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The importance of single floral visit of *Chalicodoma cincta cincta* Fabricius 1871 (Hymenoptera : Megachilidae) in the pollination and yield of *Vigna unguiculata* (L.) Walp. 1843 (Fabaceae) in Cameroon.

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Key words: *Vigna unguiculata*, flowers, *Chalicodoma cincta cincta,* pollination, yields.

Abstract

Experiments were made in Yaoundé to determine the effects of a *Chalicodoma cincta cincta* Fabricius 1871 visit on the pollination and the productivity of cowpea, *Vigna unguiculata* (L.) Walp. 1843. Two treatments for each experiment were used on 40 randomly-selected plants each. These included Autonomous Self-Pollination (ASP) with flowers from which insects visit, with airborne pollen flow excluded (treatments 1 and 3), flowers that received a single bee visit (SBV) of *C. cincta cincta* (treatments 2 and 4). This wild bee mainly foraged for nectar and pollen resources. The mean foraging speed was 10.82 flowers/min and the duration of visits was 13.35 sec to collect nectar and pollen. All flowers produced pod with or without insect visits. *Chalicodoma cincta cincta* was effective pollinator, and of course their visits increased pod production (fruiting rate), number of seeds (number of seeds/pod) and normal seed (percentage of normal seed). *Chalicodoma cincta cincta* foraging resulted in a significant increment of the fruiting rate by 31.98 %, as well as the number of seeds/pod by 30.76 % and the percentage of normal seeds by 15.27 % in Yaoundé-Cameroon. Conservation of *C. cincta cincta* nests close to *V. unguiculata* fields could be recommended to increase pod and seed production in the region.

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Introduction

Cowpea, *Vigna unguiculata* (L.) Walp, is the third important grain legume in Cameroon after groundnut and dry beans (MINADER, 2012). It is a major source of cheap plant protein to most poor families, provides regular income to farmers for the sale of grain and fodder, and a good source of animal fodder. Mature cowpea seeds averagely content 23 to 25 % of protein, 50 to 67 % of starch and vitamin B (Ntoukam *et al.,* 1993), and its ability to fix atmospheric nitrogen, which allows it to grow on and improve poor soil (Muleba *et al.,* 1997). At present, this species is the second most important pulse crop (Marechal *et al.,* 1978) and in Cameroon, it is generally cultivated for its seed (shelled green or dried), pods and leaves which are consumed in fresh firm as green vegetables while snacks and mail meal dishes are prepared from the dried grain (Pando *et al.,* 2013). All the plant parts used for food are nutritious, making it extremely valuable where many people cannot afford protein foods such as meat and fish (Dabire, 2001). The seed is valued as a nutritional supplement to cereals and an extender of animal proteins (Coetzee, 1995). It is very palatable, highly nutritious and relatively free of metabolites or other toxins (Quass, 1996). The rest of the cowpea plant, after pods are harvested, is also used as a nutritious livestock fodder (Tarawali *et al.,* 1997).

The flower is hermaphroditic, open early in the morning, close before noon (Asiwe, 2009, Ige *et al.,* 2011) and it produces nectar and pollen which attract insects (Tchuenguem *et al.,* 2009, Egho, 2011, Pando *et al.,* 2013, Kwapong et al., 2013). In Cameroon, the quantity of *V. unguiculata* available to consumers is very low (153675.5 tones/years for 243070 hectares: MINADER, 2012), the demand for cowpea seeds is high (184744 tones/years: DSCE, 2009), and its pod and seed yields are low (MINADER, 2012). It is therefore important to investigate how the production of this plant could be increased in the country. The relationships between *V. unguiculata* and anthophilous insects have not been well studied in Cameroon. In other countries of sub-Sahara Africa such as Benin, Nigeria and Ghana, Pauly *et al.* (2009), Ige *et al.* (2011) and Kwapong *et al.* (2013) reported that megachile bees manipulated the flower by landing on the keel and grappling with it in the process of seeking nectar and pollen. The studies by Pando (2013) have shown that *Chalicodoma cincta cincta* visits *V. unguiculata* flowers to harvest both nectar and pollen and increases its pollination possibilities. There has been no previous research reported on the pollination efficiency of *C. cincta cincta* on *V. unguiculata* flowers in Cameroon. The size of *C. cincta cincta* (Pauly *el al.,* 2009) played a positive role in crop pollination. According to Kwapong *et al.* (2013), when collecting nectar and/or pollen, *Chalicodoma* shook flowers and this movement could facilitate the liberation of pollen by anthers, for the optimal occupation of the stigma. This bee can be managed for pollination (Roubik, 1995; Abrol, 2012). Also, Pando (2013) reported that pollen loads on the *C. cincta cincta* were observed to be abundant and the ability of this bee to disperse swiftly across large areas suggests that it is able to cross-pollinate the cowpea flowers regularly and efficiently. This current work was aimed at assessing the activity period, the foraging activity of *C. cincta cincta* foragers on *V. unguiculata* and the effects of pollination efficiency of this bee on yields. The information gained on the interaction of cowpea flowers and *C. cincta cincta* will enable farmers to develop management plans that will increase the overall quality and quantity of cowpea yield.

Materials and methods

Study site, experimental plot and biological material

The experiment was carried out twice, first from August to November 2008 and then September to December 2009 at Nkolbisson (3°51.800′N, 11°27.450′E, 726 m above sea level),Yaoundé, central Cameroon. This region belongs to the forest agroecological zone, with bimodal rainfall pattern. The climate is of the Guinea type, characterized by four seasons: a brief rainy season (March to June), a short dry season (July to August), a longer rainy season (September to November) and a more extended dry season (November to March). The annual rainfall varies from 1500 to 2000 mm. The average annual temperature is 25°C, while the mean annual relative humidity is 75%. The experimental plot was 26 x 16 m where in seeds of *V. unguiculata* purchased from the local Bafia seed outlets were planted.

Sowing and weeding

On the 21 August 2008 and 20 September 2009 the experimental plot was divided into four subplots (10 x 5 m each). The sowing was done on ten lines per subplot, each line with ten holes and in each hole, three seeds were placed. The space was 75 cm between holes and 75 cm between lines. Weeding was performed manually as necessary to maintain weed-free plots. Direct observations on flowers were made daily during 20 days of the blooming period and between 7:00 and 11:00 am (local time) since preliminary observations indicated that cowpea flowers were fully visited by *C. cincta cincta* between 6.00 and 15.00 am (unpubl. data). At least, ten specimens of *C. cincta cincta* were captured with the pliers and were conserved in a box containing 70% of ethanol for future taxonomy. Bee identification was done by Dr. Alain Pauly, Department of Entomology Royal Belgian Institute of Natural Sciences of Brussels in Belgium.

Activity of Chalicodoma cincta cincta on Vigna unguiculata flowers

Floral products harvested

The floral products (nectar or pollen) harvested by *C. cincta cincta* during each floral visit were registered based on its foraging behavior. Nectar foragers were seen extending their proboscises to the base of the corolla while pollen gatherers scratched anthers with the mandibles or the legs. During the same time that *C. cincta cincta* encounters on flowers were registered, we noted the type of floral products collected by this bee. This parameter was measured to determine whether *C. cincta cincta* was strictly gathering pollen, nectar or both, as this has implications for its efficacy as a pollinator. This could give an idea of its implication as a cross-pollinator of *V. unguiculata*.

Duration of visits and foraging speed

During the same days as for the registration of visits, the duration of the individual flower visits was recorded (using a stopwatch) at least three times: 7.00–8.00 hours, 9.00–10.00 hours, 11.00-12.00 hours and 13.00–14.00 hours. Moreover, the foraging speed, according to Jacob-Remacle (1989), is the number of flowers visited by a bee per min. According to Tchuenguem *et al.* (2004), the foraging speed could be calculated by this formula: $Vb = (Fi / di) \times 60$ where di is the time (s) given by a stopwatch and *Fi* is the number of flowers visited during *di*.

Assessment of the pollination efficiency of Chalicodoma cincta cincta on Vigna unguiculata.

To assess of the pollination efficiency of *C. cincta cincta,* for each experiment, two treatments were done: (1) Autonomous self-pollination (ASP) in which flower buds were isolated with hydrophilic bags (Figure 1) (12 × 16 cm; Osmolux®, Pantek France, Montesson) a day before anthesis to prevent anthophilous insect visitation and airborne pollen flow the following day (2008: treatments 1 and 2009: treatment 3). These bags were removed on the day following anthesis. (2) Single bee visit (SBV) in which flower buds were isolated (2008: treatment 2 and 2009: treatment 4). Between 7.00 hours and 11.00 hours of each observation date, the hydrophilic bags were delicately removed from each inflorescence carrying new opened flowers and this inflorescence observed for up to 20 min by four-man observer team was positioned in the study field. The flowers visited by *C. cincta cincta* were marked and the new opened flowers that were not visited were eliminated. Each flower was monitored until it received a single visit by *C. cincta cincta*.

Fig. 1. bagged flowers of *V. unguiculata*

Fig. 2. *C. cincta cincta* collecting on *V. unguiculata*

After a *C. cincta cincta* visit, the flower was bagged with a hydrophilic plastic bag (12 × 16 cm) until the next day to avoid any additional insect visitation (Vaissière *et al.,* 1996, Gingras *et al.,* 1999), after which the flower and the equivalent plant were also tagged. At maturity, pods were harvested from each treatment and the number of seeds per pod counted. The mean number of seeds per pod and the percentage of normal seeds (well-developed seeds) were then calculated for each treatment. The fruiting rate due to the influence of foraging *C. c. cincta* (*Fri*) was calculated by the formula:

$$Fri = \{[(Fr_Y - Fr_X) / Fr_Y] \times 100\},$$

where Fr_Y and Fr_X were the fruiting rate in treatment Y (protected flowers and visited exclusively by *C. c. cincta*) and treatment X (protected flowers).

$$Fr = [(F_2 / F_1) \times 100]$$

where F_2 is the number of pods formed and F_1 the number of viable flowers initially set. The impact of flowering *C. cincta cincta* on seed yields was evaluated using the same method as mentioned above for fruiting rate (Tchuenguem *et al.,* 2004).

Influence of neighbouring floral

During the study period, flowers of several other plant species including *Aspilia africana* (Pers.) C.D. Adams, 1956; *Mimosa pudica* (Linneaus, 1753), *Cajanus cajan* (Linnaeus) Millsp., 1900; *Senna javanica* (Linnaeus, 1753); *Phaseolus coccineus* (Linnaeus, 1753); *Ipomoea involucrata* (P. Beauv., 1817) and *Psidium guajava* (Linnaeus, 1753) in bloom in the vicinity of the experimental plot were observed to attract *C.cincta cincta.*

Data analysis

Data were analyzed using descriptive statistics, Student's *t*-test for the comparison of means of the two samples, chi-square (χ^2) for the comparison of two percentages using SPSS statistical software (version 19.0; SPSS, Inc. Chicago, Illinois, USA) and Microsoft Excel 2010.

Results

Activity of Chalicodoma cincta cincta on Vigna unguiculata flowers

From this study's field observations, *C. cincta cincta* foragers were found to collect nectar and pollen on *V. unguiculata* flowers (Fig. 2). Nectar and pollen simultaneous collection was intensive and regular (more than 61.88 % of visits each year). Other individuals would collect either nectar or pollen only whereas nectar (32.77 %) or pollen (05.35 %) collection only was very low (Table 1). The activity of the megachile bee observed foraging on cowpea flowers was mainly for pollen and nectar gathering base to their foraging behaviour.

Table 1. Products harvested by *Chalicodoma cincta cincta* on the inflorescences of *Vigna unguiculata* in 2008 and 2009

Year	Number of visits studies	Visits for nectar harvest		Visits for pollen harvest		Visits for nectar and pollen harvest	
		Number	%	Nombre	%	Nombre	%
2008	239	102	42.68	13	05.44	124	51.88
2009	527	149	28.27	28	05.31	350	66.42
total	**766**	**251**	**32.77**	**41**	**05.35**	**474**	**61.88**

The mean duration of *C. c. cincta* visits per *V. unguiculata* flower varied significantly according to the type of food harvested. In 2008, the mean duration of a visit was 8.893 s (n = 95; s = 1.07), with a maximum of 16 s for nectar harvest, against 4.27 s (n = 95; s = 1.22), with a maximum of 9 s for pollen collection. In 2009, the corresponding figures were 8.76 s (n = 160; s = 2.70), with a maximum of 17 s for nectar, against 4.14 s (n = 160; s = 1.42), with a maximum of 8 s for pollen harvest. The difference between the duration of the visit to harvest nectar and pollen is highly significant (t_{2008} = -17.36 [df = 253, P < 0.001]; t_{2009} = -19.12 [df = 253, P < 0.001]). Furthermore, the difference between the duration of the visit to harvest nectar in 2008 and 2009 is not significant (t = 0.52 [df = 253; P > 0.05]), whereas the difference between the duration of visit for pollen in 2008 and 2009 is highly significant (t = 2.37 [df = 253; P < 0.01]).

On the experimental plot of *V. unguiculata, C. cincta cinca* visited between three and 19 flowers/min in 2008 and between three and 20 flowers/min in 2009. The mean foraging speed was 9.92 flowers/min (n = 95; s = 3.03) in 2008 and 11.71 flowers/min (n = 113; s = 3.46) in 2009, which was significantly different (t = -3.93 [df = 206; P < 0.001]).

During the observation period, flowers of many other plant species growing near *V. unguiculata* were visited by *C. cincta cincta* for nectar (N) and/or pollen (P). Among these plants were *Aspilia africana* (Asteraceae; N and P), *Mimosa pudica* (Mimosaceae, N and P), *Cajanus cajan*, *Senna javanica* and *Phaseolus coccineus* (Fabaceae; N and P) *Ipomoea involucrata* (Convovulaceae, N) and *Psidium guajava* (Myrtaceae; P). During one foraging trip, an individual bees foraging on *V. unguicuilata* was not observed moving from *V. unguiculata* to the neighbouring plant and vice versa.

Pollination efficiency of Chalicodoma cincta cincta on Vigna unguiculata

During the collection of pollen or nectar on flowers, foragers regularly contacted anthers, stigma and carried pollen. With this pollen, they flew frequently from flower to flower. The percentage of the total number of visits during which forager bees came into contact with the stigma of the visited flowers was 100.00 % for pollen harvest and 92.83 % for nectar harvest (Table 2). Thus *C. cincta cincta* greatly increased the pollination possibilities of *V. unguiculata* flowers. Table 3 shows the fruiting rate, the mean number of seeds per pod and the percentage of normal seed in treatments 1 and 3 (ASP) and treatments 2 and 4 (SBV). It appeared from this table that each flower turned into a pod, regardless of the treatment it received.

Table 2. Number and frequency of contacts between *Chalicodoma cincta cincta* and the stigma during floral visits to *Vigna unguiculata*

Products harvested	November 2008			December 2009			Total		
	Number of studied visits	Visits with stigmatic contacts		Number of studied visits	Visits with stigmatic contacts		Number of studied visits	Visits with stigmatic contacts	
		Number	%		Number	%		Number	%
Nectar	226	2O1	88.94	499	472	94.59	725	673	92.83
Pollen	137	137	100	378	378	100	515	515	100

- The fruiting rate ranged from 69.00 % in treatment 1 to 94.00 % in treatment 2 in 2008 and from 57.00 % in treatment 3 to 90.00 % in treatment 4 in 2009. The comparison of the fruiting rate showed that the differences observed were highly significant between treatments 1 and 2 (χ^2 = 20.73 [df = 1; P < 0.001]), treatments 3 and 4 (χ^2 = 30.04 [df = 1; P < 0.001]) and not significant between treatments 2 and 4 (χ^2 = 0.65 [df = 1; P > 0.05]). Consequently, the fruiting rate of flowers bagged and visited exclusively by *C. cincta cincta* (treatments 2 and 4) was higher than that of flowers bagged during their flowering period (treatments 1 and 3). The percentage of the fruiting rate due to *C. cincta cincta* activity was 31.98 %.

- The mean numbers of seeds per pod were 13.35, 20.33, 14.47 and 19.87 in treatments 1, 2, 3 and 4 respectively. The difference was significant between treatments 1 and 2 (t = 25.81 [df = 161; P < 0.001]), 3 and 4 (t = 31.45 [df = 146; P < 0.001]). Consequently, the number of seed yields per pod of flowers bagged and visited exclusively by *C. cincta cincta* (treatments 2 and 4) was higher than that of flowers bagged during their flowering period (treatments 1 and 3). The contribution of *C. cincta cincta* to the increment of the number of seeds per pod was 30.76 %.

- The percentage of normal seed was 81.54 %, 98.06 %, 84.97 % and 98.51 % in treatments 1, 2, 3 and 4 respectively. The difference between treatments 1 and 2 (χ^2 = 250.40 [df = 1; P < 0.001]), and treatments 3 and 4 (χ^2 = 192.03 [df = 1; P < 0.001]) was highly significant and was not significant between treatments 2 and 4 (χ^2 = 1.08 [df = 1; P > 0.05]). Consequently, the percentage of normal seeds of floral access to only *C. cincta cincta* (treatments 2 and 4) was higher than that of flowers bagged during their opening period (treatments 1 and 3). This may show high pollination deficit on the crop, indicating need for *C. cincta cincta* management to increase developed seeds. The contribution of *C. cincta cincta* to the increment of the percentage of normal seed was 15.27 %.

Discussion

Our study indicates that *C. cincta cincta* was visited cowpea flowers to collected nectar or/and pollen. The attractiveness of *V. unguiculata* nectar could be partially explained by its reported high production and total sugar concentration (34.13–54.27 %: Tchuenguem *et al.,* 2009), compared to range 15–75% in which most of the plant species fall (Proctor *et al.,* 1996). According to Asiwe (2009) and Egho (2011) in Nigeria; Pauly *et al.* (2009) in Bénin; Kwapong *et al.* (2013) in Ghana; Tchuenguem *et al.* (2009) and Pando *et al.* (2013) in Cameroon, nectar produced by *V. unguiculata* attracts various insects in natural conditions.

The significant difference observed between the duration of pollen harvest visits and that of nectar collection visits could be explained by the accessibility of each of these floral products. Floral morphology of this crop ensures high protection of the nectary such that the keel is forcefully opened and this ensures tripping of the flowers, resulting to pollen release. Under these conditions an individual bee must spend much more time on flowers to obtain its nectar load, compared to the time she needs for pollen load. This result confirms other findings reported by Pando *et al.* (2011) on *Cajanus cajan* in Cameroon. The present study shows that during one foraging trip, an individual bee foraging on a given plant species scarcely visited another plant species. This result indicates that *C. cincta cincta* shows flower constancy (Basualdo *et al.,* 2000) for the flowers of each of the plant studied. *Chalicodoma cincta cincta* has been previously reported as good constancy visitor as such; effective pollinators (Pauly *et al.,* 2009, Abrol, 2012, Kwapong *et al.,* 2013, Pando *et al.,* 2013). Flower constancy is an important aspect in management of pollination and this shows *C. cincta cincta* can provide the advantages of pollination management for *V. unguiculata*. Investment in *C. cincta cincta* management may provide high returns to investment on this crop. During the collection of nectar and pollen on each flower, *C. cincta cincta* regularly comes into contact with the stigma. It could enhance auto-pollination, which has been demonstrated in the past (Pauly *et al.,* 2009, Ige *et al.,* 2011). *Chalicodoma cincta cincta* would provide allogamous pollination through carrying of pollen within their furs, legs and mouth accessories, which is consequently deposited on another flower belonging to different plant of same species. Pauly *et al.* (2009), Egho (2011) and Pando *et al.* (2011) have also been observed by other studies in Benin, Nigeria and Cameroon respectively.

The positive and significant contribution of *C. cincta cincta* in the pod and seeds yields of *V. unguiculata* is justified by the action of this forager bee on self-pollination and cross-pollination. During foraging behaviour on flowers of *V. unguiculata*, *C. cincta cincta* played a positive role: when collecting nectar and/or pollen, *C. cincta cincta* shook flowers and this movement could facilitate the liberation of pollen by anthers, for the optimal occupation of the stigma. Nevertheless, the morphology of *V. unguiculata* flowers seems to avoid auto-pollination and seems to favour cross-pollination (Ige *et al.,* 2011, Pando et *al.,* 2013). Pollen grains of cowpea are heavy and sticky and could not be readily transferred by wind, therefore insects are responsible for transfer of pollen grains and consequently cross pollination in cowpea plant. *Chalicodoma cincta cincta* has been previously reported as good pollen collectors and as such; effective pollinators (Pauly *et al.,* 2009, Abrol, 2O12, Kwapong *et al.,* 2013). During our investigations, the falling of pollen carried by the foragers and the deposition of this pollen on the stigma and stamens of the flowers to be visited by the action of gravity and that of wind have been observed. Such pollen losses by bees are frequent at the end of single flower or inflorescence visits, especially during the hovering flight of foragers above these organs. The flowers that were exposed exclusively to *C. cincta cincta* provided more pods, more seeds per pod with the heavier seeds and of better shape than the bagged flowers, in agreement to previous results reported on *Cajanus cajan* (Pando *et al.,* 2011, Abrol, 2012). The higher productivity of floral access to only *C. cincta cincta* visits compared with bagged flowers explains that insects' visits were effective in increasing cross-pollination. Our results confirm those of Tchuenguem *et al.* (2009) in Ngaoundéré-Cameroon and Ige *et al.* (2011) in Nigeria that *V. unguiculata* flowers set few pods in the absence of insect pollinators (Table 3). Abrol (2012) and Pando *et al.* (2013) showed that self-pollination of *V. unguiculata* flowers produced fewer set pods than cross-pollination.

Table 3. Fruiting rate, mean number of seeds yield per pod and percentage of normal seeds according to the treatments of *Vigna unguiculata*

Year	Treatment	Flowers	Pods	Fruiting rate	Seeds/pod		Total seeds	Normal seeds	% Normal seeds
					Mean	SD			
2008	1 (Bagged flowers)	100	69	69.00	13.35	3.16	921	751	81.54
	*2 (*Floral access to only *C. cincta cincta*)	100	94	94.00	20.33	1.29	1911	1874	98.06
2009	3 (Bagged flowers)	100	57	57.00	14.47	2.71	825	701	84.97
	4 (Floral access to only *C. cincta cincta*)	100	91	90.00	19.87	1.38	1808	1781	98.51

Conclusion

In Yaoundé-Cameroon, *V. unguiculata* benefits highly from pollination by *C. cincta cincta*. The comparison of pods and seeds set of protected inflorescences with that of inflorescences visited exclusively by *C. cincta cincta* underscores the value of this bee in increasing pod and seed set as well as seed quality. The study thus shows investment in management of *C. cincta cincta* in terms of nest provision at the proximity of *V. unguiculata* field is worthy while for growers. The conservation and/or the kept of *C. cincta cincta* nest at the proximity of *V. unguiculata* plots should be recommended for Cameroonian farmers and horticulturists to increase pods and seeds yields. Cowpea should to be cultured more in Cameroon to contribute to the food supply and to favor populations of *C. cincta cincta*.

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