



(RESEARCH ARTICLE)



Insect pollinators and productivity of soybean [*Glycine max* (L.) Merr. 1917] at Maroua, Far North, Cameroon

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Abstract

To determine the insect species richness, relative frequency, food products, potential pollinators and their impact on the yields, the flowers of *Glycine max* (Fabaceae) were observed from September 3rd to 27th in 2015 (Mayel-Ibbé) and from September 6th to October 10th in 2018 (Wourndé). Two hundred plant flower clusters in 2015 and in 2018 they are divided in two treatments, differentiated according to the presence or absence of protection against insect's activities. The diversity of the flowering insects of soybean was 19 and 12 species respectively in Mayel-Ibbé and Wourndé. These insects visited the flowers of the soybean from 6 am to 5 pm with the activity peak situated between 10 am and 11am. These insects developed and elaborated behaviour when they collected the nectar and/or pollen. Thus, they can be grouped into major pollinators, minor pollinators and occasional pollinators. By comparing yield of unprotected plants to yield of protected plants from insect visits, it is appeared that insects have a positive impact on this yield. The influence of the insects on the increasing of soybean yield is estimated at 39.29%, 11.70%, 22.88% and 03.76% for the pod/plant, the number of seeds/pod, the percentage of the mass of seeds and the percentage of normal seeds respectively. In order to improve the yield of *G. max*, it is advisable to preserve the flower-dwelling insects in the farm by avoiding pesticide treatments during the period of flowering when they are not justified.

Keywords: *Glycine max*; Flowers; Insects; Pollination; Yields

1. Introduction

Animal-mediated pollination is a regulating ecosystem service of vital importance for nature, agriculture, and human well-being [1]. This service is provided by pollinators, namely by managed bees, wild bees, other insects such as flies, moths, butterflies and beetles, as well as vertebrates such as bats, birds and some primates [1]. The IPBES assessment report on Pollinators, Pollination, and Food Production underscores the role of pollinators in multiple respects [2]. Nearly 90% of the world's wild flowering plant species depend, entirely or at least in part, on animal pollination [3]. These plants are essential for the functioning of ecosystems through the provision of food, habitat and other resources to other species. More than three fourth of the leading food crops benefit to some extent from animal pollination, with an estimated annual market value of US\$235-\$577 billion in 2015 [2]. In addition to that, even auto-pollinated crops like soybean can benefit from enhanced productivity by animal pollinators [4]

Soybean (*Glycine max*) is a legume native to East Asia, but presently cultivated worldwide for its bean which has a variety of uses from animal to human food, industrial application of its oil and biofuel production [5]. Nowadays, soybean is one of the most traded commodity and important revenue for exporter countries as well as food security of importer countries. The world production reached 264.9 million tons in 2010 for 102.5 million cultivated hectares [6].

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In Cameroon, the quantity of *G. max* available to consumers is very low 12,544 tons/years [7], the demand for soybean seeds is high (22,544 tons/years: [8]), and its pod and seed yields are low [7]. It is therefore important to investigate how the production of this plant could be increased in the country.

The relationships between *G. max* and anthophilous insects have not been well studied in Cameroon [9, 10]. In other countries such as Mexico, Nogueira-Couto *et al.*, [11]; USA, Rortais *et al.*, [12]; Brazil, Chiari *et al.*, [13] and Milfont *et al.*, [4] reported that insects manipulate the flower by landing on the keel and grappling with it in the process of seeking nectar and pollen. Investigations have shown greater yield in soybean crops when insect pollinated [13]. Nevertheless, soybean is listed among the crops which show some dependence to insect pollination to increase their yield [3]. In fact, this work shows that in the presence of pollinating insects, yield Fabaceae increase in quantity and quality. Despite the economic importance of *G. max*, little data exist on its relationship with flowering insects in other countries in general and particular in Cameroon. Yet, it is known that the impact of flowering insects on pollination and plant yields can vary with space and time [14]. Hence, it is important to carry out studies in this region to complete existing data. The present work is a contribution of the knowledge of *G. max* pollinators in order to use their eco-systemic service in the resolution of soybean yield in Cameroon. There are four specific objectives to: (a) determine the diversity and abundance of the main insect visitors of soybean flowers; (b) study their foraging activities; (c) determine the potential pollinator of this plant; (d) assess the impact of pollination by insects on pod and seed yields of *G. max*. The information gained on the interaction of soybean flowers and insect floral visitors will enable farmers to develop management plans that will increase the overall quality and quantity of soybean yield.

2. Material and methods

2.1. Site and biological materials

The studies were carried out in Mayel-Ibbé (10°62'69"N, 14°33'96"E and 394.08 m) in 2015 and Wourndé (10°38'15.7"N, 14°18'40.4"E and 437 m) in 2018, two localities of Maroua (Far North; Cameroon: Fig. 1).

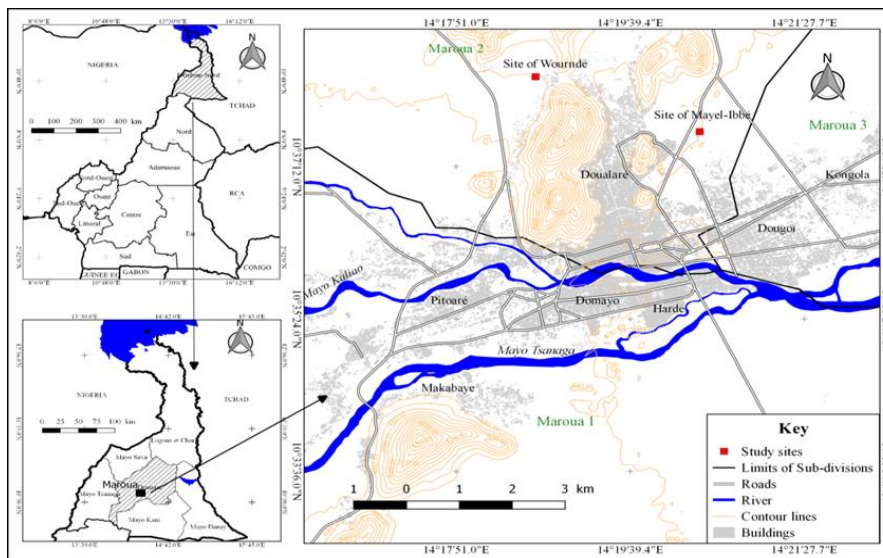


Figure 1 Map of Maroua town locating the experimental fields

This Region belongs to the ecological zone with three phytogeographical areas (Sahel-Sudanian, Sahelian and Sudanian altitude) periodically flooded, with unimodal rainfall [15]. It has a Sahel-Sudanian climate type, characterized by two annual seasons: a long dry season (November to May) and a short rainy season (June to October); August is the wettest month of the year [16]. Annual rainfall varies from 400 to 1100 mm [16]. The annual average temperature varies between 29°C and 38°C and a daily temperature range between 6°C and 7°C [16]. The experimental plot area was 400 m². The animal material was represented by insects naturally present in the environment. Vegetation was represented by wild species and cultivated plants. The plant material was represented by the seeds of *Glycine max* provided by IRAD (Institute of Agricultural Research for Development).

2.2. Sowing and weeding

On June 18th, 2015 in Mayel–Ibbé and June 15th, 2018 in Wourndé, sowing was done on the plot having 30 lines with 40 seed holes per line. 4 - 3 seeds were sown per seed hole. The spacing was 30 cm between rows and 20 cm on rows. Each hole was 3 cm in depth. From germination (which occurred from June 22nd, 2015 and June 22nd, 2018) to the development of the first flower (2nd September, 2015 and 4th September, 2018), the field was regularly weeded with a hoe. Two weeks after germination, the plants were thinned and only two were left per hole. Weeding was performed manually as necessary to maintain the plot weeds-free.

2.3. Estimation of the diversity and relative frequency of *Glycine max* flowering insects in each locality

On the 1st September, 2015 and 4th September, 2018, 200 plants and 200 plants of *Glycine max* at the bud stage were labeled respectively, among the 200 plants, 100 plants were left unattended (Fig. 2) and 100 plants were bagged to prevent visit of insects (Fig. 3).



Figure 2 Plants of *Glycine max* unprotected



Figure 3 Plants of *Glycine max* bagged to prevent visitors.

The frequency of insects in the flowers of *G. max* was determined based on observations on flowers of treatment 1, four days per week, from September 3rd to 27th, 2015 (Mayel–Ibbé) and from September 6th to October 10th, 2018 (Wourndé), at 6 am-7 am, 8 am-9 am, 10 am-11am, 12 am-1pm, 2 pm-3 pm and 4 pm-5 pm. Flowers typically were completely opened at 6 am and closed before 5 pm.

For each time slot, each plot was observed for 15 minutes. At each passage, the different insects encountered on the blooming flowers were identified by a code and counted. The insects not being marked, the cumulated results were expressed by the number of visits [17]. The set of various species of insects listed on the flowers of *G. max* constituted the specific diversity of each locality. The frequency of visits of insect *i* (F_i) to *G. max* flowers was calculated using the following formula: $F_i = (n_i/N*100)$, where n_i is number of insect visits *i* on the flowers and N the number of visits of all the insects to the same flowers [17].

Active insects on *G. max* plants were captured by hand, or by entomological net. In the field, the captured insects were kept in vials containing 70% ethanol, except for Lepidoptera which were kept in foil, as recommended by Borror & White [18] for identification with the aid of the author name identification keys [19, 20].

The Shannon diversity indices (H) and Piéluou equitability (EQ) were calculated using the formulas: $H = -\sum_{i=1}^S pi(\log_2 pi)$ and $EQ = \frac{H}{\log_2(S)}$; where $pi = n_i/N$; n_i : number of individuals of *i* (corresponding to the number of visits of *i*); N : total number of individuals (corresponding to the total number of visits) and S : total number of species observed. The Jacard index (J) was calculated to determine the similarity between the two sites. $J = \frac{c}{a+b-c}$; where a = number of species from list *a* (record A), b = number of species in list *b* (survey B) and c = number of species common to surveys A and B.

2.4. Foraging activities and resources of the insects on *Glycine max* flowers

Daily observations were made between 6 am and 5 pm on flowers of *G. max* for foragers, the resources collected (nectar foragers were seen introducing the head between the stigma or the anther and the corolla, while pollen gatherers directly scratched the anthers with the mandibles or the legs), their abundance, the foraging behaviour and the

disruption of the activity of foragers by competitors. All the insects that visited the *G. max* flowers were collected using insects sweep net. Collected flower visitor insects were sorted out into their various species and their bodies examined to check the presence of pollen.

2.5. Evaluation of the rhythm of visits according time slots and the daily frequency of visits

The number of visits of each species of flowering insects of *G. max* by time and by day of observation made it possible to determine the rhythm of visits according to the time periods and the days of observation. The results are expressed as a percentage (P): $P = (n_v/N)*100$ [17]; where n_v is the number of visits per time slot (or per observation day) and N is the total number of insect visits recorded during the observation period.

These operations made it possible to determine for each species of insect the highest percentage of visits per time slot corresponding to the peak activity of this insect [17]. The daily frequency of visits ($f(\%)$) which is the percentage of the number of days that the insect was observed in relation to the total number of observation days was evaluated using the formula: $f(\%) = (n_i/N)*100$; where, n_i is the number of days of presence of insects during N observation days.

2.6. Evaluation of the effect of insects on *Glycine max* yield

This evaluation was based on the impact of insects visiting flowers on pollination, the impact of pollination on fructification of *G. max* and the comparison of yield (Number of pod/plant, mean number of seeds/pod, mean mass of seeds and percentage of normal seeds) of treatment X (unprotected plants) and treatment Y (protected plants). The number of pod/plant due to the influence of foraging insects (Fr_i) was calculated by the formula: $Fr_i = \{[(Fr_x - Fr_y) / Fr_x] * 100\}$ [17]; where Fr_x and Fr_y were the number of pod in treatment X and treatment Y. The fruiting rate of a treatment (Fr) is: $Fr = [(F_2/F_1)*100]$ [17]; where F_2 is the number of pods formed and F_1 the number of viable flowers initially set. At maturity, pods were harvested from each lot and the number of seeds per pod counted. The mean number of seeds per pod and the percentage of normal seeds (well-developed seeds) were then calculated for each treatment. The impact of flowering insects on seed yields was evaluated using the same method as mentioned above for fruiting rate.

2.7. Data analysis

Data were analyzed using:

- Descriptive statistics (for calculating averages, standard deviations and percentages);
- Three tests: the Chi-square (χ^2) for the comparison of the percentages; the Z test for the comparison of the averages of two samples; Student's t modified for comparison of site-specific diversity indices was calculated by the formula:

$$t = \frac{|H_1 - H_2|}{\sqrt{\text{Var}(H_1) + \text{Var}(H_2)}} \text{ re } H_1 \text{ and } H_2 \text{ the Shannon-Wiener to sites 1 and 2 respectively.}$$

- The XLSTAT 14.1 software.

3. Results and discussion

3.1. Diversity and frequency of floral entomofauna of *Glycine max*

Twenty and twelve insect species have made 2588 and 666 visits on *G. max* flowers in 2015 and 2018 respectively at Mayel-Ibbé and Wourndé (Table 1).

Table 1 Diversity and frequency of *Glycine max* flowering insects at Mayel-Ibbé in 2015 and Wourndé in 2018.

| Order | Family | Species | Mayel-Ibbé 2015 | | Wourndé 2018 | | Total | |
|-----------------------|----------------|-----------------------------------|--------------------|--------------------|-----------------|--------------------|----------------|--------------------|
| | | | n ₁ | P ₁ (%) | n ₂ | P ₂ (%) | n _T | P _T (%) |
| Hymenoptera | Halictidae | <i>Lipotriches collaris</i> | 610 | 23.58 | 39 | 05.86 | 649 | 19.94 |
| | | <i>Lasioglossum albipes</i> | 341 | 13.18 | 58 | 08.71 | 399 | 12.26 |
| | | <i>Lasioglossum</i> sp. | 193 | 07.46 | 54 | 08.11 | 247 | 07.59 |
| | | <i>Seladonia</i> sp. | 94 | 03.63 | / | / | 94 | 02.89 |
| | Apidae | <i>Apis mellifera adansonii</i> | 54 | 02.09 | / | / | 54 | 01.66 |
| | | <i>Amegilla</i> sp. | 12 | 00.46 | / | / | 12 | 00.37 |
| | Vespidae | <i>Belonogaster juncea juncea</i> | 32 | 01.24 | 13 | 01.95 | 45 | 01.38 |
| | Megachilidae | <i>Chalicodoma cincta cincta</i> | 12 | 00.46 | / | / | 12 | 00.37 |
| | | <i>Megachile bituberculata</i> | 8 | 00.31 | / | / | 8 | 00.24 |
| | | <i>Megachile angularum</i> | 2 | 00.04 | / | / | 2 | 00.06 |
| | | Sphecidae | sp. | 3 | 00.12 | / | / | 3 |
| Total | 5 | 11 | 1361 | 52.59 | 164 | 24.62 | 1525 | 46.87 |
| Lepidoptera | Nymphalidae | <i>Acraea ranavalana</i> | 24 | 00.93 | / | / | 24 | 00.74 |
| | | <i>Hemiargus hanno</i> | 340 | 13.14 | 98 | 14.71 | 438 | 13.46 |
| | | <i>Acraca serena</i> | 249 | 09.63 | / | / | 249 | 07.65 |
| | | <i>Eurena</i> sp. | 217 | 08.39 | / | / | 217 | 06.67 |
| | | <i>Danaus chrysipus</i> | 196 | 07.58 | / | / | 196 | 06.02 |
| | | <i>Chitoria sordida</i> | / | / | 53 | 07.81 | 53 | 01.63 |
| | Lycaenidae | <i>Leptotes pirithous</i> | / | / | 182 | 27.33 | 182 | 05.59 |
| | | <i>Pelopidas</i> sp. | 158 | 06.10 | / | / | 158 | 04.86 |
| | | <i>Deudorix epijarbas</i> | / | / | 12 | 01.80 | 12 | 00.37 |
| | | <i>Mesosemia asa</i> | / | / | 78 | 11.71 | 78 | 02.39 |
| | Pieridae | <i>Catopsilia florella</i> | 28 | 01.08 | / | / | 28 | 00.86 |
| <i>Vanessa cardui</i> | | 8 | 00.31 | 34 | 05.11 | 42 | 01.29 | |
| Total | 3 | 12 | 1220 | 47.14 | 457 | 68.62 | 1677 | 51.54 |
| Diptera | Bombyllidae | <i>Anthrax aterrimus</i> | / | / | 27 | 04.05 | 27 | 00.83 |
| | Mycetophilidae | <i>Cordyla</i> sp. | / | / | 18 | 02.70 | 18 | 00.55 |
| Total | 2 | 2 | / | / | 45 | 06.76 | 45 | 01.38 |
| Coleoptera | Coccinellidea | <i>Henospilachna</i> sp. | 7 | 00.27 | / | / | 7 | 00.21 |
| Total | 1 | 1 | 7 | 00.27 | / | / | 7 | 00.21 |
| Total | 11 | 26 | 2588 | 100.0 | 666 | 100.0 | 3254 | 100.0 |
| | | | | 0 | | 0 | | 0 |

n₁: number of visits to flowers in 14 days in 2015; n₂: number of visits to flowers in 14 days in 2018; n_T: number of flower visits in 28 days in 2015 and 2018; P₁: visit percentage in 2015, P₂: visit percentage in 2018; P_T: visit percentage in 2015 and 2018 with $P_1 = (n_1/2588)*100$; $P_2 = (n_2/666)*100$; $P_T = (n_T/3254)*100$.

The diversity indices of Shannon-Weaver (H₁) was 3.36 at Mayel-Ibbé and 3.17 (H₂) at Wourndé. The difference between the Shannon-Weaver diversity indices of the two sites is significant ($t = 4.03$ [$df = 1234$; $p < 0.05$]). Piélou's equitability (EQ) was 0.77 and 0.88 respectively in Mayel-Ibbé and Wourndé. The Piélou equitabilities of the two sites being very close, this would suggest that the two sites have nearly the same environmental conditions. The significant

difference between the diversity indices could be justified by a disturbance in the Wourndé site. This disturbance was due to the use of insecticides in the cowpea field that was close to the experimental plot. In fact this cowpea was extensively attacked by the pest *Hycleus senegalensis*. The calculation of the Jacard index ($J = 0.23$) reveals that the flowering insect groups of Mayel-Ibbé and Wourndé are dissimilar; further evidence that environmental conditions are not similar in both habitats. It is also reported that a significant difference between the diversity indices of two observation sites is often due by environmental disturbance.

The total species richness of *G. max*'s flowering insects was 26 at Maroua. This specific richness is far superior to that found by Taimanga and Tchuenguem [22] which was 15 species at Douala. The comparison between the two specific richness is highly significant ($t = 56.08$ [$df = 8211$; $P < 0.001$]). For Mayel-Ibbe site, Tchuenguem and Dounia [9] found 28 species of insects and the diversity indice of Shannon-Weaver was 3.50. The difference between of the indices in this site is significant ($t = 2.54$ [$df = 1536$; $p < 0.05$]). This confirms the work of Roubik [14], which reported that the species diversity of a plant's flowering entomofauna may vary in space and time. Table 1 shows that:

(a) Lepidoptera is the most important order with 51.54 % of visits. They were mainly represented by Nymphalidae with 70.18 %, *Hemiargus hanno* ranked first with 37.21 % followed by *Acraca serena* with 21.16 %. The insect species recoded to Pieridae family were less than 8 %. These results are different from those obtained by Kengni *et al.*, [10] in Ngaoundéré and Taimanga and Tchuenguem [22] in Douala where the most abundant order was Hymenoptera, with a percentage of 98.07% and 73.02% respectively.

(b) Hymenoptera was the second most important order with 46.87 %. They were mainly represented by Halictidae family with 91.08%, among Halictidae species, *Lipotriches collaris* (Fig. 4) was the most frequent species with 46.72 %, followed by *Lasioglossum albipes* (28.73 %), *Lasioglossum* sp. and *Seladonia* sp. (6.77%). These results confirmed those of Taimanga and Tchuenguem [22] where revealed that the Halictidae family was the most frequent family and different from those obtained by Tchuenguem & Dounia [9] in Maroua which observed that Apidae family was the most abundant family. The genus *Lasioglossum* was also found on the flowers of other plant species as reported by Otiobo *et al.*, [23] on *Oxalis barrelieri* in Bamenda.

(c) Diptera and Coleoptera were the less orders with 2 % of visits registered by Bombyllidae family, species *Anthax aterrimus*; Mycetophilidae family, species *Cordyla* sp. and Coccinellidae family, species *Henospilachna* sp. respectively. Hence, as reported by Roubik [14], the diversity of flowering insects varies with plant species and with the time for the same species.



Figure 4 *Lipotriches collaris* harvesting nectar on *Glycine max* flower.

3.2. Floral products harvested and seasonal frequencies of visits

According to table 2, on *G. max* flowers, each of the insects harvested nectar and/or pollen.

Table 2 Number, percentage of days during which different insects visited and product harvested on flowers of *Glycine max* at Mayel-Ibbé and Wourndé.

| Insects species | Localities | | | | | | Floral product harvested |
|-----------------------------------|------------|-----------|---------|-----------|-------|-----------|-----------------------------|
| | Mayel-Ibbé | | Wourndé | | Total | | |
| | n_1 | f_1 (%) | n_2 | f_2 (%) | f_t | f_t (%) | |
| <i>Lipotriches collaris</i> | 08 | 57.14 | 05 | 35.71 | 13 | 46.43 | NP |
| <i>Lasioglossum albipes</i> | 06 | 42.86 | 07 | 50.00 | 13 | 46.43 | NP |
| <i>Lasioglossum</i> sp. | 01 | 07.14 | 06 | 42.86 | 07 | 25.00 | NP |
| <i>Seladonia</i> sp. | 02 | 14.29 | / | / | 02 | 07.14 | NP |
| <i>Apis mellifera adansonii</i> | 04 | 28.57 | / | / | 04 | 14.29 | NP |
| <i>Amegilla</i> sp. | 01 | 07.14 | / | / | 01 | 03.57 | NP |
| <i>Belonogaster juncea juncea</i> | 02 | 14.29 | 03 | 21.43 | 05 | 17.86 | NP |
| <i>Chalicodoma cincta cincta</i> | 01 | 07.14 | / | / | 01 | 03.57 | NP |
| <i>Megachile bituberculata</i> | 01 | 07.14 | / | / | 01 | 03.57 | NP |
| <i>Megachile angularum</i> | 01 | 07.14 | / | / | 01 | 03.57 | NP |
| (Sphecidae) sp. | 02 | 14.29 | / | / | 02 | 07.14 | P |
| <i>Catopsilia florella</i> | 03 | 21.43 | / | / | 03 | 10.71 | N |
| <i>Acraea ranavalana</i> | 02 | 14.29 | / | / | 02 | 07.14 | N |
| <i>Hemiargus hanno</i> | 07 | 50.00 | 07 | 50.00 | 14 | 50.00 | N |
| <i>Acraca serena</i> | 03 | 21.43 | / | / | 03 | 10.71 | N |
| <i>Eurena</i> sp. | 05 | 35.71 | / | / | 05 | 17.86 | N |
| <i>Danaus chrysipus</i> | 02 | 14.29 | / | / | 02 | 07.14 | N |
| <i>Chitoria sordida</i> | / | / | 03 | 21.43 | 03 | 10.71 | N |
| <i>Vanessa cardui</i> | 03 | 21.43 | 02 | 14.29 | 05 | 17.86 | N |
| <i>Leptotes pirithous</i> | / | / | 09 | 64.29 | 09 | 32.14 | N |
| <i>Pelopidas</i> sp. | 02 | 14.29 | / | / | 02 | 07.14 | N |
| <i>Deudorix epijarbas</i> | / | / | 02 | 14.29 | 02 | 07.14 | N |
| <i>Mesosemia asa</i> | / | / | 04 | 28.57 | 04 | 14.29 | N |
| <i>Anthrax aterrimus</i> | / | / | 04 | 28.57 | 04 | 14.29 | P |
| <i>Cordyla</i> sp. | / | / | 02 | 14.29 | 02 | 07.14 | N |
| <i>Henospilachna</i> sp. | 01 | 07.14 | / | / | 01 | 03.57 | P |

n_1 : number of days of presence of insects during N_1 observation days in 2015; n_2 : number of days of presence of insects during N_2 observation days in 2018; n_t : number of days of presence of insects during N_t observation days in 2015 and 2018; f_1 (%): Relative frequency of insect visits (n_1/N_1)*100; f_2 (%): Relative frequency of insect visits (n_2/N_2)*100; f_t (%): Relative frequency of insect visits (n_t/N_t)*100; $N_1 = 14$, $N_2 = 14$, $N_t = 28$; N: nectar; P: pollen; NP: nectar and/or pollen

It appears from table 2 that there are three categories of insects: (a) species exclusively in search of pollen which was represented by *Anthrax aterrimus* and the Sphecidae (one species); (b) species exclusively in search of nectar which was represented by *Leptotes pirithous*, *Mesosemia asa*, *Hemiargus hanno*, *Deudorix epijarbas*, *Vanessa cardui*, *Catopsilia florella*, *Acraea ranavalana*, *Acraca serena*, *Eurena* sp., *Danaus chrysipus*, *Pelopidas* sp., *Cordyla* sp. and *Chitoria sordida*. All the above species belong to Lepidoptera order. These results confirm that of Benachour [24] which reported that Lepidopteran are exclusively nectar foragers. Many authors noticed that apart from Lepidopteran which harvested exclusively nectar on this plant something is lacking in this sentence, Tchuenguem and Dounia [9]; Kengni *et al.*, [10] reported that *Apis mellifera adansonii* harvest exclusively nectar on *G. max*. (c) Species in search of nectar and pollen

were represented by: *Lipotriches collaris*, *Lasioglossum* sp., *Lasioglossum albipes*, *Belonogaster juncea juncea*, *Seladonia* sp., *A. m. adansonii*, *Amegilla* sp., *Chalicodoma cincta cincta*, *Megachile bituberculata* and *Megachile angularum*. This can be justified in fact that, these species have a high aptitude in collecting nectar and pollen especially when available. These observations were according with those of Tchuenguem and Dounia [9] in Maroua and Kengni *et al.*, [10] in Ngaoundéré.

During 28 days of observation, the frequency of each insect species is varied. We obtained three categories of frequencies (Table 2): (a) frequent visitors ($50 < f < 100\%$): no insect; (b) visitors with average frequencies ($15 < f \leq 50\%$): *Vanessa cardui*, *Eurena* sp., *Hemiargus hanno*, *Lasioglossum* sp., *Lasioglossum albipes* and *Lipotriches collaris*; (c) Rare visitors ($f \leq 15\%$) represented by other insects. The high frequency of some species is due to their attachment to the pollen and/or nectar of *G. max*. Halictidae family species, pollen is indispensable for their nutrition Roubik [25]. However, the pollen of *G. max* is very accessible to insects. In addition, the attractive nature of its flowers with insects is due to the color of the flowers which is purple the most attractive color according to Faegri and Piji [26].

3.3. Rhythm of visits according to time and observation days

Insects visited *G. max* flowers from 6 am to 5 pm, the daily foraging period varied with insects species as shown in Table 3 in both survey sites and the peak of activity situated between 10 am and 11 am. During this period of the day, the mean hygrometry (70.18%) along with the mean temperature (28.78°C) are high and could therefore be favorable to the high availability of nectar that attract insects [27]. These conditions might partially justify the highest frequency of insect visits during that time frame. Table 3 presents the rhythm of visits according to observation time periods.

Table 3 Rhythm of visits according to observation time periods.

| Insects species | Daily periods | | | | | | | | | | | | Total (A) |
|-----------------------------------|---------------|-------|------|-------|--------|-------|--------|-------|--------|-------|--------|-------|--------------|
| | 6-7h | | 8-9h | | 10-11h | | 12-13h | | 14-15h | | 16-17h | | |
| | N | P (%) | N | P (%) | N | P (%) | N | P (%) | N | P (%) | N | P (%) | |
| <i>L. collaris</i> | 17 | 02.62 | 126 | 19.41 | 230 | 35.44 | 178 | 27.43 | 84 | 12.94 | 14 | 02.16 | 649 |
| <i>L. albipes</i> | 10 | 02.51 | 86 | 21.55 | 189 | 47.37 | 79 | 19.80 | 29 | 07.27 | 06 | 01.50 | 399 |
| <i>Lasioglossum</i> sp. | 07 | 02.83 | 35 | 14.17 | 119 | 48.18 | 49 | 19.84 | 35 | 14.17 | 02 | 00.81 | 247 |
| <i>Seladonia</i> sp. | 04 | 04.26 | 22 | 23.40 | 40 | 42.55 | 23 | 24.47 | 05 | 05.34 | / | / | 94 |
| <i>A. m. adansonii</i> | 04 | 07.41 | 09 | 16.67 | 21 | 38.89 | 11 | 20.37 | 07 | 12.96 | 02 | 03.70 | 54 |
| <i>Amegilla</i> sp. | / | / | 02 | 16.67 | 06 | 50.00 | 03 | 25.00 | 01 | 08.33 | / | / | 12 |
| <i>B. juncea juncea</i> | 02 | 04.44 | 10 | 22.22 | 30 | 66.67 | 02 | 04.44 | 01 | 02.23 | / | / | 45 |
| <i>C. c. cincta</i> | 01 | 08.33 | 02 | 16.67 | 01 | 08.33 | 06 | 50.00 | 01 | 08.33 | 01 | 08.33 | 12 |
| <i>M.</i> <i>bituberculata</i> | 02 | 25.00 | 03 | 37.50 | 01 | 12.50 | 01 | 12.50 | 01 | 12.50 | / | / | 08 |
| <i>M. angularum</i> | / | / | / | / | 01 | 50.00 | 01 | 50.00 | / | / | / | / | 02 |
| (Sphecidae) sp. | / | / | 01 | 33.33 | 02 | 66.67 | / | / | / | / | / | / | 03 |
| <i>C. florella</i> | 02 | 07.14 | 07 | 25.00 | 11 | 39.29 | 06 | 21.43 | 02 | 07.14 | / | / | 28 |
| <i>A. ranavalana</i> | 03 | 12.50 | 05 | 20.83 | 09 | 37.50 | 04 | 16.67 | 02 | 08.33 | 01 | 04.17 | 24 |
| <i>H. hanno</i> | 30 | 06.85 | 151 | 34.47 | 131 | 29.91 | 71 | 16.21 | 46 | 10.50 | 09 | 02.06 | 438 |
| <i>A. serena</i> | 08 | 03.21 | 49 | 19.68 | 82 | 32.93 | 66 | 26.51 | 36 | 14.46 | 08 | 03.21 | 249 |
| <i>Eurena</i> sp. | 15 | 06.91 | 58 | 26.73 | 80 | 36.87 | 31 | 14.29 | 25 | 11.52 | 08 | 03.69 | 217 |
| <i>D. chrysipus</i> | 06 | 03.06 | 48 | 24.49 | 64 | 32.65 | 49 | 25.00 | 24 | 12.25 | 05 | 02.55 | 196 |
| <i>C. sordida</i> | 01 | 01.89 | 07 | 13.21 | 11 | 20.75 | 23 | 43.40 | 09 | 16.98 | 02 | 03.77 | 53 |
| <i>V. cardui</i> | 02 | 04.76 | 17 | 40.48 | 16 | 38.09 | 03 | 07.14 | 03 | 07.14 | 01 | 02.38 | 42 |

| | | | | | | | | | | | | | |
|--------------------------|-----|-------|-----|-------|------|-------|-----|-------|-----|-------|----|-------|------|
| <i>L. pirthous</i> | 04 | 02.20 | 84 | 46.15 | 40 | 21.98 | 32 | 17.58 | 16 | 08.79 | 06 | 03.30 | 182 |
| <i>Pelopidas</i> sp. | 06 | 03.80 | 27 | 17.09 | 65 | 41.14 | 40 | 25.34 | 16 | 10.13 | 04 | 02.53 | 158 |
| <i>D. epijarbas</i> | / | / | 06 | 50.00 | 03 | 25.00 | 02 | 16.67 | 01 | 08.33 | / | / | 12 |
| <i>M. asa</i> | 01 | 01.28 | 21 | 26.92 | 20 | 25.64 | 29 | 37.18 | 06 | 07.69 | 01 | 01.28 | 78 |
| <i>A. aterrimus</i> | / | / | 07 | 25.93 | 08 | 29.63 | 12 | 44.44 | / | / | / | / | 27 |
| <i>Cordyla</i> sp. | 01 | 05.56 | 04 | 22.22 | 06 | 33.33 | 07 | 38.89 | / | / | / | / | 18 |
| <i>Henospilachna</i> sp. | / | / | 02 | 28.57 | 04 | 57.14 | 01 | 14.29 | / | / | / | / | 07 |
| Total | 126 | 03.87 | 789 | 24.25 | 1190 | 36.57 | 729 | 22.40 | 350 | 10.76 | 70 | 02.15 | 3254 |

N: number of visits on *Glycine max* flowers in 28 days; *A*: total number of visit of the insects in 28 days; *P* (%): percentage of visit of the insect = $(N/A) * 100$. (**Bold**): peak of activities

From table 3, it is also observed that the active and peak periods vary with insect species: (a) *Lipotriches collaris*, *L. albipes*, *Lasioglossum* sp., *A. m. adansonii*, *A. ranavalana*, *A. serena*, *Eurena* sp., *D. chrysipus*, *Pelopidas* sp. and *M. asa* visited *G. max* flowers from 6 am to 5 pm and the peak of activities was situated between 10 am and 11am; (b) *V. cardui*, *H. hanno* and *L. pirthous* collected nectar of *G. max* from 6 am to 5 pm and the peak of activities was situated between 8 am and 9 am; (c) *Seladonia* sp., *B. juncea juncea* and *C. florella* harvested pollen/or nectar from 6 am to 4 pm and the peak of visits was situated between 10 am and 11 am; (d) *C. cincta cincta*, *M. asa* and *C. sordida* visited flowers from 6 am to 5 pm and the peak of activities was situated between 12 am and 1 pm; (e) *D. epijarbas* and *Amegilla* sp. collected nectar and/or pollen from 8 am to 3 pm and the peak of activity was situated between 8 am and 9am, and 10 am and 11am respectively; (f) *A. aterrimus* and *Henospilachna* sp. visited flowers of *G. max* from 8 am to 1pm and the peak of visits were situated between 10 am and 11am and 12 am and 1pm respectively; (g) *M. bituberculata* harvested nectar and pollen from 6 am to 3 pm and the peak of visits situated between 8 am and 9 am; (h) *Cordyla* sp., one species of Sphecidae (1 sp.) and *M. angularum* collected pollen and/nectar from 6 am to 1 pm, 8 am to 11 am and 10 am to 1 pm, and the peak of activity situated between 12 am and 1 pm, 10 am and 11 am and 10 am and 1 pm respectively.

The reduction of insects activity observed on flowers after 3 pm could be linked to the low quantity and/or quality of their respective floral products and to the increase of the temperature on the experimental area. Although foragers preferred warm or sunny days for the good floral activity, the negative influence of the up temperature is higher on the plant as pollen and nectar producer than on the foragers. Thus, the temperature allows floral anthesis, and accelerates flower wilting or closing when raising [28]. In the same order, the rainfall was documented as an environmental factor that can disrupt the floral insect activity [27]. According to Kasper *et al.*, [29], when the floral products are not easily reached or when its quantity and/or quality decrease, foragers reduce their activity on flowers (to make the working energy lower than that of harvesting products energy). Same observations were reported by Pando *et al.*, [30] on *Cajanus cajan* foraging by insects in Maroua. In fact, these insect species does not visit *C. cajan* flowers when they are poor in nectar and pollen after 3 pm. Moreover, according to Bramel *et al.*, [27], a higher temperature along with a very weak relative humidity has a negative influence on the activity of pollinators on flowers.

3.4. Impact of insects on *Glycine max* pollination

When harvesting pollen and/or nectar on flowers of *G. max*, insects were frequently in contact with the anthers and the stigma of visited flowers. They could therefore be directly involved in self-pollination, by putting pollen of one flower on to the stigma of the same flower. Table 4 shows the frequency of contacts between insect, anther and stigma of *G. max*. It appears on that table that all the 26 insect species that visited the flowers had contact with the anthers and/or stigmas: (a) nine of these insect species have a frequency of contact with the anthers of 100%, eleven have an incidence of contact with the anthers of between $50\% \leq f < 100\%$ and six have a frequency of contact with the anthers of between $25\% \leq f < 50\%$; (b) six of these insect species have a frequency of contact with the stigma of 100%, twelve have an incidence of contact with the stigma of between $50\% \leq f < 100\%$ and eight have a frequency of contact with the stigma of between $25\% \leq f < 50\%$. Individuals of each studied bee species were seen carrying pollen of *G. max* from flower to flower, using the legs, mouthparts, thorax and abdomen. Therefore, they were likely playing a positive role in geitogamy [31] by putting the pollen of one flower to the stigma of another flower of the same plant species. The foragers passing from flower to flower on different plants were seen carrying pollen from one plant to another. They could therefore allowed xenogamy [32], by putting the pollen of plant species to the stigma of another plant species. Several flowering insects in general and Apoidea family in particular were reported as being part of the pollinating entomofauna of *G. max* [4, 10].

Table 4 Regularity index, numbers and percentage of insect visits in contact with the anthers of *Glycine max* flowers at Maroua

| Insectes | Mayel-Ibbé | Wourndé | Total | n_t | NCV | | | |
|-----------------------------------|------------|---------|--------|-------|--------|-------|--------|-------|
| | | | | | Anther | | Stigma | |
| | R_1 | R_2 | R_T | | n_a | p_a | n_s | p_s |
| <i>Lipotriches collaris</i> | 0.1347 | 0.0209 | 0.0925 | 649 | 649 | 100 | 541 | 83.36 |
| <i>Lasioglossum albipes</i> | 0.0584 | 0.0435 | 0.0569 | 399 | 399 | 100 | 243 | 60.90 |
| <i>Lasioglossum</i> sp. | 0.0053 | 0.0347 | 0.0189 | 247 | 247 | 100 | 211 | 85.42 |
| <i>Seladonia</i> sp. | 0.0051 | / | 0.0020 | 94 | 89 | 94.68 | 65 | 69.15 |
| <i>Apis mellifera adansonii</i> | 0.0059 | / | 0.0023 | 54 | 54 | 100 | 54 | 100 |
| <i>Amegilla</i> sp. | 0.0003 | / | 0.0001 | 12 | 12 | 100 | 12 | 100 |
| <i>Belonogaster juncea juncea</i> | 0.0018 | 0.0042 | 0.0024 | 45 | 31 | 68.89 | 21 | 46.67 |
| <i>Chalicodoma cincta cincta</i> | 0.0003 | / | 0.0001 | 12 | 12 | 100 | 12 | 100 |
| <i>Megachile bituberculata</i> | 0.0002 | / | 0.0000 | 8 | 8 | 100 | 12 | 100 |
| <i>Megachile angelarum</i> | 0.0000 | / | 0.0000 | 2 | 2 | 100 | 2 | 100 |
| (Sphecidae) sp. | 0.0001 | / | 0.0000 | 3 | 1 | 33.33 | 2 | 66.67 |
| <i>Catopsilia florella</i> | 0.0003 | / | 0.0009 | 28 | 15 | 53.57 | 9 | 32.14 |
| <i>Acraea ranavalana</i> | 0.0013 | / | 0.0005 | 24 | 21 | 87.50 | 17 | 70.83 |
| <i>Hemiaragus hanno</i> | 0.0657 | 0.0735 | 0.0673 | 438 | 297 | 67.81 | 281 | 64.16 |
| <i>Acraea serena</i> | 0.0206 | / | 0.0081 | 249 | 107 | 42.97 | 201 | 80.72 |
| <i>Eurena</i> sp. | 0.0299 | / | 0.0119 | 217 | 99 | 45.21 | 121 | 55.76 |
| <i>Danaus chrysipus</i> | 0.0108 | / | 0.0043 | 196 | 101 | 51.53 | 89 | 45.41 |
| <i>Chitoria sordida</i> | / | 0.0167 | 0.0017 | 53 | 21 | 39.62 | 34 | 64.15 |
| <i>Vanessa cardui</i> | 0.0006 | 0.0073 | 0.0023 | 42 | 18 | 42.86 | 37 | 88.09 |
| <i>Leptotes pirithous</i> | / | 0.1757 | 0.0179 | 182 | 101 | 55.49 | 89 | 48.90 |
| <i>Pelopidas</i> sp. | 0.0087 | / | 0.0034 | 158 | 78 | 49.37 | 91 | 57.59 |
| <i>Deudorix epijarbas</i> | / | 0.0025 | 0.0002 | 12 | 8 | 66.67 | 12 | 100 |
| <i>Mesosemia asa</i> | / | 0.0334 | 0.0034 | 78 | 43 | 55.12 | 31 | 39.74 |
| <i>Anthrax aterrimus</i> | / | 0.0115 | 0.0169 | 27 | 19 | 70.37 | 6 | 22.22 |
| <i>Cordyla</i> sp. | / | 0.0038 | 0.0003 | 18 | 14 | 77.78 | 8 | 44.44 |
| <i>Henospilachna</i> sp. | 0.0002 | / | 0.0000 | 7 | 7 | 100 | 2 | 28.57 |

$R = (P_n/100)*(f_n/100)$; P_n : percentage of insect visits (Table I); f_n : Relative frequency of insect visits $(n_t/28)*100$; n_t : number of visits studied; NCV: number of contact visits, n_t' : Number of total visits, n_a : number of contact anther visits; P_a : percentage of anther contact visits; n_s : number of contact stigma visits; p_s : percentage of stigma contact visits

According to table 4, the different insect species found on *G. max* flowers can be classified into three categories of pollinators: (a) major pollinators which are characterized by a high regulatory index ($R > 0.05$) and has a high pollen harvesting rate; (b) minor pollinators which are characterized by a low regulatory index ($0.05 < R < 0.01$). This could be explained by the low number of the species present in the experimental field or the species were preferentially in search of nectar. (c) Occasional pollinators which are characterized with a very weak regulatory index ($R < 0.01$) and absence of behaviour link to the search of pollen and/or nectar but may have a destructive attitude. All these species of insect carry out foraging activities on the flowers of *G. max* thus contribute to auto pollination and/or cross pollination. These therefore ensure the diversity of the species and increase the seeds yield and produce.

3.5. Evaluation of the impact of insects on pods and seeds yield

Table 5 presents the number of pods per plant, number of seeds per pod, mass of seed per pod and percentage of normal seed in the different treatments.

Table 5 Yield of pods and seeds of *Glycine max* in different treatments.

| Parameters | Traitments (A, A') | Traitments (B, B') | Comparison (A and B, A' and B') |
|--------------------|----------------------------|----------------------------|---|
| Gp ₂₀₁₅ | 40.88 (n = 120, s = 22.28) | 29.33 (n = 120, s = 15.47) | Z ₂₀₁₅ = 4.85 [df = 238; P < 0.05] * |
| Ng ₂₀₁₅ | 2.57 (n = 100, s = 0.5) | 2.42 (n = 100, s = 0.5) | Z ₂₀₁₅ = 1.34 [df = 198; P > 0.05] |
| Mg ₂₀₁₅ | 0.20 (n = 100, s = 0.04) | 0.17 (n = 100, s = 0.05) | Z ₂₀₁₅ = 2.77 [df = 198; P < 0.05] * |
| Gn ₂₀₁₅ | 98.97 % | 95.14% | χ ² ₂₀₁₅ = 14.74 [df = 1; P < 0.01] * |
| Tf ₂₀₁₈ | 51.74 (n = 120, s = 3.98) | 25.70 (n = 120, s = 15.47) | Z ₂₀₁₈ = 60.23 [df = 238; P < 0.01] * |
| Ng ₂₀₁₈ | 2.96 (n = 100, s = 0.5) | 2.44 (n = 100, s = 0.37) | Z ₂₀₁₈ = 8.39 [df = 198; P < 0.01] * |
| Mg ₂₀₁₈ | 0.13 (n = 100, s = 0.15) | 0.09 (n = 100, s = 0.02) | Z ₂₀₁₈ = 2.84 [df = 198; P < 0.05] * |
| Gn ₂₀₁₈ | 95.50 % | 92.00% | χ ² ₂₀₁₈ = 93.8 [df = 1; P < 0.01] * |

Gp: number of pod/plant, Ng: number of seeds/pod, Mg: mass of seed, Gn: percentage of normal seed

*: Significant at P < 0.05.

From this table, we documented the following:

(a) the comparison of the pod/plant revealed that the differences observed were significant between treatment A and treatment B (Z₂₀₁₅ = 4.85 [df = 238; P < 0.05]) and treatment A' and treatment B' (Z₂₀₁₈ = 60.23 [df = 238; P < 0.01]). The pod formation was higher in unprotected flowers for unlimited visits (A and A') than in the bagged flowers (B and B'). The percentage of pod/plant attributed to insect activity was 39.29%.

(b) The difference between treatments A and B was not significant (Z₂₀₁₅ = 1.34 [df = 198; P > 0.05]) and significant (Z₂₀₁₈ = 8.39 [df = 198; P < 0.01]). The mean number of seeds/pod was higher in unprotected flowers for unlimited visits (A and A') than in the bagged flowers (B and B'). Similar results were obtained in USA and Brazil by Rortais *et al.*, [12] and Milfront *et al.*, [4] respectively. The percentage of the number of seeds/pod due to insects was 11.70%. Kengni *et al.*, [10] also were obtained the corresponding result with 32.87% in Ngaoundéré which is superior. This could be due to absence or its lower abundance of the same major pollinators.

(c) The difference between treatments A and B (Z₂₀₁₅ = 2.77 [df = 198; P < 0.05]) and treatments A' and B' (Z₂₀₁₈ = 8.39 [df = 198; P < 0.01]) were significant. The Mass of seed yield in unprotected flowers for unlimited visits (A and A') was higher than that in the bagged flowers (B and B'). The percentage of mean mass of seeds due to insects was 22.88 %. This result is in accordance with that obtained by Rortais *et al.*, [12] in USA.

(d) The comparison of the percentages of normal seeds revealed that the differences observed were highly significant between treatments A and B (χ²₂₀₁₅ = 14.74 [df = 1; P < 0.01]) and treatments A' and B' (χ²₂₀₁₈ = 93.8 [df = 1; P < 0.01]). The Normal seed yield in unprotected flowers for unlimited visits (A and A') was higher than that in the bagged flowers (B and B'). The percentage of normal seeds attributed to the influence of insects was 03.76%. This result is inferior to what was obtained by Tchuenguem & Dounia [9] which was 21.61% and Taimanga and Tchuenguem [23] which was 23.31%. The difference in this value could be explained by the presence of more pollinating species in their experimental area.

4. Conclusion

In Maroua, 26 species of insects distributed in 12 families and four orders visited the flowers of *Glycine max* to harvest nectar and/or pollen. Lepidoptera were the most frequent order with 51.54% followed by Hymenoptera (46.87%), Diptera (1.38%) and Coleoptera (0.21%). The Shannon diversity indices are 3.36 and 3.17 at Mayel-Ibbé and Wourndé respectively, and Jacard's index $J = 0.23$ reveals that the flowering insect groups of Mayel-Ibbé and Wourndé are not similar. These insects foraged the flowers of this Fabaceae from 6 am to 5 pm, with a peak activity between 10 am and 11am, where 36.57% of visits are observed at that time. Thus, these insects can be classified into major pollinators, minor pollinators and occasional pollinators. Comparing the yield of unprotected flowers with insect-protected flowers,

it is observed that insect visits increase the number of fruits per plant, the average number of seeds per fruit, the seed mass and the percentage of normal seeds 39.29%, 11.70%, 22.88% and 03.76% respectively. The treatment of soybean plants with chemical pesticides should be avoided during the flowering period in order to benefit from the ecosystem service of pollinating insects.

Compliance with ethical standards

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