



Impact of single visit of *Lipotriches collaris* Vachal 1903 (Hymenoptera: Halictidae) on *Phaseolus vulgaris* (Fabaceae) flowers at Maroua (Cameroon)

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ABSTRACT

To evaluate the impact of single visit of *Lipotriches collaris* on pod and seed yields of *Phaseolus vulgaris* (Red and Small Seeds), its foraging and pollinating activities were studied in Maroua, during 2010, 2011 and 2013 cropping seasons. Treatments included bagged flowers to avoid all visits and bagged flowers limited on a single visit of *L. collaris*. Observations were made on 147, 139 and 138 flowers in 2010, 2011 and 2013 respectively. The foraging behavior on flowers and its pollination efficiency (fruiting rate, number of seeds/pod and percentage of normal seeds) were recorded. *Lipotriches collaris* foraged pollen and nectar. The foraging activities of *L. collaris* increase the fruiting rate by 39.49 % and the number of seeds/pod by 14.65 %. Conservation of *L. collaris* nest close to *P. vulgaris* crop fields should be recommended to improve pod and seed production in the region.

1. INTRODUCTION

Phaseolus vulgaris is a plant that originated from South and Central America [1]. Bean plants are bushy or upright (40 to 60 cm); Climbing stems are slightly branched; the leaves are stalked; alternate and compound trifoliate; green or purple [2]. Flowering starts 28-35 days after sowing; 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 (Ngaoundere-Cameroon) the activities of *Xylocopa olivacea* on flowers of *P. vulgaris* increase the fruiting rate by 63.30 %, the number of seeds/pod by 18.98 % and the normal seeds by 26.96 % [4]. Recent research conducted in Maroua in 2013 by Douka and Tchuenguem [5] has revealed that *Apis mellifera adansonii* visiting *P. vulgaris* (Red and Small Seeds) flowers this insect behavior 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 by insects is generally observed [4-5-6-7] and this plant is autogam/allogam [2-4-5]. In Cameroon, *P. vulgaris* can be consumed as vegetable raw or cooked, or transformed into flour,

while the stems and leaves are used to feed livestock [3]; the production of *P. vulgaris* is 353,729 tons, but the projections of production is more than 500,000 tons [8]. Therefore, it is important to investigate on the possibilities of increasing the production of this plant in Cameroon. The main objective of this research was to gather data on the relationships between *P. vulgaris* and *L. collaris* in Maroua, for optimal management of pollination services in Cameroon. Specific objectives were the registration of the activities and this efficiency pollination on this plant.

2. MATERIALS AND METHODS

2.1. Study site, experimental plot and biological material

The studies were conducted from June-September in 2010, 2011 and 2013 respectively in the locality of Maroua (Latitude 10° 62'N, Longitude 14° 33'E and altitude 400m), Far North Region of Cameroon. This Region belongs to the ecological zone with three phytogeographical areas: Sahel-Sudanian, Sahelian and Sudanian altitude, which is periodically flooded with unimodal rainfall [9]. 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; annual rainfall varies from 400 to 1100 mm; the annual average temperature varies between 29 °C and 38 °C and a daily temperature range between 6 °C and 7 °C [10].

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The experimental plot is an area of 156 m². The insect in this study was *Lipotriches collaris* Vachal 1903 (Hymenoptera: Halictidae). The plant was *Phaseolus vulgaris* (Red and Small Seeds). The seeds of this plant were provided by IRAD (Institut de Recherche Agricole pour le Développement) of Maroua.

2.2. Sowing and weeding

On June 12, 2010, June 15, 2011 and July 13, 2013, the experimental plot was cleaned and divided into 24 subplots, each measuring 1m × 1.5m. Two seeds were sown in 2 lines per subplot, each of which had 5 holes per line. Holes were separated 30 cm from each other, while lines were 80 cm apart. Weeding was performed manually as necessary to maintain plots in a weed-free state.

2.3. Foraging activities and Pollination efficiency of *Lipotriches collaris* on *Phaseolus vulgaris*

From July 8 to 2nd August 2010, July 16 to 7 August 2011 and August 15 to 5 September 2013, 147, 139 and 138 flowers were isolated at bud stage respectively and for each year two treatments were made. Treatment 1 was made by bagged flowers with gauze bag to avoid all visits (figure 1) and treatment 2 was constituted by bagged flowers limited on a single visit of *L. collaris*.



Fig. 1: Plant of *Phaseolus vulgaris* showing a flower isolated from insects..

On treatment 2 between 6 and 9h am of each observation date and each year; the gauze bag was delicately removed from each opened flower and this flower observed for up to 10 min. The flowers visited by *L. collaris* were labeled after this manipulation (Figure 2).

For foraging activities observations were conducted at the same date and the same time (6- 9h am).

The floral rewards (nectar or pollen) harvested by *L. collaris* during each floral visit were registered based on its foraging behavior. Nectar foragers were expected to extend their proboscis to the base of the corolla and the stigma, while pollen gatherers were expected to scratch the anthers with their mandibles or legs [11]. In the morning of each day, the number of opened flowers on *P. vulgaris* plant was counted.

For the abundances of *L. collaris*, large numbers of the insect were found to be simultaneously active per flower and per 1000 flowers, 1000 (A_{1000}) were recorded. The first parameter was

recorded as a result of direct counts. For A_{1000} , *L. collaris* were counted on a known number of open flowers. A_{1000} was then calculated using the formula: $A_{1000} = [(Ax/Fx) 1000]$, where Fx and Ax are the number of opened flowers and the number of *L. collaris* effectively counted on these flowers at time x respectively [12-13].

The duration of individual flower visits was recorded (using a stopwatch).



Fig. 2: Steps of manipulation on *Phaseolus vulgaris* flowers from treatment 2. (A) Plant showing bagged flowers of *P. vulgaris*, (B) Opened flowers after delicate removal from the gauze bag, (C) a single visit of *L. collaris* on the opened flower, (D) After a single visit of *L. collaris* the flower was labeled.

Two week after the end of observation (16 August 2010, 21 August 2011 and 19 September 2013) for each treatment the number of pod and the number of seeds per pod were counted then the quality of seed was also appreciated. The contribution ($Frlc$) of *L. collaris* to fruiting was calculated by the formula: $Frlc = \{[(Fr2 - Fr1) / Fr2] \times 100\}$. Where $Fr1$ and $Fr2$ are the fruiting rates in treatment 1 (protected flowers) and in treatment 2 (protected flowers and visited exclusively by *L. collaris*). For the fruiting rates (Fr) was then calculated as described by Tchuenguem *et al.* [12]: $Fr = F2/F1$. Where $F2$ is the number of pods formed and $F1$ the number of viable flowers initially set). The mean number of seeds per pod and the percentage of normal seeds were then calculated for each treatment.

2.4. Data analysis

Data were analyzed using descriptive statistics with Microsoft Excel 2007, Student's (t) test for the comparison of the average number of seeds per boll between treatments 1 (bagged flowers to avoid all visits) and treatment 2 (bagged flowers limited on a single visit of *L. collaris*). Correlation coefficient (r) was used to study of the association between the variables of abundance of *L. collaris* and opened flowers. Chi - Square (χ^2) test was used for the comparison of fruiting rates and the percentage of normal seeds between treatments 1 and 2. Comparison of the means of

duration of visits and the abundance of *L. collaris* of 2010, 2011 and 2013 was done using ANOVA (*F*).

3. RESULTS

3.1. Activity of *Lipotriches collaris* on *Phaseolus vulgaris* flowers

3.1.1. Floral rewards harvested

From our field observations, *L. collaris* were found to collect pollen and nectar on *P. vulgaris* flowers. Pollen collection was regular and intensive whereas nectar collection was regular but less intensive. In 62, 52 and 34 visits counted on flowers, respectively in 2010, 2011 and 2013, 45 (72.58%), 37 (71.15%) and 16 (47.05%) were for pollen collection and 17 (27.41%), 15 (28.84%) and 18 (52.94%) for nectar collection, respectively in 2010, 2011 and 2013 (Table 1).

Table 1: Products harvested by *Lipotriches collaris* on flowers of *Phaseolus vulgaris* in 2010, 2011 and 2013 in Maroua.

Year	Number of visits	Visits for pollen harvest		Visits for nectar harvest	
		Number	%	Number	%
2010	62	45	72.58	17	27.41
2011	52	37	71.15	15	28.84
2013	34	16	47.05	18	52.94
Total	148	98	66.22	50	33.78

Comparison of the percentage of harvest visits for the three years of investigation: Nectar/ Pollen: $\chi^2 = 7.27$ (*ddl* = 2; *P* < 0.05).

3.1.2. Relationship between visits and flowering stages

Positive and significant correlation was found between the number of *P. vulgaris* opened flowers and the number of *L. collaris* visits in 2010 (*r* = 0.68; *df* = 19; *p* < 0.001), in 2011 (*r* = 0.66; *df* = 19; *p* < 0.001) and in 2013 (*r* = 0.62; *df* = 19; *p* < 0.01) (Figure 3).

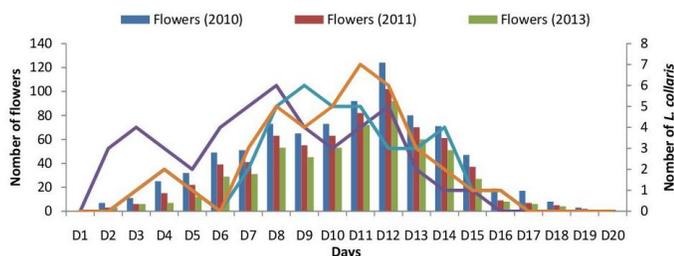


Fig. 3: Variation of number of flowers and number of *Lipotriches collaris* on flowers of *Phaseolus vulgaris* in 2010, 2011 and 2013 in Maroua.

3.1.3. Abundance of *Lipotriches collaris*

In 2010, the highest average number of *L. collaris* simultaneously active was one per flower (*n* = 30, *s* = 0) and 132.76 per 1000 flowers (*n* = 37, *s* = 121.55, *max* = 400). In 2011, the corresponding figures were 1 per flower (*n* = 30, *s* = 0) and 147.92 per 1000 flowers (*n* = 45, *s* = 100.55, *max* = 500). In 2013, the results were 1 per flower (*n* = 30, *s* = 0) and 119.23 per 1000 flowers (*n* = 42, *s* = 98.14, *max* = 456). The difference between the average number of *L. collaris* per 1000 flowers in 2010, 2011 and 2013 is significant (*F* = 2.71 [*df* = 122, *P* < 0.05]).

3.1.4. Duration of visits per flower

The average duration of a visit of *L. collaris* per flower of *P. vulgaris* varied significantly depending on the substance taken. In 2010, the average duration of a visit for pollen collection was 1.84 sec (*n* = 45, *s* = 0.57, *max* = 8); for the collection of nectar, it was 1.45 sec (*n* = 71, *s* = 0.51, *max* = 5). In 2011, the corresponding results were 1.94 sec (*n* = 37, *s* = 0.78, *max* = 7) and 1.77 sec (*n* = 15, *s* = 0.81, *max* = 5) for pollen and nectar harvest respectively. In 2013, we have found 1.58 sec (*n* = 16, *s* = 0.95, *max* = 9) for pollen and 1.32 sec (*n* = 18, *s* = 0.47, *max* = 6) for nectar.

The difference between the three means durations is significant for nectar harvest (*F* = 3.37 [*df* = 48, *P* < 0.05]) and for pollen collecting (*F* = 4.09, [*df* = 96, *P* < 0.05]).

3.2. Pollination efficiency of *Lipotriches collaris* on *Phaseolus vulgaris*

During pollen and nectar harvest on *P. vulgaris*, *L. collaris* always shook flowers and are regularly in contact with the anthers and stigma, increasing cross-pollination possibility of *P. vulgaris* fruiting rate and number of seeds per pod in different treatments (Table 2).

a) The difference observed between the fruiting rate of treatment 2 and that of treatment 1 was significant in 2010 ($\chi^2 = 4.61$ [*df* = 1, *P* < 0.05]), in 2011 ($\chi^2 = 5.34$ [*df* = 1, *P* < 0.05]) and in 2013 ($\chi^2 = 5.40$ [*df* = 1, *P* < 0.05]). Therefore, the rate of fruit set of flowers isolated and visited exclusively by *L. collaris* (treatments 2) is higher than that of protected flowers (treatments 1). In 2010, 2011 and 2013, the percentages of fruiting rate attributed to the efficiency of pollinating of *L. collaris* were 39.55%, 37.77% and 41.17% respectively. For the three years of experiments, the percentage is 39.49 %.

b) There was a highly significant difference between the number of seeds per pod of treatments 2 and 1 (*t* = -5.08 [*df* = 49, *P* < 0.001]) in the first year, the second year (*t* = -11.61 [*df* = 58, *P* < 0.001]) and the third year (*t* = -3.83 [*df* = 51, *P* < 0.001]). High mean number of seeds per pod of flowers of treatment 2 was noticed compared to flowers of treatment 1. Consequently, in 2010, 2011 and 2013, the number of seeds per pod of flowers isolated and visited exclusively by *L. collaris* (treatments 2) was higher than that of protected flowers (treatments 1). Percentages of the number of seeds per pod affected to the pollination efficiency of *L. collaris* were 14.39%, 19.13% and 10.45% respectively in 2010, 2011 and 2013. For the three seasons of study, this percentage was 14.65%.

c) There was no significant difference between the percentage of normal seed of treatment 2 and that of treatment 1 in the first year ($\chi^2 = 3.19$ [*df* = 1, *P* > 0.05]), the second year ($\chi^2 = 0.30$ [*df* = 1, *P* > 0.05]) and the third year ($\chi^2 = 0.63$ [*df* = 1, *P* > 0.05]). Therefore, in 2010, 2011 and 2013, the percentage of normal seeds from flowers isolated and visited exclusively by *L. collaris* (treatment 2) was not higher than that of protected flowers (treatment 1).

Table 2: *Phaseolus vulgaris* yields under pollination treatments.

Treatments	Year	F Flowers	Boll	Fruiting Rate	Seeds/Boll		Total Seeds	Normal Seeds	% Normal Seeds
					mean	sd			
1 (Fi)	2010	108	32	29,62	3,33	1,39	99	84	84,84
2 (FvI)		39	19	49,00	3,89	1,09	152	140	92,11
1 (Fi)	2011	108	41	37,96	3,93	1,05	173	154	89,01
2 (FvI)		31	19	61,00	4,86	0,96	122	111	90,98
1 (Fi)	2013	108	36	33,33	3,17	1,18	126	109	86,50
2 (FvI)		30	17	56,66	3,54	0,88	54	49	90,74

Fi: isolated flowers ; FvI: isolated flowers and then visited exclusively by *Lipotriches collaris*.

4. DISCUSSION

4.1. Activity of *Lipotriches collaris* on *Phaseolus vulgaris* flowers

4.1.1. Floral rewards harvested

During each of the three flowering periods of *P. vulgaris*, *L. collaris* intensively and regularly harvested pollen or nectar. This could be attributed to the needs of this bee during the flowering period and it indicates that *L. collaris* can provide benefits to pollination of *P. vulgaris* in Maroua [14]. Similar observations were found on flowers of some Fabaceae with *A. m. adansonii* (Hymenoptera: Apidae) workers foraging on flowers of *Entada africana*, *Glycine max*, *Vigna unguiculata* [15-16-17-18], *Chalicodoma cincta cincta* (Hymenoptera: Megachilidae) foraging on *Cajanus cajan* flowers [19] and *Xylocopa olivacea* (Hymenoptera: Apidae) workers foraging *P. vulgaris* flowers [4]. The weight of *L. collaris* played a positive role during pollen and nectar collection. *Lipotriches collaris* shook flowers, facilitating the liberation of pollen by anthers for the optimal occupation of the stigma [14].

4.1.2. Relationship between visits and flowering stages

Lipotriches collaris was the main floral visitor of *P. vulgaris* flowers during the observation period. Halictidae have been reported as the main floral visitor of *P. vulgaris*, *P. coccineus* and *Glycine max* flowers in Cameroon [4,15,20]. The significant difference between the percentage visits of *L. collaris* within the years of study could be attributed to the experimental site variation. In field conditions, the activity of bee on flowers was dependent on the site and in the same conditions this activity varied with years, the climatic factors and the availability of pollen and nectar on flowers [15-20].

4.1.3. Abundance of *Lipotriches collaris*

The abundance of *L. collaris* foragers on 1000 flowers and the positive and significant correlation between the number of *P. vulgaris* flowers (as bloom), as well as, the number of *L. collaris* visits indicated the attractiveness of *P. vulgaris* pollen and nectar. In fact, weather during bloom was demonstrated to affect the abundance and foraging of pollinator insects [21].

Similar observations were made in Maroua with *A. m. adansonii* and *Macronomia vulpina* on *Alum cepa*, *Gossypium hirsutum* and *G. max* flowers [22-24].

4.1.4. Duration of visits per flower

The significant difference between the duration of visits in 2010, 2011 and 2013 could be attributed to the availability of floral products or the variation of diversity of flowering insects from one year to another. During each of the three flowering periods of *P. vulgaris*, *L. collaris* intensively and regularly harvested nectar and pollen. This could be attributed to the needs of individuals at flowering period. Similar observations were made for *A. m. adansonii* workers foraging on *Entada africana* and *Vigna unguiculata* (Fabaceae) flowers [17-18], and for *Chalicodoma cincta* (Hymenoptera: Megachilidae) foraging on *Cajanus cajan* (Fabaceae) flowers [20].

4.2. Pollination efficiency of *Lipotriches collaris* on *Phaseolus vulgaris*

During the collection of nectar and pollen on each flower, *L. collaris* foragers regularly come into contact with the stigma. 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), to the same flower (autogamy) or to that of another plant (xenogamy).

The significant contribution of *L. collaris* in pods and seed yield of *P. vulgaris* is in agreement with similar findings in Ngaoundere (Cameroon) [4], England [25] and United State of America [2] which showed that *P. vulgaris* flowers produce fewer seeds per pod in the absence of efficient pollinators.

The contribution of *L. collaris* to *P. vulgaris* production through its pollination efficiency was significantly higher than that of isolated flowers. The weight of *L. collaris* played a positive role during nectar and pollen collection. *Lipotriches collaris* shook flowers facilitating the liberation of pollen by anthers for the optimal occupation of the stigma. Our results confirmed those of Azo'o et al. [26] who revealed that the development of fruits from *Abelmoschus esculentus* (L.) Moench (Malvaceae) flowers that have received a single visit of *Eucara macrognatha* to the increment of the fruit length is 6.85 % and that of *Tetralonia fraterna* is 0.90 %. This phenomenon was also reported by Vanderborcht and Rasmont [27] for *Xylocopa bariwal*, an efficient *P. coccineus* pollinator.

5. CONCLUSION

This study revealed that plants from Small and Red Seed of *P. vulgaris* obtained benefits from pollination by *L. collaris*.

The comparison of pods and seeds between sets of protected flowers with those of flowers visited only by *L. collaris* underscores the value of this bee in increasing pods and seed yields. The installation of *L. collaris* nest at the proximity of *P. vulgaris* fields is recommended for the increase of pods and seed yields of this valuable crop.

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