



E-ISSN: 2320-7078

P-ISSN: 2349-6800

JEZS 2019; 7(1): 645-651

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Received: 18-11-2018

Accepted: 21-12-2018

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Foraging and pollination behaviour of *Xylocopa olivacea* (Hymenoptera: Apidae) on *Phaseolus vulgaris* (Fabaceae) flowers at Doyaba (Sarh, Tchad)

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Abstract

To evaluate the impact of single visit of *Xylocopa olivacea* on pod and seed yields of *Phaseolus vulgaris* Large White Seeds variety, its foraging and pollinating activities were studied in Sarh, during 2015 and 2016 rainy seasons. Treatments included unlimited flowers access by all visitors, bagged flowers, flowers limited visits by *X. olivacea* only and flowers bagged, opened and closed without insect or other organism visits. The foraging behavior of *X. olivacea* on flowers and its pollination efficiency were recorded. *Xylocopa olivacea* was the most frequent visitor and it intensely and exclusively collected nectar. The fruiting rate of unprotected flowers is significantly higher than that of protected flowers. Through its pollination efficiency, *X. olivacea* provoked a significant increase of the fruiting rate by 52.27%, the number of seeds/pod by 30.79% and the percentage of normal seeds by 84.03%. Conservation of *X. olivacea* nest close to *P. vulgaris* (Large White Seeds variety) fields is recommended to improve pod and seed production in the region.

Keywords: *Phaseolus vulgaris*, *Xylocopa olivacea*, flowers, yield, pollination

Introduction

Phaseolus vulgaris is an annual plant originated from South and Central America [1]. Flowering starts 28-35 days after sowing [2]; the flower is pink, but can vary from white to purple depending on the different varieties [3] and produces nectar/pollen which attract insects [4, 5]. *Phaseolus vulgaris* flowers were reported to produce fewer seeds per pod in the absence of efficient pollinators in the United States of America [2]. In Dang (Ngaoundéré, Cameroon) the activities of *Xylocopa olivacea* on flowers of *P. vulgaris* (Black Seed Outlets variety) increase the fruiting rate by 63.30%, the number of seeds/pod by 18.98% and the normal seeds by 26.96% [4]. Research conducted in Maroua in 2013 by Douka and Tchuenguem [5] has revealed that *Apis mellifera* visits *P. vulgaris* (Red and Small Seeds 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 [4, 5, 6] and this plant is autogamous/allogamous [2, 4, 5]. Prior to this studies, no previous research has been reported on the relationships between *P. vulgaris* Large White Seeds variety and *X. olivacea* in Doyaba (Moyen-chari Region, Chad), although, the activity and diversity of flowering insects of a plant species vary with varieties [4].

In Tchad, *P. vulgaris* is consumed as vegetable, raw or cooked, or transformed into flour while the stems and leaves are used to feed livestock; the domestic production of edible fruits was about 138 088 and 144 070 tons annually in 2015 and 2016 respectively (Direction of the Agricultural Statistics in Chad, 2018), but the projections of production is more than 4500,000 tons. Therefore, it is important to investigate on the possibilities of increasing the production of this plant in this country.

The general objective of this work is to contribute to the understanding of the relationships between *P. vulgaris* (Large White Seeds variety) and *X. olivacea*, for their optimal management. Specific objectives were to: (a) determine the place of *X. olivacea* in the *P. vulgaris* floral entomofauna; (b) study the activity of this Apidae on flowers of this Fabaceae;

(c) evaluate the impact of the flowering insects including *X. olivacea* on pollination and pods and seeds yields of *P. vulgaris*; (d) estimate the pollination efficiency of *X. olivacea* on this plant species.

2. Materials and Methods

2.1. Study site, experimental plot and biological material

The studies were conducted from June to September, in 2015 and 2016, in the locality at Doyaba (latitude of 09° 04.875' N, a longitude of 018° 25.721' E, an altitude 363.3 m.a.s.l.), a village located in the southern of the city of Sarh in the Moyen - Chari Region of Tchad. The climate is of Sudanian type, characterized by two annual seasons: a long dry season (November to April) and a short rainy season (May to October); August is the wettest month of the year; the average annual rainfall is 1100 mm. The average temperature is 28 °C with a maximum of 33 °C and a minimum of 22 °C [7]. The humidity, very low in February and March, increases gradually from April with the rise of the Intertropical Front, peaking in August [7].

The experimental plot was an area of 437 m². The animal material was mainly represented by insects naturally present in the environment and 15 nests of *Xylocopa olivacea* (Hymenoptera: Apidae) located close to the experimental field. The plant material was *P. vulgaris* Large White Seeds variety collected in the surrounding of the Unit for Apply Apidology of the University of Ngaoundéré, Cameroon.

2.2. Sowing and Weeding

On June 26th, 2015 and July 14th, 2016, the experimental plot was cleaned and divided into eight subplots, each measuring 8*4.5 m². Two seeds were sown in six lines per subplots, each of which had 16 holes per line. Holes were separated 50 cm from each other, while lines were 75 cm apart [8]. Weeding was performed manually as necessary to maintain plots weedfree.

2.3. Determination of the reproduction mode of *Phaseolus vulgaris*

On August 12th, 2015, 240 flowers from untreated subplots at the budding stage were labeled among which 120 flowers were left unprotected (treatment 1), while 120 others were bagged using gauze bags (treatment 2) to prevent visiting insects [9]. In similar subplots, on August 24th, 2016, 240 flowers at the budding stage were labeled of which 120 flowers were unprotected (treatment 3), while 120 were bagged (treatment 4). For each cropping year, two weeks after shedding of the last labeled flower, the number of pods was assessed in each treatment. The podding index was then calculated as described by [9]: $Pi = Fb/Fa$, where Fb is the number of pods formed and Fa the number of viable flowers initially set. The allogamy rate (TC) from which derives the autogamy rate (TA) was expressed as the difference in fruiting indexes between treatment X (unprotected flowers) and treatment Y (bagged flowers) [10]. $TC = [(PiX - PiY) / PiX] * 100$, where PiX and PiY are respectively the mean fruiting indexes of treatment X and treatment Y ; $TA = 100 - TC$.

2.4. Study of the foraging activity of *Xylocopa olivacea* on *Phaseolus vulgaris* flowers

Observations were conducted on flowers of treatments 1 and 3, from the opening of the first flower bud (13th August 2015 and 25th August 2016) to the fading of the last flower (18th August 2015 and 30th August 2016), according to six daily time frames: 6 - 7 h, 8 - 9 h, 10 - 11 h, 12 - 13 h, 14 - 15 h and

16 - 17 h. The identity of all insects that visited *P. vulgaris* flowers was recorded at each daily time frame. All insects encountered on flowers were recorded and the cumulated results expressed in number of visits have been used to determine the relative frequency of *X. olivacea* (Fx) in the anthophilous fauna of *P. vulgaris*.

For each year of study, $Fx = [(Vx / Vi) * 100]$, where Vx is the number of visits of *X. olivacea* on flowers of free treatment and Vi , the total number of insect visits on flowers of the same treatment [9].

During each day of investigation, before starting visit counts, the number of open flowers was counted. The same days as for the registration of frequency of visits, the floral products (nectar and/or pollen) collected by the carpenter bee were recorded for the same date and daily time frame as that of insects' counts. The study of this parameter indicates whether *X. olivacea* is strictly polliniferous and/or nectariferous on *P. vulgaris* flowers [11]. This can give an idea of its involvement in the pollination of this plant. The duration of the individual flower visits was recorded (using a stopwatch) according to six daily time frames: 7 - 8 h, 9 - 10 h, 11 - 12 h, 13 - 14 h, 15 - 16 h and 17 - 18 h [12]. The foraging speed (number of flowers visited by a carpenter bee per minute according to [13]) was calculated using the following formula [12]: $Vb = (Fi/di) * 60$ where di is the time (s) given by a stopwatch and Fi is the number of flowers visited during di .

The abundance of foragers (highest number of individuals foraging simultaneously) per flower or per 1000 flowers ($A1000$) were recorded on the same dates and time slots as the registration of the duration of visits [9]. Abundance per flower was recorded as a result of direct counting. For determining the abundance per 1000 flowers, some 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 x [14]. The disruption of the activity of foragers by competitors or predators and the attractiveness exerted by other plant species on this insect was assessed by direct observations. For the second parameter, the number of times the carpenter bee went from *P. vulgaris* flowers to other plant species and vice versa was noted throughout the periods of investigation. During each observation date, temperature and relative humidity in the station were registered after every 30 minutes using a mobile thermo-hygrometer (HT-9227) installed in the shade [12].

2.5. Evaluation of the effect of *Xylocopa olivacea* and other insects on *Phaseolus vulgaris* yields

This evaluation was based on the impact of visiting insects on pollination, the impact of pollination on fructing of *P. vulgaris*, and the comparison of yields (podding rate, mean number of seed per pod and percentage of normal or well developed seeds) of treatments 1 (unprotected flowers) and 2 (bagged flowers). The podding 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 podding rate in treatments X and Y . The podding rate (Fr) is: $Fr = [(Fb/Fa) * 100]$ where Fb is the number of pods formed and Fa the number of opened flowers initially set [4].

At maturity, pods were harvested and counted from each treatment. The mean number of seeds per pod and the percentage of normal seeds were then calculated for each treatment.

2.6. Evaluation of the pollination efficiency of *Xylocopa olivacea* on *Phaseolus vulgaris*

In parallel to the constitution of treatments 1, 2, 3 and 4, 600 flowers were protected in 2015 and 2016, and two treatments were formed:

- treatments 5 in 2015 or 7 in 2016: 200 flowers protected using gauze bags to prevent insect visitors and destined exclusively to be visited by *X. olivacea*. As soon as the flowers was opened, each flowers of treatments 5 and 7 was inspected. Hence, the gauze bag was delicately removed and this flowers was observed for up to 10 minutes; the flowers visited by *X. olivacea* was marked and then protected once more [4];

- treatments 6 in 2015 or 8 in 2016: 100 flowers destined to opening and closing without the visit of insects or any other organisms. As soon as the flowers was opened, each flowers of treatments 4 and 8 was inspected. Hence, the gauze bag was delicately removed and this flower was observed for up to 10 minutes while avoiding the visit of insect or any other organism.

For each observation period, the contribution of *X. olivacea* in the podding rate, the number of seeds per pod and the percentage of normal seeds were calculated for each

treatment.

For each observation year, the contribution of *X. olivacea* in the podding rate (FrX) was calculated using the formula: $FrX = \{[(Fra-Frb)/Fra]*100\}$, where Fra and Frb are podding rates in treatment a (flowers visited exclusively by *X. olivacea*) and treatments b (flowers bagged than opened and closed without insect or other organism visits).

At the maturity, pods were harvested and counted from each treatments. The podding rate, the percentage of pods with seeds and the percentage of normal seeds were then calculated for each treatment.

2.7. Statistical analysis

Data were subjected to descriptive statistics, student's t -test for the comparison of means of the two samples, Pearson correlation coefficient (r) for the study of the association between two variables, and chi-square (χ^2) for the comparison of percentages, using Microsoft Excel 2013 software.

3. Results

3.1. Reproduction mode of *Phaseolus vulgaris*

The podding indexes of *P. vulgaris* were 0.95, 0.21, 0.94 and 0.45 for treatments 1, 2, 3 and 4 respectively (Table 1).

Table 1: Allogamy and autogamy rates of *Phaseolus vulgaris* in 2015 and 2016.

Years	Treatments	Number of flowers	Number pods	Podding indexes	Autogamy rate	Allogamy rate
2015	1 (unprotected flowers)	120	114	95	21.93	78.07
	2 (bagged flowers)	120	25	20.83		
2016	3 (unprotected flowers)	120	113	94.16	47.8	52.2
	4 (bagged flowers)	120	54	45		
2015 /2016	Total	480	306	63.74	34.86	65.13

Thus, in 2015, the autogamy rate was 21.93%, whereas the allogamy rate was 78.07%. In 2016, the corresponding figures were 47.8% and 52.2%. For the two cumulative years, the autogamy rate was 34.86% and the allogamy rate was 65.13%. It appears that *P. vulgaris* Large White Seeds variety has a mixed reproduction mode with the predominance of allogamy over autogamy.

3.2. Activity of *Xylocopa olivacea* on *Phaseolus vulgaris* flowers

3.2.1. Frequency of visits

Amongst the 620 and 648 visits of 15 and 16 insects species recorded on the flowers of *P. vulgaris* in 2015 and 2016 respectively, *X. olivacea* was the most represented insect with 136 visits (21.94%) and 143 visits (22.07%) in 2015 and 2016 respectively (Table 2). The difference between these two percentages is not significant ($\chi^2 = 0.00$; $df = 1$; $P > 0.05$).

Table 2: Diversity of insects on *Phaseolus vulgaris* flowers in 2015 and 2016 at Doyaba, number and percentage of visits of different insects.

Order	Family	Insects Genus and species	2015		2016		2015/2016		
			n_1	p_1 (%)	n_2	p_2 (%)	n_T	p_T (%)	
Coleoptera	Meloidae	<i>Coryna</i> sp. (fc)	47	7.58	49	7.56	96	7.57	
Hymenoptera	Apidae	<i>Apis mellifera</i> (ne)	49	7.9	53	8.18	102	8.04	
		<i>Amegilla</i> sp. 1 (ne)	38	6.13	44	6.79	82	6.47	
		<i>Amegilla</i> sp. 2 (ne)	14	2.26	9	1.39	23	1.81	
		<i>Xylocopa inconstans</i> (ne)	21	3.39	21	3.24	42	3.31	
		<i>Xylocopa olivacea</i> (ne)	136	21.94	143	22.07	279	22	
		<i>Xylocopa</i> sp. 1 (ne)	35	5.65	30	4.63	65	5.13	
	Formicidae	<i>Camponotus flavomarginatus</i> (ne)	24	3.88	20	3.09	44	3.47	
	Megachilidae	<i>Chalicodoma cincta</i> (ne)	64	10.32	70	10.8	134	10.57	
		<i>Chalicodoma rufipes</i> (ne)	40	6.45	38	5.86	78	6.15	
		<i>Megachile</i> sp. 1 (ne)	24	3.87	16	2.47	40	3.15	
		<i>Megachile torrida</i> (ne)	19	3.06	16	2.47	35	2.76	
	Vespidae	<i>Belonogaster juncea</i> (ne)	31	5	39	6.02	70	5.52	
		(1 sp.) (ne)	-	-	17	2.62	17	1.34	
Lepidoptera	Nymphalidae	(1 sp.) (ne)	24	3.87	20	3.09	44	3.47	
	Pieridae	<i>Eurema</i> sp. (ne)	54	8.7	63	9.72	117	9.23	
		Total	visits	620	100	648	100	1268	100
			species		15		16		16

n_1 and n_2 : number of visits on 120 flowers in 12 days, p_1 and p_2 : percentages of visits, $p_1 = (n_1/620) * 100$; $p_2 = (n_2/648) * 100$. Comparison of percentages of *Xylocopa olivacea* visits (2015/2016): $\chi^2 = 0.00$; $df = 1$; $P > 0.05$; fc : consumption of flowers; ne: collection of nectar; sp. : unidentified species.

3.2.2. Floral products harvested

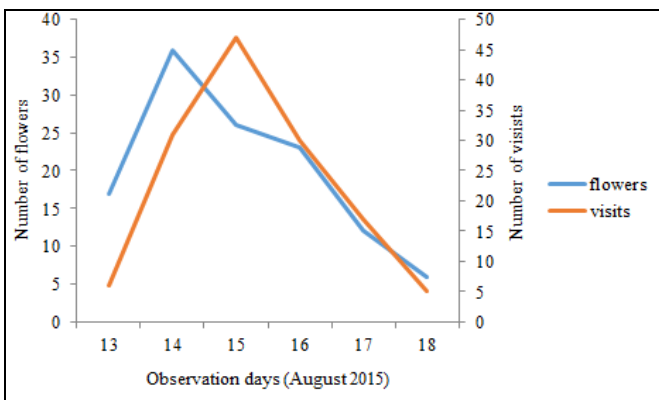
From our field observations and during the two flowering periods, individual *X. olivacea* were found to intensively and regularly collect only nectar on flowers from *P. vulgaris* (Figure 1).



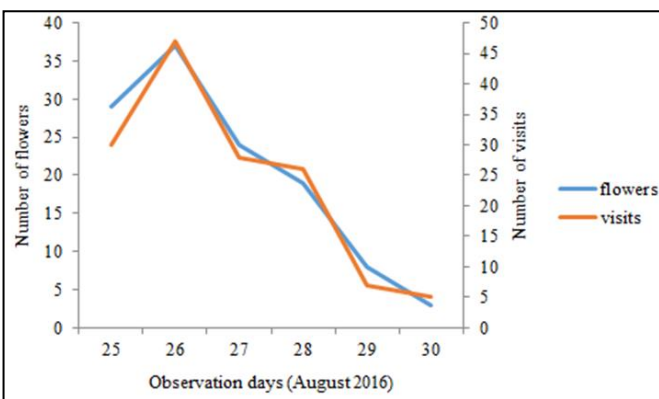
Fig 1: *Xylocopa olivacea* collecting nectar in a flower of *Phaseolus vulgaris*

3.2.3 Relationship between visits and flowering stages

Overall, visits of *X. olivacea* were more numerous on treatment 1 and 3 when the number of opened flowers was highest (Figures 2A and B). The correlation was highly significant between the number of *P. vulgaris* opened flowers and the number of *X. olivacea* visits in 2015 ($r = 0.74$; $df = 4$; $p < 0.001$) as well as in 2016 ($r = 0.98$; $df = 4$; $p < 0.001$).



A

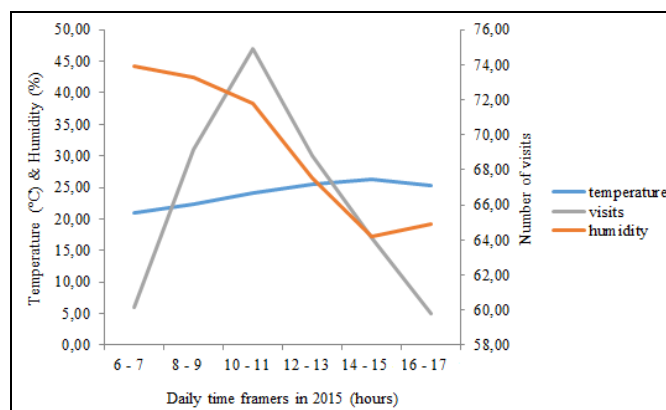


B

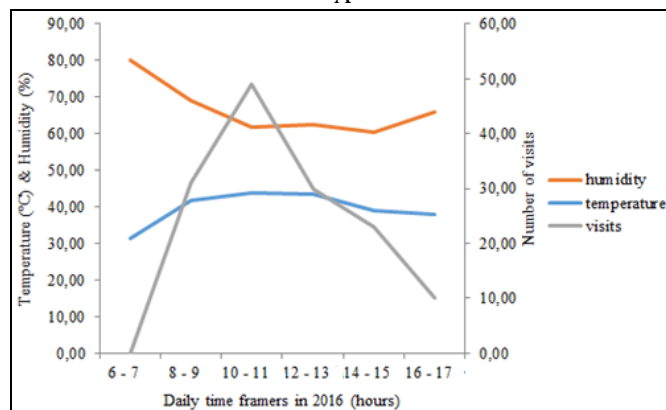
Fig 2: Seasonal variation of the number of *Phaseolus vulgaris* opened flowers and the number of *Xylocopa olivacea* visits in 2015 (A) and 2016 (B) at Doyaba.

3.2.4. Diurnal flower visits

The carpenter bee was active on *P. vulgaris* flowers throughout the day, with a peak of activity observed between 10 and 11 a.m. in 2015 and 2016 (Table 3). The correlation was not significant between the number of *X. olivacea* visits and relative humidity in 2015 ($r = 0.31$; $df = 4$; $P > 0.05$) as well as in 2016 ($r = 0.70$; $df = 4$; $P > 0.05$) (Figures 3A and B). The correlation was not significant between the number of *X. olivacea* visits and the temperature in 2015 ($r = 0.08$; $df = 4$; $P > 0.05$) whereas it was highly significant in 2016 ($r = 0.91$; $df = 4$; $P < 0.001$).



A



B

Fig 3: Daily distribution of *Xylocopa olivacea* visits on *Phaseolus vulgaris* flowers over 12 days in 2015 (A) and 2016 (B) as influenced by mean temperature and mean humidity at Doyaba.

3.2.5. Duration of a visit per flower

In 2015, the mean duration of a flower visit was 6.99 sec ($n = 432$; $s = 8.92$; $maxi = 60$ sec), while in 2016, the corresponding figure was 7.2 sec ($n = 323$; $s = 13.6$; $maxi = 215$), giving a highly significant difference ($t = 3.47$; $P < 0.001$) between the two sampling years. For the two cumulated years, the mean duration of a flower visit was 7.09 sec.

3.2.6. Abundance of Xylocopa olivacea

In 2015, the highest mean number of *X. olivacea* simultaneously active was one per flower ($n = 39$, $s = 0$) and 112 per 1000 flowers ($n = 239$, $s = 88.62$, $maxi = 524$). In 2016, the corresponding figures were 1 per flower ($n = 39$, $s = 0$) and 120 per 1000 flowers ($n = 179$, $s = 94.35$, $maxi = 568$). The difference between the mean number of *X. olivacea* per 1000 flowers in 2015 and that in 2016 is highly significant ($t = - 8.96$; $P < 0.001$).

3.2.7. Foraging speed of *Xylocopa olivacea* on *Phaseolus vulgaris* flowers

In *Phaseolus vulgaris* field, *X. olivacea* visited between 1 and 20 flowers/min in 2015 and between 1 and 35 flowers/min in 2016. The mean foraging speed was 9 flowers/min ($n = 120$, $s = 4.55$) in 2015 and 14 flowers/min ($n = 170$, $s = 22.52$) in 2016. The difference between these means is highly significant ($t = -20.56$; $df = 288$; $P < 0.001$). For the two cumulated years, the mean foraging speed was 11.5 flowers/min.

3.2.8. Influence of neighboring floral

During each observation periods, 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: *Abelmoschus esculentus* (po), *Arachis hypogaea* (ne), *Bidens pilosa* (ne and po), *Cajanus cajan* (ne), *Gossypium hirsutum* (ne and po), *Helianthus annuus* (ne and po), *Phaseolus coccineus* (ne), *Psidium guajava* (ne and po), *Sesamum indicum* (ne), *Vigna unguiculata* (ne and po) and *Zea mays* (po). During the whole observation periods individual carpenter bee foraging on *P. vulgaris* were not observed moving to a neighboring plant species and vice versa.

3.2.9. Influence of wildlife

Individuals of *X. olivacea* were disturbed in their foraging by other arthropods that were competitors for the search of pollen or nectar. These disturbances resulted in the interruption of certain *X. olivacea* visits. In 2015, for 632 visits of *X. olivacea*, 21 (3.32%) were interrupted by *Chalicodoma cincta*. In 2016, for 291 visits of *X. olivacea*, 16 (5.5%) was interrupted by *Apis mellifera*. 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 anthophilous insects including *Xylocopa olivacea* on the pollination, pod and seed yields of *Phaseolus vulgaris*

During nectar harvest on *P. vulgaris*, some foraging insects always shake flowers and contact anthers and stigma, increasing the cross pollination possibilities of this Fabaceae. The comparison of the podding rate (Table 4) showed that the differences observed were highly significant between treatments 1 and 2 ($\chi^2 = 135.41$; $df = 1$; $P < 0.001$) and treatments 3 and 4 ($\chi^2 = 68.53$; $df = 1$; $P < 0.001$). Consequently, in 2015 and 2016, the podding rate of unprotected flowers (treatments 1 and 3 respectively) was higher than that of flowers protected during their flowering period (treatments 2 and 4 respectively).

Table 4: Podding rate, number of seeds per pod and percentage of normal seeds according to different treatments of *Phaseolus vulgaris* in 2015 and 2016 at Doyaba.

Years	Treatments	NF	NFP	PrR (%)	Seeds/pod		TNS	NS	% NS
					<i>m</i>	<i>sd</i>			
2015	1 (unprotected flowers)	120	114	95	7.27	2.53	828	762	92.02
	2 (bagged flowers)	120	25	20.83	3.64	1.55	91	30	32.97
2016	3 (unprotected flowers)	120	113	94.16	6.32	1.29	714	661	92.58
	4 (bagged flowers)	120	54	45	5.43	1.96	293	71	24.23

NF: Number of flowers; NFP: Number of formed pod; PrR: Podding rate; TNS: Total number of seeds; NS: Normal seeds; % NS: Percentage of normal seeds; *m*: mean; *sd*: standard deviation.

The comparison of the mean number of seeds per pod (Table 4) showed that the difference observed were highly significant between treatments 1 and 2 ($t = 31$; $P < 0.001$) and treatments 3 and 4 ($t = 21$; $P < 0.001$). As a matter of fact, in 2015 and 2016, the mean number of seeds per pod in opened flowers was higher than that of flowers bagged during their flowering period.

The comparison of the percentage of normal seeds (Table 4) showed that the difference observed were highly significant between treatments 1 and 2 ($\chi^2 = 240.15$; $df = 1$; $P < 0.001$) and treatments 3 and 4 ($\chi^2 = 488.84$; $df = 1$; $P < 0.001$). Hence, in 2015 as well as 2016, the percentage of normal seeds of exposed flowers was higher than that of flowers bagged during their flowering period.

In 2015, the numeric contributions of anthophilous insects on the podding rate, the mean number of seeds per pod and the percentage of normal seeds were 78.07%, 49.93% and 64.17% respectively. In 2016, the corresponding figures were 52.2%, 14.08% and 73.83% respectively. For the two

cumulate years, the numeric contributions of flowering insects were 65.13%, 32% and 69% on the podding rate, the number of seeds per pod and the normal seeds, respectively.

3.4. Pollination efficiency of *Xylocopa olivacea* on *Phaseolus vulgaris*

During the nectar harvest from flowers, individuals of *X. olivacea* were always in contact with the stigma and the anthers. Thus, this carpenter bee highly increased the pollination of *P. vulgaris* flowers.

The comparison of the podding rate (Table 5) shows that the differences observed were highly significant between treatments 5 and 6 ($\chi^2 = 13.52$; $P < 0.001$) and between treatments 7 and 8 ($\chi^2 = 79.31$; $P < 0.001$). Hence in 2015 and 2016 the podding rate of flowers exclusively visited by *X. olivacea* (treatment 5 and 7 respectively) was significantly higher than that of flowers bagged, opened and closed without insect or any other organism visit during their flowering period (treatment 6 and 8) respectively.

Table 5: Podding rate, number of seed per pod and percentage of normal seeds according to different treatments of *Phaseolus vulgaris* in 2015 and 2016 at Doyaba.

Years	Treatments	NF	NFP	PrR (%)	Seeds/pod		TNS	NS	% NS
					<i>m</i>	<i>sd</i>			
2015	5 (flowers visited exclusively by <i>X. olivacea</i>)	200	111	55.5	3.99	1.91	443	355	80.14
	6 (bagged flowers, opened and closed without visit)	100	33	33	1.99	0.84	65	7	10.77

2016	7(flowers visited exclusively by <i>X. olivacea</i>)	200	150	75	6.19	1.3	929	830	89.34
	8 (bagged flowers, opened and closed without visit)	100	21	21	5.48	1.91	115	19	16.52

NF: Number of flowers; NFP: Number of formed pod; PrR: Podding rate; TNS: Total number of seeds; NS: Normal seeds;% NS: Percentage of normal seeds; *m*: mean; *sd*: standard deviation.

The comparison of the mean number of seeds per pod (Table 5) showed that the difference observed were highly significant between treatments 5 and 6 ($t = 29.30$; $P < 0.001$) and treatments 7 and 8 ($t = 9.36$; $P < 0.001$). For the two years, the difference was highly significant between the mean number of seeds per pod of flowers bagged and visited exclusively by *X. olivacea* (treatment 5 and 7 respectively) and those of flowers bagged, then opened and closed without insect or any other organism visit (treatment 6 and 8 respectively).

The comparison of the percentage of normal seeds (Table 5) showed that the differences observed were highly significant between treatments 5 and 6 ($\chi^2 = 133.17$; $df = 1$; $P < 0.001$) and treatments 7 and 8 ($\chi^2 = 357.27$; $df = 1$; $P < 0.001$). Hence, in 2015 as well as in 2016, the percentage of normal seeds of flowers exclusively visited by *X. olivacea* (treatment 5 and 7 respectively) was significantly higher than that of flowers bagged, opened and closed without insect or any other organism visit during their opening period (treatment 6 and 8 respectively).

In 2015, the numeric contributions of *X. olivacea* on the podding rate, the number of seeds per pod and the normal seeds via a single flower visit were 40.54%, 50.12% and 86.56% respectively. In 2016, the corresponding figures were 72%, 11.47% and 81.51% respectively. For the two cumulate years, the corresponding figures were 56.27%, 30.79% and 84.03% respectively.

4. Discussion

Xylocopa olivacea was the main floral visitor of *P. vulgaris* during the observation period. This carpenter bee is known as an insect flower visitor of *V. paradoxa* in Ghana [15] and in the Adamaoua region of Cameroon [16]. *Xylocopa olivacea* has been shown to be the most abundant floral visitor of *P. vulgaris* Black Seed Outlets variety [4], *Vigna unguiculata* [17] and *P. coccineus* [18, 19]. The significant difference between the percentage visits of *X. olivacea* within studied years could be explained by the presence of several nests of *X. olivacea* near the experimental plot in 2015 when compared to that of 2016. The peak of *X. olivacea* activity on *P. vulgaris* flowers was located between 10.00 am and 11.00 am, which correlated with the period of highest availability of nectar on this Fabaceae. The abundance of *X. olivacea* individuals per 1000 flowers and the positive and highly significant correlation between the number of *P. vulgaris* flowers in bloom as well as the number of *X. olivacea* visit indicates the high attractiveness of nectar with respect to this carpenter bee.

During each of the two flowering periods of *P. vulgaris*, *X. olivacea* intensively and regularly harvested nectar. This could be attributed to the needs of individual carpenter bees during the flowering period of the Fabaceae. The disruptions of 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 *P. coccineus* [19] in Yaoundé and on flowers of *P. vulgaris* in Ngaoundéré [4].

During the collection of nectar on each flower, *X. olivacea* individuals regularly come into contact with the stigma and anthers. They carried pollen with their hairs, legs, thorax, abdomen and mouth accessories from a flower of one plant to,

to that of the same plant or to that of another plant. Individuals of this carpenter bee thus influence self-pollination and cross-pollination [20, 21]. Similar observations have been made for *X. olivacea* foraging on flowers of *P. vulgaris* (Red and Small Seeds variety) in Western Kenya [22].

The weight of *X. olivacea* played a positive role in the self-pollination; when collecting nectar, *X. olivacea* shakes flowers; this movement could facilitate the liberation of pollen by anthers, for optimal occupation of the stigma [23]. Results of the present study confirm those of the studies carried out by Tchuenguem *et al.* and Pando *et al.* [18, 19] on *P. coccineus* in yaoundé and Ngaoundéré respectively [4] and on *P. vulgaris* (Red and Small Seeds variety) in Ngaoundéré. The present study demonstrates that during one foraging trip, an individual bee foraging on *P. vulgaris* scarcely visits other plant species.

The higher productivity of pods in unlimited visits when compared with bagged flowers showed that insect visits were effective in increasing cross and/or self-pollination. Higher productivity of flowers exposed to visits by *X. olivacea* compared with those under unlimited visits by all kinds of visitors shows that this carpenter bee is an important pollinator of *P. vulgaris* and thus can be targeted for the managed pollination of this plant. Our results confirmed those of the studies carried out by Webster *et al.*, Wells *et al.* and Kingha *et al.* [24, 25, 4] in England, Brazil and Ngaoundéré respectively who revealed that *P. vulgaris* flowers set little pods in the absence of insect pollinators.

The positive and significant contribution of *X. olivacea* to the yields through its pollination efficiency is in agreement with similar findings in Yaoundé on *P. coccineus* [18] and in Ngaoundéré on *P. vulgaris* [4].

5. Conclusion

This study reveals that in Sarh, *P. vulgaris* White and Large Seeds variety is a highly nectariferous bee plant that obtained benefits from the pollination by insects among which *X. olivacea* is the most important. The comparison of pod and seed sets of unprotected flowers with those of flowers exclusively visited by *X. olivacea* underscores the value of this carpenter bee in increasing podding rate, number of seeds/pod and percentage of normal seeds of *P. vulgaris*. The installation of *X. olivacea* nests at the proximity of *P. vulgaris* White and Large Seeds variety is recommended for the increase of pods and seed yields of this valuable crop.

6. References

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