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Diversity and impact of insects on pod and seed production of *Vigna subterranea* (L.) Verdcourt white variety (Fabaceae) at Ngaoundere (Cameroon)

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Abstract
Experiments were conducted to study the biodiversity of the floral entomofauna of *Vigna subterranea* and assess its impact on yields. Two treatments of 240 plants were differentiated according to the presence or the absence of their protection regarding insect visits. The insect species richness, the relative abundance, the frequency of visits were recorded and yields were compared between treatments. In 2013 and 2014, 1402 and 1622 visits of seven insect species were recorded on *V. subterranea* flowers respectively. *Paragus borbonicus* was the most frequent insect species, followed by *Eurema eximia*. The comparison of yields of the two treatments showed that insects significantly increased the podding rate by 62.05%, as well as the number of seeds per pod by 15.98% and the percentage of normal seeds by 18.11%. This study provided some knowledge about the anthophilous insect's diversity of this Fabaceae, which can be exploited to improve pod production.

Keywords: *Vigna subterranea*, *Eurema eximia*, *Paragus borbonicus*, flowers, yield, pollination

1. Introduction

Bambara groundnut (*Vigna subterranea* (L.) Verdcourt) is an indigenous African leguminous crop which has been cultivated in the tropical regions of sub-Saharan Africa and Madagascar for many centuries [1]. It is the third most important legume crop after cowpea and groundnut [2, 3] described bambara groundnut seeds as a complete balanced diet, making it a good supplement to cereal-based diets. Nutritionally, it contains sufficient quantities of proteins (19%), carbohydrates (63%), fats (6.5%) and essential amino acids [4] and minerals like calcium (95.5-99 mg/100 mg) and iron (5.1-9 mg/100 mg) [5]. The fresh seeds may be boiled and eaten; the dry seeds may be ground into flour and made into paste [6], then fried and eaten [7-9].

In Cameroon, *V. subterranea* is cultivated mainly in the Northern Region for food and income [10, 11]. Currently, the production of *V. subterranea* in this country is low, whereas the demand for its seeds is high [12]. Therefore, it is important to investigate on the possibilities of increasing the production of this valuable plant in the country. This can be done sustainably if flowering insects of *V. subterranea* in this region are well known and exploited [13].

The role of pollinators for many plant species is well known throughout the world and their activities are essential to ecosystem functioning and agriculture [14]. In Cameroon, research on anthophilous insects of many plants species has been increased because of their vital importance in the pollination of food crops [15, 16]. Prior to this study in the Adamawa Region, no previous research on the relationships between anthophilous insects and yield production of the white variety *V. subterranea* has been reported.

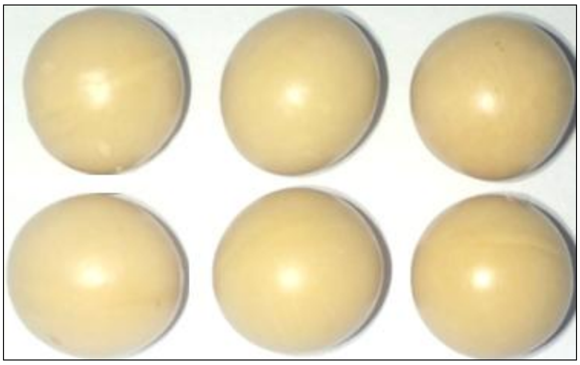
The main objective of this research was to gather more data on the relationships between *V. subterranea* and flower visiting insects, for the optimal management of the pollination services. The registration of the activity of insects on *V. subterranea* white variety flowers, the evaluation of the impact of flower visiting insects on pollination, pod and seed yields of this Fabaceae are addressed.

2. Materials and methods

2.1 Study site, experimental plot and biological material

The experiment was carried out from May to August 2013 and 2014 at Dang, within the experimental field of the Unit for Apply Apidology (latitude 07°42.26'N, longitude 13°53.94'E

and altitude 1106 m above sea level) of the Faculty of Science, University of Ngaoundere, Cameroon. The site belongs to the high altitude Guinean savannah agroecological zone [17]. The climate is characterized by a rainy season (April to October) and a dry season (November to March), with an annual rainfall of approximately 1500 mm. The mean annual temperature is 22°C, while the mean annual relative humidity is 70% [17]. The animal material was mainly represented by insects naturally present in the environment and 48 colonies of *A. mellifera* Linnaeus (Hymenoptera: Apidae) located close to the experimental field. The flora surrounding *V. subterranea* field had various unmanaged and cultivated species.
Vigna subterranea seeds of white variety (Figure 1) were provided by the Institute of Research for Agricultural Development (IRAD) at Garoua.



1 cm

Fig 1: *Vigna subterranea* white variety seeds.

2.2 Sowing and weeding

In April 4 and April 2 respectively in 2013 and 2014, the experimental plot was prepared and divided into 8 subplots (8 m long * 4.5 m wide each). In May 5 and 2 May respectively, one seed was sown per hole in 20 lines per subplot. Each line had 18 holes. Holes were separated 40 cm from each other, while lines were 20 cm apart, according to the technical sheet of Institute of IRAD. Weeding was performed manually as necessary to maintain subplots weed-free.

2.3 Determination of the reproduction mode of *Vigna subterranea*

From June to July, 2013, eight subplots carrying 240 plants were labeled among which 120 plants with 4320 flowers were left opened to insects visit (treatment 1) (Figure 2) while 120 other plants with 3960 flowers were bagged using gauze bags (1 mm^2 mesh) to prevent visiting insects (treatment 2) (Figure 3). Between June and July 2014, the experiment was repeated. In treatment 3, there were eight subplots carrying 120 plants with 4989 flowers, while in treatment 4, the eight subplots with 120 plants had 3023 flowers. For each cropping year, thirty days after shading of the last flower, the number of pods was assessed in each treatment. The podding index (*Pi*) was then calculated as described by [18]: $Pi = F_b/F_a$, where F_b is the number of pods formed and F_a the number of viable flowers initially set. The allogamy rate (*TC*) from which derives the autogamy rate (*TA*) was expressed as the difference in podding indexes between treatment *X* (unprotected flowers) and treatment *Y* (bagged flowers) [19]. $TC = [(PiX - PiY) / PiX] * 100$, where *PiX* and *PiY* are respectively the podding average indexes of treatment *X* and treatment *Y*. $TA = 100 - TC$.

Fig 2: Plant of *Vigna subterranea* at left in free pollination.

Fig 3: Plant of *Vigna subterranea* at protected from insects.

2.4 Assessment of the foraging activity of insects on *Vigna subterranea* flowers

Observations were conducted on flowers of treatments 1 and 3, each day, from Juin 15th to July 12th 2013 and from Juin 14th to July 18th 2014 at 09.00 - 10.00 am, 11.00 - 12.00 am, 13.00 - 14.00 pm and 15.00 - 16.00 pm. All insect visits were recorded onthe flowers each of these treatments. Specimens of all insect taxa (3 to 5 per species) were caught on flowers using an insect net, conserved in 70% ethanol, except Lepidoptera that were kept in curls for subsequent taxonomy determination [20]. All insects encountered on flowers were registered and the cumulated results expressed in number of visits to determine the relative frequency of each insect in the anthophilous entomofauna of *V. subterranea* [21].
In addition to the determination of the relative frequency of all insect visitors, direct observations of the foraging activity on flowers were made on insect fauna in the experimental field. The floral rewards (nectar or pollen) harvested by each

insect species during each floral visit were registered based on its foraging behavior. Nectar foragers were expected to extend their proboscis or head to the base of the corolla and the stigma, while pollen gatherers were expected to scratch the anthers with their mouth ports or legs [22]. On each sampling day, the number of opened flowers was counted in each treatment. The same days as for the frequency of visits, the duration of individual flower visits was recorded (using a stopwatch) at least three times during each of the following daily time frames: 10.00 - 11.00 a.m., 12.00 a.m. - 13.00 p.m. and 14.00 a.m.-15 p.m.

Moreover, the number of pollinating visits, the abundance of foragers [23] and the foraging speed referring to the number of flowers visited by an insect per minute [24] were evaluated. Abundance per flower was recorded by direct counting on the same dates and during the same daily periods as for the recording of the duration of visits. For determining the abundance per 1000 flowers (A_{1000}), some foragers were counted on a known number of flowers. $A_{1000} = ((Ax/Fx)*1000)$, where Fx and Ax are the number of opened flowers and the number of insects effectively counted on these flowers at time x [23]. The foraging speed Vb was calculated by the formula: $Vb = (Fi/di)*60$, where di is the time (sec) given by a stopwatch, and Fi, the number of flowers visited during di. The disruption of the activity of individual foraging insects by competitors or predators and the attractiveness of other plant species to *Eurema eximia* and *Paragus borbonicus* were assessed. At each observation date, every 30 minutes, ambient temperature and relative humidity were recorded using a portable thermo-hygrometer (HT-9227).

2.5 Evaluation of the apicultural value of *Vigna subterranea*

The apicultural value of *V. subterranea* was assessed as in other plant species [23, 25, 26], using data on the plant flowering intensity and the attractiveness of *Apis mellifera* workers with respect to nectar and pollen.

2.6 Assessment of the effects of anthophilous insects on *Vigna subterranea* yields

The comparison of yields (podding rate, mean number of seeds per pod and percentage of normal seeds) of treatment 1 and 3 to those of treatments 2 and 4 were assessed.

2.7 Data analysis

To analyze the data we used Microsoft Excel 2010 and tree tests: Student's (t) for comparison of means of two samples, Pearson correlation coefficient (r) for the determination of the linear relationship between two variables and Chi-square ($\chi 2$) for the comparison of percentages.

3. Results

3.1 Reproduction mode of *Vigna subterranea*

The podding indexes of *V. subterranea* were 0.69, 0.26, 0.71 and 0.26 for treatments 1, 2, 3, and 4 respectively (Table 1). Thus, in 2013 allogamy rate was 62.31%, whereas autogamy rate was 37.69%. In 2014, the corresponding figures were 63.38% and 36.61%. For the two cumulative years, the allogamy rate was 62.84% and the autogamy rate was 37.15%. Consequently, the *V. subterranea* white variety used in our experiment has a mixed autogamous-allogamous reproduction mode with the predominance of allogamy.

Table 1: Allogamy and autogamy rates of *Vigna subterranea* in 2013 and 2014 at Dang.

Years	Traitments	Number of flowers	Number of pods	Podding index	Allogamous rate (%)	Autogamous rate (%)
2013	1 (Unprotected plants)	4320	2983	0.69	62.31	37.69
	2 (Bagged plants)	3960	1036	0.26		
2014	3 (Unprotected plants)	4989	3577	0.71	63.38	36.61
	4 (Bagged plants)	3023	813	0.26		

3.2 Frequency of floral entomofauna of *Vigna subterranea*

Among the 1402 and 1622 insects visits of 6 and 7 insect species recorded on 4320 and 4989 flowers in 2013 and 2014 respectively, *Paragus borbonicus* and *Eurema eximia* were the most represented insect species with 467 visits (33.30%) and 532 visits (37.94%) in 2013 respectively. The correspondind figures were 438 visits (27%) and 350 visits (21.57%) in 2014 (Table 2).

Table 2: Diversity of insects visiting *Vigna subterranea* in 2013 and 2014 at Dang, number and percentage of visits of different insects.

Insects			2013		2014		Total$_{2013/2014}$	
Order	Familly	Genus, Species	n_1	P_1 (%)	n_2	P_2 (%)	n_T	P_T (%)
Hymenoptera	Apidae	*Apis mellifera* (ne)	35	2.46	89	5.48	124	4.10
	Halictidae	*Lasioglossum* sp. (po)	-	-	219	13.50	219	7.24
		Total Hymenoptera	35	2.46	308	18.98	343	11.34
Diptera	Syrphidae	(sp. 1) (po)	143	10.19	159	9.80	302	9.98
		(sp. 2) (po)	133	9.48	149	9.19	282	9.32
		Paragus borbonicus (po)	467	33.30	438	27.00	905	29.92
		Total Diptera	743	52.97	746	45.99	1489	49.22
Lepidoptera	Pieridae	*Eurema eximia* (ne)	532	37.94	350	21.57	882	29.16
	Lycaenidae	(sp. 1) (ne)	92	6.56	218	35.01	310	10.25
		Total Lepidoptera	624	44.50	568	31.68	1192	39.41
Total		Visits	1402	100	1622	100	3024	100
		Species	6		7		7	

sp.: species not determined; n_1: cumulated number of visits of an insect on 4320 flowers; n_2: cumulated number of visits of an insect on 4989 flowers; n_T: total number of visits; P: percentage of visits; $P_1 = (n_1/1402) * 100$; $P_2 = (n_2/3024) * 100$; ne: collection of nectar; po: collection of pollen.

3.3 Activity of insects on *Vigna subterranea* flowers

The abundance, the foraging speed and the duration of insect visits shall be focused on *Apis mellifera* and the two major flower visiting insects, *Eurema eximia* and *Paragus borbonicus*.

3.3.1 Floral products harvested
Flowers of *V. subterranea* were visited by Halictidae (*Lasioglossum*), Syrphidae (*Paragus borbonicus* and two unidentified species) to collect pollen. Pieridae (*Eurema eximia*), Lycanidae (sp.1) and Apidae (*Apis mellifera*) were found to collect only nectar.

3.3.2 Relationships between insect visits and flowering stages of the plant
Overall, insect visits were more numerous on treatments 1 and 3 when the number of open flowers was highest (Figure 4). A positive and significant correlation was found between the number of opened flowers and the number of insect visits in 2013 (r = 0.94; df = 27; P<0.001) as well as in 2014 (r = 0.97; df = 34; P<0.001).

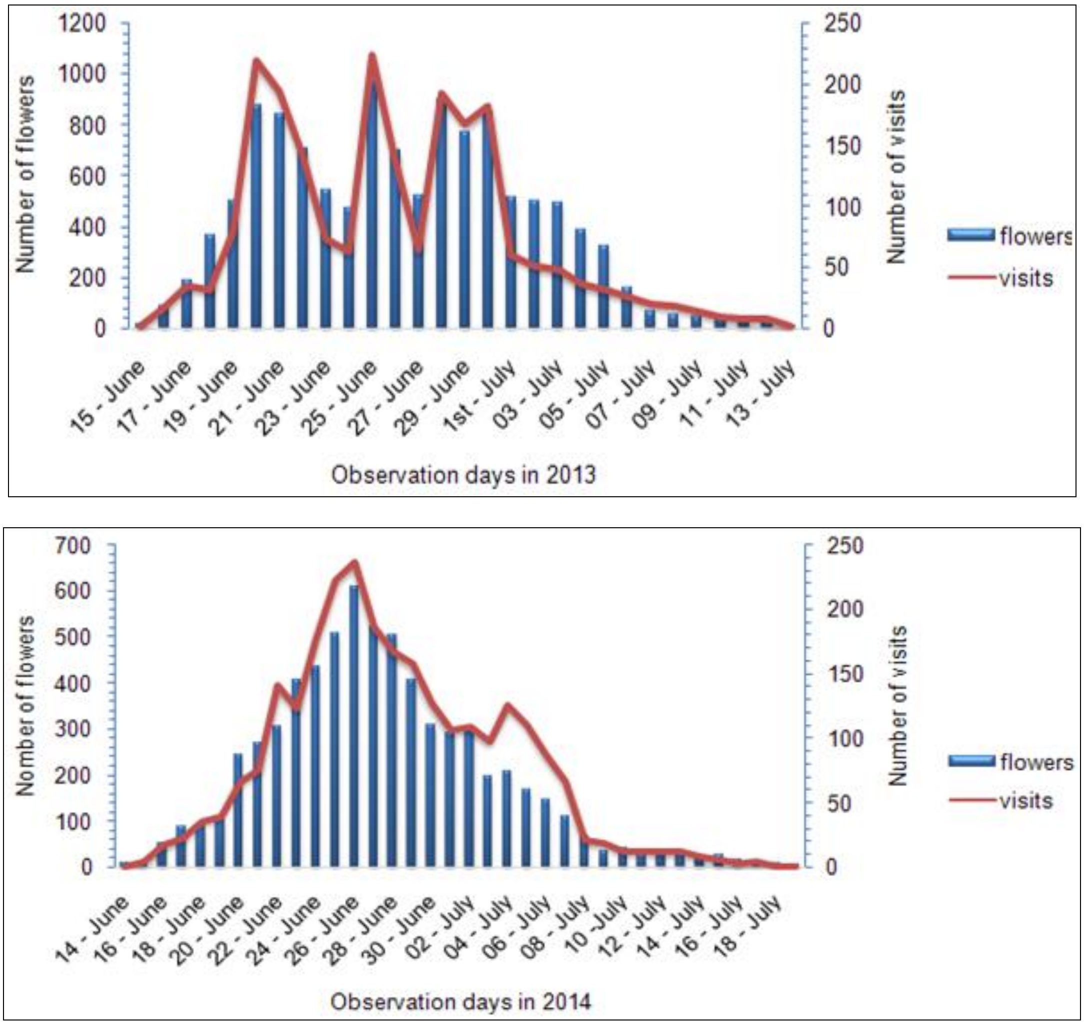


Fig 4: Variations of the number of *Vigna subterranea* opened flowers and the number of insect visits according to the observation days in 2013 (A) and 2014 (B) at Dang.

3.3.3 Daily rhythm of visits
Insects foraged on *V. subterranea* flowers daily and throughout the flowering period, with a peak of activity between 10.00 a.m. and 11.00 a.m. (Tables 3 and 4). The correlation between the number of insects visits and the temperature was not significant in 2013 (r = -0.74; df = 2; P>0.05) and in 2014 (r = -0.50; df = 2; P>0.05). Concerning the relative humidity, the correlation between this parameter and the number of insect visits was equally not significant in 2013 (r = 0.59; df = 2; P>0.05) and in 2014 (r = 0.23; df = 2; P>0.05) (Figure 5).

Table 3: Number and frequency of insect visits on flowers of *Vigna subterranea* according to the daily period of observation in 2013 at Dang.

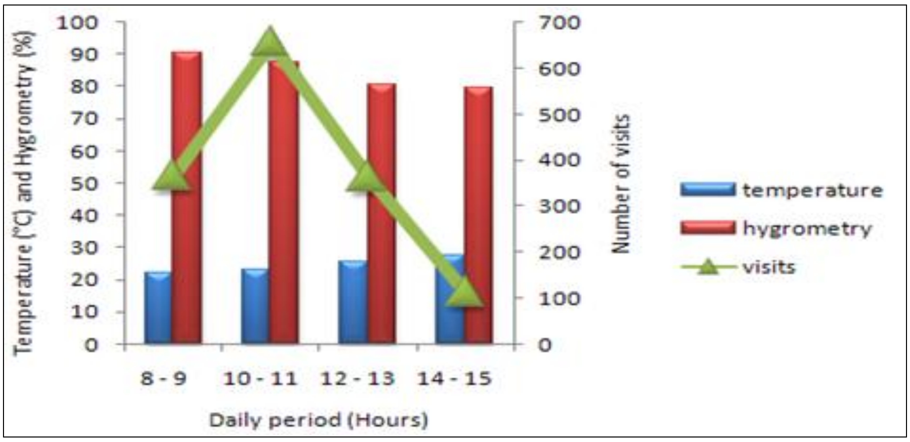
Insects	Daily period (hours)								
	8 - 9		10 - 11		12 - 13		14 - 15		A
	n	***P* (%)**	***n***	***P* (%)**	***n***	***P* (%)**	***n***	***P* (%)**	
Apis mellifera	13	37.14	17	48.57♣	10	28.57	5	14.28	35
Syrphidae (sp. 1)	35	24.47	57	39.86♣	34	23.77	17	11.88	143
Syrphidae (sp. 2)	25	18.79	51	38.34♣	43	32.33	14	10.52	133
Paragus borbonicus	117	25.05	167	35.76♣	128	27.40	55	11.77	467
Eurema eximia	112	21.05	197	37.03♣	159	29.88	64	12.03	532
Lycaenidae (sp. 1)	21	22.82	60	65.21♣	9	9.78	2	2.17	92
Total	323	23.03	549	39.15♣	383	27.31	157	11.19	1402

n: number of visits in 36 days, *p*: percentage of visits, *p* = (*n*/A) * 100, *A*: total number of insect visits, ♣: daily peak of visits.

Table 4: Number and frequency of insect visits on flowers of *Vigna subterranea* according to daily period of observation in 2014 at Dang.

Insects	Daily period (hours)								
	8 - 9		10 - 11		12 - 13		14 - 15		A
	n	***P* (%)**	***n***	***P* (%)**	***n***	***P* (%)**	***n***	***P* (%)**	
Apis mellifera	28	31.46	31	34.83♣	23	25.84	7	7.86	89
Lasioglossum	24	10.95	97	44.29♣	51	23.28	47	21.46	219
Syrphidae (sp. 1)	19	11.94	60	37.73♣	41	25.78	39	24.52	159
Syrphidae (sp. 2)	17	11.40	41	27.51	53	35.57♣	38	25.50	149
Paragus borbonicus	103	23.51	91	20.77	153	34.93♣	91	20.77	438
Eurema eximia	73	20.85	151	43.14♣	81	23.14	45	12.85	350
Lycaenidae (sp. 1)	65	29.81	103	47.24♣	30	13.76	20	9.17	218
Total	329	20.28	574	35.38♣	432	26.63	287	17.69	1622

n: number of visits in 34 days, *p*: percentage of visits, $p = (n/A) * 100$, *A*: total number of visits, ♣: daily peak of visits.



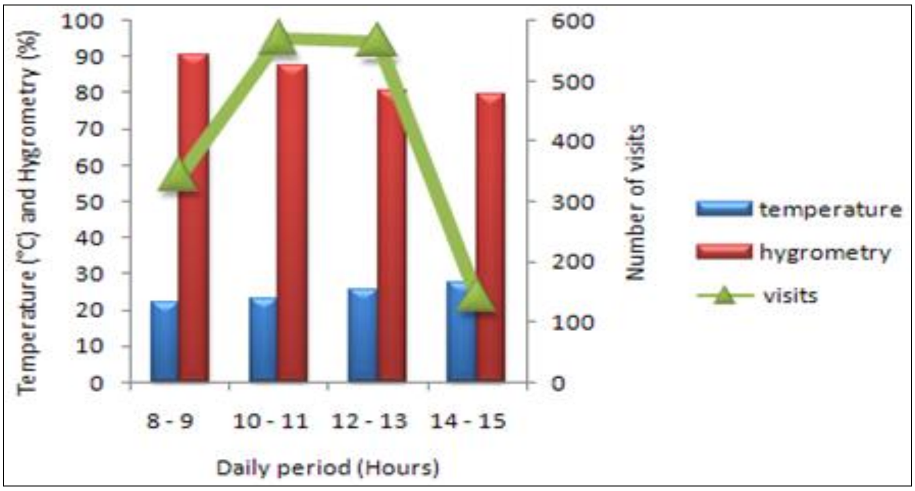


Fig 5: Daily distribution of insect visits on *Vigna subterranea* flowers over 36 days in 2013 (A) and 34 days in 2014 (B) as influenced by mean temperature and mean humidity at Dang.

3.3.4 Abundance of foraging insects

In 2013, the highest mean number of *E. eximia* simultaneously in activity was 1 per flower and 67.65 per 1000 flowers (Table 5). In 2014, the corresponding values were 1 per flower and 31.22 per 1000 flowers. The highest mean number of *P. borbonicus* simultaneously in activity in 2013 was 1 per flower and 67.59 per 1000 flowers; in 2014, the corresponding values were 1 per flower and 106.07 per 1000 flowers. For *Apis mellifera*, in 2013, the highest mean number of foragers simultaneously in activity was 1 per flower and 8.89 per 1000 flowers; in 2014, the corresponding values were 1 per flower and 5.23 per 1000 flowers.

Table 5: Abundance of insect on flowers of *Vigna subterranea* at Dang in 2013 and 2014.

Insects	Years	*n*	Abundance per 1000 flowers				
			m	***sd***	***maxi***	***mini***	**Comparison of means**
Apis mellifera	2013 2014	11 8	8.89 5.23	3.40 2.25	13.97 7.23	2.87 1.83	$t = 5.39$ ($df = 17$; $P<0.001$)
Paragus borbonicus	2013 2014	150 105	67.65 106.07	67.59 29.77	240.99 70.63	4.21 41.70	$t = -42.78$ ($df = 253$; $P<0.001$)
Eurema eximia	2013 2014	96 146	76.01 31.22	50.62 21.96	171.63 101.79	4.75 4.22	$t = 74.44$ ($df = 240$; $P<0.001$)

n: sample size; *m*: mean; *sd*: standard deviation; *maxi*: maximum; *mini*: minimum.

3.3.5 Duration of insect visits per flower
In 2013, the mean duration of one *E. eximia* visit on a flower of *V. subterranea* was 5.22 sec. In 2014, the corresponding values were 3.66 sec. The mean duration of *P. borbonicus* visit on flowers was 3.84 sec in 2013 and 2.98 sec in 2014. For *Apis mellifera* foragers the mean duration of a visit was 13.80 in 2013 and 11.89 in 2014 (Table 6).

Table 6: Duration of insect visits on *Vigna subterranea* flower at Dang in 2013 and 2014

Insects	Years		Duration of visits per flower				
		n	*m*	*sd*	*maxi*	*mini*	Comparison of means
Apis mellifera	2013 2014	35 89	13.80 11.89	14.71 11.36	16 14	1 1	$t = 3.83$ ($df = 122$; $P<0.001$)
Paragus borbonicus	2013 2014	421 411	3.84 2.98	2.20 1.48	10 7	1 1	$t = 95.06$ ($df = 830$; $P<0.001$)
Eurema eximia	2013 2014	486 323	5.22 3.66	2.76 2.35	13 9	1 1	$t = 116.09$ ($df = 807$; $P<0.001$)

n: sample size; *m*: mean; *sd*: standard deviation; *maxi*: maximum; *mini*: minimum.

3.3.6 Foraging speed of insects on *Vigna subterranea* flowers
In 2013, the mean foraging speed of *E. eximia* was 13 flowers/min. In 2014, the corresponding values were 11.32 flowers/min. The mean foraging speed of *P. borbonicus* was 12.92 flowers/min in 2013 and 10.62 flowers/min in 2014. The mean foraging speed of *Apis mellifera* was 11 flowers/min in 2013 and 10.92 flowers/min in 2014 (Table 7).

Table 7: Foraging speed of insects on *Vigna subterranea* flower at Dang in 2013 and 2014.

Insects	Years	Foraging speed (flowers/min)					
		n	*m*	*sd*	*max*	*mini*	Comparaison des moyennes
Apis mellifera	2013 2014	33 44	11 10.92	3.55 4.99	23.33 36.00	6.08 1.06	$t = 0.33$ ($df = 75$; $P>0.001$)
Paragus borbonicus	2013 2014	95 156	12.92 10.62	4.91 5.01	36.00 28.42	4.86 2.72	$t = 27.20$ ($df = 249$; $P<0.001$)
Eurema eximia	2013 2014	102 149	13.00 11.32	9.42 3.72	54.00 26.56	1.00 4.29	$t = 15.22$ ($df = 249$; $P<0.001$)

n: sample size; *m*: mean; *sd*: standard deviation; *maxi*: maximum; *mini*: minimum

3.3.7 Influence of wildlife
Eurema eximia and *P. borbonicus* were disturbed in their foraging activity by other arthropods, which were either predators or competitors for the search of pollen or nectar. These disturbances have resulted in the interruption of certain visits. For 486 and 323 visits of *E. eximia*, 93 (19.13%) and 90 (27.86%) were interrupted by the same insect and other arthropods in 2013 and 2014 respectively. For 421 and 411 visits of *P. borbonicus*, 4 (2.7%) and 114 (27.73%) were interrupted by the same insect and other arthropods in 2013 and 2014 respectively. For their load of pollen or nectar, individuals who suffered such disturbances were forced to visit more flowers and / or plants during the corresponding foraging trip. In pollen foragers, these disturbances resulted in partial loss of carried pollen. During the study periods, workers of *A. mellifera* were not disturbed in their foraging activity on *V. subterranea*.

3.3.8 Influence of neighboring flora
During the observation period, flowers of other plant species growing in the study area were visited by *A. mellifera*, *E. eximia* and *P. borbonicus* for either nectar (ne) or pollen (po). Amongst these plant species were, *Cosmos sulphureus* (Asteraceae, ne and po), *Cajanus cajan* (Fabaceae, ne), *Tithonia diversifolia* (Asteraceae, ne and po) and *Helianthus annuus* (Asteraceae, ne and po). During the entire observation period, *A. mellifera*, *E. eximia* and *P. borbonicus* foraging on *V. subterranea* flowers were not observed moving to a neighboring plant of a different species and vice versa.

3.3.9 Apicultural value of *Vigna subterranea*
During *V. subterranea* flowering period, a weak nectar collection activity of *A. mellifera* was observed on *V. subterranea* flowers. No pollen collection by this honey bee was noted. On 1402 visits recorded in 2013, Only 35 (2.46%) visits of *A. mellifera* were registered and on 1622 visits recorded in 2014, only 89 (5.48%) visits of *A. mellifera* were registered (Table 2). These data show the low attractivity of *V. subterranea* nectar to *A. mellifera*. They allow the classification of this crop legume species among the slightly nectariferous bee plant.

3.3.10 Impact of insects on the pollination, pod and seed yields of *Vigna subterranea*
During pollen and nectar harvest from *V. subterranea*, foraging insects were always in contact with the anthers and stigma. Thus these insects increased the pollination possibilities of this plant species. Table 8 presents the results on the podding rate, the number of seeds per pod and the percentage of normal seeds in different treatments. From this table, we documented the following:

a) in 2013, pod and seed yields from flowers of plants visited by insects (treatment 1) was higher than that of flowers protected from insects (treatment 2); in 2014, pod and seed yields from flowers of plants visited by insects (treatment 3) was greater than that of flowers protected from insect visits (treatment 4);

b) The differences observed between the four podding rates were highly significant ($\chi^2_{Global} = 3098.64$; $df = 3$; $P<0.001$). The difference between the podding rates of treatments 1 and 2 ($\chi^2 = 1521.52$; $df = 1$; $P<0,001$) and between the podding rates of treatments 3 and 4 ($\chi^2 = 1525.06$; $df = 1$; $P<0,001$) were highly significant. Consequently, in 2013 and 2014, the podding rate of protected flowers was highly from that of flowers bagged during their flowering period. The podding rate due to the action of flowering insects was 61.62% in 2013 and 62.49% in 2014. For the two years of investigations, the podding rate due to the influence of the flowering insects is 62.05%;

c) The differences observed between the four mean number of seeds were highly significant (F = 224; df_1 = 3; df_2 = 476; P<0.001). The difference between the mean number of seeds of treatments 1 and 2 (t = 17.34; df = 4716; P<0.001) and between the mean number of seeds of treatments 3 and 4 (t = 13.95; df = 4388; P<0.001) were highly significant. Consequently, the number of seeds per pod of exposed flowers was higher than that of protected flowers. The number of seeds per pod attributed to the activity of flowering insects was 15.42% in 2013 and 16.54% in 2014. For both years of study, this percentage is 15.98%;

d) The differences observed between the four percentage of normal seeds indicate were highly significant (χ^2_{Global} = 336.62; df = 3; P<0.001). The difference between the percentage of normal seeds of treatments 1 and 2 (χ^2 = 165.31; df = 1; P<0,001) and between the percentage of normal seeds of treatments 3 and 4 (χ^2 = 160.32; df = 1; P<0,001) were highly significant. Thus, the percentage of normal seeds in opened flowers was higher than that of protected flowers in 2013 and 2014. The percentage of the normal seeds due to the action of insects was 16.41% in 2013 and 19.82% in 2014. For two cumulative years, this percentage is 18.11%.

Table 8: Yield components of different treatments as influenced by protection of *Vigna subterranea* flowers from insects in 2013 and 2014 at Dang.

Years	Traitements	NF	NFP	PrR (%)	NS / P		TNS	NS	% NS
					m	***sd***			
2013	1(Unprotected plants)	4320	2983	69,05	1.29	0.46	4766	4216	88.45
	2(Bagged plants)	3960	1036	26,16	1.08	0.28	1220	902	73.93
2014	3(Unprotected plants)	4989	3577	71,69	1.39	0.55	5092	4588	90.10
	4(Bagged plants)	3023	813	26,89	1.16	0.39	1272	979	72.24

NF: Number of flowers; NFP: Number of formed pod; PrR: Podding rate; NS / P: Number of seeds per pod; TNS: Total number of seeds; NNS: Number of normal seeds; % NS: Percentage of normal seeds; *m*: mean; *sd*: standard deviation.

Comparison of the podding rates: χ^2_{Global} = 3098.64 (df = 3, P<0.001); $T1_{2013}/T2_{2013}$: χ^2 = 1521.52 (df = 1, P<0.001); $T1_{2013}/T3_{2014}$: χ^2 = 7.79 (df = 1, P<0.001); $T1_{2013}/T4_{2014}$: χ^2 = 1265.76 (df = 1, P<0.001); $T2_{2013}/T3_{2014}$: χ^2 = 1832.83 (df = 1, P<0.001); $T2_{2013}/T4_{2014}$: χ^2 = 0.47 (df = 1, P>0.05); $T3_{2014}/T4_{2014}$: χ^2 = 1525.50 (df = 1, P<0.001).

Comparison of the mean number of the seeds per pod: F = 224 (df_1 = 3, df_2 = 476, P<0.001); $T1_{2013}/T2_{2013}$: t = 383.43 (df = 4017, P<0.001); $T1_{2013}/T3_{2014}$: t = - 318.23 (df = 6558, P<0.001); $T1_{2013}/T4_{2014}$: t = 186.20 (df = 3794, P<0.001); $T2_{2013}/T3_{2014}$: t = - 495.81 (df = 4611, P<0.001); $T2_{2013}/T4_{2014}$: t = - 109.42 (df = 184, P<0.001); $T3_{2014}/T4_{2014}$: t = 290.66 (df = 4388, P<0.001).

Comparison of the percentage of normal seeds: χ^2_{Global} = 336.62 (df = 3, P<0.001); $T1_{2013}/T2_{2013}$: χ^2 = 165.31 (df = 1, P<0.001); $T1_{2013}/T3_{2014}$: χ^2 = 6.95 (df = 1, P<0.01); $T1_{2013}/T4_{2014}$: χ^2 = 110.43 (df = 1, P<0.001); $T2_{2013}/T3_{2014}$: χ^2 = 227.13 (df = 1, P<0.001); $T2_{2013}/T4_{2014}$: χ^2 = 3.09 (df = 1, P>0.05); $T3_{2014}/T4_{2014}$: χ^2 = 160.32 (df = 1, P<0.001).

4. Discussion

Our study indicates that, during cropping season, the main anthophilous insects visiting *V. subterranea* were of the order of Diptera, the family Syrphidae being the preponderant. Findings in Ngaoundere (Cameroon) on the same plant species revealed, Lepidoptera as the most abundant [13]. These differences could be due to the low presence of the population of these insects at the level of the investigation stations. The presence of other attractive flowering plants to these Lepidoptera in the study site may limit their abundance on the flowers of the plant studied. The diversity and abundance of the flower-visiting entomofauna have been reported to vary with time and space [21] with plant species [27].

The shift of the daily peaks of activity of certain flowering insects in 2014, would allow them to limit interspecific competition for the collection of pollen and / or nectar of this plant (table 4).

The weak abundance of insects per 1000 flowers indicated the low attractivity of the nectar and / or the pollen of *V. subterranea* to various insects. As far as *A. mellifera* on *V. subterranea* is concerned, it could be related to more interesting and easily accessible food source for this honeybee in the environment of *V. subterranea* during its flowering period. Similar results were reported on another variety of *V. subterranea* (Creams ivory variety with fine brown spots) at Dang [13] and on *A. hypogaea* at Nkolbisson [28]. The significant difference observed between the duration of visits in 2013 and 2014 could be attributed to the availability of nectar and pollen or the variation in the diversity of insect visitors from one year to another.

The peak activity of insects on *V. subterranea* flowers was between 10.00 p.m. and 11.00 p.m., which corresponds probably to the period of highest availability of nectar and / or pollen in the flowers of this crop. The working visiting time of insects on flowers was reported to depend on the availability of pollen [29] or nectar [30- 32].

During our investigations, the disruption of visits by other insects reduced the duration of some *E. eximia* and *P. borbonocus* visits.

The diversity and richness of the neighbouring flora to *V. subterranea* could explain the weak frequency of visits of several insect species on flowers of this plant.

During the collection of nectar on each flower, insect's foragers regularly come into contact with the stigma. They were also able to carry pollen with their hairs, legs and mouth accessories from a flower of one plant to stigma of another flower of the same plant (geitonogamy), to the same flower (autogamy) or to that of another plant (xenogamy) [23]. The significant contribution of insects in pod and seed yield of *V. subterranea* is in agreement with similar findings in Ngaoundere [13], which showed that *V. subterranea* White variety flowers produce fewer seeds per pod in the absence of pollinating insects.

The high yield in pod and seed from treatments visited by insects compared to treatment bagged indicate that insects were effective in increasing cross-pollination or selfpollination. In our experiment, the uses of both flowering insects highly improved the seed and pod yields of *V. subterranea*. Flowering insects contributed to facilitate the liberation of pollen from anthers for an optimal occupation of stigma, thus increasing pollination [33].

5. Conclusion

From our study, *V. subterranea* White variety is a plant that highly benefits from pollination by insects. The comparison of the pod and seed sets of protected flowering plant with those of flowering plant visited by insects underscores the value of this these insects in increasing pod and seed yields, as well as improving seed quality. Our results suggest that preserving harmless anthophilous insects close *V. subterranea* field significantly improve the pods and seed production of this important legume in the region.

6. References

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