International Journal of Science and Research Archive

eISSN: 2582-8185 CODEN (USA): IJSRO2

Cross Ref DOI: 10.30574/ijsra

Journal homepage: https://ijsra.net/

(RESEARCH ARTICLE)

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Pollination efficiency of a single flower visit of *Graphium angolanus* Goeze (Lepidoptera: Papilionidae) on *Psorospermum febrifugum* Spach (Hypericaceae) at Dang (Ngaoundere, Cameroon)

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International Journal of Science and Research Archive, 2023, 10(01), 090–096

Publication history: Received on 17 July 2023; revised on 30 August 2023; accepted on 02 September 2023

Article DOI: https://doi.org/10.30574/ijsra.2023.10.1.0695

Abstract

To determine the impact of *Graphium angolanus* on fruit and seed production of *Psorospermum febrifugum*, its foraging and pollinating activities were studied at Dang, from March 1st to 8th, in 2016 and 2017. For each cropping period, two treatments were set up through labelling of 100 to 200 flowers: one treatment made up of flowers that were protected then uncovered, exposed to a single visit of *G. angolanus* and reprotected; another treatment with at flowers protected then uncovered and reprotected without insect or any other organism visit. The foraging behavior and pollination efficiency of *G. angolanus* were measured. The fruiting rate, the mean number of seeds per fruit and the percentage of normal seeds of *P. febrifugum* were evaluated. The fruiting rate, the mean number of seeds per fruit and the percentage of normal seeds were evaluated. Results show that, *G. angolanus* foraged on *P. febrifugum* flowers throughout its whole blooming period. On flowers, individual of *G. angolanus* intensely harvested nectar. The mean number of individuals foraging simultaneously on 1000 flowers was 19, the mean duration of a visit per flower was 1.57 second and the mean foraging speed was 33.42 flowers per minute. For the two years, through the pollination efficiency of a single flower visit, *G. angolanus* provoked a significant increment of the fruiting rate by 47.95%, the mean number of seeds per fruit by 15.23% and the percentage of normal seeds by 26.32%. The protection of populations of *P. febrifugum* is important to maintain the butterfly *G. angolanus* in the environment so that it contributes to the plant covert essential for the conservation of biodiversity, and to improve fruit production and seed quality of this Hypericaceae.

Keywords: *Graphium angolanus*; *Psorospermum febrifugum*; Flowers; Pollination efficiency; Yields

1. Introduction

Pollination insects are a group comprising a very large number of species; the socials bees are undoubtedly the best known species, but it is not to forget the other species such as solitary bees, some flies, certain beetles and the Lepidoptera order [1]. Butterflies are among the regular visitors of the flowers, in search of food [2]. They are phytophagous insects which include more than 160000 species in the world; the best known are the rhopaloceres [1]. What most distinguishes butterflies from other pollinators insects of many plant species cultivated or spontaneous is their proboscis, constituted of a set of buccal pieces very well suited for harvesting nectar [3]. Thus, a butterfly is able to search for nectar from any angle and the movement of proboscis in the flower causes the deposition of pollen grains on stigma of the visited flower [1]. *Graphium angolanus* is one of the species, originary of Angola and belonging to the family of Papilionidae [4].

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Psorospermum febrifugum is a spontaneous plant from Hypericaceae family, native to tropical Africa [5]. It is a shrub 1 to 6 m high and is found in the Sudanian savannhs where it flowers in the dry season; the inflorescence is a terminal, short-stalked corymb 5 to 7 mm long [5]. The flower is sweet smelling, cream-white, usually densely hairy and glandular, with five sepals, five hairy petals and many stamens [6]. The edible fruits round are berries of 6 - 10 mm, bright to dark red, in terminal clusters [6]. The roots and leaves are used in Uganda to treat diarrhea [7]. In Cameroon, the leaves are used to treat epilepsy [8]. In Nigeria, leaf and stem extracts have shown anioxidant and antiacne activities [9]. Its root bark is used in Benin to treat anaemia [10].

For better increasing the fruiting rate of this Hypericaceae in Cameroon, observations were carried out on the *G*. *angolanus* flora at Dang village located in Ngaoundere III Subdivision, in the Adamawa region. In this country, the demand of fruits and seeds of *P. febrifugum* is high, whereas its yields are weak, notably because of the absence of published data on the relations between *P*. *febrifugum* and anthophilous insects such as *G*. *angolanus* in the different regions. Referring to the best of our knowledge, no previous work has been reported on the foraging and pollination activity of *G*. *angolanus* on *P*. *febrifugum*.

The present work is a contribution to the understanding of the relationships between *P*. *febrifugum* and *G*. *angolanus*, for their optimal management in Cameroon. It has two main specific objectives: study the activity of *G*. *angolanus* on *P*. *febrifugum* flowers; assess the pollination efficiency of *G*. *angolanus* on this plant.

2. Material and methods

2.1. Biological material and site

The animal material consisted of *G*. *angolanus* naturally found in the environment of the study site and the plant material consisted of the flowering plants of *P*. *febrifugum* grown as spontaneous plants.

Field observations were conducted during two seasons from February to May in 2016 and 2017 in order to identify insect visitors of *P*. *febrifugum*. These periods correspond to the peak of flowering season of *P*. *febrifugum*, in Dang, a village in the Vina Division, Adamawa Region of Cameroon. This Region is located within the high-altitude Guinean savannah agro-ecological zone; the climate is characterized by a rainy season (April to October) and a dry season (November to March), with an average annual rainfall of about 1500 mm; the mean annual temperature is 22 ^{o}C, while the mean annual relative humidity is 70% [11]. The vegetation in the study site was represented by crops, ornamentals, hedge and native plants of savannah and gallery forests.

2.2. Study of the activity of *Graphium angolanus* on *Psorospermum febrifugum* flowers

In addition to the determination of the flower visiting insect frequency, direct observation of the foraging activity of *G. angolanus* on flowers was made in the experimental field. The floral product (nectar) harvested by *G. angolanus* during each visit were registered based on its foraging behavior. Nectar foragers were seen extending their proboscis in the corolla [12].

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 three time frames:

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 butterflies and stigma [13], the abundance of foragers (highest number of individuals foraging simultaneously per flower and per 1000 flowers) [14] and the foraging speed (number of flowers visited by individual of *G. angolanus* per minute [13]) were recorded during the same dates and daily periods as the registration of the duration of visits. The foraging speed (*Fs*) was calculated using the following formula:

$Fs = (Nf / dv) * 60$, 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 [14].

The attractiveness exerted by other plant species on *G. angolanus* was 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 9 am to 4 pm.

2.3. Assessment of the pollination efficiency of *Graphium angolanus* on *Psorospermum febrifugum*

The contribution of *G. angolanus* in the fruiting rate, the mean number of seeds per fruit and the percentage of normal seeds was calculated using data of treatments 1 and 2 for 2016 and those of treatments 3 and 4 for 2017.

For each observation year, the contribution of *G. angolanus* in the fruiting rate (*FrG*) was calculated using the formula *FrG* = {[(*FG* - *FZ*) / *FG*] * 100}, where *FG* and *FZ* are the fruiting rate in treatment *G* (flowers protected then uncovered and visited once by *G. angolanus*) and *Z* (flowers protected then uncovered and reprotected, without insect or any other organism visit) respectively [15].

The number of seeds per fruit and the percentage of normal seeds dues to *G. angolanus* were evaluated using the above method as mentioned for fruiting rate.

2.4. Data analysis

Data were analyzed using descriptive statistics, ANOVA (*F*) for the comparison of means of more than two samples, student's *t*-test for the comparison of means of two samples, Chi-square (χ^2) for the comparison of percentages, Pearson correlation coefficient (*r*) for the study of the association between two variables performed by SPSS 18.5.2.0.

3. Results and discussion

3.1. Activity of *Graphium angolanus* on *Psorospermum febrifugum* flowers

3.1.1. Floral product harvested

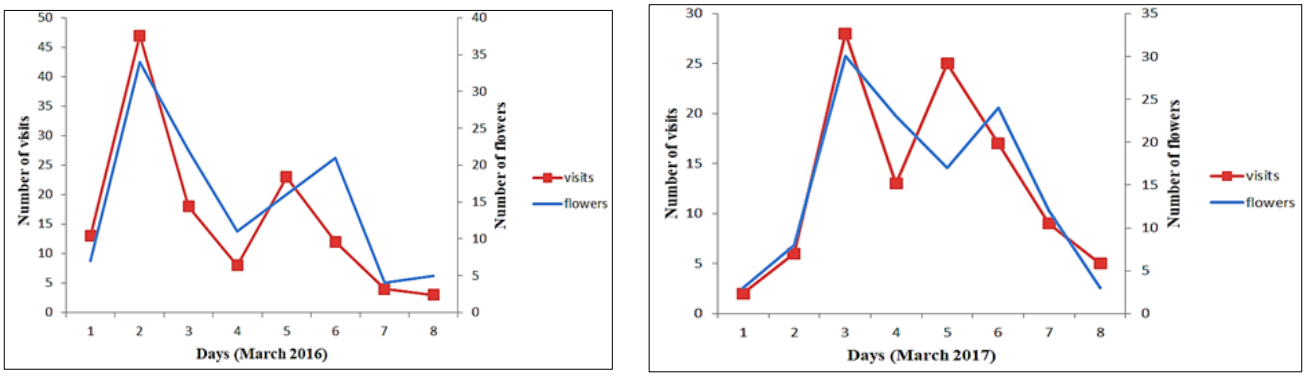
During each flowering season, *G. angolanus* foragers were seen exclusively collecting nectar (Figure 1) in *P. febrifugum* flowers.

Figure 1 *Graphium angolanus* collecting nectar in *Psorospermum febrifugum* flowers at Dang in 2017

During each of the two flowering periods of *P. febrifugum* (355 and 140 visits recorded in 2016 and 2017 respectively), *G. angolanus* intensely and exclusively harvested nectar. Similar observations were made by Kudum and Kwapong (2010) on *Ananas comosus* [16], Yapo *et al*. (2018) on *Anacardium occidentale* [17], Louabé *et al*. (2020, 2022) on *Lantana camara* [18, 19] in Ghana, Ivory Coast and Cameroon respectively.

3.1.2. Rhythm of visits according to the flowering stages

Graphium angolanus visits were more numerous on *P. febrifugum* individual plant when their number of opened flowers was high (Figure 2).



A B

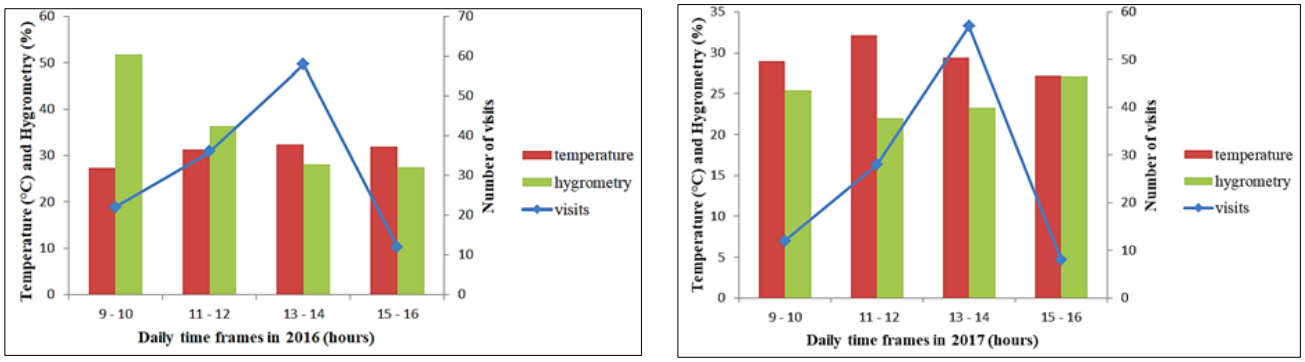
Figure 2 Seasonal variation of the number of *Psorospermum febrifugum* opened flowers and the number of *Graphium angolanus* visits on these organs, in 2016 (A) and 2017 (B) at Dang

Furthermore, we found a positive and significant correlation between the number of opened flowers and the number of visits in 2016 (r = 0.87; df = 6; P < 0.01) as well as in 2017 (r = 0.84; df = 6; P < 0.01). This result indicates the good attractiveness of *P. febrifugum* nectar with respect to *G. angolanus*.

This difference could also be explained by the fact that butterflies with high wing loading have greater energy demands and longer proboscises increase potential access to a wider range of nectar flowers [20].

3.1.3. Daily rhythm of visits

Graphium angolanus was active on *P. febrifugum* flowers from 9 am to 4 pm and throughout its blooming period, with a peak of visits between 1 and 2 pm in 2016 as well as in 2017 (Figure 3).



A B

Figure 3 Daily variation of *Graphium angolanus* 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 on this Hypericaceae.

The correlation was not significant between the number of *G. angolanus* visits and the temperature (r = 0.40; df = 2; P > 0.05) as well as between the relative humidity and the same number of visits (r = - 0.28; df = 2; P > 0.05) in 2016. Moreover, the correlation was not also significant between the number of *G. angolanus* visits and the temperature (r = 0.37; df = 2; P < 0.05) and between the number of visits and the relative humidity (r = - 0.67; df = 2; P > 0.05) in 2017.

3.1.4. Abundance of Graphium angolanus

In 2016, the highest mean number of *G. angolanus* individuals simultaneously in activity was 1 per flower (n = 170; s = 0) and 19 per 1000 flowers (n = 166; s = 7.48; *mini* = 10; *maxi* = 30). In 2017, the corresponding values were 1 individual per flower (n = 214; s = 0) and 20 individuals per 1000 flowers (n = 151; s = 9.74; *mini* = 5; *maxi* = 40). The difference between these two means is not significant (t = 1.59; df = 315; P > 0.05).

3.1.5. Duration of visits per flower

In 2016 and 2017, the mean duration of a *G. angolanus* visit per flower for nectar harvest exclusively was 1.66 sec (n = 355; s = 1.28; *mini* = 1; *maxi* = 12) and 1.47 sec (n = 140; s = 1.08; *mini* = 1; *maxi* = 6) respectively. The difference between these two means is not significant (t = 1.55; df = 493; P > 0.05).

3.1.6. Foraging speed

On *P. febrifugum*, *G. angolanus* between 6 and 60 flowers per minute in 2016 and between 12 and 54 flowers per minute in 2017. The mean foraging speed was 33.88 flowers per minute (n = 162; s = 11.85) in 2016 and 32.96 flowers per minute (n = 217; s = 9.67) in 2017. The difference between these two means is not significant (t = 0.83; df = 377; P > 0.05).

3.1.7. Influence of neighboring flora

During the flowering period of *P. febrifugum*, flowers of one other plant species surrounding *P. febrifugum* blooming individuals were visited by *G. angolanus* foragers, for nectar. This plant was *Lantana camara* (Verbenaceae) (Figure 4).

Figure 4 *Graphium angolanus* collecting nectar in *Lantana camara* flowers during the flowering period of *Psorospermum febrifugum* at Dang in 2017

During the two years of study, no passage of *G. angolanus* from *P. febrifugum* flowers to this Verbenaceae was observed and vice versa. Thus during foraging trips on *P. febrifugum*, individuals of *G. angolanus* were faithful to this plant species. Floral constancy is an important aspect in management of pollination [21]. Attributes of plants such as flower color, scent and morphology largely determine the associated pollinators guilds and insects typically acquire preferences for particular flowering plants [20].

3.2. Pollination efficiency of *Graphium angolanus* on *Psorospermum febrifugum*

During nectar harvest on *P. febrifugum*, individuals of *G. angolanus* always shook flowers and regularly contacted anthers and stigma (100% of visits in 2016 as well as in 2017), increasing self-pollination and / or cross-pollination possibilities of this plant species.

The fruiting rates were 42.30%, 24.00%, 48.57% and 23.00% in treatments 1 to 4 respectively. The differences between these four percentages are highly significant (χ^2 = 13.56; df = 3; P < 0.001). Two to two comparisons showed that the difference observed is highly significant between treatments 1 and 2 (χ^2 = 26.54; df = 1; P < 0.001) as well as between treatments 3 and 4 (χ^2 = 40.58; df = 1; P < 0.001).

The mean numbers of seeds per fruit were 3.27, 2.83, 3.35 and 2.78 in treatments 1 to 4 respectively. The differences between these four means are not significant (F = 1.96; df_1 = 3; df_2 = 8 2 ; P < 0.001).

The percentages of normal seeds were 80.55%, 60.29%, 75.43% and 54.68% in treatments 1 to 4 respectively. The differences between these four percentages are highly significant (χ^2 = 13.69; *df* = 3; *P* < 0.001). Two to two comparisons showed that the difference observed is highly significant between treatments 1 and 2 (χ^2 = 16.51; *df* = 1; *P* < 0.001) as well as between treatments 3 and 4 (χ^2 = 12.50; *df* = 1; *P* < 0.001).

In 2016, the numeric contribution of a single flower visit of *G. angolanus* in the fruiting rate, the mean number of seeds per fruit and the percentage of normal seeds of *P. febrifugum* were 43.27%, 13.45% and 25.15% respectively. In 2017, the corresponding figures were 52.64%, 17.01% and 27.50% respectively. For the two cumulate years, the numeric contributions of flowering insects were 47.95%, 15.23% and 26.32% for the fruiting rate, the mean number of seeds per fruit and the percentage of normal seeds respectively.

The positive and significant contribution of *G. angolanus* 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 *G. angolanus* on the pollination of visited flowers.

4. Conclusion

At Dang, *P. febrifugum* is a plant species that highly benefits from pollination by insects, among which *G. angolanus* is one of the most important. On flowers, individual of *G. angolanus* exclusively harvested nectar. Via the pollination efficiency of a single flower visit, *G. angolanus* increase the fruit and seed yields of *P. febrifugum*. The protection of populations of *P. febrifugum* is important to maintain the butterfly *G. angolanus* in the environment so that it contributes to the plant covert essential for the conservation of biodiversity, and improve fruit production and seed quality of this Hypericaceae.

Compliance with ethical standards

Disclosure of conflict of interest

The corresponding author and the other authors, declare that there is no conflict of interest.

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