

## Full Length Research Paper

# Variation in nectar volume and sugar content in male flowers of *Musa* cultivars grown in Rwanda and their non-effect on the numbers of visiting key diurnal insect vectors of banana *Xanthomonas* wilt

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Insects are a major mode of banana *Xanthomonas* wilt (XW) spread. High insect activity has been blamed for the high XW incidence in 'Kayinja' (ABB-genome) dominated banana landscapes across east and central Africa. 'Kayinja' male bud nectar composition reportedly contributes to high insect activity. The variation in nectar composition with agro-ecological zones and banana cultivars and its influence on the number of visiting insects in Rwanda were assessed. Three male buds were collected per cultivar for nectar extraction and analysis using a high performance liquid chromatography. Nectar volume and sugar concentrations varied ( $P < 0.001$ ) across 27 banana cultivars, annual seasons and agro-ecological zone. The highest nectar volume was recorded among the East African highland cooking cultivars (AAA-genome) in the high altitude site and the short-heavy rainy season. Nectar contained three sugars: glucose, fructose and sucrose, though hexose (glucose and fructose) was dominant. The three sugars varied significantly ( $P < 0.001$ ) within each cultivar. The total nectar-sugar concentration ranged from 2.3–32%, with the highest among dessert cultivars 'Kamaramasenge' (AAB-genome) and 'Gisukari' (AAA-genome). No strong correlation occurred between insect population and total nectar sugar concentration or nectar volume. Insect populations were rather influenced by the weather conditions, the long rainy season characterized by moderate well distributed rainfall recording the highest insect populations as compared to the short rainy season (with heavy rainfall) and the dry seasons.

**Key words:** Banana, insects, nectar, vectors, *Xanthomonas campestris* pv. *musacearum*

## INTRODUCTION

Insects play an important role in the pollination of many plant species. In many plant-insect pollination systems, the plant produces a 'reward', usually in the form of

nectar (Willson et al., 1996). Though parthenocarpic, bananas attract a diversity of insect species that obtain nectar and collect pollen from the male flowers. This

relationship has been reported as a major mechanism for the spread of bacterial diseases in banana plants (Harrison, 1980). The role of insect vectors, mainly bees, flies and fruit flies (drosophilids) in the spread of *Xanthomonas* wilt of banana (XW) (causal agent *Xanthomonas campestris* pv. *musacearum* (*Xcm*)) has been confirmed (Tinzaara et al., 2006; Shimelash et al., 2008; Rutikanga et al., 2015).

Nectar sugar concentration may also affect visitor preference or association (Hainsworth and Wolf, 1976; Bolten and Feinsinger, 1978; Schondube and Martinez, 2003); for example, 'Kayinja' (*Musa* ABB) is reported to attract more insects than east African highland banana cultivars (*Musa* AAA), owing to its nectar sugars (Karamura et al., 2008). The observed field susceptibility of *Musa* cultivar 'Kayinja' to infection by *Xcm* has been attributed to its susceptibility to insect vector spread (Blomme et al., 2005; Ocimati et al., 2013). This banana cultivar has not only been decimated in central Uganda, but has also been blamed for the rapid spread of XW disease in central Uganda during 2002-2006 (Njeri, 2008).

Measuring the volume of nectar and its sugar concentration is common in the study of many ecological processes (Dungan et al., 2004), in particular, the study of plant-animal interactions (Bolten and Feinsinger, 1978; Kearns and Inouye, 1993) and can permit the calculation of carrying capacity for nectarivores (Petit and Pors, 1996). However, studies to assess the variability of nectar volume and sugar concentration of major banana cultivars has not yet been carried out in east and central Africa, where XW is now endemic.

Nectar concentration is highly influenced by environmental factors, especially temperature and humidity (Nicolson and Nepi, 2005). The differences in mean nectar concentration between plants can also be explained by contrasting environmental particularities of regions (Forcone et al., 1997; Bernardello et al., 1999). For example, the relatively low mean nectar concentration of some plant species could be related to the lower mean maximum temperatures and higher precipitation characteristics of their environment (Barros et al., 1983).

The environmental differences of the different agro-ecologies in east and central Africa could therefore have influences on nectar concentrations of *Musa* cultivars and therefore the prevalence of insect-vector mediated XW in the cultivars in different agro-ecologies and could influence decisions regarding deployment of cultivars in the different agro-ecologies in the region. However, the potential effect of the variable environment in east and central Africa on nectar concentrations and volumes in *Musa* cultivars is not known.

This study therefore sought to improve knowledge of the interaction of insect vectors (of *Xcm*) and agro-ecologies through determining: (i) male flower nectar variability in terms of volumes and sugar concentration across banana cultivars, seasons and altitudes in Rwanda and (ii) the influence of the variation in male flower nectar volume and sugar content on the population of insect vectors of *Xcm*.

## MATERIALS AND METHODS

### Description and selection of the study sites

This study was conducted in four banana growing agro-ecological zones of Rwanda, categorized into three altitude ranges: low 800-1,400 m above sea level (masl), medium (1,450-1,650 masl) and high (1,700-2,200 masl) (Table 1). The districts in the Lake Kivu border region, with a medium altitude (1,410-1,642 masl) were given special attention due to the high mean rainfall (compared with other medium altitude sites) and incidence of XW that has devastated bananas in the area (Table 1). Detailed information on the biophysical conditions of the four agro-ecologies is documented in Table 1. This study was conducted in 2012 at the middle of each of the four annual seasons prevailing in Rwanda (that is, (1) the short dry season (SdS) from January to February, (2) the short rainy season (SrS) characterized by heavy rains from March to May, (3) the long dry season (LdS) from June to August and (4) the long rainy season (LrS) from September to December). In each agro-ecological zone, three districts (administrative divisions) with XW were purposively selected and a highly infected sector (administrative division under district) chosen for data collection through interaction with the agricultural staff (Table 1). In each sector, three villages with the highest XW incidence (based on sector records on the disease incidence) were purposively sampled, from which a XW-infected banana field was selected for data collection.

### Nectar sampling and laboratory analysis

Three plants per banana cultivar were selected in each field, their male buds were harvested and flowers kept intact until analysis in the laboratory. Male buds of more or less the same age, with at least one open male bract exposed below the last female hand, were sampled. To avoid nectar fermentation, collected flowers were carried in an ice-cold container and kept under refrigeration at -8°C in the laboratory (Petit and Freeman, 1997).

Laboratory analysis was performed in the analytical chemistry laboratory of the Kigali Institute of Science and Technology. The male flowers were allowed to thaw at room temperature while still attached to the male buds. Nectar extraction was performed by rinsing each male flower (under the most recently open male bud bract) four times with 0.5 mL of distilled water using a pipette and this was repeated for seven male flowers from three male buds of the same cultivar (Nunez, 1977; Mallick, 2000).

With the help of the pipette, efforts were made to recover all the nectar-water mixture from the flowers. The nectar and distilled water solutions were thoroughly mixed using a vortex mixer. The volume of nectar for the banana cultivars was calculated as the

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**Table 1.** Description of the study areas.

Category of altitude	District	Sector	Altitude (masl)	Type of soil	Mean rainfall (mm)				Mean temperature (°C)				Mean relative humidity (%)			
					SdS	SrS	LdS	LrS	SdS	SrS	LdS	LrS	SdS	SrS	LdS	LrS
Low	1.Kayonza	Mukarange	1,300	Ferralsols	34.0	175.0	35.6	117.0	20.6	19.4	19.4	19.0	70.9	81.0	70.7	81.2
	2.Gatsibo	Kiziguro	1,400	Ferralsols	14.0	107.0	9.2	111.0	20.3	17.5	21.9	20.5	69.6	79.3	60.4	78.7
	3.Nyagatare	Tabarwe	1,380	Regosols	21.6	119.0	17.0	109.0	21.4	21.4	20.8	21.1	71.3	80.0	70.5	80.2
	Averages				23.2	133.7	20.6	112.3								
Medium	1.Ruhango	Bunyogombe	1,600	Lexisols	43.3	174.0	25.8	108.0	19.8	18.9	18.3	19.2	68.4	79.9	70.0	78.1
	2.Huye	Mukura	1,650	Ferralsols	84.9	197.0	10.5	171.0	20.0	19.1	19.3	19.1	71.0	80.0	60.0	85.0
	3.Gisagara	Save	1,600	Acrisols	7.6	62.2	30.9	57.4	19.9	19.0	18.8	19.2	75.0	82.0	65.0	88.0
	Averages				45.3	144.4	22.4	112.1								
High	1.Rulindo	Rusiga	1,887	Cambisols	48.9	155.0	44.9	189.0	17.5	17.0	17.4	17.4	80.0	85.0	76.0	89.0
	2.Gakenke	Nemba	2,112	Cambisols	67.6	187.0	15.5	253.0	18.5	18.1	18.1	18.4	82.0	86.0	77.0	90.0
	3.Burera	Kinoni	2,167	Luvissols	69.3	166.0	27.2	164.0	18.0	17.1	19.0	16.3	70.1	87.0	67.4	90.0
	Averages				61.9	169.3	29.2	202.0								
Lake Kivu border (medium)	1.Rubavu	Rugerero	1,600	Andosols	36.1	164.0	54.2	158.0	20.7	20.2	19.9	20.2	75.0	80.0	68.1	75.3
	2.Rutsiro	Mushyonyi	1,642	Alisols	78.1	139.0	27.3	119.0	20.3	20.1	19.7	20.1	70.0	80.0	65.0	82.0
	3.Karongi	Bwishyura	1,596	Ferralsols	75.3	166.0	40.5	113.0	19.8	19.9	19.4	20.0	73.2	82.0	68.0	83.7
	Averages				63.2	156.3	40.7	130.0								

Source: Climatic data: Rwanda Meteorology Agency (2012) and soil information: Carte Pédologique du Rwanda. SdS, SrS, LdS and LrS, respectively, denote: short dry season (from January to February), short rainy season (March to May), long dry season (June to August) and long rainy season (September to December).

difference between the total volume of liquid within 3 mL test tubes (51 x 12 mm) and the volume of distilled water added during rinsing. Smaller volumes (0.01-0.7 mL) were measured using manual injection syringes from Waters U6K injection valve. High performance liquid chromatography (HPLC) was used to quantify the specific sugars in a nectar sample (Kearns and Inouye, 1993).

Acetonitrile (HPLC grade) was used as a solvent and the mobile phase was made with a solution of acetonitrile and deionized water in the ratio 75:25 at a flow rate of 1.25 mL/min. The column used was NH<sub>2</sub> (25 cm x 4 mm). A UV detector set at 193 nm and a recorder of 50 mV with a flow of 5 mm per minute were also used to perform the analysis. A mixed stock standard (100 mL) of fructose (10 mg/mL), glucose (100 mg/mL) and sucrose (100 mg/mL) was prepared using deionized water. An injection of 20 µl of the sample was made using an injector loop (full loading loop technique).

Each analysis was run for 45 min. Sugar ratios appeared

in the form of curves with peaks. Curves were integrated to quantify and convert the sugar ratios (in %) using a computer-assisted digital microprocessor, the Baseline 815 software from Waters.

#### **Insect collection from banana male buds**

To determine the effect of nectar concentration and volumes on insect vector abundance, insects on the male buds of different cultivars were captured at the different altitudes and in the middle of each of the four seasons. Insects were captured as described by Rutikanga et al. (2012), Tinzaara et al. (2006) and Fiaboe et al. (2008). Flying insects were captured using separate sweep nets.

Insects were captured in the morning (7-9 am), between 10 am and 12 noon, in the afternoon (1-3 pm) and in the evening (4-6 pm). These insects were sorted into different broad taxa, separately enumerated 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 University of Rwanda, College of Agriculture. The identification exercise was supported by use of an electron microscope and a data base for insect species available on the internet (Castner, 2000; Iowa State University, 2014). Only the major insects groups reported by Tinzaara et al. (2006), Fiaboe et al. (2008) and Rutikanga et al. (2015) were focused on in this study.

#### **Data analysis**

Data were subjected to analysis of variance using GenStat, 5<sup>th</sup> edition, and the means separated using Fishers least significance difference at 5%. Correlations of nectar sugar content with nectar volume and of nectar sugar content and volume with insect populations across the four

seasons were obtained using MS Excel.

## RESULTS

### Nectar volume

Significant differences ( $P < 0.05$ ) in nectar volume were observed between the 27 banana cultivars (Table 2, Figures 1 to 4). Generally, higher nectar volumes were recorded among the east African highland cooking banana cultivars (*Musa* AAA-EA), with mean highest volumes recorded in 'Injagi' (0.49 mL/male flower in the low altitudes), 'Incakara' (0.59 mL in the Lake Kivu border area) and 'Barabeshya' (0.53 and 0.68 mL in the medium and high altitudes) (Table 2, Figures 1 to 4). In contrast, lower nectar volumes (0.26 mL) were recorded in the brewing banana cultivars (AAA-EA and ABB genotypes) and the least (0.13 mL) in the dessert banana cultivars (Table 2, Figures 1 to 4).

Nectar volumes significantly increased ( $R^2 > 0.05$ ) with the total amount of rainfall in all the banana cultivars (Figure 5). The lowest volumes were recorded during LDS (40.7 mm of rainfall) while the highest was in the SRS (156.3 mm) that is characterized by heavier rainfall (Figures 1 to 5). For example, nectar volumes of 0.01-0.26 mL in the LDS and 0.03-0.37 mL in the SDS (63.3 mm of rainfall), when compared with 0.07-0.42 mL in LRS (130 mm) and 0.13-0.49 mL in the SRS were recorded in the low altitude zone. Similarly, 0.07-0.48 and 0.12-0.55 mL, when compared with 0.17-0.61 and 0.21-0.68 mL, respectively were recorded in the high altitude zone. This was a general trend for all *Musa* groups investigated and other altitudes (agro-ecologies) in this study (Figures 1 to 5).

Nectar volumes generally increased with altitude in this study (Figure 6). For example, nectar yields during the SRS varied from 0.13 – 0.49 mL in the low altitude zones, 0.14 – 0.53 mL in the medium altitudes, 0.16 to 0.59 mL in the Lake Kivu Border region with a medium altitude and 0.21 to 0.68 mL (mean = 0.45 mL) in the high altitude (Figures 1 to 4).

### Nectar sugar concentration

Laboratory nectar analysis revealed the presence of three sugars: glucose, fructose and sucrose. Nectar from the different banana genotypes/cultivars was hexose-dominant (glucose and fructose which is dominant) (Figures 7 to 10). The percentage of the three sugars varied significantly ( $P < 0.001$ ) within each banana cultivar. For example, in the cultivar, 'Kamaramasenge' the level of glucose was 13.60%, fructose: 9.51% and sucrose: 7.73% in the low altitude zone during the long dry season. The total nectar sugar concentration was in the range of 3 and 32% across all banana cultivars and agro-ecological zones. The total nectar sugar concentration

also varied significantly ( $P < 0.001$ ) among banana cultivars and cultivar groups (Table 2 and Figure 11). A case in point is the situation in low altitudes during the long dry season where higher total nectar sugar concentration (mean = 30.2%) was noted in dessert cultivars when compared with 24.8% in brewing cultivars and 16.8% in cooking cultivars (Figure 11). The dessert banana cultivars 'Kamaramasenge' (*Musa* AAB), 'Igisukari' (*Musa* AAA), 'Gros Michel' (*Musa* AAA) and 'Poyo' (*Musa* AAA) had the highest nectar sugar concentrations and this was consistent for all the four agro-ecological zones and cropping seasons (Figures 7 to 10). Brewing banana cultivars 'Ingumba' (*Musa* AAA), 'Nyiramabuye' (*Musa* AAA) and 'Kayinja' (*Musa* ABB) (Figures 7 to 10) contained sugars in almost the same concentration range as dessert bananas.

Nectar sugar concentration significantly ( $R^2 > 0.5$ ) declined with an increase in the total amount of rainfall (mm) and an increase in altitude (Figure 12) in all the banana cultivars. It should be noted that the precipitation also increased with an increase in altitude in the study sites. For example, the mean total sugar concentration across the altitudes declined significantly ( $R^2 = 0.788$ ) from 25% in the cultivar 'Igisukari' at a low rainfall of 28 mm to 12% at 150 mm rainfall (Figures 12). Similarly, the mean sugar concentration across seasons for 'Igisukari' significantly ( $R^2 = 0.85$ ) declined from 23% at 1350 masl to 14% at 2027 masl. Similar trends were observed for cultivars when disaggregated by seasons and/or altitudes.

Nectar sugar concentration was observed to decline with an increase in the nectar volume and this was consistent across all altitudes and seasons. For example, a negative linear relationship ( $R^2 = 0.5923$ ) was observed between banana nectar volume and the concentration of sugars at high altitude and in the LRS (Figure 13). Similarly, the east African highland *Musa* cultivars having the highest nectar volume had the lowest sugar concentration, while the dessert types with the least nectar volumes had the highest sugar content.

### Variation in the number of insects per male bud with nectar volume and sugar concentration

Fruit flies in the family of Drosophilidae and Tephritidae followed by bees (Hymenoptera, Apidae) were the most dominant insects collected from banana male flowers in this study (Table 2). Other insects captured in smaller numbers included different species in the families of Lonchaedae, Muscidae, Neriidae and Sarcophagidae, Vespidae, Formicidae, Nitidulidae, Tenebrionida and Staphylinidae.

Some banana cultivars such as the beer cultivars 'Intuntu' (AAA-EA), 'Ingame' (AAA-EA) and 'Kayinja' (ABB) and the cooking cultivars 'Barabeshya' (AAA-EA), 'Incakara' (AAA-EA) and 'Injagi' (AAA-EA) (Table 2)

**Table 2.** Nectar volume and sugar concentration, and the respective insect populations for different cultivars averaged across all seasons and altitudes. The letter 'D' denotes: dessert type; 'B', beer; 'Ck', cooking and 'M', multiple use.

Cultivar	Nectar features (Average)		Major Captured Insect Population (Average)		
	Volume [ml]	Sugar concentration (%)	Fruit flies (Diptera: Drosophilidae & Tephritidae)	Bees (Hymenoptera, Apidae)	Other flies (Diptera: Lonchaedae, Muscidae, Neriidae, Sarcophagidae)
'Gros Michel'(D)	0.2±0.02	16.4±4.9	9.6±3.2	6.1±1.0	1.5±0.6
'Igisukari'(D)	0.1±0.01	17.7±6.2	9.9±3.2	6.2±1.6	1.6±0.6
'Kamaramasenge'(D)	0.1±0.01	17.6±6.2	9.9±2.9	5.7±2.4	1.5±0.5
'Poyo'(D)	0.2±0.04	16.1±5.2	9.2±2.1	5.8±1.2	1.7±0.6
'Barabeshya'(Ck)	0.3±0.01	9.6±3.9	12.8±4.6	6.9±1.3	1.8±0.6
'Incakara'(Ck)	0.3±0.01	9.7±4.0	12.9±4.7	6.9±1.4	1.8±0.6
'Ingagara'(Ck)	0.4±0.07	10.0±3.5	10.3±3.2	5.6±1.1	1.6±1.1
'Ingaju'(Ck)	0.3±0.07	13.4±6.5	12.5±2.9	6.6±1.4	1.3±0.6
'Ingenge'(Ck)	0.3±0.09	11.8±5.1	11.1±2.8	6.1±0.9	1.4±0.7
'Injagi'(Ck)	0.3±0.01	9.9±4.1	13.1±4.8	7.0±1.3	2.0±0.6
'Intokatoke'(Ck)	0.5±0.02	8.4±3.8	10.9±3.3	6.3±1.5	2.0±0.5
'Inzirabahima'(Ck)	0.4±0.1	8.9±4.8	10.8±2.2	5.5±0.7	1.7±0.8
'Inzirabushera'(Ck)	0.4±0.09	8.8±5.0	11.3±2.6	6.6±0.6	1.9±0.7
'Kibuzi' (Ck)	0.4±0.08	9.3±5.0	11.4±3.2	6.6±0.6	2.1±0.9
'Nkazikamwe' (Ck)	0.3±0.02	9.3±4.0	3.8±2.0	0.4±0.3	0.5±0.4
'Intutsi' (Ck)	0.4±0.09	13.3±6.5	11.0±5.2	2.3±3.5	0.8±1.3
'Impura' (B)	0.3±0.1	9.2±2.3	2.8±1.6	0.4±0.3	0.7±0.6
'Ingame'(B)	0.3±0.02	9.6±2.8	11.8±3.1	7.4±1.1	2.1±0.5
'Ingenge'(B)	0.3±0.07	12.1±5.9	12.1±3.2	6.7±1.4	2.0±0.5
'Ingumba'(B)	0.3±0.05	20.0±10.1	11.1±4.2	7.0±2.3	1.3±0.6
'Intuntu'(B)	0.3±0.08	12.0±4.4	12.7±4.0	7.2±1.9	1.7±0.9
'Kayinja'(B)	0.3±0.02	14.9±5.8	12.7±4.3	7.4±2.1	2.2±0.6
'Nyiramabuye' (B)	0.3±0.04	15.6±6.4	11.8±4.3	7.5±1.9	2.0±0.6
'Umuzibo' (B)	0.3±0.01	15.7±5.8	10.4±4.4	6.1±1.2	2.2±0.9
'FHIA17'(M)	0.3±0.02	9.2±3.4	9.8±2.2	5.8±1.0	1.3±0.6
'FHIA25'(M)	0.3±0.02	9.9±3.3	9.3±2.5	5.4±1.2	1.8±0.6
CV%	10.97	24.4	22.8	19.8	21.9

attracted more insects across all the agro-ecologies and especially during the LRS. 'Kayinja' a model cultivar in this study, had total nectar sugar and insect populations in levels comparable to those of AAA-EA beer and cooking types. However, no strong positive or negative correlation ( $R^2 = 0.0004 - 0.4$ ) was observed between insect population and either the total nectar sugar concentration or total nectar volume, at the insect family group level, across all the altitudes and rainy seasons (Figures 14 to 17). For instance, dessert cultivars, though with the highest levels of total nectar sugar, generally attracted fewer insects than the beer (both ABB and AAA-EA) and cooking AAA-EA banana cultivars (Table 2).

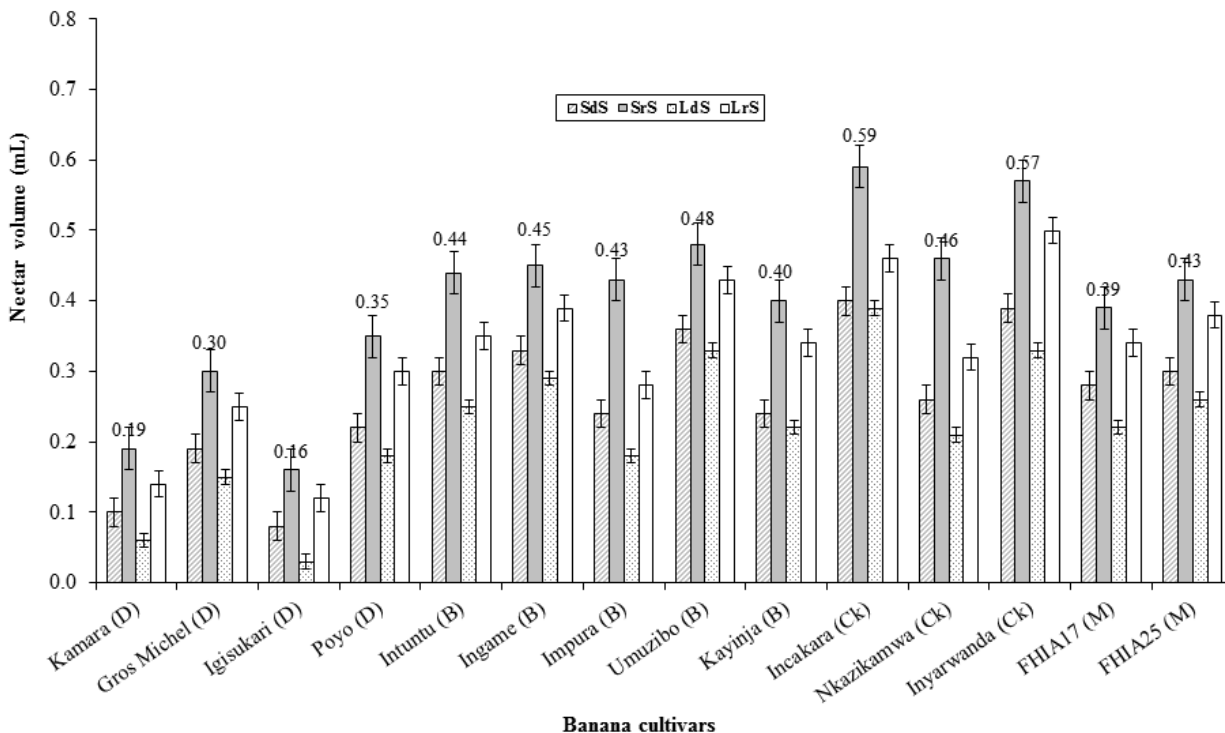
More insects were generally observed in the long rainy season, characterized by moderate rainfall spread over a longer period of time.

## DISCUSSION

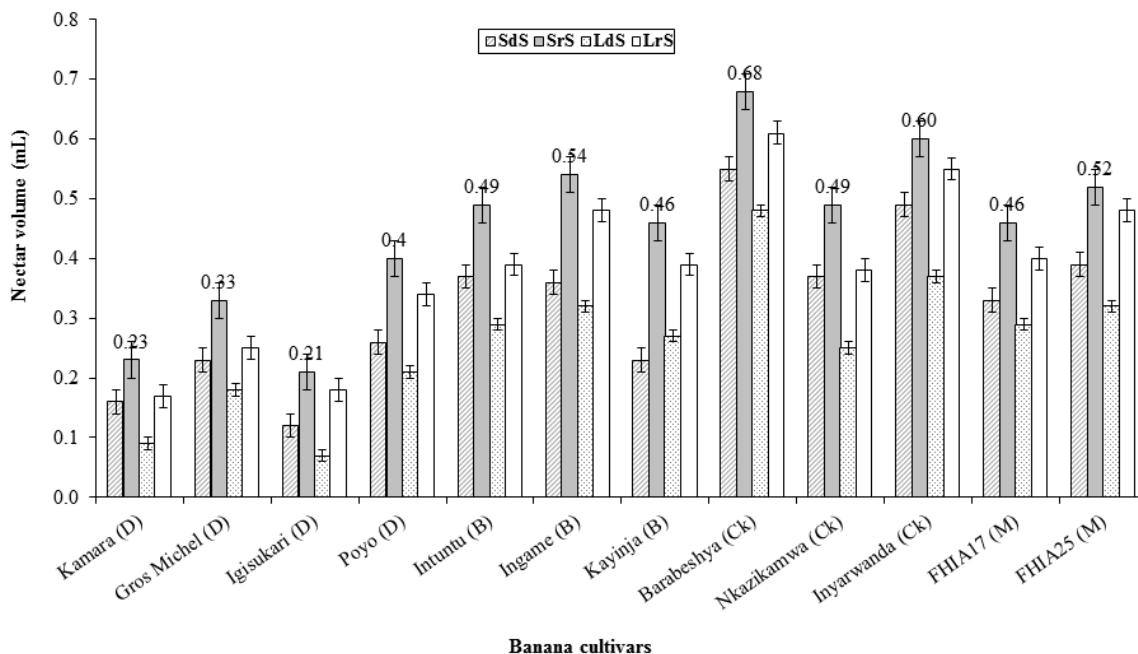
This study assessed the variation in the population of insect vectors of Xcm with nectar volume and sugar content across banana cultivars, at different altitudes and seasons in Rwanda. Nectar features (volume and sugar content) were influenced by the banana genotype/cultivar, characteristics of the seasons and altitude. A negative linear relationship was observed between nectar volume and sugar content i.e. the higher the nectar volume, the lower was the sugar content.

### Variation in nectar volume and sugar content across the study sites

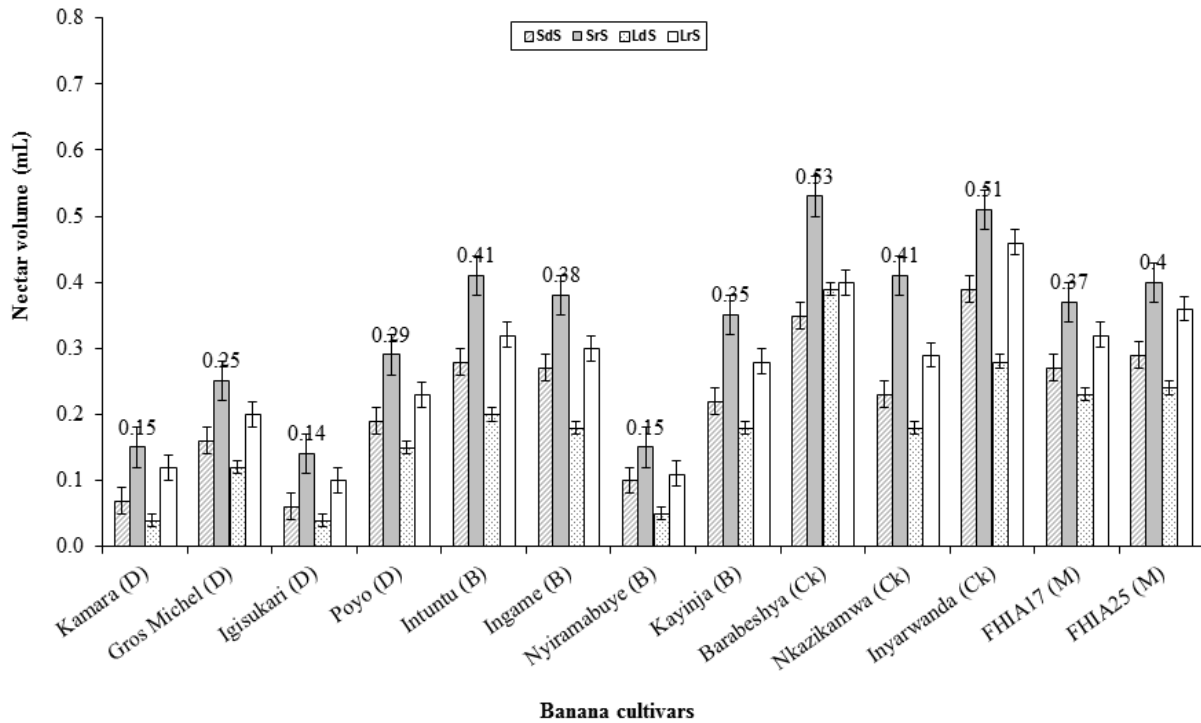
Higher nectar volumes were recorded from the high



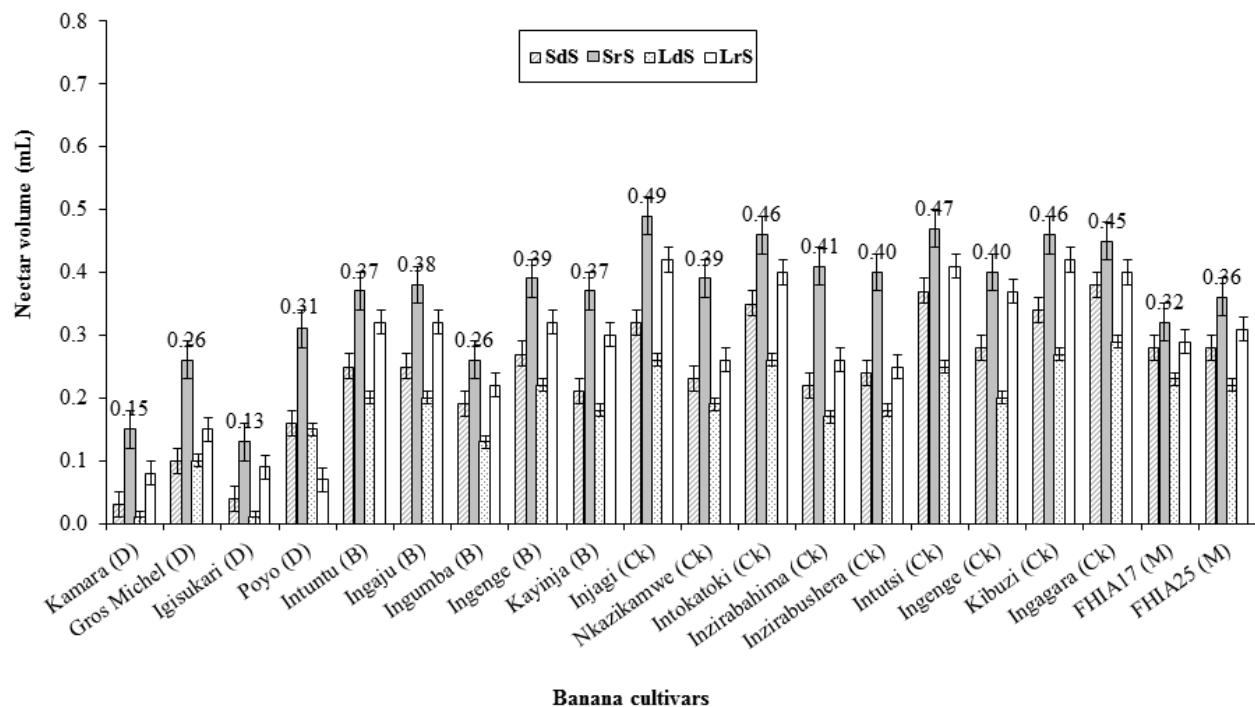
**Figure 1.** Nectar volume across banana cultivars in the Lake Kivu border region (with a medium altitude). Letters in brackets stand for D: dessert, B: beer, Ck: cooking and M: multipurpose. SdS, SrS, LdS and LrS respectively, denote short dry season (63.3 mm of rainfall), short rainy season (156.3 mm), long dry season (40.7 mm) and long rainy season, (130.0 mm). Error bars indicate the standard error of the mean.



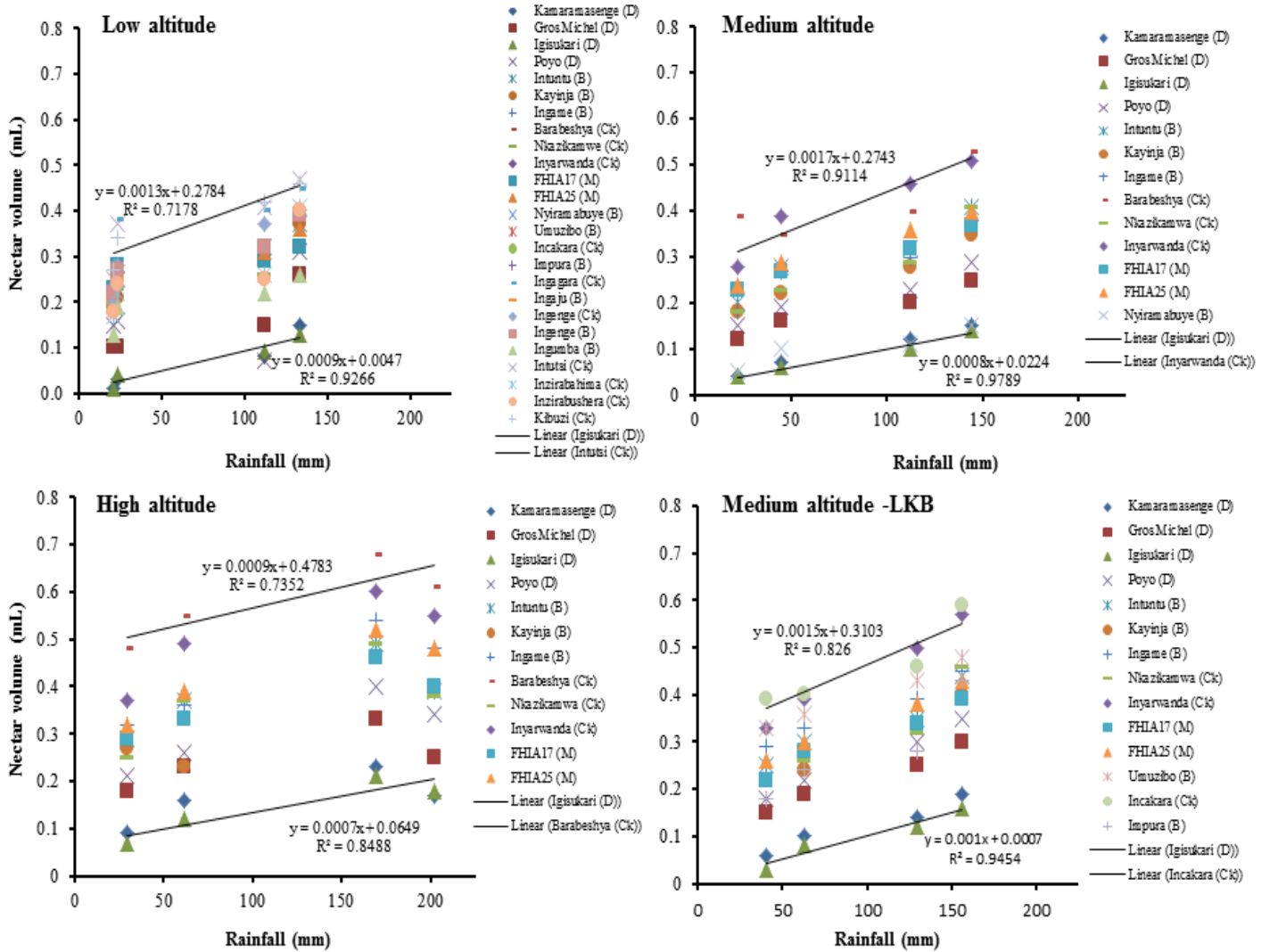
**Figure 2.** Nectar volume across banana cultivars in the high altitude areas. Letters between brackets stand for D: dessert, B: beer, Ck: cooking and M: multipurpose. SdS, SrS, LdS and LrS respectively, denote short dry season (61.9 mm of rainfall), short rainy season (169.3 mm), long dry season (29.2 mm) and long rainy season (202 mm). Error bars indicate the standard error of the mean.



**Figure 3.** Nectar volume across banana cultivars in the medium altitude areas. Letters between brackets stand for D: dessert, B: beer, Ck: cooking and M: multipurpose. SdS, SrS, LdS and LrS, respectively, denote short dry season (45.3 mm of rainfall), short rainy season (144.4 mm), long dry season (22.4 mm) and long rainy season (112.1 mm). Error bars indicate the standard error of the mean.



**Figure 4.** Nectar volume across banana cultivars in the low altitude areas. Letters between brackets stand for D: dessert, B: beer, Ck: cooking and M: multipurpose. SdS, SrS, LdS and LrS, respectively, denote short dry season (23.2 mm of rainfall), short rainy season (133.7 mm), long dry season (20.6 mm) and long rainy season (112.3 mm). Error bars indicate the standard error of the mean.



**Figure 5.** Variation in nectar volume (mL) in male flowers of different banana cultivars with amount of rainfall (mm) at four altitudes of 1300 - 1400 masl (low), 1596 - 1642 (Medium, Lake Kivu Border region), 1600 - 1650 (medium) and 1887 -2167 (high altitude).

altitude areas and the Lake Kivu border regions that are characterized by heavy rains and low mean temperatures (Atlapedia online, 2013). The good water supply to the roots, coupled with the low evapo-transpiration, probably influenced the amount of water in the nectaries. This justifies the higher nectar volumes recorded during the rainy seasons when compared with the dry seasons. Higher nectar sugar content was also noted in the low altitude sites characterized by lower rainfall levels. Low mean nectar concentration of some plant species has been reported to be related to lower mean maximum temperatures and higher precipitation (Barros et al., 1983).

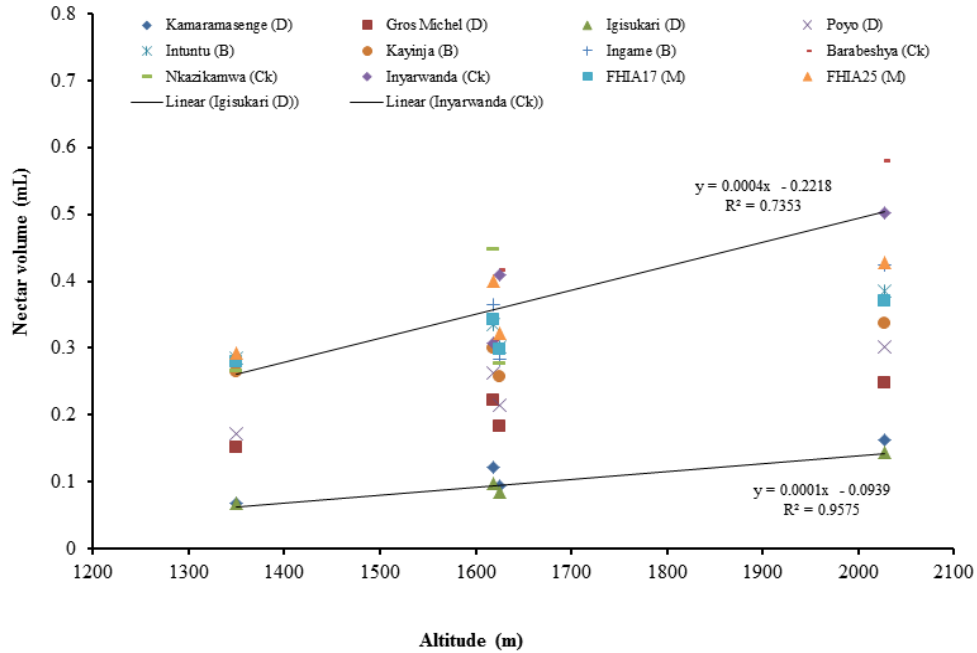
Similarly, Nicolson and Nepi (2005) reported a decrease in nectar volume and increased concentration due to high temperatures. Nicolson and Nepi (2005) also

reported that substantial amounts of genetic variation can be swamped in the field due to the environmental factors surrounding the plant.

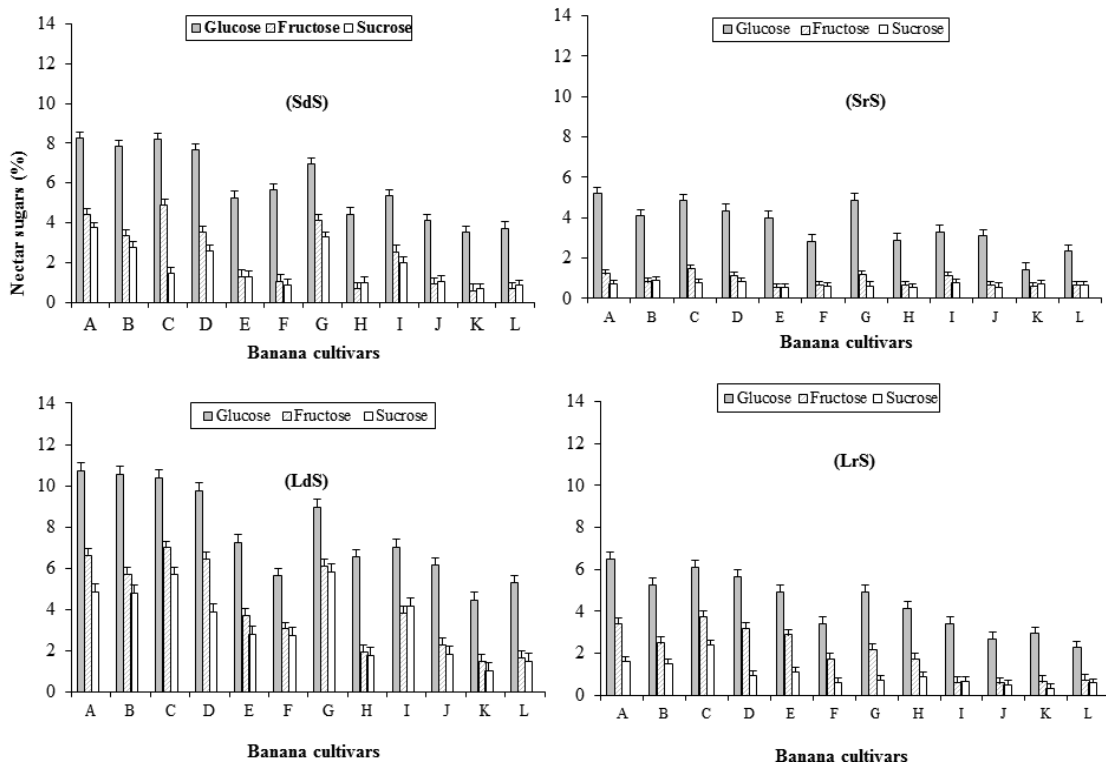
**Variation in nectar volume and sugar content across banana cultivars**

East African highland cooking banana cultivars (AAA genome) generally contained a higher nectar volume as compared to the dessert and beer types. In contrast, nectar sugar content in AAA-EA cooking banana cultivars was relatively low when compared with the dessert and beer (ABB and AAA) types. These observations suggest that nectar volumes and sugar content in bananas were influenced by the interaction between the genotypes of

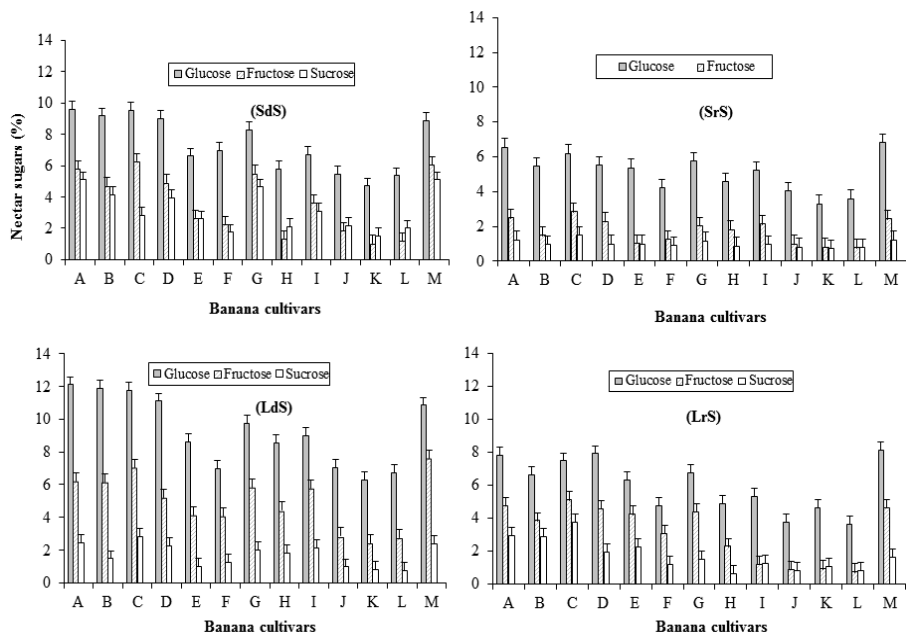




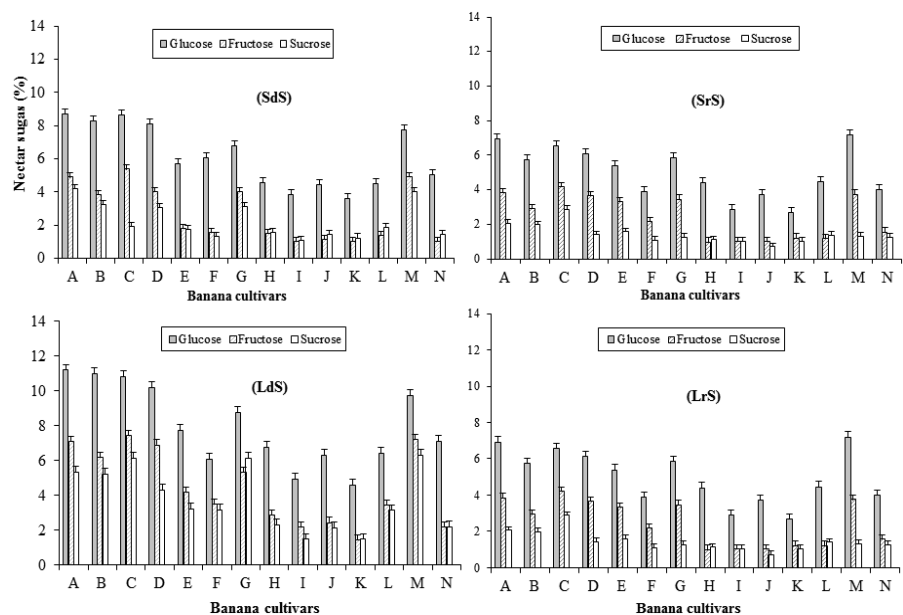
**Figure 6.** Variation in nectar volume (mL) in male flowers of different banana cultivars with altitude (masl) across all the rainfall seasons.



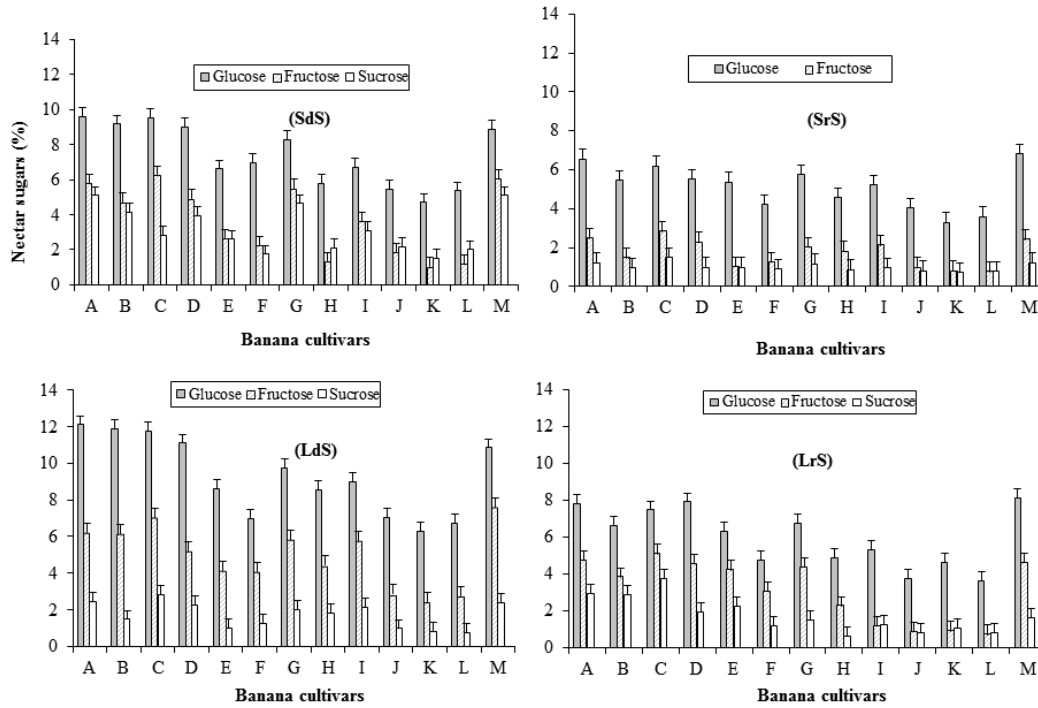
**Figure 7.** Nectar sugar content (%) in banana cultivars grown in the high altitude areas during the short dry season (SdS, 62 mm of rainfall, January to February), short rainy season (SrS, 169 mm, March to May), long dry season (LdS, 29 mm, June to August ) and the long rainy season (LrS, 202 mm, September to December). Error bars indicate the standard error of the mean. Letters on the X axis stand for: (A) Kamara, (B) Gros Michel, (C) Igisukari, (D) Poyo, (E) Intuntu, (F) Ingame, (G) Kayinja, (H) Incakara, (I) Nkazikamwe, (J) Inyiwanda, (K) FHIA17, (L) FHIA25.



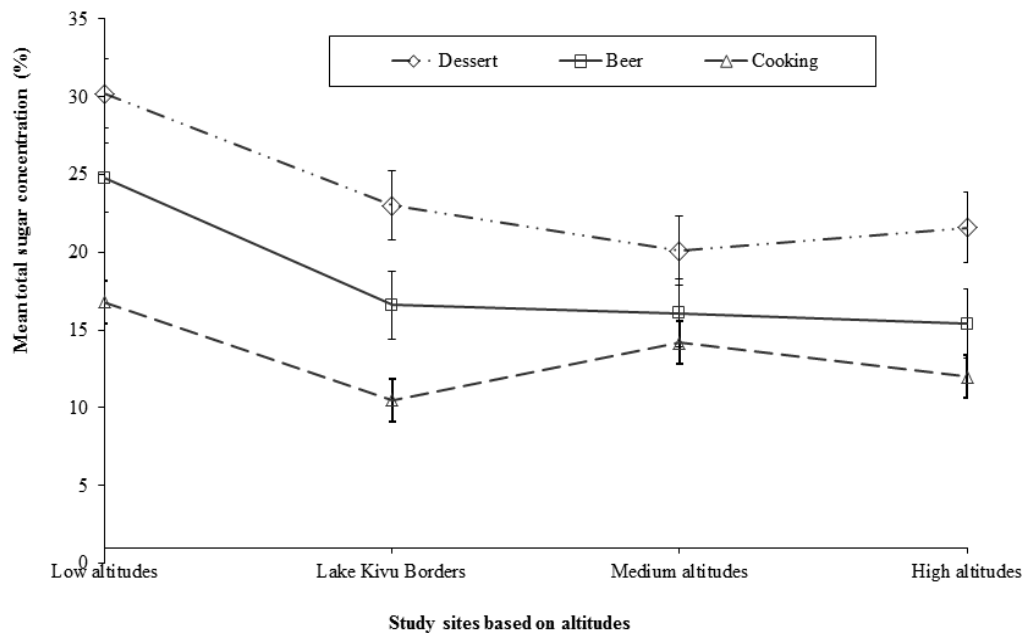
**Figure 8.** Nectar sugar content (%) in banana cultivars grown in the Medium Altitude areas during the short dry season (SdS, 45 mm of rainfall, January to February), short rainy season (SrS, 144 mm, March to May), long dry season (LdS, 22 mm, June to August) and the long rainy season (LrS, 112 mm, September to December). Error bars indicate the standard error of the mean. 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.



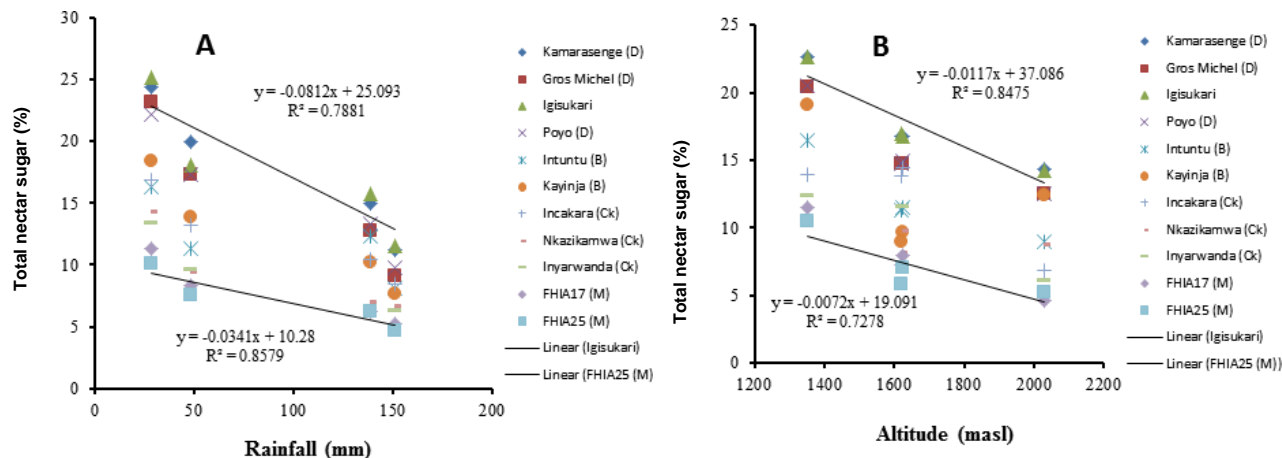
**Figure 9.** Nectar sugar content (%) in banana cultivars grown in the Kivu Lake Border (medium altitude) during the short dry season (SdS, 63 mm of rainfall, January to February), short rainy season (SrS, 156 mm, March to May), long dry season (LdS, 41 mm, June to August) and the long rainy season (LrS, 130mm, September to December). Error bars indicate the standard error of the mean. 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) Umuzibo and (N) Impura.



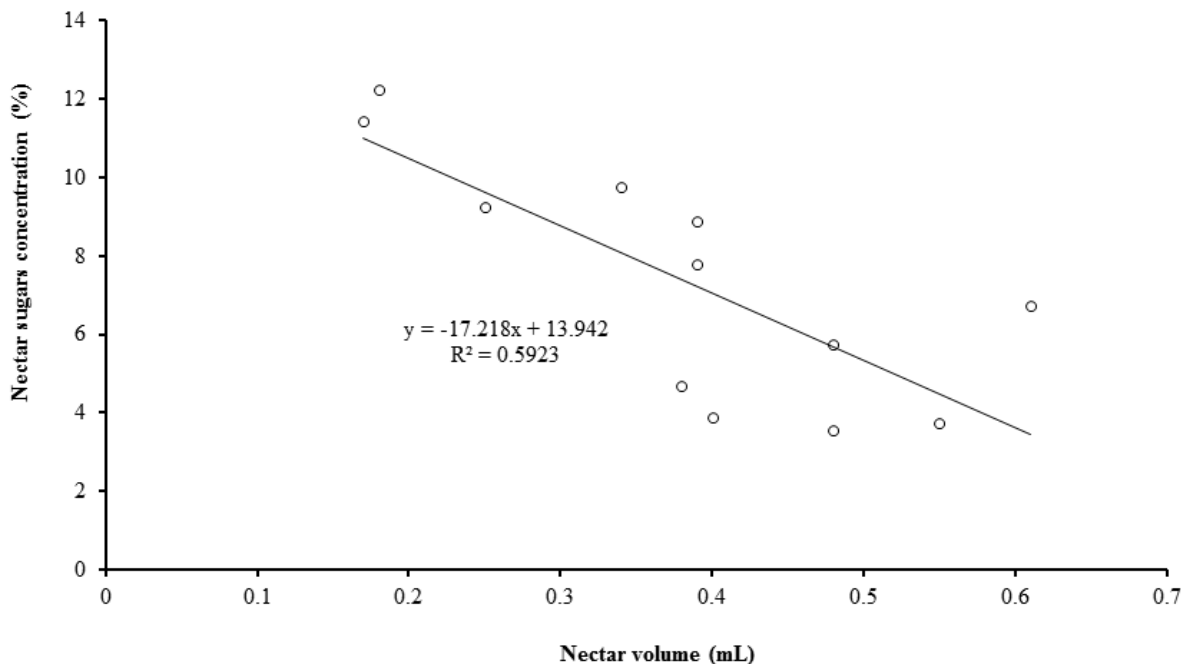
**Figure 10.** Nectar sugar content (%) in banana cultivars grown in the low altitude during the short dry season (SdS, 23 mm of rainfall, January to February), short rainy season (SrS, 134 mm, March to May), long dry season (LdS, 21 mm, June to August) and the long rainy season (LrS, 112 mm, September to December). Error bars indicate the standard error of the mean. 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, (P) Ingaju, (Q) Ingenge-cooking, (R) Ingenge-beer, (S) Ingumba, (T) Intutsi, (U) Inzirabahima, (V) Inzirabushera, (W) Kibuzi.



**Figure 11.** Mean total sugar concentration (%) for the three groups of banana cultivars across the study sites selected based on altitudes during the long dry season. Low altitude varied between 1300 and 1400 masl; Lake Kivu Borders -1596 to 1642; medium altitude – 1600 to 1650; and the high altitude site – 1887 and 2167 masl. Error bars indicate the standard error of the means.



**Figure 12.** Variation in mean total nectar sugar (%) with rainfall (A) and altitude (B) for different banana cultivars in four agro-ecologies of Rwanda.



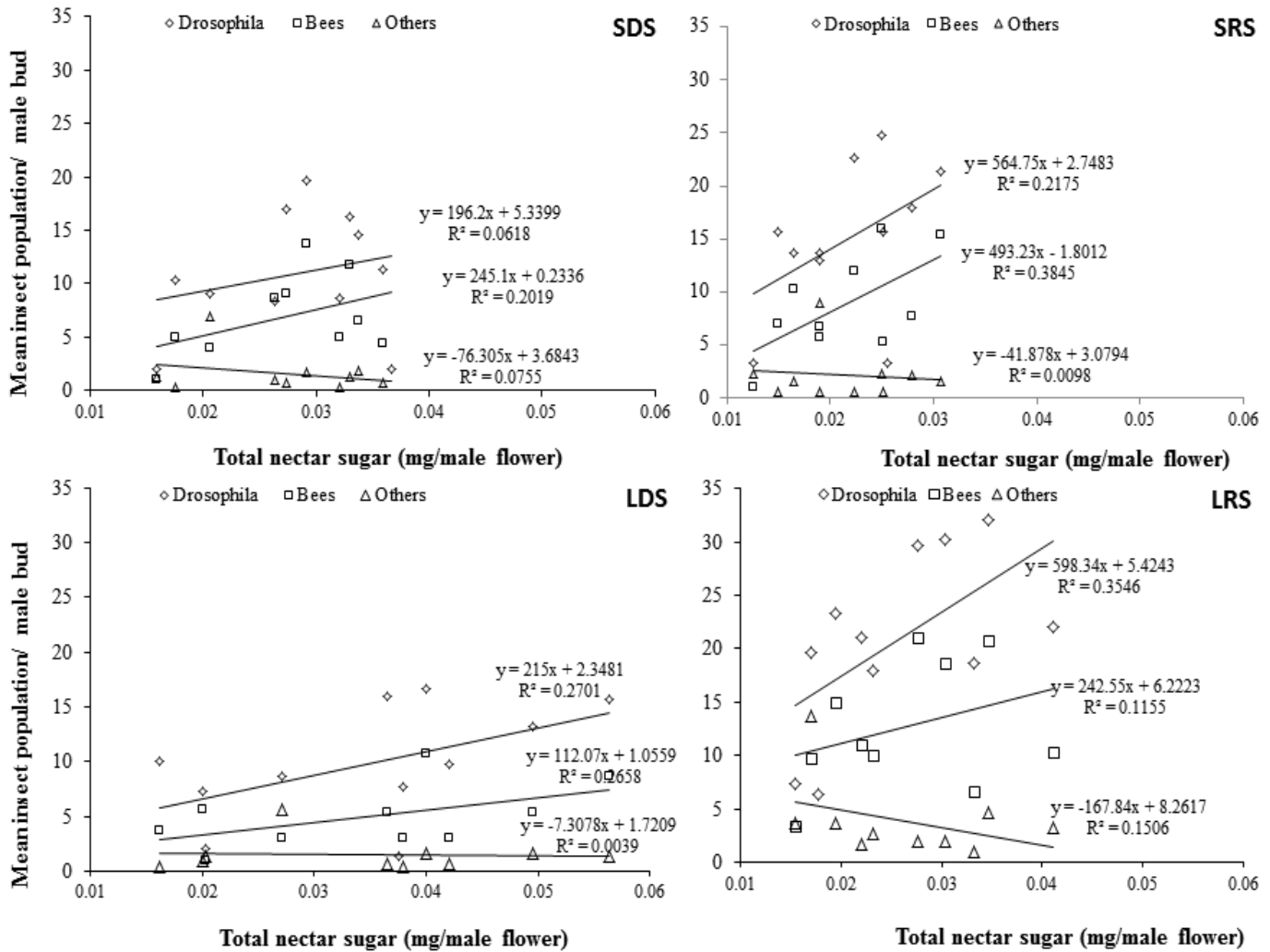
**Figure 13.** Variation of the total nectar sugars (%) with nectar volume for the major banana cultivars grown in the high altitude areas during the long rainy season.

the cultivars and their environmental conditions. Nectar is a complex mixture of substances dissolved in water, with water as the most abundant component (~30 to 90%) (Nicolson, 2002; Nicolson and Fleming, 2003).

**Nectar sugar composition and proportion for the banana cultivars identified in the study areas**

The nectar in banana is chemically a very specific

secretion made of 68% water and 32% dry weight (mainly sugars that make up about 90%) (Luttege, 1977). Three types of sugars, glucose, fructose and sucrose were detected in the banana cultivars. Hexose sugar was the predominant type of sugar in all the banana cultivars. Percival (1961) reported nectar to contain basically a sugar solution composed of one disaccharide (sucrose) and one monosaccharide-hexose (glucose and fructose), the amount and relative concentrations of which vary among species. The total amount of sugar content and

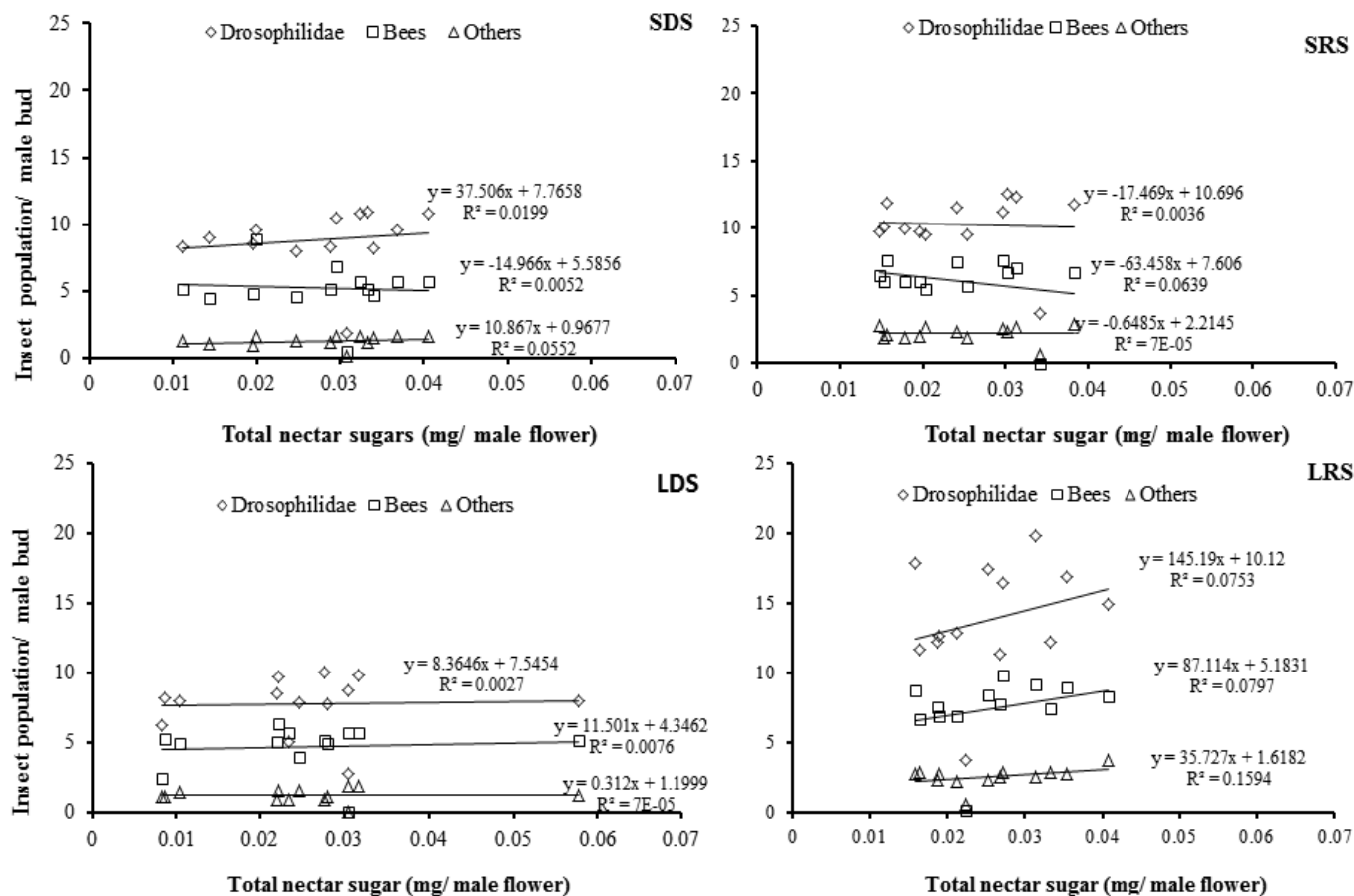


**Figure 14.** Variation of the total number of insects with total nectar sugar concentration for different banana cultivars at the high altitude areas during the short dry season (SDS), short rainy season (SRS), long dry season (LDS) and long rainy season (LRS).

the proportion of the sugars varied among banana cultivars. Within a cultivar, the proportion of the sugars in the nectar was observed to be consistent irrespective of the altitude and seasons. Luttege (1977) reported that sugar proportions in nectar tend to be constant within a species and in other cases within a genus or a family, revealing a clear phylogenetic influence on nectar sugar profiles. However, divergent nectar features have also been reported in plant taxa from the same lineage that has maintained a close relationship with different pollinator guilds (Temeles and Kress, 2003). Cawoy et al. (2008) reported minor changes in sugar proportions due to differences in age, variations among inflorescences and plants when compared with the distinct differences observed among species. The age of the male buds is therefore unlikely to have affected the results of this study, though efforts were also made to obtain male buds that were closely of the same age.

### Prevailing insect species on banana male flowers and their association with nectar sugars

In the current study, insects collected from banana male flowers were mostly fruit flies and bees. This is consistent with previous literature (Tinzaara et al., 2006; Rutikanga et al., 2015). These insects have been confirmed to be vectors of *Xcm* that causes XW disease in bananas (Tinzaara et al., 2006; Rutikanga et al., 2015). The proportions of the three sugars (glucose, fructose and sucrose) have been linked with different classes of pollinators. Sucrose-rich nectar has been found mostly in flowers pollinated by insects with long mouth parts (long-tongued bees, moths and butterflies), whereas hexose-rich nectar has been found in flowers pollinated by short-tongued bees, bats, perching birds and flies (Baker and Baker, 1983; Elisens and Freeman, 1988; Baker and Baker, 1990; Baker et al., 1998). Bats and birds have



**Figure 15.** Variation of the total number of insects with nectar volume for different banana cultivars at the high altitude areas during the short dry season (SDS), short rainy season (SRS), long dry season (LDS) and long rainy season (LRS).

also been observed to suck nectar from banana male flowers (Buregyeya et al., 2014).

High nectar sugar content has been hypothesized to be responsible for a high prevalence of insects on the male inflorescence of some cultivars and thus the high XW incidence in them. For example, the ABB cultivar, 'Kayinja', which is amongst the cultivars with high nectar sugar content in this study, has previously been reported to be more susceptible to insect vector-mediated transmission (Blomme et al., 2005; Rutikanga et al., 2015). However, in this study, no strong positive correlation was observed between insect population and the nectar sugar concentration or the nectar volume. The dessert types with the highest sugar concentrations attracted the least number of insects.

#### Prevalence of insect vectors of *Xcm* versus cropping seasons

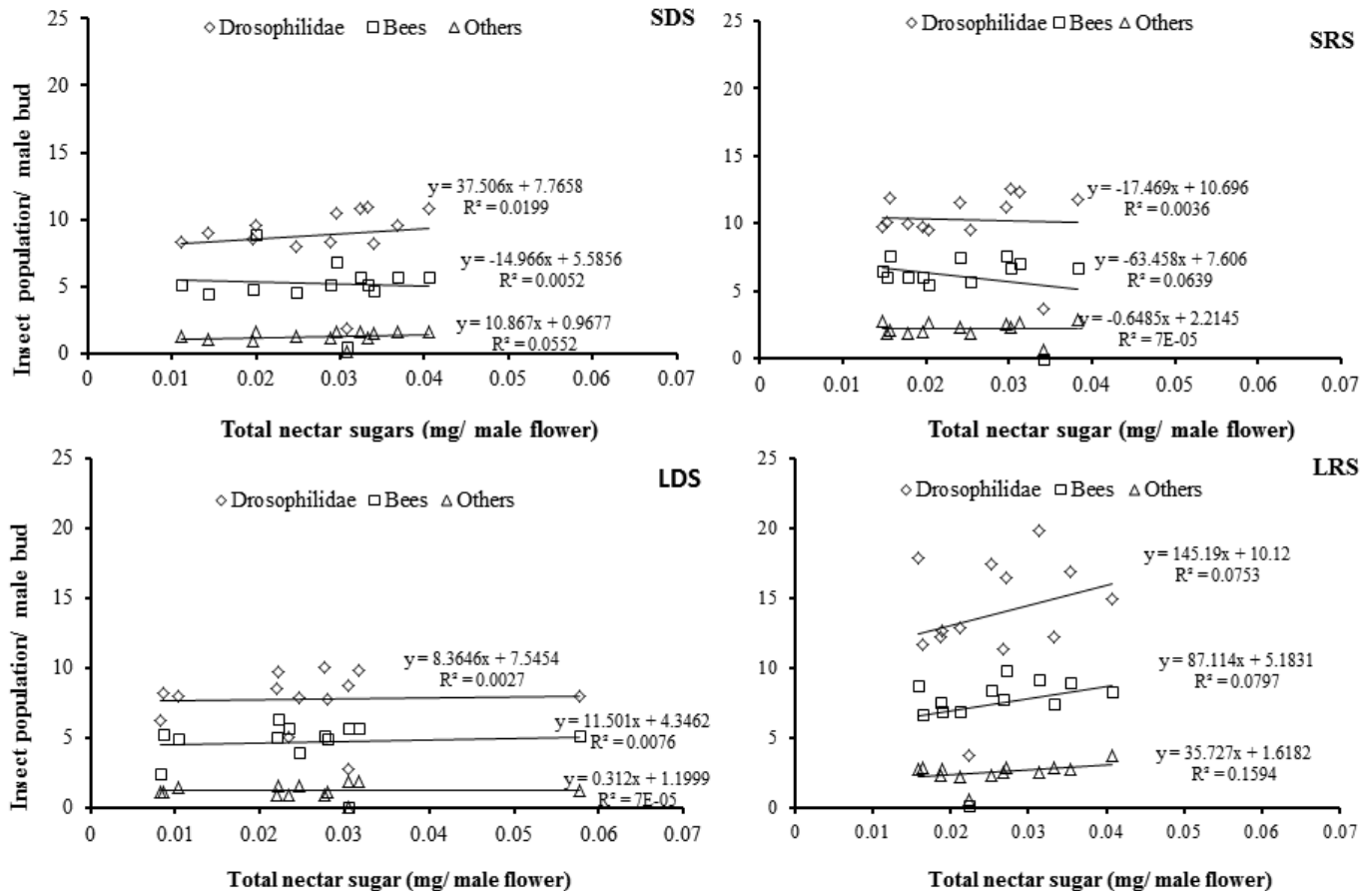
More insects were noted in the long rainy season to have moderate and well distributed rainfall. During the rainy seasons, nectar volumes are high but with lower

concentrations when compared with the dry seasons. These observations suggest that, rather than the concentration of nectar sugars, the prevalence of insects was influenced by the prevailing weather and environmental conditions that could have supported insect survival and activity, and possibly other stimuli other than nectar concentration.

According to Altieri and Letourneau (1984), the visual and chemical stimuli from host plants affect insect colonization and behaviour. In addition, the presence of other plants (not investigated in this study) that are often cultivated in the rainy seasons could also have influenced the number of insects.

#### CONCLUSION AND RECOMMENDATIONS

The findings of the current study revealed that the attractiveness of a banana cultivar to insect vectors was not influenced by the nectar concentration and volume. AAA dessert cultivars generally had a higher content of nectar sugars across the altitudes and seasons though sugar content did not correlate to insect activity in the



**Figure 16.** Variation of the total number of insects with nectar sugar concentration for different banana cultivars at the low altitude areas during the short dry season (SDS), short rainy season (SRS), long dry season (LDS) and long rainy season (LRS).

different study sites. Insect population and activity was mainly influenced by the prevailing weather conditions (dry vs wet season). It has been hypothesized that the high nectar sugar concentration in 'Kayinja', a highly susceptible cultivar to insect-mediated XW infections contributed to its attractiveness to insects and thus the observed susceptibility. On the basis of the above findings, this study rejects this hypothesis. In addition to the weather conditions, other factors not investigated in this study such as male inflorescence behavior (e.g. persistent vs. non-persistent bracts), appearance (e.g. flower shape and colour), smell and shape; and presence of other crops within or around the banana plantations in the rainy seasons could have also influenced insect activity. For example, cultivars with morphological forms of resistance to insect transmission, such as persistent male bracts and neuter flowers have been reported to escape insect mediated infections. The contribution of other stimuli such as inflorescence color and semiochemicals to the attraction of insect vectors of *X cm* needs to be investigated. The number of flowering plants in the studied farms/seasons and other crops in the

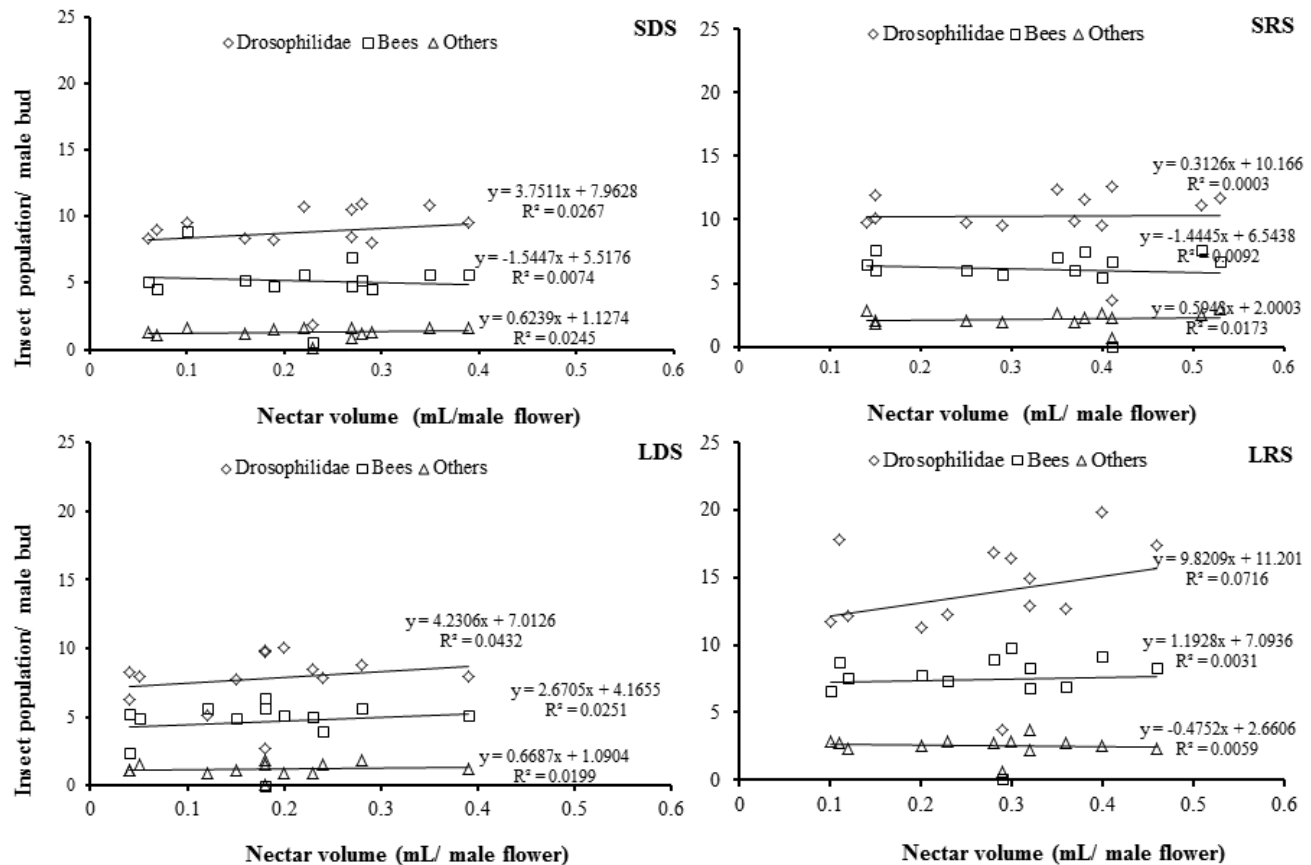
vicinity of the farms or in the agro-ecologies could have also influenced insect activity across fields or farms, yet this aspect was not evaluated in this current study.

### Conflict of interests

The authors have not declared any conflict of interests.

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**Figure 17.** Variation of the total number of insects with nectar volume for different banana cultivars at the low altitude areas during the short dry season (SDS), short rainy season (SRS), long dry season (LDS) and long rainy season (LRS).

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