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SPATIAL AND TEMPORAL DISTRIBUTION OF INSECT VECTORS OF *Xanthomonas campestris* pv. *musacearum* AND THEIR ACTIVITY ACROSS BANANA CULTIVARS GROWN IN RWANDA

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ABSTRACT

Insect vectors of *Xanthomonas campestris pv musacearum (Xcm)* have played a major role in long distance and plant to plant transmission of Xanthomonas wilt of banana (XW). The prevalence of insects has been reported to vary in space and time. Some banana cultivars have also been reported to attract more insect vectors of *Xcm* than others. The present study was conducted to determine the spatial and temporal distribution of insect vectors of *Xcm* and assess their activity across banana cultivars grown in Rwanda. The study was carried out in four banana growing areas of Rwanda selected on the basis of their altitude (i.e.Low, Medium and High). The Kivu Lake Border region was selected as a fourth site due to the high prevalence of XW. Insects were sampled in the four annual seasons (short dry, short rainy, long dry and long rainy) and at different times of the day. During sampling of insects, the incidence of XW-male bud infection was also recorded. Collected insects were immediately sorted into taxonomic groups and conserved in vials containing 70% ethanol for further identification to genus and species level. Five insect specimens in each taxon were put aside for the isolation of *Xcm* on their external body parts. There was a high prevalence of fruit flies, honey bees and other flies (in other families than Drosophilidae and Tephritidae) compared with wasps, ants and beetles. More insects were recorded in the low altitude area and during the long rainy season. These findings correlated with the observed high incidence of XW in the wet seasons. Incidence of floral infections was higher in the low altitudes declining with the increase in altitude, correlating with the decline in insect activity as altitude increased. The activity of insects on banana male buds varied among banana cultivars, with more activity on beer (AAA-East African Highland (EAH) and ABB types) and dessert banana cultivars compared with cooking or mixed use cultivars. Among the cooking types only 'Injagi' and its clone sets 'Barabeshya' and 'Incakara' attracted large insect populations. Banana cultivars 'Nkazikamwe' (cooking AAA-EAH), 'Impura' (beer AAA-EAH) and 'Ikinyangurube' (dessert AAA) possessed persistent male bracts and neuter flowers and were less attractive to flower visitors. These cultivars could be promoted in areas prone to insect vector infections. Timely and proper de-budding should be emphasized with special attention during the rainy seasons and for banana cultivars with non-persistent male buds.

Keywords: Xanthomonas wilt incidence, insect vectors, banana cultivars**,** growing areas and period

INTRODUCTION

Xanthomonas wilt of banana (XW) has become endemic in the east and central African region, since its first official reports in 2001 in the Democratic Republic of Congo and Uganda. The bacterial disease has drastically reduced banana productivity and is severely affecting farmer livelihoods (Carter *et al.,* 2010, Mbaka, *et al.,* 2009, Mgenzi *et al.,* 2006, Tushemereirwe et al., 2004; Mwangi, 2009; Ndungo et al, 2006) and (Reeder *et al*., 2007). No curative treatment has so far proven effective in controlling XW. Only cultural practices are recommended as preventive measures for the containment of the disease (Karamura *et al*., 2008). These are effective if sources of inoculum are removed and opportunities for spread are reduced or eliminated (Eden-Green, 2004 and Blomme *et al.,* 2005);). Insect vectors are one of the main mechanism of dispersal of *Xanthomonas campestris* pv. *musacearum* (*Xcm*)*,* the causal agent of XW (Tinzaara *et al.,* 2006; Yirgou *et al*.; 1974;Yirgou *et al*., 1968). Insects are thought to play a major role in long distance and plant to plant transmission of the disease (Buregyeya *et al*., 2008; Fiaboe *et al*., 2008; Shimelash *et al.,* 2008). There is a symbiotic relationship between bananas and some insect species based on the fact that bananas serve as source of food (nectar and pollen) for insects and in return, insects act as the pollinators of bananas (Willson *et al.,* 1996). In the search for nectar, insects spread bacterial diseases from plant to plant. XW transmission by insect vectors is influenced by ecological conditions and bio-physical factors including the predominant cultivars grown. Ecological conditions greatly influence the life history and distribution of insect species. Climate conditions affect insect biology and behavior. These are dictated by variations in altitude whereby prevailing temperature, humidity and rainfall regime are determining factors in insect population fluctuations throughout the year (Salin *et al*., 1999).

For example, altitudes above 1,700 masl are reported to have less insect vector activity and thus lower floral XW infections (Shimelash *et al.,* 2008) than lower altitudes. Bio-physical conditions also dictate distribution of crop cultivars. This is the case in Rwanda where different banana (*Musa* spp.) cultivars grow across a range of altitudes with different climatic and soil conditions. The predominant banana cultivars in a cropping system greatly influence the extent of insect transmission of XW. For example, higher incidences of insect mediated floral XW infections have been reported in areas dominated by 'Pisang awak' (*Musa* ABB genome) in East Africa and Ethiopia (Addis *et al.,* 2004; Blomme *et al.,* 2005). In Uganda, predominant insect species reported on banana inflorescences include fruit flies (Drosophilidae), stingless bees (*Plebeina denotti*) and grass flies (Chloropidae). Based on field experiments, *P. denotti* and grass flies were reported to possibly spread XW from infected to healthy plants (Tinzaara *et al.,* 2006). The spread of XW is influenced by the predominant insect species. For example, fruit flies are present in large numbers but spend most of their lives on a few banana mats and thus spread the disease over short distances, whereas larger insects (e.g. bees) visit larger geographical areas/ numbers of flowering banana plants each day and can spread the disease over long distances. Understanding of the dynamics of insect species in different environments can improve our understanding of the dynamics of Xanthomonas wilt. This would in turn help improve management of the disease. The present study aimed at investigating the spatial and temporal distribution of insect vectors of *Xcm* and their activity on inflorescences of different banana cultivars grown in Rwanda.

MATERIALS AND METHODS

This study was conducted in major banana growing areas as distributed across the three main ranges of altitude prevailing in Rwanda: (i) low (800- 1,400 masl), (ii) medium (1,450-1,650 masl) and (iii) high altitude (1,700-2,200 masl) (Table 1). Districts touching on Lake Kivu borders were given special attention due to high XW infection that has devastated bananas in the area. The Lake Kivu border has a medium altitude in the range of (1,410-1,647 masl) but a higher rainfall and somewhat different soils compared with the three districts in the other group within the medium altitudes (Table 1). Across the growing areas, increasing altitude is associated with increased annual rainfall, lower annual temperatures, higher humidity and different soil groups (Table 1). The study covered the four annual seasons in Rwanda during 2012. Data were collected once per week for four weeks of the typical month characteristic of the season.

For the short dry season (January-February) sampling was done in January, for the short rainy season (March-May), it was done in April, for the long dry season (June-August), it was done in July and for the long rainy season (September-December), the sampling was done in October. In each altitude category, three districts with Xanthomonas Wilt disease of banana (XW) were selected and a highly infected Sector (administrative division below District) chosen for data collection (Table 1). Four XW-infected banana fields were selected in each Sector for the collection of insects from three plants per banana cultivar selected based on cultivar availability in each field. It is worthy to note that different plants of the same cultivar, preferably on the same mat/stool, were considered for each sampling time. Sweep nets were used to capture flying insects on the male inflorescence of individual plants. Other insects such as ants and small beetles were handpicked and put into vials. It is important to note that separate nets were used to collect flying insects that were used for *Xcm* isolation so as to avoid *Xcm* contamination among insects from different taxa.

This was done by waving insect nets in the air around male flowers of individual plants. For this purpose, bees, flies and wasps were scared off the male buds of the plants by a simple touch and individual insects were trapped into the net as they tried to escape. In contrast, fruit flies did not fly far away from the floral parts and were easily trapped by shaking them off into the net. To understand the distribution of insect species during the day, insect sampling was done on the same day between: i) 7 and 9 am; ii) 10 am and 12 noon; iii) 1 and 3 pm and iv) 4 and 6 pm. The weather conditions at the time of sampling were also noted and sampling during extreme weather conditions such as during or soon after rains was avoided. Captured insects were immediately sorted into their respective taxonomical orders, counted and put into labeled vials containing 70% ethanol for further identification to family and species level. Identification to family level was performed in the entomology laboratory of the Higher Institute of Agriculture and Animal Husbandry (ISAE-Busogo) in Rwanda. The identification exercise was supported by use of electron microscope and a data base for insect species available on the internet (i.e. Canadian Journal of Arthropod Identification 1911-2173; http:// bugguide.net/node/view and Castner *et al.,* 2000). Five specimens of insects that were morphologically similar (e.g. five bees of the same species) were put together in vials for isolation of *Xcm* from their external body parts.

Bacterial isolation was done by washing the insect body with distilled water. Ten µL of the resulting suspension was cultured by spreading in a petri-dish containing a semi-selective medium of Cellobiose Cephalexin Agar composed of yeast extract (1 gL^{-1}), glucose (1 gL^{-1}), peptone (1 gL^{-1}), NH_4Cl (1 gL^{-1}), $MgSO_4.7H_2O$ (1 gL^{-1}), K_2HPO_4 (3 gL^{-1}), beef extract (1 gL^{-1}), cellobiose (10 gL^{-1}), agar (14 gL^{-1}), cephalaxin (40 mg), 5-fluorouracil (10 mg) and cycloheximide (120 mg). Cultures were incubated for a period of 72 hours at 25°C (Mwangi *et al.,* 2007; Tripathi *et al.,* 2007). The resulting colonies were compared with pure cultures isolated from *Xcm*-infected banana plants and used in pathogenicity tests that were performed by inoculating healthy banana tissue culture plantlets. In the fields selected for sampling, the morphology of the male bud (persistence/non-persistence of the male bud bracts) and XW incidence of plants showing signs characteristic of insect vector transmission (i.e. wilting of the male bud) on three plants, purposively selected per cultivar, were also recorded. The data (for XW incidence) were square root transformed; GenStat 11th Edition (VSN International Ltd, 2008) statistical package used to generate the Analysis of variance (ANOVA) and the means separated using the Least Significant Difference at 5%.

RESULTS

Investigations on the current banana cultivars, their distribution and the prevalence of XW-floral infection across the study areas formed a basis to determine the spatial and temporal distribution of insect vectors of *Xcm*.

Table 1. Description of the study areas

Category of altitude	District	Sector	Altitude (masl)	Type of soil	Annual rainfall (mm)	Mean Annual temperature (^{0}C)	Relative Humidity (%)
Low	1.Kayonza	Mukarange	1,300	Ferrisols	800-1,000	25	60
	2.Gatsibo	Kiziguro	1,400	Ferrisols	800-1,000	24	70
	3.Nyagatare	Tabagwe	1,380	Ferrisols	800-1,100	25	50
Medium	1.Ruhango	Bunyogombe	1,600	Granites	1,000-1,100	21	70
	2.Huye	Mukura	1,650	Paragneiss	1,000-1,100	20	80
	3.Gisagara	Save	1,600	Orthogneis	1,000-1,100	20	75
High	1.Rulindo	Rusiga	1,887	Granites	1,680-1,970	17	90
	2.Gakenke	Nemba	2,112	Granites	1,700-2,200	16	92
	3.Burera	Kinoni	2,167	Volcanic	1,800-2,500	13.2	95
Kivu lake border (Medium)	1.Rubavu	Rugerero	1,600	Volcanic	1,200-1,350	21	75
	2.Rutsiro	Mushyonyi	1,642	Granites	1,200-1,350	20	85
	3.Karongi	Bwishyura	1,596	Ferrisols	1,200-1,350	20	80

Banana cultivar distribution in the study areas

A total of 27 banana cultivars grouped into four categories (based on their main end use); dessert, beer, cooking and multipurpose bananas, were identified in this study (Table 2). The most dominant banana cultivars across all the study sites were the cooking (41%) and the beer (33%) banana cultivars, while the dessert and multipurpose banana cultivars represented only 19% and 7% of the cultivars, respectively (Table 3). Most of the cooking (45%) banana cultivars were observed in the low altitudes zone. The cultivars in the other use groups were more or less evenly distributed across the altitudes (Table 2, Table 3). Though the dessert banana cultivars 'Kamaramasenge' elsewhere known as 'Sukali ndizi', 'Gros Michel', 'Igisukari'; and the beer cultivar 'Kayinja' (Pisang awak) and other two cultivars ('Kivuvu' (Bluggoe) and 'Gisubi') in the same clone set were recorded from all the studied altitudes, their occurrence was sporadic. The beer bananas, 'Impura' and 'Umuzibo' both belonging to the *Musa* AAA genotype were found exclusively grown in the Lake Kivu Border region (medium altitude), while the beer banana 'Nyiramabuye' (*Musa* AAA) was only grown in Ruhango District (medium altitude). The beer bananas 'Ingumba', 'Ingenge' and 'Ingaju' were only found

Table 2. Distribution of banana cultivars in the study areas and their description based on vernacular names, synonyms, the morphology of the male bud, genome group and the main use. Abbreviations for districts are: Kay: Kayonza, Gat: Gatsibo, Nya: Nyagatare, Ruh: Ruhango, Gis: Gisagara, Huy: Huye, Rub: Rubavu, Rut: Rutsiro, Kar: Karongi, Rul: Rulindo, Gak: Gakenke, Bur: Burera. The signs '+' and '-' means the presence and the absence of the banana variety, respectively, in the study area.

Description of banana cultivars					Distribution of banana cultivars in the study areas											
Cultivar	Synonyms (farmers)	Morphology of male bud	Genome group	Main Use	Low altitude			Medium altitude			Kivu Lake border			High altitude		
					Kay	Gat	Nya	Ruh	Gis	Huy	Rub	Rut	Kar	Rul	Gak	Bur
'Barabeshya'	'Injagi', 'Incakara'	Not persistent	AAA	Cooking	-	-	-	+	+	+	+	+	+	+	+	+
'FHIA17'		Not persistent	AAAA	Multiple	+	+	+	+	+	+	+	+	+	+	+	+
'FHIA25'		Not persistent	AAB	Multiple	+	+	+	+	+	+	+	+	+	+	+	+
'Gros Michel'	'Mbogoya'	Not persistent	AAA	Dessert	+	+	+	+	+	+	+	+	+	+	+	+
'Igisukari'		Not persistent	AAA	Dessert	+	+	+	+	+	+	+	+	+	+	+	+
'Ikinyangurube'	Dwarf Cavendish	Persistent	AAA	Dessert	-	-	-	-	-	-	+	+	+	-	-	-
'Impura'	'Bakenga'	Persistent	AAA	Beer	-	-	-	-	-	-	+	+	+	-	-	-
'Incakara'	'Injagi', 'Barabeshya' Impundahunde	Not persistent	AAA	Cooking	-	-	-	-	-	-	+	+	+	-	-	-
'Ingagara'		Not persistent	AAA	Cooking	+	+	+	-	-	-	-	-	-	-	-	-
'Ingaju'		Not persistent	AAA	Beer	+	+	+	-	-	-	-	-	-	-	-	-
'Ingame'	'Indaya', 'Yangambi Km^5'	Not persistent	AAA	Beer	-	-	-	+	+	+	+	+	+	+	+	+
'Ingenge'		Not persistent	AAA	Beer	+	+	+	-	-	-	-	-	-	-	-	-
'Ingenge'		Not persistent	AAA	Cooking	+	+	+	-	-	-	-	-	-	-	-	-
'Ingumba'		Not persistent	AAA	Beer	+	+	+	-	-	-	-	-	-	-	-	-
'Injagi'	'Incakara','Barabeshya'	Not persistent	AAA	Cooking	+	+	+	-	-	-	+	+	+	-	-	-
'Intokatoke'	'Inyarwanda'	Not persistent	AAA	Cooking	+	+	+	+	+	+	+	+	+	+	+	+
'Intuntu'		Not persistent	AAA	Beer	+	+	+	+	+	+	+	+	+	+	+	+
'Intusi'		Not persistent	AAA	Cooking	+	+	+	-	-	-	-	-	-	-	-	-
'Inzirabahima'		Not persistent	AAA	Cooking	+	+	+	-	-	-	-	-	-	-	-	-
'Inzirabushera'		Not persistent	AAA	Cooking	+	+	+	-	-	-	-	-	-	-	-	-
'Kamaramasenge'	'Kamara', Sukali ndizi	Not persistent	AAB	Dessert	+	+	+	+	+	+	+	+	+	+	+	+
'Kayinja' (Pisang awak)	'Kivuvu'(Bluggoe), 'Gisubi'	Not persistent	ABB	Beer	+	+	+	+	+	+	+	+	+	+	+	+
'Kibuzi'		Not persistent	AAA	Cooking	+	+	+	-	-	-	-	-	-	-	-	-
'Nkazikamwe'	'Mbwazirume', 'Kiryumukungu'	Persistent	AAA	Cooking	+	+	+	+	+	+	+	+	+	+	+	+
'Nyiramabuye'		Not persistent	AAA	Beer	-	-	-	+	-	-	-	-	-	-	-	-
'Poyo'	'Cavendish'	Not persistent	AAA	Dessert	+	+	+	+	+	+	+	+	+	+	+	+
'Umuzibo'		Not persistent	AAA	Beer	-	-	-	-	-	-	+	+	+	-	-	-

Table 3. Distribution of banana cultivars across the districts based on end use (dessert, beer, cooking and multipurpose) and distribution among altitude groups.

Banana Cultivars groups	Distribution across all districts (%)	Distribution per altitude group (%)			
		Low altitude	Medium altitude	High altitude	Lake Kivu Border
Dessert	19	25	25	25	25
Beer	33	29	24	18	29
Cooking	41	45	15	15	25
Multipurpose	7	25	25	25	25

grown in the low altitude region. The cooking banana cultivars 'Ingenge', Inzirabushera', 'Inzirabahima', 'Intutsi', 'Kibuzi' and 'Ingagara' were also only observed in the low altitudes. Of all the 27 banana cultivars, only three cultivars 'Impura' (also known as 'Bakenga'), 'Ikinyangurube' and 'Nkazikamwe' (elsewhere known as 'Mbwazirume') had persistent male bud bracts (Table 2). All the banana cultivars sampled except 'Kamaramasenge' (*Musa* AAB), FHIA25 (*Musa* AAB), FHIA (*Musa* AAAA) and 'Kayinja' (*Musa* ABB) belonged to the *Musa* AAA genomic group (Table 2).

Incidence of XW-floral infection in the study areas

The incidence of Xanthomonas Wilt of banana based on floral symptoms varied with the altitude, season and cultivars. However, no case of XW-male bud infection was recorded in the high altitude. Relatively higher incidences were recorded in Lake Kivu Border region and in the low altitudes. The incidence seemed to vary with cultivar distribution within each study area (agro-ecology). In the Lake Kivu Border region, the beer banana 'Ingame' (*Musa* AAA) elsewhere known as 'Yangambi Km5, showed the highest (5.66%) XW-male bud infection.

Other highly susceptible banana cultivars in this area were the beer banana 'Kayinja' (*Musa* ABB), the dessert bananas 'Kamaramasenge' (*Musa* AAB) and 'Poyo' (*Musa* AAA), and the cooking banana 'Incakara' (*Musa* AAA). In the low altitude, the beer banana 'Ingumba' (*Musa* AAA) was recorded with the highest incidence (5.79%) of male bud infection (Table 4). Xanthomonas Wilt-male bud infection was recorded across all annual seasons with higher prevalence observed during the rainy seasons.

Table 4. Incidence of XW-floral infection (square root transformed data) across major banana cultivars grown in Rwanda, respective cultivar genomes and end use in the four agro-ecologies and during the four annual seasons: Long dry Season (LdS), Long rainy Season (LrS), Short dry Season (SdS) and Short rainy Season (SrS).: Low altitude (Low), Medium, High altitude (High), and Lake Kivu Border (LKB) with altitude (Medium). Means with same letter within a row are not significantly different at P< 0.05.

Cultivar	Genome	Use	XW incidence (%) across agro-ecologies				XW incidence (%) across annual seasons			
			Low	Medium	High	LKB	LdS	LrS	SdS	SrS
'Barabeshya'	AAA	Cooking	-	2.00b	0a	0a	0a	1.17b	0a	0.83b
'FHIA17'	AAAA	Multipurpose	2.83b	0a	0a	0a	0a	1.17b	0.83b	0.83b
'FHIA25'	AAB	Multipurpose	0.83a	0a	0a	0b	0a	0.83b	0a	0a
'Gros Michel'	AAA	Dessert	2.83b	0a	0a	4.27c	0a	2.83c	2.00b	2.27b
'Igisukari'	AAA	Dessert	2.49b	0a	0a	2.49b	0a	1.66b	1.66b	1.66b
'Ikinyangurube'	AAA	Dessert	-	-	-	0	0a	0a	0a	0a
'Impura'	AAA	Beer	-	-	-	0	0a	0a	0a	0a
'Incakara'	AAA	Cooking	-	-	0a	5.10b	0.83a	1.66b	1.17a	1.44b
'Ingagara'	AAA	Cooking	4.27	-	-	-	0.83a	1.44b	0.83a	1.17ab
'Ingaju'	AAA	Beer	1.66	-	-	-	0a	0.83b	0a	0.83b
'Ingame'	AAA	Beer	-	1.65b	0a	5.66c	1.17a	2.49b	1.17a	2.49b
'Ingenge'	AAA	Beer	3.59	-	-	-	1.33a	1.17b	0.83ab	0.83b
'Ingenge'	AAA	Cooking	5.39	-	-	-	0a	1.44b	1.17b	1.44b
'Ingumba'	AAA	Beer	5.79	-	-	-	0.82a	1.87b	1.44b	1.66b
'Injagi'	AAA	Cooking	5.07	-	-	3.31b	0.82a	2.00b	0.83a	1.66b
'Intokatoke'	AAA	Cooking	2.00a	-	-	-	0.83a	1.66b	1.17b	1.44b
'Intuntu'	AAA	Beer	4.00b	0a	0a	4.95c	2.00a	2.61b	2.00a	2.35ab
'Intutsi'	AAA	Cooking	4.87	-	-	-	0.83a	1.44b	1.17ab	1.44b
'Inzirabahima'	AAA	Cooking	2.49	-	-	-	0a	0.83b	0.83b	0.83b
'Inzirabushera'	AAA	Cooking	2.00	-	-	-	0a	1.00c	0.42ab	0.83bc
'Kamaramasenge'	AAB	Dessert	5.31c	1.66b	0a	5.10c	1.66a	4.36d	2.35b	3.70c
'Kayinja'	ABB	Beer	5.10c	2.49b	0a	5.10c	1.66a	4.15d	3.174b	3.701c
'Kibuzi'	AAA	Cooking	3.31	-	-	-	0.83a	0.83a	0.83a	0.83a
'Nkazikamwe'	AAA	Cooking	0a	0a	0a	0.81b	0a	0a	0.83b	0a
'Nyiramabuye'	AAA	Beer	-	4.92	-	-	0a	2.05b	1.44b	1.44b
'Poyo'	AAA	Dessert	3.44c	0.83b	0a	5.18d	1.17a	3.92c	2.00b	2.35b
'Umuzibo'	AAA	Beer	-	-	0a	3.44b	0a	1.44c	0.83b	1.17bc
LSD						0.27				0.55
Cv%						86.5				17.8

The highest incidences (4.36% & 4.15% for 'Kamaramasenge' and 'Kayinja', respectively) were recorded during the long rainy season (LrS). Second to this were incidence levels noted during the short rainy season (SrS) (3.70) for both the varieties 'Kamaramasenge' (*Musa* AAB) and 'Kayinja' (*Musa* ABB), respectively (Table 4).

Identification of insect species visiting banana male buds and assessment of their ability to carry Xcm.

Seventeen insect species were collected across the different banana cultivars grown in the different agro-ecologies. Of these, there was two species of bees (Hymenoptera: Apidae), nine species of flies (Diptera: Drosophilidae, Tephritidae , Lonchaedae, Muscidae, Neriidae and Sarcophagidae), two species of wasps (Hymenoptera: Vespidae), three species of beetles (Coleoptera: Nitudulidae, Tenebrionidae, Staphylinidae) and one species of ant (Hymenoptera: Formicidae) (Table 5). *Xanthomonas campestris* pv. *musacearum* was isolated from the external body parts of all groups of insects collected from banana plants.. Light yellow, mucoid bacterial colonies characteristic of *Xcm* were observed to grow on culture media after plating 10 µl of suspension from insect bodies on a semi-selective media. However, fewer ants and beetles compared with the other insect groups carried the bacteria (Table 5). It is worthy to note that 'Fruit fly' will be used here as a common name for flies in the families 'Tephritidae and Drosophilidae' while other flies will indicate true flies in the families other than Tephritidae and Drosophilidae.

Activity of insect species across agro-ecological zones

All the identified insect species were observed across all the study areas. However, their prevalence differed. More insects were collected from the low altitude and the Lake Kivu Border region than in mid and high altitude zones (Figures 1-8). Among them the fruit flies dominated. Bee species (Hymenoptera: Apidae) came in second position in terms of number while the true fly species took the third position. Wasps, ants and beetles were present in small numbers and the trend for the proportion of insect prevalence was almost the same across all the study areas (Figures 1-8).

Table 5. Taxonomical identification of collected insects and the proportion (%) of them that carried *Xcm* on their external body parts in relation to the season of the year when they were captured.

Taxonomical Identification				Proportion (%) of typical colonies of Xcm isolated from external body parts of insects			
Common name	Order	Family	Species	Short dry	Short rainy	Long dry	Long rainy
1.Fruit fly	Diptera	Tephritidae	*Ceratitis rosaKarsch*	40±3.4	60±3.5	20±3.2	80±2.8
2.Bee	Hymenoptera	Apidae	*Apis sp*	40±3.4	60±3.5	20±3.2	80±2.8
3.Bee	Hymenoptera	Apidae	*NI	20±3.4	40±3.5	40±3.2	100±2.8
4. Fruit fly	Diptera	Drosophilidae	*Drosophila sp.*	40±3.4	60±3.5	20±3.2	80±2.8
5. Fruit fly	Diptera	Drosophilidae	*Zaprionus sp.*	60±3.4	20±3.5	40±3.2	100±2.8
6. Fruit fly	Diptera	Drosophilidae	*Leucophanga sp.*	40±3.4	40±3.5	20±3.2	60±2.8
7.Fly	Diptera	Lonchaedae	*Silba sp.*	20±3.4	60±3.5	40±3.2	80±2.8
8.Fly	Diptera	Muscidae	*Neomyia rudissima (Loew)*	40±3.4	40±3.5	40±3.2	100±2.8
9.Fly	Diptera	Neriidae	*Carpophilus sp.*	20±3.4	20±3.5	40±3.2	80±2.8
10.Fly	Diptera	Sarcophagidae	*Sarcophaga sp.*	40±3.4	40±3.5	20±3.2	60±2.8
11.Fly	Diptera	Muscidae	*Musca domestica*	40±3.4	60±3.5	20±3.2	80±2.8
12.Wasps	Hymenoptera	Vespidae	*NI*	20±3.4	20±3.5	40±3.2	40±2.8
13.Wasps	Hymenoptera	Vespidae	*NI*	20±3.4	0±3.5	40±3.2	60±2.8
14.Ant	Hymenoptera	Formicidae	*NI*	20±3.4	20±3.5	0±3.2	20±2.8
15.Beetle	Coleoptera	Nitidulidae	*NI*	0±3.4	20±3.5	20±3.2	20±2.8
16.Beetle	Coleoptera	Tenebrionidae	*NI*	20±3.4	20±3.5	0±3.2	20±2.8
17.Beetle	Coleoptera	Staphylinidae	*NI*	20±3.4	0±3.5	20±3.2	20±2.8
Average				29.4±3.4	34.1±3.5	25.9±3.2	63.5±2.8

**NI: Not identified*

Variation of insect activity with time of the day

A highly significant difference (P < 0.001) was observed in the population of insect species collected throughout the four times of day and in all the three altitude zones (Figures 1 – 4). In all altitudes, fruit flies were more dominant, followed by bees and other flies (Figures 1 – 4). The other insects (wasps, ants and beetles) were relatively less prevalent and active on banana inflorescences in all surveyed areas in Rwanda (Figures 1- 4). Fruit flies were more prevalent in the morning hours (7 am to 9 am),

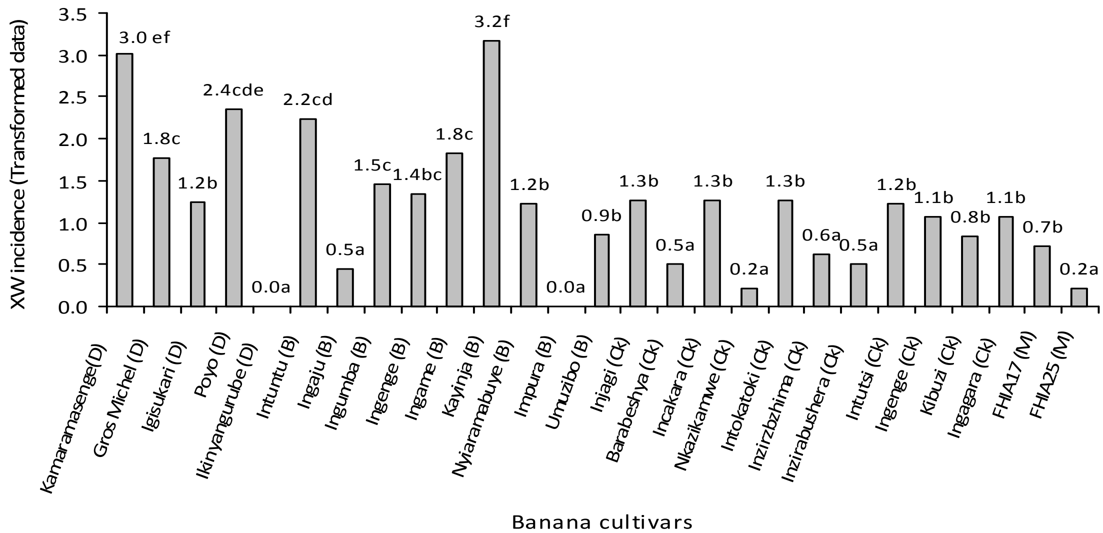

Figure 1. Xanthomonas wilt (XW) incidence (square root transformed data) with male bud infection across banana cultivars grown in Rwanda. Means followed by the same letter are not significantly different at $p \leq 0.05$

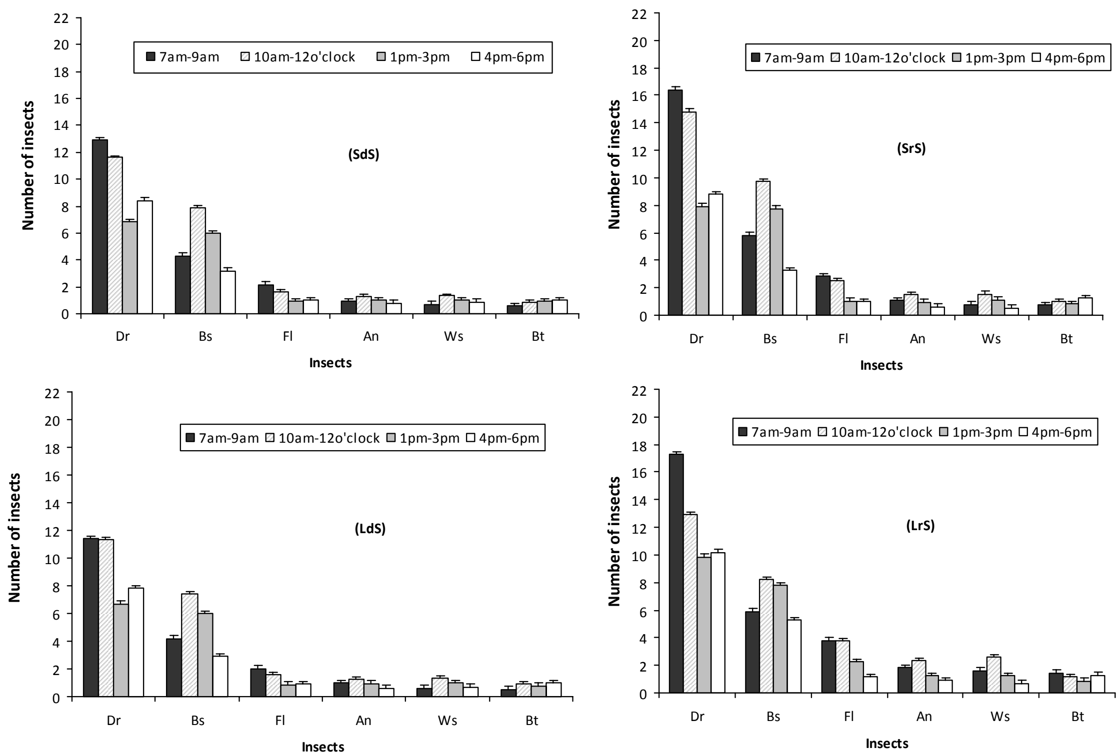

Figure 2. Insect activity during different time of the day in the High Altitude zone during the Short dry Season (SdS), Short rainy Season (SrS), Long dry Season (LdS).Error bars indicate the SEM. Letters at the 'X' axis stand for: (Dr) Drosophilidae, (Bs) Bees, (Fl) Flies, (An) Ants, (Ws) Wasps and Beetles (Bt). The error bars indicate the Standard Error of Means

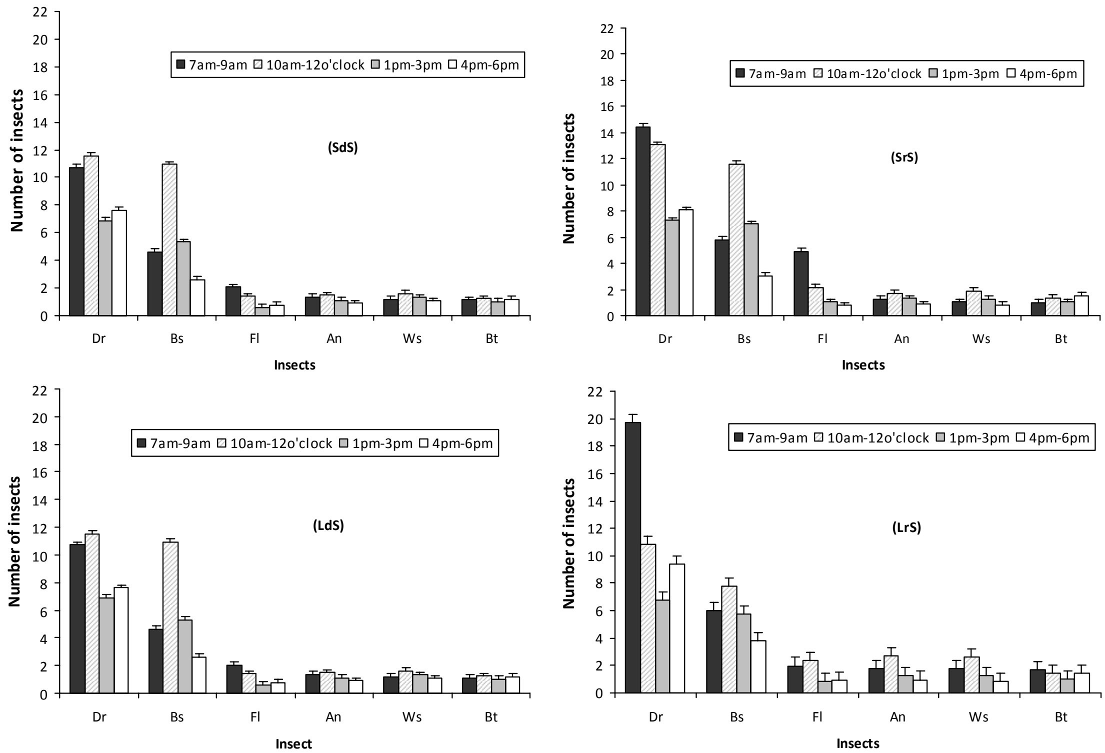

Figure 3. Insect activity during different time of the day in the Medium Altitude during the Short dry Season (SdS), Short rainy Season (SrS), Long dry Season (LdS) and the Long rainy Season (LrS). Error bars indicate the SEM. Letters at the 'X' axis stand for: (Dr) Drosophilidae, (Bs) Bees, (Fl) Flies, (An) Ants, (Ws) Wasps and Beetles (Bt). The error bars indicate the Standard Error of Means

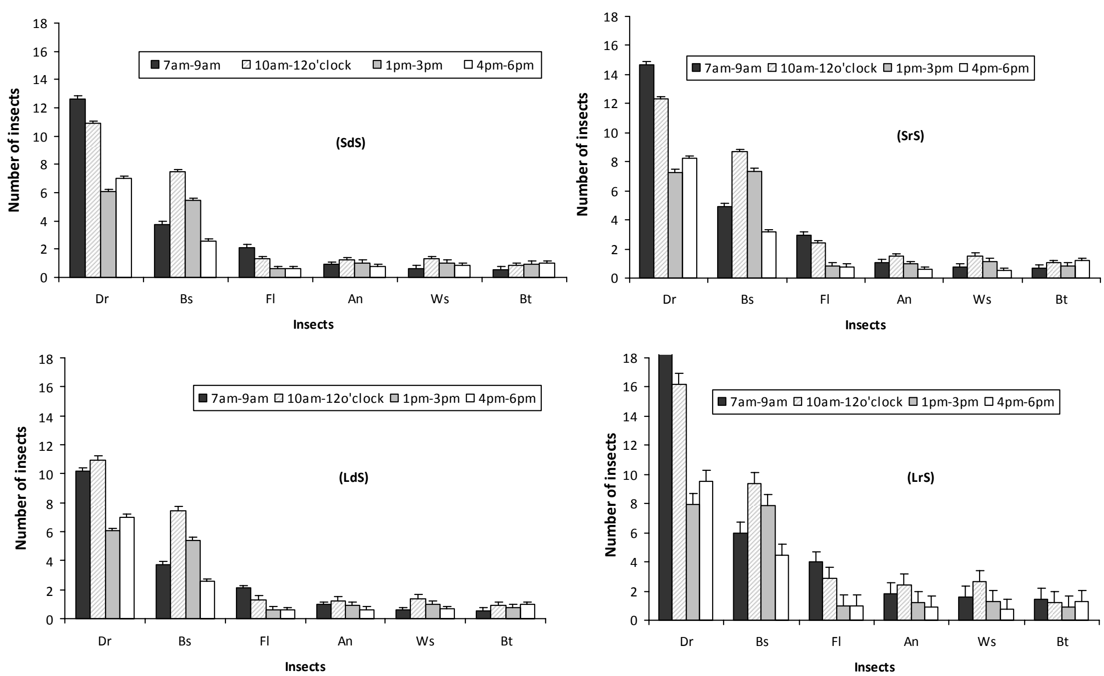

Figure 4. Insect activity during different time of the day in the Kivu Lake Border during the Short dry Season (SdS), Short rainy Season (SrS), Long dry Season (LdS) and the Long rainy Season (LrS). Error bars indicate the SEM. Letters at the 'X' axis stand for: (Dr) Drosophilidae, (Bs) Bees, (Fl) Flies, (An) Ants, (Ws) Wasps, (Bt) Beetles.The error bars indicate the Standard Error of Means

declining from mid-morning (10 am) to the late afternoon (3 pm) and rising slightly in the evening (4 pm to 6 pm) (Figures 1- 4). Higher populations of flies were also recorded in the morning, declining during the day. This trend was consistent in all agro-ecologies and seasons (Figures 1 – 4).

However, the difference between the number of flies captured in the morning and in the period ranging from mid-morning to mid-day was not significantly different (P< 0.005). Similarly, no significant differences were noted between numbers of flies collected in the afternoon (1 pm-3 pm) or in the evening (4pm-6pm) (Figures 1- 4). Bees were less active on banana inflorescences in the morning (7 am – 9 am), peaking between 10 am and 12 noon, and declining towards the evening (Figures 1 – 4). The average number of wasps, ants and beetles per banana plant was in general relatively low across all altitudes (Figures 1 – 4). Wasps and ants were observed to be higher in the morning (7 am-9 am) while beetles were more active during the morning and evening (7 am-9 am and 4 pm-6 pm) (Figures 1-4).

Activity of insect species across seasons

Prevalence of insects was significantly different (P < 0.001) between the annual seasons. Lower insect numbers were recorded in the long dry season, while the largest number occurred during the long rainy season (Figures 1 –8). During rainy days the population of ants doubled compared with fine days with excessively high activity during early and late evening. Flies were also more active during rainy days compared to sunny days. No correlation was observed between bee activity and weather patterns. Similarly the activity of beetles was consistent across seasons.

Activity of insect species on banana cultivars

Insect activity (based on the population of insect species collected) on male buds of different banana cultivars (dessert, beer, cooking and multipurpose) grown in the four agro-ecologies of Rwanda, differed significantly (P<.001). Beer and dessert banana cultivars in general attracted more insects compared with cooking and multipurpose banana genotypes (Figures 5-8). The beer banana 'Kayinja' (*Musa* ABB), the dessert banana 'Kamaramasenge' (*Musa* AAB) and the East African Highland cooking banana 'Injagi' (*Musa* AAA), were the leading cultivars in attracting insects within their respective use groups and this was consistent in all altitudes (Figures 5- 8).

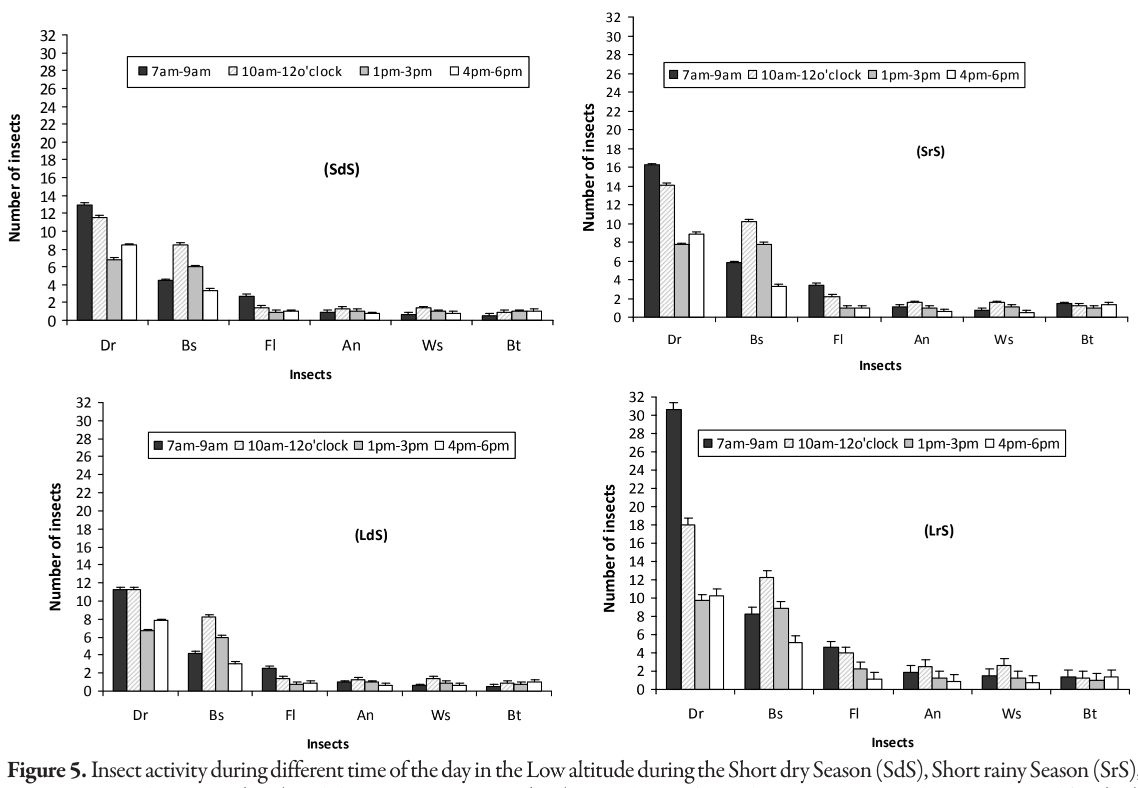

Figure 5. Insect activity during different time of the day in the Low altitude during the Short dry Season (SdS), Short rainy Season (SrS), Long dry Season (LdS) and the Long rainy Season (LrS). Error bars indicate the SEM. Letters at the 'X' axis stand for: (Dr) Drosophilidae, (Bs) Bees, (Fl) Flies, (An) Ants, (Ws) Wasps, (Bt) Beetles. The error bars indicate the Standard error of Means

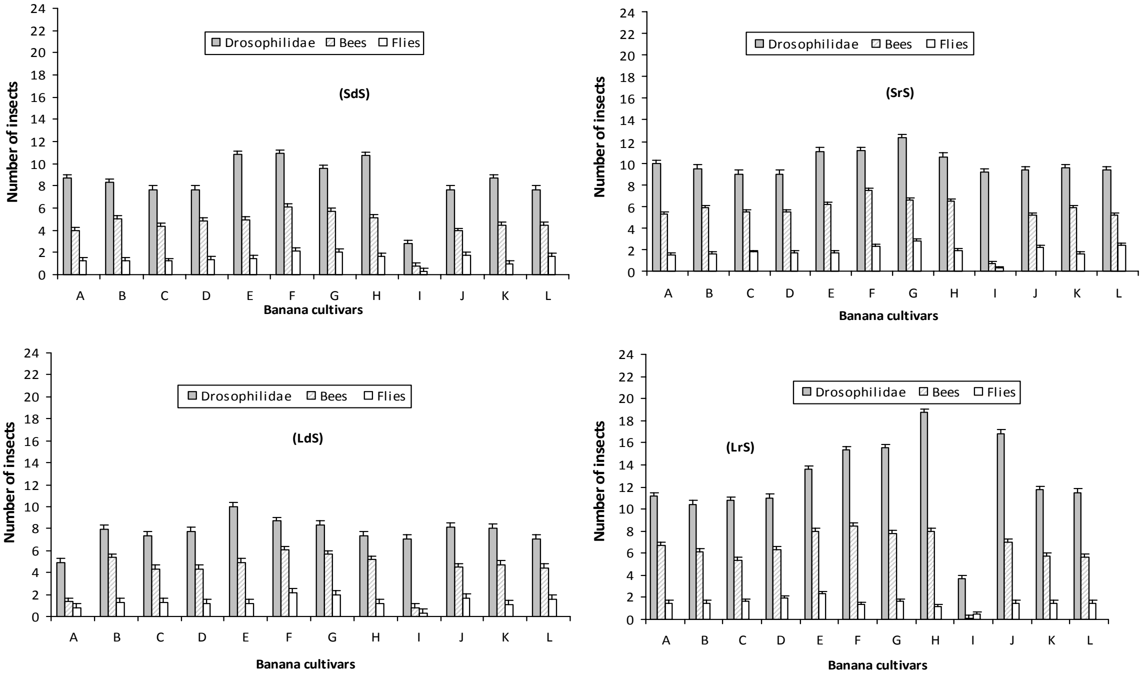

Figure 6. Prevalence of insects across banana cultivars grown in the High Altitude during the Short dry Season (SdS), Short rainy Season (SrS), Long dry Season (LdS) and the Long rainy Season (LrS). Error bars indicate the SEM. Letters at the 'X' axis stand for: (A) Kamaramasenge, (B) Gros Michel, (C) Igisukari, (D) Poyo, (E) Intuntu, (F) Ingame, (G) Kayinja, (H) Incakara, (I) Nkazikamwe, (J) Inyarwanda, (K) FHIA17, (L) FHIA25

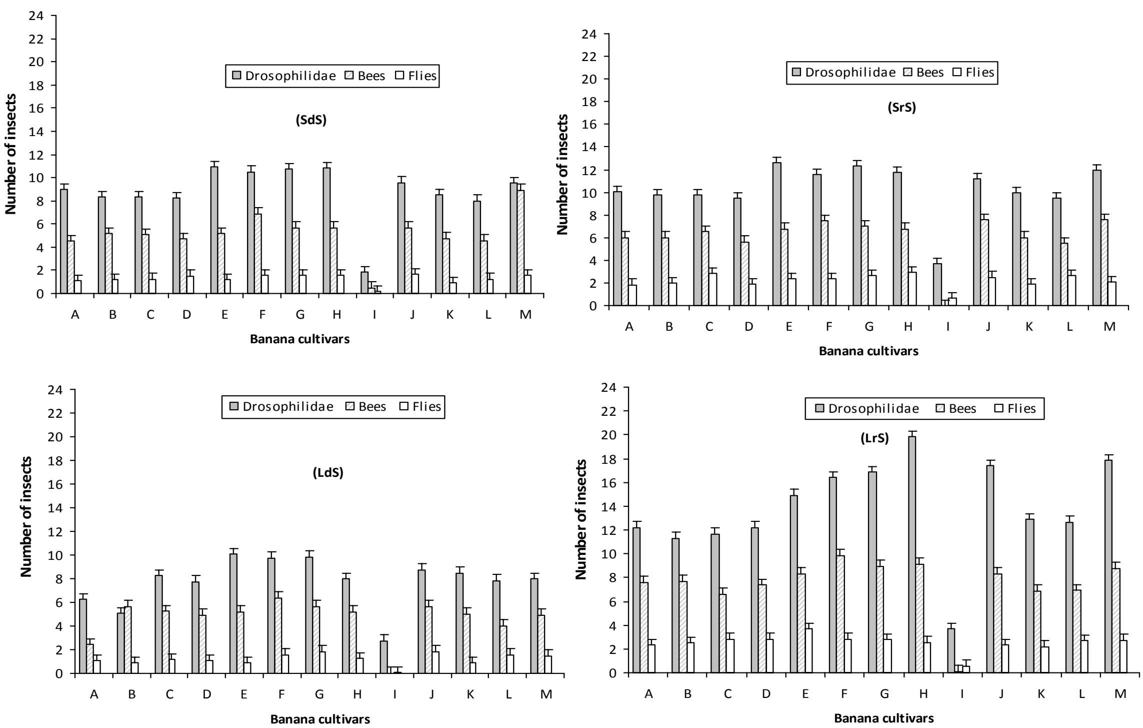

Figure 7. Prevalence of insects across banana cultivars grown in the Medium Altitude during the Short dry Season (SdS), Short rainy Season (SrS), Long dry Season (LdS) and the Long rainy Season (LrS). Error bars indicate the SEM. Letters at the 'X' axis stand for: (A) Kamaramasenge, (B) Gros Michel, (C) Igisukari, (D) Poyo, (E) Intuntu, (F) Ingame, (G) Kayinja, (H) Incakara, (I) Nkazikamwe, (J) Inyarwanda, (K) FHIA17, (L) FHIA25, (M) Nyiramabuye

As per previous scenarios (activity of insects vis- a -vis time of day and seasons of year), fruit flies, bees and other flies were the most predominant insect species across all the banana genotypes in all the study areas. The fruit flies were most attracted by beer and cooking banana cultivars, while dessert banana cultivars attracted more bees compared with other insect species (Figures 5 - 8). Higher prevalence of fruit flies was recorded on the beer banana 'Intuntu' and the cooking banana variety 'Injagi', while bees were more active on the beer cultivars 'Ingumba' 'Kayinja' 'Intuntu' and 'Nyiramabuye'. No significant differences were noted on the prevalence of flies. The activity of the three insect species was very low on the banana cultivars 'Nkazikamwe', 'Impura' and 'Ikinyangurube' (Figures 5 – 8) that have persistent floral bracts.

DISCUSSION

The present study was carried out in an attempt to understand the dynamics of insect vectors of Xanthomonas wilt of banana across four main agro-ecologies and banana cultivars grown in Rwanda. It is expected that knowledge of these aspects will help in designing improved management practices for the disease. Results showed that insect vectors of XW varied horizontally (with the four agro-ecologies) and vertically (with annual seasons and time of the day). The prevalence of insects also varied across banana cultivars grown in Rwanda. All the factors considered for the present study influenced the disease (XW) incidence as they showed significant influence on the dynamics of insect vectors of XW. According to current findings, banana cultivar distribution varied across banana growing areas. Similar results were reported from Kenya (Nguthi, 1998). A relatively higher diversity of banana cultivars was recorded in the low altitude and the Lake Kivu border region. Similar findings were also reported in two studies conducted on banana cultivar distribution in Rwanda in 2001 (Nsabimana *et al*., 2008) and in 2007 (Ocimati *et al.,* 2014).

Whereas all banana cultivars in East Africa are susceptible to XW disease (Ssekiwoko *et al*., 2006), susceptibility to floral infections was observed to vary with banana cultivars. High incidence of male bud infections were observed among beer bananas 'Kayinja' (ABB), 'Ingumba' (AAA-EA), 'Nyiramabuye' (AAA-EA) and the dessert banana cultivar 'Kamaramasenge' (AAB; elsewhere known as 'Sukari ndizi'). This study confirms the role of insects in the high XW incidence banana production systems.

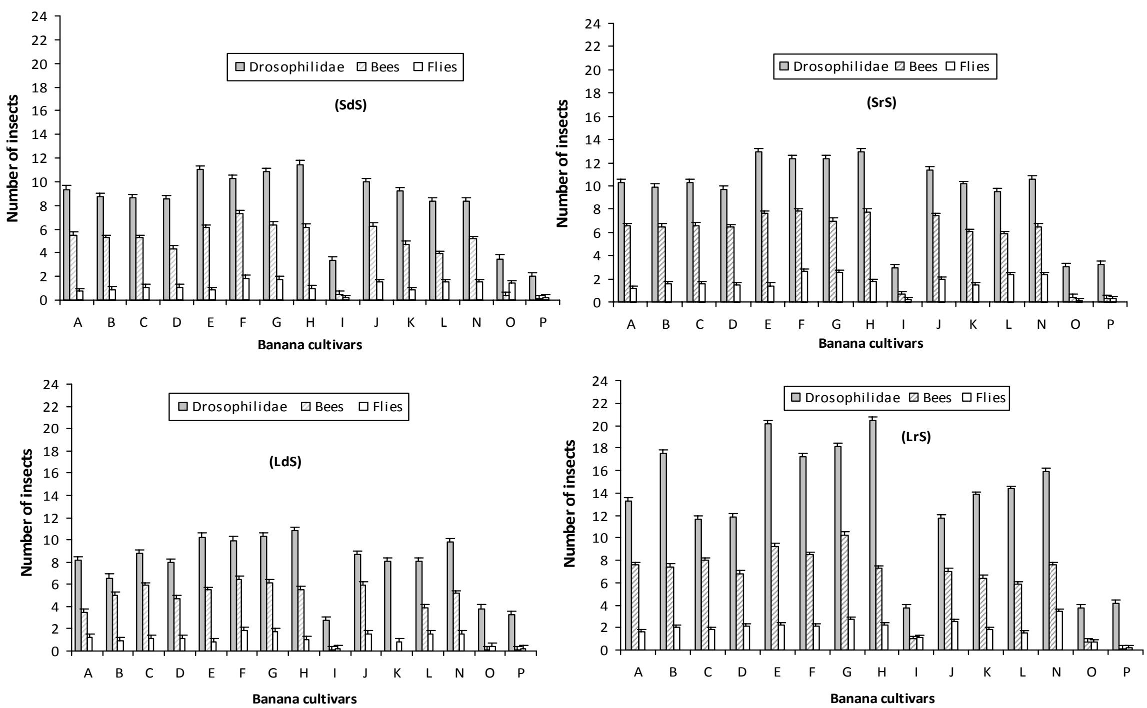

Figure 8. Prevalence of insects across banana cultivars grown in the Kivu Lake Border during the Short dry Season (SdS), Short rainy Season (SrS), Long dry Season (LdS) and the Long rainy Season (LrS). Error bars indicate the SEM. Letters at the 'X' axis stand for: (A) Kamaramasenge, (B) Gros Michel, (C) Igisukari, (D) Poyo, (E) Intuntu, (F) Ingame, (G) Kayinja, (H) Incakara, (I) Nkazikamwe, (J) Inyarwanda, (K) FHIA17, (L) FHIA25, (N) Umuzibo and (O) Impura, (P) Ikinyangurube

Higher male bud infections have been reported in the beer banana variety 'Kayinja' (*Musa* ABB) compared with a 'Dwarf Cavendish' in Ethiopia and the east African highland bananas in Uganda (Biruma *et al.*, 2007;Tinzaara *et al.,* 2006; Shimelash *et al.,* 2008;). Higher incidence of insect mediated floral XW infection was also reported in 'Kayinja' dominated banana areas (Addis *et al.,* 2004). This study however, reveals cultivars other than 'Kayinja', including east African highland banana cultivars (e.g.: 'Ingumba', 'Nyiramabuye', 'Ingame') and dessert cultivar 'Kamaramasenge' to also be highly susceptible to insect mediated floral infections. The presence of these cultivars, just like 'Kayinja', in a system may also drive insect mediated infections.

Generally, the incidence of Xanthomonas wilt of banana varied significantly (P<0.001) across banana cultivars grown in Rwanda. Higher disease incidence was recorded among dessert and beer bananas. The beer banana 'Kayinja' (*Musa* ABB) and the dessert banana 'Kamaramasenge' (*Musa* AAB) showed high susceptibility to XW-male bud infection with 3.2% and 3.0%, respectively (Figure 9). These results revealed other additional cultivars similar to 'Kayinja' were susceptible to insect mediated infections. Banana cultivars 'Ikinyangurube' (AAA dessert), 'Impura' (AAA-EA) and 'Nkazikamwe' (AAA-EA) had persistent male bud bracts and neuter flowers and were thus not susceptible to XW male bud infection. Cultivars with persistent male bracts and flowers have long been reported to escape insect transmission (Biruma *et al.*, 2007).

However, few 'Nkazikamwe' plants were observed to show floral symptoms, which could be attributed to tool infections. Late floral symptoms were observed in plants that were inoculated through the corm using farm tools (Ocimati *et al.,* 2013). A diversity of insects was recorded across the four study areas and annual seasons. The dynamics of insect communities (size, density, and distribution of insect populations) have been reported to vary in space and time and this is strongly influenced by environmental heterogeneity (Dobzhansky and Pavan, 1950), (Valadao *et al.*, 2010 and Wolda *et al.*, 1978). The most prevalent insects recorded on banana male buds were fruit flies, other flies, bees and wasps. Similar observations were noted in studies conducted in Uganda and Ethiopia (Shimelash *et al.,* 2008) and (Tinzaara *et al.,* 2006). The activity of insects generally differed with the time of the day.

Fruit flies were more active on male buds in the morning and evening. Such results were also reported in a similar study (Masanori, 1973). The crepuscular activity of these insects is a behavioral adaptation controlled by light intensity to avoid desiccation (Pavan *et al.*, 1950 ; Mitchell and Epling, 1951);(. The activity of flies was similar to

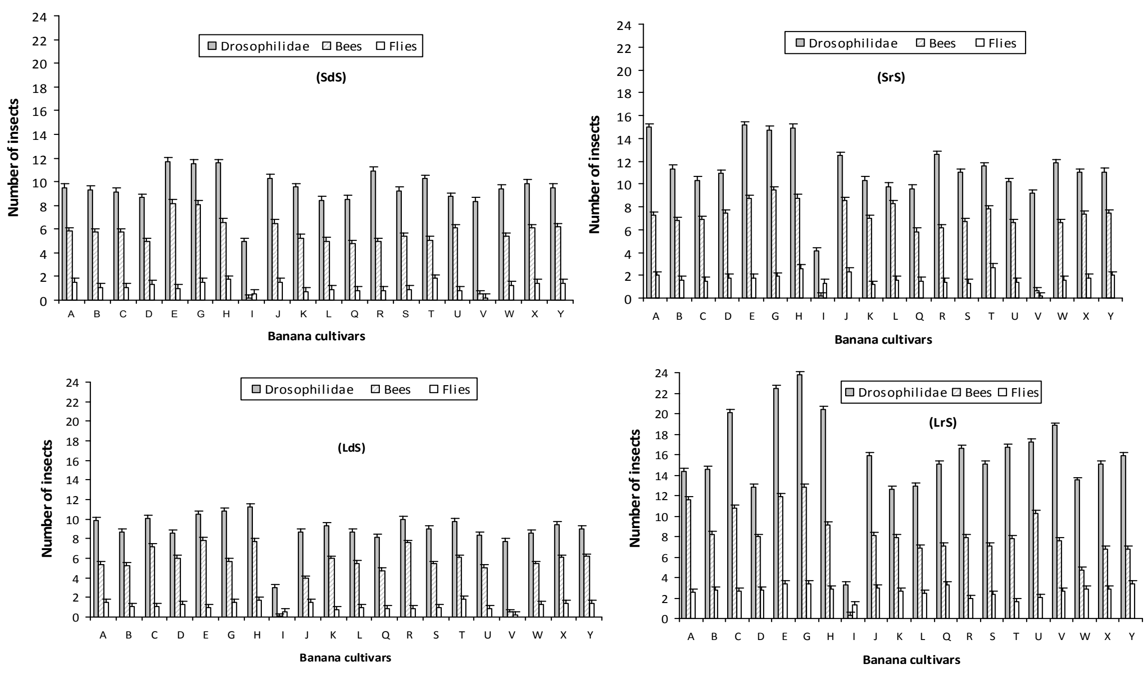

Figure 9. Prevalence of insects across banana cultivars grown in the Low Altitude during the Short dry Season (SdS), Short rainy Season (SrS), Long dry Season (LdS) and the Long rainy Season (LrS). Error bars indicate the SEM. Letters at the 'X' axis stand for: (A) Kamaramasenge, (B) Gros Michel, (C) Igisukari, (D) Poyo, (E) Intuntu, (G) Kayinja, (H) Injagi, (I) Nkazikamwe, (J) Inyarwanda, (K) FHIA17, (L) FHIA25, (P) Ingagara, (Q) Ingaju, (R) Ingenge-cooking, (S) Ingenge-beer, (T) Ingumba, (U) Intutsi, (V) Inzirabahima, (W) Inzirabushera, (X) Kibuzi

one recorded for fruit flies. Normally, fly species are reported more active on nectar plants with peaks in the morning and evening (Heatwole *et al*., 1981). Some banana cultivars have a peak of nectar production near midday and midnight but most flower opening occurs near dawn and sunset. The total resource available to pollinators will be a function of the rate of nectar production and the numbers of flowers open (Liu, 2002a). It has been also reported that the greatest amount of resource is available from 8 a.m. to 12 noon and from 10 p.m. to 2 a.m (Liu *et al.,* 2002b). This matches the timing of most visits. More bees were captured in the period between 10 am and 12 o'clock. Similar observation were made in previous studies in which, bees had unimodal peaks centering on midday (Heatwole *et al*., 1981). Wasps were more active between 10 am and 3 pm. It has been reported that the temperature is the most important factor that influences foraging of wasp species (Canevazzi *et al*., 2011). The activity of ants was consistent throughout the day. Ants in general are reported active at all hours of the day and night and time of activity peaks depend on the weather conditions (Heatwole *et al*., 1981).

Beetles are mostly active during twilight and at night or bimodally (morning and evening) except in the early rainy season when they are strongly day-active (Kenagy and Stevenson, 1982). However, no correlation was observed between beetle activity and weather patterns in the current study. Similar observations have been reported (Heatwole *et al*., 1981). This could be because beetles are strongly day-active in the early rainy season and mostly active during twilight and at night or bimodally (morning and evening) during other seasons (Kenagy and Stevenson, 1982). It was generally noted that insects were more active during the rainy season. The tendency for several insect species to be active during rainy weather especially in morning and evening may be linked to the fact that nectar resources are most heavily utilized at such time (Heatwole *et al*., 1981) Higher XW incidence was observed in the long rainy season in this study. Similar observations have been reported elsewhere (Biruma *et al*., 2007). The observed high activity (frequency and population) of insect vectors of *Xcm* during the rainy season could partially explain the high XW incidence in the rainy season. More insects were captured from the low altitude and the Lake Kivu border regions.

A higher prevalence of floral infections was observed in the low altitude and Lake Kivu border regions while no floral XW infections were recorded in the high altitude area. The high floral XW incidence in these areas correlates with the high insect activity. Fewer male bud infections were reported at altitudes above 1,700 masl compared with a lower altitude in Ethiopia (Shimelash *et al.,* 2008). The observed high activity of insect vectors of *Xcm* in the low altitude areas could partially explain the high XW incidence at the lower altitudes. In the current study, much of AAA-EA highland bananas are grown in the high altitude areas that do not support high numbers of the insect vectors, which could have also contributed to the observed low incidence of floral infections on these cultivars. Yellow colonies characteristic of *Xcm* were isolated from all the captured insect groups, with a higher frequency and load isolated from fruit flies, true flies, bees and wasps. Though ants and beetles carried the bacteria, their ability to spread it seems to be rare. These results are consistent with findings of similar studies in the DR Congo (Fiaboe *et al.,* 2008) Ethiopia (Shimelash *et al.,* 2008) and in Uganda (Tinzaara *et al.,* 2006). It should however be noted that insects such as the ants and fruit flies spend much of their life time on a few plants and may only cause localized spread of the disease.

A diversity of insect species was observed visiting banana inflorescences. The role of insects in the spread of XW disease in banana genotypes with non-persistent bracts has been demonstrated. Among the identified insect species, relatively higher populations of fruit flies, bees, other flies and wasps were observed active on male buds of banana cultivars in this study. The other insect species (i.e. ants and beetles) occurred in low numbers on banana male buds. The *Xcm* bacteria were isolated from all the insects species sampled, though ants and beetles carried low bacterial load compared with other insect species. The low number of ants and beetles carrying *Xcm* and their low activity suggest that their contribution in the spread of *Xcm* is likely to be very low. This study confirms bees, fruit flies, other flies and wasps as key insect vectors of XW disease.

Insect population varied with banana cultivars, with 'Kayinja', Nyiramabuye', Ingumba', 'Injagi' and other cultivars in the same clone set ('Incakara' and 'Barabeshya'), and 'Kamaramasenge' attracting more insects than other cultivars. Higher XW incidence has been reported in 'Kayinja' dominated areas in central Uganda. However, the distribution of this cultivar and others such as 'Kivuvu' (*Musa* ABB) and 'Gisubi' (*Musa* ABB) and 'Kamaramasenge' were sporadic. It is also eminent that several other cultivars, such as 'Ingumba' and 'Injagi' as observed in this studycan, like 'Kayinja' significantly influence XW spread. The banana cultivars 'Impura', 'Nkazikamwa' and 'Ikinyangurube' were recorded to possess persistent male buds and hence were not susceptible to transmission of *Xcm* by insect vectors. Insect vectors of *Xcm* also varied with the altitude, seasons and the time of the day. Higher insect activity was recorded in the low altitude zone in this study. Incidentally this correlated with the highest incidence of floral infections, suggesting that disease prevalence was influenced by the high activity of the insects. Insect population and disease incidence declined with an increase in altitude.

The highest insect activity occurred during the long rainy season and least in the long dry season. This could explain the high XW incidence that has been reported in the rainy seasons. fruit flies and other flies were specifically more active during the early morning while bees and wasps were more active in the period ranging between 10 pm and 12 noon. Further evaluation of the severely affected germplasm in this study for susceptibility to insect mediated infection under controlled conditions in regions with high XW disease pressure and at a low altitude is recommended.It is also recommended that, banana cultivars with persistent male buds be promoted as they were observed to escape XW infections via insect transmission. Genetic improvement for banana cultivars such as 'Ikinyangurube' and 'Impura' should be envisaged as the two cultivars are currently not preferred by farmers due to their low yields. The lack of the yellow color at ripening in the dessert banana 'Ikinyangurube' is also blamed for its low adoption by farmers. Timely and proper de-budding (removal of the male floral bud) should contine to be advocated and emphasized during periods and in areas with high prevalence of insect vectors of *Xcm*.

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