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# Spatio-temporal dispersion patterns of *Bactrocera musae* Tryon (Diptera: Tephritidae: Dacinae) in Vudal agroecosystem, East New Britain

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## Abstract

Banana fruit fly, *Bactrocera musae* Tryon is endemic to Australia and mainland Papua New Guinea (PNG) and remains an important pest in tropical orchards. *B. musae* is classified as polyphagous since it feeds on several families in Musaceae, Caricaceae and Myrtaceae while preferring banana as its primary economic host. Methyl eugenol was successful in trapping very high number of adults: primary forest (100%), secondary forest (99%) and cocoa plantation (94%). Abundance trapped with cue-lure was very low, 6% in cocoa plantation and 1% in secondary forest. Significant difference was detected between abundance in cocoa plantation and primary forest ($p<0.01$), and also with secondary forest ($p<0.05$). However, there was no significant difference between primary and secondary forest ($p>0.05$). The striking differences can be attributed to both biotic and abiotic factors, and their interactions. The population of *B. musae* demonstrated a dynamic pattern on a temporal scale (fig. 3). Our 6 months of intensive sampling detected the lowest population in June (159, 17.7±8.4, $p<0.05$) and the highest in October (620, 91.2±37.2, $p>0.05$). Wet season recorded higher numbers while high air temperature reduces the activity of adult *B. musae*. Host availability and fruiting season contributed positively to peak numbers of fruit flies. Finally, the height, canopy structure and different architecture of vegetation in primary and secondary forests provided a suitable resting site for adults of *B. musae.*

**Keywords:** *Bactrocera musae,* polyphagous, methyl eugenol, cue-lure, abundance, resting site

## Introduction

The banana fruit fly, *Bactrocera musae* Tryon (Diptera: Tephritidae: Dacinae) is widely distributed in the Oceania region. It has been recorded in Queensland (Australia), Islands region of Papua New Guinea (Bismarck Archipelago), Solomon Islands and West Papua (Indonesia) (Drew 1989; Tenakanai 1997; Schutze et al. 2018) [12, 57, 50]. *B. musae* is endemic to Australia and mainland Papua New Guinea (PNG) and remains an important pest in tropical orchards (Noushini et al. 2020) [38]. The primary economic host of *B. musae* is banana (Musaceae: *Musa* spp.) however it can also utilize other host plants in the family of Caricaceae (i.e papaya) and Myrtaceae (i.e. guava) (Mararuai et al. 2002; Schutze et al. 2018) [33, 50]. Field sampling in mid-1999 recorded an incursion of *B. musae* in Gazelle Peninsula of East New Britain (ENB) (Mararuai et al. 2002) [33]. In order to protect banana fruits against *B. musae*, a traditional method of wrapping developing bunches in banana leaves is done to improve fruit quality (Waterhouse 1997) [64]. Females oviposit eggs into unripe banana fruits and then the subsequent larvae penetrate the flesh causing fruit destruction and premature fruit detachments (Mararuai et al. 2002) [33].

Most studies on *B. musae* in PNG was done by using the male parapheromone, methyl eugenol (ME) as an attractant (Sar et al. 2001; Novotny et al. 2005b; Novotny et al. 2007; Royer et al. 2018) [49, 39, 41]. A “attract and kill” system in which a toxicant is incorporated has proven to be effective in sampling male fruit flies (Khan et al. 2015) [20]. Although a few studies were done on the distribution of *B. musae* in Gazelle Peninsula and East New Britain, their dispersion patterns and occurrence with a tropical agroecosystem has not been investigated. The dispersion of polyphagous pests like *B. musae* within an agroecosystem containing orchards, forests and crop fields is important to determine their occurrence within the crop environment on a spatial and temporal scale (Balagawi et al. 2014) [4]. The occurrence of fruit flies (i.e. *B. musae)* at a given time and place are determine by both biotic and abiotic factors (Duyck et al. 2004) [13]. Biotic factors such as interspecific competition, natural enemies and host plant (i.e. fruiting season) can determine the dispersion of fruit flies (Hendrichs et al. 1991; Duyck et al. 2004; Rwomushana et al. 2009) [24, 14, 47]. Climatic factors such as temperature, relative humidity and rainfall can also influence the occurrence of fruit flies (Duyck et al. 2004; Duyck et al. 2006; Gutierrez et al. 2009; Ganie et al. 2013) [14, 15, 22, 20]. Temperature influences the developmental activity of fruit flies therefore studies have been conducted to determine the minimum development thresholds and thermal constants for various species (Messenger & Flitters 1958; Delrio 1986; Kasana & AliNiazee 1994; Yang et al. 1994; Vargas et al. 1996; Brévault & Quilici 2000; Duyck & Quilici 2002) [34, 11, 29, 13, 65, 59]. The effect of relative humidity on the developmental stages (larvae to pupa) is exerted mainly by the host fruit than by climatical conditions (Neilson 1964; Shoukry & Hafez 1979; Eskafi & Fernandez 1990; Teruya 1990) [37, 55, 17, 58]. Sufficient rainfall increases soil and air humidity thus favoring survival of both larvae and adults while superabundant rainfall raises the soil humidity, which can lower the pupating ability and subsequent emergence of neonate fly (Ye & Liu 2007) [66].

Our study was based on *B. musae* collected with the male parapheromones, Methyl eugenol (ME) and Cue-lure (Cue). We examined the monthly abundance of fruit flies in three vegetation types located within Vudal agroecosystem: (1) cocoa plantation, (2) primary forest and (3) secondary forest. The data collected was used to quantify the population dynamics of *B. musae*, investigate niche partitioning, assess host plant availability (i.e. fruiting season) and correlating population to climatic factors. We also evaluated the interaction of climatic and biotic factors on the response of *B. musae* abundance.

## Materials and methods

The *B. musae* study was done at PNG University of Natural Resources and Environment (PNG UNRE) which is located within Vudal area of Gazelle Peninsula in Papua New Guinea (PNG). PNG UNRE is located at an elevation of 51 meters above sea level and approximately 4º21’01.90” S and 152º 54 00’33.44” E (Iamba & Yoba 2019) [27]. The soil is more calcareous in nature and relatively sandy loam with high alkalinity (Iamba & Malapa 2020) [26]. The sampled vegetation sites included (1) Crops field, (2) Secondary forest, and (3) Primary forest reserve. The crops field constitute of cocoa (*Theobroma cacao*), teak (*Tectona grandis*) and balsa (*Ochroma pyramidale*) plantations to the north-east, vegetable gardens to the east and a cattle farm and paddock situated to the west. Predominant vegetables grown here are cabbages, tomatoes and capsicum, grown all year round. Bananas (*Musa* spp.), mangoes (*Mangifera indica*), star fruits (*Averrhoa carambola*), breadfruit (*Artocarpus altilis*) and other fruit trees are also growing in the vicinity. Cocoa plantation is approximately 30km$^2$ and represents an agricultural monoculture system. Most of the cocoa trees are clonal varieties that has some level of tolerance to pest attacks. The secondary forest contains vegetation regrowth after disturbances from gardening and clear cutting. The common tree species found here are breadfruit (*Artocarpus altilis*), ton (*Pometia pinnata*), tulip tree (*Spathodea campanulata*) and bamboos (*Bambusa* sp.) distributed in approximately 30km$^2$. The primary forest is actually a reserved forest that has been preserved for its vital ecosystem services such as waste water filtration, conservation site for fauna and flora, and research site for academic studies. It has an approximate area of 200km$^2$. Common tree species in primary forest include *Pometia pinnata*, *Alstonia scholaris*, *Canarium indicum*, *Inocarpus fagifer*, and *Maniltoa* sp. The three study sites selected are a good representation of Vudal agroecosystem and an example of a tropical agroecosystem.

### *Fruit fly sampling*

Adult fruit flies were attracted to two male parapheromone lures, cue-lure (Cue) and methyl eugenol (ME), exhibited in modified Steiner traps. Six traps were placed along a transect with minimum distance of 10m between traps at each study site; 3 ME traps and 3 Cue traps. The study sites were more than 500m apart from each other to avoid overlapping of attractant cues. A total of 18 baited traps were used in this study and were placed strategically to capture the vegetation type which were not continuous but fragmented. Each trap used the lure-and-kill technique (Balagawi et al. 2014; Khan et al. 2015) [33, 30] where Dichlorvos (DDVP [2, 2-dichlorovinyl dimethyl phosphate]) cubes were used as a toxicant (Senior 2016; Broughton & Rahman 2017) [51, 7]. For all lures, each trap contained a 15mm DDVP cube (Dichlorvos; BioTrap Australia Pty Ltd, Victoria, Australia) that was used as killing agent (Vargas et al. 2010a) [60]. About 2 to 3 ml of each parapheromone were soaked in a cotton wick and placed inside each trap at the back along with one DDVP cube and hung from understory tree branches 1.5 m above the ground using string ropes. A new pair of laboratory gloves were used to handle individual types of lures, and the lures were separately done in the laboratory before taking them out to the field. All traps at all three study sites were emptied at weekly intervals from 18th June – 16th November 2019. All flies were collected into vials containing 70% ethanol and identified to species using (Schutze et al. 2018) [50]. Environmental factors were taken into consideration as they can have adverse effect on distribution. Therefore, daily recordings of temperature, relative humidity and rainfall were recorded at the weather station at UNRE campus. Weekly mean values of each factor were then calculated and used for analysis.

We used the R function *aov* (R Core Team, 2013) for Analysis of Variance (ANOVA) to analyze effect of vegetation types and attractants on abundance of *B. musae*. Principal Component Analysis (PCA) was used (function *prcomp*, package vegan) to analyze the relationship of different biotic and abiotic variables on the distribution of *B. musae* to vegetation types on an ordination scale. For correlation between variables such as abundance, temperature, rainfall and relative humidity, we used function *cor.test* (package ggplot2) for Pearson correlation, and function ggbarplot (package ggpubr) for species abundance against months (time). In addition, we used function *stat_cor* and *stat_regline_equation* (package ggpmisc) to construct linear regression equations between abundance and environmental factors.

## Results

We collected a total of 2716 *B. musae* individuals from 151 trap-days during sampling. The highest abundance was recorded in primary forest (1260), followed by secondary forest (1238) and lowest in cocoa plantation (218). Most *B. musae* was caught with Methyl eugenol (ME) with extremely very low numbers trapped in cue-lure (Cue) (fig. 1). Cue-lure attracted only 1% of *B. musae* individuals in Cocoa plantation and 6% in secondary forest. *B. musae* was only attracted to ME in primary forest (100%) meaning none was caught with cue-lure. ME also attracted 94% of individuals in cocoa plantation and 99% in secondary forest (fig. 1). *B. musae* is highly responsive to ME therefore it is an effective attractant to be considered in monitoring studies.

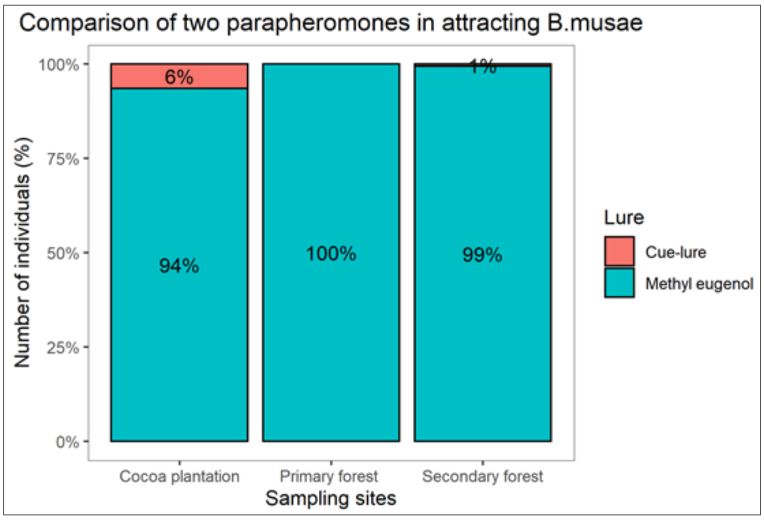

**Fig 1:** B. musae individuals were mainly caught with methyl eugenol (ME) while extremely very low numbers were recorded in cue-lure. All individuals caught in primary forest were from ME-baited traps while none was recorded in cue-lure. Adults of B. musae were very responsive to ME within all study sites.

Overall, pairwise comparison of the mean abundance in the three study sites showed significant difference ($p<0.01$) (fig.2). The means were separated using Tukey HSD test to detect the existence of the differences. Significant difference was detected between *B. musae* abundance in cocoa plantation and primary forest ($p<0.01$) (fig. 2). Significant result was also recorded between cocoa plantation and secondary forest ($p<0.05$). However, there was no significant difference between primary and secondary forest ($p>0.05$). The striking differences can be attributed to both biotic and abiotic factors, and their interactions. Since it an ecology-based study, several factors can play essential roles in determining the population dynamics of *B. musae*. Even though the study sites are located within the same biogeographical ecosystem, their abundance varied on a spatial and temporal scale.

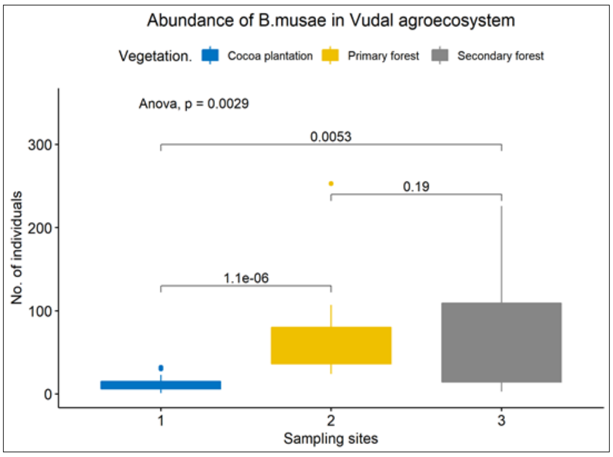

**Fig 2:** The overall mean of abundance was significantly different in this study. Separation of means using Tukey HSD test showed that significant differences occurred between cocoa plantation and the other two sites, primary forest ($p<0.05$) and secondary forest ($p<0.01$). However, there was no significant difference between primary and secondary forest.

The population of *B. musae* demonstrated a dynamic pattern on a temporal scale (fig. 3). According to our 6 months of intensive sampling, the abundance of *B. musae* was the lowest in the month of June (159, 17.7±8.4, $p<0.05$). A steady increase was observed in the month of July (374, 31.2±6.4, $p<0.05$) and also in August (547, 36.5±7.4, $p<0.01$). The increasing population trend continued to September (490, 81.7±39.8, $p>0.05$) and reached its highest peak in October (620, 91.2±37.2, $p>0.05$). There was a decline in the population after October with November having a reduction in abundance (526, 87.7±33.4, $p>0.05$). The population trend generally follows a hill or bell shape meaning that there is only one peak point (i.e. October) where population reaches its highest and then starts to decline.

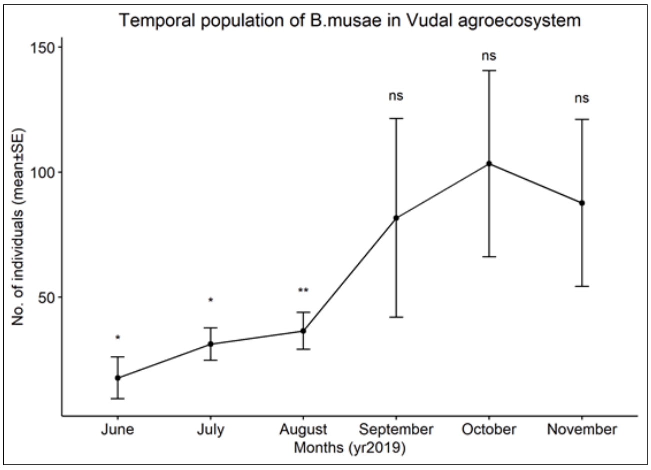

**Fig 3:** Population of B. musae was the lowest in month of June and reached its highest in October. Abundance of B. musae was low in June and July (*p <0.05) then slightly increase in August (**p<0.01). There was a steep increased in September then it peaked in October and eventually decreased in November. The population dynamics of B.musae is attributed to both biotic and abiotic factors that influences the fluctuations the abundance and distribution.

The values of temperature (ºC) and relative humidity (%) are positively correlated ($r = 0.38$, $p<0.01$). An increase in temperature generally contributes to rise in relative humidity (RH). A significant negative correlation existed between temperature and rainfall (mm) ($r = -0.51$, $p<0.001$). A negative correlation was also detected between RH and rainfall (RF) ($r = -0.22$, $p>0.05$). Therefore, we imply that the interaction of temperature and relative humidity is insignificant to cause changes in rainfall. A significant negative correlation was detected between temperature and abundance ($r = -0.28$, $p<0.05$) meaning temperature influences population. Abundance of *B. musae* had negative correlation with RH ($r = -0.12$, $p>0.05$) and positive correlation with RF ($r = 0.17$, $p>0.21$) however the relationships were not significant. There is a significant positive strong correlation between abundance and methyl eugenol because most *B. musae* were trapped with ME ($r= 0.99$, $p<0.001$). The relationship between abundance and cue-lure is very weak ($r = -0.12$, $p>0.05$) since very low numbers of *B. musae* were trapped with cue. Vegetation also had a significant effect on abundance ($p<0.05$) however the correlation was weakly correlated ($r = 0.3$).

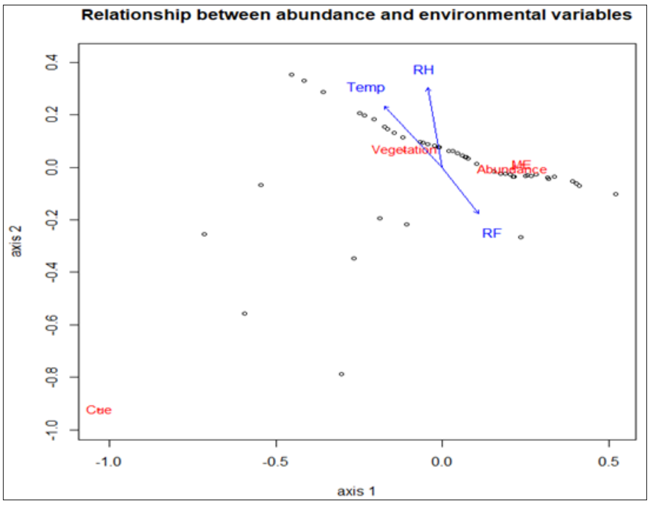

**Fig 4:** Three (3) environmental factors were measured and correlations drawn between abundance and vegetation types (study sites). There was a positive correlation between temperature (°C) and relative humidity (%) ($r= 0.38$). Both temperature and relative humidity (RH) had negative correlation with rainfall (mm). The abundance of B. musae trapped in methyl eugenol and cue-lure was significantly different ($p<0.01$). The correlation between abundance and vegetation types were positively correlated ($r= 0.39$).

## Discussion

We recorded high abundance of *B. musae* in primary reserved forest and secondary forest while very low population in cocoa plantation. Both primary and secondary forests provide vegetation around field margins with optimal height and canopy structure that is utilized by fruit flies as their resting sites (Balagawi et al. 2014) [4]. Even though secondary forest is a disturbed vegetation, we recorded significantly higher number of *B. musae* (Virgilio et al. 2011) [63]. The horticultural orchards in an agroecosystem provides suitable habitats for fruit flies to strive while trading of agricultural products by humans contributes to the dispersal of tephritid pests (Prokopy et al. 2000; Malacrida et al. 2007) [42, 32]. As the abundance was quantified using two types of lure traps, we found that methyl eugenol (ME) attracted higher number of fruit flies than cue-lure (Cue) (Shelly et al. 2004; Shelly & Kurashima 2016; Biasazin 2017; Royer et al. 2019) [52, 53].

Both Methyl eugenol and Cue-lure are contained phenyl propanoids which is a class of organic compounds having a structure of a C6– C3 skeleton (Friedrich 1976) [19]. Most species of Dacinae fruit flies (Diptera: Tephritidae) are readily attracted to Phenyl propanoids (Vargas et al. 2010b) [61]. Methyl eugenol occurs naturally in plants while cue-lure is an artificial compound isolated from plant-borne substances (Fletcher 1987; Robinson & Hooper 1989) [18, 44]. Cue-lure attracted individuals from three (3) genera; *Zeugodacus* (i.e. *Z. cucurbitae), Bactrocera* (i.e. *B. fraunfeldi),* and *Dacus* (i.e. *D. longicornis)* including cryptic species like *B. kirki* and *B. psiidi* (table 1). Our findings support Metcalf (1990) [35] that male Dacine fruit flies from genera *Bactrocera* Macquart and *Dacus* Fabricius are attracted to cue-lure while other species to ME. In general, fruit fly species show preference to one attractant, either ME or cue-lure, and the chemical cue is detected by specific antennal receptor sites (Metcalf & Metcalf 1992) [35]. Our results confirms that mature males in genera *Bactrocera* (i.e. *Bactrocera musae)* responds quickly to ME therefore it is generally recognized as a more powerful lure than cue-lure (Shelly et al. 2010) [54]. According to ancestral host-hypothesis, lures originated as plant kairomones serving as engagement stimulants to foster mating, while sexual selection-hypothesis suggests that the lures serve as male pheromone progenitor that affects the role of female in choice of mates (Raghu 2004) [43].

*Bactrocera* genera (i.e. *B. musae)* is commonly found in tropical agroecosystems and can easily be sampled with methyl eugenol (Gnanvossou et al. 2017) [21]. Our study affirms the findings of Gnanvossou et al. (2017) [21] that abundance of *Bactrocera* species positively correlates with rainfall. We also found that there is negative correlation between fruit fly abundance and temperature whereas positively correlated with rainfall (Laskar & Chatterjee 2010) [31]. As the temperature increases, the number of fruit flies decreases and wet season boosted the population of *B. musae*. Backing our findings, Tan and Serit (1994) [56] recorded peak numbers of *B. dorsalis* males within wet season when starfruit, *Averrhoa carambola,* was fruiting. Starfruit is a common fruit tree grown around Vudal area and generally starts fruiting in September and reaches peak fruit season in October which falls in wet season. Monthly rainfalls have been found to synthetically influenced the population dynamics of *B. dorsalis* (Chen & Ye 2007) [8]. Host plant availability and suitability as well as meteorological factors have proven to have an impact on fruit fly population dynamics and occurrence patterns (Hodkinson 2005; Mwatawala et al. 2006; Rwomushana et al. 2008; Vayssieres et al. 2008; Aidoo et al. 2014) [25, 36, 48]. Fruiting season of starfruit (*Averrhoa carambola*), breadfruit (*Artocarpus altilis),* mangoes (*Mangifera indica)* and bananas (*Musa* spp.) in the months of September, October and November (fig. 3) also resulted in peak numbers of *B. musae.* Fruit flies can adjust their resource searching patterns in response to changes in seasonal distribution of food, spatial and temporal factors (Hendrichs et al. 1991) [24]. The banana fruit fly, *B. musae,* had the highest abundance due to the study sites being dominated with banana plants growing around the vicinity. The population of *B. musae* also peaked in the month of September and October which is the fruiting season for most fruit trees. Studies in spatio-temporal dispersion patterns showed that fruit flies can move between habitats on a daily cycle to satisfy their feeding, mating and ovipositional requirements (Hendrichs et al. 1991; Danjuma 2013) [24]. Temperature was the abiotic factor inversely regulating the temporal population of *B. musae* in our study. High air temperatures can cause physiological stress to adult fruit flies and heavy rains can influence activities of fruit flies (Hedström 1991).

The distribution of *B. musae* and its population within our three study sites can be attributed to several factors. *B. musae* was trapped in all three vegetation types in considerable numbers suggesting they move between habitats in search for food, mating and refugia (Hendrichs et al. 1991; Danjuma 2013; Balagawi et al. 2014) [24, 4]. Searching for these resources in fruit flies can be dynamic (Hendrichs et al. 1991; Aluja & Birke 1993; Aluja et al. 1993) [24]. Interspecific niche partitioning also plays an important role in the distribution of fruit fly species (i.e. *B. musae)*. According to ecology theory, organisms differ in their use of shared, limiting resources if they are to coexist (Danjuma 2013) thus specialization is needed to reduce interspecific competition and promote species coexistence through niche partitioning (Dyer et al. 2007). Very high number of *B. musae* was recorded in primary forest (100%) (Fig. 1) which we attributed to the niche being utilized as refugium (Hendrichs et al. 1991; Danjuma 2013; Balagawi et al. 2014) [24, 4]. *B. musae* numbers peaked in secondary forest which is mainly due to sufficient availability of host plants, bananas (*Musa* spp.) starfruit (*Averrhoa carambola*), and mangoes (*Mangifera indica)* (Hendrichs et al. 1991; Aluja & Birke 1993; Aluja et al. 1993) [24] and mating purposes as fruiting season also signals congregating females (Hendrichs et al. 1991; Danjuma 2013) [24].

Frugivorous insects like fruit flies can be categorized as monophagous (utilizing a single host plant species); stenophagous (a few closely related species under a single plant family); oligophagous (two or few plant families); or polyphagous (utilizing many plant families). *B. musae* is classified as polyphagous since it feeds on several families in Musaceae, Caricaceae and Myrtaceae with banana as its primary economic host but occasionally feeds on papaya and guava (Schutze et al. 2018) [50]. Another example is *B. dorsalis* which is polyphagous or generalist, but shows preference to mango and guava over other fruits, *Z. cucurbitae* (polyphagous) prefers cucurbitaceous plants, but oviposits on other fruits, and *B. oleae* (monophagous) prefer only olives for their development (Biasazin 2017). According to Novotny et al. (2005a) [40], fruit flies exhibited low host specificity with respect to congeneric plant species but much higher, and similar with respect to both confamilial genera and interfamilial.

A complex of cues from the host plants within the three agro-habitats, parapheromones (Cue/ME), and meteorological factors played an essential role in determining the spatio-temporal population of *B. musae*. Since we were using parapheromones for trapping sexually matured male *B. musae*, it has to be correlated with changes in their olfactory circuitry (Biasazin 2017). Through evolution, generalist species might have conserved olfactory receptor neurons while specialists would have lost some of these neurons on the expense of others (Dekker et al. 2006; Ibba et al. 2010). In our study, *B. musae* abundance had positive correlation ($r = +0.17$, $p > 0.05$) with rainfall suggesting that they respond better to olfactory cues during wet season. Negative correlation between abundance and temperature ($r = -0.28$, $p < 0.05$) suggests that increase in temperature might have caused physiological stress in adult fruit flies thus lowering their population (Hedström 1991).

## Conclusion

This study finds that there is high abundance of *B. musae* in primary and secondary forests and lower population in disturbed area of cocoa plantation. Several factors influenced the abundance of *B. musae* within each habitat. Environmental factors such as temperature and to lesser extent rainfall and relative humidity exerted significant impact on *B. musae* population and their distribution among the three study sites. Wet season recorded higher numbers while high air temperature reduces the activity of fruit flies. Host availability and fruiting season contributed positively to peak numbers of fruit flies. Most of the fruit fly species recorded their highest abundance in the month of September and October which signifies the fruiting season. Specialization and generalization are needed to reduce interspecific competition and promote species coexistence through niche partitioning (Dyer et al. 2007). The matured males are able to utilize different plant resources and cues for feeding and mating. The luring of matured males to parapheromone, ME, is often correlated with changes in their olfactory circuitry. Finally, the height, canopy structure and different architecture of vegetation in primary and secondary forests provided a suitable resting site for adults of *B. musae.*

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