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Pollination and yield attributes of (cowpea) *Vigna unguiculata* L. Walp. (Fabaceae)as influenced by the foraging activity of *Xylocopa olivacea* Fabricius (Hymenoptera: Apidae) and inoculation with *Rhizobium* in Ngaoundere, Cameroon

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Key words: *Xylocopa olivacea*, *Rhizobium*, *Vigna unguiculata,* Pollination efficiency, Yields.

Abstract

To determine *Xylocopa olivacea impact* and *Rhizobium* on *Vigna unguiculata* , field trials were carried out in 2011 and 2012 during the same cropping season. Hence, 480 flowers were labeled each year and divided into five treatments, differentiated according to whether plots were inoculated with *Rhizobium* or not, or plants were protected from insects activities or not and the last treatment with flowers isolated then opened only to *X. olivacea*. The effects of inoculums on nodulation, plant biomass and seed yield, as well as the foraging behavior of *X. olivacea* on flowers, the number of seeds per pod and the normal seeds rate were evaluated. Results indicate that inoculation significantly increased the number of flowers ($p<0.0001$), root nodules ($p<0.001$), plant biomass ($p<0.0001$), pod and seeds yields in inoculated plots. *X. olivacea* foraged on *V. unguiculata* flowers from 06.00 am to 11.00 am and throughout the whole blooming period. *X. olivacea* intensely harvested nectar and pollen. By comparing the yields of unprotected flowers to those of flowers isolated then open to *X. olivacea*, 67.89 % increase fructification index, and 13.58 % increase in the number of seeds per pod due to this bee were recorded. The synergistic activity of insects and *Rhizobium* increased the number of seeds per pod by 62.28 % and the percentage of normal seeds by 87.15%. Our results reveal that inoculation of cowpea plant at sowing with *Rhizobium* and installation of *X. olivacea* nest close to the field could be recommended for a sustainable pods and seed yield improvement of this crop.

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Introduction

Cowpea, *Vigna unguiculata* (L.) Walp. is an annual plant originated from occidental Africa (Graham *et al.*, 1997). It is an economically important grain legume and cash crop with high quality protein (24%) to supplement the staple food crops in most African communities (Singh *et al.*, 2011). In the worldwide production of 3 million tons seed yield harvested from 12.5 million hectares, Africa accounts for about 68 % and Cameroon only 0.4% of this production (Singh *et al.*, 1997). Cowpea is a far from negligible item of internal trade, but it is an important component of some dietary staples in Cameroon. There is a real need to increase its production in order to improve on human nutrition. Cowpea yield are generally high (1.5 to 3 $t.ha^{-1}$), but the actual yields on small-scale farm in developing countries is just averagely 0.2 to 0.4 $t.ha^{-1}$(Murdock and Kitch, 1993). Insects pests attack, diseases and deficiency of soils in nutrients, particularly phosphorus and nitrogen are the major constraints of yields deficits. Though chemical fertilizers are often used, they are toxic to soil micro-organisms or hazardous to human (Margni *et al.,* 2002). One way to circumvent this may be the use of microbial inoculants such as rhizobia and AMF, which have a direct beneficial effect on the host plant (Arshad et Frankenberger, 1993; Denison and Kiers, 2004; Ngakou *et al.*, 2007a; 2007b; 2009).

In Cameroon, researches on African bees is increasing due to their vital importance in the pollination of food crops (Tchuenguem *et al.*, 2009a, 2009b ; Azo'o *et al.*, 2010, 2011, 2012a, 2012b ; Pando *et al.*, 2011a, 2011b ; Kingha *et al.*, 2012; Fameni *et al.*,2013, 2014). It is well known that anthophilous insects including bees usually increase fruit and seed yields of many plant species, through pollination provision (Sabbahi *et al.*, 2005, Klein *et al.*, 2007, Tchuenguem Fohouo *et al.*, 2009a).

In Cameroon investigation of the activities of pollinators' insects such as *Apis mellifera adansonii* in Ngaoundéré on *V. unguiculata* was made by Tchuenguem *et al.*, (2009).The contribution of arbuscular Mycorrhizal Fungi (AMF), Rhizobia and *metarhizium anisopliae* to cowpea production in Cameroon was investigated by Ngakou *et al.* (2007a; 2007b; 2009; 2011).

Prior to these studies, no previous research has been reported on the relationships between *Rhizobium*, anthophilous insects and yield production of *V. unguiculata*. *X. olivacea* is one of the common carpenter bees in Cameroon. During preliminary investigations on flower-insect relationships in Ngaoundere in 2009 (Tchuenguem *et al.*, 2009), *X. olivacea* was revealed to intensively visit flowers of *V. unguiculata*.

The main objective of this research was to gather more data on the relationships between *V. unguiculata*, *Rhizobium* and flower visiting insects for the optimal management of pollination services. The registration of the activity of *X. olivacea* on *V. unguiculata* flowers, the evaluation of the impact of visiting insects on pollination, pods and seeds yields of this Fabaceae, the estimation of pollination efficiency of *X. olivacea* on this plant, the estimation of impact of *Rhizobium* on cowpea and the evaluation of the impact of the cumulative action of *Rhizobium* and flowers visiting insects especially *X. olivacea* are discussed.

Material and methods

Study site, experimental plot and biological material

The experiment was carried out in the field from April to August 2011 and repeated from March to September 2012 at Dang (07°42.260' N, 13°53.585' E, 1112 m above sea level), Ngaoundéré, Cameroon.

This region is within the high altitude of Guinean savannah agro-ecological zone (Tchuenguem *et al.*, 2007). The climate is characterized by a distinct rainy season (April to October) and dry season (November to March), with an annual rainfall about of 1500 mm. The mean annual temperature is 22°C, while the mean annual relative humidity is 70% (Tchuenguem *et al.*, 2007). The animal material was represented by insects naturally present in the environment and many colonies of *Apis mellifera adansonii* Latreille

(Hymenoptera: Apidae) located at 3 km in diameter around the experimental site.

The vegetation was represented by crops, ornamental plants, hedge plants and native plants of savannah and gallery forest. The vegetation near the *V. unguiculata* field had various unmanaged and cultivated species. *Rhizobium* was produced at IRAD (The Institute of Reasearch for Agricultural Development), center of Wakwa (Ngaoundere). The plant material was represented by small brown seeds of *V. unguiculata*, with a lifecycle of 85 to 95 days was provided by IRAD of Wakwa.

Sowing and weeding

On May 4, 2011 and May 16, 2012, the experimental plot was prepared and divided into 8 subplots, each measuring 36 m^2. Four subplots were inoculated (treatment a) and four left uninoculated (treatment b). Two seeds were sown in 6 lines per subplot, each of which had 30 holes. Holes were separated 25 cm from each other, while lines were 75 cm apart. Cowpea seeds were inoculated as described by Ngakou *et al.* (2007). Weeding was performed manually as necessary to maintain plots weed-free.

Assessment of the influence of inoculation on nodulation and biomass of V. unguiculata

For each plot, 10 plants (40 plants for each treatments a and b) were labeled. Then nodules per plant were harvested at 35 days after planting (DAP), counted, sun dried, stored in envelopes, and weighed. Plants were dried in an oven at 72°C for 12 hours and weighed (Ngakou, 2007). Biomass and nodulation were evaluated on the same 40 individual plants.

Determination of the reproduction system of V. unguiculata:

On August 11th 2011, 120 flowers at the budding stage were labeled among which 60 flowers from uninoculated plot were left un-attended (treatment 1) and 60 flowers from uninoculated plot were bagged (treatment 2) to prevent visiting insects. On August 13th 2012, 120 flowers of *V. unguiculata* at the budding stage were labeled of which 60 flowers from uninoculated plot were left un-attended (treatment 3), while 60 others from uninoculated plot were bagged (treatment 4) to prevent visiting insects. For each year, ten days after shedding of the last labeled flowers, the number of pods was assessed in each treatment. The podding index was then calculated as described by Tchuenguem *et al.*, (2001): $Pi = F_2/F_1$; where F_2 is the number of pods formed and F_1 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] x 100 where PiX and PiY are respectively the podding average indexes of treatment X and treatment Y. Atr = 100 – Alr.

Evaluation of the impact of Rhizobium on yield of V. unguiculata

To evaluate the impact of *Rhizobium* on yield of *V. unguiculata* 240 flowers were labeled and isolated to form treatment 5 (2011) and 6 (2012) (like those of treatments 2 and 4) on the inoculated plots. Comparison of yields, fruiting rate, percentage of normal seeds and number of seeds per pod of lots 2 and 5 for the first year and 4 and 6 for the second year was done to evaluate the impact of *Rhizobium* on yield of *V. unguiculata*.

Estimation of the frequency of X. olivacea on V. unguiculata flowers

The frequency of *X. olivacea* on *V. unguiculata* flowers was determined based on observations on treatments 1 and 3, every day, from August 2nd to August 16th 2011 and from August 16th to August 29th 2012, from 06.00–07.00 a.m., 08.00–09.00 a.m. and 10.00– 11.00 a.m. Flowers were completely opened at 06.00 a.m. and closed before 12.00 am. By observing labeled flowers of treatments 1 and 3, all visits of insects were recorded. Specimens of all insect taxa (3 to 5 per species) were caught with an insect net on unlabeled flowers. 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 *X. olivacea* in the anthophilous entomofauna of *V. unguiculata*. In addition to the determination of the floral insects' frequency, direct observations of the foraging activity on flowers were made on insect pollinators in the experimental field. The floral products (nectar or pollen) harvested by *X. olivacea* 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 and legs. In the morning of each sampling date, the number of opened flowers was counted, while the duration of the individual flower visits was recorded (using a stopwatch) at least three times: 07.00 and 12.00 am.

Moreover, the number of pollinating visits (the bee came into contact with the stigma: Tchuenguem Fohouo, 2005), 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 (number of flowers visited by a bee per minute: Jacob-Remacle, 1989) were measured. Abundance per flower was recorded following the direct counting, on the same dates and daily periods as for the registration of the duration of visits. 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, 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 *X. olivacea* was assessed.

During each observation date, temperature and relative humidity were registered after every 30 minutes using a mobile thermo-hygrometer.

Assessment of the pollination efficiency of X. olivacea on V. unguiculata

To assess the pollination efficiency of *X. olivacea*, 120 flowers were bagged (treatment 5) in 2011 and 2012 (treatment 6) on uninoculated plot parallel to the constitution of treatment 2 and 4. Between 8 and 9 am of each observation date, the gauze bag was delicately removed from each inflorescence carrying new opened flowers and observed for up to 20 minutes. Flowers visited by *X. olivacea* were marked and new unvisited flowers opened flowers were eliminated. After this flowers were protected again.

The contribution (Frx) of *X. olivacea* in fruiting rate was calculated by the formula: Frx = {[(FrZ- FrY)/ FrZ] x 100}, where FrZ and FrY are fruiting rate in treatment 5 or 6 (bagged flowers and visited exclusively by *X. olivacea*) and treatment 2 and 4 (bagged flowers). At the maturity, pods were harvested from treatment 5 and 6 and the number of seeds per pod was counted. The mean number of seeds per pod and the percentage of normal seeds were then calculated for each treatment. The impact of *X. olivacea* on seed yields was evaluated using the above method as mentioned for fruiting rate.

Assessment of the cumulative action of insects and Rhizobium on V. unguiculata yields

This evaluation was based on the impact of both *Rhizobium* and insects on *V. unguiculata* yield. The comparison of yields (fruiting rate, mean number of seed per pod and percentage of normal seeds of treatment 7 and 8 with those of treatments 2 and 4 was assessed. The contribution of cumulative action of insects and *Rhizobium* on *V. unguiculata* in fruiting rate , mean number of seeds per pod and the percentage of normal seeds was calculated by the Frx above formula.

Data analysis

Data were subjected to 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 (χ^2) for the comparison of two percentages and ANOVA for the comparison of means of more than two samples. SPPSS statistical program was used to assess the correlation between the pod and yield parameters.

Results

Reproduction system of V. unguiculata

In each of treatments 1, 2, 3, 4 respectively in 2011 and 2012, 120 flowers were investigated. In 2011, the podding index was 0.53 for treatment 1 and 0.43 for treatment 2, while in 2012, it was instead 0.8 for treatment 3 and 0.56 for treatment 4. Hence, the allogamy rate (TC) and the autogamy rate (TA) were respectively 18.13% and 81.13% in 2011, against 30% and 70% in 2012. It appears from these results that *V. unguiculata* variety used for our experiment had a mixed mating system, allogamous and autogamous, with the predominance of autogamy over allogamy. Our results line with those of Tchuenguem *et al.*, (2009b), who reported the predominance of autogamy (79.03%) over the allogamy (20.90%) on *V. unguiculata* in Ngaoundéré.

Table 1. Number of nodules, weight of dry nodules and Plant biomass of *V.unguicalata* in uninoculated and inoculated plot in 2011 and 2012.

Years	Treat	Nodules(per/plant)	Flowers (per plot)	weight of dry nodules (g/plant)	Plant biomass (g/plant)
2011	Uninoculated	(6.8± 1.00)a	(66.47± 19.37)a	(0.030±0.06)a	(10.90±2.07)a
	Inoculated	(16.25±1.00)b	(120.23 ± 31.53)b	(0.26±0.06)b	(27.16±2.07)b
	P. value	<0.0001	<0.0001	0.01	<0.0001
	LSD (5%)	9.45	53,76	0.23	16.23
2012	Uninoculated	(7.1± 1.02)a	(90.08 ± 19.66)a	(0.033±0.06)a	(13.31±2.13)a
	Inoculated	(16.4±1.02)b	(149.75 ± 39.60)a	(0.26±0.06)b	(27.58±2.13)b
	P. value	<0.0001	<0.0001	0.01	<0.0001
	LSD (5%)	9.3	59.66	0.23	14.26

Values in the same column followed by the same letter are not significantly different at 5% level.

Therefore, it is suggested that the mating system of *V. unguiculata* may not vary from one agroecological zone to another or one studied area to another.

Effect of rhizobial inoculation on nodulation, flowers and biomass of V. unguiculata

Number and dry weight of nodules, as well as numbers of flowers and Plant biomass were assessed on *V. unguicalata* (Table 1). Plants inoculated at sowing produced a significantly higher number of nodules ($p<0.001$), greater nodule dry weight ($p<0.01$) and biomass of plant ($p<0.001$). The highest plant biomass in 2011 (27.16g) and in 2012 (27.58g) was obtained from inoculated plants. Inoculated plants also produced more flowers; although it was significant ($p<0.001$) for both years.

Root nodules were formed by both inoculated and uninoculated plants. But inoculated plant significantly formed ($p<0.001$) more nodules than the uninoculated control during the two cropping years. Similar finding were obtained after fields trials on cowpea in four agroecological zones of Cameroon: zone I: Soudano-sahelian zone, zone II: Guinea savannah zone, zone IV: Humid forest zone with monomodal rainfall and zone V: humid forest zone with bimodal rainfall (Ngakou, 2007).

Frequency of floral entomofauna of V. unguiculata

Among the 539 and 692 visits of 12 and 10 insect species recorded respectively on uninoculated flowers in 2011 and 2012, and 555 and 414 visits of 12 and 9 insect species found on inoculated flowers of *V. unguiculata*, *X. olivacea* was ranked second as the most represented insect species: on uninoculated flowers, 109 visits (20.22%) and 149 visits (21.53%) respectively in 2011 and 2012; similary,120 (21.62%) and 85 (20.53%) of its visits in 2011 and 2012 respectively, were recorded on inoculated flowers (Table 2). However, no significant difference each year in the percentage between flowers from uninoculated and inoculated plants ($\chi^2 = 0.05$; *df= 1;* $p> 0.05$, 2011); ($\chi^2 = 0.04$; *df= 1*; $p> 0.05$, 2012). Flowers of *V. unguiculata* are visited by Apidae (*A. m. adansonii* and *X. olivacea*), Coleoptera (Pentatomidae) and Megachilidae (*Chalicodoma*

cinta cinta and *Croscisaspidia chandleri*) to collect nectar and pollen. Dipteral (*M. autumnalis* and *M. domestica*), Formicidae (*Camponotus acvapimensis* and *Myrmicaria opaciventris*), Lepidoptera (*Eurema* sp.1 and *P. mathias*) and Syrphidae (*Episyrphus* sp.) were found to collect only nectar. Besides, *Apis mellifera adansonii* was the most represented insect on *V. unguiculata* flowers.

Activity of X. olivacea on V. unguiculata flowers floral rewards harvested

Floral products harvested

From our field observations and during each of the two flowering periods, *X. olivacea* foragers were found to intensively and regularly collect nectar and pollen on both inoculated and uninoculated flowers of *V. unguiculata*. On Figure 1, *X. olivacea* is shown to collect nectar on *V. unguiculata* inoculated flower. Simultaneous, nectar and pollen collection was intensive and regular (100 % of visits each year).

Table 2. Diversity of insects on *Vigna unguiculata* flowers *from* uninoculated and inoculated with *Rhizobium* in 2011 and 2012 at Dang, number and percentage of visits of different insects.

Insects			2011				2012				Total 2011 /2012	
			Uninoculated		Inoculated		Uninoculated		Inoculated			
Order	Family	Genus, species, Sub-species	n_1	P_1(%)	n_2	P_2(%)	n_3	P_3(%)	n_4	P_4(%)	nT	PT(%)
Coleoptera	Pentatomidae	(1 sp.) (P)	2	0.37	1	0.18	-		-		3	0.13
	Meloidae	*Coryna* sp. (eat flowers)	6	1.11	8	1.44	5	0.72	4	0.97	23	1.06
	Total Coloptera	8	0.74	9	0.81	5	0.72	4	0.97	26	0.59	
Diptera	Muscidae	*Musca autumnalis*(N)	3	0.56	1	0.18	-		-		4	0.18
		Musca domestica (N)	3	0.56	2	0.36	1	0.14	-		6	0.26
	Syrphidae	*Episyrphus* sp. (N, P)	-		-		2	0.29	1	0.24	3	0.13
	Total Diptera	6	0.56	3	0.27	3	0.21	1	0.24	14	0.19	
Hymenoptera	Apidae	*Apis mellifera adansonii* (N, P)	334	61.97	367	66.13	457	66.04	267	64.49	1425	64.65
		Xylocopa olivacea (N, P)	109	20.22	120	21.62	149	21.53	85	20.53	463	20.97
		Total Apidae	334	33.06	487	43.87	606	43.78	352	42.51	1888	42.81
	Formicidae	*Camponotus acvapimensis* (N)	5	0.93	4	0.72	4	0.58	5	1.21	18	0.86
		Myrmicaria opaciventris (N)	6	1.11	3	0.54	-		-		9	0.41
		Total Formicidae	11	1.02	7	0.63	4	0.58	5	1.21	27	0.86
	Megachilidae	*Chalicodoma cinta cinta* (N, P)	56	10.39	35	6.31	61	8.83	42	10.14	194	8.91
		Crocisaspidia chandleri (N, P)	9	1.67	10	1.80	5	0.72	4	0.97	28	1.29
		Total Megachilidae	65	6.03	45	4.05	66	4.77	46	5.55	222	5.1
	Total Hymenoptera		519	96.29	539	97.12	676	97.7	403	97.34	2137	97.11
Lepidoptera	Pieridae	*Eurema* sp.1 (N)	4	0.74	2	0.36	5	0.72	6	1.45	17	0.81
	Hespiridae	*Pelopidas mathias*(N)	2	0.37	2	0.36	3	0.43	-		7	0.29
	Total Lepidoptera		6	0.55	4	0.36	8	0.57	6	1.45	24	0.55
Total			539 (12)	100	555 (12)	100	692 (10)	100	414 (9)	100	2200 (13)	100

n_1,n_2, n_3 et n_4: number of visits on 120 flowers in 12 days; sp.: undetermined species; P_1,P_2, P_3 et P_4: visits percentages: P_1= (n_1/539) x 100; P_2= (n_2/555) x 100; P_3= (n_3/682) x 100; P_4= (n_4/414) x 100. Comparison of percentages visits: (*Xylocopa olivacea*$_{2011/2012}$) χ^2 = 0, 04; *P*> 0, 05; N: visitor collecting nectar; P: visitor collecting pollen; NP: visitor collecting both nectar and pollen.

Relationship between insect visits and flowering stages of the plant

The number of visits was elevated when the number of opened flowers was highest on both uninoculated and inoculated flowers (fig. 2). A positive and significant correlation was found between the number of uninoculated *V. unguiculata* opened flowers and the number of *X. olivacea* visits in 2011 (r = 0.72; df = 11; p < 0.001), and 2012 (r = 0.88; df = 11; p < 0.001), as well as for inoculated flowers in 2011 (r = 0.83; df = 9; p < 0.001) and 2012 (r = 0.76; df = 8; p < 0.05).

Diurnal flower visits

X. olivacea foraged on *V. unguiculata* flowers

throughout the flowering period, with a peak activity between 8.00 and 9.00 a.m. daily (Fig. 3). This activity was not influenced by temperature but by hygrometry. The correlation between the number of *X. olivacea* visits and relative humidity was positive and significant on uninocultated *V. unguiculata* flowers (r = 0.81; df = 3; p< 0.05) and inocultated *V. unguiculata* flowers (r = 0.74; df = 3; p< 0.05).

Table 3. Fruiting rate, mean number of seeds yield per pod and percentage of normal seeds as influenced by protection of flowers and *Xylocopa olivacea in* 2011 and 2012**.**

Years	Treatments	Flowers	Pods	Fruiting rate (%)	Seeds/Pods		Total seeds	Normal seeds	% Normal seeds
					Mean	SD			
2011	1 (unlimited visits)	60	32	53.33	14.48	2.42	467	449	96.14
	2 (bagged flowers)	60	13	21.66	5.38	2.53	87	70	80.45
2012	3 (unlimited visits)	60	37	61.66	14.88	2.49	555	536	96.57
	4 (bagged flowers)	60	14	23.33	8.64	1.69	134	121	90.29
2011	5 (*X. olivacea* visits on flowers)	120	72	60	18.65	1.47	1361	1343	98.67
2012	6 (*X. olivacea* visits on flowers)	120	83	69.16	18.66	1.50	1567	1549	98.85

Abundance of X. olivacea

In 2011, the highest mean number of *X. olivacea* simultaneously in activity was 1 per flower (n = 108; s = 0) and 111 per 1000 flowers (n = 108*;* s = 54.03*;* maxi = 300) on uninoculated flowers and 1 per flower (n = 80; s = 0) and 89 per 1000 flowers (n = 108*;* s = 40.17*;* maxi = 200) on inoculated flowers. In 2012, the corresponding values were 1 (n = 135; s = 0) and 166 per 1000 flowers (n = 135*;* s = 96.01*;* maxi = 454) on uninoculated flowers and 1 (n = 125; s = 0) and 106 per 1000 flowers (n = 125*;* s = 52.01*;* maxi = 266) on inoculated flowers. The difference between the mean number of foragers per 1000 flowers in 2011 and 2012 was highly significant (t = 60.49; p < 0.001) on uninoculated flowers, and on inoculated flowers (t = 20.48; p < 0.001).

Table 4. Fruiting rate, mean number of seeds yield per pod and percentage of normal seeds according to the uninoculated flowers (protected) and inoculated unprotected flowers (visited by insects) of *Vigna unguiculata* in 2011 and 2012.

Years	Treatments	Flowers	Pods	Fruiting rate (%)	Seeds/pods			Total seeds	Normal seeds	%Normal seeds
					n	mean	SD			
2011	2 (bagged flowers)	60	13	21,66	13	5,38	2,53	87	70	80,45
	7 (inoculated flowers open to insect)	60	32	53,33	32	18,45	1,28	575	572	99,47
2012	4(bagged flowers)	60	14	23,33	14	8,64	1,69	134	121	90,29
	8 (inoculated flowers open to insect)	60	48	80	48	18,75	1,42	916	900	98,25

Duration of X. olivacea visits per flower

In 2011, the mean duration of a flower visit was 9.31 sec (n = 82*;* s = 2.63; maxi = 15 sec) on uninoculated flowers and 9.26 sec (n = 60*;* s =2.52; maxi = 13 sec) on inoculated flowers, whereas in 2012, the visit lasted for 10.23 s (n = 117; s = 2.45; maxi = 18 sec) on uninoculated flowers and 9.86 sec (n = 160*;* s = 2.90; maxi = 17 sec) on inoculated flowers. The difference was highly significant (t = 17.47; df = 197; p < 0.001) on uninoculated plots and on inoculated plots (t = 9.30; df = 218; p < 0.001) between the two sample years. For the two cumulated years, the mean duration of a flower visit was 9.77sec on uninoculated flowers and 9.56 sec on inoculated flowers.

Foraging speed of X. olivacea on V. unguiculata flowers

In *V. unguiculata* field*, X. olivacea* visited between 5.45 and 24 flowers/ min on uninoculated plots and between 6.66 and 24 flowers/min on inoculated

flowers in 2011. In 2012, *X. olivacea* visited between 5 and 24 flowers/ min on uninoculated plotsand 5.66 and 24 flowers/min on inoculated plots. The mean foraging speed was 13.35 flowers/min (n = 165; s = 3.76) on uninoculated and 12.56 flowers/min (n = 104; s = 3.37) on inoculated flowers in 2011. In 2012, the foraging speed was 13.96 flowers/min (n = 125; s = 3.88) on uninoculated plots and 12.28 flowers/min (n = 89; s = 3.26) on inoculated plots. The difference between these means was not significant neither for uninoculated flowers (t = 2.23; p > 0.05) nor inoculated ones (t = 4.02; p > 0.05). For the two cumulated years, the mean foraging speed was 13.41 flowers/min for uninoculated plots and 12.42 flowers/min for inoculated ones.

Fig. 1. *Xylocopa olivacea* collecting nectar on a flower of *Vigna unguiculata* at Dang-Ngaoundere.

Influence of neighboring flora

During the observation period, flowers of many other plant species growing in the study area were visited by *X. olivacea* individuals, for nectar (ne) and/or pollen (po). Amongst these plants were *Arachis hypogea* (Fabaceae, ne and po), *Bidens pilosa* (Asteraceae, ne and po), *Callistermon rigidus* (Myrtaceae, ne and po), *Gossypium hirsutum* (Malvaceae, po), *Phaseolus coccineus* (Fabaceae, ne and po), *Phaseolus vulgaris* (Fabaceae, ne) and *Senna mimosoides* (Mimosaceae, ne and po). During the entire observation period, individual *X. olivacea* foraging *V. unguiculata* flowers were not observed moving to a neighbouring plant of a different species and vice versa. Foragers of *X. olivacea* were regularly interrupted by other foragers or by other bees collecting *V. unguiculata* floral products such as *Apis mellifera adansonii* (ne or po) and *Chalicodoma cinta cinta* (ne and po).

Impact of X. olivacea on pollination, pod/set and seed yields of V. unguiculata

The comparison of the fruiting rate (Table 3) shows that differences observed were highly significant between treatments 2 and 5 ($\chi 2$ = 23.58; df = 1; P < 0.001) , and treatments 4 and 6 ($\chi 2$ = 33.82; df = 1; P < 0.001). Hence, the fruiting rate of unprotected flowers (treatments 2, 2011 and treatment 4, 2012) was higher than that of flowers protected and opened exclusively to *X. olivacea* visits (treatment 5, 2011 and treatment 6, 2012).

The comparison of the mean number of seeds per fruit (Table 3) shows that the differences observed were highly significant between treatments 2 and 5 (t = 86.14; p< 0.001) and treatments 4 and 6 (t = 77.69; P < 0.001). As a matter of fact, in 2011, seed yield per fruit of flowers protected and visited exclusively by *X. olivacea* (treatment 5) was higher than those protected from insects (treatment 2). In 2012, seed yield per fruit of flowers protected and visited exclusively by *X. olivacea* (treatment 6) was higher than that of protected from insect visits (treatment 4). The comparison of the percentages of normal seeds (Table 3) shows that the differences were highly significant between treatments 2 and 5 ($\chi 2$ = 115.06; df = 1; p< 0.001) and treatments 4 and 6 ($\chi 2$ = 50.47; df = 1; P < 0.001).Thus, the percentage of normal seeds of flowers protected and visited exclusively by *X. olivacea* (treatments 5, 2011 and 6, 2012) was higher than that of flowers protected during their opening period (treatments 2, 2011 and 4, 2012).

The fruiting rate (%) due to *X. olivacea* activity was 63.90% in 2011 and 71.89% in 2012. For the two years of study, the fruiting rate percentage attributed to *X. olivacea* was 67.89%. The number of seeds per fruit (%) due to *X. olivacea* was 71.15% in 2011 and 53.69 % in 2012. For the two years of study, the number of seeds per pod (%) attributed to *X. olivacea* was

13.55%.The number of normal seeds (%) due to *X. olivacea* was 18.46% in 2011 and 8.65% in 2012. For the two years of study, the number normal seeds per pod attributable to *X. olivacea* were 13.56%. In short, the influence of *X. olivacea* on pod and seeds yields was positive and significant.

Furthermore, we found a positive and significant correlation between the number of uninoculated *V. unguiculata* opened flowers and the number of *X. olivacea* visits in 2011 (r = 0.72; df = 11; p < 0.001), and in 2012 (r = 0.88; df = 11; p < 0.001).

Cumulative impact of insects and Rhizobium on the pollination, pod and seed yields of V. unguiculata

The comparison of the fruiting rate (Table 4) shows that differences observed were highly significant between treatments 7 and 2 ($\chi 2$ = 12.84; df = 1; p< 0.001) , and treatments 8 and 4 ($\chi 2$ = 38.58; df = 1; P < 0.001). Therefore, in 2011, the fruiting rate from flowers inoculated and opened to insects (treatment 7) was higher than that of protected flowers (treatment 2) where as in 2012; the fruiting rate of the inoculated flowers opened to insects (treatment 8) was higher than that of protected flowers (treatment 4).

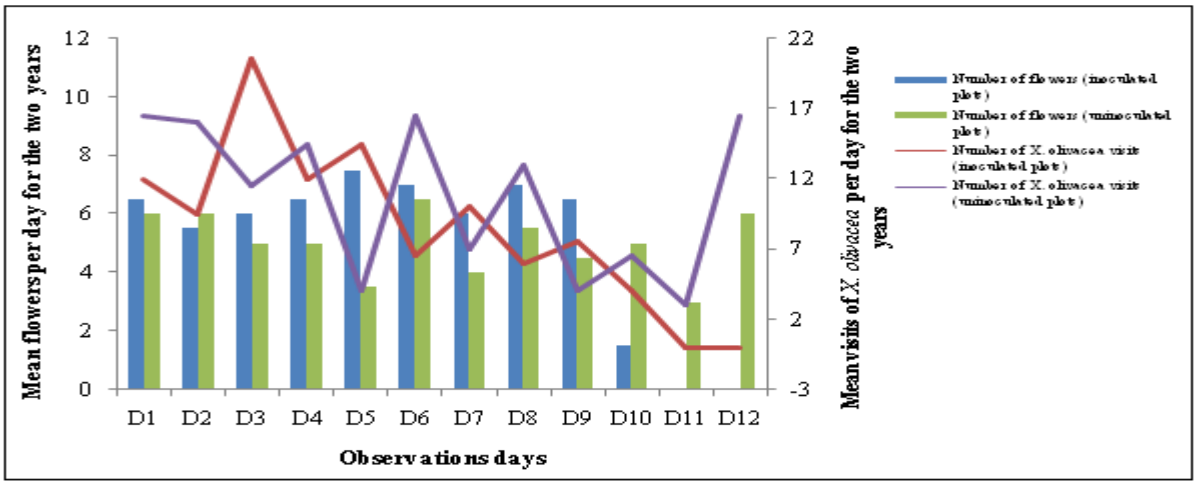

Fig. 2. Variations of the number of *Vigna unguiculata* opened flowers and the number of *Xylocopa olivacea* as a function of observation dates in 2011 and 2012 at Dang-ngaoundere.

The comparison of the mean number of seeds per fruit (Table 4) shows that the differences observed were highly significant between treatments 7 and 2 (t = 68.02; P < 0.001), as well as treatments 8 and 4 (t = 72.57; P < 0.001). Thus, in 2011, the number of seeds per fruit from flowers of inoculated plots and opened to insects (treatment 7) was higher than those protected from insect visits (treatment 2). In 2012, the number of seeds per fruit of inoculated flowers opened to insects (treatment 8) was higher than those protected from insect visits (treatment 4).

The comparison of the percentages of normal seeds (Table 3) shows that the differences were highly significant between treatments 7 and 2 ($\chi 2$ = 93.29; df = 1; p< 0.001), treatments 8and 4($\chi 2$ = 27.54; df = 1; p< 0.001). In 2011, the normal seeds (%) from flowers inoculated plots and open to insects (treatment 7) was higher than that of flowers protected from insect visits (treatment 2), while in 2012, the normal seeds (%) from flowers of inoculated plots and opened to insects (treatment 8) was higher than that of flowers protected from insect visits (treatment 4).

The fruiting rate (%) due to cumulative effect of insects and *Rhizobium* activity was 53.38% in 2011 and 71.25% in 2012. For the two years of study, the fruiting rate (%) attributed to the influence of both insects and *Rhizobium* was 65.31%.

The number of seeds per fruit (%) due to cumulative impact of insects and *Rhizobium* was 70.84% in 2011 and 53.92 % in 2012. For the two years of study, the percentage of the number of seeds per pod attributed to the cumulative influence of insects and *Rhizobium*

was 62.38%.The cumulative effect of insects and *Rhizobium* on normal seed was 87.76% in 2011, 86.55% in 2012, and 87.15% for the two cumulative years. Hence, the cumulative influence of insects and *Rhizobium* on pod and seeds yields was positive and highly significant.

Discussion

Rhizobia including *Rhizobium* and *Bradyrhizobium* are best known as biological nitrogen fixers in root nodules of legumes (Kiers *et al.*, 2003; Denison and Kiers, 2004; Ngakou *et al.,* 2007). In the Guinea-savannah zone of Cameroon, the number of nodule formed by cowpea was already reported to be low in the absence of inoculation (Ngakou, 2007), with nodules starting to degenerate as from 45 days after planting. The positive correlations between nodule and plant dry weight was in accordance with findings of other authors (Thiagarajan *et al.*, 1992; Hungria *et al.*, 2001). There was a significant correlation between nodulation and the plant biomass, supporting the improved nitrogen fixation potential of the host grain legume that usually lead to increased soil fertility(Ngakou *et al.*, 2008).

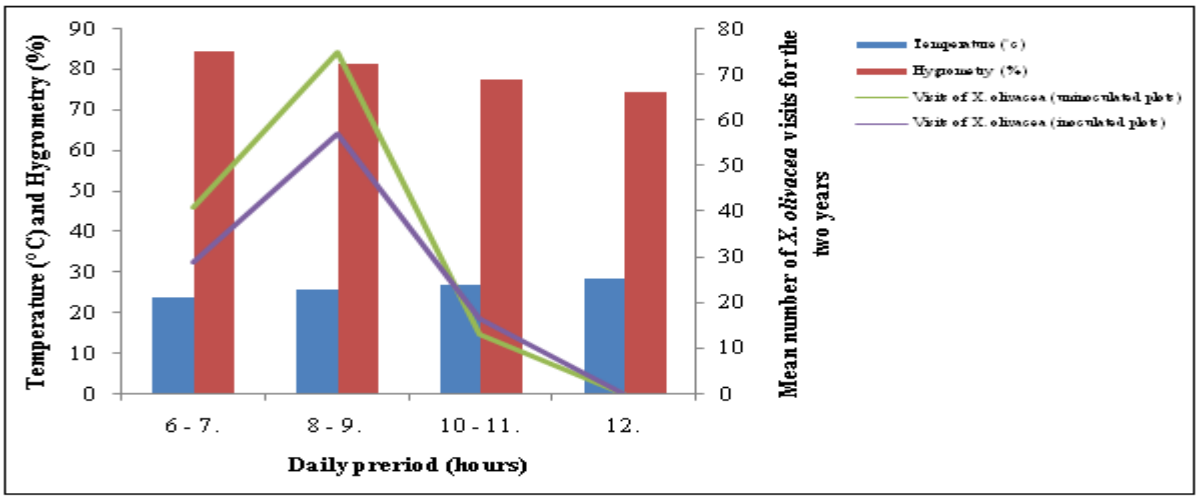

Fig. 3. Daily distribution of *Xylocopa olivacea* visits on 120 *Vigna unguiculata* flowers over 12 days in 2011 and 2012 as influenced by mean temperature and humidity of the study site at Dang-Ngaoundere.

A. mellifera adansonii was the most preponderant, and *X. olivacea* was the second insect on cowpea at Dang-cameroon. This result confirm those already reported by Tchuenguem *et al*. (2009b),who revealed *A. mellifera adansonii* as the most frequent insect on flowers of the same plant. However, this result did not confirm those of Pando *et al.,* (2013) who found *X. olivacea* as the most preponderant insect in Yaounde . Previous researches have revealed *X. olivacea* foraging on *V. unguiculata* (Pando *et al.,* 2011), *Luffa aegyptiaca* (Mensah & Kudom, 2011), *Phaseolus vulgaris* (Kingha *et al.,* 2012) and *Phaseolus coccineus* (Fameni *et al*., 2012). *X. olivacea* foraged *V. unguiculata* flowers for nectar and pollen. *X. olivacea* has been reported as the main floral visitors of this crop (Pando *et al*., 2013) in Yaounde. This rank could be due to relative abundance of *X. olivacea* in different study areas, though the abundance and diversity of floral insects to a plant varies depending on the region (Roubik, 2000). The significant difference between the percentages of floral visits by *X. olivacea* during the two study years and the percentages of *X. olivacea* visits could be explained by the presence of their natural nests close to the experimental plot. It is known that the anthophilous insects of a plant vary over time (Elfawal *et al*., 1976; Tchuenguem, 2005). The peak of *X. olivacea* activity on flowers in the morning corresponds to the period of high availability of pollen and nectar on *V. unguiculata* flowers. The decreased in the activity of *X. olivacea* between 11.00 a.m. could be related to elevated temperature and the closure of flowers in the experimental field. Although, foragers preferred warm or sun rays for maximum floral activity (Kasper

et al., 2008), high temperature negatively affect insect activity on foraged flowers. Similarly, rainfall has been documented as an environmental factor that can disrupt the floral insect activity (McGregor, 1976).The abundance of *X. olivacea* foragers on 1000 flowers, as well as the positive and highly significant correlation between the number of *V. unguiculata* flowers at bloom and the number of *X. olivacea* visits indicates the attractiveness of *V. unguiculata* nectar and pollen with respect to this bee. In fact, weather conditions during bloom were revealed to affect the abundance and foraging of pollinator insects as *Apis mellifera Ligustica* and *Bombus impatiens* in Michigan (Julianna and Rufus, 2010).The significant difference between the duration of visits in 2011 and 2012 could be attributed to the availability of floral products or the diversity of flowering insects from one year to another. During each of the two flowering periods of the host plant, *X. olivacea* intensely and regularly harvested nectar and pollen. This could be linked to the needs of individuals at the flowering period. The disruptions of visits by other insects reduced the duration of certain *X. olivacea*. This has obliged some bees to visit more flowers during a foraging trip in order to maximize their pollen loads. Similar observations were made for *Chalicodoma cincta cincta* (Hymenoptera: Megachilidae) foraging on *Cajanus cajan* (Fabaceae) flowers (Pando *et al*., 2011b), *Xylocopa olivacea* workers foraging *Phaseolus vulgaris* of Small Black Seeds flowers (Kingha *et al*., 2012), and *Xylocopa calens* foraging on flowers of *P. coccineus* (Pando *et al*.,2011a). *X. olivacea* foragers had a high affinity with respect to *V. unguiculata* when compared to the neighboring plant species, indicating their faithfulness to this fabaceae, a phenomenon known as "floral constancy" (Louveaux,1984; Backhaus, 1993; Basualdo *et al*., 2000). Flower constancy is an important aspect in the management of pollination. For this research, it indicates that *X. olivacea* can provide benefits to pollination management of *V. unguiculata*. During the collection of nectar and pollen on each flower, *X. olivacea* individuals regularly come into contact with the stigma. They were also able to carry pollen with their hairs, legs and mouth accessories from a flower of one plant to stigma of another flower of the same plant (geitonogamy) and to the same flower (autogamy). The foragers might thus have influenced self- pollination and cross-pollination as early reported (Moffett *et al*., 1975; Rao *et al*., 1969).

The significant contribution of *X. olivacea* in pod and seed yields of *V. unguiculata* lines with findings of Pando *et al.* (2013). This high productivity of pod and seeds in unlimited visits when compared with bagged flowers showed that insect visits were effective in increasing cross-pollination. Our results confirmed those of Pando *et al*., (2013) at Yaounde and Kingha *et al*., (2012) in Ngaoundere who revealed that *V. unguiculata* and *P. vulgaris* flowers set little pods in the absence of insect pollinators. The contribution of *X. olivacea* to *V. unguiculata* production through its pollination efficiency was significantly higher than that of all insects visiting the exposed flowers. The weight of *X. olivacea* might have played a positive role during nectar and pollen collection. In fact, *X. olivacea* shook flowers when landing on plants, facilitating the liberation of pollen by the anthers for optimal occupation of the stigma. Similar experiments in England (Free, 1966) and in Brazil (Free, 1993) have shown that pollination by insects was not always needed. Thus, pollination requirements might differ between regions or countries.

In our experiment, the uses of both pollinating insects and *Rhizobium* have highly improved the seeds and pods yields of *V. unguiculata***.** Insects have facilitated the liberation of pollen from anthers for optimal occupation of the stigma, thus increasing pollination (Mensah and Kundom, 2011), while *Rhizobium* have improved nitrogen fixation potential of the host grain legume that usually lead to increased plant growth and yield (Ngakou *et al*., 2007). Our result are supported by those of Summers and Mondor (2011) who have predicted that bean plants harboring *Rhizobium leguminosarum* would produce more extrafloral nectar (EFN) upon leave damage than plants lacking symbionts.

Conclusion

From our observations, *Vigna unguiculata* is a plant species that highly benefits from pollination by insects, of which is *Xylocopa olivacea* that harvests nectar and pollen. *Xylocopa olivacea* foragers regularly contacted anthers and carried pollen. The comparison of the pod and seed sets of unprotected flowers with those of flowers exclusively visited by *X. olivacea* underscores the value of this bee in increasing pod and seed yields, as well as improving seed quality. Futhermore, the comparison of pod and seeds sets of uninoculated and bagged flowers with those of flowers inoculated and visited by insects indicates the value of cumulative activity of insects and *Rhizobium* in increasing pod and seeds yields. Our results suggest that sowing of *V. unguiculata* seeds with *Rhizobium* and the installation of *X. olivacea* nests close to *V. unguiculata* fields could be encouraged to significantly improve the pods and seeds production of this valuable crop, and keep *X. olivacea* population active in the environment.

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