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## Pollination efficiency of *Dactylurina staudingeri* (Hymenoptera: Apidae) on *Psorospermum febrifugum* (Hypericaceae) at Dang (Ngaoundéré, Cameroon)

**Zra Ganava Venceslas, Mazi Sanda and Tchuenguem Fohouo Fernand-Nestor**

**Abstract**

To evaluate the impact of *Dactylurina staudingeri* on fruit and seed yields of *Psorospermum febrifugum*, its foraging and pollinating activities were studied in Ngaoundéré, from March 1<sup>st</sup> to 8<sup>th</sup>, in 2016 and 2017. The experiments were carried out on 540 flowers divided in four treatments: two treatments differentiated according to whether flower buds were protected or not against insect visits; two other treatments made up of flowers that were protected then exclusively exposed to *D. staudingeri*, or unbagged and reprotected without insect or any other organism visit. Stingless bee seasonal rhythm of activity, its foraging behavior on flowers, its pollination efficiency, the fruiting rate, the number of seeds per fruit and the percentage of normal seeds were evaluated. Results show that, *D. staudingeri* foraged on *P. febrifugum* flowers throughout its whole blooming period. Among the 37 insect species recorded on *P. febrifugum* flowers, *D. staudingeri* was the most frequent insect with 17.95% of 2373 visits. On flowers, individual bee intensely harvested nectar and slightly collected pollen. The mean foraging speed was 5.03 flowers / min. For the two years, through its pollination efficiency, *D. staudingeri* provoked a significant increment of the fruiting rate by 66.02%, as well as the percentage of normal seeds by 29.97%. Conservation of *D. staudingeri* nests close to *P. febrifugum* populations is recommended to improve fruit production, seed quality and stingless bee honey yield in the region.

**Keywords:** *Dactylurina staudingeri*, *Psorospermum febrifugum*, flowers, pollination efficiency, yields

**Introduction**

Many species of stingless bees have been kept by farmers for their products (honey, propolis and pollen) (Roubik, 1995) <sup>[1]</sup> and also for providing pollination services for fruits and vegetables in the tropics (Heard, 1999) <sup>[2]</sup>. Through pollination, bees contribute to the preservation and maintenance of the genetic diversity of flowering plants (Bradbear, 2010) <sup>[3]</sup>. *Psorospermum febrifugum* is a shrub of 1.5 - 6 m high (Ruffo *et al.*, 2002) <sup>[4]</sup>. The flower is sweet smelling, cream-white, usually densely hairy and glandular, with 5 sepals, 5 hairy petals and many stamens (Ruffo *et al.*, 2002) <sup>[4]</sup>. The edible fruits round are berries of 6 - 10 mm, bright to dark red, in terminal clusters (Ruffo *et al.*, 2002) <sup>[4]</sup>. Roots and leaves are used to treat leprosy; roots are also used as a mouthwash for tongue diseases (Ruffo *et al.*, 2002) <sup>[4]</sup>.

For a sustainable development of meliponiculture in Cameroon, investigations are carried out on the stingless bee flora in Ngaoundéré. In this country, the demand for fruits and seeds of *P. febrifugum* is high, whereas its production is weak, notably because of the absence of published data on the relations between this Hypericaceae and flower visiting insects such as *D. staudingeri* in the various localities.

Before our research, no previous work has been reported on the foraging and pollination activity of *D. staudingeri* on *P. febrifugum*.

The present work is a contribution to the understanding of the relationships between *P. febrifugum* and *D. staudingeri*, for their optimal management in Cameroon. It has five main specific objectives: (1) determine the place of *D. staudingeri* in *P. febrifugum* floral entomofauna; (2) study the activity of *D. staudingeri* on *P. febrifugum* flowers; (3) estimate the meliponicultural value of this plant; (4) assess the impact of the flowering insects including *D. staudingeri* on fruit and seed yields of this Hypericaceae; (5) evaluate the pollination efficiency of *D. staudingeri* on *P. febrifugum*.

## Materials and Methods

### Study site

Experiments were carried out from February to May, in 2016 and 2017 at Dang (Latitude 7°25.365 N, Longitude 13°32.572 E and Altitude 1083 m), a village located in the Adamawa region of Cameroon. This region belongs to the high altitude guinean savannah agro-ecological zone; the climate is characterized by two seasons: a rainy season (April to October) and a dry season (November to March). The annual rainfall is about 1500 mm; the mean annual temperature is 22 °C; the mean annual relative humidity is 70% (Amougou *et al.*, 2015) [5]. The vegetation is represented by crops, ornamental plants, hedge plants and native plant species of the savannah and gallery forests.

### Biological materials

The plant material was represented by *P. febrifugum* naturally presents in the study site. The animal material included many insects species naturally present in the environment. The number of *D. staudingeri* colonies located in the study site was six in 2016 as well as in 2017.

### Determining the reproduction mode of *Psorospermum febrifugum*

On February 29<sup>th</sup>, 2016, 240 flowers of *P. febrifugum* in bud stage were labelled among which 120 were left unprotected (treatment 1) and 120 were bagged using gauze bags (treatment 2) to prevent insect visits (Delaplane *et al.*, 2013) [6].

On February 28<sup>th</sup>, 2017, 240 flowers in bud stage were labelled among which 120 were left unprotected (treatment 3) and 120 were bagged using gauze bags to prevent insect visits (treatment 4).

For each studied year, eight days after the shedding of the last labelled flower, the number of fruits was assessed in each treatment. The fruiting index ( $F_i$ ) was then calculated as described by Tchuenguem *et al.* (2001) [7]:  $F_i = F_b / F_a$ , where  $F_b$  is the number of formed fruits and  $F_a$  the number of viable flowers initially set.

The allogamy rate ( $Alr$ ) from which derives the autogamy rate ( $Atr$ ) was expressed as the difference in fruiting indexes between treatment X (unprotected flowers) and treatment Y (protected flowers) (Demarly, 1977) [8].

$Alr = [(F_{iX} - F_{iY}) / F_{iX}] * 100$ , where  $F_{iX}$  and  $F_{iY}$  are respectively the mean fruiting indexes in treatments X and Y.  $Atr = 100 - Alr$ .

### Determination of the place of *Dactylurina staudingeri* in *Psorospermum febrifugum* floral entomofauna

The determination of the frequency of *D. staudingeri* visits on *P. febrifugum* flowers was based on observations of flowers of treatments 1 and 3, every day, from March 1<sup>st</sup> to 8<sup>th</sup>, in 2016 and 2017 respectively. Data were taken according to five daily time frames: 7 - 8 h, 9 - 10 h, 11 - 12 h, 13 - 14 h and 15 - 16 h. In a slow walk along all labelled flowers of treatments 1 and 3, the identity of all insects that visited *P. febrifugum* flowers was recorded (Tchuenguem, 2005) [9]. Specimens of all insect taxa were caught using insect net on unlabeled flowers and conserved in 70% ethanol, excluding butterflies that were preserved dry (Borror and White, 1991) [10], for subsequent taxonomic identification. All insects encountered on flowers were registered and the cumulated results expressed as the number of visits to determine the relative frequency of *D. staudingeri* in the anthophilous

entomofauna of *P. febrifugum* (Tchuenguem, 2005) [9]. Data obtained were used to determine the frequency of visits ( $F_i$ ) of each insect species on *P. febrifugum* flowers; for each studied period,  $F_i = \{[V_i / V_t] * 100\}$ , with  $V_i$  the number of visits of insect  $i$  on flowers of treatment with unprotected flowers, and  $V_t$  the total number of visits of all recorded insect species on these flowers (Tchuenguem, 2005) [9].

### Study of the activity of *Dactylurina staudingeri* on *Psorospermum febrifugum* flowers

In addition to the determination of the flower visiting insect frequency, direct observation of the foraging activity of *D. staudingeri* on flowers was made in the experimental field. The floral products (nectar or pollen) harvested by *D. staudingeri* during each visit were registered based on its foraging behavior. Nectar foragers were seen extending their proboscis in the corolla, while pollen gatherers scratched the anthers using their mandibles and their legs (Jean-Prost, 1987) [11].

In the morning of each sampling day, the number of opened flowers was counted in treatments 1 and 3. During the same days as for the frequency of visits, the duration of individual flower visits was recorded (using a stopwatch) according to four time frames: 8 - 9 h, 10 - 11 h, 12 - 13 h and 14 - 15 h.

Moreover, the number of pollinating visits which was defined as visits with contact between the bees and stigma (Jacob-Remacle, 1989) [12] the abundance of foragers (highest number of individuals foraging simultaneously per flower and per 1000 flowers) (Tchuenguem, 2005) [9] and the foraging speed (number of flowers visited by individual bee per minute (Jacob-Remacle, 1989) [12]) were recorded during the same dates and daily periods as the registration of the duration of visits. The foraging speed ( $F_s$ ) was calculated using the following formula:  $F_s = (Nf / dv) * 60$ , where  $dv$  is the duration (sec) given by a stopwatch and  $Nf$  the number of flowers visited during  $dv$ .

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. The abundance per 1000 flowers ( $A_{1000}$ ) was also recorded: some foragers were counted on a known number of flowers.  $A_{1000}$  was then calculated using the formula:  $A_{1000} = ((Ax / Fx) * 1000)$ , where  $Fx$  and  $Ax$  are the number of opened flowers and the number of foragers effectively counted on these flowers at time  $x$  (Tchuenguem, 2005) [9].

The disruption of the activity of foragers by competitors or predators and the attractiveness exerted by other plant species on *D. staudingeri* were assessed. During each daily period of investigation, a mobile thermo-hygrometer was used to register the temperature and the relative humidity of the study station every 30 min, from 7 am to 4 pm.

### Assessment of the concentration in total sugars of *Psorospermum febrifugum* nectar

The concentration in total sugars of *P. febrifugum* nectar was determined using a handheld refractometer (0 - 50% Brix). From March 1<sup>st</sup> to 8<sup>th</sup>, in 2016 and 2017, at least three times during each of the following daily time frames: 7 - 8 h, 9 - 10 h, 11 - 12 h, 13 - 14 h and 15 - 16 h. Since the nectar of *P. febrifugum* is less directly accessible to the investigator, *Apis mellifera* workers in full activity of nectar harvest were captured on the flowers of this Hypericaceae. Thus collected individuals were anesthetized by their introduction in a small bottle containing cotton moistened with chloroform

(Tchuenguem *et al.*, 2007) <sup>[13]</sup>. Then, by small pressures on the bee abdomen placed between the thumb and the forefinger of the experimenter, the nectar of the crop was expelled and its concentration in total sugars (g/100 g dry matter) measured (Tchuenguem *et al.*, 2007) <sup>[13]</sup>. The registered values were corrected according to the ambient temperature, using a table provided by the device leaflet (Tchuenguem *et al.*, 2007) <sup>[13]</sup>.

#### Evaluation of the meliponicultural value of *Psorospermum febrifugum*

The meliponicultural value of *P. febrifugum* was assessed using data on the plant flowering intensity and the attractiveness of *D. staudingeri* foragers with respect to nectar and pollen.

#### Evaluation of the effect of insects including *Dactylurina staudingeri* on *Psorospermum febrifugum* yields

For each investigation year, this evaluation was based on the impact of flowering insects on pollination, the impact of pollination on *P. febrifugum* fruiting, and the comparison of yields [fruiting rate, number of seeds per fruit and percentage of normal (that is well developed) seeds] of unprotected flowers (treatment X) to that of protected flowers (treatment Y) (Roubik, 1995) <sup>[1]</sup>.

For each observation period, the fruiting rate due to foraging insects including *D. staudingeri* (*Fri*) was calculated using the formula:  $Fri = \{[(Frx - Fry) / Frx] * 100\}$  where *Frx* and *Fry* were the fruiting rate in treatment X and treatment Y respectively. The fruiting rate of a treatment (*Fr*) is:

$Fr = [(Fb / Fa) * 100]$ , where *Fb* is the number of fruits formed and *Fa* the number of viable flowers initially set (Tchuenguem *et al.*, 2001) <sup>[7]</sup>.

At maturity, fruits were harvested from each treatment and the number of seeds per fruit as well as the number of normal seeds were counted. The fruiting rate, the number of seeds per fruit and the percentage of normal seeds were then calculated for each treatment. The impact of flower visiting insects on seed yields was evaluated using the same method as mentioned above for the fruiting rate.

#### Assessment of the pollination efficiency of *Dactylurina staudingeri* on *Psorospermum febrifugum*

In 2016, along with the layout of treatments 1 and 2, 158 flowers at bud stage were protected and two treatments were formed: treatment 5, with 58 flowers protected using gauze bags to prevent insect visitors and destined exclusively to be visited by *D. staudingeri*; treatment 6, with 100 flowers protected from insects then unbagged and reprotected without *D. staudingeri* or any other organism visit.

In 2017, along with the layout of treatments 3 and 4, 164 flowers at the bud stage were labeled and two treatments were formed: treatment 7 with 64 flowers protected from insects using gauze bags and destined exclusively to be visited by *D. staudingeri*; treatment 8 with 100 flowers protected from insects then unbagged and reprotected without *D. staudingeri* or any other organism visit.

As soon as each flower was opened in treatments 5 and 7, the gauze bags were removed and the flower was observed for up to 10 min. Flowers visited once by *D. staudingeri* was marked and then reprotected. For treatment 6 and 8, as soon as each flower was opened, the gauze bag was removed and the flower was observed for up to 10 min while avoiding the visit

by *D. staudingeri* or other organism.

For each observation period, the contribution of *D. staudingeri* in the fruiting rate (*Fr<sub>d</sub>*) was calculated using the formula:  $Fr_d = \{[(f_z - f_w) / f_z] * 100\}$  where *f<sub>z</sub>* and *f<sub>w</sub>* are the fruiting rates in treatments Z (flowers protected and visited exclusively by *D. staudingeri*) and W (flowers protected then unbagged and reprotected without visit of *D. staudingeri* or any other organism) (Tchuenguem *et al.*, 2018) <sup>[14]</sup>. At their maturity, fruits were harvested and counted from treatments Z and W. The fruiting rate, the number of seeds per fruit and the percentage of normal seeds were then calculated for each of these two treatments. The impact of *D. staudingeri* on seed yields was evaluated using the above method as mentioned for fruiting rate.

#### Data analysis

Data were analyzed using descriptive statistics, Student's *t*-test for the comparison of means of two samples, ANOVA (*F*) for the comparison of means of more than two samples, Pearson correlation coefficient (*r*) for the study of the association between two variables, Chi-square ( $\chi^2$ ) for the comparison of percentages and Microsoft Excel 2010.

#### Results and Discussion

##### Reproduction mode of *Psorospermum febrifugum*

The number of fruits was 100, 17, 97 and 14 in treatments 1, 2, 3 and 4 respectively. Thus, in 2016, the fruiting index was 0.83 for treatment 1 and 0.14 for treatment 2, while in 2017, it was 0.80 for treatment 3 and 0.11 for treatment 4. Hence, *Alr* and *Atr* were 83.00% and 17.00% in 2016 against 85.57% and 14.43% in 2017. For the two cumulated years, *Alr* was 84.28% and *Atr* was 15.72%. Consequently, *P. febrifugum* has a mixed mating mode, allogamous and autogamous, with the predominance of allogamy.

##### Place of *Dactylurina staudingeri* in *Psorospermum febrifugum* floral entomofauna

Among 1128 and 1245 visits of 36 and 34 insect species recorded on *P. febrifugum* flowers in 2016 and 2017 respectively, *D. staudingeri* was the most represented with 164 visits (14.54%) and 262 visits (21.04%), in 2016 and 2017 respectively (Table 1). The difference between these two percentages is highly significant ( $\chi^2 = 17.00$ ; *df* = 1; *P* < 0.001).

This difference could be attributed to a combination of climatic factors and seasonal variation in floral resources availability. It is known that in stingless bee colonies, food collection is influenced by both abiotic factors, such as temperature and rainfall (Kajobe and Echazarreta, 2005; Figueiredo-Mecca *et al.*, 2013) <sup>[15, 16]</sup> and differences in floral resource availability (Aleixo *et al.*, 2017) <sup>[17]</sup>. Other researches have revealed *D. staudingeri* among least frequent insects on *Dacryodes edulis* (Tchuenguem *et al.*, 2001) <sup>[7]</sup>, *Zea mays* (Tchuenguem *et al.*, 2002) <sup>[18]</sup>, *Phaseolus coccineus* (Pando *et al.*, 2011a) <sup>[19]</sup>, *Cajanus cajan* (Pando *et al.*, 2011b) <sup>[20]</sup>, *Cucumeropsis mannii* (Azo'o and Messi, 2012) <sup>[21]</sup>, *Vigna unguiculata* (Pando *et al.*, 2013) <sup>[22]</sup>, *Physalis micrantha* (Otiobo *et al.*, 2015) <sup>[23]</sup>, *Physalis minima* (Djakbé *et al.*, 2017) <sup>[24]</sup>, *Ceratotheca sesamoides* (Tchuenguem *et al.*, 2018) <sup>[14]</sup>, *Luffa cylindrica* (Farda and Tchuenguem, 2018) <sup>[25]</sup> and *Helianthus annuus* (Egono *et al.*, 2018) <sup>[26]</sup>.

**Table 1:** Diversity of flowering insects on *Psorospermum febrifugum* in 2016 and 2017 at Dang, number and percentage of visits of different insects.

Insects			2016		2017		Total 2016/2017		
Order	Family	Genus and species	$n_1$	$p_1$ (%)	$n_2$	$p_2$ (%)	$n_T$	$p_T$ (%)	
Coleoptera	Scarabeidae	(sp. 1) (ne)	8	0.71	-	-	8	0.34	
		(sp. 2) (ne)	22	1.95	18	1.45	40	1.69	
		(sp. 3) (ne)	-	-	6	0.48	6	0.25	
Diptera	Bombyliidae	(sp.) (ne)	5	0.44	-	-	5	0.21	
	Calliphoridae	<i>Calliphora</i> sp. (ne)	18	1.60	12	0.96	30	1.26	
	Syrphidae	(sp.) (ne)	5	0.44	8	0.64	13	0.55	
Hemiptera	Alydidae	<i>Riptortus dentipes</i> (ne)	8	0.71	5	0.40	13	0.55	
	Pyrrhocoridae	<i>Dysdercus voelkeri</i> (ne)	9	0.80	11	0.88	20	0.84	
Hymenoptera	Apidae	<i>Apis mellifera</i> (ne, po)	144	12.77	185	14.86	329	13.86	
		<i>Ceratina</i> sp. 1 (ne, po)	39	3.46	48	3.86	87	3.67	
		<i>Ceratina</i> sp. 2 (ne, po)	11	0.98	17	1.37	28	1.18	
		<i>Dactylurina staudingeri</i> (ne, po)	164	14.54	262	21.04	426	17.95	
		<i>Meliponula ferruginea</i> (ne, po)	38	3.37	23	1.85	61	2.57	
		Formicidae	<i>Camponotus brutus</i> (ne)	14	1.24	22	1.77	36	1.52
			<i>Polyrhachis</i> sp. (ne)	12	1.06	14	1.12	26	1.10
		Halictidae	<i>Crocisaspida chandleri</i> (ne)	4	0.35	2	0.16	6	0.25
			<i>Lasioglossum</i> sp. (po)	26	2.30	45	3.61	71	2.99
			(sp.) (po)	6	0.53	10	0.80	16	0.67
		Megachilidae	<i>Coelioxys circumscriptus</i> (ne)	11	0.98	23	1.85	34	1.43
			<i>Megachile torrida</i> (po)	28	2.48	36	2.89	64	2.70
			<i>Megachile</i> sp. 1 (ne)	23	2.04	31	2.49	54	2.28
			<i>Megachile</i> sp. 2 (po)	17	1.51	22	1.77	39	1.64
		Scoliidae	(sp.) (ne)	6	0.53	-	-	6	0.25
Crabronidae	<i>Bembix</i> sp. (ne)	26	2.30	17	1.37	43	1.81		
	<i>Philanthus triangulum</i> (ne)	12	1.06	23	1.85	35	1.47		
Vespidae	<i>Belonogaster juncea</i> (ne)	12	1.06	8	0.64	20	0.84		
	(sp.) (ne)	24	2.13	16	1.29	40	1.69		
Lepidoptera	Hesperiidae	(sp.) (ne)	5	0.44	9	0.72	14	0.59	
		(sp.) (ne)	17	1.51	7	0.56	24	1.01	
	Lycaenidae	<i>Danaus chrysippus</i> (ne)	18	1.60	11	0.88	29	1.22	
		<i>Hypolimnas misippus</i> (ne)	52	4.61	42	3.37	94	3.96	
		<i>Junonia hierta</i> (ne)	4	0.35	10	0.80	14	0.59	
	Papilionidae	<i>Graphium angolanus</i> (ne)	128	11.35	105	8.43	233	9.82	
		<i>Papilio demodocus</i> (ne)	82	7.27	66	5.30	148	6.24	
	Pieridae	<i>Catopsilia florella</i> (ne)	68	6.03	77	6.18	145	6.11	
<i>Eurema</i> sp. (ne)		26	2.30	31	2.49	57	2.40		
	<i>Mylothris chloris</i> (ne)	36	3.19	23	1.85	59	2.49		
Total		37 species	1128	100	1245	100	2373	100	

$n_1$  and  $n_2$ : number of visits on 120 flowers in 8 days in 2016 and 2017 respectively;  $n_T$ : total number of visits on 240 flowers in 16 days; sp.: undetermined species; ne: visitor collected nectar; po: visitor collected pollen;  $p_1$  and  $p_2$ : percentages of visits in 2016 and 2017 respectively;  $p_T$ : total percentage of visits;  $p_1 = (n_1 / 1128) * 100$ ;  $p_2 = (n_2 / 1245) * 100$ ;  $p_T = (n_T / 2373) * 100$ . Comparison of percentages of *Dactylurina staudingeri* visits for the two years:  $\chi^2 = 17.00$  ( $df = 1, P < 0.001$ ).

### Activity of *Dactylurina staudingeri* on *Psorospermum febrifugum* flowers

#### Floral products harvested

During each flowering season, *D. staudingeri* foragers were seen collecting nectar (Fig. 1, a) and pollen (Fig. 1, b) on *P. febrifugum* flowers.



(a)

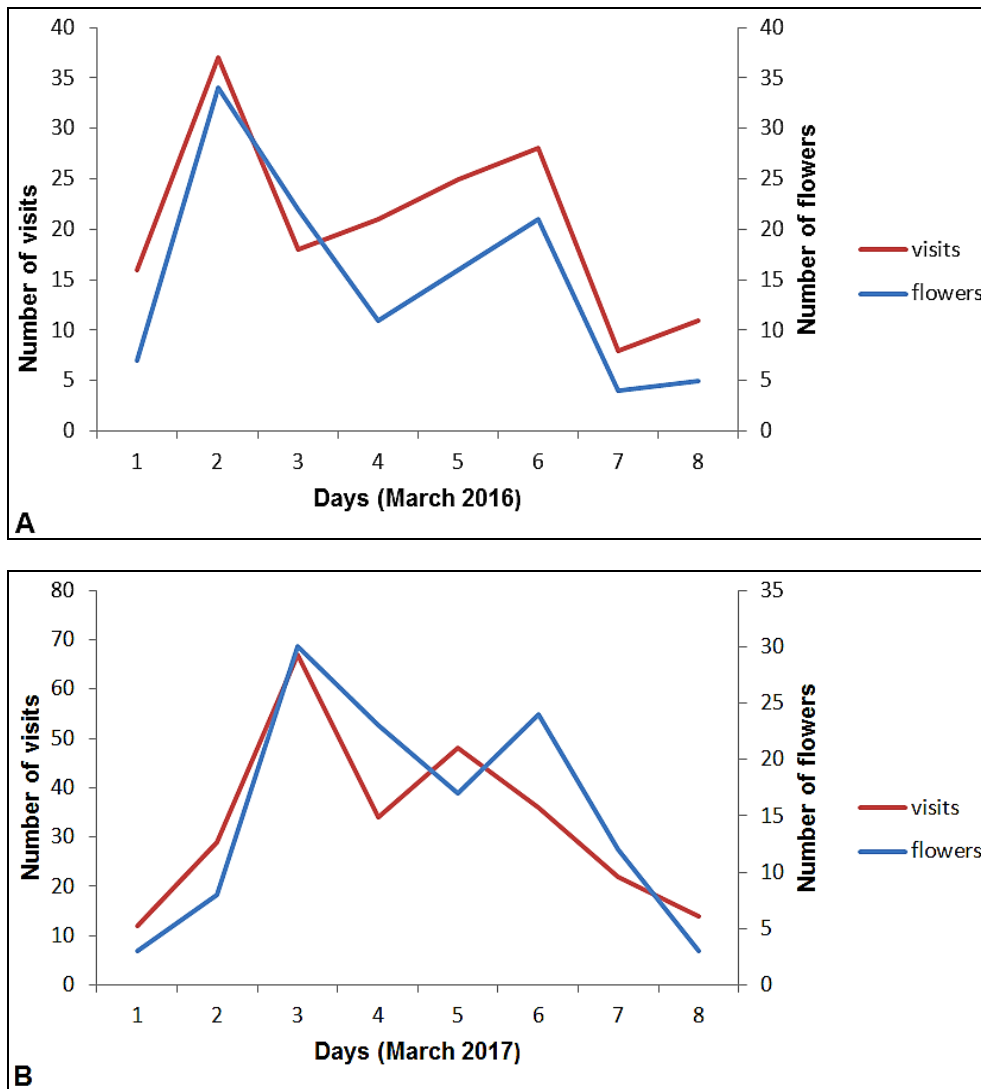
(b)

**Fig. 1.** *Dactylurina staudingeri* collecting nectar (a) and pollen (b) on *Psorospermum febrifugum* flowers at Dang in 2017.

For 486 visits recorded in 2016, 330 (67.90%) were devoted to nectar harvest and 156 (32.10%) for pollen; in 2017, for 562 visits registered, 418 (74.38%) were devoted to nectar harvest and 144 (25.62%) to pollen collection. Thus during each of the two flowering periods of *P. febrifugum*, *D. staudingeri* intensely and regularly harvested nectar compared to the pollen which was slightly harvested. Similar observations were made by Azo'o *et al.* (2010) [27] on *Citrullus lanatus*, Pando *et al.* (2011b) [20] on *Cajanus cajan*, Pando *et al.* (2013) [22] on *Vigna unguiculata*, Otiobo *et al.* (2015) [23] on *Physalis micrantha* and Egono *et al.* (2018) [26] on *Helianthus annuus*. This could be attributed to the needs of *D. staudingeri* colonies during the flowering period of *P. febrifugum*.

#### Rhythm of visits according to the flowering stages

*Dactylurina staudingeri* visits were more numerous on *P. febrifugum* individual plant when their number of opened flowers was highest (Fig. 2).

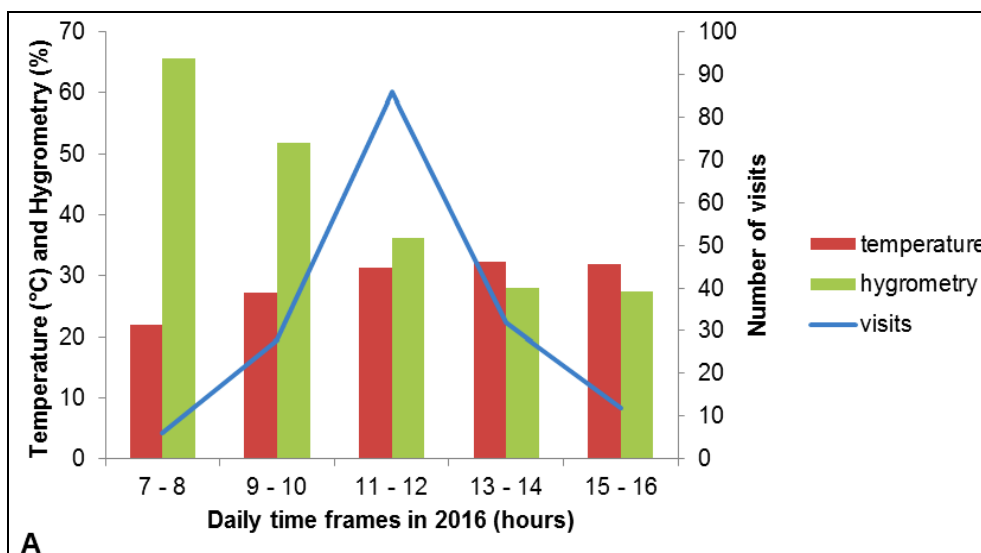


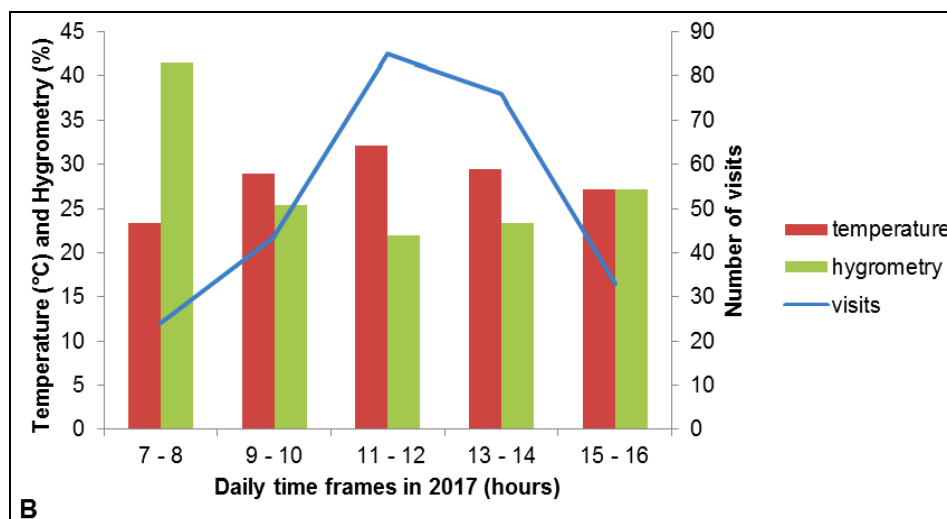
**Fig 2:** Seasonal variation of the number of *Psorospermum febrifugum* opened flowers and the number of *Dactylurina staudingeri* visits on these organs, in 2016 (A) and 2017 (B) at Dang.

Furthermore, we found a positive and significant correlation between the number of opened flowers and the number of visits in 2016 ( $r = 0.89$ ;  $df = 6$ ;  $P < 0.01$ ) as well as in 2017 ( $r = 0.86$ ;  $df = 6$ ;  $P < 0.01$ ). This result indicates the good attractiveness of *P. febrifugum* nectar and / or pollen with respect to *D. staudingeri*.

**Daily rhythm of visits**

*Dactylurina staudingeri* was active on *P. febrifugum* flowers from 7 am to 4 pm and throughout its blooming period, with a peak of visits between 11 and 12 am in 2016 as well as in 2017 (Fig. 3).





**Fig 3:** Daily variation of *Dactylurina staudingeri* visits on *Psorospermum febrifugum* flowers in 2016 (A) and 2017 (B) at Dang, mean temperature and mean humidity of the study station.

This peak of activity could be linked to the period of the highest availability of nectar or pollen on this Hypericaceae. The same peak of visits has been reported by Tchuenguem *et al.* (2002) [18] on *Zea mays* in Yaoundé. Our results are not in line with those obtained with the same stingless bee by Azo'o and Messi (2012) [21] on *Cucumeropsis mannii* in Yaoundé. According to these authors, *D. staudingeri* had a peak of activity situated between 9 and 10 am.

In 2016, the correlation was not significant between the number of *D. staudingeri* visits and the temperature ( $r = 0.45$ ;  $df = 3$ ;  $P > 0.05$ ) as well as between the relative humidity and the same number of visits ( $r = -0.32$ ;  $df = 3$ ;  $P > 0.05$ ). In 2017, the correlation was significant between the number of *D. staudingeri* visits and the temperature ( $r = 0.88$ ;  $df = 3$ ;  $P < 0.05$ ), while it was not significant between the number of visits and the relative humidity ( $r = -0.77$ ;  $df = 3$ ;  $P > 0.05$ ).

#### Abundance of *Dactylurina staudingeri*

In 2016, the highest mean number of *D. staudingeri* individuals simultaneously in activity was 1 per flower ( $n = 212$ ;  $s = 0.70$ ) and 39.20 per 1000 flowers ( $n = 240$ ;  $s = 21.53$ ). In 2017, the corresponding values were 1 individual per flower ( $n = 355$ ;  $s = 0$ ) and 60.80 individuals per 1000 flowers ( $n = 206$ ;  $s = 28.28$ ). The difference between these two means is highly significant ( $t = 9.14$ ;  $df = 444$ ;  $P < 0.001$ ). The highly abundance of *D. staudingeri* foragers on 1000 flowers indicates the good attractiveness of *P. febrifugum* nectar and / or pollen with respect to this stingless bee. The highly significant difference between the first and the second year could be explained by the variation in the foraging behavior of flowering insects, the biotic and abiotic factors as well as floral resources.

#### Duration of visits per flower

In 2016 and 2017, the mean duration of a *D. staudingeri* visit per flower for nectar harvest was 14.77 sec ( $n = 330$ ;  $s = 12.47$ ) and 15.48 sec ( $n = 418$ ;  $s = 12.09$ ) respectively. The difference between these two means is not significant ( $t = 0.79$ ;  $df = 746$ ;  $P > 0.05$ ). For pollen, the corresponding figures were 14.18 sec ( $n = 156$ ;  $s = 11.55$ ) and 13.68 sec ( $n = 144$ ;  $s = 11.66$ ), in 2016 and 2017 respectively. The difference between these two means is not significant ( $t = 0.37$ ;  $df = 298$ ;  $P > 0.05$ ).

For the two cumulated years, the mean duration of a visit per

flower was 15.13 sec ( $n = 374$ ;  $s = 12.28$ ) for nectar collection and 13.93 sec ( $n = 150$ ;  $s = 11.61$ ) for pollen harvest. The difference between these two means is not significant ( $t = 1.03$ ;  $df = 522$ ;  $P > 0.05$ ).

In 2008, Tchuenguem *et al.* [28] have noted 5.25 sec for *A. mellifera* visit devoted to the nectar harvest on the same plant. Tchuenguem *et al.* (2002) [18] found 3.46 sec as duration of a visit for pollen harvest by *D. staudingeri* on *Zea mays*. These durations of visit are smaller than that recorded on *P. febrifugum*. Thus the duration of a visit on a flower for the harvest of a floral product varies with social bee and plant species.

#### Foraging speed

On *P. febrifugum*, *D. staudingeri* visited between 1 and 26 flowers per minute in 2016 and between 1 and 30 flowers per minute in 2017. The mean foraging speed was 4.94 flowers per minute ( $n = 186$ ;  $s = 3.42$ ) in 2016 and 5.12 flowers per minute ( $n = 256$ ;  $s = 4.14$ ) in 2017. The difference between these two means is not significant ( $t = 0.48$ ;  $df = 440$ ;  $P > 0.05$ ).

#### Influence of the fauna

In 2016, for 522 visits of *D. staudingeri*, 14 (2.68%) was interrupted by *D. staudingeri*, 11 (2.11%) by *Apis mellifera*, 6 (1.15%) by *Megachile torrida* and 5 (0.96%) by *Graphium angolanus*. In 2017, for 609 visits of *D. staudingeri*, 21 (3.45%) was interrupted by *D. staudingeri*, 18 (2.95%) by *A. mellifera*, 6 (0.99%) by *M. torrida* and 2 (0.33%) by *G. angolanus*. In order to obtain their optimal nectar or pollen loads, individuals of *D. staudingeri* who suffered from such disturbances were forced to visit more flowers during the corresponding foraging trip.

#### Influence of neighboring flora

During the flowering period of *P. febrifugum*, flowers of many other plant species surrounding *P. febrifugum* blooming individuals were visited by *D. staudingeri* foragers, for nectar (ne) and / or pollen (po). Among these plants were: *Euphorbia milii* (Euphorbiaceae, ne and po), *Jatropha gossypifolia* (Euphorbiaceae, ne and po) and *Vernonia amygdalina* (Asteraceae, po). During the two years of study, we observed no passage of *D. staudingeri* from *P. febrifugum* flowers to another plant species and vice versa. Thus during

foraging trips on *P. febrifugum*, individuals of *D. staudingeri* were faithful to this Hypericaceae.

### Concentration in total sugars of *Psorospermum febrifugum* nectar

The mean concentration in total sugars of *P. febrifugum* nectar was 36.63% ( $n = 50$ ;  $s = 9.65$ ) in 2016 and 37.58% ( $n = 50$ ;  $s = 8.81$ ) in 2017. The difference between these means is not significant ( $t = 0.51$ ;  $df = 98$ ;  $P > 0.05$ ). For the two years, the mean concentration in total sugars of *P. febrifugum* nectar was 37.11%.

The mean concentration in total sugars of *P. febrifugum* nectar (37.11%) was high, compared to the range of 5 to 80% for several plant species (Philippe, 1991) [29] and could justify the good attractiveness of the flowers of this Hypericaceae with respect to *D. staudingeri*.

### Meliponicultural value of *Psorospermum febrifugum*

During the two flowering seasons, a well elaborated activity of *D. staudingeri* foragers was registered on *P. febrifugum* flowers. In particular, there were a good daily and seasonal frequency of visits, a high density of foragers per 1000 flowers, a good nectar harvest, a slight pollen collection and the fidelity of the foragers to *P. febrifugum* flowers during foraging bouts. Furthermore, each *P. febrifugum* plant could produce 6000 to more than 10000 flowers. In addition, according to our investigations, during eight days ( $n = 100$ ;  $s = 9.23$ ), each *P. febrifugum* flower produced nectar that was rich in sugars (up to 37.11%) and easy for *D. staudingeri* to harvest.

These data highlight the good attractiveness of *P. febrifugum* nectar and slight attractiveness of its pollen to *D. staudingeri*. Therefore, *P. febrifugum* is a highly nectariferous and slightly polliniferous stingless bee plant.

### Impact of flowering insects including *Dactylurina staudingeri* on *Psorospermum febrifugum* yields

The table 2 indicates the fruiting rate, the mean number of seeds per fruit and the percentage of normal seeds in the different treatments of *P. febrifugum*.

**Table 2:** Fruiting rate, mean number of seeds per fruit and percentage of normal seeds according to different treatments of *Psorospermum febrifugum* in 2016 and 2017 at Dang.

Years	Treatments	NF	Nfr	FrR (%)	seeds / fruit		TNS	NS	% NS
					m	sd			
2016	1 (Uf)	120	100	83.33	3.43	1.25	343	312	90.96
	2 (Pf)	120	17	14.16	2.41	1.06	41	16	39.02
	5 (Fpvd)	58	38	65.51	3.36	0.91	128	110	85.93
	6 (Fpwv)	100	24	24.00	2.83	0.96	68	41	60.29
2017	3 (Uf)	120	97	80.83	3.50	1.25	340	308	90.58
	4 (Pf)	120	14	11.66	2.42	1.08	34	13	38.23
	7 (Fpvd)	64	47	73.44	3.42	1.15	161	126	78.26
	8 (Fpwv)	100	23	23.00	2.78	0.73	64	35	54.68

NF: number of flowers; Nfr: number of fruits; FrR: fruiting rate; TNS: total number of seeds; NS: number of normal seeds; % NS: percentage of normal seeds; m: mean; sd: standard deviation; Uf: unprotected flowers; Pf: protected flowers; Fpvd: flowers protected unbagged, exclusively visited once by *Dactylurina staudingeri* and rebagged; Fpwv: flowers protected then unbagged and rebagged without visit by insects or any other organism.

### This table shows that

a) The fruiting rates were 83.33%, 14.16%, 80.83%, 11.66%, 65.51%, 24.00%, 73.44% and 23.00% in

treatments 1 to 8 respectively. The difference between all these percentages is highly significant ( $\chi^2 = 301.75$ ;  $df = 7$ ;  $P < 0.001$ ). The two to two comparisons show that the difference observed is highly significant between treatments 1 and 2 ( $\chi^2 = 114.89$ ;  $df = 1$ ;  $P < 0.001$ ) and treatments 3 and 4 ( $\chi^2 = 115.47$ ;  $df = 1$ ;  $P < 0.001$ ). Consequently, in 2016 and 2017, the fruiting rate of exposed flowers (treatments 1 and 3) was higher than that of flowers protected during their opening period (treatments 2 and 4).

b) The mean numbers of seeds per fruit were 3.43, 2.41, 3.50, 2.42, 3.36, 2.83, 3.42 and 2.78 in Treatments 1 to 8 respectively. The difference between all these means is highly significant ( $F = 4.52$ ;  $df_1 = 7$ ;  $df_2 = 352$ ;  $P < 0.001$ ). The two to two comparison shows that the difference is highly significant between treatments 1 and 2 ( $t = 3.17$ ;  $df = 115$ ;  $P < 0.01$ ) and treatments 3 and 4 ( $t = 3.06$ ;  $df = 109$ ;  $P < 0.01$ ). Consequently, in 2016 as well as in 2017, the mean number of seeds per fruit of unprotected flowers was higher than that of protected flowers.

c) The percentages of normal seeds were 90.96%, 39.02%, 90.58%, 38.23%, 85.93%, 60.29%, 78.26% and 54.68% in treatments 1 to 8 respectively. The difference between all these percentages is highly significant ( $\chi^2 = 183.93$ ;  $df = 7$ ;  $P < 0.001$ ). The two to two comparisons show that the difference observed is highly significant between treatments 1 and 2 ( $\chi^2 = 79.31$ ;  $df = 1$ ;  $P < 0.001$ ) and treatments 3 and 4 ( $\chi^2 = 69.65$ ;  $df = 1$ ;  $P < 0.001$ ). Hence, in 2016 and 2017, the percentage of normal seeds of exposed flowers was higher than that of flowers protected during their opening period.

In 2016, the numeric contributions of anthophilous insects on the fruiting rate, the number of seeds per fruit and the percentage of normal seeds were 83.00%, 29.73% and 57.10% respectively. In 2017, the corresponding figures were 85.57%, 30.85% and 57.79%.

For the two cumulate years, the numeric contributions of flowering insects were 84.28%, 30.29% and 57.44% for the fruiting rate, the number of seeds per fruit and the percentage of normal seeds, respectively.

### Pollination efficiency of *Dactylurina staudingeri* on *Psorospermum febrifugum*

During pollen and / or nectar harvest in flowers, individuals of *D. staudingeri* always came into contact with anthers and stigma (100% of visits in 2016 as well as in 2017) and thus increasing the possibilities of *P. febrifugum* pollination.

The comparison of the fruiting rates (Table 2) shows that the difference observed is highly significant between treatments 5 and 6 ( $\chi^2 = 26.54$ ;  $df = 1$ ;  $P < 0.001$ ) and treatments 7 and 8 ( $\chi^2 = 40.58$ ;  $df = 1$ ;  $P < 0.001$ ). Hence, in 2016 and 2017, the fruiting rate of flowers protected and visited exclusively by *D. staudingeri* was higher than that of flowers protected then unbagged and reprotected without visit of this stingless bee or any other organism.

The fruiting rate due to *D. staudingeri* was 63.36% in 2016, 68.68% in 2017 and 66.02% for the two cumulated years.

The comparison of the mean number of seeds per fruit (Table 2) shows that the difference observed is significant between treatments 5 and 6 ( $t = 2.18$ ;  $df = 60$ ;  $P < 0.05$ ) and treatments 7 and 8 ( $t = 2.43$ ;  $df = 68$ ;  $P < 0.05$ ).

The number of seeds per fruit due to *D. staudingeri* was 15.77% in 2016, 18.71% in 2017 and 17.24% for the two cumulated years.

The comparison of the percentage of normal seeds (Table 2) shows that the difference is highly significant between treatments 5 and 6 ( $\chi^2 = 16.51$ ;  $df = 1$ ;  $P < 0.001$ ) and treatments 7 and 8 ( $\chi^2 = 12.50$ ;  $df = 1$ ;  $P < 0.001$ ). Our observations pointed out that flowers visited by *D. staudingeri* have the highest number of normal seeds compare to those protected then unbagged and reprotected without visit of this stingless bee or any other organism.

The percentage of normal seeds due to *D. staudingeri* was 29.83% in 2016, 30.12% in 2017 and 29.97% for the two cumulated years.

The positive and significant contribution of *D. staudingeri* in the fruiting rate, the number of seeds per fruit and the percentage of normal seeds of *P. febrifugum* is justified by the action of the stingless bees on the pollination of visited flowers.

### Conclusion

From our observations, *P. febrifugum* is a plant species that highly benefits from pollination by insect, among which *D. staudingeri* is one of the most important and harvest nectar and pollen. The comparison of fruit and seed yields of flowers visited exclusively by *D. staudingeri* with those protected from insects then uncovered and reprotected without the visit of insect or any other organism demonstrates the value of this bee in increasing fruit production as well as seed quality. *Psorospermum febrifugum* is a highly nectariferous and slightly polliniferous stingless bee plant, that should be planted and protected to increase stingless bee honey production. Conservation of *D. staudingeri* nests close to *P. febrifugum* population is recommended to improve its fruit and seed productions as well as its seed quality in the Adamawa region.

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