p=0.008$ in greenhouse) with variety CO421 producing the tallest cane. There was significant interaction between temperature and varieties in height ($p<0.001$) in both lowland and upland.

There was a significant difference in cane girth among the temperatures ($p<0.001$) in both sites (Table 4.7). Canes treated at 45, 50 and 52° had thicker stalks compared to the control (25°C) and temperature 55°C. Cane girth was not significantly different at 45, 50 and 52°C. However, in both varieties canes treatments at 50°C were thickest. Varieties were significantly different ($p<0.001$ in lowland, $p=0.002$ in the upland and $p=0.002$ in the greenhouse) over cane girth. Variety D8484 produced the thickest cane. There was a significant interaction between temperature and varieties in the upland and lowland sites.

Results (Table 4.8) show that, both in lowland and upland there was no significant difference in the number of internodes for the canes treated at 45, 50, 52°C and control (25°C) all of which were different from temperature 55°C which produced the lowest number of internodes. In the greenhouse there was no significant difference in the number of internodes at 25, 45 and 52°C. Varieties were significantly different over the number of internodes ($p=0.003$ lowland, $P=0.054$ in the upland, $p=0.01$in the greenhouse). Variety D8484 and CO 421 produced the highest number of internodes. There was also effect of interaction between temperature and varieties in the field trial.

Table.4.5. Number of millable stalks for three sugarcane varieties treated at different temperatures under field and greenhouse conditions

Site	variety	Temperature °C 25	45	50	52	55	Mean
Lowland site							
	D8484	65_{de}	67_{de}	69_{de}	66_{de}	37_{ef}	61_{b}
	CO421	90_{cd}	101_{bcd}	114_{abc}	113_{abc}	76_{cd}	99_{a}
	KEN83737	$98b_{cd}$	137_{ab}	143_{a}	131_{ab}	0_{f}	102_{a}
	Mean	85_{b}	102_{ab}	108_{a}	104_{ab}	39_{c}	
	LSD $_{(p\leq 0.05):}$ Temp 22.37, variety17.33, temp*variety 38.74. CV (%) = 26.5						
Upland site							
	D8484	33_{bc}	55_{cd}	62_{de}	54_{cd}	7_{a}	42_{a}
	CO421	80_{efg}	93_{fgh}	90_{fgh}	73_{def}	31_{b}	73_{b}
	KEN83737	92_{fgh}	108_{h}	99_{gh}	88_{fgh}	0_{a}	77_{b}
	Mean	68_{b}	85_{d}	84_{cd}	72_{bc}	12_{a}	
	LSD $_{(p\leq 0.05):}$ Temp 12.86, variety9.96 , temp*variety 22.2, CV(%) =20.7						
Greenhouse							
	D8484	10_{ns}	10_{ns}	14_{ns}	12_{ns}	4_{ns}	10_{a}
	CO421	10_{ns}	13_{ns}	16_{ns}	15_{ns}	8_{ns}	12_{b}
	KEN83737	7_{ns}	10_{ns}	13_{ns}	10_{ns}	3_{ns}	9_{a}
	Mean	9_{b}	11_{bc}	14_{d}	12_{dc}	5_{a}	
	LSD $_{(p\leq 0.05):}$ Temp 3, variety 2.3, temp*variety5. CV(%) = 29.8						

Values followed by the same letters within and columns the rows are not significantly different.
LSD= Least significant difference, CV= Coefficient of variation, Temp=Temperature, ns=Not significant

Table 4.6. Height (cm) for three sugarcane varieties treated at different temperatures under field and greenhouse conditions

		Temperature °C					
Site	variety	25	45	50	52	55	Mean
Lowland site							
	D8484	144.9_{b}	186.0_{bc}	258.5_{def}	213.0_{cd}	127.4_{b}	186_{a}
	CO421	183.7_{bc}	221.3_{cde}	283.7_{ef}	269.5_{def}	242.5_{cdef}	240.1_{b}
	KEN83737	234.0_{cde}	252.7_{def}	300.0_{f}	258.8_{def}	0.0_{a}	209.1_{a}
	Mean	187.6_{c}	220_{bc}	280.7_{a}	247.1_{ab}	123.3_{d}	
	$LSD_{(p\leq 0.05):}$ Temp 37.11, variety 28.74, temp*variety 64.67. C.V (%) =18.1						
Up land site							
	D8484	122.5_{b}	144.3_{bc}	194.5_{cdefg}	184.1_{cdef}	48.5_{a}	138.8_{a}
	CO421	169.3_{bcd}	174.7_{bcde}	245.2_{g}	221.7_{defg}	187.0_{cdef}	199.6_{c}
	KEN83737	207.6_{defg}	210.3_{defg}	230.3_{fg}	226.7_{efg}	0.0_{a}	175.0_{b}
	Mean	166.5_{b}	176.4_{b}	223.3_{c}	210.8_{c}	78.5_{a}	
	$LSD_{(p\leq 0.05):}$ Temp 31.50, variety24.40, temp*variety 54.56. C.V (%) =19.1						
Greenhouse							
	D8484	142.9_{ns}	127.8_{ns}	181.3_{ns}	137.3_{ns}	54.3_{ns}	128.7_{ab}
	CO421	139.1_{ns}	152.0_{ns}	196.6_{ns}	181.0_{ns}	93.4_{ns}	152.4_{b}
	KEN83737	92.3_{ns}	117.4_{ns}	142.3_{ns}	117.7_{ns}	39.3_{ns}	101.8_{a}
	Mean	124.8_{b}	132.4_{b}	173.4_{c}	145.3_{bc}	62.4_{a}	
	$LSD_{(p\leq 0.05):}$ Temp 39.53, variety 30.62, temp*variety 68.47. C.V (%)= 32.2						

Values followed by the same letters within the rows and columns are not significantly different.
LSD= Least significant difference, CV= Coefficient of variation, Temp=Temperature, ns=Not significant

Table 4.7. Cane girth (cm) for three sugarcane varieties treated at different temperatures under field and greenhouse conditions.

	Temperature °C					
variety	25	45	50	52	55	Mean
Low land site						
D8484	2.41_{abcd}	2.88_{ab}	2.97_{a}	2.80_{abc}	1.60_{f}	2.53_{a}
CO421	1.83_{def}	2.25_{cde}	2.35_{abcd}	2.28_{bcd}	2.40_{abcd}	2.22_{b}
KEN83737	1.63_{ef}	2.12_{def}	2.37_{abcd}	2.24_{cde}	0.00_{g}	1.67_{c}
Mean	1.96_{b}	2.42_{a}	2.56_{a}	2.44_{a}	1.33_{c}	
LSD $_{(p\leq 0.05)}$: Temp 0.37, variety 0.28, temp*variety 0.63. C.V (%) = 17.6						
Upland site						
D8484	2.11_{cd}	2.71_{cde}	3.60_{e}	3.01_{de}	1.15_{b}	2.51_{b}
CO421	2.10_{cd}	2.16_{cd}	2.54_{cd}	2.48_{cd}	2.32_{cd}	2.32_{b}
KEN83737	2.00_{bc}	2.06_{bc}	2.05_{bc}	2.42_{cd}	0.00_{a}	1.71_{a}
Mean	2.07_{b}	2.31_{bc}	2.73_{c}	2.64_{c}	1.16_{a}	
LSD $_{(p\leq 0.05)}$: Temp = 0.53, variety 0.41, temp*variety 0.92. C.V(%)= 25.1						
Greenhouse						
D8484	1.68_{ns}	2.11_{ns}	2.67_{ns}	2.30_{ns}	0.83_{ns}	1.92 $_{b}$
CO421	1.39_{ns}	1.46_{ns}	1.61_{ns}	1.50_{ns}	1.10_{ns}	1.41 $_{a}$
KEN83737	1.17_{ns}	1.32_{ns}	2.29_{ns}	1.73_{ns}	0.65_{ns}	1.43 $_{a}$
Mean	1.42_{b}	1.63_{b}	2.19_{c}	1.85_{bc}	0.86_{a}	
LSD $_{(p\leq 0.05)}$: Temp 0.55, variety 0.43, temp*variety 0.96. C.V(%) = 36.3						

Values followed by the same letters within the rows and columns are not significantly different.

LSD= Least significant difference, CV= Coefficient of variation, Temp=Temperature, ns=Not significant

Table 4.8. Internodes numbers for three sugarcane varieties treated at different temperatures under field and greenhouse conditions.

	Temperature °C					
variety	25	45	50	52	55	Mean
Lowland site						
D8484	17_{c}	13_{c}	13_{c}	16_{c}	7_{b}	12_{b}
CO421	11_{c}	11_{c}	12_{c}	13_{c}	11_{c}	11_{b}
KEN83737	12_{c}	12_{c}	12_{c}	11_{c}	0_{a}	9_{a}
Mean	12_{b}	12_{b}	12_{b}	12_{b}	6_{a}	
LSD $_{(p\leq 0.05):}$ Temp 1.7, variety 1.3, temp*variety 3.0 C.V(%) = 16.4						
Upland site						
D8484	12_{c}	12_{c}	11_{bc}	13_{c}	3_{a}	10_{ns}
CO421	12_{c}	11_{c}	12_{c}	13_{c}	8_{b}	11_{ns}
KEN83737	11_{c}	13_{c}	11_{bc}	11_{c}	0_{a}	9_{ns}
Mean	12_{b}	12_{b}	11_{b}	12_{b}	4_{a}	
LSD $_{(p\leq 0.05):}$ Temp 1.8, variety 1.4, temp*variety 3.2 C.V(%)=18.7						
Greenhouse						
D8484	10_{ns}	13_{ns}	15_{ns}	12_{ns}	4_{ns}	11_{ab}
CO421	14_{ns}	11_{ns}	15_{ns}	13_{ns}	8_{ns}	12_{b}
KEN83737	8_{ns}	8_{ns}	12_{ns}	12_{ns}	2_{ns}	8_{a}
Mean	11_{b}	11_{b}	14_{c}	12_{bc}	5_{a}	
LSD $_{(p\leq 0.05):}$ Temp3.1, variety2.4, temp*variety 5.3. CV(%)= 30.5						

Values followed by the same letters within the rows and columns are not significantly different.
LSD= Least significant difference, CV= Coefficient of variation, Temp=Temperature, ns= Not significant

4.5.4. Effect of hot water treatment on cane yield

Hot water treatment had effect on cane yield and there was a significant difference between the treatment temperatures. In both sites, the cane weight was significantly different among the treatment temperatures ($p<0.001$). Cane treated at 45, 50 and 52°C in both field and greenhouse trial produced higher average yield compared to control (25°C). However, average cane yield was highest at 50°C and lowest at 55°C for both varieties (Table 4.9). In the lowland and upland, average cane yield at 50°C and 52°C were not significantly different. There was a significant difference among the varieties on cane yield ($p<0.001$ in the lowland, $p=0.002$ in the upland, $p=0.018$ in the greenhouse) with variety D8484 producing the highest mean yield (Table 4.9). Significant interaction occurred between temperature and varieties at the lowland site ($p<0.001$).

4.5.5. Effect of hot water treatment on cane quality

There was no significant difference in sucrose content in canes treated at 45, 50, 52°C and the control 25°C (Table 4.10). However, cane treated at 50°C and 52°C in both sites produced the highest sucrose content. In the lowland, varieties were significant ($p<0.001$) with variety D8484 producing the highest sucrose content. There was also interaction between temperature and variety. Variety D8484 and KEN 83737 produced higher sucrose at temperature 50°C while CO421 gave higher sucrose content at 52° C in the lowland. However in the upland and greenhouse, varietal effect and interactions between variety and temperature were not significant over sucrose content.

Table.4.9. Cane weight (tonnes/ha) for three sugarcane varieties treated at different temperatures under field conditions and in the greenhouse (kg/pot).

	Temperature °C					
variety	25	45	50	52	55	Mean
Lowland site						
D8484	109_{cdef}	136_{fgh}	161_{h}	149_{gh}	54_{b}	122.4_{b}
CO421	57.8_{b}	92_{cd}	106_{cdef}	114_{cdef}	83_{bc}	91.0_{a}
KEN83737	104_{cde}	119_{defg}	151_{h}	131_{efgh}	0_{a}	101.4_{a}
Mean	90.6_{b}	116.2_{c}	139.9_{d}	132_{cd}	46_{a}	
$LSD_{(p\leq 0.05):}$ Temp 18.5, variety 14.3, temp*variety 32, C.V(%) = 18.2						
Up land site						
D8484	95.9_{ns}	116.6_{ns}	152.2_{ns}	129.6_{ns}	26.0_{ns}	104.1_{b}
CO421	60.1_{ns}	100.4_{ns}	103.0_{ns}	100.6_{ns}	30.5_{ns}	78.9_{a}
KEN83737	79.8_{ns}	101.7_{ns}	119.3_{ns}	113.5_{ns}	2.7_{ns}	83.4_{a}
Mean	78.6_{b}	106.2_{c}	124.8_{d}	114.6_{cd}	19.8_{a}	
$LSD_{(p\leq 0.05):}$ Temp 18.0, variety 14, temp*variety 31.2, C.V(%) = 21						
Greenhouse(Kg/pot)						
D8484	1.9_{ns}	2.1_{ns}	2.7_{ns}	1.9_{ns}	0.6_{ns}	1.9_{ns}
CO421	1.4_{ns}	1.3_{ns}	1.8_{ns}	1.5_{ns}	0.7_{ns}	1.4_{ns}
KEN83737	1.1_{ns}	1.4_{ns}	2.5_{ns}	2.1_{ns}	0.5_{ns}	1.5_{ns}
Mean	1.5_{b}	1.6_{b}	2.3_{c}	1.8_{b}	0.6_{a}	
$LSD_{(p\leq 0.05):}$ temp 0.4, variety 0.3, temp*variety 0.7, CV (%)= 28.6						

Values followed by the same letters within the rows and columns are not significantly different.
LSD= Least significant difference, CV= Coefficient of variation, Temp=Temperature, ns=Not significant

Table 4.10. Sucrose content (%) for three sugarcane varieties treated at different temperatures under field and greenhouse conditions

		Temperature °C					
	variety	25	45	50	52	55	Mean
Lowland							
	D8484	16.4_{cde}	17.2_{de}	18.7_{e}	17.3_{de}	9.7_{b}	15.9_{c}
	CO421	12.8_{bc}	13.4_{bc}	14.8_{cd}	15.3_{cde}	13.8_{cd}	14.0_{b}
	KEN83737	14.3_{cd}	15.1_{cde}	15.5_{cde}	15.2_{cde}	0.0_{a}	12.1_{a}
	Mean	14.5_{b}	15.3_{b}	16.3_{b}	16_{b}	7.9_{a}	
	LSD $_{(p\leq 0.05)}$: Temp 2.2, variety 1.7, temp*variety 3.8, CV(%) = 16.2						
Up land							
	D8484	14.7_{ns}	15.5_{ns}	16.7_{ns}	16.8_{ns}	5.4_{ns}	13.8_{ns}
	CO421	12.3_{ns}	13.6_{ns}	14.9_{ns}	14.9_{ns}	14.3_{ns}	14_{ns}
	KEN83737	13.0_{ns}	13.7_{ns}	14.5_{ns}	14.1_{ns}	4.5_{ns}	12_{ns}
	Mean	13.3_{b}	14.23_{b}	15.3_{b}	15.3_{b}	8.1_{a}	
	LSD $_{(p\leq 0.05)}$: Temp 3.2, variety 2.5, temp*variety 5.5, CV(%) = 24.7						
Greenhouse							
	D8484	16.7_{ns}	16.3_{ns}	18.2_{ns}	15.2_{ns}	4.7_{ns}	14.2_{ns}
	CO421	13.4_{ns}	11.4_{ns}	15.9_{ns}	14.1_{ns}	9.7_{ns}	12.9_{ns}
	KEN83737	13.9_{ns}	14.5_{ns}	17.5_{ns}	14.5_{ns}	4.9_{ns}	13.0_{ns}
	Mean	14.7_{b}	14.0_{b}	17.2_{b}	14.6_{b}	6.4_{a}	
	LSD $_{(p\leq 0.05)}$: Temp 3.9, variety 3.0, temp*variety 6.7, CV(%) = 30						

Values followed by the same letters within the rows and columns are not significantly different.
LSD= Least significant difference, CV= Coefficient of variation, Temp=Temperature, ns=Not significant

4.6. Discussion

The study found that hot water treatment had effect on sugarcane germination and growth. Hot water treatment at 45, 50 and 52°C significantly increased germination and tiller growth compared to the untreated (25°C) whereas treatment at 55°C adversely affected germination and tiller growth. Germination and tiller growth was highest at 50°C. These results are in agreement with Damann *et al*., (1983), Ongoma, (1992); Jualia louse, (1998); Johnson and Tyagi, (2010) who found that hot water treatment of sugarcane setts increased germination and growth of varieties. Higher germination and tiller growth at 50°C, confirmed results by Johnson and Tyagi (2010) who found hot water treatment of cane at 50°C for 2hrs as the most effective RSD control strategy.

The increase in germination and growth in the treated setts were probably due to change in hormonal balance, auxin levels may have been reduced during heat treatment, breaking bud dormancy. Raven *et al*.,1999 showed that auxin hormone, indol-3-acetic acid (IAA) produced at the apex of the stem is responsible for lateral bud dormancy by causing cells in the lateral buds to produce another hormone, ethylene which is a growth inhibitor. Ratoon stunting bacteria within the treated setts was reduced by hot water treatment, improving germination (John and Tyagi, 2010; Viswanathan, 2012).

Low germination and tiller count in the untreated setts could be attributed to effect of ratoon stunting disease. Research conducted by McFarlane (2002) and Zvoutete (2004) and showed that setts inoculated with ratoon stunting bacteria had reduced germination and growth compared to healthy setts. Hot water treatment at 55°C may have killed the buds resulting to poor germination and subsequent low tiller count. A study by Damayanti *et al.*, (2010) showed that cane setts could not withstand elevated temperatures of 55°C and 60°C and these temperatures

caused the buds of all treated setts to die. High germination and growth for CO 421 at 50°C and 52°C compared to D8484 and KEN83737 could be due to the bud orientation or its tough coat. In an earlier study by Ongoma, (1992) and Julia louse, (1998) it was found that results of heat treatment depended on variety, stalk width, cane quality, cane age and growing conditions.

Hot water treatment significantly increased the cane growth, overall yield and sucrose content. The study found that cane treated at 45, 50 and 52°C had taller and thicker stalks with more internodes and millable stalks, higher cane weight and sucrose content compared to control (25°C). These results are in agreement with Johnson and Tyagi, (2010) who found hot water treated cane had higher cane and sugar yield than the untreated. Hot water treatment could have reduced ratoon stunting disease resulting to high germination and subsequent high yields in the treated cane. Heat treatment kills or eliminates the bacteria present in the sugarcane setts (Gillaspie and Davis, 1992; Viswanathan, 2012). James (2005) reported that hot water treatment does not totally eliminate ratoon stunting infection but reduces the infection to a level that enable the grower establish a nursery with very low level of disease infection. The high cane yield and sucrose content at 50°C, confirmed findings by Johnson and Tyagi, (2010), who found that hot water treatment at 50°C for 2 hrs, gave higher cane and sucrose yield and was the most effective. Treatment at 55°C adversely affected cane yield. Perhaps the high temperature of 55°C may have killed the buds leading to low germination and thus low plant population resulting to low yields (Johnson *et al*., 2006).

The production of shorter - thinner stalks, low cane and sucrose yield in untreated cane (25°C) may have been due to effect of ratoon stunting disease. Research conducted by Grisham (1991) and Grisham *et al* (2009) showed ratoon stunting disease reduced cane and sucrose yield of sugarcane cultivars tested in four to six crop cycles of three year plantings. In related studies by

Zvoutete, 2004; Comstock, (2008) and Chen Ming-hui *et al*., (2013) they showed that plant height, stalk diameter, internode length, cane weight and sucrose content was decreased in RSD infected cane compared to healthy crop. Chen Ming- hui *et al*., (2013) found that chloroplast and mitochondria in RSD diseased leaves were abnormal and deformed, electron dense substances accumulated in the infected stalk cells and the xylem cell walls were degraded and broken in different degrees. Their results indicate that sugarcane quality and yield reduction due to RSD infection may be associated with the disorder of water and nutrition transportation and decline in photosynthetic efficiency in plants.

Hot water treatment significantly reduced RSD bacteria. The percent of stalks with RSD bacteria was lower in the treated cane compared to control (untreated). Treatment at 55°C completely cured ratoon stunting disease and had the lowest percent of infected stalks. The findings are in agreement with Julia louse (1998) and Johnson and Tyagi (2010) who reported that hot water treatment reduced ratoon stunting bacteria in infected sugarcane. Gillaspie and Davis (1992) and Comstock and Lentini (2005) and reported that heat treatment of seed cane before planting eliminates ratoon stunting disease in infected sugarcane. Though treatment at 55°C completely eliminated ratoon stunting bacteria, its recommendation to growers is highly unlikely because it adversely affected germination, growth and yield. Hot water treatment at 50°C and 52°C effectively controlled ratoon stunting disease, but treatment at 50°C gave higher overall cane yield compared to 52°C for both varieties. The optimum temperature for hot water treatment in the control of ratoon stunting disease has been found to be 50°C for 2 hours (Gillaspie and Davis, 1992; Johnson and Tyagi, 2010). Variety D8484 produced higher cane yield at all levels of hot water treatment compared to KEN83737 which yielded more than CO421. Improved commercial cane varieties such as D8484 and KEN 83737 are high yielding compared to old

varieties commonly used by cane farmers such as CO421, CO 617, EAK 69-47, EAK 70-97, KEN 71-402. The improved sugarcane varieties are early maturing, high yielding, highly sugared and are resistant to major sugarcane diseases (KESREF, 2013; KEPHIS, 2015)

The reduction of the disease and increase in cane yield and sucrose content in treated cane at 45, 50 and 52°C indicate the effectiveness of hot water treatment in the control of ratoon stunting disease. Hot water treatment at 50°C was the most effective and produced the highest cane yield which was not significantly different from temperature 52°C.

CHAPTER FIVE

GENERAL CONCLUSION AND RECOMMENDATIONS

5.1. Conclusions

The results of this study indicate that sugarcane production in Nyando is done by small scale farmers. Most farmers grew old commercial sugarcane varieties and their main source of seed cane was neighbours and own crop which could lead to spread of sugarcane diseases and low cane production. Awareness on ratoon stunting disease was low and this could contribute to spread of the disease unknowingly through infected cuttings or farm implements. The survey confirmed the presence of ratoon stunting disease in Nyando sugar belt. The mean disease prevalence was 67% while the mean disease incidence was 20%. The use of seed cane from neighbours and own crop in addition to low awareness on ratoon stunting disease might be contributing to high disease prevalence in Nyando sugar belt.

The study found that hot water treatment reduced ratoon stunting bacteria in the diseased cane. Hot water treatment at 45, 50 and 52°C reduced the RSD pathogen and increased cane germination resulting to high cane and sucrose yield which indicates the effectiveness of hot water treatment in the control of ratoon stunting disease. Treatment at 45°C, 50°C and 52°C reduced ratoon stunting disease without detrimental effect on cane. Although high temperatures of 55°C for two hours completely eliminated the disease it may not be useful because it was detrimental on cane. Among the treatment temperatures 50°C was the most effective since it controlled the disease and produced the highest yield. However results showed that yields at temperature 50°C and 52°C were not significantly different thus either temperatures can be

recommended for management of ratoon stunting disease. Varieties D8484 and KEN 83737 were more sensitive to high temperatures compared to variety CO421.

5.2. Recommendations

From the findings in this study the following recommendations are proposed:

1. Sensitization of farmers on ratoon stunting disease and its management strategies like planting healthy seed cane, disinfection of farm implements and destruction of plant debris and old crop before fields is replanted since these acts as a source of inoculums.
2. Development of strategies to produce and supply healthy initial seed cane through hot water treatment to aid in arresting the spread of ratoon stunting disease in sugarcane producing areas and continuous screenings of new sugarcane varieties for RSD susceptibility before are released.
3. Training of sugarcane farmers on hot water treatment at 50°C or 52°C for two hours to manage ratoon stunting diseases for increased cane productivity.
4. Further research to determine the mechanism of survival and characterize RSD bacterium.
5. Further research to determine varietal tolerance to ratoon stunting disease.

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APPENDICES

APPENDIX.1. Questionnaire on sugarcane production practices in Nyando Sugar belt.

1. What is your total farm size?

a) < 1acres…….. b) 1-2 acres …… c) 2-5 acres…… d) > 5 acres ……

2. How many years have you practiced sugarcane production?.....

3. Acreage under sugarcane (acres)

a) < 1 acres…….. b) 1 - 2 …… c) 2-5 acres…… d) > 5 acres ……

3. Varieties grown a)……. b) ……..…… c) ……

4. What are the other major crops you grow in your farm?

…… , ……, ……, ……

5. Where do you source planting material/seed cane from?

Own ( )

Neighbours ( )

Milling companies ( )

KESREF ( )

6. How old is your cane…….months and how many weeding's have you carried since last harvesting……..

7. Which cycle is your crop? a) Ratoon crop……. b) plant crop…….

8. Do you apply any fertilizer to your cane?

a) Yes…….. b) No……

9. What is the average yield of your cane (tonnes) for the last three years?

a) 2011…….. b) 2012……. C) 2013……..

10. What are the major diseases affecting your sugarcane crop? (Rank)

a) …… b) …….

c) …… c) …….

11. What methods do you use to manage the diseases?

a) …… b) ……

c) …….. c) ……

d) …….. e) ……

12. Show farmers the RSD photos. Have you seen such symptoms in your farm?

a) Yes……. b) No……..

If yes, how long the disease has been present on your farm? .....

. How is the spread of the disease in your farm? 1) Few plants ( ) 2. Spots ( )

3) Whole field ( )

How does the disease affect the crop?

……

……

……

……

Does it affect all the sugarcane varieties? .....

13. a) what methods do you use to control the disease?

……

……

b) Where do you Source information on disease management? ……..

……..

Interviewer observations on each farm.

1. Presence /absence of the RSD on the farm. …….

2. Distribution of the disease within the farm. None =0

Few plants =1

Spots =2

Whole field =3

4. Incidence of the disease within the farm <10 %( ) 10-20 %( ) 20-30% ( ) 50 %( ) >50% ( ).

5. Record observed symptoms on diseased cane……

……..

Collect samples from each farm.