

Pollination and yield responses of pigeon pea (*Cajanus cajan* L. Mill sp.) to the foraging activity of *Chalicodoma cincta cincta* (Hymenoptera: Megachilidae) in Yaoundé (Cameroon)

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1 SUMMARY

To assess the impact of the Megachile bee, Chalicodoma cincta cincta on pod and seed set of Cajanus cajan L. Mill sp, its foraging and pollinating activities were studied in Yaoundé, for two seasons (September - October 2008 and July - August 2009). Observations were made on 80 inflorescences per treatment. The treatments included unlimited floral access by all visitors, bagged flowers to deny all visits, and, limited visits by *C. c. cincta* only. In addition, all flower visitors were recorded. The Megachile bee's seasonal rhythm of activity, its foraging behaviour on flowers, its pollination efficiency, the fruiting rate, the number of seeds per pod were recorded. Individuals from 18 species of insects were recorded visiting flowers of C. cajan in the two years. Chalicodoma cincta cincta was the most frequent, followed by Xylocopa calens, Apis mellifera adansonii, Megachile bituberculata, Camponotus flavomarginatus and Xylocopa albiceps. Apart from bees, wasps were also recorded as likely predators. Chalicodoma cincta cincta mainly foraged for nectar and pollen resource. The mean foraging speed was 10.33 flowers/min. Flowers visited by C. c. cincta had higher fruiting rate compared with the others while those bagged had the lowest. In addition, seed formation was higher in C. c. cincta visited flowers compared with all others. The results show that this crop experiences pollination deficit even under normal circumstances, considering that flowers visited by C. c. cincta had higher yields compared with those under unlimited access by all visitors. The fruiting rate, the number of seeds/ pod and the percentage of normal seeds of unprotected inflorescences were significantly higher than those of inflorescences protected from insects. Chalicodoma cincta cincta foraging resulted to a significant increment of the fruiting rate by 19.65 %, as well as the number of seeds/per pod by 24.33 % and the percentage of normal seeds by 11.52 %. Conservation of C. c. cincta nests close to C. cajan fields could be recommended to improve pod and seeds production in the region.

2 INTRODUCTION

Pigeon pea, *Cajanus cajan* L. Mill sp is one of the major grain legume crops grown in the tropics and subtropics (Saxena et al., 2002). Most of

the evidence points to India as the place where pigeon pea originated because of the presence of several wild relatives, the large diversity of



the crop gene pool, ample linguistic evidence, a few archaeological remains, and the wide usage in daily cuisine (Van der Maesen, 1990). Cajanus cajan offers many benefits to subsistence farmers as a food and cash crop and also ensures stable crop yields in times of drought (Nene and Sheila, 1990). As a food source, C. cajan offers a cheap source of valuable protein to people. Its protein content averages 21%, although some high-protein lines are being bred with up to 30% protein (Gupta et al., 2001; Saxena et al., 2002). It has more minerals, ten times more fat, five times more vitamin A, and three times more vitamin C than ordinary peas 1995). The World (Madeley, Health Organization (WHO) recommends 0.75 g of protein daily for each kg of body weight to meet the needs of most of the general world population (Shils et al., 1994; Garrison and Somer, 1995).

Cajanus cajan, as a legume, improves soil fertility through biological nitrogen fixation. It is reported to contribute approximately 40 kg N ha⁻¹ (Kumar Rao et al., 1990). Leaf fall at maturity not only adds to the organic matter in the soil, but also provides additional nitrogen. This also benefits subsequent cereal crops when grown in a mixture with maize and sorghum (Yun et al., 2001; Arya et al., 2002). The deep root system of pigeon pea is reported to break plough pans, thus improving the soil structure (Nene and Sheila, 1990). The extensive ground cover provided by C. cajan prevents soil erosion by wind and water, encourages infiltration, minimizes sedimentation, and smothers weeds (Nene and Sheila, 1990). Cajanus cajan has many traditional medicinal uses. Dry roots, leaves, flowers, and seeds are used in different countries to treat a wide range of ailments of the skin, liver, lungs, and kidney (Nene and Sheila, 1990). The roots are used to treat febrile diseases and relieve fever, constrict tissue to controlling bleeding, and destroy internal worms. The leaves can be used to treat jaundice, trauma, cough, burn infection, and bedsores, (Shiving et al., 2001).

In Cameroon, very little information exists on the relationships between flowering insects and many plants species. Nevertheless, it is known that generally anthophilous insects and bees in particular usually increase the fruit and seed yields of many plants species, through pollinisation provision (Fluri and Frick, 2005; Sabbahi et al., 2005; Klein et al., 2007). Cajanus cajan flowers have bright corollae and produce nectar and pollen. These traits suggest that C. cajan would be attractive and possibly be pollinated by bees (Grewal et al., 1990; Saxena et. al., 1990; Reddy et al., 2004; Sarah et al., 2010). The pollen and nectar in its flowers are, however, also accessible to insects other than bees, requiring the separation of pollinators from other visitors. Cajanus cajan is one of many plants for which information on insect pollination in Africa, particularly in Cameroon are still lacking. Then, it is a greatest necessity to carry out further researches in the insect pollination of this crop plant to provide new baseline information in this country. In Cameroon, C. cajan is cultivated as a vegetable and can be consumed raw or cooked. Its pods are sold when fresh (green beans) and seeds can be transformed into flour while its stems and leaves are used as livestock feed. Moreover, the demand for C. cajan pods and seeds is higher but its yields are very low. It is therefore important to investigate on how the production of this plant could be increased in Cameroon to satisfy the demand of the consumer. There has been no previous research report on the relationship of C. cajan and its anthophilous insects. This study carried out to assess the effects of

foraging behavior of C. c. cincta on yields of C. cajan. Chalicodoma cincta cincta is one of the Megachile bee in Cameroon. During preliminary investigations on flower-insect relationships in Yaoundé before 2008 (unpublished data), C. c. cincta have been seeing visiting flowers of C. cajan intensively. This bee can be managed for pollination.



3 MATERIALS AND METHODS

3.1 Study site, experimental plot and biological material: The experiment was carried out twice, first on April to December 2008 and then March to September 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 agro ecological 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 (26×16) m where seeds of C. cajan purchased from the local seed outlets were planted. Two Kenya Top Bar Hives (KTBH) with Apis mellifera adansonii Latreille (Hymenoptera: Apidae) colonies were installed close to the experimental plot. The bee C. c. cincta digs its nest in the trunks of the trees and floor in the natural conditions. The vegetation near C. cajan field had various spontaneous and cultivated species

3.2 Sowing and weeding: On the 4 April 2008 the experimental plot was divided into four subplots each (10×5) m. The sowing was done on five lines per subplot, each line with 10 holes and on each hole 2 seeds were placed. The space was 1 m between holes and 1 m between lines. Weeding was manually performed as necessary to maintain plots weeds free.

3.3 Determination of the reproduction system of C. cajan: On 30th August 2008, 200 inflorescences of C. cajan at the bud stage were labeled among which 100 inflorescences (3188 flowers) were left un-attended and 100 inflorescences (3154 flowers) bagged to prevent visitors. On 10th July 2009, 200 inflorescences of C. cajan with flowers at the bud stage were labeled among which 100 inflorescences (3313 flowers) left for unlimited visits and 100 inflorescences (3118 flowers) were bagged.

Ten days after shedding of the last flower of the labeled inflorescences, the number of pods was assessed in each treatment. The podding index was then calculated as described by Tchuenguem et al. (2001): Pi = F2/F1 Where F2 is the number of pods formed and F1 the number of viable flowers initially set.

The allogamy rate (Alr) from which derives the

autogamy rate (Atr) was expressed as the difference in podding indexes between treatment X (unprotected flowers) and treatment Y (bagged flowers) (Demarly, 1977).

 $Alr = [(PiX - PiY) / PiX] \times 100$ where PiX and PiY are respectively the podding average indexes of treatment X and treatment Y. Atr = 100 – Alr.

Estimation of the frequency of C. c. 3.4 cincta in the flowers of C. cajan: The frequency of C. cincta in the flowers of C. cajan was determined based on observations on flowers of treatment 1 and treatment 3, every two days per three, from 2nd September to 17th October 2008 and from 13th July to 26th August 2009, at 7 - 8h, 9 - 10h, 11 - 12h, 13 - 14h and 15 - 16h. Flowers typically were completely opened at 7 and closed before 16h. In a slow walk along all labeled inflorescences of treatment 1 and treatment 3, the identity of all insects that visited C. cajan was recorded. Specimens of all insect taxa were caught with an insect net on unlabeled inflorescences, for each species 3 - 5 specimens were captured. These specimens were conserved in 70% ethanol for subsequent taxonomy determination. All insects encountered on flowers were registered and the cumulated results expressed in number of visits to determine the relative frequency of C. c. cincta in the anthophilous entomofauna of C. cajan.

In addition to the determination of the floral insects frequency, direct observation of the foraging activity on flowers were made on insect pollinator fauna in the experimental field. The floral products (nectar or pollen) harvested by C. c. cincta during each floral visit were registered based on its foraging behaviour. 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. c. cincta encountered on flowers were registered, the types of floral products collected by this bee were noted. This parameter was measured to determine if C. c. cincta is strictly a pollenivore, nectarivore or pollenivore and nectarivore. This could give an idea on its implication as a cross pollinator of C. cajan. In the morning of each sampling day, the number of opened flowers carried by each labeled inflorescence was counted.

During the same days as for the frequency of visits, the duration of the individual flower visits was recorded (using a stopwatch) at least three times: 8 -



9h, 10 - 11h, 12 -13h and 14 - 15h.

Moreover, the number of pollinating visits (the bee came into contact with the stigma), the abundance of foragers (highest number of individuals foraging simultaneously on a flower or on 1000 flowers: Tchuenguem et al., 2004) and the foraging speed, according to Jacob-Remacle (1989) is number of flower visited by a bee per min. According to Tchuenguem (2005), the foraging speed could be calculated by this formula: Vb = (Fi/di) x 60 where di is the time (s) given by a stopwatch and Fi is the number of flowers visited during di. The disruption of the activity of foragers by competitors or predators and the attractiveness exerted by other plant species on *C. c. cincta* was assessed.

During each daily period of observations, the temperature and relative humidity at the station was registered using a mobile thermo-hygrometer every 30 minutes.

3.5 Evaluation of the impact of C. c. cincta and other insects on C. cajan yields: This evaluation was based on the impact of insect visiting flowers on pollination, the impact of pollination on fructification of C. cajan, and the comparison of yields (fruiting rate, mean number of seed per pod and percentage of normal seeds) of treatment X (unprotected flowers) and treatment Y (bagged flowers). The fruiting rate due to the influence of foraging insects (Fri) was calculated by the formula: $Fri = \{[(FrX-FrY) / FrX] \ge 100\}$ where FrX and FrY were the fruiting rate in treatment X and treatment Y. The fruiting rate of a treatment (Fr) is $Fr = [(F2/F1) \times 100]$ where F2 is the number of pods formed and F1 the number of viable flowers initially set. 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

4 **RESULTS**

4.1 Reproduction system of pigeon pea: The podding index of *C. cajan* was 0.88, 0.81, 0.91 and 0.77, respectively for treatment 1, treatment 2, treatment 3 and treatment 4. Thus, in 2008 allogamy rate was 07.95 % and autogamy rate was 92.05 %; where in 2009, allogamy rate was 15.39 % and autogamy rate was 84.61 %. It appears that the variety of *C. cajan* used in these experiments had a mixed reproduction regime with the predominance of autogamy over allogamy. developed seeds) were then calculated for each treatment. The impact of flowering insects on seed yields was evaluated using the same method as mentioned above for fruiting rate (Tchuenguem et al., 2004).

3.6 Assessment of the pollination efficiency of *C. c. cincta* on *C. cajan:* To assess of the pollination efficiency of *C. c. cincta*, 80 inflorescences were bagged (treatment 5) in 2008 and 80 inflorescences were bagged (treatment 6) in 2009. Between 9 am and 11 am of each observation date, the gauze bag was delicately removed from each inflorescence carrying new opened flowers and this inflorescence observed for up to 20 minutes. The flowers visited by *C. c. cincta* were marked and the new opened flowers that were not visited were eliminated. The inflorescence was protected once more.

The contribution (Frx) of *C. c. cincta* in the fruiting was calculated by the formula: $Frx = \{[(FrZ-FrY) / FrZ] x 100\}$ where FrZ and FrY were the fruiting rate in treatment Z (bagged flowers and visited exclusively by *C. c. cincta*) and treatment Y (bagged flowers). At the maturity, pods were harvested from treatment 5 and treatment 6 and the number of seeds per pod counted. The mean number of seeds per pod and the percentage of normal seeds were then calculated for each treatment. The impact of *C. c. cincta* on seed yields was evaluated using the same method as mentioned above for fruiting rate (Tchuenguem et al., 2004).

3.7 Data analysis: Data were analyzed using descriptive statistics, student's t-test for the comparison of means of the two samples, correlation coefficient (r) for the study of the association between two variables, chi-square (X²) for the comparison of two percentages using SPSS statistical software and Microsoft Excel

4.2 Frequency of *C. c. cincta* in the floral entomofauna of *C. cajan:* Amongst the 5531 and 6917 visits of 13 and 17 insect species recorded on *C. cajan* flower in 2008 and 2009, respectively, *C. c. cincta* was the most represented insect with 1621 visits (29.30 %) and 1984 visits (28.68 %), in 2008 and 2009 respectively (Table1). The difference between these two percentages is not significant (X² = 0.58; df = 1; P > 0.05).



	Inse	cts	2008		2009	
Order	Family	Genre, specie, sub-specie	<i>n</i> ₁	<i>p</i> ₁ (%)	<i>n</i> ₂	$p_2(\%)$
Hymenoptera	Apidae	Xylocopa calens **	1182	21,37	1571	22,71
	-	Xylocopa torrida **	187	03,38	169	02,44
		Xylocopa albiceps **	262	04,74	263	03,81
		Xylocopa nigrita **	83	01,50	82	01,19
		Apis mellifera adansonii **	650	11,75	766	11,07
		Dactylurina staudingeri **	293	05,30	112	01,62
		Meliponula erythra **			176	02,54
		(1 sp.) *			36	00,52
	Megachilidae	idae Chalicodoma cincta cincu **		29,30	1984	28,68
		Chalicodoma rufipes **			270	03,91
		Chalicodoma torrida **			160	02,31
		Chalicodoma rufipennis **			58	00,84
		Megachile bituberculata **	541	09,78	645	09,32
	Halictidae	Crocisaspidia chandlerie **			201	02,91
	Formicidae	Camponotus flavomarginatus *	478	08,64	253	03,66
	Sphecidae	Philanthus Triangulum ***	96	01,74	35	00,51
	Xymonidae	Synagris cornuta $\overset{_{\overline{*}}}{*}$	85	01,54	136	01,96
	Vespidae	Belonogaster juncea juncea *	53	00,96		
Total		- · · ·	5531	100,00	6917	100,00

Table 1: Diversity of floral insects on *C. cajan* inflorescences in 2008 and 2009, number and percentage of visits of different insects

 $\mathbf{n_1}$: number of visits on 100 inflorescences in 31 days ; $\mathbf{n_2}$: number of visits on 100 inflorescences in 31 days $\mathbf{p_1}$ et $\mathbf{p_2}$: visits percentages; $\mathbf{p_1} = (\mathbf{n_1} / 5531) \times 100$; $\mathbf{p_2} = (\mathbf{n_2} / 6917) \times 100$; comparison of visit percentage of *Chalicodoma cincta cincta* for two years: $X^2 = 0.58$; P > 0.05 (* Visitor collected nectar, ** Visitor collected nectar and

pollen, ***Visitor collected nectar or predation, 1 sp: Undetermined species)

In 2008, the highest mean number of *C. c. cincta* simultaneous in activity was 1 per flower (n = 50; s = 0), 1.81 per inflorescence (n = 100; s = 0.66; maxi = 4) and 181.22 per 1000 flowers (n = 96; s = 63.55; maxi = 334). In 2009, the corresponding numbers were 1 (n = 50; s = 0), 1.99 (n =100; s = 0.88; maxi = 4) and 186.14 (n = 96; s = 60.21; maxi = 353). The difference between the mean number of foragers per 1000 flowers in 2008 and 2009 is not significant (t = -0.55; df = 190; P = 0.58).

In fact, flowers of *C. cajan* were visited by seven families: Apidae (46.85 %), Megachilidae (42.41 %), Formicidae (05.87 %), Xymonidae (1.77 %), Halictidae (1.62 %), Sphecidae (1.05 %) and Vespidae (0.40 %). The most frequent species within the family Megachilidae were the bees *Chalicodoma cincta cincta* (28.99 %), *Megachile bituberculata* (9.55 %), *Chalicodoma rufipes* (3.91 %) and *Chalicodoma torrida* (2.31 %). The most frequent species within the family Apidae were the bees *Xylocopa calens* (22.04 %), *Apis mellifera adansonii* (11.41 %), *Xylocopa albiceps* (4.28 %), *Dactylurina* staudingeri (3.46 %), Xylocopa torrida (2.91 %), Meliponula erythra (2.54 %) and Xylocopa nigrita (1.35 %). All Megachilidae and Apidae visiting *C. cajan* flowers collected nectar and pollen only 1 sp. harvested nectar. *Camponotus flavomarginatus* (Formicidae), *Synagris cornuta* (Xymonidae), and *Belonogaster juncea juncea* (Vespidae) collected nectar. *Philanthus Triangulum* (Sphecidae) collected nectar or captured honey bee and *Dactylurina staudingeri*. An unlimited visit implies that all this diversity of visitors was present.

4.3 Activity of *C. c. cincta* on *C. cajan* flowers

4.3.1 Floral products harvested: From this study's field observations, *C. c. cincta* foragers were found to collect nectar and pollen on *C. cajan* flowers. Nectar and pollen simultaneous collection was intensive and regular (more than 54.45 % of visits each year). Other individuals would collect either nectar or pollen only whereas nectar (42.06%) or pollen (3.50%) collection only was very low (Table 2).



Voor	Number of visite Visite for motion Visite for Visite for							
Tear	Inumber of visits		visits for nectar		VISIUS IOF		visits for flectar and	
	studies	harvest		pollen harvest		pollen harvest		
		number	%	number	%	number	%	
2008	1621	607	37.45	83	05.12	931	57.43	
2009	1984	926	46.67	37	01.87	1021	51.46	

Table 2: Products harvested by C. c. cincta on the inflorescences of C. cajan in 2008 and 2009

4.3.2 Rhythm of visits according to the flowering stages: Visits were most numerous when the number of open flowers was highest (Figure 1). Furthermore, a positive and significant correlation

was found between the number of *C. cajan* opened flowers and the number of *C. c. cincta* visits in 2008 (r = 0.71; df = 29; P < 0.001) as well as in 2009 (r = 0.63; df = 29; P < 0.001).



Figure 1: Variations of the number of *C. cajan* opened flowers and the number of *C. c. cincta* in function of days of observation in 2008 and 2009

4.3.3 Daily rhythm of visits: Chalicodoma cincta cincta foraged on C. cajan flowers throughout the blooming period, with a peak of activity situated between 9 and 10 am daily (Table 3). Climatic conditions influenced the activity of C. c. cincta. The correlation was negative and significant ($r_{2008} = -$ 0.48; df = 155; P < 0.01 and r_{2009} = -0.19; df = 155; P = 0.02) between the number of C. c. cincta visits on C. cajan flowers and the temperature. It was positive and significant ($r_{2008} = 0.58$; df = 155; P < 0.01 and $r_{2009} = 0.42$; df = 155; P < 0.01) between the number of visits and relative humidity. Duration of visits per flower: In 2008 4.3.4 and 2009, the mean duration of C. c. cincta visit was 10.08 sec (n = 160; s = 5.59; max = 32 sec) and

7.64 sec (n = 160; s = 1.83; max = 18) for nectar harvest respectively. The difference between the duration of the visit to harvest nectar in 2008 and 2009 is higher significant (t = 4.10; df = 318; P < 0.001). For pollen, the corresponding number were 5.58 sec (n = 160; s = 2.44; max = 15 sec) and 3.19 sec (n = 160; s = 1.01; max = 5 sec) in 2008 and 2009 respectively. The difference between duration of visit for pollen in 2008 and 2009 is significant (t = 8.12; df = 318; P < 0.001). The mean duration of *C*. *c. cincta* visits per *C. cajan* flower varied significantly according to the type of food harvested ($t_{2008} = -9.33$; df = 318; P < 0.001 and $t_{2009} = -26.91$; df = 318; P < 0.001).



Year	Parameter registered	Daily period						
		7 - 8 h	9 - 10 h	11 - 12 h	13 - 14 h	15 - 16 h		
2008	Number of visits	298	702	412	169	39		
	Temperature (°C)	23.45	25.13	26.94	28.82	28.89		
	Hygrometry (%)	82.64	77.98	71.63	64.23	59.74		
2009	Number of visits	283	854	547	249	51		
	Temperature (°C)	22.92	25.98	29.19	30.16	28.97		
	Hygrometry (%)	80.55	75.76	70.48	65.05	60.18		

Table 3: Daily distribution of *C. c. cincta* visits on 100 and 100 *C. cajan* inflorescences over 31 days in 2008 and 31 days 2009 respective, mean temperature and mean humidity of study site

Temperature and hygrometry, each figure represents the means of two observations per hourly period and per day, within 31 days.

4.4 Foraging speed of *C. c. cincta* on *C. cajan* flowers: On the experimental plot of *C. cajan*, *C. c. cincta* visited between 2 and 20 flowers/min in 2008 and between 3 and 20 flowers/min in 2009. The mean foraging speed was 10.76 flowers/min (n = 159; s = 4.33) in 2008 and 9.89 flowers/min (n = 160; s = 4.71) in 2009, which is not significantly different (t = 1.7; df = 317; P = 0.08).

4.5 Influence of neighboring floral and foraging ecology: During the observation period, flowers of many others plant species growing near C. cajan were visited by C. c. cincta, for nectar (n) and/or pollen (p). Amongst these plants were Chromolaena odorata (Asteraceae; n and p), Thithonia diversiforia (Asteraceae; n and p), Mimosa pudica (Fabaceae; p), Manguifera indica (Anacardiaceae; n and p), Citrullus lanatus (Cucurbitaceae; n and p) and Dacryodes edulis (Bursaceae; n and p). During one foraging trip, an individual bee foraging on C. cajan was not observed moving from C. cajan to the neighbouring plant and vice versa. Foragers of C. c. cincta were regularly interrupted by other foragers or by other bees collecting C. cajan floral products such as Xylocopa torrida (n and p) Xylocopa calens (n and p), Xylocopa albiceps (n and p) and Megachile bituberculata (n and p) or C. c. cincta predator such as Mantis religiosa.

4.6 Impact of insect activity on pollination and pollination efficiency of *C. c. cincta* on yield of *C. cajan:* During nectar and/or pollen harvest on *C. cajan,* foraging insects always shook flowers and regularly contacted anthers and stigma, increasing cross pollination possibility of *C. cajan.* The comparison of the fruiting rate (table 4) showed the differences observed were highly significant between treatments 1 and 2 ($X^2 = 62.01$; df = 1; P < 0.001) and treatments 3 and 4 ($X^2 =$ 241.5; df = 1; P < 0.001). The difference between treatments 1 and 3 was significantly ($X^2 = 19.21$; df = 1; P < 0.01). On all visited flowers, C. c. cincta contacted anthers and carried pollen. With this pollen, the Megachile bee flew frequently from flower to flowers of same species. Chalicodoma cincta cincta came into contact with visited flowers during 100% of visits. Thus this bee highly increased the pollination possibilities of C. cajan flowers. The comparison of the fruiting rate (table 4) showed that the differences observed were highly significant between treatments 2 and 5 ($X^2 = 13.44$; df = 1; P < 0.001) and treatments 4 and 6 ($X^2 =$ 21.86; df = 1; P < 0.001). The difference between the treatments 5 and 6 was not significant (X^2 = 0.10; df = 1; P > 0.05). Table 4, documented the high fruiting rate or pod formation during unlimited visits (where high diversity of insects were observed) compared with bagged flowers. The percentages of the fruiting rate attributed to the insects were 08.19 % in 2008 and 15.45 % in 2009. For all of the inflorescences studied, the percentage of the fruiting rate attributed to the influence of insects was 11.82 %. Also the highest fruiting on C. c. cincta visited flowers than even the unlimited visits flowers. This suggests high pollination deficit in the crop, indicating need for C. c. cincta management to increase fruiting. The percentage of the fruiting rate due to C. c. cincta activity was 19.55 %;



Treatments	Years	Flowers	pods	Fruiting	Seeds/pod		Total	Normal	% normal	
				rate			seeds	seeds	seeds	
					mean	sd				
1 (unlimited visits)	2008	3188	2801	87.86	5.93	0.72	15271	14503	94.97	
2 (bagged flowers)	2008	3154	2544	80.66	4.96	0.65	11707	10191	87.05	
3 (unlimited visits)	2009	3313	3021	91.19	5.99	0.59	16818	15971	94.96	
4 (bagged flowers)	2009	3118	2404	77.10	4.75	0.72	11211	9653	86.10	
5 (C. c. cincta flowers)	2008	67	66	98.51	6.44	0.50	425	413	96.94	
6 (C. c. cincta flowers)	2009	91	89	97.80	6.39	0.59	569	562	98.77	

Table 4: Fruiting rate, mean number of seeds yield per pod and percentage of normal seeds according to the treatments of *C. cajan*.

There were a high mean number of seeds per pod in unlimited visits compared with bagged flowers. The comparison of the mean number of seeds per pod has shown that the difference observed was highly significant between treatments 1 and 2 (t =22.39; P < 0.01) and treatments 3 and 4 (t = 29.66; P< 0.001). The percentage of the number seeds per pod due to the action of insects was 16.36 % in 2008 and 20.70 % in 2009. For all of the inflorescences studied, the percentage of the seeds production attributed to the influence of insects was 18.53 %.

Seeds per pod were also highest in *C. c. cincta* and lowest in bagged, showing though *C. cajan* has high autogamous tendency, allogamy would increase yields significantly higher. The comparison of the mean number of seeds per pod has shown that the differences observed were highly significant between treatments 2 and 5 (t = -17.61; df = 564; P < 0.001) and treatments 4 and 6 (t = -17.38; df = 564; P < 0.001). The percentage of the number of seeds per pod attributable to the influence of *C. c. cincta* was 24.33 %. The higher normal seed yield unlimited visits treatment compared with bagged flowers. The comparison of the percentage of

DISCUSSION

reproduction mixed regime with the А predominance of autogamy over allogamy could be explained by the structure of the flower. The flowers are self-compatible and usually selfpollinated (Smartt, 1976). Although the structure is most suited for autogamy, in pigeon pea a certain amount of allogamy does occur with insect visitations (Saxena et. al., 1990; Reddy et al., 2004). Out-crossing rate of 3 to 26 % has been reported by Reddy et al. (2004) and ours results were included to this range

normal seeds showed that the observed highly significant between differences was treatments 1 and 2 ($X^2 = 536.45$; df = 1; P < 0.001) and treatments 3 and 4 ($X^2 = 673.3$; df = 1; P <0.001). The percentage of the normal seeds attributed to influence of insects was 08.84 %. The highest normal seeds yield in C. c. cincta visited flowers than even the unlimited visits flowers. This may show high pollination deficit on the crop, indicating need for C. c. cincta management to increased developed seeds. The comparison of the percentages of normal seeds has shown that the differences were highly significant between treatments 2 and 5 ($X^2 = 38.20$; df = 1; P < 0.001) and treatments 4 and 6 ($X^2 = 75.42$; df = 1; P <0.001). The percentage of the normal seed yields attributed to the influence of C. c. cincta was 11.52 %. In short, the influence of C. c. cincta on pod and seeds yields was positive and significant. A positive and highly significant correlation was found between the number of seeds and the number of C. c. cincta visits on C. cajan inflorescences, in 2008 (r =0.51; df = 98; P < 0.001) as well as in 2009 (r = 0.77; df = 98; P < 0.001).

Chalicodoma cincta cincta was the main floral visitor of *C. cajan* during the observation periods. However, in other parts of the world such as the United States of America (Grewal et al., 1990), other bees Megachilidae *Megachile* sp. and *Chalicodoma* sp., have been reported as the main floral visitors of this crop. This could be due to absence of this bee in this country or its lower abundance. Through the abundance and diversity of insects floral of a plant vary depending on the regions (Roubik, 2000). The not significant difference between the percentages



of C. c. cincta visit for the two years of study could be explained by the constancy presence of the nest of this insect near the experimental plot. The activity peak of C. c. cincta on C. cajan inflorescences was in the morning, which correlates to the period of highest availability of the nectar and/or pollen on C. cajan flowers as reported by Saxena and Kumar (2010). This is likewise the period in which the stigma of C. cajan has optimal receptivity to pollen or the weather conditions. In fact, Bramel et al. (2004) and Julianna and Rufus (2010) reported that the weather during bloom affects abundance and foraging of insect pollinators. The high abundance of C. c. cincta on 1000 flowers and the positive and significant correlation between the number of C. cajan flowers bloom and number of C. c. cincta visits, underscores the attractiveness of C. cajan floral rewards to this bee. For 18 species of insects visits the flowers of C. cajan; C. c. cincta is the most abundant species (28.99 %), followed by Xylocopa calens (22.04 %), Apis mellifera adansonii (11.41 %) and Megachile bituberculata (09.55 %), 6 species have a frequency of less (2 %) and 8 species have a frequency between 2 and 8 %.

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. The weight of C. c. cincta played a positive role: when collecting nectar and/or pollen, C. c. cincta shook flowers and this movement could facilitate the liberation of pollen by anthers, for the optimal occupation of the stigma. Similar phenomenon was also reported by Vanderborght and Rasmont (1987) for X. bariwal on Phaseolus coccineus. 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. Similar results were found in A. m. adansonii workers foraging on flowers of Helianthus annuus (Tchuenguem et al., 2009a), Citrullus lanatus (Azo'o Ela et al., 2010), Vernonia amygdalina (Tchuenguem et al., 2010), and in Meliponula erythra foraging on Dacryodes edulis flowers (Tchuenguem et al., 2001).

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. c. cincta* shows flower

constancy (Basualdo et al., 2000) for the flowers of each of the plant specie studied. Flower constancy is an important aspect in management of pollination and this shows C. c. cincta can provide the advantages of pollination management for C. cajan. Investment in C. c. cincta management may provide high returns to investment on this crop. The disruptions of visits by other insects reduced the duration of certain C. c. cincta visits. This obliged some workers to visit more flowers during a foraging trip, in order to maximize their pollen or nectar loads. Similar observations have been made in A. m. adansonii workers foraging on flowers of Callistemon rigidus (Tchuenguem et al., 2004), Entada africana (Tchuenguem et al., 2007), Vitellaria paradoxa (Tchuenguem et al., 2008), Vigna unguiculata (Tchuenguem et al., 2009b), and in Meliponula erythra foraging on Dacryodes edulis flowers (Kengue et al., 2002).

During the collection of nectar and pollen on each flower, *C. c. cincta* foragers regularly come into contact with the stigma. They could enhance autopollination, which has been demonstrated in the past (Saxena et al., 1990; Singh et al., 1990; Reddy et al., 2004; Sarah et al., 2010). *Chalicodoma cincta cincta* would provide allogamous pollination through carrying of pollen with their furs, legs and mouth accessories, which is consequently deposited on another flower belonging to different plant of same species. This has also been observed by other studies (Grewal et al., 1990; Saxena et al., 1990; Reddy et al., 2004).

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. Thus in addition to their direct pollination role, C. c. cincta foragers also indirectly effected self-pollination and crosspollination of C. cajan flowers. Similar observations have been made in A. m. adansonii workers foraging on flowers of Callistemon rigidus (Tchuenguem et al., 2004) and Brassica napus (Pierre et al., 2010) that airborne pollen released by honeybee foraging mark easier a wind assisted pollination of these plant species.

The positive and significant contribution of *C. c. cincta* in the pod and seeds yields of *C. cajan* is justified by the action of this bee on pollination.



The most important yield (pods, seeds, percentage of normal seeds in pods) recorded in unlimited visits can be attributed to the important role the pollinating insects. The flowers that were exposed to pollinators provided more pods per inflorescence, more seeds per pod with the heavier seeds and of better shape than the bagged flowers, in agreement to previous results reported on soybean (Benachour et al., 2007) and cowpea (Tchuenguem et al., 2009b). The high fruiting rate observed in 2009 compared to 2008 could be explained not only by the important number of species insects in 2009, but also by the high number of visitation of principal pollinators.

The significant contribution of *C. c. cincta* and other insects in the pod and seeds yields of *C. cajan* is similar to the findings in Great Britain (Kendall & Smith, 1976) and United State of America (Ibarra-Perez et al., 1999) which showed that *P. coccineus* flowers produce less seeds per pod in the absence of efficient pollinators. Higher fruiting of flowers exposed to visits by *C. c. cincta* only compared with those flowers under unlimited visits by all kind of visitors show that this bee is a main

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REFERENCES

- Arya RLKP, Niranjan A. and Singh JB: 2002. Food - fodder cropping systems with nutrient management in agro forestry systems under rainfed conditions. *Indian Journal of Soil Conservation* 30:166-171.
- Azo'o Ela M, Messi J, Tchuenguem Fohouo F-N, Tamesse JL, Kekeunou S. and Pando J B: 2010. Foraging behaviour of *Apis mellifera adansonii* and its impact on pollination, fruit and seed yields of *Citrullus lanatus* at Nkolbisson (Yaoundé, Cameroun). *Cameroon Journal Experimental Biology* 6 (1): 41 – 48.
- Benachour K, Louadi K. and Terzo M: 2007. Role of wild and honey bees (Hymenoptera: Apoidea) in the pollination of *Vicia faba* L. var. major (Fabaceae) in Constantine area

pollinator of *C. cajan* and thus can be targeted for managed pollination of this crop. This result is similar other findings reported by Kendall & Smith (1976) shown that in Great Britain, the multiplicity of insects pollinators visits on *P. coccineus* did not ameliorate the production of pods.

In Yaoundé, Cameroon, C. cajan benefits highly from pollination by insects, among which C. c. cincta is the most important pollinator. The comparison of pods and seeds set of unprotected inflorescences with that of inflorescences visited exclusively by C. c. 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. c. cincta in terms of nest provision at the proximity of C. cajan field is worthy while for growers. The conservation and/or the kept of C. c. cincta nest at the proximity of C. cajan plots should be recommended for Cameroonian farmers and horticulturists to increase pods and seeds yields. Pigeon pea should to be cultured more in Cameroon to contribute to the food supply and to favor populations of C. c. cincta.

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(Algeria). Annales de la Société Entomologique de France 43 (2): 213–219.

- Basualdo M, Bedascarrasbure E. and De Jong D: 2000. Africanized Honey Bees (Hymenoptera: Apidae) Have a Greater Fidelity to Sunflowers than European Bees. *Journal of Economic Entomology* 93(2): 304-307.
- Bramel J, Kiran S, Reddy J, Ford-Lloyd B. and Chandra S: 2004. Degree and distribution of pigeon pea landrace morphological diversity in traditional cropping systems in Andhra Pradesh. In: Bramel PJ, editor. Assessing the risk of losses of biodiversity in traditional cropping systems: A case study of pigeon pea in Andhra Pradesh. *International crop Research Institute for the semiarid Tropics*, pp. 1-45. Pantacheru Andhra Pradesh, Indian.



- Demarly: 1977. Génétique et amélioration des plantes. Masson, Paris. 577 p.
- Fluri P. and Frick R: 2005. L'apiculture en Suisse: état et perspectives. Revue Suisse d'Agriculture 37: 81-86.
- Garrison RJ. and Somer E: 1995. The nutrition desk reference. 3rd edition. Keats Publishing Inc., New Canaan, Connecticut, USA.
- Grewal GS, Singh G. and Kandoria JL: 1990. Insect pollinators of pigeon pea (*Cajanus cajan*) around Ludhiana. *Indian Journal of Agricultural Science* 60: 227-228.
- Gupta DN, Parab AA, Mehta HR. and Goyal R: 2001. Evaluation of nutritive value of promising genotypes and horsegram in Konkan. *Journal of Phytological Research* 14:143-146.
- Ibarra-Perez FJ, Barnhart D, Ehdaie B, Knio KM. and Waines J G: 1999. Effects of insect tripping on seed yield of common beans. *Crop Science* 39: 428-433.
- Jacob-Remacle A: 1989. Comportement de butinage de l'abeille domestique et des abeilles sauvages dans des vergers de pommiers en Belgique. *Apidologie* 20: 271-285.
- Julianna KT. and Rufus I: 2010. Weather during affects pollination and yield of highbush blueberry. *Journal of Economic Entomology* 103 (3): 557 – 562.
- Kendal DA. and Smith BD: 1976. The pollinating efficiency of honey bee and Bumblebee visits to flowers of runner bean *Phaseolus* coccineus. Journal of Applied Ecology 13(3): 749-752.
- Kengue J, Tchuenguem Fohouo FN. and Adewusi HG: 2002. Towards the improvement of safou (*Dacryodes edulis*): population variation and reproductive biology. *Forests, Trees and Livelihoods* 12: 73 - 84.
- Klein AM, Vaissière BE, Cane JH, Steffan-Dewenter I, Cunnigham SA, Kremen C. and Tscharntke T: 2007. Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society* 274: 303-313.
- Kumar Rao JVDK: 1990. Pigeon pea: Nitrogen fixation. In: Nene YL, Hall SD, Sheila VK, editors. *The pigeon pea. CAB International*, pp.233-256. Wallingford, Oxon, UK.

- Madeley J: 1995. Sahel's salvation: African farming and food processing, May/June 1995. Alain Charles Publishing Ltd, London, UK.
- Nene YL. and Sheila VK: 1990. Pigeon pea: Geography and importance. In Nene YL, Hall SD, Sheila VK, editors. The pigeon pea. CAB International, pp.1-14. Wallingford, Oxon, UK
- Proctor M, Yeo P. and Lack A: 1996. *The natural history of pollination*. Corbet SA, Walters SM, Richard W, Streeter D, editors, Harper collings, p. 462.
- Pierre J, Vaissière B, Vallée P. and Renard M: 2010. Efficiency of airborne pollen released by honeybee foraging on pollination in oilseed rape: a wind insect-assisted pollination. *Apidologie*, 41, 109-115.
- Reddy LJ, Chandra S, Pooni H. and Bramel PJ: 2004. Rate of outcrossing in pigeon pea under intercropped conditions. In: Bramel PJ, editor. Assessing the Risk of Losses in Biodiversity in Traditional Cropping Systems: A Case Study of Pigeon pea in Andhra Pradesh. International Crops Research Institute for the Semi-Arid Tropics, pp. 133– 141. Patancheru 502 -324, Andhra Pradesh, India.
- Roubik DW: 2000. Pollination system stability in Tropical America. *Conservative Biology*, 14: 1235-1236.
- Sabbahi R, De Oliveira D. and Marceau J: 2005. Influence of honey bee (Hymenoptera: Apidae) density on the production of canola (Crucifera: Brassicacae). *Journal of Economic Entomology* 98: 367-372.
- Sarah S, Moray F, Anne WM. and Said S: 2010. Genetic diversity in pigeon pea [Cajanus cajan (L.) Millsp.] Landraces as revealed by simple sequence repeat markets. African journal of Biotechnology 9 (22): 3231 – 3241.
- Saxena KB, Singh L. and Gupta MD: 1990. Variation for natural out-crossing in pigeon pea. *Euphytica* 46:143–148.
- Saxena K.B, Kumar RV. and Rao PV: 2002. Pigeon pea nutrition and its improvement. *Journal* of Crop Production 5: 227-260.
- Saxena KB. and Kumar RV: 2010. Insect aided natural out-crossing in four wild relatives of pigeon pea. *Euphytica* 173: 329 335.
- Shils ME, Olson JA. and Shike M: 1994. Modern nutrition in health and disease, 9th edition. Williams and Wilkins, Baltimore, London.



- ShiyingY, Pang Wen, Zong Xuxiao, Li Zhenghong, Zhou Chaohong, Saxena KB. and Liang Hanchao: 2001. Pigeon pea: A potential fodder crop for Guangxi Province of China. International Chickpea and Pigeon pea Newsletter 8: 54-55.
- Singh L, Gupta SC. and Faris DG: 1990. Pigeon pea: Breeding. *In* Nene YL. Hall SD. and Sheila VK. (eds). The pigeon pea. CAB International, Wallingford, Oxon, UK, p. 375-399.
- Smartt J: 1976. Tropical pulses. Longman Group Limited, London. 348 p.
- Tchuenguem Fohouo FN, Messi J. and Pauly A: 2001. Activité de *Meliponula erythra* sur les fleurs de *Dacryodes edulis* et son impact sur la fructification. *Fruits* 56 : 179–188.
- Tchuenguem Fohouo FN, Messi J, Brückner D, Bouba B, Mbofung G. and Hentchoya Hemo J: 2004. Foraging and pollination behaviour of the African honey bee (*Apis mellifera adansonii*) on *Callistemon rigidus* flowers at Ngaoundéré (Cameroon). *Journal* of the Cameroon Academy of Sciences 4: 133-140.
- Tchuenguem Fohouo FN: 2005. Activité de butinage et de pollinisation d'Apis mellifera adansonii Latreille (Hymenoptera : Apidae, Apinae) sur les fleurs de trois plantes à Ngaoundéré (Cameroun) : Callistemon rigidus (Myrtaceae), Syzygium guineense var. macrocarpum (Myrtaceae) et Voacanga africana (Apocynaceae). Thèse de Doctorat d'Etat, Université de Yaoundé I, 103 p.
- Tchuenguem Fohouo FN, Djonwangwe D, Messi J. and Brückner D: 2007. Exploitation des fleurs de Entada africana, Eucalyptus camaldulensis, Psidium guajava et Trichillia emetica par Apis mellifera adansonii à Dang (Ngaoundéré, Cameroun). Cameroon Journal of Experimental Biology 3(2): 50 – 60.
- Tchuenguem Fohouo FN, Djonwangwe D, Messi J. and Brückner D: 2008. Exploitation of Dichrostachys cinerea, Vitellaria paradoxa, Persea americana and Securidaca longepedunculata flowers by Apis mellifera adansonii Latreille (Hymenoptera: Apidae) at Dang (Ngaoundéré, Cameroon). International Journal of Tropical Insect Science 28 (4): 225-233.
- Tchuenguem Fohouo FN, Djonwangwe D, Messi J. and Brückner D: 2009a. Activité de butinage et de pollinisation de *Apis mellifera*

adansonii sur les fleurs de Helianthus annus (Asteraceae) flowers à Ngaoundéré (Cameroun). Cameroon Journal of Experimental Biology 5: 1-9.

- Tchuenguem Fohouo FN, Ngakou A. and Kegni BS: 2009b. Pollination and yield responses of cowpea (*Vigna unguiculata* L. Walp.) to the foraging activity of *Apis mellifera adansonii* (Hymenoptera: Apidae) at Ngaoundéré (Cameroon). *African Journal of Biotechnology* 8: 1988-1996.
- Tchuenguem Fohouo FN, Fameni Tope S, Pharaon Mbianda A, Messi J. and Brückner D: 2010. behaviour of Apis mellifera Foraging (Hymenoptera: adansonii Apoidae) on nigricans, Eryhrina Combretum sigmoidea, Lannea kerstingii and Vernonia amygdalina flowers at Dang (Ngaoundéré, Cameroon). International Journal of Tropical Insect Science 30 (1): 40 - 47.
- Van der Maesen LJG: 1990. Pigeon pea: Origin, history, evolution and taxonomy. In: Nene YL, Hall SD, Sheila VK, editors. *The pigeon pea CAB International*, pp. 15-46.Wallingford, Oxon, UK.
- Vanderborght I. and Rasmont P: 1987. Utilisation de l'abeille domestique (*Apis mellifera* L.) et de la Xylocope (*Xylocopa barinval* MAIDL) (Hymenoptera, Apidae) pour la pollinisation en cage de *Phaseolus coccineus* L. *Bulletin de Recherche Agronomique de Gembloux* 22(4): 279-284.
- Yun ZS, Gu Yong, Yang Shi Ying and Zong XuXiao: 2001. Traditional and alternative uses of pigeon pea in China. *International Chickpea and Pigeon pea Newsletter*, 8: 55-57.