Diversity of cocoa pollinators in Cameroon

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The studies on cacao pollinators diversity and their efficiency in pollination dated back from 70's and 80's did not bring to a general consensus. Who are the true pollinators of cocoa? How do the pollinators vary according to agro ecological conditions in the plots? Considering the disparity of knowledge on the main pollinators' families and ecology it is not possible to design systems with the most favorable habitats conditions for the pollinators communities, neither to quantify precisely losses due to the absence of some pollinators. Here we propose to analyze potential pollinators fauna and their pollination activities in a Cameroonian plantation. Insect traffic on cocoa flowers were followed up under different shade conditions with different litter characteristics. Many methods (i.e. direct observation or by a community approach through global fauna collection) in the plot contributed to establish a functional diversity of pollinators insects and to characterize pollination activities. A high diversity of insects visit cocoa flowers (Formicidae, Milichidae, Psilidae). Most of them spend less than one minute on the flower without exploring the interior of the flower. Species from Ceratopogonidae family were not found visiting the cocoa flowers. The addition of banana stem rot in the litter increase the abundance of some species (like ants) but decreases overall species richness and modify pollinators functional diversity.

Introduction

Cacao (*Theobroma cacao* L., Malvaceae) is one of the most important cash crop species in the world. Cacao tree produces lots of flowers (Toledo-Hernandez et al., 2017) but pollination rate is low (less than 5 % of the flowers set fruit in cacao plantations). Indeed, cacao pollination is very specific. The flowers that are very tiny are receptive for 1 day after the anthesis; stigma and style receptivity is high in the morning and early afternoon (Young et al. 1986). Unpollinated flowers drop after about 2 days. Cacao is generally considered to be mainly pollinated by tiny midges (Ceratopogonidae). Species belonging to Forcipomyia genus are described as the most effective (Young 1982). However, many cacao pollinator surveys have often found low abundances of these midges (Toledo-Hernandez et al., 2017). Many other insects attracted to cacao flowers are visiting the flowers. They belong to Dipterian families such as Cecidomyiid midges, Phoridae, Sciaridae and Drosophilidae. The role of ants (Formicidae) as cocoa pollinators is controversial since they often visit the flowers but their efficiency in pollen transportation is questioned. Many studies conducted mainly in Latin America showed that cocoa pollinator populations depend on the systems characteristics to provide nesting and resting sites for potential pollinators. In that sense, the microclimate provided by shade trees, the litter characteristics are important elements to favor pollinators visitors abondance and diversity. Enhancement of ground litter with pod husks or rotten banana stems should favor Ceratopogonidae development sites (Forbes and Northfield 2017, Young et al. 1982). Some studies clearly demonstrate a positive correlation between the population of ceratopogonid midges and fruit set in cacao (Groeneveld et al. 2010, Frimpong et al. 2011, Forbes and Northfield 2017). In Africa, most of recent studies on cocoa pollination come from Ghana (Frimpong et al. 2009, 2011). This work presents an update of the composition of pollination community and their activity in a cocoa plantation in Cameroon. The objectives of our study were to identify floral visitors and to describe their pollination activity and efficiency. One of the hypothesis of the study was that the pollinator assemblages (taxonomy and abundance) and the pollination efficiency differ under the action of the treatment received.

Material and methods

This work was conducted at Irad (Agricultural Research Institute for Development) Cocoa Collection set up in 2006 in Nkolbisson, Cameroon. In this region, the climate is equatorial with a bimodal rainfall pattern. Daily temperature is more or less constant throughout the year, with an average of about 26°C. Rainfall occurs mainly during two rainy seasons, from April to June and from September to November. In this area, the main flowering season is in March-April-May. We worked with clones that were auto incompatible inter compatible.

The study took place in a 2100m² plot within the cocoa collection. The plot was divided into four blocks which contained 30 healthy cocoa trees with high amount of flower buds. Each block was characterized for the shade density, the species richness in associated trees and for the density of planted cocoa trees (Fig. 1). Shade density was estimated with a densitometer, as the average of shade cover on 12 points of the block. Two out of the four blocks (Block 1 and Block 4) received a treatment with addition of discs of rotten banana trees ("with rotten banana stems" treated). The treatment consisted in addition of 255g of banana stems rots at the base of 30 cocoa trees one and a half month before the beginning of the experiment. The "with rotten banana stems" treated plots were spaced at a minimum of 25 m away from the "no banana stem" treated plots to reduce the possibility of treatment effect spillover.

For the study, 16 trees were selected from the four blocks (4 trees per block). A total of 160 flowers (10 per tree) were observed for three hours during different time slots from 16.05.205 to 14.06.2015 between 7 am to 1 pm. All the observed flowers were isolated with a mosquito net the day before the observation just before the anthesis.

The day of the observation, the mosquito net was removed and two flowers that were well opened and ready to be pollinated were observed. Two flowers were observed by one observer for three hours at three different time slots (from 7 am to 8 am; from 9 am to 10 am; and from 11am to 12pm). During these different time slots, the observer stayed in front of the two flowers observing and recording the activity (time duration, interruption…) of all the visiting insects on the flower. At the same time a second observer was following the insect that visits the flower to collect it, when it went far from the observed flowers. In fact, he did not the collection straight on the flower since some insects can come visit the flower and move on the pedicel and come back on the flower. During the non-observation phase, the flower stayed opened to limit its manipulation. At the end of the observation phase, these flowers were covered again and followed up for fruiting rate. In the text these flowers will be referenced as "isolated flowers". All the observed flowers were marked at the end of the experiment so that it was easy to follow the fructification rate. 48h-72h after the observations of the flowers, fruit formation was followed for the isolated flowers in two blocks (Block 2 and Block 4). Then cherel set was determined one week after the observation. Pods formation, three months after the set-up of the experiment was recorded on the marked flowers. The fruit set rate (number of cherels/number of flowers followed) and the fruiting rate (number of pods/number of flowers followed) were determined for the 40 marked flowers per block.

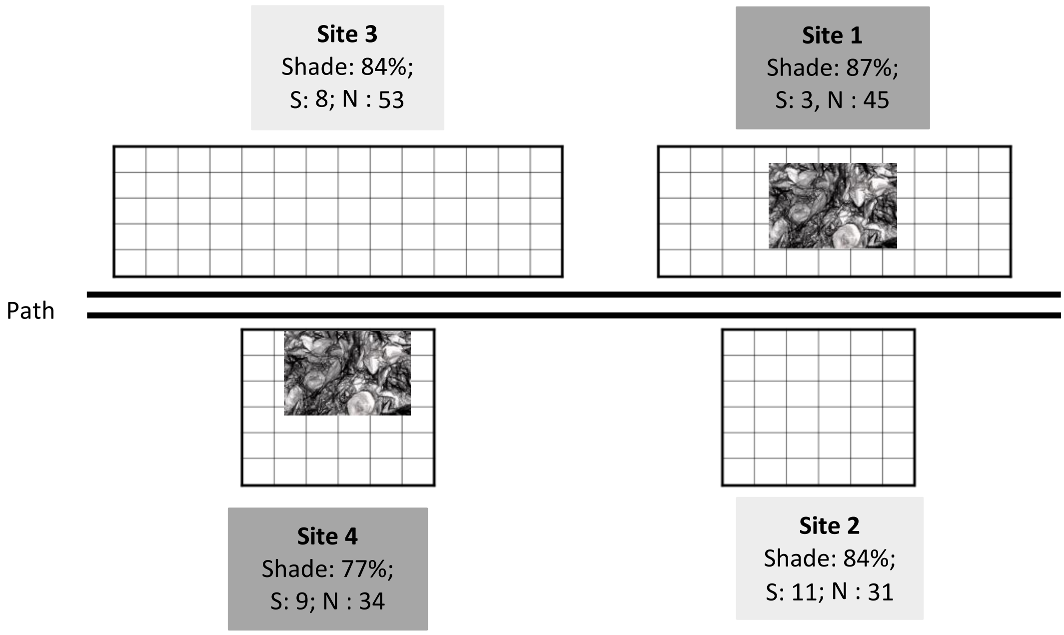

Figure 1: Study sites characteristics: Shade estimated by densitometer, S: Species richness of associated trees, N: number of cocoa trees; sites 1 and 4 with addition of discs of rotten banana stems

At the end of the observation work in the blocks, a follow up of the global population of insects were done. Two methods were done at the same time. A Malaise trap was disposed in each block for 24 hours to collect small flying insects of the blocks. Then, to also estimate flying insect population, samples were taken with a sweep net used for 30 seconds around each selected cocoa tree at two different moments (8 am and 10 am). At the end of the experiment, all collected insects were determined to insect family level (Delvare & Aberlenc 1989). A morpho-species approach was also done to approximate the species richness of the blocks.

Binomial tests and Fisher tests were conducted to compare relative abundance of species and visiting rate per flowers in the different treatments.

Results

Richness and diversity of visitors

41/160 (20 in rotten banana stem treated block and 21 in non-treated block) flowers were visited. 70 visits on cocoa flowers were recorded and analyzed. There was no difference between the number of visits in rotten banana stem treated blocks (31) and in non-treated blocks (39) (Binomial test, p= 0.07). A total of 11 families were observed on the marked cocoa flowers (Fig.2). Formicidae was the most frequently observed. The diversity of families that visited the flowers were significantly linked with the addition of rotten banana stems in the blocks (Fisher test, p=0.002). In fact, there were significantly more families that visited the flowers in the plots without rotten banana stems (10/11) in comparison with the treated plots (5/11). In treated blocks, ants represent more than 65% of observed insects and dipterans (only "flies") represent only 9% of the visitors. In comparison, in the non-treated plots, Hymenopterans included ants represented in average 38% whereas Dipteran families represented 56% (with at least 18% of small "mosquitoes"). There was no effect of shade in the blocks nor of associated trees richness on the diversity of visitors encountered.

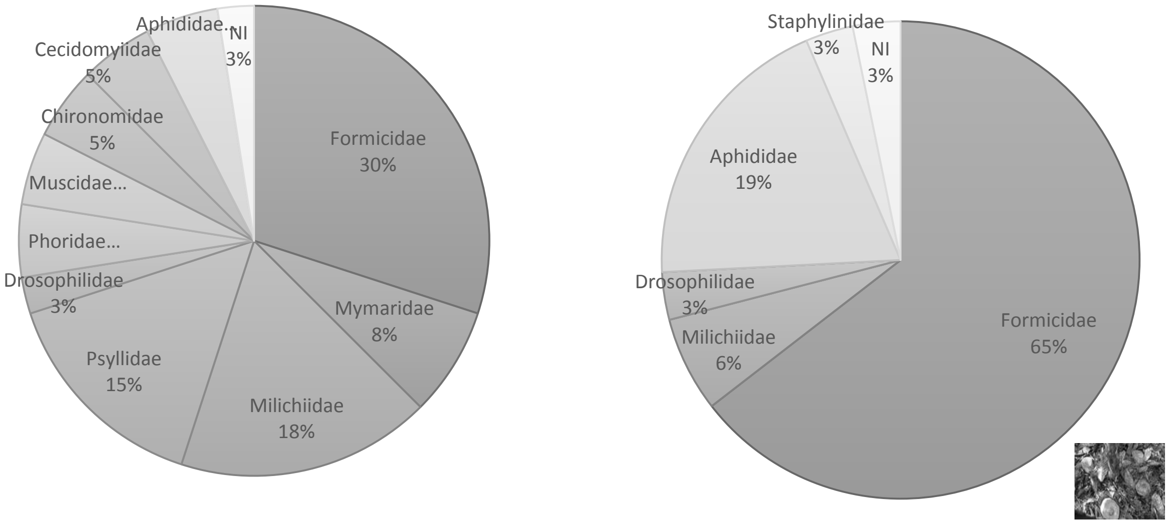

Fig. 2. Diversity of visitors on marked flowers in the different blocks with addition of rotten banana stems and without addition.

Insects species richness collected with Malaise traps and sweep nets were higher in the rotten banana stems treated blocks reaching around 81 different morphospecies in comparison with 59 morphospecies for the other blocks. Globally the Shannon diversity index was also higher in these blocks (4.32 in comparison with 4.05). The global proportion of cocoa flowers visitors were slightly but non significantly more important in the treated blocks (Binomial test, p=0.51) since ants' richness was slightly higher (Binomial test, p=0.14). Considering the richness of dipterans, it was significantly higher in the non-treated blocks (Binomial test, p=0.048) and more diverse (H=2.80; 2.62 respectively in non-treated and treated blocks). 10 insects belonging to the Ceratopogonidae family were collected in the blocks (4 in the treated blocks and 6 in the non-treated blocks).

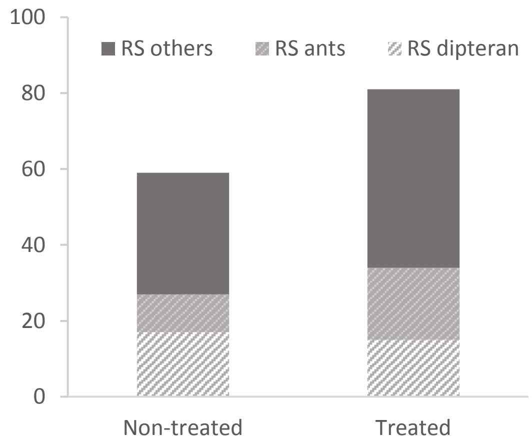

Fig.3. Insects' species richness collected with Malaise Trap and sweep nets in the treated and non-treated sites (In hashed were represented the potential visitors as the ones identified on the flowers)

Fruiting rate

In the rotten banana stems treated block, the fruit set rate was 32.5% (13 cherels out of 40 marked) and fruiting rate was 20% (8 pods out of 40 marked flowers). In this block two cherels were obtained from two isolated flowers from one tree that were visited only by small ants. In the non–treated blocks, the fruit set rate and the fruiting rate was 5% (2 cherels and 2 pods out of 40 marked flowers). Even if the number of followed flowers and fruit set are low, the fruiting rate was better in the treated blocks (binomial test; $P < 0,05$). The two cherels obtained from isolated flowers were visited only by one ant species that was also the most common species seen on the flowers (9 times out of 70 visits). On these two flowers, this ant visited at least twice the flower and spent almost three minutes within the flower. There were also some aphids that were present on the peduncle of the flowers that seemed to attract the ants.

Pollination activity

The maximum of the pollination activity was observed between 11-12 pm with on average 1.9 insects that were visited the flower. But the peak of visits recorded between 9 to 10 am (21 vs 12 between 11-12pm). Only one visit was recorded between 7 to 8 am in the morning. On one cocoa tree, one visitor whatever the species and family, can visit between one to five flowers during one hour depending on the distance between the flowers. The ants generally visit between 1 to 3 flowers on the tree during one hour. Sometimes, they came back on a previously visited flower. In general, the insects go from one flower to another flying except for Formicidae that walk and barely go on other trees during one hour. During the observation, some insects visiting the flowers seem to look for nectar or pollen.

Most of the visitors, spent less than one minute of the flower per visiting sequence. Those insects belong to Aphididae, Myrmaridae, Phoridae and some Formicidae. Other insects like: other Formicidae, Milichidae, Cecidomyiidae, Drosophilidae, Phoridae et Psyllidae spend between 1 to 5 minutes on one flower during one sequence. Those insects that spent more time on one flower were sometimes interrupted during their activity by other species or by conspecifics. For the ants for example, when more than three individuals were observed on one flower, there were interactions between them and exclusion of one individual at least. We never observed presence of two different species on one flower at the time.

Discussion Conclusion

A great diversity of insects can visit cocoa flowers. In our study, the addition of rotten banana stems had direct effect on insects' populations visiting the cocoa flowers. Although it increased the overall diversity and richness of potential pollinators it also increased ants' activities on the flowers. This activity on cocoa flower appears to have prevented the visit of the flowers by other species. Ants appear to exclude the activity of other insects but its high presence also has a positive effect on the overall pollination in the plot at the same time. More works are needed to better understand the functional role of the diversity of insects present in the cocoa systems. It is not only important to increase the presence but also to better the interactions between the species.

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