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Pollination Efficiency of *Xylocopa olivacea* (Hymenoptera: Apidae) on *Phaseolus vulgaris* Large White Seed Variety (Fabaceae) Flowers in Ngaoundéré (Cameroon)

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Abstract

To evaluate the impact of a single flower visit of *Xylocopa olivacea* on the production of *Phaseolus vulgaris* Large White Seed variety, its foraging and pollinating activities were studied at Dang in June 2017 and 2018. The experiments were carried out on 540 flowers labeled at bud stage and divided in four treatments: two differentiated according to the presence or absence of flowers protection regarding insect visits; the third protected and uncovered when flowers were opened, to allow *X. olivacea* visits and the fourth with the flowers protected, uncovered when they were opened, then rebagged without any visit. Results indicate that among seven insect species recorded on flowers, *X. olivacea* ranked first and harvested nectar. Throughout the pollination efficiency of a single flower visit, *X. olivacea* provoked a significant increase of the podding rate and the percentage of normal seeds by 30.45 % and 32.81 % respectively. The conservation and installation of *X. olivacea* nests close to *P. vulgaris* var. Large White Seed fields is recommended to improve its pod and seed productions.

Keywords: *Xylocopa olivacea*, *Phaseolus vulgaris* var. Large White Seed, Pollination Efficiency, Flowers, Yields

1. Introduction

Pollinators play an important role in the sustainability of many agricultural ecosystems (Klein *et al*., 2007) [16]. Nearly 80 % of the commercial crops are pollinated by the insects (Free, 1993) [10]. The main groups of pollinating insects are bees, wasps, butterflies, moths, flies and beetles (Mbaikoua, 2015) [18]. Wild pollinators, in particular wild bees, contribute significantly to the pollination of a large array of crops (Winfree *et al*., 2008) [23].

The common bean, *Phaseolus vulgaris* is native of South and Central America (Graham & Ranalli, 1997) [11]. The plant is bushy or upright (40 to 60 cm) with climbing stem which is slightly branched (Ibarra-Perez *et al*., 1999) [12]. Green or purple, leaves are stalked, alternate and compound trifoliate (Ibarra-Perez *et al*., 1999) [12]. Flowering starts 28-35 days after sowing (Debouck, 1991) [3]. The flower is pink, but can vary from white to purple depending on the variety ((Debouck, 1991) [3]. Flowers produce nectar and pollen which attract insects (Deli *et al*., 2020; Douka & Tchuenguem, 2013) [4, 9].

Phaseolus vulgaris flowers were reported to produce fewer seeds per pod in the absence of efficient pollinators in the United States of America (Mbaikoua, 2015; Ibarra-Perez *et al*., 1999) [18, 12]. In Chad, Mainkété *et al*. [17] have found that, throughout its foraging and pollination activities, *X. olivacea* increased significantly the podding rate, the number of seeds per pod and the percentage of normal seeds of *P. vulgaris* Large White Seeds variety (var. LWS) by 52.27%, 30.79% and 84.03% respectively. At Dang (Ngaoundéré, Cameroon) the activity of *X. olivacea* on flowers of *P. vulgaris* (Bigarre variety) increase the fruiting rate by 39.48 %, the number of seeds/pod by 18.19 % and the normal seeds by 49.62 % (Deli *et al*., 2020) [4]. In 2013, research conducted in Maroua by Douka & Tchuenguem [9] has revealed that *Apis mellifera* visits *P. vulgaris* (Red and Small Seed variety) flowers for nectar and pollen and increase the fruiting rate by 55.32 %, the number of seeds/pod by 19.10 % and the

normal seeds by 7.71 %. Cross-pollination of *P. vulgaris* by insects is generally observed (Deli *et al*., 2020; Douka & Tchuenguem, 2013) [4, 9] and this plant is autogamous/allogamous (Ibarra-Perez *et al*., 1999; Deli *et al*., 2020; Douka & Tchuenguem, 2013) [12, 4, 9]. Up to date, no previous research has been reported on the relationships between *P. vulgaris* var. LWS and *X. olivacea* in Cameroon. Besides, the activity and diversity of flowering insects of a plant vary with place, period and variety (Deli *et al*., 2020) [4].

Therefore, it is important to investigate on the possibilities of increasing the production of *P. vulgaris* var. LWS in Ngaoundéré.

The main objective of this study was to contribute to the understanding of the relationships between *P. vulgaris* var. LWS and *X. olivacea* for their optimal management. Considering this plant, specific objectives were to: (a) determine the place of *X. olivacea* in floral entomofauna; (b) study of the activity of this carpenter bee on flowers; (c) assess the impact of flowering insects including *X. olivacea* on pod and seed productions; (d) evaluate the pollination efficiency of a single visit of this Apidae.

2. Materials and Methods

2.1 Study Site

The experiment was carried out in June 2017 and 2018 at Dang, in the experimental fields of the Unit for Apply Apidology (latitude 07°25.38'N, longitude 13°32.37'E and altitude1092 m above sea level) of the Faculty of Science, University of Ngaoundéré, Adamawa Region of Cameroon. This region belongs to the high-altitude Guinean savanna agro-ecological zone (Djoufack *et al*., 2012) [8]. The climate is characterized by a rainy season (April to October) and a dry season (November to March), with an annual rainfall of about 1500 mm (Djoufack *et al*., 2012) [8]. The mean temperature is 22 °C, while the mean relative humidity is 70 % (Amougou *et al*., 2015) [1]. The vegetation is represented by crops, ornamental, hedge and native plants of savanna and gallery forests.

2.2 Biological Materials

The animal material was mainly represented by insects naturally present in the environment and nine artificial and active nests of *Xylocopa olivacea* (Hymenoptera: Apidae) located close to the experimental field.

The plant material was *P. vulgaris* var. LWS (Fig 1) bought at the local market of Dang.

Fig 1: Seeds of *Phaseolus vulgaris* Large White Seeds variety bought at the local market of Dang in 2017

2.3 Methods

2.3.1 Preparation of Experimental Plot, Sowing and Weeding

From July 31th to August 03rd 2017 then from 22th to 31th July in 2018, the experimental plot was delimited, ploughed and divided into eight subplots, measuring 8*4.5 m^2 each (Kingha *et al*., 2012) [15]. On August 05th 2017 then August 02th 2018, sowing was done on six lines per subplot (Kingha *et al*., 2012) [15], each of which had 16 holes per line [10]. Four seeds were sown per hole. Holes were separated 50 cm from each other, while lines were 75 cm apart (Kingha *et al*., 2012) [15]. Weeding was performed manually as necessary to maintain plots weeds-free.

2.3.2 Determination of the Reproduction Mode of *Phaseolus vulgaris*

On September 10th 2017 as on September 20th 2018, 240 flowers at bud stage were labeled and divided in two treatments: 120 unprotected flowers [(treatment X: 1 (2017) or 5 (2018)] and 120 flowers bagged using gauze bags net to avoid insect visits [(treatment Y: 2 (2017) or 6 (2018)]. For each cropping year, one week after shedding of the last labeled flower, the number of pods was assessed in each treatment. The podding index (*Pi*) was then calculated as described by Tchuenguem *et al*. [20]:

$$Pi = Fb/Fa,$$

Where *Fa* is the number of flowers initially considered and *Fb* the number of the formed pods.

The allogamy rate (*Alr*) from which derives the autogamy rate (*Atr*) was expressed as the difference in fruiting indexes between treatment *X* (unprotected flowers) and treatment *Y* (bagged flowers) (Demarly, 1977) [5]:

$$Atr = \{[(PiX - PiY)/ PiX] * 100\},$$

Where

PiX and *PiY* are the fruiting indexes in treatments *X* and *Y* respectively;

$$Alr = 100 - Atr.$$

2.3.3 Determination of the Place of *Xylocopa olivacea* on *Phaseolus vulgaris* Entomofauna

Observations were conducted on flowers of treatments X, every day, from 12th to 18th September 2017 and from 22th to 27th September 2018. During each observation day, before starting visit counts, the number of opened flowers in each treatment was registered. Data were taken according to six daily time frames: 6 - 7 am, 8 - 9 am, 10 - 11 am, 12 - 13 pm, 14 - 15 pm and 16 - 17 pm. In a slow walk along all labeled flowers of treatments X, the identity of insects that visited *P. vulgaris* flowers was recorded (Tchuenguem *et al*., 2001) [20]. All insects encountered on flowers were registered and the cumulated results expressed as the number of visits to determine the relative frequency of each insect species in anthophilous entomofauna of *P. vulgaris* (Tchuenguem, 2005) [22]. Data obtained were used to determine the frequency of visits (*Fi*) of each insect species on *P. vulgaris* flowers. For each study period:

$Fi = [(Vi / Vt) * 100]$, with *Vi* the number of visits of insect *i* on flowers of treatment X and *Vt* the total number of insect visits of all recorded insect species on these flowers (Kingha *et al*., 2012) [15].

Specimens (3 to 4) of each insect taxa, excluded *Apis mellifera* were caught using insect net on unlabeled flowers and conserved in 70 % ethanol, excluding butterflies that

were preserved dry (Borror & White, 1991) [2], for subsequent taxonomic identification.

2.3.4 Study of the Foraging Activity of *Xylocopa olivacea* on *Phaseolus vulgaris* flowers

2.3.4.1 Floral Product Harvested

The floral products (nectar or pollen) harvested by *X. olivacea* during each floral visit were recorded based on its foraging behavior. Nectar foragers were expected to extend their proboscis in the corolla, while pollen gatherers were supposed to scratch anthers using mandibles and legs (Jean-Prost, 1987) [14]. During the same time that *X. olivacea* visits on flowers were registered, the type of floral product collected by this carpenter bee was noted (Tchuenguem, 2005) [22].

2.3.4.2 Duration of Visits Per Flower

During the same days of taking data on frequency of visits, the duration of individual flower visits was recorded (using stopwatch) according to six-time frames: 7 - 8 am, 9 - 10 am, 11 - 12 am, 13 - 14 pm, 15 - 16 pm and 17 - 18 pm. The stopwatch, previously on zero was switched on as soon as an insect landed on a flower. It was stopped when the insect leaves the flower. The related duration of visit corresponds to the value red on the stop watch (Tchuenguem *et al*., 2004) [19].

2.3.4.3 Foraging Speed

Concerning the foraging speed (*Fs*) which is the number of flowers visited by an individual carpenter bee per minute (Jacob-Remacle, 1989) [13], data were registered during the same dates and according to same time frames and daily periods as for the duration of visits. The stopwatch, previously set to zero was switched on as soon as an individual landed on a flower and the number of visited flowers was concomitancy counted. The stopwatch was stopped as soon as the visitor was lost to sight or when it left *P. vulgaris* flowers for another plant species. The foraging speed was calculated using the following formula (Jacob-Remacle, 1989) [13]:

$$Fs = (Nf / dv) * 60,$$

Where *dv* is the time (in sec) given by a stopwatch and *Nf* the number of flowers visited during *dv.*

During the observation period, when a forager returns to previously visited flower, counting was performed as on two different flowers (Jacob-Remacle, 1989) [13].

The disruption of the activity of foragers by competitors or predators and the attractiveness exerted by other plant species on *X. olivacea* was assessed by direct observations. For the second parameter, the number of times that this carpenter bee left *P. vulgaris* flowers to other plant species and vice versa was noted through the investigation period (Tchuenguem, 2005) [22].

2.3.4.4 Abundance Per Flower and Per 1000 Flowers

The abundance of foragers (highest number of individuals foraging simultaneously) per flower and per 1000 flowers (*A1000*) were recorded on the same dates and daily periods as the registration of the duration of visits. Abundance per flower was recorded as a result of direct counting. For determining the abundance per 1000 flowers, foragers were counted on a known number of opened flowers and *A1000* was calculated using the following formula:

$$A1000 = [(Ax / Fx) * 1000],$$

Where *Fx* and *Ax* are respectively the number of flowers and the number of foragers effectively counted on these flowers at time (Tchuenguem *et al*., 2004) [19].

During each daily period of investigation, ambient temperature and relative humidity in the station were registered every 30 minutes using a mobile thermo-hygrometer (HT9227) *x* (Tchuenguem, 2005) [22] installed in the shade.

2.3.5 Evaluation of the Impact of the Flowering Insects Including *Xylocopa olivacea* on *Phaseolus vulgaris* Production

For each year, parallel to the constitution of treatments X and Y, 300 flowers at bud stage were protected and two treatments were formed:

- treatment C: 200 flowers protected using gauze bag nets to prevent insect visits and destined to be visited once by *X. olivacea*; as soon as the flowers were opened, each flower of treatments C were inspected. Hence, gauze bag was delicately removed and this flower was observed for up to 10 minutes; the flower visited by *X. olivacea* were marked and then reprotected (Tchuenguem *et al*., 2001) [20];
- treatment Z: 100 flowers were protected using gauze bag nets and destined to be uncovered then rebagged without the visit of insects or any other organism; as soon as each flower of treatments Z was opened, the gauze bag was removed and the flower was observed for up to 10 minutes while avoiding the visit by *X. olivacea* or any other organism (Djakbé *et al*., 2017) [7].

At maturity, pods were harvested and counted from each treatment. The mean number of seeds per pods, the percentage of normal (well developed) (Tchuenguem *et al*., 2009) [21] seeds were then evaluated.

For each observed year, the fruiting rate due to the flowering insects including *X. olivacea* (*Fri*) was calculated using the following formula (Diguir *et al*., 2020) [6]:

$$Fri = \{[(FX - FZ) / (FX + FY - FZ)] * 100\},$$

Where *FX*, *FY* and *FZ* are the fruiting rates in treatment *X* (unprotected flowers), treatment *Y* (flowers protected from all insect visits) and treatment *Z* (flowers bagged then uncovered and rebagged without insect or any other organism visit).

The fruiting rate of a treatment (*Fr)* is giving by the following formula:

$$Fr = [(b / a) * 100],$$

Where *a* is the number of flowers initially considered and *b* the number of formed pods (Tchuenguem *et al*., 2001) [20].

The impact of flower visiting insects including *X. olivacea* on the number of seeds per pod and the percentage of normal seeds were evaluated using the same method as mentioned above for the fruiting rate.

2.3.6 Assessment of the Pollination Efficiency of *Xylocopa olivacea* on *Phaseolus vulgaris*

For each observation year, the contribution of *X. olivacea* on the fruiting rate, the number of seeds per pod and the percentage of normal seeds was calculated using the data of treatments C and Z.

The contribution of *X. olivacea* on the fruiting rate *(FrX)* was calculated using the following formula:

$$FrX = \{[(FC - FZ) / FC] * 100\},$$

Where *PC* is the fruiting rate in treatment *C* (flowers visited once by *X. olivacea*) and *FZ* the fruiting rate in treatment *Z* (flowers uncovered then rebagged without the visit of insects or any other organism) (Djakbé *et al*., 2017) [7].

The impact of *X. olivacea* on the number of seeds per pods and the percentage of normal seeds were evaluated using the same method as mentioned above for the fruiting rate.

2.3.7 Data Analysis

Data were analyzed using descriptive statistics (means, standard deviation and percentages), ANOVA (*F*) for the general comparison of means of more than two samples, student's *t*-test for the comparison of means of two samples, Pearson correlation coefficient (*r*) for the study of the association between two variables and chi-square ($\chi 2$) for the comparison of percentages, using Microsoft Excel 2013 software and R commander version i386 3.2.0.

3. Results

3.1 Reproduction Mode of *Phaseolus vulgaris*

The fruiting indexes of *P. vulgaris* were 0.80, 0.40, 0.89 and 0.54 for treatments 1, 2, 5 and 6 respectively (Table 1). Thus, in 2017, the allogamy rate was 40.00% whereas the autogamy rate was 60.00 %. In 2018, the corresponding figures were 39.00 % and 61.00%. For the two cumulative years, the allogamy rate was 39.50% and the autogamy rate was 61.50%. It appears that *P. vulgaris* var. LWS has a mixed reproduction mode, allogamous and autogamous, with the predominance of autogamy over allogamy.

3.2 Activity of *Xylocopa olivacea* on *Phaseolus vulgaris* Flowers

3.2.1 Frequency of Visits

Amongst the 263 and 275 visits of seven and nine insect species recorded on *P. vulgaris* flowers in 2017 and 2018 respectively, *X. olivacea* ranked first with 44.11% and 32.26% of visits (Table 1). The difference between these two percentages is highly significant ($\chi 2 = 7.86$; $df = 1$; $P < 0.01$).

3.2.2 Floral Products Harvested

During each flowering period, individuals of *X. olivacea* were found to harvest exclusively nectar (Fig 2) on *P. vulgaris* flowers, intensively.

3.2.3 Relationship between Visits and Flowering Stages

The visits of *X. olivacea* were more numerous in the *P. vulgaris* field when the number of opened flowers was highest (Fig 3).

The correlation was highly significant between the number of *P. vulgaris* var. LWS opened flowers and the number of *X. olivacea* visits in 2017 ($r = 0.96$; $df = 5$; $P < 0.001$) and in 2018 ($r = 0.97$; $df = 5$; $P < 0.01$).

Table 1: Diversity of insects recorded on *Phaseolus vulgaris* flowers in 2017 and 2018 at Dang, number and percentage of visits of different insects

Insects			2017		2018		Total	
Order	**Family**	**Genus et Species**	***n1***	***p1* (%)**	***n2***	***p2* (%)**	***nt***	***pt* (%)**
Hymenoptera	Apidae	*Amegilla* sp. (ne)	29	11.03	28	10.18	57	10.59
		Apis mellifera (ne, po)	18	6.84	18	6.55	36	6.69
		Ceratina sp.	-	-	13	4.73	13	2.42
		Xylocopa inconstans (ne)	32	12.17	40	14.55	72	13.38
		Xylocopa olivacea (ne)	116	44.11	89	32.36	205	38.10
	Megachilidae	*Chalicodoma cincta* (ne)	54	20.53	56	20.36	110	20.45
		Chalicodoma rufipes (ne)	12	4.56	15	5.45	27	5.02
Lepidoptera	Pieridae	*Eurema* sp. (ne)	2	0.76	6	2.18	8	1.49
	Hesperridae	(1 genus sp.)	-	-	10	3.64	10	1.86
Total		**Visites**	**263**	**100**	**275**	**100**	**538**	**100,00**
		Espèces	**7**		**9**		**9**	

n1, *n2*, *nt*: number of visits on 120 flowers in 7 days, *p1*, *p2*, *pt*: percentages of visits, *p1* = (*n1*/263)∗100; *p2* = (*n*2/275)∗100; *pt* = (*nt*/538)∗100

ne: collection of nectar; pe: collection of pollen; genus sp.: unidentified genus; sp.: unidentified species

Fig 2: *Xylocopa olivacea* collecting nectar in a *Phaseolus vulgaris* flower at Dang in 2017

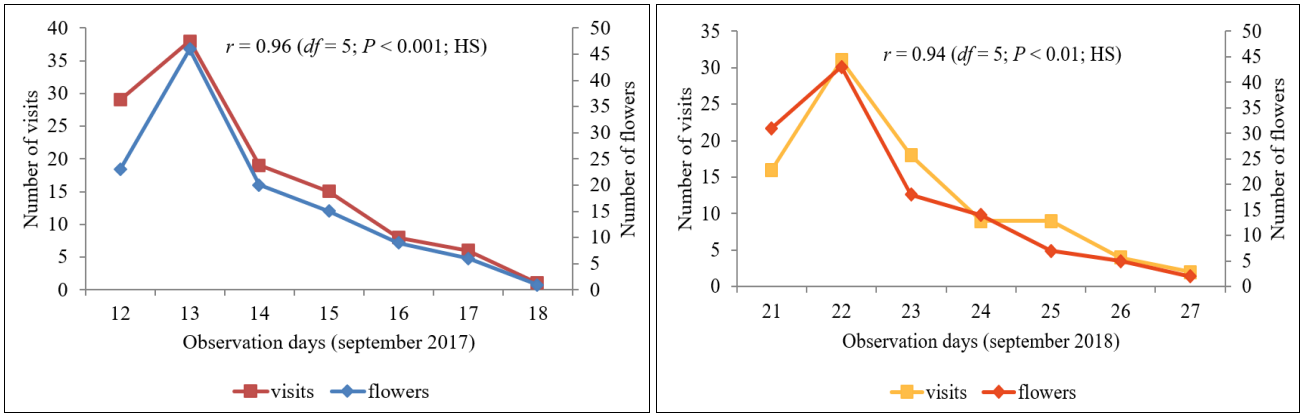

Fig 3: Seasonal variations of the number of *Phaseolus vulgaris* opened flowers and the number of *Xylocopa olivacea* visits on these organs in 2017 and 2018 in Ngaoundere.
r: correlation coefficient between the number of flowers and the number of visits; *ddl*: degrees of freedom; *P*: significance threshold; HS: highly significant

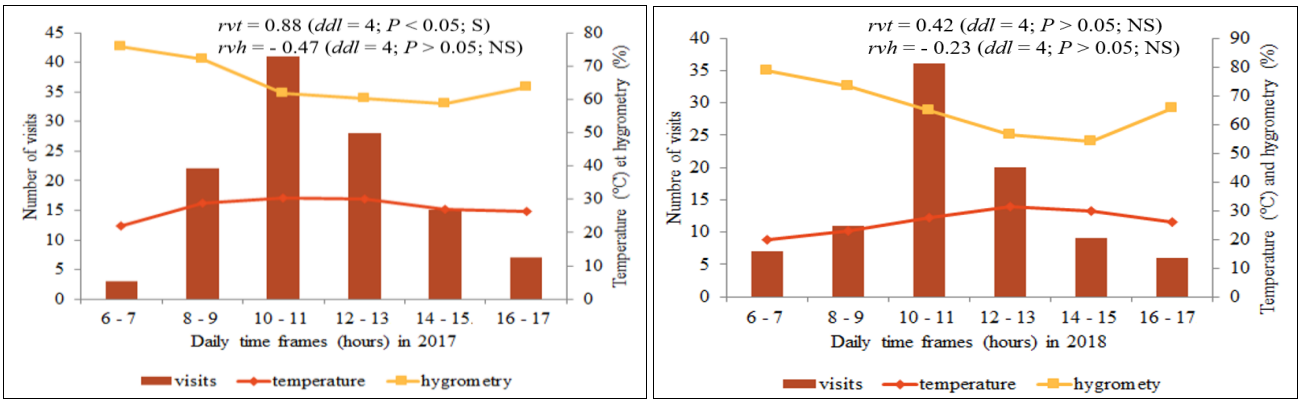

Fig 4: Variation of number of *Xylocopa olivacea* visits on *Phaseolus vulgaris* flowers according to daily time frames in 2017 and 2018 in Ngaoundere *rvt*: correlation coefficient between the number of visits and temperature; *rvh*: correlation coefficient between the number of visits and hygrometry; *ddl*: degrees of freedom; *P*: significance threshold; S: significant; NS: non significant

3.2.4 Daily Visits

Xylocopa olivacea individuals were active on *P. vulgaris* var. LWS flowers throughout the day, with the peak of visits situated between 10 and 11 am in 2017 as well as in 2018 (Fig 4).

In 2017, the correlation was significant between the number of *X. olivacea* visits and the temperature (r = 0.88; df = 4; P < 0.05) and not significant between the same number of visits and relative humidity (r = - 0.47; df = 4; P > 0.05). In 2018, the correlation was not significant between the number of *X. olivacea* visits and the temperature (r = 0.42; df = 4; P > 0.05) and between the number of these visits and relative humidity (r = -0.23; df = 4; P > 0.05).

3.2.5 Duration of a Visit Per Flower

In 2017 and 2018, the mean duration of *X. olivacea* visit per flower was 4.49 sec (n = 612; s = 2.50; *mini* = 2; *maxi* = 12) and 5.73 sec (n = 1165; s = 2.90; *mini* = 2; *maxi* = 16) respectively. The difference between these two means is highly significant (t = 9.66; df = 1775; P < 0.001). For the two cumulated years the mean duration per flower was 5.11 sec.

3.2.6 Abundances of *Xylocopa olivacea*

The highest mean number of *X. olivacea* simultaneously in activity was 1 per flower in 2017 (n = 184; s = 0.79) as well as in 2018 (n = 171; s = 0.4). In 2017 and 2018, the mean numbers of *X. olivacea* per 1000 flowers were 13.58 (n =146; s = 9.07; *mini* = 5; *maxi* = 50) and 18.81 (n = 204; s = 13.01; *mini* = 5; *maxi* = 75) respectively. The difference between these two mean is highly significant (t = 9.61; df = 373; P < 0.001).

3.2.7 Foraging Speed

On the experimental plots of *P. vulgaris*, *X. olivacea* visited between 1 and 14 flowers/min in 2017 and between 4 and 12 flowers/min in 2018. The mean foraging speed was 8.04 flowers/min (n = 227, s = 1.94) in 2017 and 6.47 flowers/min (n = 332; s = 1.28; *mini* = 4) in 2018. The difference between these means is highly significant (t = 3.27; df = 557; P < 0.01). For the two cumulated years, the mean foraging speed was 7.34 flowers/min.

3.2.8 Influence of the Neighboring Flora

During each observation period, flowers of many other plant species growing in the study area were visited by *X. olivacea* individuals, for nectar (ne) and/or pollen (po). Among these plants were: *Cajanus cajan* (Fabaceae; ne), *Crotalaria goreensis* (Fabaceae; ne), *Crotalaria juncea* (Fabaceae; ne), *Crotalaria retusa* (Fabaceae; ne), *Hibiscus sabdariffa* (Mavaceae; ne) and *Phaseolus vulgaris* var. Bigarre (Fabaceae; ne). During the whole observation periods, individual of *X. olivacea* foraging on *P. vulgaris* var. LWS were never observed moving from this Fabaceae to a neighboring plant species and vice versa.

3.2.9 Influence of the Fauna

Individuals of *X. olivacea* were disturbed in their foraging activity by bees from the same species or those from other species, which were competitors for *P. vulgaris* nectar. In 2017, for 612 visits, two (1.8 %) were interrupted by *Chalicodoma cincta* (Megachilidae), three (0.49%) by *Amegilla* sp. (Apidae) and six (0.26%) by *X. olivacea*. In 2018, for 1165 visits, 13 (1.12%) were interrupted by *X. olivacea*, five (0.43%) by *Xylocopa inconstans*, three (0.26%) by *Amegilla* sp., two (0.17 %) by *Apis mellifera* and two (0.17%) by *Chalicodoma cincta*. For their load of nectar, some individuals of *X. olivacea* who suffered such disturbances were forced to visit more flowers and/or plants during the corresponding foraging trip.

3.3 Impact of Flowering Insects including *Xylocopa olivacea* on *Phaseolus vulgaris* Production

During collection of nectar in *P. vulgaris* flower, all the insect species recorded were always in contact with the anthers, stigma and carried pollen. Thus, they greatly increased the possibilities of cross-pollination of *P. vulgaris*. Table 1 summarizes the data on the fruiting rate, the average number of seeds per pod and the percentage of normal seeds in each treatment of *P. vulgaris*.

Table 1: Podding rate, mean number of seeds per pod and percentage of normal seeds of a seed according to the different treatments of *Phaseolus vulgaris*, in 2017 and 2018 in Ngaoundere

Treatments	Years	NF	NP	Pr (%)	Number of seeds/pod			TNS	NS	% NS
					n	*m*	*sd*			
1 (UF)	2017	120	97	80.83	97	3.39	0.55	329	293	89.06
2 (PF)		120	48	40.00	48	2.63	0.73	126	57	45.23
3 (PFX)		200	139	69.50	139	3.09	0.74	429	350	81.59
4 (PFN)		100	54	54.00	54	2.80	0.83	151	81	53.64
5 (UF)	2018	120	107	89.16	107	3.44	0.63	368	323	87.77
6 (PF)		120	65	54.17	65	2.63	0.72	171	83	48.54
7 (PFX)		200	153	76.50	153	3.24	0.64	496	395	79.64
8 (PFN)		100	62	62.00	62	2.76	0.64	171	102	59.65

NF: number of flowers; NP: number of pods; Pr: podding rate; TNS: total number of seeds; NS: number of normal seeds; %NS: percentage of normal seeds; *n*: sample size; *m*: mean; *sd*: standard deviation; UF: unprotected flowers; PF: protected flowers; PFX: flowers protected, uncovered, visited once by *Xylocopa olivacea* and rebagged; PFN: flowers bagged then uncovered and rebagged without visit by insect or any other organism

It emerges from this Table that:

1. The podding rates were 80.83%, 40.00%, 65.50%, 54.00%, 89.16%, 54.17%, 76.50% and 62.00% in treatments 1 to 8 respectively. The differences between these eight percentages are globally highly significant (χ^2 = 103.26; *df* = 7; *P* < 0,001). The two - by – two comparisons show that the difference is highly significant between treatments 1 and 2 (χ^2 = 41.83; *df* = 1; *P* < 0.001) and between treatments 5 and 6 ($\chi 2$ = 36.20; *df* = 1; *P* < 0.001). Hence, in 2017 and 2018, the podding rate of unprotected flowers (treatments 1 and 5) and of was higher than that of protected flowers (treatments 2 and 6);
2. The mean number of seeds per pod were 3.39, 2.63, 3.09, 2.80, 3.44, 2.63, 3.24 and 2.76 in treatments 1 to 8 respectively. The differences between these eight means are globally highly significant (*F* = 18.99; *df1* = 7; *df2* = 717; *P* < 0.001). The two - by - two comparisons show that the difference observed is not significant between treatments 1 and 2 (*t* = 1.37 *df* = 143; *P* > 0.05) and significant between 5 and 6 (*t* = 2.05; *df* = 170; *P* < 0.05). Consequently, in 2018, the mean number of seeds per pod of unprotected flowers was higher than that of protected flowers;
3. The percentages of normal seeds were 89.06%, 45.23%, 81.59%, 53.64%, 87.77%, 48.54%, 79.64% and 59.65% in treatments 1 to 8 respectively. The differences between these eight percentages are globally highly significant (χ^2 = 264.91; *df* = 7; *P* < 0.001). Pairwise comparisons showed that the difference is highly significant between treatments 1 and 2 (χ^2 = 86.13; *df* = 1; *P* < 0.001) as well as between treatments 5 and 6 (χ^2 = 49.74; *df* = 1; *P* < 0.001). Hence in 2017 as well as in 2018, the percentage of normal seeds of unprotected flowers was higher than that of protected flowers.

In 2017, the numeric contributions of anthophilous insects including *X. olivacea* on the fruiting rate and the percentage of normal seeds were 40.15% and 43.92% respectively. In 2018, the fruiting rate, the number of seeds per pod and the percentage of the normal seeds 29.47%, 24.47% and 36.68% respectively. For the two years of cumulative experimentation, the fruiting rate and the percentage of normal seeds due anthophilous insects were 34.76% and 40.30% respectively.

3.4 Pollination Efficiency of *Xylocopa olivacea* on *Phaseolus Vulgaris*

During the nectar harvest, individuals of *X. olivacea* always came into contact with anthers and stigma. Thus they increased self-pollination and cross-pollination possibilities of visited flowers.

The comparison of podding rates (Table 1) shows that, the difference was highly significant between treatments 3 and 4 ($\chi 2$ = 6.98; *df* = 1; *P* < 0.01) as well as between treatments 7 and 8 ($\chi 2$ = 6.90; *df* = 1; *P* < 0.01). The podding rates due to *X. olivacea* were 22.30% in 2017, 14.08% in 2018 and 32.92% for the two cumulated years. Therefore, in 2017 and 2018, the podding rate of flowers visited by *X. olivacea* was higher than that of flowers protected, uncovered and rebagged without the visit of insect or any other organism.

The comparison of the mean number of seeds per pod (Table 1) shows that the difference was not significant between treatments 3 and 4 (*t* = 0.80; *df* = 191; *P* > 0.05) as well as between treatments 7 and 8 (t = 1.29; *df* = 213; *P* > 0.05).

The comparison of the percentage of normal seeds (Table 1) shows that the difference was highly significant between treatments 3 and 4 ($\chi 2$ = 45.65; *df* = 1; *P* < 0.001) as well as between treatments 7 and 8 ($\chi 2$ = 26.75; *df* = 1; *P* < 0.001). The percentage of normal seeds due to *X. olivacea* was 34.26% in 2017, 25.10% in 2018 and 29.68% for the two cumulated years.

4. Discussion

Results obtained from experiments indicated that *P. vulgaris* Large White Seeds has a mixed reproduction mode that is allogamous-autogamous with the predominance of autogamy over allogamy. Our result is not on line with those obtained by Mainkété *et al.* [17] at Sarh (Chad) on the same *P. vulgaris* variety. According to these authors, allogamy predomines over autogamy. This could be explained by the difference between the diversity of anthophilous insects,

which is one of the factors that can influence the reproduction mode of a plant (Jean-Prost, 1987) [14].

Xylocopa olivacea was the first insect visitor of *P. vulgaris* flowers in 2017 as well in 2018 and it intensely and exclusively collected nectar. In Chad, this carpenter bee also occupied the first place with 22% visits of all insect species of the same variety of the Fabaceae (Mainkété *et al*., 2019) [17]. At Dang, this bee ranked second (23.05%) in floral entomofauna of *P. vulgaris* var. Bigarre (Deli *et al*., 2020) [4].

During each observation period of *P. vulgaris*, *X. olivacea* intensively and regularly harvested only nectar. This could be attributed to the needs of individual of this carpenter bees during the flowering period of the Fabaceae (Mainkété *et al*., 2019) [19].

The peak of activity of *X. olivacea* was situated between 10 and 11 am, which could be the daily period of highest availability of nectar in *P. vulgaris* flowers. Our result corroborates those of Mainkété *et al*. [17] in Chad who reported the same peak of *X. olivacea* visits on the flowers of the same plant species. Similarly, this peak was demonstrated by Deli *et al*. [4] for the same bee species on *P. vulgaris* var. Biggare flowers, in the same study site at Ngaoundere.

The high abundance of foragers per 1000 flowers, and the positive and significant correlation between the number of *P. vulgaris* flowers and the number of *X. olivacea* visits underscore the attractiveness of *P. vulgaris* nectar for *X. olivacea*. The attractiveness for *P. vulgaris* nectar could be partially explained by its highest production and the accessibility or quality of this product (Deli *et al*., 2020; Douka & Tchuenguem, 2013; Mainkété *et al*., 2019) [4, 9, 17].

The significant difference observed between the mean durations of visit per flower could be explained by the availability of nectar or the variation of diversity of flowering insects in 2017 and 2018. In Chad, according to Mainkété *et al*. [17], the mean duration of *X. olivacea* visit per flower was 7.06 sec on this same variety of *P. vulgaris* against 5.38 sec recorded during our observations. The difference between these two means is highly significant (t = 4.77; df = 2530; $P < 0.001$).

The disruptions of *X. olivacea* visits by other insects reduced the duration of certain *X. olivacea* visits. Similar observations were made for the same carpenter bee foraging on flowers of the same Fabaceae in Chad (Mainkété *et al*., 2019) [17].

The higher production of pods and seeds in unprotected flowers when compared with bagged flowers showed that insect visits were effective in increasing self-pollination and/or cross-pollination of flowers visited. Our results confirmed those of Douka & Tchuenguem [9] and Deli *et al*. [4] in Maroua and Ngaoundere respectively in Cameroon, who revealed that *P. vulgaris* flowers set little pods in the absence of pollinating insects. Overall, flowering insects increased the fruiting rate and the percentage of the normal seeds of *P. vulgaris* Large White Seeds. In Chad, anthophilous insects boosted the fruiting rate and the percentage of the normal seeds of the same variety of *P. vulgaris* by 65.13% and 69% respectively (Mainkété *et al*., 2019) [17].

During the collection of nectar on each flower, *X. olivacea* individuals regularly come into contact with stigma and anthers. They 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) or to that of other plant (xenogamy) (Tchuenguem *et al*., 2001) [20]. The foragers could thus influence self-pollination and cross-pollination (Tchuenguem *et al*., 2001; Tchuenguem *et al*., 2004) [20, 19]. In Chad, Mainkété *et al*. [17] indicated that, throughout the pollination efficiency of a single flower visit, *X. olivacea* increased the fruiting rate and the percentage of the normal seeds of *P. vulgaris* var. Large White Seeds by 56.27% and 84.03% respectively against 32,92% and 36,68% obtained in our work.

5. Conclusion

The results obtained from this study reveal that *P. vulgaris* Large White Seeds variety is a plant that benefits from the pollination by insects, among which *Xylocopa olivacea* is one of the most important and harvest exclusively nectar. The comparison of pod and seed sets of flowers bagged then uncovered, visited once by *X. olivacea* and rebagged with those of flowers bagged then uncovered and reprotected without the visit of insects or any other organism underscores the value of this carpenter bee in increasing the fruiting rate and the percentage of normal seeds. Thus conservation and installation of *X. olivacea* nests close to *P. vulgaris* Large White Seed variety is recommended to improve its pod production as well as its seed quality and to favor the population of this carpenter bee in the Adamaoua Region.

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7. References

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