Original Research Article

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

ABSTRACT

Banana Xanthomonas Wilt (BXW) caused by "*Xanthomonas campestris* pv. musacearum" (Xcm) 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 upto 100%. The BXW disease spread on banana occurs mainly through insect vectors which ~~visit~~ use 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 determining the diversity of insects associated with banana in BXW epidermic areas in western Kenya ~~namely~~ including Busia, Kakamega and Siaya counties. A collection of all types of insects visiting inflorecence parts of banana and on the same inflorescence DNA samples were captured by 2minutes dipstick to confirm the presence of Xcm using PCR procedures ~~upto~~ and eclectroforesis gel picture. Insects were positively identified by entomologist with aid of a dicotomous 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

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frequently recorded in banana ~~plantations~~ farms. 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 inflorecence samples, positvely confimed the presence of Xcm through eclectroforesis gel picture in the study area in western Kenya. Insect are potential vectors of Xcm.

Comment [H2]: Conclusion at the end of abstraction incomplete and needs rerwiting.

***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 producting 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].

Comment [H3]: 2006? It is old, now is 2022, please present new data.

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~~ from 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 been spread to Uganda, the Democratic Republic of Congo, Rwanda, Burundi, Tanzania and Kenya [8]. In Kenya the disease was first reported in 2006 ~~in~~ from Bungoma, Busia, and Teso Counties and ~~a year~~ later year 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~~ is transmitted 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,].

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The study was in Western in Kenya where the dieases 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 disease management.

2. MATERIALS AND METHODS

2.1 Study area

Insects were collcted from three counties in western Kenya namely;including: Busia in Agroecological zone Low Midland 1 (LM_1); Kakamega (LM_1) and Siaya (LM_1 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 investiagated and at each farm ten mats were sampled randamly randomly whether BXW affected or not.

2.2 Insect collection procedure

All types of insects visiting floral parts of banana were collected to establish identifiy 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 including healthy banana, banana diseased with BXW, Yellow Sigatoka (*Mycosphaerell musicola)* and panama (*Fusaruim oxyporrum* fsp. cubencis) 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

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Comment [H6]: This word used frequently, and is not suitable for describing insect contact with bananas, it should be replaced with a proper word.

Comment [H7]: Were these diseases are part of your research?

gr~~u~~abbing 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~~n~~ 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~~ identify 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.

Comment [H8]: Department or college, …?

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

Two hundred and fifty banana farms visited in western Kenya, ten plants were randoamly sampled for isolation of BXW pathogen (*Xanthomonas campestris* pv musacearum) from infloresence where the insects were collected. The samlpes were from different banana cultivars, farmer-managed fields in Busia 100, ~~100 samples,~~ Kakamega 70, and Siaya 80 samples. A small piece of 2cm^2 was cut from male flower ~~part~~ for PCR ~~procedures~~ amplification, 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 Resarch Iintitute) BecA laboratory in Kenya for PCR ~~procedures to confirm the disease~~ amplification. The objective was to diagnise and cofirm the presence of Xcm pathogen in the study area.

Comment [H9]: Investigated or surveyed?

2.5 Extraction of Two minutes DNA dipstick samples for Xcm

DNA was extracted from the infloresence from a small piece cut of 2cm^2. 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. Fter 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 mm^2 disc was taken from the sample dipsticks and eluted in wells for PCR procedure for DNA extraction.

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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 dignostic Primer-Xcm-38..5R / F was used in the PCR ~~procedure~~ amplification. A conventional PCR amplification was performed using a Gene Amplification PCR System 9700 Thermal cycler 10 µl volume~~s~~ 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 H_2O-9ul, Primer-Xcm-38F-0.5ul, Primer-Xcm-38R0-0.5 ul and DNA template 1(2mm) disc making a total reaction for 10ul. This was set up and ruan as follows; initial denaturing at temperatures 94^{0}C for 5 minutes, denaturing at 94^{0}C for 20 secs that ruan for 40 cycles, annealing at 60^{0}C for 20 secs for 40 cycles, extension at 72^{0}C for 1 minute 40 cycles and lastly Final extension at 72^{0}C for 10 minutes. The product of the captured DNA of Xcm 2minutes dipstick was ruan on agarose 2% percentage, (100 ml of 1 X TBE buffer plus 2g of Agarrose gel) this was boiled in microwave cooled. For tracking 2ul gel red was added ran in electrophoresis machine at 120 volts for 30 minutes then into a in UV illuminator and a gel picture was captured ~~read and reported~~.

Comment [H11]: It is better to bring this section as a table

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2.7 Data analysis

Data on insect divesity were clustered acording to; the counties insects were collected from, infloresence parts of banana from either suspected healthy or diseased banana plants, type of the banana whether dessert or cooking, various banana varieties and topograpical elevation the alttitude 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 comparision ~~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 interprated for confirmation of *Xanthomonas campestris* pv musacearum in the study area.~~

3. RESULTS

3.1 Insect diversity and ~~abundance~~ frequency

The ~~divesity of insects collected~~ collected insects 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 files, beetles and black ants on banana plant. More than three times insects were obsevred in low altitudes of 1224-1282 meters above sea level(~~masl~~ 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~~frequent insects in dessert banana cultivars as compared to cooking cultivars. Bees had higher mean followed by fruit flies, butteflies. ~~Banana variety Ngombe had signficantly 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 populations of banana fruit flies ~~populations~~ observed on FHIA17, Valery and Mysore. Other insects such as beetles and black ants were few (Figures 1 to 7).

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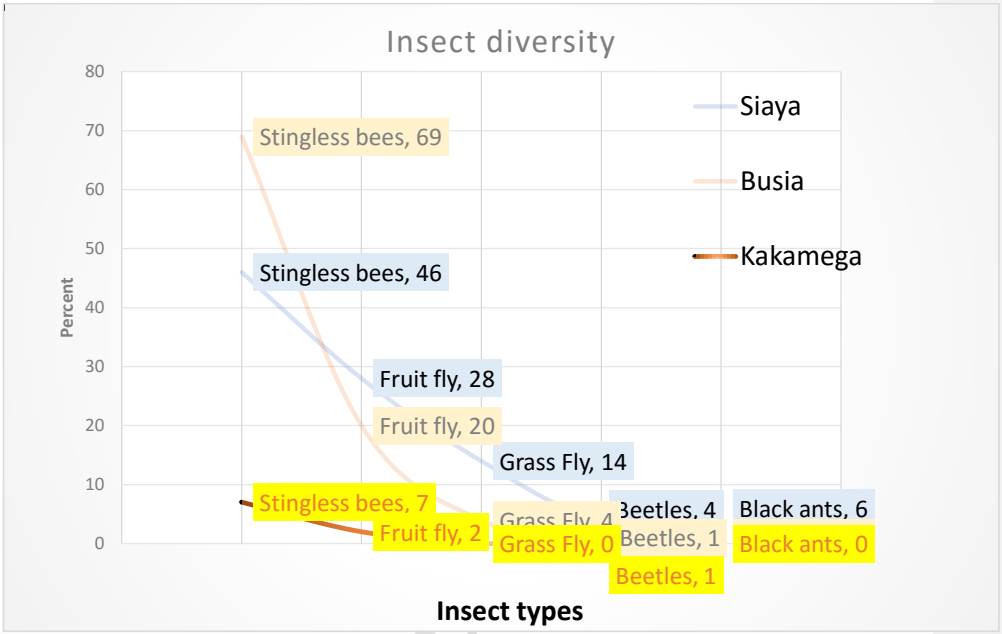

Figure 1: Percent diversity of insects associated with banana plants in BXW epidemic areas in Siaya, Busia and Kakamega counties

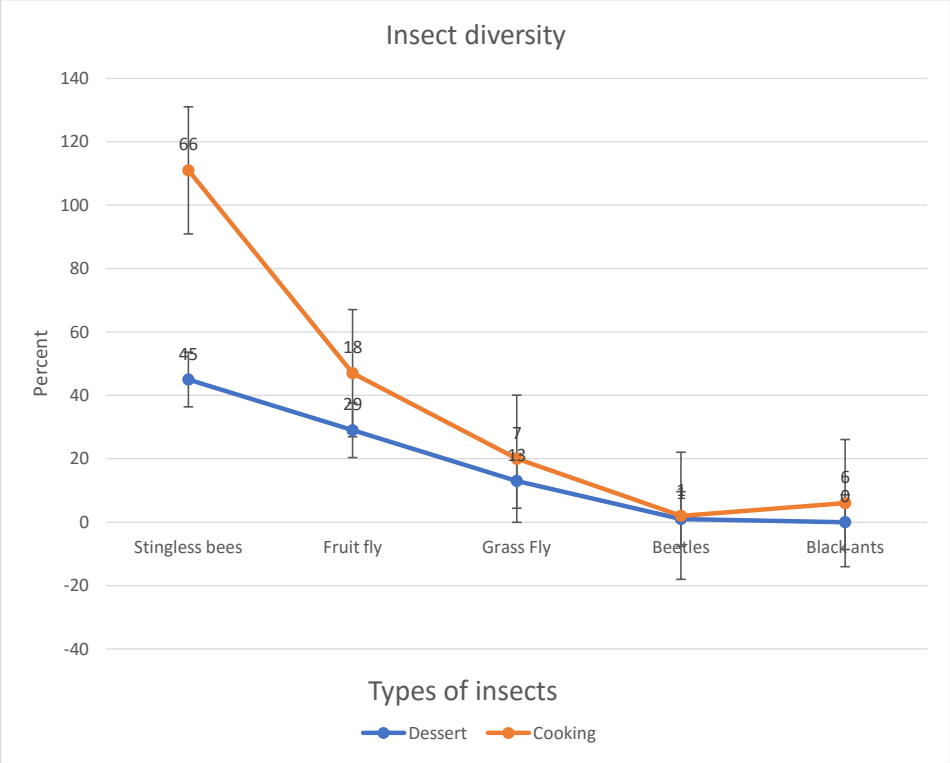

Figure 2: Diversity of insects associated with banana plants in BXW epidemic areas according to banana usage

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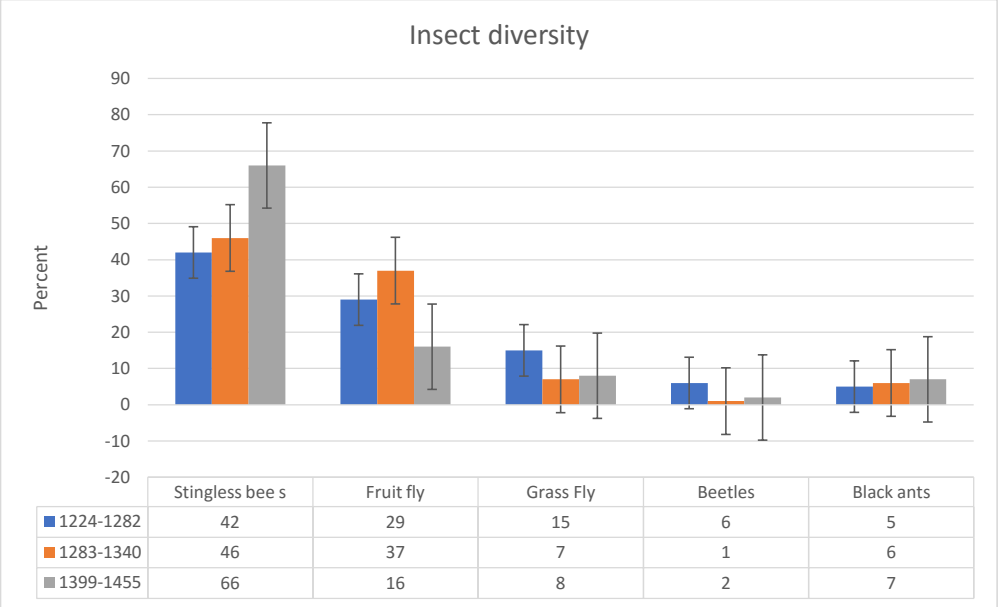
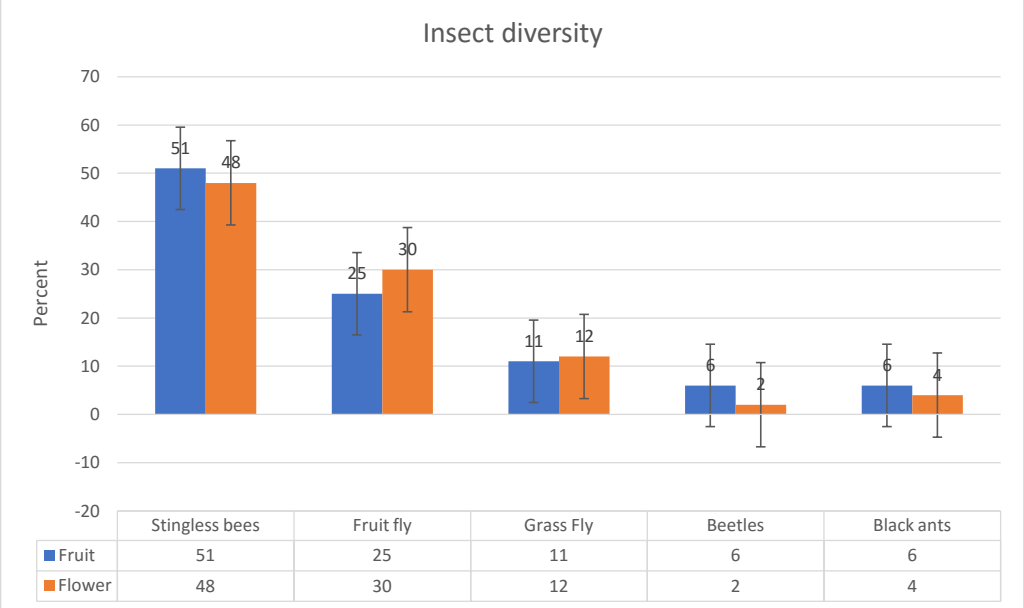


	Stingless bee s	Fruit fly	Grass Fly	Beetles	Black ants
1224-1282	42	29	15	6	5
1283-1340	46	37	7	1	6
1399-1455	66	16	8	2	7

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



	Stingless bees	Fruit fly	Grass Fly	Beetles	Black ants
Fruit	51	25	11	6	6
Flower	48	30	12	2	4

Figure 4: Percent insect diversity from flower or fruit ~~part~~ organ of the banana in BXWepidemisareas homonas wilt epidemic areas

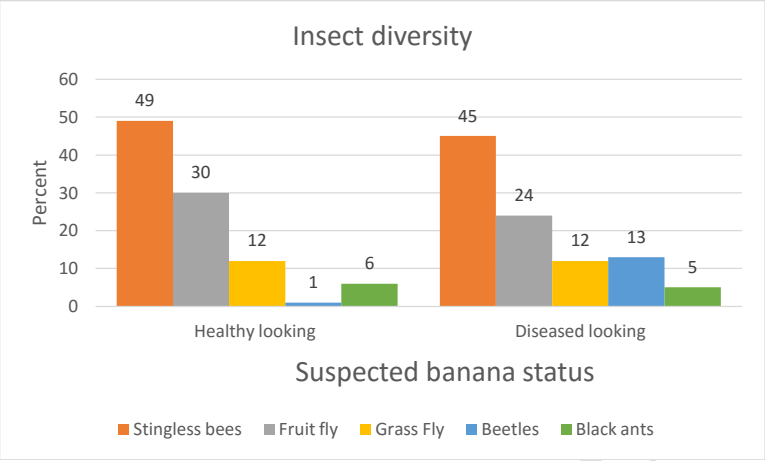


Figure 5: Percent insect diversity according to health status of banana plant in BXW epidemic areas

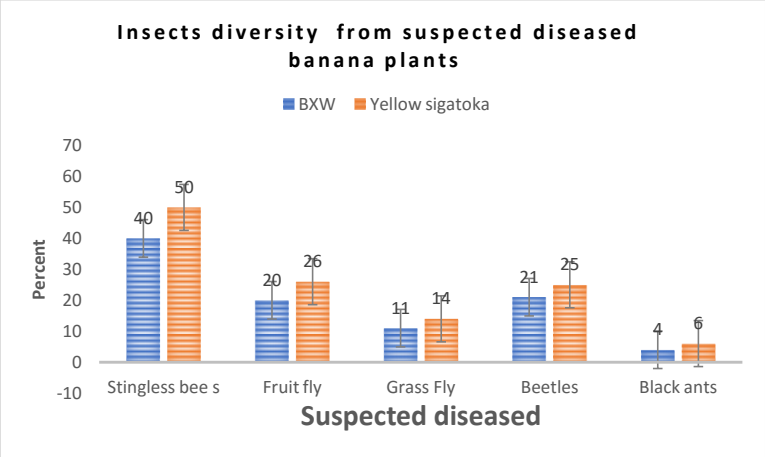


Figure 6: Percent diversity of insects fromr suspected diseased banana plants in BXW epidemic areas

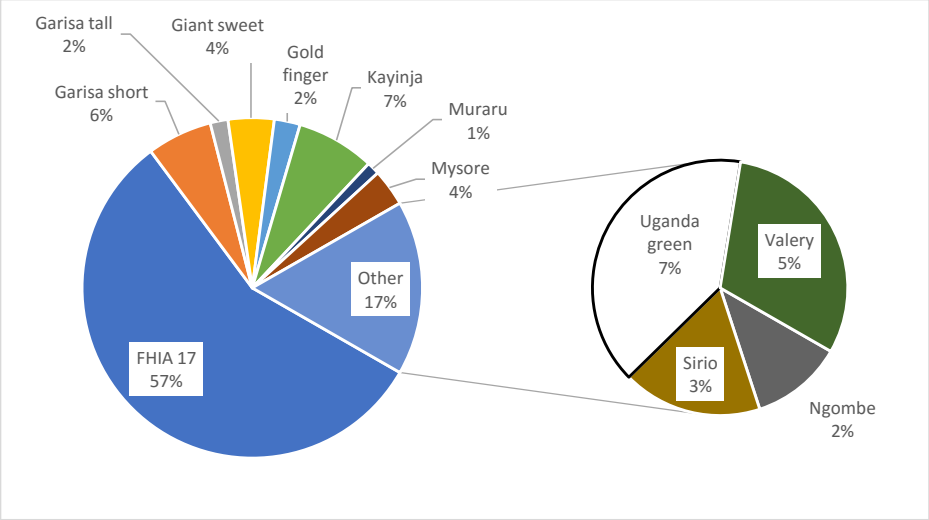


Figure 7: Percent diversity of insects from various banana cultivars in banana Xanthomonas wilt epidemic areas.

3. 2 Confimation of BXW in study areas

Banana *Xanthomonas* wilt disease was ~~positively~~ confirmed through PCR procedure ~~from isolates~~ of collected ~~-~~samples~~d~~ from the stud~~y~~ied area (Fig 8 and 9). The results of amplfication were positive with a mea~~m~~n 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~~ samles produced band~~s~~ ~~at~~ with a size of 650 bp ~~size with ladder M at 100bp~~ that cofirmed the presence of *Xanthomonas campestris* pv musacearum in ~~the~~ studied~~y~~ area(Fig. 8).

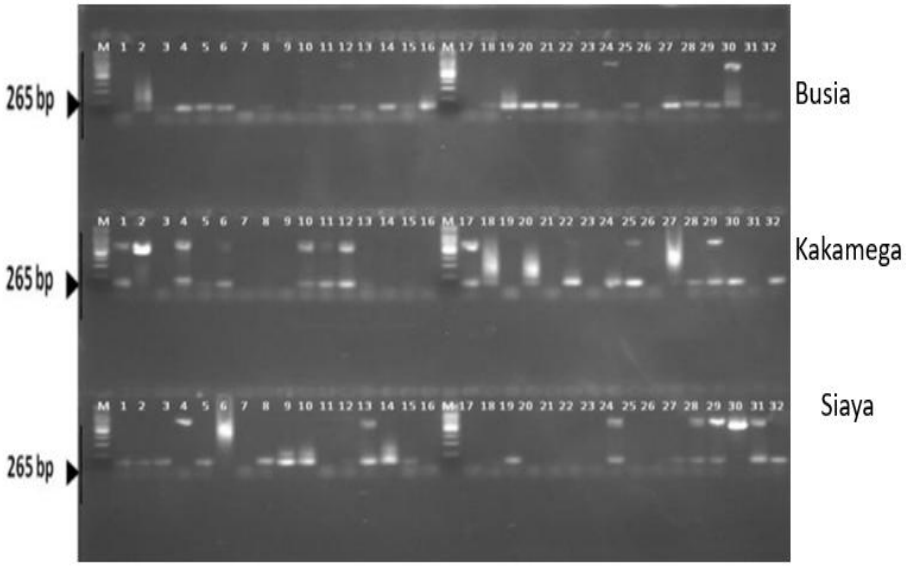


Fig. 8 : Gel images of 2minutes dipstick DNA capture kit isolates from banana infloresence in Busia, Kakamega and Siaya; at bp=265 Primer base pair, M=100bp Ladder

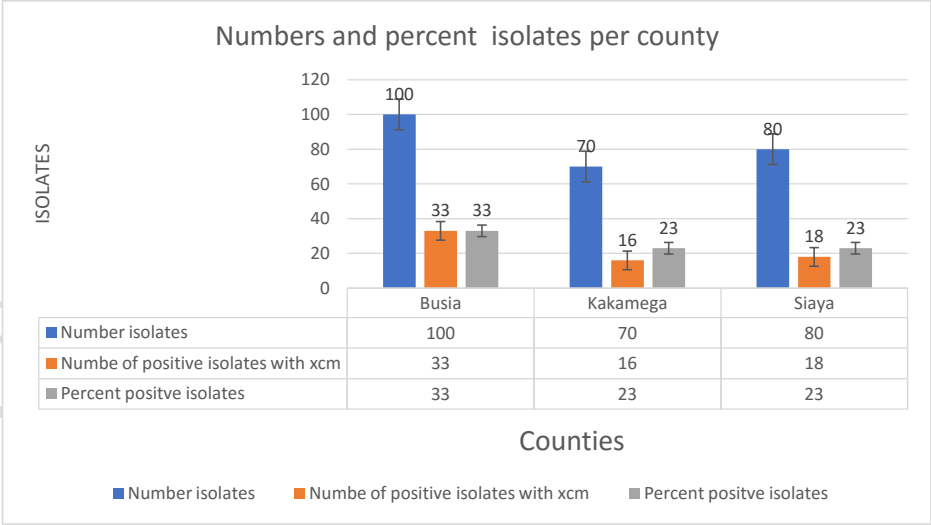


Figure 9: Positve PCR results of Xcm DNA isolates captured by 2 minutes dip stick from Busia, Kakamega and Siaya Counties in BXW epidemic areas

4. DISSCUSSION

4.1 Insect diversity and ~~abundance~~ frequency

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 most frequent~~highest~~ 48% followed by banana fruit flies 27% and grass fly 12%(Figures 1 and 6)**:**. These observations ~~concurs~~ agree with studies in Ethiopia and Uganda [17, 19, 21]. The ~~study confirmed~~ more insects were observed in low altitude areas of 1224 ~~masl~~ MASL ~~of 60% of the total insects populations observed~~ than in high altitudes areas(60% of the total populations insects) and ~~concurs~~ agrees with [22] that more insects population around the lower elevations of Lake Victoria at altitude 1135 ~~masl~~ MASL than at 1700 ~~masl~~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 confimed that there were more insects on the flowers and fruits, due to the fact that they collect nector as has been reported by other researchers [19, 23, 24]. Significantly more insects were observed in the banana dessert cultivars cavendish group ~~namely;~~including: Gros Michel, Valery, FHIA 17, Garisa and Gold finger cultivars than the cooking type Uganda green ~~concuring~~ agreement with studies in Ethiopia[19], that Dwarf Cavendish hosted the highest diverstity of insect families. Dessert banana had more nector, produce more pollen and

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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 variesties.

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~~ of insects associated with banana flowers may be hypothezised to provide opprtunity for picking Xcm from infected plants and transmitting to the non infected plants thereby acting as vectors.

There were diversified ~~and abundance of~~ inscects that were associated with banana plant in BXW in epidemic areas ~~namely~~ such as Bee, Grass Fly, Banana fruit fly, Fly, Wasp, Butterfly Beetles, Spider and Black ants. ~~More b~~Bees were recorded as dominant population followed by flies insects ~~these were insects that~~ due to feed on or collect nector~~, thus might account for the higher numbers~~. Conversly some insects like spiders may be concerned with trapping ~~some of these~~others insects like wasps, ants, flies and moths for their food and might have no direct activity with banana flowers thus ~~accounts for~~ their ~~numbers~~ numbers is low in the infloresence. The Drosophilidae, Apinae and Tephritidae families were most frequent in banana plantations.

The study area lied at altitude of 1224-1455~~masl~~ MASL. These are ~~areas~~ with warm climatical conditions and insects diversity and activity are high as confirmed by this study and ~~concurs with~~ previous studies [17, 19]. At lower altitude, ~~areas~~ below 1700~~masl~~ MASL there were more insects ~~abundance~~ than higher altitude

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~~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 nector 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~~ varieties and, this might be due to the higher sugar content in them as compared to the cooking banana varieties. ~~Banana varietal differences like some dessert~~ Dessert bananas may produce more pollen than the cooking varieties and may be attribute to more insects attraction than others. ~~Virous b~~Banana varieties has differences in their floral colours, some has 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~~s~~ ~~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~~ contact male bract and flower scars [24], futher more infection of Xcm may occurs via infected inflorescences and ~~mab 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~~ lesion due to Xcm pathogen, easily ooze out of infected inflorescences and

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most likely ~~the~~ insects pick up bacteria from these wounds mechanically, during feeding and or collecting nector [18]. As the insect carry their activities there are chances for them of picking the Xcm fron 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 insectcs involved in the floral activities it is advategious to practice debudding.

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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.

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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 Hondurus 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 hypothezised 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).

Comment [H22]: It has problems in terms of writing and sentence structure rerwiting

5. CONCLUSION

More insect divesity and abundance were observed in the study. Bees were the most abundant insects on banana plantation. Xcm was positvely 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

Comment [H23]: is not enough and desirable

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