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Research Paper

Impact of the foraging activity of *Apis mellifera adansonii* Latreille (Hymenoptera: Apidae) and *Bradyrhizobium* fertilizer on pollination and yield components of *Glycine max* L. (Fabaceae) in the field

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Abstract

To determine the impact of *Apis mellifera adansonii* and *Bradyrhizobium* on pod and seed yields of *Glycine max*, field trials were carried out during 2012 and 2013 cropping seasons. Hence, 120 to 25658 flowers were labeled each year and divided into five treatments, differentiated according to whether plots were inoculated with *Bradyrhizobium* or not, or plants were protected from insects activities or not and the last treatment with flowers isolated then opened only to *A. m. adansonii*. The effects of *Bradyrhizobium* on nodulation, plant biomass and seed yield, as well as the foraging behavior of *A. m. adansonii* on flowers, the number of seeds per pod and the normal seeds' rate were evaluated. Results indicate that *Bradyrhizobium* significantly increased the number of flowers (P<0.001), root nodules (P<0.0001), plant biomass (P<0.0001), pod and seeds yields in inoculated plots. *A. m. adansonii* foraged on *G. max* flowers from 09.00 a.m. to 16.00 p.m. and throughout the whole blooming period. This insect intensely harvested only nectar. By comparing the yields of unprotected flowers to those of flowers isolated then opened to *A. m. adansonii*, 35.85% increase fructification index, and 73.09% increase in the number of seeds per pod due to this bee were recorded. The synergistic activity of insects and *Bradyrhizobium* increased the number of seeds per pod by 32.16% and the percentage of normal seeds by 32.87%. Our results reveal that inoculation of soybean plant at sowing with *Bradyrhizobium* and installation of hives close to the field could be recommended for a sustainable pods and seed yield improvement of this crop.

Keywords: *Apis mellifera adansonii*; *Bradyrhizobium*; Foraging activity; *Glycine max;* Yields.

1. Introduction

Soybean [*Glycine max* (L.) Merrill] is the world's leading source of oil and protein (Ikeogu&Nwofia 2013). It has the highest protein content of all crops legume (38-40%) and is second only to groundnut in terms of oil content among food legumes (Tien et al. 2002, Alghamdi 2004, Ikeogu&Nwofia 2013).The plant is an annual, herbaceous, erected crop, and can reach a height of 1.5 m. Soybean cultivars have been reported to be indeterminate, determinate and semi-determinate in growth habit (Gallais&Bannerot 1992). The first leaves are simple, opposite and swallowed, while the following ones are trifoliate and alternate (Hymowitz& Harlan 1983). Pods are straight or slightly curved, with a length of 2-7 cm; the seed is generally oval, but may vary depending on the cultivar, almost spherical, elongated or flattened (Hymowitz& Harlan 1983). Flowers are grouped by two 2-8 on a short racemes inserted on the stem axile sheets and are purple or white (Boyeldieu 1991). Each flower has a tubular calyx of 5 sepals, a corolla of 5 petals, a single carpel and 10 stamens, 9 of which are welded while the tenth is free (Hymowitz&Harlan 1983). Each flowers produce nectar and pollen which attract insects (Milfont et al. 2013). Soybean is grown primarily for its seeds, which have many uses in the food and industrial sectors (Diers et al. 1992). Currently the production of *G. max* in Cameroon is low whereas the demand for seeds is high (MINADER 2012). Therefore, it is important to investigate on the possibilities of increasing the production of this valuable plant in the country. This can be achieved if *G. max* flowering insects in each region are well-known and exploited (Milfont et al. 2013) or by introducing microbial inoculants, which can have been applied to legume crops for over 120 years as bio-fertilizers (Ormeno-orrillo et al. 2012), which have a direct beneficial effect on the host plant (Denison & Kiers 2004, Ngakou et al. 2009, Kengni et al. 2015).

Unfortunately no research has been reported on the relationships between *G. max*, inoculation with *Bradyrhizobium* and its anthophilous insects in Cameroon. In Ngaoundere *A. m. adansonii* visit flowers of *G. max* (unpublished data), and this study is carried out to assess the effects of foraging activities of *A. m. adansonii* on yields of *G. max*. In Maroua, *A. m. adansonii* has recently been reported to visit *G. max* flowers by Tchuenguem&Dounia (2014) resulting in a significant increment in fruiting rate, number of seeds and percentage of normal seeds respectively by 14.14%, 36.95% and 32.61%.

The main objective of this research was to gather more data on the relationships between *G. max*, *Bradyrhizobium* and flowers visiting insects for the optimal management of pollination services. The registration of the activity of *A. m. adansonii* on *G. max*

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flowers, the evaluation of the impact of visiting insects on pollination, pods and seeds yields of this Fabaceae, the estimation of pollination efficiency of *A. m. adansonii* on this plant, the estimation of the impact of *Bradyrhizobium* on soybean and the evaluation of the impact of the cumulative action of *Bradyrhizobium* and flowers visiting insects especially *A. m. adansonii* are discussed.

2. Material and methods

2.1. Study site, experimental plot and biological material

The experimental was carried out in the field from march to september, in 2012 and 2013 at Beka-Hossere (07°31.547'N, 13°54.659'E, 1117 m above sea level) in Ngaoundéré, Adamaoua region of Cameroon. This region belongs to the high-altitude guinean savannah agro-ecological zone (Tchuenguem et al. 2007). The climate is characterized by a distinct rainy (april to october) and dry (november to march) seasons, with an annual rainfall of about 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, many colonies of *Apis mellifera adansonii* Latreille (Hymenoptera: Apidae) located about 3 km in diameter around the experimental site and a hive of *Apis mellifera adansonii* installed at 1m of the experimental site in 2013.
The vegetation was represented by crops, ornamental plants and native plants of savannah and gallery forest. The vegetation near *G. max* field had various unmanaged and cultivated species.
Microbial inoculum of the genus *Bradyrhizobium* was produced at IRAD (The Institute of Research for Agricultural Development) Wakwa Ngaoundéré, while *G. max* seeds were from Maïscam-Ngaoundere Agro-industry (life cycle of 155 to 160 days).

2.2. Sowing and weeding

On may 5, 2012 and may 12, 2013, experimental plot was cleaned and divided into 18 subplots, each measuring $3m^2$. Nine subplots were inoculated and nine uninoculated. Three seeds were sown in 3 lines per subplot, each of which had 12 holes per line. Holes were separated 25 cm from each other, while lines were 30 cm apart. Soybean seeds were inoculated as described by Ngakou et al. (2007). Weeding was performed manually as necessary to maintain plots weed-free.

2.3. Determination of the reproduction system of *Glycine max*

On july 22, 2012, six subplots carrying 216 plants with 29254 flowers at the bud stage were labeled. Three subplots carrying 108 plants with 14247 flowers were left open to be pollinated (treatment 1) (Figure 1) and three others carrying 15007 flowers were protected with gauze mesh to prevent insect or other pollinating animals visits (treatment 2) (Figure 2). On july 25, 2013, the experiment was repeated. For treatment 3, four subplots carrying 108 plants with 20352 flowers and for treatment 4 four subplots carrying 108 plants with 18867 flowers.
Twenty 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 Tchuenguem et al. (2004): Pi = F2/F1, where F2 is the number of pods formed and F1 the number of viable flowers initially set. The allogamy rate (Alr) from which derives the autogamy rate (Atr) was expressed as the difference in podding indexes between treatment X (unprotected flowers) and treatment Y (bagged flowers) as follows (Demarly, 1977): Alr = [(PiX - PiY) / PiX] * 100, Where PiX and PiY are respectively the podding average indexes of treatments X and Y. Atr = 100 – Alr.

2.4. Assessment of the influence of inoculation on nodulation and biomass of *Glycine max*

For each subplot, 18 plants for each treatment a (uninoculated subplot) and b (inoculated subplot) were labeled. Then nodules per plant were harvested at 60 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 36 individual plants of treatments a and b.

2.5. Estimation of the frequency of *Apis mellifera adansonii* on *Glycine max* flowers

The frequency of *A. m. adansonii* on *G. max* flowers was determined based on observations on treatments 1 and 3, every day, from july 16^{th} to august 16^{th} 2012 and from july 26^{th}, to august 25^{th} 2013, during four daily time frames: 09.00 – 10.00 a.m., 11.00 – 12.00 a.m., 13.00 – 14.00 p.m. and 15.00 – 16.00 p.m. Flowers were completely opened at 06.00 a.m. and closed before 06.00 p.m., but insects activity started at 9.00 a.m and stopped at 04.00 p.m.
By observing labeled flowers of treatments 1 and 3, all insect visits were recorded. Specimens of all insect taxa (3 to 5 per species) were caught with an insect net on flowers of unlabeled subplots. 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 *A. m. adansonii* in the anthophilous entomofauna of *G. max*. 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. Nectar harvested by *A. m. adansonii* during each floral visit was registered based on its foraging behavior. Nectar foragers were observed extending their proboscis to the base of the corolla. Every sampling date, the number of opened flowers was counted, while the duration of the individual flower visits were recorded (using a stopwatch) at least three times: 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 bee came into contact with the stigma: Tchuenguem 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 referring to the 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) * 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 foragers by competitors or predators and the attractiveness exerted by other plant species around experimental plot on *A. m. adansonii* was assessed.
During each observation date, temperature and relative humidity were registered after every 30 minutes using a mobile thermo-hygrometer.



Fig. 1: *Glycine max* subplot showing unprotected plants.



Fig. 2: *Glycine max* subplot showing isolated plants.

2.6. Assessment of the cumulative effects of insects and *Bradyrhizobium* on *Glycine max* yields

This evaluation was based on the impact of both *Bradyrhizobium* and insects on *G. max* 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 were assessed. The contribution of cumulative action of insects and *Bradyhizobium* on *G. max* fruiting rate, mean number of seeds per pod and the percentage of normal seeds was calculated by the Frx above formula.

2.7. Data analysis techniques

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.

3. Results

3.1. Reproduction system of *Glycine max*

According to table 1, allogamy, rate was 21.43% and 29.07% respectively in 2012 and 2013; whereas the autogamous rate was 78.57% and 79.93% respectively in 2012 and 2013. *Glycine max* used in our experiments, had a mixed reproduction system antogamous-allogamous, with the predominance of autogamy. Our results line with those of Tchuenguem&Dounia, 2014, who reported the predominance of autogamy (94.01%) over allogamy (5.98%) on G. max in Maroua. Therefore, it is suggested that the mating system of *G. max* may not vary from one agroecological zone to another.

Table 1: Reproduction system of *Glycine max* in years 2012 and 2013

Years	Autogamous rate (%)	Allogamy rate (%)
2012	78.57	21.43
2013	79.93	29.07
Mean (2012/2013)	79.25	25.25

3.2. Effect of *Bradyrhizobium* on numbers of flowers, nodulation and biomass of *Glycine max*

The number and dry weight of nodules per plant, the numbers of flowers per subplot and plant biomass were assessed on *G. max* (Table 2) in 2012 and 2013. Plants inoculated at sowing produced a significantly higher numbers of nodules ($P<0.0001$), greater nodule dry weight ($P<0.0001$) and biomass of plant ($P<0.0001$). The highest plant biomass in 2012 (36.55 g) and in 2013 (36.95 g) was obtained from inoculated plants. Inoculated plants also produced a significant numbers of flowers ($P<0.0001$) 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: Soudano-sahelian, Guinea savannah, humid forest with monomodal rainfall, humid forest with bimodal rainfall (Ngakou 2007).

3.3. Frequency of floral entomofauna of glycine max

Among the 330 and 1844 visits of6 insect species recorded respectively on uninoculated flowers in 2012 and 2013, and378and2132 visits of7 insect species recorded respectively on inoculated flowers of G. max, A. m. adansoniiwas ranked second as the most represented insect species the first year on both uninoculated and inoculated flowers. In the second year this bee was first on both uninoculated and inoculated flowers, with 1428 visits (77.44%) on uninoculated flowers and 1608 visits (75.42%) on inoculates ones (Table 3). Significant difference in the percentage of visits between flowers from uninoculated ($\chi^2=29.51$; df=1; $P<0.001$) and inoculated ($\chi^2=35.74$; df=1; $P<0.001$) plants were observed for the two years. Flowers of *G. max* were visited by Apidae (*A. m. adansonii* and *Ceratina* sp.), Formicidae (*Camponotus acvapimensis*), Halictidae (*Lasioglossum* sp.), Meloidae (*Coryna* sp.), Muscidae (*Musca domestica*) and Syrphidae (*Epysyrphus balteatus*). These insects were found to collect only nectar. Besides, *Ceratina* sp. was the most representative insect the first year against *A. m. adansonii* the second year. For both years *A. m. adansonii* was the most representative insect on *G. max* flowers.

Table 2: Number of nodules, plant biomass and number of flowers of *Glycine max* as affected by *Bradyrhizobium* in 2012 and 2013

Years	Treat	Nodules (per/plant)	Flowers (per/subplot)	Weight of dry nodules (g/plant)	Plant biomass (g/plant)
2012	Uninoculated	(10.61±0.86) a	(1299.14±230.268) a	(0.59±0.04) a	(15.38±1.19) a
	Inoculated	(23.6±0.86) b	(2182.94±263.80) b	(1.37±0.04) b	(36.55±1.19) b
	P. value	<0.0001	<0.001	<0.0001	<0.0001
2013	Uninoculated	(8.7±1.04) a	(1211.26±218.48) a	(0.48±0.05) a	(20.07±1.11) a
	Inoculated	(27.72±1.04)b	(2078.9±218.45)b	(1.50±0.05)b	(36.95±1.11)b
	P. value	<0.0001	<0.001	<0.0001	<0.0001

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

Table 3: Diversity of insects on *Glycine max* from uninoculated and inoculated flowers with *Bradyrhizobium* in 2012 and 2013 at Beka-Hossere, number and percentage of visits of different insects.

Insects			2012				2013				Total 2012/2013	
			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	Meloidae	*Coryna* sp. (eat flowers)	17	5.17	15	3.97	22	1.19	10	0.47	64	1.36
		Total Coloptera	17	5.17	15	3.97	22	1.19	10	0.47	64	1.36
Diptera	Muscidae	*Musca domestica* (N)	6	1.82	6	1.59	3	0.16	1	0.05	16	99.44
	Syrphidae	*Epysyrphus balteatus* (N)	-	-	-	-	4	0.22	6	0.28	10	0.34
		Total Diptera	6	1.82	6	1.59	7	0.38	7	0.33	26	0.56
	Apidae	*Apis mellifera adansonii* (N)	64	19.39	87	23.02	1428	77.44	1608	75.42	3187	68.04
		Ceratina sp. (N)	230	69.70	262	69.31	382	20.72	492	23.08	1366	29.16
		Total Apidae	294	89.09	349	92.33	1810	98.16	2100	98.50	4553	97.20
Hymenoptera	Formicidae	*Camponotus acvapimensis* (N)	7	2.12	4	1.06	5	0.27	3	0.14	19	0.40
		Total Formicidae	7	2.12	4	1.06	5	0.27	3	0.14	19	0.40
	Halictidae	*Lasioglossum* sp. (N)	6	1.82	4	1.06	-	-	12	0.56	22	0.46
		Total Halictidae	6	1.82	4	1.06	0	0	12	0.56	22	0.46
	Total Hymenoptera		307	93.03	357	94.44	1815	98.42	2115	99.20	4594	98.07
Total			330 (6)	100	378 (6)	100	1844 (7)	100	2132 (7)	100	4684 (7)	100

n_1, n_2, $n_3$et n_4: number of visits on 30260 flowers in 22 days; sp.: undetermined species; P_1, P_2, P_3 et P_4: visits percentages P_1= (n_1/330) * 100; P_2= (n_2/378) * 100; P_3= (n_3/1844) * 100; P_4= (n_4/2132) * 100. Comparison of percentages visits: (Apis mellifera adansonii $_{2012/2013}$) χ^2 = 61.13; P< 0.001; N: visitor collecting nectar.

3.4. Activity of *Apis mellifera adansonii* on *Glycine max* flowers

3.4.1. Floral products harvested

From our field observations and during the two flowering periods, *A. m. adansonii* foragers were found to intensively and regularly collect only nectar on flowers of both inoculated and uninoculated *G. max* plants as shown on Figure 3.



Fig. 3: *Apis mellifera adansonii* collecting nectar on a flower of *Glycine max* at Beka-Hossere

3.4.2. Relationships between insect visits and flowering stages of the plant

The number of visits was elevated when the number of opened flowers was higher on flowers of both uninoculated and inoculated plants (Figure 4). This was consistent up to day 9 (optimum), then decreased with time till days 21. A positive and significant correlation was found between the numbers of opened flowers of uninoculated *G. max* plants and the number of *A. m. adansonii* (r=0.88; ddl=21; P<0.001) in 2012 and 2013, as well as for flowers of inoculated plants in 2012 and 2013 (r=0.87; ddl=21; P<0.001).

3.4.3. Diurnal flower visits

Apis mellifera adansonii foraged on *G. max* flowers daily and throughout the flowering period, with a peak of activity between 01.00 p.m. and 02.00 p.m. (Figure 5). This activity was influenced by temperature and hygrometry. The correlation between the number of *A. m. adansonii* visits and the temperature was positive and significant on uninoculated *G. max* flowers (r=0.96; ddl=3; P<0.001) and inoculated ones (r=0.96; ddl=3; P< 0.001) for the both years. Concerning the relative humidity, the correlation with the number of *A. m. adansonii* visits was negative and significant on uninoculated (r=-0.87; ddl=3; P<0.05) and inoculated (r=-0.86; ddl=3; P<0.05) flowers of this crop.

3.4.4. Abundance of *Apis mellifera adansonii*

In 2012, the highest mean number of *A. m. adansonii* simultaneous in activity was 1 per flower (n=61; s=0) and 3 per 1000 flowers (n=260; s=1.32; maxi=10) on flowers of uninoculated plot and 1 per flowers (n=90; s=0) and 3 per 1000 flowers (n=251; s=1. 63; maxi=10) on flowers of inoculated subplot. In 2013, the corresponding values were 1 (n=263; s=0) and 40 per 1000 flowers (n=673; s=10.62; maxi=92) on flowers of uninoculated subplot and 1 per flowers (n=182; s=0) and 3 per 1000 flowers (n=266; s=14.17; maxi=80) on flowers of inoculated subplot.

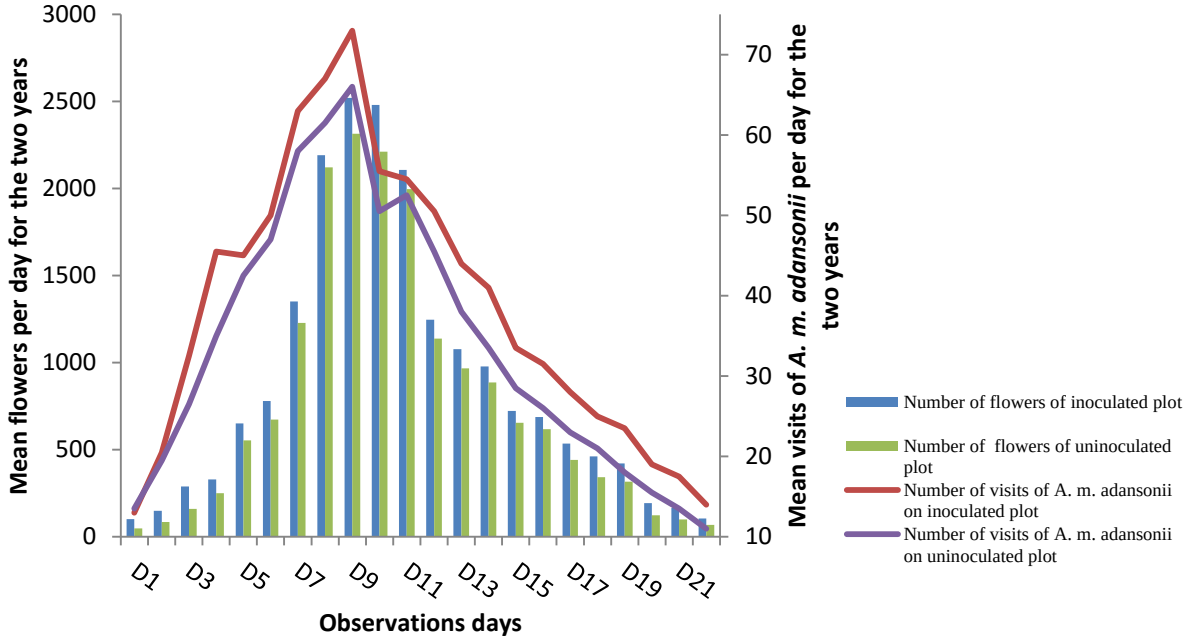


Fig. 4: Variations of the number of *Glycine max* opened flowers and the number of *Apis mellifera adansonii* visits with observation days in 2012 and 2013 at Beka-Hossere.

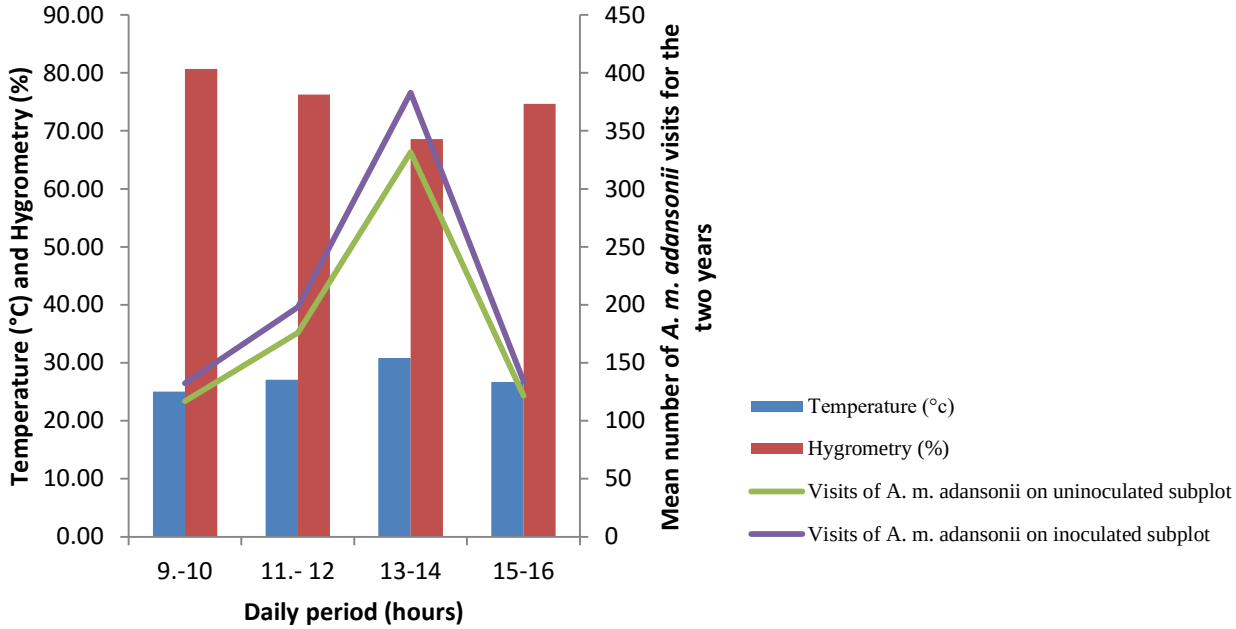


Fig. 6: Daily distribution of *Apis mellifera adansonii* visits on *Glycine max* flowers over 22 Days in 2012 and 2013 as influenced by mean temperature and mean humidity at Beka-Hossere.

3.4.5. Duration of *Apis mellifera adansonii* visits per flower

In 2012, the mean duration of a flower visit was 7.26 sec (n=346; s=4.18; max=42 sec) on flowers of uninoculated subplot and 7.42 sec (n=240; s=4.47; max=21 sec) on flowers of inoculated subplot, whereas in 2013, the visit lasted for 7.73 sec (n=613; s=2.58; max=12 sec) on flowers of uninoculated subplot and 8.79 sec (n=634; s=4.26; max=21 sec) on flowers of inoculated subplot. The difference was highly significant (t=29.3; df=957; P<0.001) between the mean duration of visits per flowers of uninoculated subplot and that flowers from inoculated subplot (t=55.16; df=872; P<0.001). For the two cumulative years, the mean duration of a flower visit was 7.49 sec for uninoculated subplot and 8.10 sec for the inoculated subplot.

3.4.6. Foraging speed of *Apis mellifera adansonii* on *Glycine max* flowers

In *Glycine max* field, *A. m. adansonii* visited between 3.24 and 33 flowers/min on uninoculated subplot and on inoculated subplot between 1.38 and 30 flowers/min in 2012. In 2013, *A. m. adansonii* visited between 1.85 and 45 flowers/min on uninoculated subplot and 8.18 and 30 flowers/min on inoculated subplot. The mean foraging speed was 10.66 flowers/min (n=182, s=5.29) on uninoculated subplot and 9.41 flowers/min (n=175; s=5.99) on inoculated subplot in 2012. In 2013, the mean foraging speed was 14.90 flowers/min (n=486; s=9.24) on uninoculated subplot and 17.10 flowers/min (n=355, s=6.02) on inoculated subplot. The difference between these means was highly significant (t=67.12; P<0.001) for uninoculated subplot or inoculated ones (t=149.69; P<0.001). For the two cumulated years, the mean foraging speed was 12.78

flowers/min for uninoculated plot and 13.25 flowers/min for inoculated ones.

3.4.7. Influence of neighboring flora

During the observation period, flowers of many other plant species growing in the study area were visited by A. m. adansonii for either nectar (ne) or pollen (po). Amongst those plant species were *Arachis hypogea* (Fabaceae, ne), *Bidens pilosa* (Asteraceae, ne and po), *Cucumeropsis manii* (Cucurbitaceae, ne), *Cucumis melo* (Cucurbitaceae, ne and po), *Hypomea batatas* (Convolvulaceae, ne), *Phaseolus coccineus* (Fabaceae, ne and po), *Phaseolus vulgaris* (Fabaceae, ne and po), *Senna mimosoides* (Mimosaceae, ne and po), *Vigna unguiculata* (Fabaceae, ne and po) and *Zea mays* (Poaceae, po). During the entire observation period, *A. m. adansonii* foragers on *G. max* flowers were not observed moving to a neighboring plant of a different species and vice versa. That bee's foragers were regularly interrupted by other foragers or by other bees collecting *G. max* floral products such as *Ceratina* sp. (ne).

3.4.8. Apicultural value of *Glycine max*

During *G. max* flowering period, a well elaborated activity of *A. m. adansonii* was registered on its flowers. In particular, there were good nectar harvest and workers faithfulness to its flowers. These data point out the good attractiveness of *G. max* nectar to *A. m. adansonii*. From this, it appears that our studied plant could be classified in as a highly nectariferous bee plant.

3.4.9. Impact of *Apis mellifera adansonii* on pollination, pod/set and seed yields of *Glycine max*

Apis mellifera adansonii foragers were always in contact with the stigma and the anthers of *G. max* (100 % for all visits). Consequently this bee increased possibilities of the pollination of *G. max* flowers.

The comparison of the fruiting rate (table 4) shows that differences observed were highly significant between treatment 2 and 3 (χ^2=40.18; df=1; P<0,001), as well as treatment 5 and 6 (χ^2=47.34; df=1; P<0,001). Hence, the fruiting rate of unprotected flowers opened exclusively to *A. m. adansonii* visits (treatment 3; 2012 and treatment 6; 2013) was higher than that of bagged flowers (treatment 2; 2012 and treatment 5; 2013)

The comparison of the mean number of seeds per pod showed that the differences were highly significant between treatment 2 and 3 (t=265.90; P<0,001) and treatments 5 and 6 (t=488.83; P<0,001). As the matter of fact, in 2012, seed yield per pod of flowers protected and visited by *A. m. adansonii* (treatment 3) was higher than that of flowers protected from insects (treatment 2). In 2013, seed yield per fruit of flowers protected and visited exclusively by *A. m. adansonii* (treatment 6) was greater than that of flowers protected from insect visits (treatment 5). The comparison of the percentages of normal seeds shows that the differences were highly significant between treatment 2 and 3 (χ^2=27.30; df=1; P<0,001), then treatments 5 and 6 (χ^2=35.45; df=1; P<0,001). Thus, the percentages of normal seeds of flowers protected and visited exclusively by *A. m. adansonii* (treatment 3) was higher than those protected from insects (treatment 2).

The fruiting rate due to *A. m. adansonii* activity was 33.46% in 2012 and 38.28% in 2013. For the two years of study, the fruiting rate percentage attributed to *A. m. adansonii* was 35.87%. The number of seeds per pod attributed to this bee activity was 76.02% in 2012 and 70.17% in 2013. For the two years of study, the proportion of seeds per pod due to *A. m. adansonii* was 73.09%. The number of normal seeds due to *A. m. adansonii* was 29.16% in 2012 and 33.04% in 2013. For the two years of study, the number of normal seeds per pod attributed to *A. m. adansonii* activity was 31.1%. In short, the influence of *A. m. adansonii* on pod and seeds yields on *G. max* was positive and significant.

Table 4: Variation of yield components between treatments as influenced by protection of *Glycine max* flowers from *Apis mellifera adansonii* in 2012 and 2013 at Beka-Hossere.

Year	Treatments	Flowers	Pod	Fruiting rate (%)	Seeds/pods Mean	SD	Total seeds	Normal seeds	%Normal seeds
2012	1 (Unlimited visits)	14247	10752	75.46	1.89	0.70	27405	24132	88.05
	2 (Bagged flowers)	15007	9987	66.54	0.76	0.47	12975	8867	68.33
	3 (*A. m. adansonii* visits on flowers)	51	51	100	3.17	0.68	170	164	96.47
2013	4 (Unlimited visits)	20352	19547	96.04	2.14	0.49	36409	33883	93.06
	5 (Bagged flowers)	18867	11645	61.72	1.05	0.6	17842	11723	65.69
	6 (*A. m. adansonii* visits on flowers)	120	120	100	3.52	0.61	478	469	98.11

4. Cumulative impact of flowering insects and *Bradyrhizobium* on the pollination, pod and seed yields of *Glycine max*

The comparison of the fruiting rate (table 5) shows that the differences observed were significant between treatments 2 and 7 (χ^2=10.81; df=1; P<0.01), and treatment 5 and 8 (χ^2=20.51; df=1; P<0.001). Therefore, in 2012, the fruiting rate of flowers inoculated and opened to insects (treatment 7) was higher than that of protected flowers (treatment 2) whereas in 2013; the fruiting rate of inoculated flowers opened to insects (treatment 8) was higher than that of flowers (treatment 5) protected from insects visits.

The number of seeds per pod from flowers of inoculated flowers and opened to insects (treatment 7) was significantly higher (t=2887.66; P<0,001) than that of flowers protected from insects visits (treatment 2). Similarly, in 2013 the number of seeds per pod of inoculated flowers opened to insects was significantly higher (t=5070.84; P<0,001) than that of flowers protected from insects visits.

The comparison of the percentages of normal seeds shows that the differences were highly significant between treatments 2 and 7 (χ^2=17.02; df=1; P<0,001), and treatments 5 and 8 (χ^2=23.60; df=1; P<0,001). In 2012, the percentage of normal seeds of flowers of inoculated plots and opened to insects (treatment 7) was higher than that of flowers protected from insect visits (treatment 2), while in 2013, the percentage of normal seeds of flowers of inoculated plots and opened to insects (treatment 8) was higher than that of flowers protected from insects visits (treatment 5).

The fruiting rate due to cumulative effects of flowering insects and *Bradyrhizobium* activity was 28.78% in 2012 and 35.54% in 2013. For the two years of study, the fruiting rate (%) influenced by both flowering insects and *Bradyrhizobium* was 32.16%.

The cumulative effect of flowering insects and *Bradyrhizobium* on the number of seeds per pod was 31.46% in 2012 and 34.28% in 2013. For the two years of study, the percentage of the number of seeds per pod due to flowering insects and *Bradyrhizobium* was 32.87%.

The number of normal seeds due to cumulative effects of flowering insects and *Bradyrhizobium* activity was 75.94% in 2012 and 70.59% in 2013. For the two years of study, the percentage of the

number of normal seeds per pod attributed to cumulative effects of flowering insects and *Bradyrhizobium* activity was 73.26%. Hence, the cumulative influence of flowering insects and *Bradyrhizobium* on pod and seeds yields of *G. max* was positive and significant.

Table 5: Variation of yield components between treatments of *Glycine max* as influenced by *Bradyrhizobium* in 2012 and 2013 at Beka-Hossere.

Year	Treatments	Flowers	Pod	Fruiting rate (%)	Seeds/pods		Total seeds	Normal seeds	%Normal seeds
					Mean	SD			
2012	2 (Bagged flowers)	15007	9987	66.54	0.76	0.47	12975	8867	68.33
	7(Inoculated flowers open to insects)	14978	12924	86.28	1.38	1.22	19583	17957	91.69
2013	5 (Bagged flowers)	18867	11645	61.72	1.05	0.6	17842	11723	65.69
	8 (Inoculated flowers open to insects)	19986	17842	89.27	1.47	0.70	27824	25989	93.40

5. Discussion

Rhizobia including *Rhizobium* and *Bradyrhizobium* are the best know as biological nitrogen fixers in root nodules of legumes (Kiers et al. 2003, Denison & Kiers 2004, Ngakou et al. 2007). In the Guinea-savannah zone of Cameroon, the number of nodule formed by cowpea was reported to be low in the absence of inoculum (Ngakou 2007), with nodules starting to degenerate as from 45 days after planting. Successful nodulation of leguminous crops by *Rhizobium* largely depends on the presence of a specific and compatible strain in the soil for a particular legume (Tamiru et al. 2012). The positive correlation between nodules and plant dry weight was in accordance with findings of others authors (Thiagarajan et al. 1992, Hungria et al. 2001, Kengni et al. 2015). 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 increase soil fertility (Ngakou et al. 2008, Kengni et al. 2015).

During the first year, *Apis mellifera adansonii* was not the preponderant insect on *Glycine max* flowers, but in the second year and with the introduction of the hive in the farm, *A. m. adansonii* came first.Cumulatively, this bee was the most frequent insect and visited soybean flowers intensively. These results confirm those already reported by Tchuenguem&Dounia (2014), who revealed *A. m. adansonii* as the most frequent insect on flowers of the same plant in Maroua. This shows that plant possess specific food ressources available to insects through flowers. This bee has been reported as the main floral visitor of this Fabaceae in USA (Rortais et al. 2005) and Brazil (Milfont et al. 2013, Chiari et al. 2005). The present work shows that honey bee visited soybean flowers only for nectar collection. This result is in accordance with those of Tchuenguem&Dounia (2014) in Maroua where this bee collected exclusively nectar on soybean flowers. The significant difference between the percentages of A. m. adansonii visits for the two studied years could be attributed to the installation of a hive the second year in the experimental site. The peak of activity of *A. m. adansonii* on *G. max* flowers was at between 01.00 p.m. and 02.00 p.m., which corresponds probably to the period of highest availability of nectar on *G. max* flowers. The positive and highly significant correlation between the number of *G. max* flowers and the number of *A. m. adansonii* visits indicates the attractiveness of *G. max* to nectar with respect to this bee. The significant difference observed between the duration of visits in 2012 and 2013 could be attributed to the availability of nectar, the floral morphology of this crop or the variation in the diversity of flowering insects from one year to another.

During our investigations, the interruption of visits by other insects or the same honey bee reduced the duration of *A. m. adansonii* visits. This result confirms other findings reported by Tchuenguem&Dounia (2014) on *G. max* in Maroua-Cameroon. The present study shows that during one forage trip, an individual bee foraging on a given plant species scarcely visited another plant species, confirming the flower constancy of *A. m. adansonii* to flower of soybean (Basualdo et al. 2000). Flower constancy is an important aspect in the management of pollination and this shows that *A. m. adansonii* can provides the advantages to the management of *G. max*. During the rainy season 2012, 2013 in Ngaoundere, *A. m. adansonii* intensely and regularly harvested nectar on the flowers of *G. max* during flowering periods. This could be attributed to the needs of colonies during the flowering period. It indicates that *A. m. adansonii* can increased soybean pollination. During the collection of nectar, *A. m. adansonii* foragers, though their weight shook *G. max* flowers. This movement played a positive role in liberation of pollen by anthers for the optimal occupation of the stigma. This phenomenon was also reported by Ahrent&Caviness (1994), Rortais et al. 2005 and Tchuenguem&Dounia (2014) on *G. max*. *Apis mellifera adansonii* foragers also affected self-pollination and cross-pollination of soybean flowers. The positive and significant contribution of *A. m. adansonii* in fruiting rate, seed yields and percentage of normal seed of *G. max* is justified by its action on pollination. Similar results were obtained in Maroua (Tchuenguem&Dounia 2014) on *G. max*; showing that self-pollination of this plant produced little seeds per pod in the absence of efficient pollinators. The higher percentage of seeds number and normal seeds in the treatment visiting exclusively by *A. m. adansonii* compared to treatment bagged, indicate that insect visits were effective in increasing cross-pollination or self-pollination. Our results confirm those of Rortais et al. (2005), Milfont et al. (2013) and Tchuenguem&Dounia (2014), who revealed that *G. max* flowers set little pods in the absence of pollinator's insects. In our experiment, the uses of both flowering insects and *Bradyrhizobium* highly improved the seed and pod yields of *G. max*, as flowering insects have facilitated the liberation of pollen from anthers for optimal occupation of the stigma, thus increasing pollination (Tchuenguem&Dounia 2014); *Bradyrhizobium* contribuated to the improvement of nitrogen fixation potential of the host legume that led to increased plant growth and yield (Ngakou et al. 2007). Same results was obtain by Kengni et al. 2015 who revealed that the use of both flowering insect and *Rhizobium* highly improved the seeds and pods yields on *Vigna unguiculata* in Ngaoundere. Investment in *A. m. adansonii* management and inoculation with *Bradyrhizobium* may provides high returns to investment on this valuable crop.

6. Conclusion

From our study, *G. max* is a plant species that highly benefits from pollination by insects, of which *A. m. adansonii* is the most important and exclusively harvest nectar. The comparison of the pod and seed sets of unprotected flowers with those of flowers exclusively visited by *A. m. adansonii* underscores the value of this bee in increasing pod and seed yields, as well as improving seed quality. Furthermore, the comparison of pod and seeds set of uninoculated and bagged flowers with those of flowers inoculated with *Bradyrhizobium* and visited by insects indicates the value of cumulative activity of insects and *Bradyrhizobium* in increasing pod and seeds yields. Our results suggest that sowing G. max seeds with *Bradyrhizobium* and the installation of *A. m. adansonii* hives close to this crop fields could be encouraged to significantly improve the pods and seeds production of this important legume.

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