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Assessment of diversity of floral insects and their contribution on yields of *Glycine max* L. (Fabaceae) in Yaoundé, Cameroon

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Abstract
To evaluate the diversity and impact of pollinators insects on pod and seed yields of *Glycine max*, the foraging and pollinating behaviour were studied in Yaoundé, during the peak and mild raining season in 2017 and 2018. Treatments included unlimited floral access by all visitors and bagged flowers to avoid all pollinators insects. For each season of study, observations were made on 15075 ± 98 flowers per treatment. The diversity of pollinators insects, its foraging behaviour, and its impact on pollination were recorded. Ten species of pollinators insects were record on *G. max* flowers. The foraging activities of those insect increased the fruiting rate, the number of seeds/pod and the normal seeds as well as in 2017 and in 2018 ($p < 0.001$). Therefore, conservation of nests and colonies of pollinators insect close to *G. max* fields should be recommended to improve pod and seed production in the region.

Keywords: Pollinators Insects, *Glycine Max*, Foraging, Pollination, Yield

1. Introduction

In Africa currently lives more than 250 million undernourished people, is 32 % of the world population (Mengoub & Ouhnini, 2020) [17]. In sub-Saharan Africa, food insecurity affects 153 million people, is 25 % of the total population (Snapp *et al.*, 2018) [23]. The prevalence of undernourishment in this continent has reached 19.1%, which means that, in general, one in five Africans suffers from malnutrition. In the world, Sub-Saharan is the only region where the problem of hunger is predicted; if drastic measures are not taken in agriculture based to the productivities of crop (Teno *et al.*, 2018; FAO, 2020) [30, 10]. The Food and Agriculture Organization of the United Nations (FAO), the International Fund for Agricultural Development (IFAD) and the World Food Program (WFP), diversify farming practices (Kumar, 1991; DSCE, 2009) [15, 8] and integrated pollinators into agricultural policies to promote increased yields (Winfree *et al.*, 2008) [31]. Yet the intervention of pollinating agents is ignored by almost all farmers in this part of the world (Eardley *et al.*, 2006) [9], notwithstanding the irreplaceable positive role that these floral insects play in agricultural production and the conservation of biodiversity. In Cameroon, the relationships between plant species and their pollinating insects are increasingly known thanks to the investigations carried out by Tchuenguem *et al.* (2002) [27]; Azo'o *et al.* (2012) [2]; Amada *et al.* (2018) [1]; Dounia *et al.* (2020) [6]; Pharaon *et al.* (2021) [21] and Douka *et al.* (2021) [5] among others. *Glycine max* L. (Fabaceae) is an annual herbaceous plant (Chevalier *et al.*, 2016) [4]. It is one of the oldest cultivated plants (Hymowitz, 1970) [11], originating from North-East China (Hymowitz, 1970; Bärtels, 1993) [11, 3]. It is cultivated in several regions and countries around the world (Chevalier *et al.*, 2016) [4]. These seeds contain significant amounts of protein, vitamins B1, B2 and B6, iron, calcium, trace elements (copper, zinc and manganese) (Pamplona-Roger, 2004) [20]. They are rich in saturated fatty acids, vegetable fibers, sugar (Chevalier *et al.*, 2016) [4] and mineral salts (potassium, phosphorus, magnesium) (Jurgonski *et al.*, 1997) [12]. Seed-based drinks promote good growth in children (Pamplona-Roger, 2004) [20]. In Cameroon, *G. max* is cultivated for its seeds. Its average annual production is estimated at 12,544 tons, for an area of 9,788 hectares (Minader/Dasa, 2012) [19]. The relationships between floral insects and *G. max* have been studied in Brazil (Milfont *et al.*, 2013) [18]; in Cameroon including tree regions Far-north (Tchuenguem & Dounia, 2014; Dounia *et al.*, 2016, 2020) [7, 6, 29], Adamaoua (Kengni *et al.*, 2015) [13] and Littoral (Taïmanga & Tchuenguem, 2018) [25]. Furthermore, the floral entomofauna and the impact of insects on pollination and the yields of a plant can vary over time and in space (Roubik, 2000; Tchuenguem, 2005) [22, 26]. Hence the need to conduct other studies in the Center Region and to complete the existing data. In general, this work aims to contribute to the understanding of the relationship between *G. max* and floral insects, for their optimal management, with a view to increasing the yield of this plant in Cameroon. Specific objectives were: (1) to inventoried the floral insects of *G. max*, (2) to study the activities of those insects on the flowers of this Fabaceae, and (3) assess the impact of anthophilic insects on pollination and production of *G. max*.

2. Materials and methods

2.1 Site and biological materials

The studies were conducted respectively in 2017 and 2018 in a field located at the campus of the Higher Teacher Training College of University of Yaoundé I (Cameroon) (Latitude: N: 3° 51.350 N, Longitude: 11° 30.380 E, Altitude: 525 m). The climate of the tropical rain forest agro-ecological zone is equatorial Guinean-type with four seasons: the peak rainy season (August to November), the peak dry season (November-March), the mild rainy season (March-July) and the mild dry season (July-August), the mean annual temperature is 25°C, while the mean annual relative humidity is 79% (Letouzey, 1968; Suchel, 1988) [16, 24]. The experimental plot was an area of 200 m^2. The animal material was represented by insects naturally present in the environment. The plant material was represented by the seeds of *G. max* var. IRAT 278 provided by the Institute of Agricultural Research for Development (Yaoundé).

2.2 Planting and maintenance of culture

In each raining seasons (2017, 2018), the experimental plot was divided into 12 blocks (1.5 x1 m). Seeds were sown on two line per block; each line had three holes and each hole received 5 seeds. The spacing was 30cm between holes and 60cm rows lines. Each hole was 4 cm depth. Two weeks after germination, the plants were thinned and only two were left per hole. Weeding was performed manually as necessary to maintain blocks weeds-free.

2.3 Diversity of floral insects on *Glycine max* flowers

The diversity of floral insects on *G. max* flowers was determined based on observations on flowers, every day, from 31 April to 22 May and to 27 October to 13 November 2017, the same manipulation was repeat in 2018. Data were taken according to fourth daily time frames (8h-9h, 11h-12h, 13h-14h and 15-16h. By observing flowers, all visits of insects on *G. max* flowers were recorded. Specimens of all insect taxa (3 to 5 per species) were caught with an insect net on flowers and conserved in 70% ethanol for later identification. All insects encountered on flowers were registered and the cumulated results expressed as the number of visits to determine the relative frequency of each floral insect in the entomofauna of *G. max* (Tchuenguem, 2005) [26].

2.4 Study of the activity of floral insects *Glycine max* flowers

Direct observations of the foraging activity of floral insects on flowers were made. The floral products (nectar and/or pollen) collected by the foragers were recorded for the same dates and time slots as that of the insect counts. Abundances (larger numbers of individuals simultaneously active) per flower and per 1000 flowers (A_{1000}) were recorded. The parameter was recorded as a result of direct counts. For the abundance per 1000 flowers, floral insects were counted on a known number of open flowers; $A_{1000} = [(Ax/Fx) * 1000]$, where *Fx* and *Ax* are respectively the number of flowers and the number of individual insects actually counted on *Fx* (Tchuenguem *et al.*, 2004) [28].

2.5 Estimation of the impact of pollinating insects on *Glycine max* yield

This estimation was based on the impact of floral insect on *G. max* yield, the impact of pollination on fruiting and the comparison of yields (fruiting rate, mean number of seeds per pod and percentage of normal) of treatment 1 (unprotected blocks) and treatment 2 (bagged blocks) (Fig 1). The fruiting rate due to the influence of foraging insects (*Fri*) was calculated using the formula: $Fri = \{[(FrX - FrY) / FrX] * 100\}$, where *FrX* and *FrY* are the fruiting rate in treatments 1 and 2 respectively. The fruiting rate of a treatment (*Fr*) is $Fr = [(F2 / F1) * 100]$, where, *F2* is the number of pods formed and *F1* the number of viable flowers initially set (Tchuenguem *et al.*, 2004) [28]. At maturity, pods were harvested from all treatments. The mean number of seeds per pod and the percentage of normal seeds were then calculated for each treatment.

Fig 1: Experimental field of *Glycine max* witch unprotected blocks and bagged blocks

3. Data analysis

SPSS software and Microsoft Excel were used for three tests: Student's (*t*) for the comparison of means, Chi-square ($\chi 2$) for the comparison of percentages.

4. Results and discussion

4.1 Frequency of the floral entomofauna of *Glycine max*

In table 1, 17714 visits of 3 orders of insects recorded on *G. max* flower in 2017 and 2018 (Diptera, Hymenoptera and Lepidoptera) and including 10 species (*Acrea acerata*, *Apis mellifera*, Calliphoridae sp., *Chalicodoma cincta cincta*, *Cotopsilia florella*, *Drosophila* sp., Formicidae sp., *Lipotriches* sp., *Meliponula togorensis* and *Xylocopa olivacea*). *Apis mellifera* and *Xylocopa olivacea* were the most represented insect with 7931 visits (44.79%) and 3679 visits (20.81%) respectively.

4.2 Activities of floral insects *Glycine max* flowers

4.2.1 Floral products harvested

During each flowering season, floral insect's foragers were intensively and regularly collecting nectar and/or pollen on *G. max* flowers. The foragers were classified on 3 categories: (1) nectar collecting (*Acrea acerata*, *Apis mellifera*, Calliphoridae sp., *Chalicodoma cincta cincta*, *Cotopsilia florella*, *Drosophila* sp., Formicidae sp., *Lipotriches* sp., *Meliponula togorensis* and *Xylocopa olivacea*) (2) pollen harvesting (*Apis mellifera*, *Chalicodoma cincta cincta*, *Meliponula togorensis* and *Xylocopa olivacea*) and (3) nectar and pollen collecting

(*Apis mellifera*, *Chalicodoma cincta cincta*, *Meliponula togorensis* and *Xylocopa olivacea*) (Fig 2).

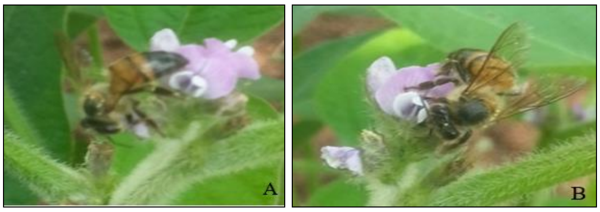

Fig 2: Pollinator insect foraging on *Glycine max* flower (*Apis mellifera* foraging pollen (A) and nectar (B))

4.2.2 Abundance of insects

The highest mean number of floral insects simultaneously in activity was 1 per flower (*n* = 100; *s* = 0) and 38.36 per 1000 flowers (*n* = 50; *s* = 9.04). In general, during the two peak rainy season, the highest mean number of floral insect simultaneously in activity was 1 per flower (*n* = 100; *s* = 0) and 35.02 per 1000 flowers (*n* = 50; *s* = 4.19). the corresponding values during the two mild rainy season were 1 per flower (*n* = 2365; *s* = 0) and 40.67 per 1000 flowers (*n* = 100; *s* = 12.87). The difference between these two means was highly significant (*t* = 29.36; *ddl* = 198; *P* < 0.001).

Table 1: Diversity of floral insects on *Glycine max* flowers in 2017 and 2018, number and percentage of visits of different insects

		Insects	**years**													
Order	**Family**	**Gender, species**	**2017**				**2018**									
			PRS		**MRS**		**PRS**		**MRS**		**TPRS**		**TMRS**		**Total**	
			n_1	p_1 (%)	n_2	p_2 (%)	n_3	p_3 (%)	n_4	p_4 (%)	n_p	p_p (%)	n_s	p_s (%)	n_T	p_T (%)
Diptera	Calliphoridae	sp. [N]	19	0.44	35	0.79	29	0.65	55	1.13	48	0.54	90	0.96	138	0.75
	Drosophilidae	*Drosophila* sp. [N]	46	1.13	66	1.49	94	2.13	79	1.63	140	1.63	145	1.56	285	1.59
Hymenoptera	Apidae	*Xylocopa olivacea* [P, N]	933	22.96	852	19.31	906	20.53	988	20.46	1839	21.74	1840	19.88	3679	20.81
		Apis mellifera [P, N]	1861	45.80	2058	46.65	1863	42.21	2149	44.52	3724	44.00	4207	45.58	7931	44.79
		Meliponula togorensis [P, N]	507	12.47	499	11.31	635	14.38	491	10.17	1142	13.42	990	10.74	2132	12.08
	Megachilidae	*Chalicodoma cincta cincta* [P, N]	-	-	103	2.33	-	-	162	3.35	-	-	265	2.84	265	1.41
	Halictidae	*Lipotriches* sp.[N]	603	14.84	728	16.50	803	18.19	844	17.48	1406	16.51	1572	16.99	2978	16.75
	Formicidae	sp. [N]	78	1.91	46	1.04	53	1.20	32	0.66	131	1.55	78	0.85	209	1.20
Lepidoptera	Acraeidae	*Acrea acerata* [N]	7	0.17	13	0.29	17	0.38	9	0.18	24	0.27	22	0.23	46	0.25
	Pieridae	*Catopsilia florella* [N]	9	0.22	11	0.24	13	0.29	18	0.37	22	0.25	29	0.30	51	0.28
Total			4063	100	4411	100	4413	100	4827	100	8476	100	9238	100	17714	100
			9 species		10 species		9 species		10 species		9 species		10 species		10 species	

PRS : Peak Rainy Season, **MRS** : Mild Rainy Season ; **TPRS** : Total of visits for the Peak Rainy Season, **TGSP** : Total of visits for the Mild Rainy Season, $\mathbf{n_1}$: number of visits on 118564 flowers in 23 days (92 hours) ; $\mathbf{n_2}$: number of visits on 89259 flowers in 18 days (72 hours) ; $\mathbf{n_3}$: number of visits on 123433 flowers in 21 days (84 hours) ; $\mathbf{n_4}$: number de visits on 130487 flowers in 22 days (88 hours) ; n_p **:** total number of visits for the two Peak Rainy Seasons ; n_s : total number of visits for two Mild Rainy seasons ; $\mathbf{p_1, p_2, p_3, p_4, p_p, p_s, p_T}$: percentages of visits ; $p_1 = (n_1 / 4063) \times 100$; $p_2 = (n_2 / 4411) \times 100$; $p_3 = (n_3 / 4413) \times 100$; $p_4 = (n_4 / 4827) \times 100$; $p_p = (n_p / 8474) \times 100$; $p_s = (n_s / 9240) \times 100$; $p_T = (n_T / 17714) \times 100$. Comparison of some percentages of visits of mayor insects: case of *A. mellifera* : PRS / MRS (2017) : $\chi^2 = 0{,}62$ ($P > 0{,}05$; NS) ; PRS / MRS (2018) : $\chi^2 = 4{,}98$ ($P < 0{,}05$; S) ; PRS $_{2017}$ / PRS $_{2018}$: $\chi^2 = 11{,}05$ ($P < 0{,}001$; THS) ; MRS $_{2017}$ / MRS $_{2018}$: $\chi^2 = 4{,}24$ ($P < 0{,}05$; S) ; PRS $_{2017}$ / MRS $_{2018}$: $\chi^2 = 1{,}47$ ($P > 0{,}05$; NS) ; MRS $_{2017}$ / PRS $_{2018}$: $\chi^2 = 17{,}61$ ($P < 0{,}001$; THS). Case of *X. olivacea* : PRS / MRS (2017) : $\chi^2 = 16{,}93$ ($P < 0{,}001$; THS) ; PRS / MRS (2018) : $\chi^2 = 0{,}01$ ($P > 0{,}05$; NS) ; PRS $_{2017}$ / PRS $_{2018}$: $\chi^2 = 7{,}37$ ($P < 0{,}05$; S) ; MRS $_{2017}$ / MRS $_{2018}$: $\chi^2 = 17{,}82$ ($P < 0{,}001$; THS) ; PRS $_{2017}$ / MRS $_{2018}$: $\chi^2 = 8{,}11$ ($P < 0{,}05$; S) ; MRS $_{2017}$ / PRS $_{2018}$: $\chi^2 = 2{,}04$ ($P > 0{,}05$; NS).

4.3 Impact of flowering insects on *Glycine max* yields

During nectar harvest on flowers, foragers regularly contacted witch anthers and pollen adhered all over its body increasing (by this action) cross pollination possibility of *G. max*. Table 2 presents the results on fruiting rate, number of seeds per fruit and percentage of normal seeds in different treatments.

a)- In 2017, the fruiting rate done by flowering insects was 90.16 % in treatment 1 (unprotected flowers) and 72.72 % in treatment 2 (bagged flowers) (Peak rainy season); it was 87.66 % in treatment 3 and 80.34 % in treatment 4 (Mild rainy season). The comparison of those percentages shows that the difference is very highly significant between treatments 1 and 2 ($\chi 2$ = 1513.46; *ddl* = 1; *P* < 0.001) as well as between treatments 3 and 4 (χ 2 =318.07; *ddl* = 1; *P* < 0.001). In 2018, the same value was 89.72 % in treatment 5 (unprotected flowers) and 63.92 % in treatment 6 (bagged flowers) (Peak rainy season); it was 85.90 % in treatment 7 and 72.40 % in treatment 8 (Mild rainy season). The comparison of those percentages shows that the difference is very highly significant between treatments 5 and 6 ($\chi 2$ = 2242.75; *ddl* = 1; *P* < 0.001) as well as between treatments 7 and 8 (χ 2 =310.76; *ddl* = 1; *P* < 0.001).

b)- In 2017 the mean number of seeds per fruit done by pollinators insects was 3.81 in treatment 1 and 2.49 in treatment 2 (Peak rainy season); the corresponding figures were 3.62 treatment 3 and 3.13 in treatment 4 (Mild rainy season). The comparison of these means shows that the difference is very highly significant between treatments 1 and 2 (*t* = 5720.71; *ddl* = 24409; *P* < 0.001) as well as in treatments 3 and 4 (*t* = 2009.95; *ddl* = 26726; *P* < 0.001). In 2018 the mean number of seeds per fruit done by pollinators insects was 3.77 in treatment 5 and 3.38 in treatment 6 (Peak rainy season); the corresponding figures were 3.91 treatment 7 and 3.59 in treatment 8 (Mild rainy season). The comparison of these means shows that the difference is very highly significant between treatments 5 and 6 (*t* = 1113.53; *ddl* = 21449; *P* < 0.001) as well as in treatments 7 and 8 (*t* =

1062.88; *ddl* = 24300; *P* < 0.001).

c)- In 2017 the percentage of normal seeds due to the action of insects was 96.15% in treatment 1 and 73.38% in treatment 2. The corresponding figures were 95.09% and 78.51% in treatments 3 and 4. The comparison of these figures shows that the difference is very highly significant between treatments 1 and 2 ($\chi 2$ = 5660.45; *ddl* = 1; *P* <0.001) as well as in treatments 4 and 5 ($\chi 2$ = 3617.23; *ddl* = 1; *P* < 0.001). In 2018 the percentage of normal seeds due to the action of insects was 94.81% in treatment 5 and 79.71% in treatment 6. The corresponding figures were 93.75% and 75.90% in treatments 7 and 8. The comparison of these figures shows that the difference is very highly significant between treatments 5 and 6 ($\chi 2$ = 2563.22; *ddl* = 1; *P* <0.001) as well as in treatments 7 and 8 ($\chi 2$ = 3390.19; *ddl* = 1; *P* < 0.001).

For the two years, the difference between the percentage of yield from treatments 1,3,5 and 7 (unprotected flowers) and yield from treatments 2, 4, 6 and 8 (bagged flowers) was very highly significant (*P* < 0.0001).

Table 2: Fruiting rate, mean number of seeds per fruit and normal seed percentage according to different treatments of *Glycine max* in 2017 and 2018 at Yaoundé

Treatments	Years	NFS	NFF	FR	Seeds/Fruit		TS	NNS	% NS
					m	*s*			
1 (UF) PRS	2017	14978	13509	90,19	3,81	1,24	29568	28431	96,15
2 (BF) PRS		14991	10902	72,72	2,49	1,56	23469	17223	73,38
3 (UF) MRS		16713	14651	87,66	3,62	1,79	31488	29942	95,09
4 (BF) MRS		15032	12077	80,34	3,13	1,37	26513	20818	78,51
5 (UF) PRS	2018	14103	12413	89,72	3,77	1,69	27271	25858	94,81
6 (BF) PRS		14137	9038	63,93	3,38	2,01	19728	15725	79,71
7 (UF) MRS		15697	13484	85,90	3,91	1,72	29022	27209	93,75
8 (BF) MRS		14941	10818	72,40	3,59	1,93	23531	17861	75,90

UF : unprotected flowers ; **BF** : bagged flowers ; **NFE** : Number of flowers shower ; **NFF** : Number of fruits formal ; **FR** : Fruiting rate (%) ; **TS** : Total Seeds ; **NNS** : Number of Normal Seeds ; **% NS** : Percentage of Normal Seeds ; **PRS** : Peak Rainy Season, **MRS** : Mild Rainy Season.

5. Discussion

At Yaoundé 10 species of floral insects are recorded on *G. max* flowers. The diversity of the floral insects of *G. max* change considerably with Agro ecological zone. 13 insect species were recorded on the flowers of the crop in Littoral region (Douala-Cameroon) (Taimanga & Tchuenguem, 2018) [25]. In Maroua, Far-north region of Cameroon on the same plant revealed 28 species (Tchuenguem & Dounia, 2014) [29] and at Ngaoundere, Adamaoua region of Cameroon 7 species (Kengni *et al.*, 2015) [13]. The number of species of Anthophilous insects of *G. max* listed in the tree agro ecological zone are different. Our results confirm other report that Hymenoptera, are the most important foragers of *G. max*. The same observations are done by Milfont *et al.* (2013) [18] in Brazil and Dounia *et al.* (2016) [7] in Maroua (Cameroon). Among the insect's species recorded on soybean flowers, nectarivorous and pollinivorous influenced yields. The floral insects visit flowers from which they obtain pollen and nectar as food resources. During their visits, they pollinate flowers. Foragers are regularly contact witch anthers and carried pollen from male organ to the stigma on the same flower or from the flower on the same plant and/or the other plant of the same species. Consequently, insects can increase the chances of pollination (Milfont *et al.*, 2013) [18]. In general, soybean is a crop that need pollinator. The data show positive effects of floral insect pollination. The presence of the abundant of floral insects in the field improved the mean number of fruits per plant, the average number of seeds per fruit and reduced significantly the abortion rate. The presence of pollinators management in treatments 1, 3, 5 and 7 decrease abortion rates. They pollinators were also able to carry pollen with their hairs, legs and mouth accessories from a flower of one plant to stigma of another flower of the same plant (geitonogamy), to the same flower (autogamy) or to the flower of another plant (xenogamy). The significant contribution of pollinating insects in pods and seed yield of *G. max* was found in Cameroon showed that *G. max* flowers produce fewer seeds per pod in the absence of pollinating insects. The weight of insect pollinators played a positive role during nectar or pollen collection, those insects shook flowers, facilitating the liberation of pollen by anthers for the optimal pollination (Klien *et al.*, 2007) [14]. This Higher productivity of pods and seeds in unlimited visits when compared with bagged flowers showed that insect visits were effective in increasing cross-pollination.

6. Conclusion

The nectar and pollen of *G. max* attract pollinator insects. This attractiveness is of benefit for the pollination process. The comparison of pods and seeds set of unprotected flowers with that of protected flowers indicated the value of these insect pollinators in increasing pods and seed yields. The installation of nests or hives of insect pollinators at the proximity of the fields should be recommended for the increase of pods and seed yields of this valuable crop.

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