International Journal of Agronomy and Agricultural Research (IJAAR)
ISSN: 2223-7054 (Print) 2225-3610 (Online)
http://www.innspub.net
Vol. 4, No. 4, p. 77-88, 2014

RESEARCH PAPER

OPEN ACCESS

Foraging and pollination behaviour of *Chalicodoma rufipes* L. (Hymenoptera: Megachilidae) on *Cajanus cajan* L. Mill sp. (Fabaceae) flowers at Dang (Ngaoundéré, Cameroon)

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Article published on April 07, 2014

Key words: *Cajanus cajan*, *Chalicodoma rufipes,* foraging, pollination.

Abstract

To evaluate *Chalicodoma rufipes* impact on fruit and seed yields of *Cajanus cajan* L., its foraging and pollinating activities were studied in Ngaoundéré for two seasons. Observations were made on 340 flowers each year and divided in three treatments. The treatments included unlimited flowers access by all visitors; bagged flowers to deny all visits and limited visits by *Ch. rufipes* only. *Chalicodoma rufipes* worker seasonal rhythm of activity, their foraging behaviour, their pollination efficiency, the fruiting rate, the number of seeds per pod and the percentage of normal seeds were evaluated. Results show that *Ch. rufipes* foraged *Ca. cajan* flowers throughout the whole blooming period. That bee intensely harvested pollen and nectar. The mean foraging speed was 11.50 flowers per minute in 2010 and 12.45 flowers per minute in 2011. The fruiting rate, the number of seeds per fruit and the percentage of normal seeds of unprotected flowers were significantly higher than those of flowers protected from insects. Through their pollination efficiency, that bee provoked a significant increment of the fruiting rate by 95.38% in 2010 and 96.72% in 2011, as well as the mean number of seeds per fruit by 04.28 in 2010 and 04.93 in 2011, and the percentage of normal (well developed) seeds by 50.72% in 2010 and 61.79% in 2011. The installation of *Ch. rufipes* nests close to *Ca. cajan* fields could be recommended to increase fruit and seed yields and to alleviate poverty in that region.

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Introduction

Cajanus cajan, also known as red gram, Congo pea, gungo pea, no eye pea, dhal, gandul, gandure, frijol de árbol, and pois cajan, occurs in several varieties, and is one of the major grain legume crops grown in the tropics and subtropics (Saxena et al., 2002; Pando et al., 2011). It belongs to the Leguminosae genus that is composed of 34 species (Kassa, 2011). Pigeonpea is the only cultivated plant among the genus, while the remaining species are wild (Kassa, 2011).

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; Pando et al., 2011). As a food crop, *Ca. cajan* offers a cheap source of valuable protein to people. Its content 21 to 30% protein, although some high-protein lines are being bred with up to 30% protein (Sharma and Green, 1980; Gupta et al., 2001; Saxena et al., 2002; Pando et al., 2011). It has more minerals, ten times more fat, five times more vitamin A, and three times more vitamin C than ordinary peas (Madeley, 1995; Changaya, 2007 ; Pando et al., 2011). The World 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 (Kumar et al., 1990; Nene & Sheila, 1990; Arya et al., 2002).

Cajanus cajan is well adapted to poor soils and semi-arid regions, this makes the plant interesting culture for people in areas where growing conditions are marginal (Niyonkuru, 2002).

Through the literature, little information exists on the relationships between flowering insects and many plants species grown in Cameroon. Nevertheless, it is known that generally enthophilous insects and bees in particular usually increase the fruit and seed yields of many plants species, through pollination provision (Fluri and Frick, 2005; Sabbahi et al., 2005; Klein et al., 2007). Pigeon pea flowers have bright corollae and produces nectar and pollen. These traits suggest that *Ca. 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 (pollinators). *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 on insect pollination of this crop plant to provide new baseline information on it in Cameroon. In this country, *Ca. 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 (Pando et al., 2011). Moreover, the demand for *Ca. 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 are lack of publication on the research report on the relationship between *Ca. cajan* and many anthophilous insects. This study was carried out to assess the effects of foraging behavior of *Chalicodoma rufipes* on *Ca. cajan* yields.

In Cameroon, before this research, no previous research has been reported in Adamawa Region on the relationships between *Ca. cajan* and its anthophilous insects in general and *Ch. rufipes* in particular.

The main objective of this work carried out in Ngaoundéré in 2010 and 2011 was to contribute to the understanding of the relationships between *Ca. cajan* and its flower visiting insects, for their optimal management. Specific objectives were: (a) the registration of the activity of *Ch. rufipes* on *Ca. cajan* flowers; (b) the evaluation of the impact of flowering insects on pollination, fruits and seeds yields of this

Fabaceae, and (c) the estimation of the pollination efficiency of *Ch. rufipes* on *Ca. cajan*.

Materials and methods

Study site, experimental plot and biological material

Experiment was carried out twice, first from December 2010 to January 2011 and then from December 2011 to January 2012 at Dang (Latitude 7°25.365 N, Longitude 13°32.572 E and Altitude 1083 masl), a village located in the North of the city of Ngaoundéré, in the Adamawa Region of Cameroon, within the university campus. This Region belongs to the high-altitude Guinean Savanna agro-ecological zone. The climate is characterized by two seasons: a rainy season (April to October) and a dry season (November to March). The annual rainfall is about 1500 mm. The mean annual temperature is 22°C while the mean annual relative humidity is 70% (Tchuenguem Fohouo, 2005). The vegetation was represented by crops, ornamental plants, hedge plants and native plants of savanna and gallery forests.

The experimental plot was a field of 416 m². The vegetation near *Ca. cajan* field was represented by wild and cultivated species. The experimental plant material was represented by *Ca. cajan* grown from seeds provided by IRAD Nkolbisson in Yaoundé.

Methods

Sowing and weeding

On the 9th June 2010 and the 12th June 2011 respectively, sowing was done per parcel on 4 lines each and 4 seeds were sown per seed holes. The space between two consecutives lines was 1m and it was 1m between two consecutives seed holes. From germination (which occurred from the 15th to 16th June 2010 and from 19th to 20th June 2011) to the development of the first flower (17 th December 2010 and 15 th December 2011), the field was regularly weeded with a hoe. After December 17 (2010) and December 15 (2011), weeding was done by hand to maintain plots weed-free. Fourteen days after germination, thinning was done and only the strongest plant was maintained for each seeds hole.

Determination of Cajanus Cajan mating system

December 21, 2010, 240 *Ca. Cajan* flowers at bud stage were labeled on 40 feet (5 plants per subplot) among which 120 were left unattended (treatment 1) and 120 were protected using gauze bags net to prevent insect visitors (Roubik, 1995) (treatment 2). December 18, 2011, 240 flowers of *Ca. Cajan* with flowers at bud stage were labelled (5 plants per subplot) among which 120 were left unattended (treatment 3) and 120 were protected using gauze bags (treatment 4).

In both years, ten days after shading of the last labelled flowers, the number of pods was assessed in each treatment. The podding index was then calculated as described by Tchuenguem Fohouo 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 (protected flowers) (Demarly, 1977).

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

$Atr = 100 - Alr$

Estimation of the frequency of Chalicodoma rufipes on Cajanus cajan flowers

On December 21, 2010, 40 *Ca. cajan* plants which had started to bloom were labelled at the experimental field. On these plants, 240 flowers at the bud stage were labelled among which 120 were left unattended (treatment 1) and 120 bagged (treatment 2) to prevent insects visitors.

On December 18, 2011, 240 flowers of *Ca. cajan* at the bud stage were labelled among which 120 were left for unprotected visits (treatment 3) and 120 bagged (treatment 4). The frequency of *Ch. rufipes* on *Ca. cajan* flowers was determined based on flowers observations of treatment 1 and treatment 3, every day, from 23rd December 2010 to 11 January 2011 and from 20th December 2011 to 14th January 2012, at 8 – 9h, 10 – 11h, 12 – 13h, 14 – 15h and 16 – 17h. In a slow walk along all labelled flowers of treatments 1 and 3, the identity of all insects that visited *Ca. cajan* flowers was recorded. Specimens of all insect taxa were caught with insect net on unlabelled flowers and conserved in 70% ethanol for further taxonomy identification. All insects encountered on flowers were registered and the cumulated results expressed as the number of visits to determine the relative frequency of *Ch. rufipes* in the anthophilous entomofauna of *Ca. cajan*.

Study of the activity of Chalicodoma rufipes on Cajanus cajan flowers

In addition to the determination of the flower visiting insect frequency, direct observation of foraging activity of insect pollinators on flowers was made in the experimental field. The floral products (nectar or pollen) harvested by *Ch. rufipes* during each floral visit were registered based on its foraging behaviour. Nectar foragers were seen extending their proboscis to the base of the corolla while pollen gatherers scratched the anthers with their mandibles or their legs. During the same time that *Ch. rufipes* visits on flowers were registered, we noted the type of floral products collected by this bee species. This parameter was measured to determine if *Ch. rufipes* is strictly a pollinivore, nectarivore or pollinivore and nectarivore. This could give us an idea of its implication on cross-pollination and/or self-pollination of *Ca. cajan*.

In the morning of each sampling day, the number of opened flowers carried by each labelled flowers was counted.

During the same days as for the frequency of visits, the duration of individual flower visits was recorded (using a stopwatch) at least six times: 9 – 10h, 11 – 12h, 13 – 14h, 15 – 16h and 17 – 18h.

Moreover, the number of pollinating visits (the bee came into contact with the anthers, stigma and anthers) (Jacob-Remacle, 1989; Freitas, 1997), the abundance of foragers (highest number of individuals foraging simultaneously per flower or per 1000 flowers (Tchuenguem et al., 2004) and the foraging speed (number of flowers visited by a bee per minute (Jacob-Remacle, 1989) were measured. The disruption of the activity of foragers by competitors or predators and the attractiveness exerted by other plant species on *Ch. rufipes* were assessed.

During each daily period of investigation, a mobile thermo-hygrometer was used to register the temperature and the relative humidity in the station.

Evaluation of the effect of Chalicodoma rufipes and other insects on Cajanus cajan yields

This evaluation was based on the impact of flowering insects on pollination, the impact of pollination on *Ca. cajan* fruiting, and the comparison of yields (fruiting rate, mean number of seed per fruit and percentage of normal seeds) of treatment X (unlimited flowers) and treatment Y (bagged flowers) (Roubik, 1995). The fruiting rate due to the influence of foraging insects (*Fri*) was calculated by the formula: $Fri = \{[(FrX - FrY) / FrX] \times 100\}$ where FrX and FrY are 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 fruits formed and $F1$ the number of viable flowers initially set (Tchuenguem et al., 2001). At maturity, fruits were harvested from each treatment and the number of seeds per fruit counted. The mean number of seeds per fruit and the percentage of normal (well developed) seeds were then calculated for each treatment. The impact of flower visiting insects on seed yields was evaluated using the same method as mentioned above for fruiting rate.

Assessment of the pollination efficiency of Chalicodoma rufipes on *Cajanus cajan*

Parallel to the constitution of treatments 1 and 2, 100 flowers were isolated (treatment 5) as those of treatment 2 (Roubik, 1995). Parallel to the constitution of treatments 3 and 4, 100 flowers were isolated (treatment 6) as those of treatment 4. Between 10 am and 12 am of each observation date, the gauze bag was delicately removed from each flower of treatments 5 and 6 carrying out new opened flowers, and these flowers were observed for up to 10 minutes. The flower visited by *Ch. rufipes* was marked and then protected once more.

For each observation period, the contribution of *Ch. rufipes* in fruiting (Frx) was calculated using Tchuenguem et al., 2004 formula: $Frx = \{[(FrZ– FrY) / FrZ] \times 100\}$ where *FrZ* and *FrY* are the fruiting rate in treatment Z (bagged flowers and exclusively visited by *Ch. rufipes*) and treatment Y (bagged flowers). At the maturity, fruits were harvested from treatment 5 and treatment 6 and the number of seeds per fruit counted. The mean number of seeds per fruit and the percentage of normal (well developed) seeds were then calculated for each treatment. *Chalicodoma rufipes* impact on seed yields was evaluated using the same method as mentioned above for fruiting rate.

Data analysis

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

Results

Cajanus cajan mating system

One hundred and twenty flowers were studied in each of the treatments 1, 2 and 3 respectively in 2010 and 2011. In 2010, the podding index was 0.93 for treatment 1, 0.63 for treatment 2 and 0.76 for treatment 3 while in 2011, it was instead 0.96 for treatment 1, 0.70 for treatment 2 and 0.78 for treatment 3. Hence, the allogamy rate (TC) and the autogamy rate (TA) were respectively 32.26% and 67.74% in 2010 against 18.75% and was 81.25% in 2011.

Table 1. Fruiting rate, mean number of seeds per pod and normal seed percentages according to different treatments of *Cajanus cajan* in 2010 and 2011 at Dang.

Treatments	Studied Years	NSF	NFFrs	FrR	Seeds/Pod		TNS	NS	%NS
					M	SD			
1 (Fl)		120	112	93.33	8.69	3.53	526	428	81.37
2(Fi)	2010	120	76	63.33	5.07	2.12	307	147	47.88
3(Fv *Ch. rufipes*)		65	62	95.38	4.28	1.15	278	141	50.72
1(Fl)		120	115	95.83	5.12	3.53	615	476	77.40
2(Fi)	2011	120	84	70.00	3.19	3.53	383	205	53.52
3(Fv *Ch. rufipes*)		61	59	96.72	4.93	1.25	301	186	61.79

Fl: Unprotected flowers; Fi: Bagged flowers; Fv *Ch. rufipes*: Flowers exclusively visited by *Chalicodoma rufipes*; NSF: Number of studied flowers; NFFrs: Number of formed fruits; FrR: Fruting rate; TNS: Total number of seeds; NS: Normal seeds; %NS: Percentage of normal seeds.

It appears from these results that *Ca. cajan* variety used for our experiment has a mixed mating system: allogamous and autogamous, with the predominance of autogamy over allogamy.

Reddy *et al.* (2004) reported the predominance of autogamy (74%) over the allogamy (26%) in India on the same plant species. Our results are in line with those of Pando et al. (2012), who reported the predominance of autogamy (92.05% in 2008 and 84.61 in 2009) over the allogamy (07.95% in 2008 and 15.39% in 2009) on *Ca. cajan* in Yaoundé-Cameroon.

Hence, the mating system of *Ca. cajan* do not vary from one agroecological zone to another or from one studied area to another.

Frequency of Chalicodoma rufipes visit on Cajanus cajan flowers

Amongst the 6531 and 7222 visits of 18 and 24 insect species recorded on *Ca. cajan* flowers during our observation periods, *Ch. rufipes* was the most represented insect with 1911 visits (29.26%) and 1958 visits (27.11%), in 2010 and 2011 respectively. The difference between these two percentages is highly significant ($\chi^2 = 7.83$; $P < 0.001$). *Ch. rufipes* was active on *Ca. cajan* flowers from 9 am to 4 pm, with a peak of visits between 8 am and 9 am in 2010 as well as in 2011 (Figure 1 A and B).

Activity of Chalicodoma rufipes on Cajanus cajan flowers

Floral products harvested

From our observations, *Ch. rufipes* were seen collecting pollen, nectar and both products on pigeon pea flowers. Nectar collection was regular and intensive whereas pollen collection was less intensive. This bee was seen actively collecting both floral products at the same time.

From 1911 visits recorded in 2010, 657 (34.38%) were devoted to exclusive nectar harvest, 405 (21.19%) to exclusive pollen harvest and 849 (44.43%) for nectar and pollen harvest; whereas in 2011, from 1958 visits recorded, 233 (11.90%) were devoted to exclusive nectar harvest, 883 (45.10%) to exclusive pollen harvest and 842 (43.00%) for both, nectar and pollen harvest.

Nectar and Pollen were harvested during all scheduled time frame.

Rhythm of visits according to the flowering stages

According to our observations, visits were most numerous when the number of open flowers was highest on *Ca. cajan* plants (Figures 2 C and D). Furthermore, we found a positive and highly significant correlation between the number of opened flowers and the number of insect visits in 2010 ($r = 0.86$; $df = 15$; $P < 0.001$) as well as in 2011; ($r = 0.96$; $df = 20$; $P < 0.001$).

Daily rhythm of visits

Chalicodoma rufipes started its daily foraging activity on *Ca. cajan* flowers around 9am and foraged throughout its blooming period, with a peak situated at the first time frame each studied year (Figures 1 A and B). Weather conditions have have not influenced *Ch. rufipes* activity (Figures 3 E and F).

In 2010, the correlation was positive and not significant ($r = 0.54$; $df = 10$; $P > 0.05$) between the number of *Ch. rufipes* visits and the temperature, and it was negative and not significant ($r = -0.25$; $df = 10$; $P > 0.05$) between the number of visits and relative humidity.

In 2011, the correlation was positive and not significant ($r = 0.57$; $df = 10$; $P > 0.05$) between the number of *Ch. rufipes* visits and the temperature, and it was negative and not significant ($r = -0.27$; $df = 10$; $P > 0.05$) between the number of visits and relative humidity.

Abundance of Chalicodoma rufipes

In 2010, the highest mean number of *Ch. rufipes* individuals simultaneous in activity was 1.02 per flower (n = 153; SD = 0.14; max = 2), 1.11 per inflorescence (n = 153; SD = 0.37; max = 3) and 214.50 per 1000 flowers (n = 153; SD = 82.93; max = 600).

In 2011, the corresponding figures were 1.10 per flower (n = 162; SD = 0.30; max= 2), 1.28 per inflorescence (n = 162; SD = 0.64; max= 4) and 223.33 (n = 162; SD = 82.31*; maxi* = 500) per 1000 flowers. The difference between the mean number of foragers per 1000 flowers in 2010 and 2011 was not significant (t = 1.246; df = 313; P *value* = 0.2138).

Duration of visits per flower

The mean duration of *Ch. rufipes* visits per *Ca. cajan* flower varied significantly according to floral product harvested. In 2010, the mean duration visit for nectar harvest was 43.15 seconds (n = 104*; SD = 26.87;* Min = 1; Max =109), and 28.00 seconds (n = 104*; SD = 21.92;* Min = 1; Max =68) for pollen harvest against 62.77 sec (n = 104*; SD = 41.01;* Min = 5; Max =135) for both floral products harvest at the same time; in 2011, the corresponding figures were 12.41 seconds (n = 121*; SD = 8.38;* Min = 1; Max =71) for nectar harvest, and 45.94 seconds (n = 121*; SD = 7.78;* Min = 1; Max =121) for pollen harvest against 45.41 sec (n = 121*; SD = 15.56;* Min = 2; Max =134) for both floral products harvest at the same time. The difference between the duration of the visit to harvest nectar in 2010 and 2011 was significant (t = 2.54*; df = 27; P value = 0.0171*), it was not significant for pollen harvest (*t = 0.97; df = 27; Pvalue = 0.3409*) as well as for nectar and pollen harvest (t = 1.11; df = 27; P = 0.2779).

Foraging speed of Chalicodoma rufipes on Cajanus cajan flowers

Chalicodoma rufipes visited between 1 and 4 flowers per minute in 2010 and between 1 and 6 flowers per minute in 2011. The mean foraging speed was 10.93 flowers/minute (n = 125; SD = 9.38) in 2010 and 3.80 flowers per minute (n = 125*; SD* = 7.24) in 2011. The difference between these two means is not significant (t = 0.62*; df* = 2*; P* > 0.05).

Influence of wildlife

Individuals of *Ch. rufipes* were disturbed in their foraging by other individuals of the same or different species, which were either predators or competitors for nectar and /or pollen (Pando et al., 2011; Dounia and Tchuenguem, 2013; Dounia and Fohouo, 2014; Mazi et al., 2013). In 2010, for 1911 visits of *Ch. rufipes,* 86 (4.50%) were interrupted whereas in 2011, for 1958 visits*,* 91 (4.65%) was interrupted. This action forces the interrupted flower visiting insects to visit a greater number of flowers during their foraging trips, to get their nectar and/or pollen load (Klein et al., 2007; Pando et al., 2011; Pando, 2013).

Influence of neighboring flora

During our observation periods, flowers of many other plant species growing around our studied area were visited by *Ch. rufipes*, for either nectar (ne) and/or pollen (po). Amongst those plant species were *Tithonia diversifolia* (Asteraceae; po); *Mimosa pudica* (Fabaceae; po), *Brachiaria brizantha* (Poaceae; po); *Mangifera indica* (Anacardiaceae; ne and po); *Senna mimosoides* (Mimosaceae; po) and *Bidens pilosa* (Asteraceae; ne and po).

Pollination efficiency of Chalicodoma rufipes on Cajanus cajan

During pollen and/or nectar harvest in pigeon pea flowers, *Ch. rufipes* foragers regularly came into contact with either anthers or stigma or both flower parts and carried pollen and/or nectar. With this pollen, the bee flew frequently from flower to flower. The percentage of the number of visits during which *Ch. rufipes* came into contact with the anthers of the visited flowers varied from 22.92% (2010) to 30.20% (2011). The percentage of the total number of visits during which this bee came into contact with the anthers and stigma of the visited flowers varied from 77.08% (2010) to 69.80% (2011). Thus, *Ch. rufipes* highly increased the pollination possibilities of *Ca. cajan* flowers.

The comparison of figures in Table 1 shows that:

a)- The fruiting rate due to flowering insects was 93.33% for treatment 1 (unattended flowers) and 63.33% for treatment 2 (bagged flowers) in 2010; it was 95.83% for treatment 1 and 70.00% for treatment

2 in 2011. The comparison of these percentages shows that the difference are highly significant between treatments 1 and 2 ($\chi2 = 31.82$; $df = 1$; $P < 0.001$) in 2010 as well as in 2011 ($\chi2 = 28.27$; $df = 1$; $P < 0.001$).

The fruiting rate due to *Ch. rufipes* was 95.38% in 2010 and 96.72% in 2011. The difference between these two percentages is significant ($\chi2 = 0.15$; $df = 1$; $P < 0.05$). In 2010, the difference between treatments 2 and 3 (flowers visited exclusively by *Ch. rufipes*) is highly significant difference ($\chi2 = 22.85$; $df = 1$; $P < 0.001$) as well as in 2011 ($\chi2 = 17.41$; $df = 1$; $P < 0.001$).

For the two studied periods, fruiting rate due to *Ch. rufipes* was higher than that of treatment 2.

b)- In 2010, the mean number of seeds per pod due to insects species was 8.69 for treatment 1 and 5.07 treatment 2; the corresponding figures were 5.12 for treatment 1 and 3.19 for treatment 2 in 2011. The comparison of these means shows that the difference is highly significant between treatments 1 and 2 ($t = 08.30$; $df = 238$; $P < 0.0001$) in 2010 as well as in 2011 ($t = 08.25$; $df = 238$; $P < 0.0001$).

The mean number of seeds per pod due to *Ch. rufipes* was 04.28 in 2010 and 04.93 in 2011. The comparison of these means shows that the difference is highly significant between treatments 3 and 2 ($t = 06.24$; $df = 183$; $P < 0.0001$) in 2010 as well as in 2011 ($t = 05.64$; $df = 183$; $P < 0.0001$).

During our observation periods, the mean number of seeds per pod in treatment exlusively visited by *Ch. rufipes* were higher than that of treatment with flowers protected from insect.

c)-In 2010, the mean number of normal seeds per pod due to flowering insects was 07.07 for treatment 1 and 02.43 for treatment 2. The corresponding figures in 2011 was 03.97 for treatment 1 and 01.71 for treatment 2. The comparison of the mean number of normal seeds per pod shows that the difference is highly significant between treatments 1 and 2 ($t = 14.70$; $df = 238$; $P < 0.0001$) in 2010 as well as in 2011 ($t = 13.98$; $df = 238$; $P < 0.0001$).

The mean number of normal seeds per pod due to *Ch. rufipes* 02.17 (2010) and 03.05 (2011). The comparison of these means shows that the difference is highly significant between treatments 3 and 2 ($t = 05.33$; $df = 150$; $P < 0.0004$ in 2010) and is highly significant between treatments 3 and 2 ($t = 06.30$; $df = 179$; $P < 0.0001$ in 2011).

Our observations pointed out that flowers visited by *Ch. rufipes* have the highest normal seed per pod compare to those protected from insects.

d)- The percentage of normal seeds due to flowering species in 2010 was 81.37% for treatment 1 and 47.88% for treatment 2. The corresponding figures were 77.40% for treatment 1 and 53.52% for treatment 2 in 2011. The comparison of these figures shows that the difference is highly significant between treatments 1 and 2 ($\chi2 = 101.67$; $df = 1$; $P < 0.001$) in 2010 and between treatments 1 and 2 ($\chi2 = 62.06$; $df = 1$; $P < 0.001$) in 2011. For the two studied years, the difference was highly significant ($\chi^2 = 81.86$; $df = 1$; $P < 0,001$).

The percentage of normal seeds per pod due to *Ch. rufipes* was 50.72% in 2010 and 61.79% in 2011. The comparison of percentages between treatments 2 and 3 shows that the difference is not significant ($\chi2 = 0.47$; $df = 1$; $P > 0.05$) in 2010 and highly significant ($\chi2 = 4.71$; $df = 1$; $P < 0.001$) in 2011. For the both studied years, the difference was highly significant ($\chi^2 = 7.21$; $df = 1$; $P < 0,001$).

Chalicodoma rufipes impact on pods and seeds yields was positive and significant. Furthermore, we found a positive and highly significant correlation coeffiscience between the number of opened flowers and the number of insect visits in 2010 (r=0.86; df=15; P<0.001) as well as in 2011 (r=0.96; df=20; P<0.001: 2011).

Discussion

Activity of Chalicodoma rufipes on Cajanus cajan flowers

During our observation periods, we have registered 18 and 24 flower visiting insect species on *Ca. cajan* flowers. Results indicated that bees are among the main insect visitor of *Ca. cajan* flowers. The same result was found in Brazil on *Couepia uiti* (Mart. and Zucc.) Benth (Chrysobalanaceae) flowers and *Adenocalymma bracteatum* (Bignoniaceae) flowers (Paulino-Neto, 2007, Almeida-Soares et al., 2010), in South Africa on *Cyrtanthus breviflorus* (Amaryllidaceae) flowers (Glanda et al., 2010) and in Cameroon on *Daniellia oliveri* (Fabaceae-Caesalpinioideae), *Delonix regia* (Fabaceae-Caesalpinioideae), *Hymenocardia acida* (Euphorbiaceae), *Terminalia mantaly* (Combretaceae) (Tchuenguem et al., 2010) and *Ximenia americana* (Olacaceae) flowers (Djonwangwe et al., 2011) where *Apis mellifera adansonii* was reported as the most frequent insect. Among all bee species observed on *Ca. cajan* flowers, *Ch. rufipes* ranked first and was the main bee species harvesting pigeon pea products all day long. The same result was reported by Pando et al. (2011) for *Chalicodoma cincta cincta* on the same plant species in Yaoundé.

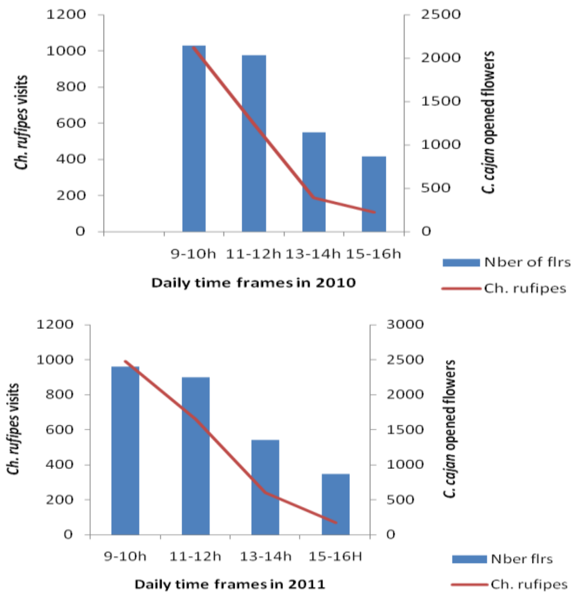

Fig. 1. Variation of number of *Cajanus cajan* flowers and *Chalicodoma rufipes* visits according to daily time frame in 2010 (A) and in 2011 (B).

The significant difference between the percentages of *Ch. rufipes* visit for the two years of study could be explained by the presence of its nests near the experimental plot.

The peak of activity of *Ch. rufipes* observed on *Ca. cajan* at the first time frame could be linked to the period of highest availability of nectar and/or pollen in this plant species flowers.

The high abundance of workers per 1000 flowers, and the positive and significant correlation between the number of *Ca. cajan* flowers and the number of *Ch. rufipes* visits, underscores the attractiveness of *Ca. cajan* nectar and pollen for *Ch. rufipes*. The attractiveness for pigeon pea nectar and pollen could be partially explained by its high production and the accessibility of these products.

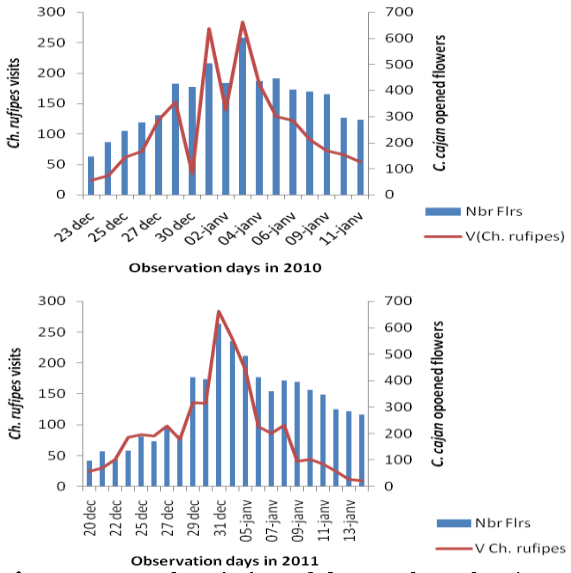

Fig. 2. Seasonal variation of the number of *Cajanus cajan* opened flowers and the number of *Chalicodoma rufipes* visits in 2010 (C) and in 2011 (D).

The significant difference observed between the duration visit during pollen harvests and that of nectar harvest could be explained by the accessibility of each of these floral products. The weight of *Ch. rufipes* played a positive role: when collecting nectar and/or pollen *Ch. rufipes* shakes flowers. This movement could facilitate the liberation of pollen by anthers, for the optimal occupation of the stigma. This phenomenon was also reported by Pando et al. in 2011 for *Xylocopa olivacea* on *Phaseolus coccineus* flowers in Yaoundé.

Impact of Chalicodoma rufipes activity on the pollination and yields of Cajanus cajan

During the collection of nectar and/or pollen on each flower, *Ch. rufipes* workers regularly come into contact with stigma and anthers. The same results were found in southwestern Brazil on *Couepia uiti* flowers (Paulino-Neto, 2007); in South Africa on *Cyrtanthus breviflorus* flowers (Glenda *et al.*, 2010); in Yaoundé on *Phaseolus coccineus* flowers (Pando et al., 2011); in Ngaoundéré on *Annona senegalensis*, *Croton macrostachyus*, *Psorospermum febrifugum* and *Syzygium guineense* var. *guineense* flowers (Tchuenguem et al., 2008), on *Ximenia americana* flowers (Djonwangwe et al., 2011), on *Phaseolus vulgaris* flowers (Kingha et al., 2012) and in Maroua on *Gossypium hirsutum* flowers (Dounia and Tchuenguem, 2014), on *Phaseolus vulgaris* and *Ricinus communis* flowers (Douka and Tchuenguem, 2013; 2014). They could thus enhance self-pollination by applying pollen of one flower on its own stigma. This is as well probable as autogamy has been demonstrated in *Ca. cajan* flowers (Pando et al., 2011). *Chalicodoma rufipes* could provide allogamous pollination through carriying of pollen with their hair, silk, legs, mouthparts, thorax and abdomen, which is consequently toughing other flowers belonging to a different plant of the same species (geitogamy). This has also been observed by others studies such as in southwestern Brazil on *Couepia uiti* flowers (Paulino-Neto, 2007); in Ngaoundéré on *Ximenia americana* (Djonwangwe et al., 2011) and in Yaoundé on *Phaseolus coccineus* (Pando et al., 2011).

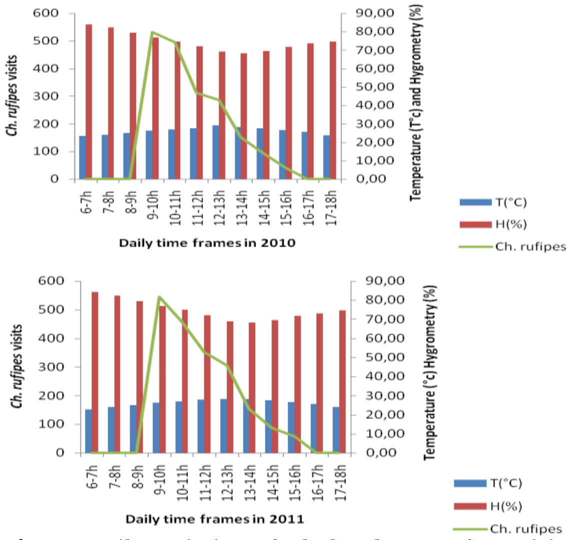

Fig. 3. Daily variation of *Chalicodoma rufipes* visits on *Cajanus cajan* flowers in 17 days in 2010 (E) and in 2011 (F), mean temperature and mean humidity of the study site.

The intervention of *Ch. rufipes* in the pollination of *Ca. cajan* is seemingly more real than its density per 1000 flowers and their foraging speed are high. Moreover, their daily period of intense activity on *Ca. cajan* flowers, which was during the first time frame, can be explained by the optimal receptivity period of the stigma of this plant species. The same observation was made in Brazil on *Adenocalymma bracteatum* flowers (Stela *et al.*, 2010).

The positive and significant contribution of *Ch. rufipes* in the fruit and seed yields of *Ca. cajan* can be justified by the action of this bee on pollination. Our results are in agreement with those obtained in Great Britain by Kendall and Smith (1976) and in the United State of America by Ibarra-Perez et al. (1999) which showed that *Phaseolus coccineus* flowers produce less seeds per pod in the absence of insect pollinators.

The numeric contribution of *Ch. rufipes* to the yields of *Ca. cajan* through its pollination efficiency was significantly higher than that of all insects on the exposed flowers. This shows that *Ch. rufipes* is one of the major insect pollinators of *Ca. cajan*. This result confirmed other findings reported by Pando et al.

(2011), Fameni et al. (2012), Kingha et al. (2012), Dounia and Tchuenguem, (2013) and Mazi et al. (2013) with *A. m. adansonii*.

Conclusion

From our observations, *Cajanus cajan* is a plant species that highly benefits from pollination by insects among which *Chalicodoma rufipes* is the most frequent pollinator which harvests nectar and pollen. The comparison of fruit and seed yields of bagged flowers, to those visited exclusively by *Ch. rufipes,* underscores the value of this bee in increasing fruit and seed yields as well as seeds quality.

The installation and/or the kept of *Ch. rufipes* nest at the proximity of pigeon pea plots should be recommended for Cameroonian farmers to increase fruit and seed yields.

Furthermore, insecticides treatments should be avoided during the flowering period of *Ca. cajan*. If these treatments are necessary, the choice of the insecticides that are less toxic for bees or the integrated pest control should be recommended to protect pollinating insects such as *Ch. rufipes*.

Acknowledgments

We would like to be grateful to the University of Ngaoundere for providing us the experimental field within the campus.

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