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COMPARATIVE EFFICACY OF *Elaeidobius kamerunicus*, *E. plagiatus*, *E. subvittatus* (COLEOPTERA: CURCULIONIDAE) AND *Microporum spp*. (COLEOPTERA: NITIDULIDAE) IN THE POLLINATION OF OIL PALM (*Elaeis guineensis*)

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ABSTRACT

Pollination capacity of *Elaeidobius kamerunicus* Faust, *E. plagiatus*, *E. subvitattus* and *Microporum spp*., was highlighted by the quanitative and qualitative analysis of the transported pollen grains and by controlled entomophilous pollination. The quantity and quality of pollen grains transported by adults of different species and the pollen grain which reached to the female inflorescence were evaluated by counting under a binocular microscope. Controlled pollination was conducted by releasing adult insects of different species on bagged female inflorescences. The fruit set and parthenocarpic were counted one month after fertilization. Results of the study revealed that *E. kamerunicus* species carries more pollen than the other three species (317 grains / individual) and this was followed by *E. plagiatus* (174 grains / individual). The males of both species carry significantly more pollen than their respective females. Quantities of pollen carried by *E. subvittatus* and *Microporum spp* did not differ significantly. *E. kamerunicus* and *E. plagiatus* carried pollen of very good quality (respective germination rate 92.7% and 77.1%). While *E. subvittatus* was transported pollen of medium quality (germination 53.87%) and *Microporum spp* vehicle pollen of poor quality (germination rate 26.8%). For the three beetles of the genus *Elaeidobius*, a significant correlation was found between the rate of fruit set and the number of individuals released on the female inflorescence of palm oil. Finally, for the same number of individuals released, *E. kamerunicus* is the species that provides the best rate of fruit set, followed by *E. plagiatus*.

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1 Introduction

Côte d'Ivoire is consider as a largest palm oil (*Elaeis guineensis*) exporter of Africa and it is expected that by the year 2020 the production will be close to 600000 tons per year (Anonymous, 2009). Effective methods of pollination and limited fruit set are the two most important constraints in the production rate for this crop (Corrado, 1985; Mariau et al, 1991). Oil palm pollination is mainly entomophilous (Syed, 1979) and its important pollinators are beetles of genus *Elaeidobius* (*E. kamerunicus, E. plagiatus*, *E. subvittatus* and *E. singularis*), *Microporum* (*M. congolense* and *M. dispar*) and *Atheta* (*A. burgeoni*) (Desmier Chenon, 1981; Mariau & Genty, 1988; Mariau et al., 1991; Hala et al., 2012). However, precise data on the average density of insects needed to ensure a good rate of fruit set are not available. In addition facing the lack of insect available for pollination, it is necessary to examine the pollination effectiveness of the species present in agro-ecosystem.

The purpose of this work was to identify the best pollinators of *E. guineensis* and their role in improving the rate of fruit set. This study was also aimed to analyze the pollination efficiency of four species of Coleopteran (*E. kamerunicus, E. plagiatus*, *E. subvittatus* and *Microporum sp.)* by assessing the quantity and quality of pollen grains carried by each species and analysis rate variations of fruit set.

2 Materials and Methods

2.1 Study area

The study was conducted in the South-eastern Côte d'Ivoire, at La Mé experimental station (5°26' Latitude North and 3°50' West Longitude). La Mé station is located in a low plateau and lowland (Fraisse, 1962) characterized by a natural rainforest vegetation, relative humidity and temperature of the study area was 70-80% & 26.9-28.8°C respectively. The available solar light per years in the study area was 2000 hours (Fraisse, 1962).

2.2 Materials

Investigations were performed on 144 inflorescences (24 female inflorescences / month for 6 months) of *E. guineensis* (figure. 1 and 2). The study plot consisted of 6.67 ha inside the plantation and was located on a low plateau. The pollinators were represented by five beetle species: *E. kamerunicus*, *E. plagiatus*, *E. subvittatus*, *M. congolense* and *M. dispar*. (Kouakou, 2009; Hala et al., 2012). Collected pollen grains were grown on a culture media consisting of 11 g of sucrose and 1.20 g of agar dissolved in 100 ml of distilled water (Bénard & Noiret, 1970: Fataye, 1984). Floral panicles were enclosed in a cage (40x40x40 cm), covered by flexible, transparent and white muslin fabric (figure. 3). Artificial fertilization bag was made in waxed Terylene (figure.4). The aspirator use to collect the pollinators consists of a pill container that keeps insects sucked. The pill container is closed by a cork perforated with two holes. A plastic pipe was introduced into each hole. The inner end of one pipe is covered with a piece of muslin to prevent the insect goes into the operators' mouth. Pruning shear, machete and pruning knife are used to clear the spathes and palm around the peduncle of the inflorescence for the installation of bags.

Figure.1 Female inflorescence in anthesis.

Figure 2 Male inflorescence in anthesis.

Figure 3 Isolation cage

Figure 4 Fertilization bag

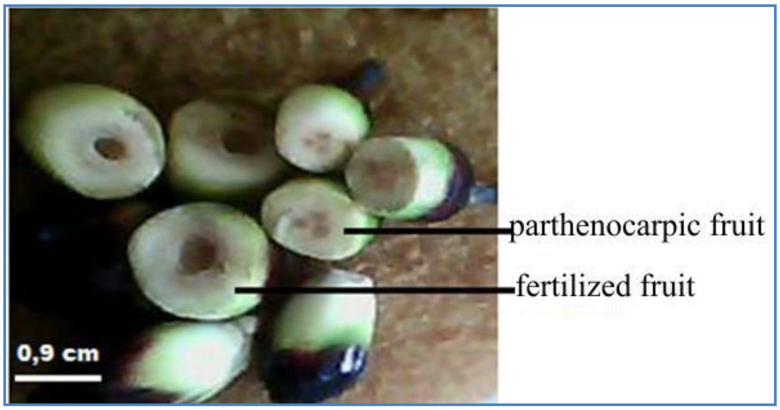

Figure 5 Fruit set and parthenocarpic fruits of palm oil a month after fertilization.

2.3 Methods

2.3-1. Evaluation of the quality and quantity of the pollen transported by insects

2.3.1.1. Capture of pollinating insects

Female inflorescences were randomly selected before flowering and were covered with a muslin cage. The day of full anthesis, ten adults of each species were captured using the aspirator. Hundred insects of each species were obtained by ten repetitions performed on a total of 10 female inflorescences randomly distributed in the plantation. These insects were placed individually in a hemolysis tube and transported to the laboratory.

2. 3.1.2. Culturing the pollen grains carried by insects

Males and females of the different species mentioned above were placed individually in petri dishes on culture medium solidified. Petri dishes were kept in an incubator at 35 °C and 90% relative humidity following the methodology of Bénard & Noiret (1970) and Fataye (1984). The living insect moves on the culture media and unwittingly sowing the pollen previously collected from the male flowers. This method of cultivation of pollen grains is similar to what takes place on the female inflorescence when insect arises there. In fact, moving on the flowers, these insect (unintentionally) pollen grains harvested from the male flowers on the stigma of the female flowers. In species *E. kamerunicus, E. plagiatus* and *Microporum spp* there is a very marked sexual dimorphism, the quantities of pollen carried by males and females were then compared.

2.3.1.3. Pollen grain germination study

After two hours spent in the incubator, cultures were observed under a binocular microscope to determine the presence and germination percentage of pollen grains. The pollen grain has a tetrahedral shape and small size (30 to 40 μ). Sprouted grains are characterized by the presence of pollen tube.

The germination rate was calculated by counted the number of germinated and non-germinated pollen grains. Three types of pollen were defined according to the recommendations of Jacquemard (1995) regarding the quality of pollen to be used in artificial insemination for seed production i.e. high quality pollen grain "for a germination rate higher than or equal to 70%"; Pollen with medium quality "for a germination rate of 50 to 70%"; Pollen with poor quality "for a germination rate below 50%".

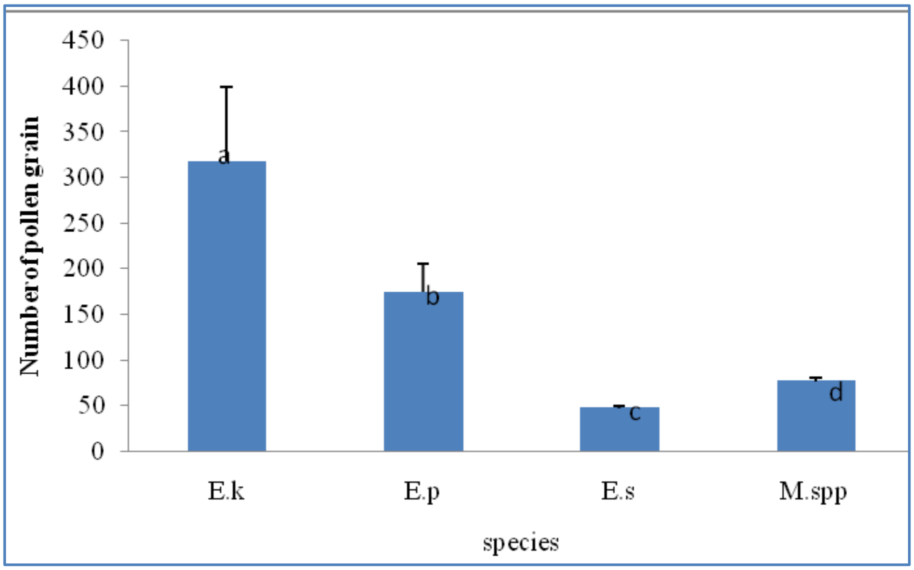

Figure 6 Number of pollen grain carried by each species (E.k: *E. kamerunicus*; E.p:*E. plagiatus*; E.s: *E.subvitattus:* M.sp and *Microporum sp*.)

2.3.2. Changes in the rate of fruit set according to insect species and the number of pollinators

Pollinating insects have been artificially introduced on previously bagged female inflorescences. This process has been finished in four steps which were the isolation of the female inflorescence, the collection of pollinators insect, the release of insects on female flowers and determination of fruit set rate.

2.3.2.1. Isolation of female inflorescence

Isolation of the female inflorescence was made before flowering and aims to prevent natural pollination. It consisted in removing the husks, to move apart the palms around the peduncle and then cap the inflorescence with a bag of artificial fertilization. Twenty-four female inflorescences have been prepared each month during 6 months. The choice of trees is done randomly throughout the extent of the plot, according to the availability of female inflorescences at the time of the experiment.

2.3.2.2. Collection of insect pollinators

When the bagged female inflorescences reached to the anthesis, one or two inflorescences from full anthesis (containing enough insects) were cut at the bottom of the peduncle carefully wrapped in fine muslin and then were transported to the laboratory. In the laboratory, the muslin was removed and shaken inflorescences above the straw mattress next to a glass window illuminated by daylight. Insects attracted by the light, fly to the window where they are captured using a vacuum cleaner. For each species, six different numbers have been established (50, 100, 300, 500, 700 and 900) and each number was replicated four times.

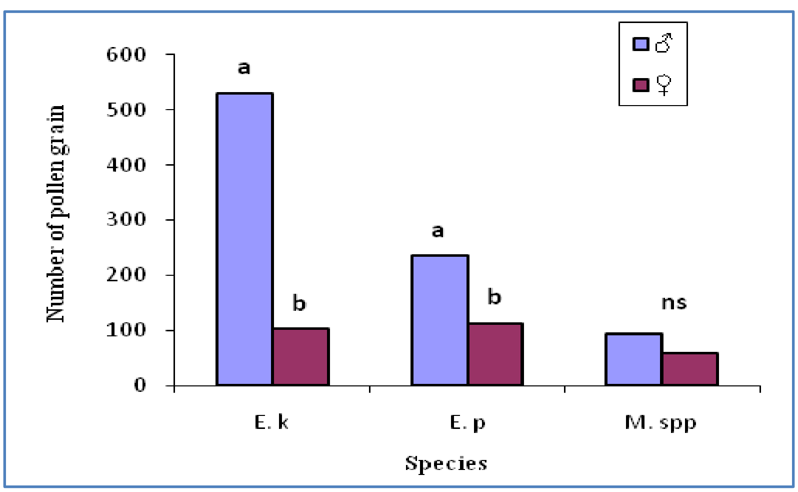

Figure 7 Number of pollen grain carried by male and female of different species.

2.3.2.3. Release of insects on the female flowers

To stretch up to the female flowers, an opening was made in the bag of artificial insemination using a razor blade and then the insects were released inside the bag. The opening was then closed with an adhesive tape. Five days after flowering, the flowers are no longer receptive to pollen (Jacquemard, 1995), and the bag was then removed to allow the inflorescence to continue its development. Two batches of controls were also established these batches contain an inflorescence bagged receiving no insect and non-bagged inflorescence, undergoing natural pollinated. Both species of *Microporum* were collected without distinction because it has been difficult to achieve the desired numbers.

2.3.2.4. Determining the rate of fruit set

The estimate of fruit set was made according to the method described by Fataye (1984), Lecoustre & De Reffey (1987) and Mariau et al. (1991): One month after pollination, bunches were harvested and spikelet were separated from the roundup. On each bunches, 50 spikelet were randomly selected, and their fruits are divided into two categories which contains fertilized fruit set and unfertilized fruit set (parthenocarpic fruits). The distinction between the two categories of fruits is based on the description given by Turner & Gillbanks (1974). After a month, knotted fruits are more developed and have a cavity containing a clear liquid which later became almond. Parthenocarpic fruits are smaller, still wrapped in their sepals. They contain no cavity when sectioned and have a pale color (figure.5). At the end of the counts, the rate of fruit set is calculated

2.4 Data Analysis

Calculations for germination and fruit set and construction of graphs were made using Excel software. Comparing the amounts of pollen grains, the germination rate and fruit set obtained with different species was performed by analysis of variance followed by the multiple comparison tests of Newman Keuls at 5%. Finally, for each species, the coefficient of linear correlation between the rate of fruit set and the number was calculated and tested to measure the link between these two factors. Statistical treatments were performed using the GenStat software version 10.1.

3 Results

3.1 Average number of pollen grains transported by different insect species

The mean values of pollen grains were 317 pollen grains per adult of *E. kamerunicus*, 175 per adult of *E. plagiatus*, 77 per adult of *Microporum spp* and 47 per adult of *E. subvittatus*, respectively. The average number of pollen grains carried by the adults of *E. kamerunicus* is significantly higher than those of the other three species. Adults of *E. plagiatus* also carry more pollen than *E. subvittatus* and *Microporum spp* (figure. 6). Males of *E. kamerunicus* and *E. plagiatus* carry more pollen than their respective females. Indeed males *of E. kamerunicus* carry about 531 pollen grains per individual against 103 for females. The males of *E. plagiatus* carry about 236 pollen grains per individual against 113 for females. The amount of pollen carried by males and females *Microporum spp* did not differ significantly each other (figure. 7).

3.2 Viability of pollen grains transported by different insect species

The average germination of pollen grains harvested by *E. kamerunicus*, *E. plagiatus*, *E. subvittatus* and *Microporum spp* were 92.70%, 77.36%, 53.87% and 27.03% respectively. Among all the tested species, *E. kamerunicus* and *E. plagiatus* were transport pollen grains of very good quality (rate of germination higher than 70%). On the other hand, the pollen conveyed by the species *E. subvittatus* was of an average quality. Only half of the pollen grains collected from this species shows germination. Pollen carries by genus *Microporum* carried pollen grain of very poor quality. Indeed, only 27% pollen grains carried by this species were viable (figure.8).

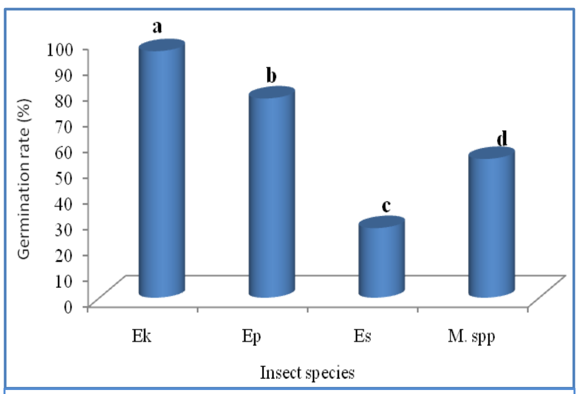

Figure 8 Germination rates of pollen grains carried by different species of insects.

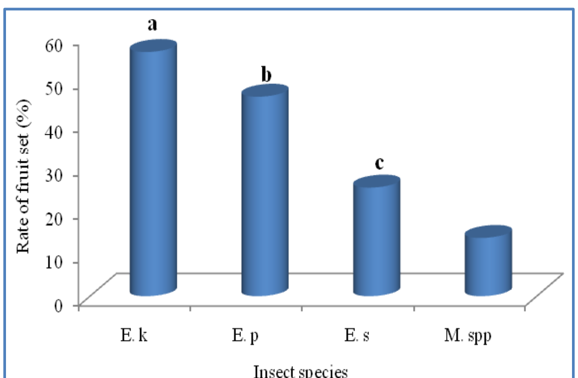

Figure 9 Fruit set rate variation as a function of the insect pollinator brought on inflorescence.

3.3 Variation in rate of fruit set according to species and the number of pollinating insects

3.3.1 Variation depending on the species

For inflorescences that have not received any insect, the fruit set rate was zero percent. The open-pollination gave an average fruit set of 88.50%, this rate is significantly higher than those obtained with the introduction of species separately. The average rate of fruit set were 56.17% for *E. kamerunicus*, 45.89% for *E. plagiatus*, 24.98% for *E. subvittatus* and 13.40% for *Microporum sp*, respectively (figure. 9).

3.3.2 Variation depending on the number of insects

Positive correlation was found between the number of species *E. kamerunicus*, *E. plagiatus* and *E. subvittatus* brought and the rate of fruit set (r = 0.8957, p = 0.0032, r = 0.9584, p = 0.0003 and r = 0.9705, p = 0.0001, respectively). The observed maximum fruit set rates were 79, 85%, 72.29% and 50% respectively with 900 specimens of *E. kamerunicus*, *E. plagiatus* and *E. subvittatus*. No significant correlation was obtained in the number of *Microporum* brought and the rate of fruit set. Moreover, even with 900 specimens of this genus, the rate of fruit set could not reach the average of 50% (figure.10).

Conclusion and Discussion

Quantitative analysis revealed that *E. kamerunicus*, *E. plagiatus*, *E. subvittatus* and *Microporum sp*. did not transported the same quantity of pollen grains. Among these, *E. kamerunicus* was the species that carries the highest number of pollen grains and it was followed by *E. plagiatus*. The males of both species were transport more pollen than females. These differences could be explained by the morphological differences between insects. According to Mariau & Genty (1988) and Mariau et al., (1991), the number of pollen grains carried by the insect is not only related to body surface area (or size), but also depend on the presence (or not) of bristles on their bodies.

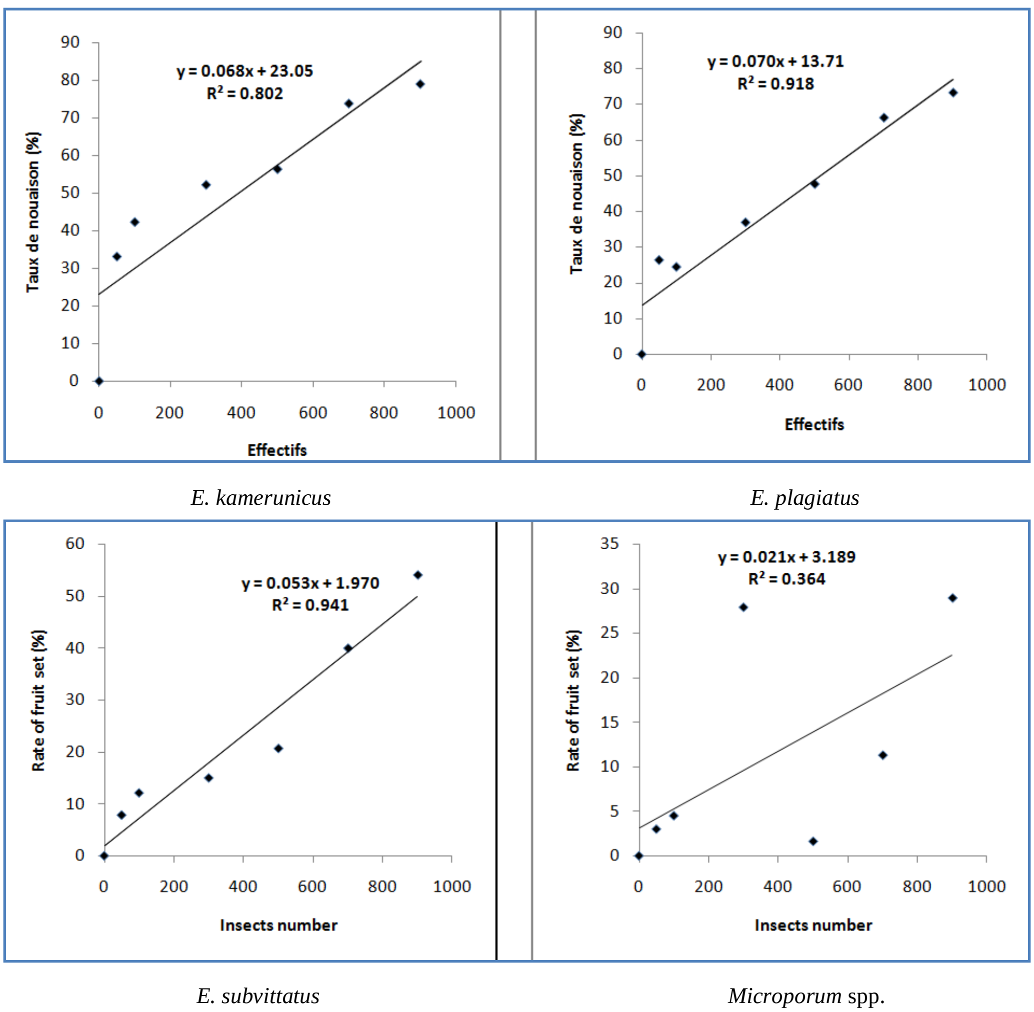

Figure 10 Evolution of fruit set rate depending on the number of pollinating insects brought upon the female inflorescence.

In *Curculionidae* family there is a direct link between the carrying capacity of pollen grains and the length and arrangement of bristles present on the bodies of insects (Baudoux, 2013). The body size of *E. kamerunicus* and *E. plagiatus* is larger than the other two species, which gives them greater carrying capacity of pollen. In addition, studies using scanning electron microscopy performed by Dhileepan (1992) on *E. kamerunicus* showed that pleural bristles, present only in males, are the bearers of the most important pollen.

It appears from the qualitative analysis that the four species carry pollen of various qualities. If *E. kamerunicus* and *E. plagiatus* conveyed pollen of very good quality, it was not the same for *E. subvittatus* especially *Microporum spp* which carry pollen grains of poor quality. The observations made at La Mé station showed that *E. kamerunicus* is more frequent in male flowers at the beginning of anthesis than at full and or end anthesis, unlike other species. This behavior would allow him to collect pollen grains fresh and viable. Furthermore, although living in the same inflorescence insects may establish niches and thus collect pollen at different strata at the base, middle or towards the top of the inflorescence. Thus, species that live and feed at the base of the inflorescence would carry pollen from lower quality. Indeed, the pollen grains at the base of the inflorescence are the first to lose their ability to germinate because the flowering begins at the base. According Bénard & Noiret (1970), this loss of viability comes from the fifth day of flowering.

The Inflorescences bagged which did not received insect, did not give bunches with fertilized fruits. This means that the inflorescences were well insulated and therefore it did not received pollen grain through the wind or insects. As the inflorescences which received different species of pollinating insects, all gave bunches with a certain percentage of fertilized fruits. Insects studied so effectively transport pollen grains deposited on the stigmas of female flowers. These results confirm those of previous studies that have identified these species as pollinators of oil palm (Syed, 1979). The rate of fruit set varies greatly depending on the species and the number of insects received by the female inflorescence. In terms of "quality of pollination" species studied can be ranked in descending order viz *E. kamerunicus*, *E. plagiatus*, *E. subvittatus* and finally *Microporum sp*. These results are in agreement with those of Mariau & Genty (1988) and Mariau et al. (1991) those have reported that *E. kamerunicus* and *E. plagiatus* were very good pollinator.

Elaedobius kamerunicus and *E. plagiatus* which carry more pollen grains gave the highest rates of fruit set. The pollinator insects' power would be partly related to their load capacity for pollen. However, while carrying approximately the same quantity of pollen, fruit set rate obtained with *E. subvittatus* and *Microporum sp.* differ significantly. Indeed, *E. subvittatus* that harvest more viable pollen gave higher than *Microporum sp.* rate of fruit set. This means that the pollinator insects' capability is related not only to their load capacity in pollen but also and especially on the quality of pollen they carry. For a given species, increasing the number of individuals providing fertilization proportionally increases the rate of fruit set. Indeed, a large number of insects also mean a greater quantity of pollen which induces an increase in number of fertilized flowers.

The inflorescences that were subjected to open pollination gave higher rate of fruit set than those pollinated by species taken separately. This could be explained by the fact that pollination results from the combined action of all pollinating insects' action, including those which have not been studied here. These inflorescences are more heavily visited throughout flowering (two to three days minimum), unlike other insects that receive at once. Observations of pollen grains transported showed that *E. kamerunicus* carries more pollen and more viable than the other three species and followed by *E. plagiatus*. These observations have also shown that there is no significant difference between the quantities of pollen carried by *E. subvittatus* and *Microporum sp.*, however, the first species listed carries significantly more viable pollen than the second. Insect pollination under controlled conditions showed that the rate of fruit set of bunches depends on the species and the number of insects that have achieved fertilization. It helped to see that for the same number of individuals released, *E. kamerunicus* is the species that provides the highest rates of fruit set.

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