

**DIVERSITY OF INSECTS ASSOCIATED WITH BANANA IN BANANA XANTHOMONAS
WILT EPIDEMIC AREAS OF WESTERN KENYA**

ABSTRACT

Banana Xanthomonas Wilt (BXW) is a disease that devastates bananas production, the causal agent is a bacterium pathogen, "*Xanthomonas campestris* pv. *musacearum*" (Xcm). The Xcm kills banana plants quickly, spreads rapidly and causes loss of up to 100%. The BXW disease spread on banana occurs mainly through insect vectors which visit banana male flower for nectar, pick Xcm bacteria on contact with the wounds left by fallen male bracts especially from infected flowers to the next uninfected flower. A study on banana insect diversity and their role as potential vectors for BXW was done in western Kenya. The objective was to determine the diversity of insects associated with banana in BXW epidemic areas in western Kenya namely Busia, Kakamega and Siaya counties. A collection of all types of insects visiting inflorescence parts of banana and on the same inflorescence DNA samples were captured by 2 minutes dipstick to confirm the presence of Xcm using PCR procedures up to electrophoresis gel picture. Insects were positively identified by entomologist with aid of a dichotomous key and pictures. Significantly a diversified insects were found to be associated with banana in BXW in epidemic areas. The Drosophilidae, Apinae and Tephritidae families were most frequently recorded in banana plantations. Bees, grass flies, banana fruit flies,

flies, wasps, butterflies, beetles, spiders and black ants formed the diversity. Significantly more bees were recorded followed by flies. Isolates from inflorescence samples, positively confirmed the presence of Xcm through electrophoresis gel picture in the study area in western Kenya. Insect are potential vectors of Xcm.

Key words: *Insect abundance banana Xanthomonas wilt Kenya.*

1. INTRODUCTION

Banana (*Musa* spp.) crop has an annual world production of around 104 million tons grown in more than 120 countries, of which a third is produced in African, Asia-Pacific, Latin American and Caribbean regions [1, 2]. East Africa is the largest banana-producing and consuming region in Africa with a per capita annual consumption of 250 kgs [3]. In Kenya banana is a major staple crop besides being the most popular eaten fruit, the cooking varieties also represent an important staple food [4, 5]. The major producing regions in Kenya during the year 2006 were; Nyanza produced 505,258 tons; Central 217,175; Western 136,266; Eastern 80,232; Coast 65,558; Rift Valley 47,613; North Eastern 5,663 and Nairobi 253 and was totally valued at 5 million US dollars [6].

Banana Xanthomonas Wilt (BXW) devastates banana production. The disease is caused by a bacterium pathogen, "*Xanthomonas campestris* pv. *musacearum*" (Xcm) and was first reported in Ethiopia in the year 1968 [7]. The pathogen kills banana plants quickly and spreads rapidly over a large area making the disease

one of the most dreaded in banana (*Musa* spp.). The disease has spread to Uganda, the Democratic Republic of Congo, Rwanda, Burundi, Tanzania and Kenya [8]. In Kenya the disease was first reported in 2006 in Bungoma, Busia, and Teso Counties and a year later in Bondo, Siaya, Mumias, Butere, Kakamega and Mt Elgon counties ([9]. Banana Xanthomonas Wilt can cause up to 100 percent loss, affecting all types of bananas ([10]. Yield losses of up to 70% in beer banana (ABB group) in central Uganda[11]. Banana Xanthomonas Wilt spread on banana occurs mainly through the use of contaminated farm tools infected, planting materials and insect vectors which visit male bract and flower scars ([12]. Infection of Xcm occurs via inflorescences transmitted by insects under field conditions [8, 13]. Insect vector transmission in banana occurs when bacterial ooze from infected banana tissues, carried by insects and also from infected peduncles to the moist cushions on the peduncle of a healthy plant as they feed on sap oozing from wounds on the inflorescences [14, 15]. Whenever there is a wound Xcm easily ooze out of infected inflorescences and most likely the insects pick up bacteria from these wounds during feeding and or collecting nector [12, 16,].

The study was in Western in Kenya where the diseases was widespread by male bud infection. Bees and wasps transmitted *Ralstonia solanacearum* bacteria in Bluggoe plantations [9, 14]. Stingless bees and grass flies were agents of Xcm transmission in banana plantations [17, 18]. The Drosophilidae and Apinae families were most frequently recorded in banana plantations [13, 19]. The

objectives of the study was, to determine the diversity of insects associated with banana in BXW epidemic areas to assist in the disease integrated management.

2. MATERIALS AND METHODS

2.1 Study area

Insects were collected from three counties in western Kenya namely; Busia in Agroecological zone Low Midland 1 (LM₁); Kakamega (LM₁) and Siaya (LM₁ and ₂) covering five sub counties namely; Butere, Butula, Emuhaya, Gem and Ugunja where BXW had been reported. During the study female and male gender households were interviewed as respondents /owners of banana orchards. A total of 250 farms were visited and at each farm ten mats were sampled randomly whether BXW affected or not.

2.2 Insect collection procedure

All types of insects visiting floral parts of banana were collected to establish the diversity and abundance of insect floral visitors on banana in BXW epidemic areas. Insects were collected from the male inflorescences using a cotton bag. The collection involved healthy banana, banana diseased with BXW, Yellow Sigatoka (*Mycosphaerell musicola*) and panama (*Fusarium oxysporum* fsp. cubensis) and any other notable disease. An insect net was put around the flower taking care not to disturb the flower and the insects on it. By grubbing the net close to its ring the insects were captured in the net. The net was then carefully withdrawn and closed not to allow insects to escape out of it. At the bottom of the net, a cotton

wool was dropped with Chloroform vapour for 1 minute to knock out the insects to ease their handling. The trapped insects were then carefully emptied in a bottle of alcohol, labelled for further identification.

2.3 Insect identification procedure

A total number of 3,165 insects were collected and further sorting and identification was done using a stereomicroscope (10-250X) at the Plant Science and Crop Protection, Entomology Laboratory at the University of Nairobi, Kenya. The identification was accomplished by an Insect Taxonomist (Entomologist) aided by a dicotomas key and pictures procedures, according to their family, genus and species level. Different insect roles/associations with the visit to the banana inflorescence were noted such as; their mode of feeding either sucking or chewing and whether their characteristics may be linked to the mechanism and mode of spread of the Xcm pathogen. The Objective was to establish the types and abundance of insects associated with spread of BXW above ground.; predators, pollinators, herbivores and sap feeders [19]. The hypothesis was that the insect characteristics may be linked to the mechanism and mode of spread of Xcm pathogen.

2.4 Sampling isolates to confirm "*Xanthomonas campestris* pv. *musacearum*"

Two hundred and fifty banana farms visited in western Kenya, ten plants were randomly sampled for isolation of BXW pathogen (*Xanthomonas campestris* pv *musacearum*) from inflorescence where the insects were collected. The samples were from different banana cultivars, farmer-managed fields in Busia, 100

samples, Kakamega 70, and Siaya 80. A small piece of 2cm² was cut from male flower part for PCR procedures aimed to confirm the presence of Xcm pathogen. The DNA of Xcm was captured using 2minutes dip stick. The samples were taken to ILRI (International Livestock Research Institute) Beca laboratory in Kenya for PCR procedures to confirm the disease. The objective was to diagnose and confirm the presence of Xcm pathogen in the study area.

2.5 Extraction of Two minutes DNA dipstick samples for Xcm

DNA was extracted from the inflorescence from a small piece cut of 2cm². This was macerated and added 2minutes-extraction buffer bottle inserted in and then the lid of the bottle closed and shaken in the bottle with the sample for 30 seconds. Four dipsticks with the glass fibre-sample pad head dipped to be in contact with the buffer and allowed the dipsticks to run for approximately 2 minutes. The dipsticks were placed on a clean paper towel and allowed to air-dry without exposing them to direct sunlight. After that the paper towels were discarded and the dipsticks were able to pick DNA. The dipsticks with DNA were dried kept. For further analysis later a single of punch 2 mm² disc was taken from the sample dipsticks and eluted in wells for PCR procedure for DNA extraction.

2.6 Procedure and methods used for running the PCR.

PCR master mix ingredients and set up for isolates from western Kenya region were prepared. A diagnostic Primer-Xcm-38..5R / F was used in the PCR procedure. A conventional PCR amplification was performed using a Gene Amplification PCR System 9700 Thermal cycler 10 µl volumes containing 1 x reaction buffer (0.5 mM KCl, 0.1 mM Tris-HCl pH 8.3, 0.15 mM MgCl₂, dNTPs at 0.25 mM), 1.0 pmol primers (Xcm 38R/F, 0.1 U of Taq DNA polymerase. Two primers were used Xcm 38-R (5'CAGCGGCGCCGGTGTATTGAGTG 3') Xcm 38-F (5'CCGCCGGTCGCAATGTGGGTAAT 3'). The premix ingredients were as follows; Distilled H₂O-9ul, Primer-Xcm-38F-0.5ul, Primer-Xcm-38R-0.5 ul and DNA

template 1(2mm) disc making a total reaction for 10ul. This was set up and ran as follows; initial denaturing at temperatures 94°C for 5minutes, denaturing at 94°C for 20 secs that ran for 40 cycles, annealing at 60°C for 20 secs for 40 cycles, extension at 72°C for 1 minute 40 cycles and lastly Final extension at 72°C for 10 minutes. The product of the captured DNA of Xcm 2minutes dipstick was ran on agarose 2% percentage, (100 ml of 1 X TBE buffer plus 2g of Agarose gel) this was boiled in microwave cooled. For tracking 2ul gel red was added ran in electrophoresis machine at 120 volts for 30minutes then into a in UV illuminator and a gel picture was captured read and reported.

2.7 Data analysis

Data on insect diversity were clustered according to; the counties insects were collected from, inflorescence parts of banana from either suspected healthy or diseased banana plants, type of the banana whether dessert or cooking, various banana varieties and topographical elevation the altitude where the crop was grown. The parameters were analysed using Stata (Statistics/Data Analysis) version 12.0. [20]. Mean, standard deviation and standard error were used for comparison of the diversity and abundance of the insects. Sample isolates were imposed to PCR electrophoresis machine procedures followed, UV illuminator and gel pictures captured, read and interpreted for confirmation of *Xanthomonas campestris* pv *musacearum* in the study area.

3. RESULTS

3.1 Insect diversity and abundance

The diversity of insects collected comprised of stingless bees (*Apis* sp), grass flies (*Drosophila* sp), banana fruit flies (*Tephritida Ceratitis*), beetles (*Neomyia ruissima* sp), black ants (*Plectroctena* sp) and beetles (*Neomyia ruissima* sp) formed the diversity. Stingless bees were the most common insects observed followed by banana fruit flies, grass flies, beetles and black ants on banana plant. More than

three times insects were observed in low altitudes of 1224-1282 meters above sea level(masl) than medium to high altitude areas. There was an increasing order from Kakamega, Siaya and Busia. Dessert types of banana had significantly more insects than cooking types. Insects observed on fruits were similar to those on flowers. There were similar insects on healthy plants as on those diseased. Stingless bees were more abundant in dessert banana cultivars as compared to cooking cultivars. Bees had higher mean followed by fruit flies, butterflies. Banana variety Ngombe had significantly the highest mean of insects. The highest mean number of insects were recorded on 'Ngombe' followed by 'Garisa short, Garisa tall, Gold finger and Valery' while the lowest numbers were found on 'Uganda green' variety (Figure 7). There were moderate banana fruit flies populations observed on FHIA17, Valery and Mysore. Other insects such as beetles and black ants were few (Figures 1 to 7).

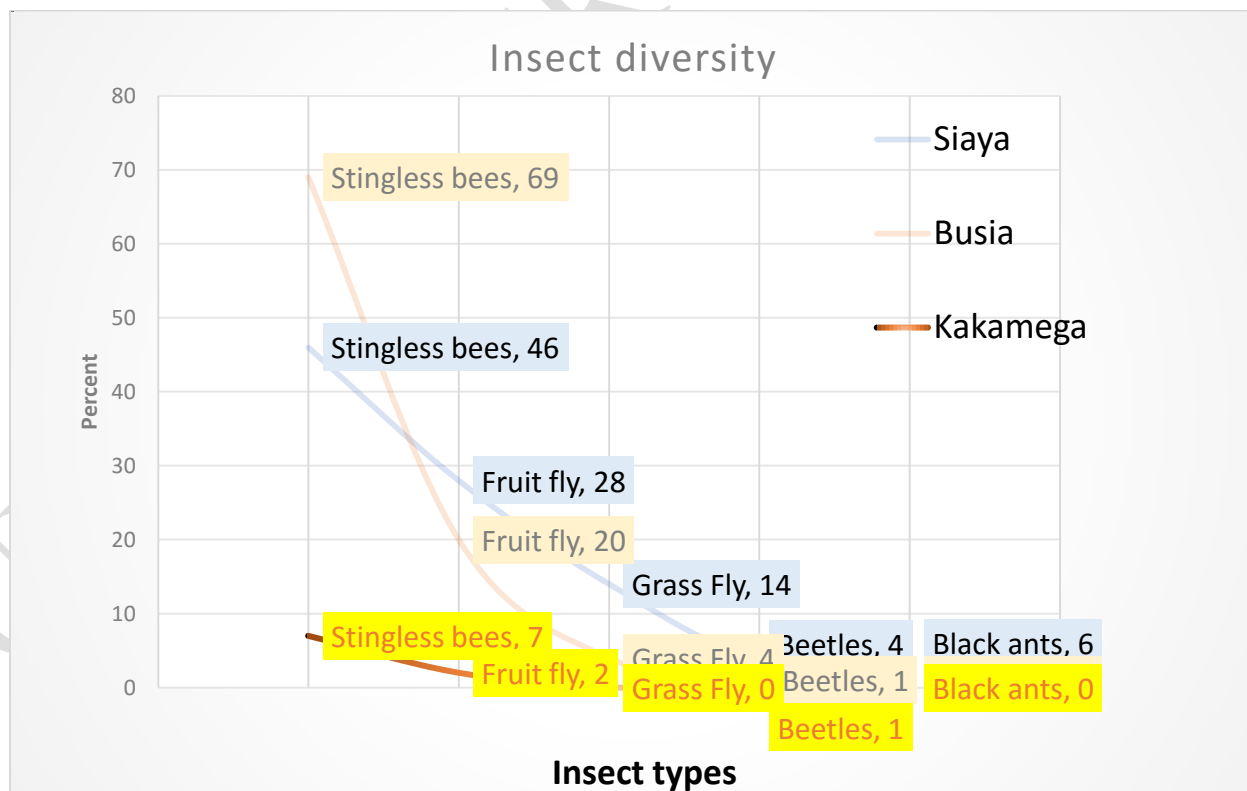


Figure 1: Percent diversity of insects associated with banana plants in BXW epidemic areas in Siaya, Busia and Kakamega counties

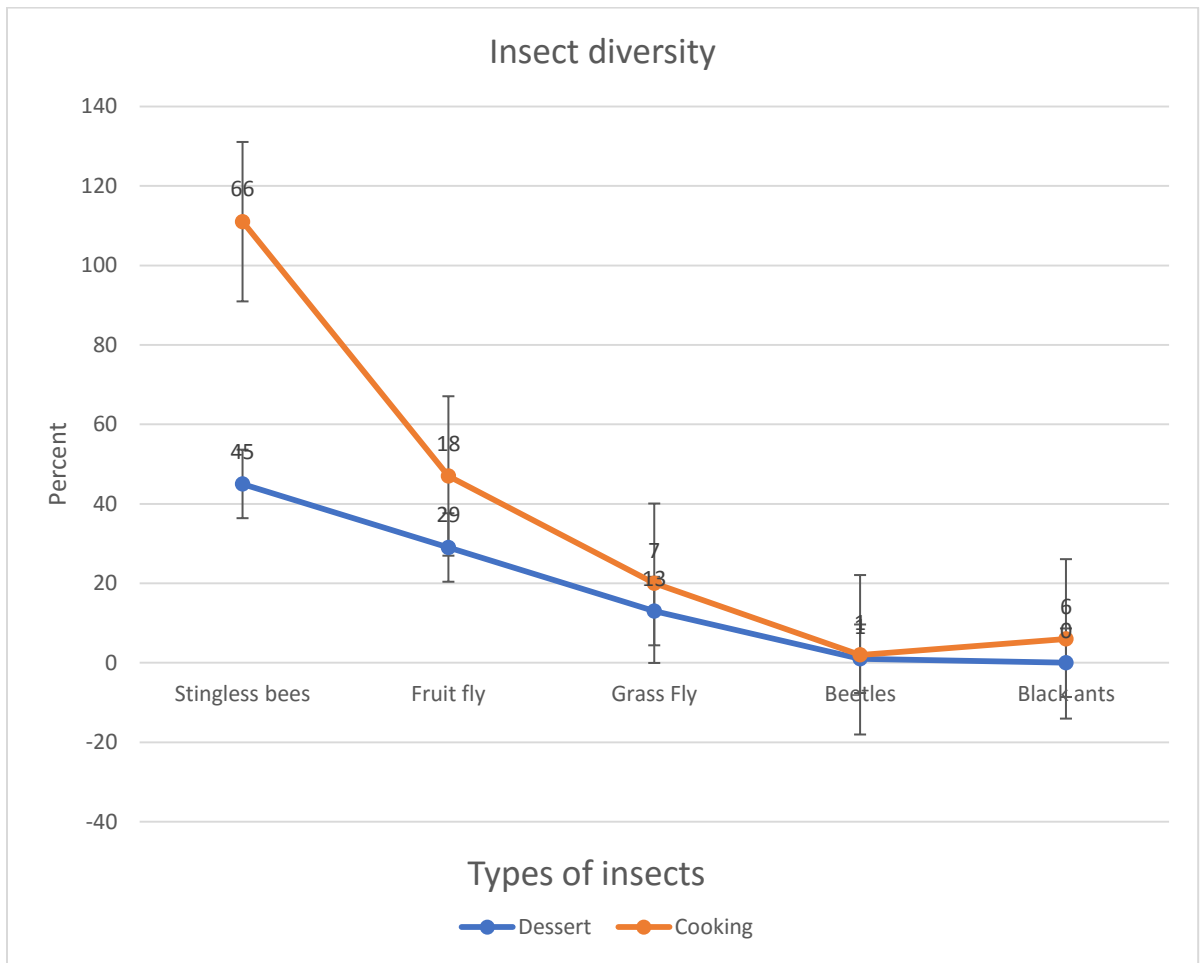


Figure 2: Diversity of insects associated with banana plants in BXW epidemic areas according to banana usage

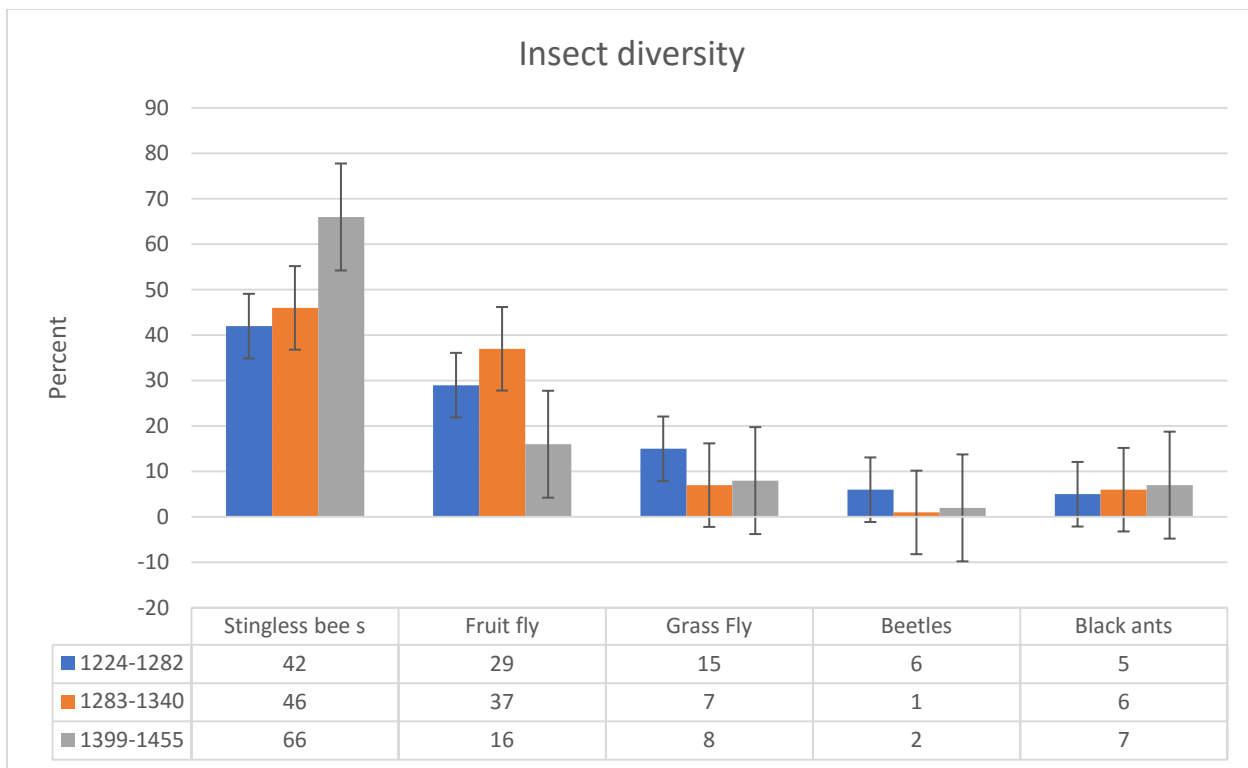


Figure 3: Percent insect diversity according to altitude in BXW epidemic areas.

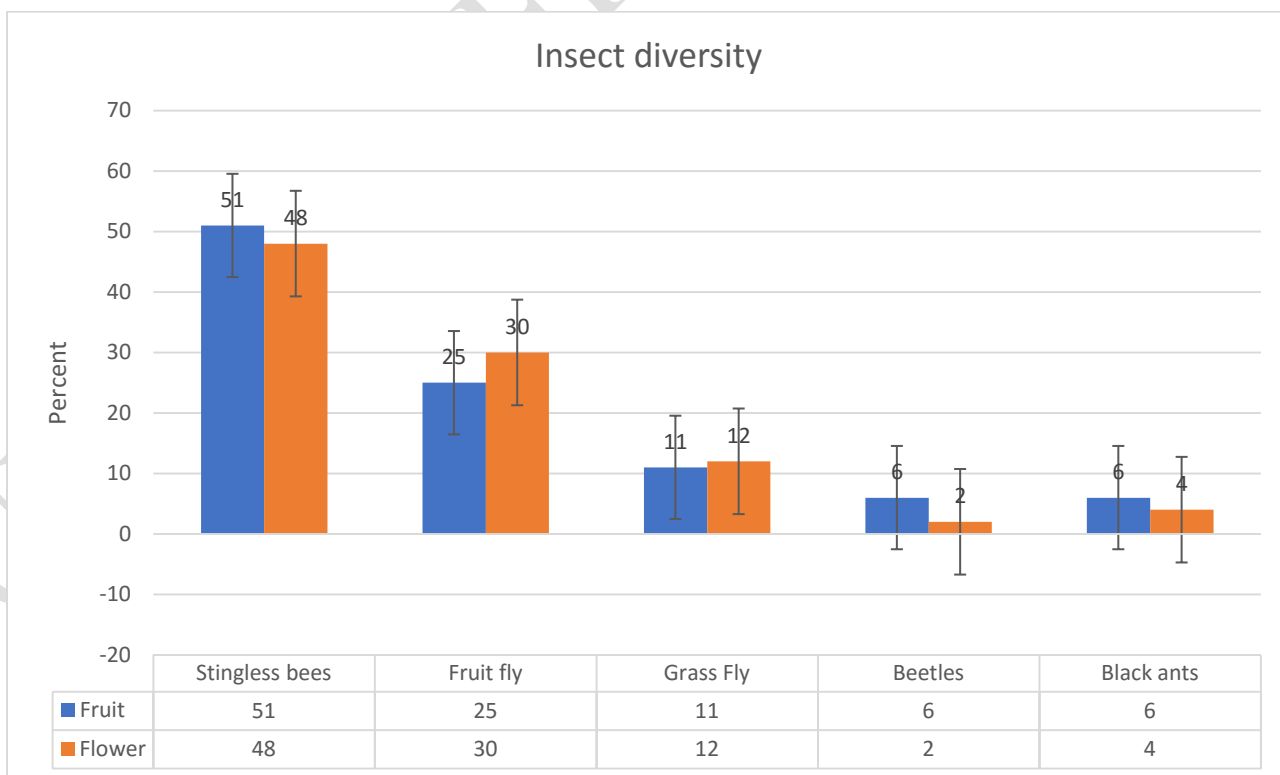


Figure 4: Percent insect diversity from flower or fruit part of the banana in BXW epidemic areas

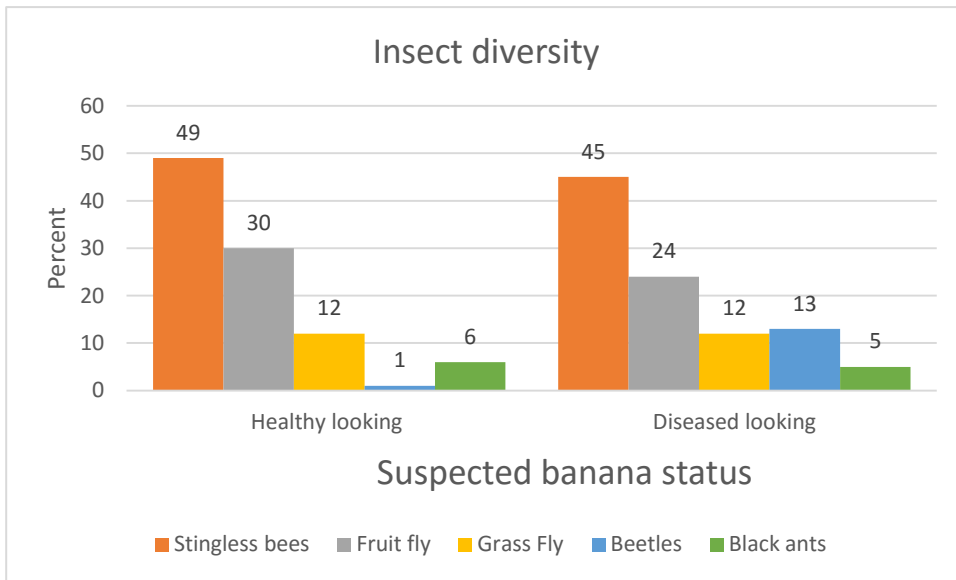


Figure 5: Percent insect diversity according to health status of banana plant in BXW epidemic areas

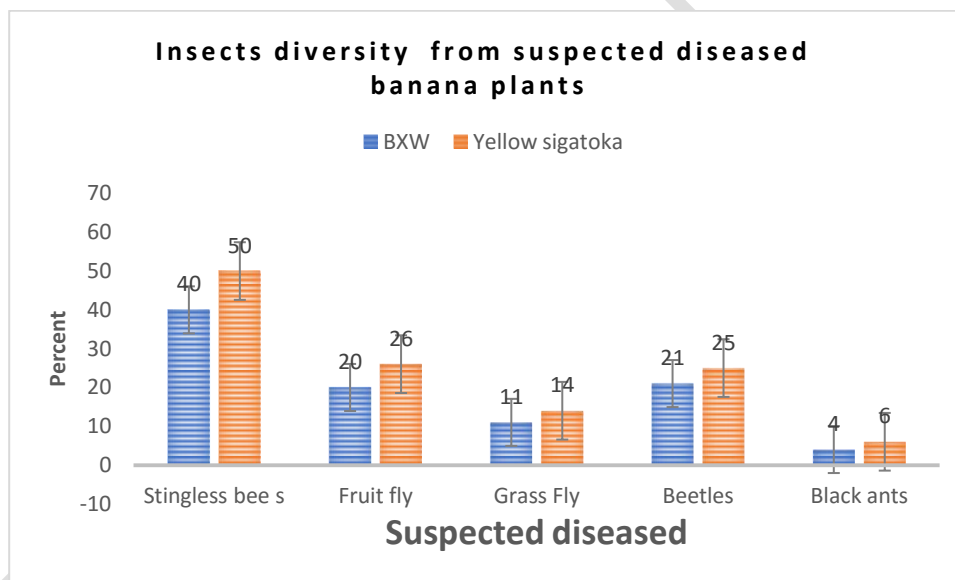


Figure 6: Percent diversity of insects fromr suspected diseased banana plants in BXW epidemic areas

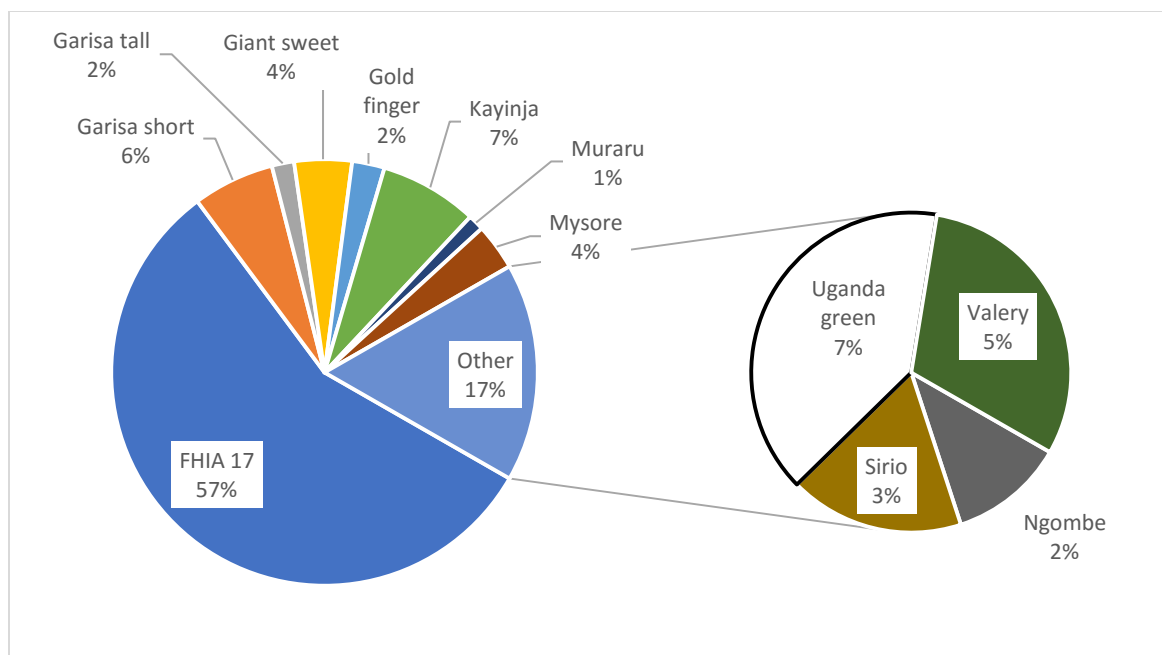


Figure 7: Percent diversity of insects from various banana cultivars in banana *Xanthomonas* wilt epidemic areas.

3. 2 Confirmation of BXW in study areas

Banana *Xanthomonas* wilt disease was positively confirmed through PCR procedure from isolates sampled from the study area (Fig 8 and 9). The amplification were positive with a mean percentage of 28%. Busia led with 33%, Siaya 18 and Kakamega 16 (Figure 9), primer Xcm 38-R (5'CAGCGGCGCCGGTGTATTGAGTG 3') Xcm 38-F (5'CCGCCGGTCGCAATGTGGGTAAT 3') was used for the 2minutes dip stick capture kit samples. The positive isolates produced bands at 650 bp size with ladder M at 100bp that confirmed the presence of *Xanthomonas campestris* pv *musacearum* in the study area.

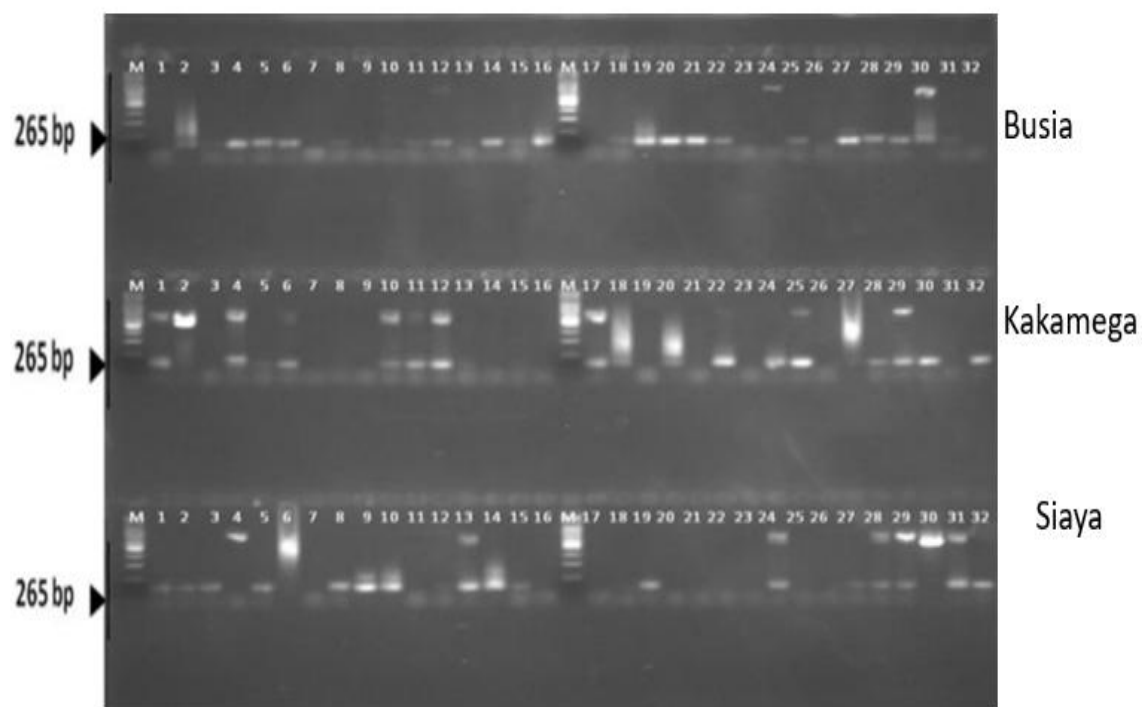


Fig. 8 : Gel images of 2minutes dipstick DNA capture kit isolates from banana inflorescence in Busia, Kakamega and Siaya; at bp=265 Primer base pair, M=100bp Ladder

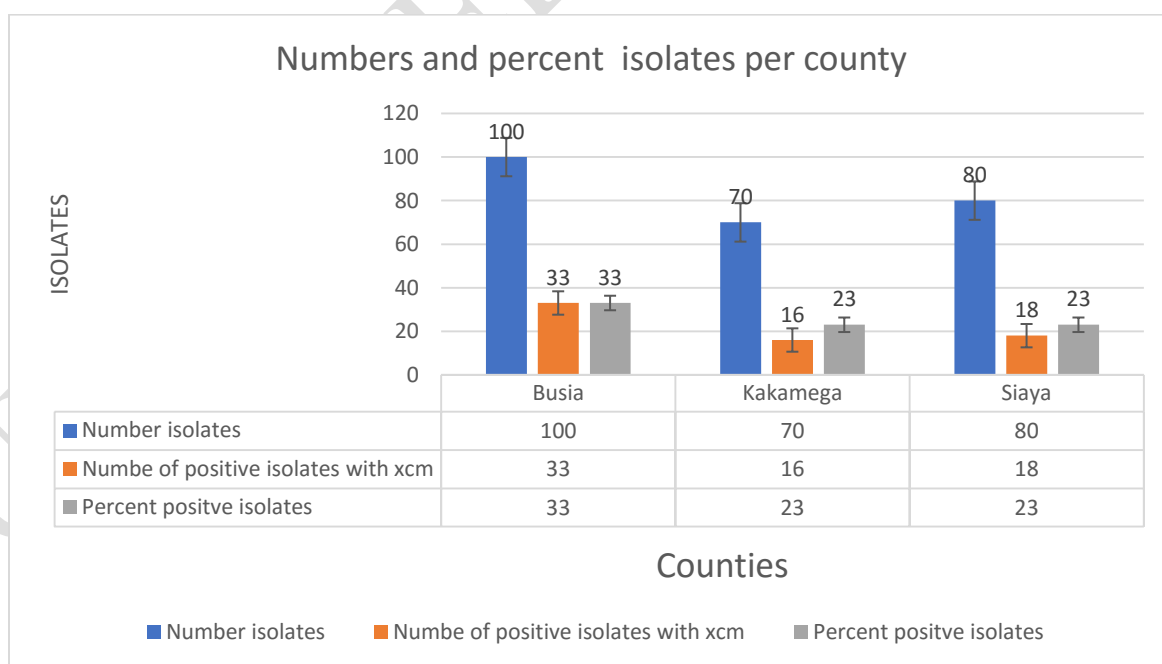


Figure 9: Positive PCR results of Xcm DNA isolates captured by 2 minutes dip stick from Busia, Kakamega and Siaya Counties in BXW epidemic areas

4. DISSCUSSION

4.1 Insect diversity and abundance

The types of insects associated with banana plant in BXW epidemic areas were stingless bee, grass fly, beetles, black ants and banana fruit fly. Stingless bees were the highest 48% followed by banana fruit flies 27% and grass fly 12% (Figures 1 and 6). These observations concurs with studies in Ethiopia and Uganda [17, 19, 21]. The study confirmed more insects were observed in low altitude areas of 1224 masl of 60% of the total insects populations observed than in high altitudes areas and concurs with [22] that more insects population around the lower elevations of Lake Victoria at altitude 1135masl than at 1700 masl. This suggests that insect activities in low altitude areas may play a role in faster disease spread than in low altitude, thus male bud removal may check the spread.

The study confirmed that there were more insects on the flowers and fruits, due to the fact that they collect nectar as has been reported by other researchers [19, 23, 24]. Significantly more insects were observed in the banana dessert cultivars cavendish group namely; Gros Michel, Valery, FHIA 17, Garisa and Gold finger cultivars than the cooking type Uganda green concurring with studies in Ethiopia [19], that Dwarf Cavendish hosted the highest diversity of insect families. Dessert banana had more nectar, produce more pollen and contains higher sugar content thus attracting more insects than the cooking banana [17]. This suggests

that disease spread may be faster in dessert banana varieties than cooking varieties.

Stingless bees, fruit flies and grass flies were abundant and may be associated with banana inflorescence as they forage for nectar or other symbiotic relationships. The forage behavior makes the insects associated with banana flowers may be hypothesized to provide opportunity for picking Xcm from infected plants and transmitting to the non infected plants thereby acting as vectors.

There were diversified and abundance of insects that were associated with banana plant in BXW in epidemic areas namely Bee, Grass Fly, Banana fruit fly, Fly, Wasp, Butterfly Beetles, Spider and Black ants. More bees were recorded followed by flies insects these were insects that feed on or collect nectar, thus might account for the higher numbers. Conversely some insects like spiders may be concerned with trapping some of these insects like wasps, ants, flies and moths for their food and might have no direct activity with banana flowers thus accounts for their numbers low in the inflorescence. The Drosophilidae Apinae and Tephritidae families were most frequent in banana plantations.

The study area lied at altitude of 1224-1455masl. These are areas with warm climatical conditions and insects diversity and activity are high as confirmed by this study and concurs with [17, 19]. At lower altitude areas below 1700masl there were more insects abundance than higher altitude zones and concurs with those reported in Uganda, Democratic Republic of Congo and Ethiopia [17, 19, 22]. There were more insects on the floweres and fruits than on banana pseudostems.

As some insects collect nectar there are others which might be feeding or making traps of other insects for their food such as wasps and spiders. The banana dessert varieties showed more insects than the cooking type this might be due to the higher sugar content in them as compared to the cooking banana varieties. Banana varietal differences like some dessert banana may produce more pollen than the cooking varieties and may attribute to more insects attraction than others. Various banana varieties have differences in their floral colours, some have brighter flowers, stronger attractive smell than others and may attract more insects than the dull coloured and less scent varieties. FHIA 17 a dessert banana variety with a very giant bunch significantly had the highest number of insects, followed by Uganda Green variety and Kayinja are well adapted in the region [5] while other varieties Gross Michel, Valery, Garisa and Gold finger were significantly low (Figure 7).

Insects act as vectors of Xcm when they visit male bract and flower scars [24], further more infection of Xcm may occur via infected inflorescences and may be transmitted by insects under field conditions [8, 17]. Insect vector transmission in banana occurs when bacterial ooze is carried by insects from infected peduncles to the moist cushions to the peduncle of a healthy plant as they feed on sap oozing from wounds on the inflorescences [14, 25, 26]. Whenever there is a wound Xcm pathogen easily oozes out of infected inflorescences and most likely the insects pick up bacteria from these wounds mechanically, during feeding and or collecting nectar [18]. As the insect carries their activities there are chances for

them of picking the Xcm from infected plant to healthy plants thereby act as vector of BXW from one plant to the next.

Breaking off the male bud has been recommended to reduce bacterial entry sites in male buds by insects and minimize the Xcm spread from farm to farm [28]. The study confirmed that there were diversified and abundance insects in western Kenya. In banana farms affected by BXW, to eliminate these insects involved in the floral activities it is advantageous to practice debudding.

4.2 Confirmation of BXW in epidemic areas of the study

In the study area stingless bees, fruit flies, grass flies, black ants and beetles were present which might be vectors for Xcm [19, 21, 26]. Although insects like bees collect nectar from male flowers of banana they have no pollination role for the formation of the edible banana fruits. Banana crop has its edible fruits formed parthenocarpically ie fruits are formed without pollination [29]. This suggests that insects or pollinators are not required in banana plantations for banana fruits formation/production. Therefore removing off the male buds [30], is recommended to reduce bacterial entry sites through male buds [26] to check Xcm spread.

Timely debudding, reduces BXW spread in banana farms infected with Xcm because the banana floral part which attracts insects activities are eliminated. Understanding the diversity of these insects associated with banana assists in the management of BXW in epidemic areas [31]. In a study on the role of insect in the transmission of BXW, Xcm cells were isolated from stingless bees, fruit flies and grass flies that were collected from male flowers of symptomatic plants [17]. The amount of Xcm cells isolated from stingless bees were twice as much as other

insects groups [17]. Stingless bees and flies were identified as important vectors in Moko disease of banana [14]. These insects causes infection through the moist cushions or scars of recently dehisced banana male flowers and floral bracts [14, 26]. While evaluating insects as potential vectors of Xcm. In Kaffa Ethiopia Xcm was successfully isolated from bees, grass flies fruit flies and wasps that were visiting banana infected male inflorescences [19]. In Honduras bees were found to carry *Ralstonia solanacearum* in Bluggoue cultivar plantations [14]. The insects associated with banana inflorescence as they forage for nectar or other symbiotic relationships roles, might likely be vectors of Xcm. The forage behavior makes the insects associated with banana plants/flowers be hypothesized to provide opportunity for picking Xcm from infected plants to non infected plants. In the study some isolates from inflorescence were confirmed positively to have Xcm using PCR procedure. We therefore deduce that insects has a role in transmission of Xcm from infected plants to clean plants (Figure 8 and 9).

5. CONCLUSION

More insect divesity and abundance were observed in the study. Bees were the most abundant insects on banana plantation. Xcm was positively cofimed in the study area in western Kenya. Removal of male flower buds checks insects activities involvement in floral parts of bananas reduces spread of BXW in epdemic areas

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