The role of carpenter bee (Xylocopa olivacea) pollination on fruit and seed yields of Lipstick tree (Bixa orellana, Bixaceae) crop in Cameroon

To evaluate the impact of a single visit of Xylocopa olivacea on fruit and seed yields of Bixa orellana, its foraging and pollinating activities were studied in Dang, during the rainy seasons of 2010 and 2011. Treatments included unlimited flowers access by all visitors, bagged flowers and flowers limited visits by X. olivacea only. The foraging behavior of X. olivacea on flowers and its pollination efficiency were recorded. Xylocopa olivacea was the third most frequent visitor and it intensely and exclusively collected pollen. The fruiting rate, the mean number of seeds per fruit and the percentage of normal seeds of unprotected flowers were significantly higher compared to protected flowers. Through its pollination efficiency, X. olivacea showed a significant increase of the fruiting rate by 35.83%, the number of seeds per fruit by 1.26% and the percentage of normal seeds by 72.49%. The conservation of X. olivacea nests close to B. orellana fields is recommended to improve fruit and seed production in the region.

**Keywords:** Bixa orellana, flowers, pollen, pollination, Xylocopa olivacea, yield.

INTRODUCTION

Over 85% of wild flowering plants depend to some extent on pollination (Betts et al., 2019). Pollination is the highest agricultural contributor to yields worldwide, contributing far beyond any other agricultural management practice (FAO, 2018). Thus, the carpenter bee such as X. olivacea and other insect pollinators make important contributions to agriculture (Pauly, 2015; OPBE, 2017). Gallai et al. (2009) estimated the total economic value of the pollination service provided by insect pollinators, to be €153 billion, which amounts to 95% of the total value of global agricultural food production.

*Xylocopa olivacea* is an important pollinator of stone fruit crops. Visits to any other flowering plants that are available and attractive to them for nectar and/or pollen including *Bixa orellana* (Pauly et al., 2015; OBPE, 2017) has been noted.

This plant contains the carotenoids bixin, norbixin and other products derived from lycopene (Addy et al., 2017). The main constituent is bixin, which represents approximately 80% of the carotenoids in the *B. orellana* colorant. Lipstick tree is widely used in the pharmaceutical, cosmetic and food industries (Yolmeh et al., 2014; Taham et al., 2015).

The flowers of *B. orellana* are perfect, fragrant, white to
light pink in color. They are born in branched terminal panicles on scaly stalks. They are 4 to 6 cm wide, resembling in wild rose, apple or peach flowers. Honeybees (*Apis mellifera*) have been recorded as their frequent visitor and could be their main pollinator in Kenya (Orwa et al., 2009).

In Brazil, in the Glória de Dourados-MS in particular, Rocha and Polatto (2017) reported that the main pollinators are Hymenopterans (*Solenopsis* sp., *Apis mellifera* and *Cephalotes* sp.). Additionally, in the Metropolitan region of Fortaleza, other bees visiting *B. orellana* flowers were observed. These included *Xylocopa frontalis*, *Xylocopa agrisense*, *Xylocopa muscaria*, *Exomalopsis analis*, *A. mellifera*, *Melipona subnitida*, *Partamona* sp., *Trigona spinipes*, *Eulaema nigrita*, *Euoglossa* sp., *Megalopta* sp., *Augochloropsis* sp. and *Oxaea* sp. (Mesquita, 2008).

Pollination is known to be positively related to activity density and diversity of insects visiting flowers which vary with regions (Roubik, 2000). Information on pollination of *Bixa orellana* by insects is lacking in Cameroon. More so, no previous research has reported the relationships between *B. orellana* and insects visiting its flowers. Therefore, it is of greatest necessity to study insect pollination of this woody plant, so as to provide new baseline information in this country.

The general objective of this work was to understand the relationships between *B. orellana* and *X. olivacea*, for their optimal management. Specific objectives were to: (a) determine the place of *X. olivacea* on its flowers; (b) study the activity of this Apidae on flowers of this Bixaceae; (c) evaluate the effect of the flower visiting insects including *X. olivacea* on pollination, fruits and seed yields of *B. orellana*; (d) estimate the pollination efficiency of *X. olivacea* on this plant species.

**MATERIALS AND METHODS**

**Study site, experimental plot and biological material**

The experiment was carried out in two seasons, from 30th June to 21st July, 2010 and 30th June to 23rd July, 2011 at Dang in N’goundéré, 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°C, while the mean annual relative humidity is 70% (Amougou et al., 2015). In this study, 285 shrubs selected for observations were located at a minimum distance of three kilometers (km) away. Kenyan top-bar hive inhabited by *Apis mellifera* (Hymenoptera: Apidae) was centrally placed in the farm. Observations made included abundances of *X. olivacea* and other insect species naturally present in the environment. The vegetation was represented by crops, ornamentals, hedge and native plants of the savannah and gallery forests.

**Determination of the reproduction mode of *Bixa orellana***

On June 30th, 2010, 240 inflorescences carried by 60 plants from untreated subplots at the budding stage were labeled, among which 120 inflorescences were left unprotected (treatment 1), while 120 others were bagged using gauze bags (treatment 2) with mesh of one square millimeter (mm²) to prevent visiting insects (Tchuenguem et al., 2001). In similar subplots, on June 30th, 2011, 240 inflorescences at the budding stage were labeled of which 120 inflorescences were unprotected (treatment 3), while 120 were bagged (treatment 4). For each cropping year, two weeks after shedding of the last labeled flower, the number of fruits set was counted in each treatment. The fruiting index was then calculated as described by Tchuenguem et al. (2001) : 

\[
Pi = \frac{Fb}{Fa} \times 100
\]

*Pi* is the number of initial viable flowers. The allogamy rate (*TC*) from which derives the autogamy rate (*TA*) was expressed as the difference in fruiting indexes between treatment *X* (unprotected flowers) and treatment *Y* (bagged flowers) (Adamou and Tchuenguem, 2014).

\[
TC = \frac{[PiX - PiY]}{PiX} \times 100
\]

*PiX* and *PiY* are respectively, the mean fruiting indexes of treatment *X* and treatment *Y*; 

\[
TA = 100 - TC
\]

**Study on the foraging activity of *Xylocopa olivacea* on *Bixa orellana* flowers**

Observations were conducted on inflorescences of treatments 1 and 3, from the opening of the first flower bud (30th June 2010 and 30th June 2011) to the fading of the last flower (21st July, 2010 and 23rd July, 2011), according to six daily time frames: 6 - 7 h, 8 - 9 h, 10 - 11 h, 12 - 13 h, 14 - 15 h and 16 - 17 h. Flowering insects that visited *B. orellana* flowers were recorded at each daily time frame during the blooming period. All insects encountered on flowers were recorded and the cumulated results expressed in number of visits. These were used to determine the relative frequency of *X. olivacea* (*Fx*) among flowering insects of *B. orellana*. For each year of study, 

\[
Fx = \frac{[Vx]}{Vi} \times 100
\]

where *Vx* is the number of visits of *X. olivacea* on flowers of free treatment and *Vi* the total number of insect visits on flowers of the same treatment (Tchuenguem et al., 2001).

During our investigations, before starting with the record of visiting pollinators, the number of open flowers was counted. Additionally, data on the frequency of visits on flowers by insects, the floral products (nectar and/or pollen) collected by a worker bee were recorded for the same date and daily time frame. This was aimed at evaluating whether *X. olivacea* is strictly polleniferous and / or nectariferous on *B. orellana* flowers (Adamou and Tchuenguem, 2014). This can give an idea of its
involvement in the pollination of this plant. The duration of the individual flower visits was recorded (using a stop watch) according to six daily time frames: 7 - 8 h, 9 - 10 h, 11 - 12 h, 13 - 14 h, 15 - 16 h and 17 - 18 h (Tchuenguem, 2005). The foraging speed expressed as: the number of flowers visited by a carpenter bee per minute according to Jacob - Remacle (1989), was calculated using the following formula: \( Vb = (Fi/di) \times 60 \) where \( di \) is the time (s) given by a stopwatch and \( Fi \) is the number of flowers visited during \( di \).

The abundance of foragers (highest number of individuals foraging simultaneously) per flower or per 1000 flowers \( (A1000) \) were recorded on the same dates and time slots as the registration of the duration of visits. Abundance per flower was recorded as a result of direct counting. For determining the abundance per 1000 flowers, some foragers were counted on a known number of opened flowers and \( A1000 \) was calculated using the following formula: \( A1000 = [(Ax / Fx) \times 1000] \), where \( Fx \) and \( Ax \) are respectively the number of flowers and the number of foragers effectively counted on these flowers at time \( x \) (Tchuenguem et al., 2004).

The disruption of the activity of foragers by competitors and / or predators and the attractiveness exerted by other plant species on this insect was assessed by direct observations. For the second parameter, the number of times the worker bee went from \( B. \) orellana flowers to another plant species and vice versa was noted throughout the study period. During each day, the temperature and relative humidity of the station were registered after every 30 minutes using a mobile thermo-hygrometer (HT-9227), installed in the shade.

**Evaluation of the effect of anthophilous insects including *Xylocopa Olivacea* on *Bixa orellana* yields**

This evaluation was based on the impact of visiting insects on pollination, the impact of pollination on fruiting of \( B. \) orellana, and the comparison of yields (fruiting rate, mean number of seeds per fruit and percentage of normal or well developed seeds) of treatments 1 (unprotected flowers) and 2 (bagged flowers). The fruiting rate due to the influence of foraging insects \( (Fri) \) was calculated using the formula: \( Fri = \left\{ \left( \frac{FrX - FrY}{FrX} \right) \times 100 \right\} \) where \( FrX \) and \( FrY \) are the fruiting rate in treatments \( X \) and \( Y \).

The fruiting rate \( (Fr) \) is: \( Fr = \left\{ \left( \frac{Fb}{Fa} \right) \times 100 \right\} \) where \( Fb \) is the number of fruits formed and \( Fa \) the number of opened flowers initially set.

At maturity, fruits were harvested and counted from each treatment. The mean number of seeds per fruit and the percentage of normal seeds were then calculated for each treatment.

**Evaluation of the pollination efficiency of *Xylocopa Olivacea* on *Bixa orellana***

Parallel to the set up of treatments 1 and 2, treatment 5 comprised of 200 inflorescences carried by 40 shrubs in 2010. These flowers were protected as those of treatment 2. As soon as the flowers were opened, the gauze bag was delicately removed from each flower and the flowers were observed for up to 10 minutes; to record a single visit of carpenter bee. The flowers visited by \( X. \) Olivacea were marked and then protected thereafter. The contribution of \( X. \) Olivacea in the fruiting rate, number of seeds per fruit and percentage of normal seeds were calculated for each treatment per studied year.

The contribution of \( X. \) Olivacea in fruiting rate \( (FrX) \) was calculated using the formula: \( FrX = \left\{ \left( \frac{Fra - Frb}{Fra} \right) \times 100 \right\} \), where \( Fra \) and \( Frb \) are fruiting rates in treatment \( a \) (flowers visited exclusively by \( X. \) Olivacea) and treatment \( b \) (flowers bagged). At maturity, fruits were harvested and counted from each treatment. The fruiting rate, the percentage of fruits with seeds and the percentage of normal seeds were then calculated for each treatment.

**Statistical analysis**

Data were analysed using descriptive statistics, student’s \( t \)-test for the comparison of means of the two samples, Pearson correlation coefficient \( (r) \) for the study of the association between two variables, and chi-square \( (\chi^2) \) for the comparison of percentages, Microsoft Excel 2013 software was also used.

**RESULTS**

**Reproduction mode of *Bixa orellana***

The fruiting indexes of \( B. \) orellana for treatments 1, 2, 3 and 4 were 0.99, 0.23, 0.80 and 0.31 respectively. Thus, in 2010, the autogamy rate was 23.23%, whereas the allogamy rate was 76.77%. In 2011, the corresponding figures were 38.75% and 61.25%. For the two cumulative years, the autogamy rate was 30.99% and the allogamy rate was 69.01%.

**Activity of *Xylocopa Olivacea* on *Bixa orellana* flowers**

**Frequency of visits**

Among the 3871 and 4430 visits of 24 and 19 insect species recorded on the flowers of \( B. \) orellana in 2010 and 2011 respectively, \( X. \) Olivacea ranked third with 343 visits (8.86%) and 293 visits (6.61%) in 2010 and 2011 respectively (Table 1). The difference between these two percentages is highly significant \( (\chi^2=14.74; df=1; P<0.001) \).

**Floral products harvested**

From our field observations and during the two flowering
Table 1. Abundance and diversity of insects collecting pollen on *Bixa orellana* flowers in Dang, Cameroon during 2010 and 2011 seasons.

<table>
<thead>
<tr>
<th>Insects</th>
<th>2010</th>
<th>2011</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Order</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coleoptera</td>
<td>Meloidae</td>
<td>Coryna sp.</td>
<td>4</td>
</tr>
<tr>
<td>Diptera</td>
<td>Muscidae</td>
<td><em>Musca domestica</em></td>
<td>38</td>
</tr>
<tr>
<td>Syrphidae</td>
<td>Episyphrus sp.</td>
<td>(sp. 1)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(sp. 2)</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(sp. 3)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(sp. 4)</td>
<td>4</td>
</tr>
<tr>
<td>Hymenoptera</td>
<td>Apidae</td>
<td><em>Apis mellifera</em></td>
<td>2389</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Xylocopa olivacea</em></td>
<td>343</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Xylocopa inconstans</em></td>
<td>26</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Ceratina</em> sp.</td>
<td>423</td>
</tr>
<tr>
<td></td>
<td>Halictidae</td>
<td><em>Halictus</em> sp. 1</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Halictus</em> sp. 2</td>
<td>237</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Lasio glossum</em> sp.</td>
<td>64</td>
</tr>
<tr>
<td>Megachilidae</td>
<td><em>Amegilla</em> sp. 1</td>
<td>102</td>
<td>2.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Amegilla</em> sp. 2</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Megachile</em> sp. 1</td>
<td>141</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Megachile</em> sp. 2</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Megachile</em> sp. 3</td>
<td>12</td>
</tr>
<tr>
<td>Vespidae</td>
<td><em>Belonogaster juncea juncea</em></td>
<td>(sp. 1)</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(sp. 2)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(sp. 3)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(sp. 4)</td>
<td>4</td>
</tr>
</tbody>
</table>

\( n_1 \): number of visits on 120 flowers in 22 days; \( n_2 \): number of visits on 120 flowers in 24 days; \( p_1 \) and \( p_2 \): percentage of visits; \( p_1 = (n_1 / 3871) \times 100 \); \( p_2 = (n_2 / 8301) \times 100 \); Comparison of percentages of *Xylocopa olivacea* visits (2010/2011): \( \chi^2 = 14.74 \); \( df = 1 \); \( P < 0.001 \); po: collection of pollen; sp.: unidentified species.

Figure 1: *Xylocopa olivacea* harvesting pollen on *Bixa orellana* flower at Dang periods, individuals of *X. olivacea* were found to intensively and regularly harvest pollen on flowers from *B. orellana* (Figure 1).

**Daily visits**

The carpenter bee was active on *B. orellana* flowers throughout the day, with a peak of activity observed between 7 and 8 am in 2010 and 2011, respectively. The correlation was not significant between the number of *X. olivacea* visits and relative humidity in 2010 \( r=0.68; df=4; P>0.05 \) as well as in 2011 \( r=0.53; df=4; P>0.05 \) (Figures 2A and B). The correlation was not significant between the number of *X. olivacea* visits and the temperature in 2010.
Figure 2: Daily distribution of Xylocopa olivacea visits on Bixa orellana flowers over 22 days in 2010 (A) and 24 days in 2011 (B) as influenced by mean temperature and mean humidity at Dang. 

(r=0.56; df=4; P>0.05) and in 2011 (r=0.31; df=4; P>0.05).

Duration of a visit per flower

In 2010, the mean duration of a flower visit was 1.04 sec (n=1541; s=0.37; maxi=8sec), while in 2011, a mean of 1.01 sec (n=1284; s=0.17; maxi=5), giving a significant difference (t=2.84; P<0.05) between the two sampling years. For the two cumulated years, the mean duration of a flower visit was 1.03 sec.

Abundance of Xylocopa olivacea

In 2010, the highest mean number of X. olivacea simultaneously active was one per flower (n=517, s=0) and 21 per 1000 flowers (n=93, s=30.68). In 2011, the corresponding figures were 1 per flower (n=497, s=0) and 13.01 per 1000 flowers (n=84, s=11.80). The difference between the mean number of X. olivacea per 1000 flowers in 2010 and that in 2011 is significant (t=2.31; P<0.05).

Foraging speed of Xylocopa olivacea on Bixa orellana flowers

During observations, X. olivacea visited between 6.31 and 180 flowers/min in 2010 and between one and 35 flowers/min in 2011. The mean foraging speed was 46.94 flowers/min (n=117, s=38.05) in 2010 and 53 flowers/min (n=91, s=41.27) in 2011. The difference between these means is not significant (t=1.01; df=206; P>0.05). For the two cumulated years, the mean foraging speed was 49.77 flowers/min.

Relationship between visits and flowering stages

The visits of X. olivacea were more numerous on treatments 1 and 3 when the number of opened flowers was high (Figures 3A and B). The correlation was significant between the number of B. orellana opened flowers and the number of X. olivacea visits in 2010 (r=0.60; df=19; P<0.05) and highly significant in 2011 (r=0.76; df=21; P<0.001).

Influence of neighbouring flowering plants on Xylocopa olivacea visitation of Bixa orellana

Other flowering plants were competing with B. orellana for visitation by X. olivacea. These included: Bidens pilosa, Cajanus cajan, Callistemon rigidus, Cosmos sulphureus, Gossypium hirsutum, Mangifera indica, Phaseolus coccineus, Psidium guajava, Tithonia diversifolia and Vigna unguiculata. During the study period, carpenter bee foraging on B. orellana were not seen flying from B. orellana flowers to neighbouring plant flowers and vice versa.

Influence of wild life on Xylocopa olivacea visitation

Foraging activity of X. olivacea on flowers was influenced by other arthropods that were either competing for floral resources or other biotic and abiotic factors. These disturbances resulted in the interruption of some visits. In 2010, 343 visits of X. olivacea, were recorded of which 21 (6.12%) were interrupted by Chalicodoma cincta (Fabricius). Of the 293 visits of X. olivacea recorded in 2011,
16 (5.46%) was interrupted by *Apis mellifera*. To collect sufficient pollen, some individuals of *X. olivacea* who suffered such disturbances were forced to visit more flowers and/or plants during the corresponding foraging trip.

**Impact of anthophilous insects including *Xylocopa olivacea* on the pollination, fruit and seed yields of *Bixa orellana***

During pollen harvest, some foraging insects always shake flowers and contact anthers and stigma, increasing the cross pollination possibilities of this Bixaceae. The comparison of the fruiting rate (Table 2) showed that the differences observed were highly significant between treatments 1 and 2 ($t=4.33; P<0.001$) and treatments 3 and 4 ($t=6.18; P<0.001$). As a matter of fact, in 2010 and 2011, the mean number of seeds per fruiting opened flower was higher than that of flowers bagged during their flowering period.

The comparison of the mean number of seeds per fruit (Table 2) showed that the difference observed was highly significant between treatments 1 and 2 ($t=4.33; P<0.001$) and treatments 3 and 4 ($t=6.18; P<0.001$). As a matter of fact, in 2010 and 2011, the mean number of seeds per fruiting opened flower was higher than that of flowers bagged during their flowering period.

A comparison of the percentage of normal seeds (Table 2) showed that the difference observed was highly significant between treatments 1 and 2 ($\chi^2=3657.50; df=1; P<0.001$) and treatments 3 and 4 ($\chi^2=2401.86; df=1; P<0.001$). Hence, in 2010 as well as 2011, the percentage of normal seeds of exposed flowers was higher than that of flowers bagged.

Figure 3: Seasonal variation of the number of *Bixa orellana* opened flowers and the number of *Xylocopa olivacea* visits in 2010 (A) and 2011 (B) at Dang.
Table 2. Fruiting rate, number of seed per fruit and percentage of normal seeds according to different treatments of *Bixa orellana* in 2010 and 2011 at Dang

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Years</th>
<th>NF</th>
<th>NFF</th>
<th>FrR</th>
<th>Seeds/fruit</th>
<th>TNS</th>
<th>NS</th>
<th>%NS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>m</td>
<td>sd</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Ff (unlimited visits)</td>
<td>2010</td>
<td>438</td>
<td>437</td>
<td>99.77</td>
<td>45.88</td>
<td>20.48</td>
<td>17071</td>
<td>13933</td>
</tr>
<tr>
<td>2 Pf (bagged flowers)</td>
<td>2010</td>
<td>1173</td>
<td>275</td>
<td>23.44</td>
<td>40.44</td>
<td>12.99</td>
<td>10946</td>
<td>5157</td>
</tr>
<tr>
<td>3 Ff (unlimited visits)</td>
<td>2011</td>
<td>689</td>
<td>553</td>
<td>80.26</td>
<td>40.50</td>
<td>16.71</td>
<td>17461</td>
<td>14357</td>
</tr>
<tr>
<td>4 Pf (bagged flowers)</td>
<td>2011</td>
<td>922</td>
<td>391</td>
<td>42.40</td>
<td>34.04</td>
<td>15.14</td>
<td>13689</td>
<td>7783</td>
</tr>
<tr>
<td>5 Fv (X. olivacea)</td>
<td>2010</td>
<td>448</td>
<td>258</td>
<td>57.59</td>
<td>33.61</td>
<td>10</td>
<td>258</td>
<td>253</td>
</tr>
</tbody>
</table>

NF: Number of flowers; NFF: Number of formed fruit; FrR: Fruit rate; TNS: Total number of seeds; NS: Normal seeds; %NS: Percentage of normal seeds; m: mean; sd: standard deviation.

Table 3. Number and frequency of contacts between *Xylocopa olivacea* and the stigma during the floral visits of *Bixa orellana* in 2010 and 2011 at Dang

<table>
<thead>
<tr>
<th>Product harvested</th>
<th>June to July 2010</th>
<th>June to July 2011</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Visits with stigmatic contacts</td>
<td>Visits with stigmatic contacts</td>
<td>Visits with stigmatic contacts</td>
</tr>
<tr>
<td>Pollen</td>
<td>No. of studied visits</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Pollen</td>
<td>1541</td>
<td>116</td>
<td>7.52</td>
</tr>
</tbody>
</table>

Table 4. Number and frequency of contacts between *Xylocopa olivacea* and anthers during the floral visits of *Bixa orellana* in 2010 and 2011 at Dang

<table>
<thead>
<tr>
<th>Product harvested</th>
<th>June to July 2010</th>
<th>June to July 2011</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Visits with anthers contacts</td>
<td>Visits with anthers contacts</td>
<td>Visits with anthers contacts</td>
</tr>
<tr>
<td>Pollen</td>
<td>No. of studied visits</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Pollen</td>
<td>1541</td>
<td>1541</td>
<td>100</td>
</tr>
</tbody>
</table>

during their flowering period.

In 2010, the contributions of anthophilous insects on the fruiting rate, the mean number of seeds per fruit and the percentage of normal seeds were 76.51, 11.86 and 42.27%, respectively. In 2011, the corresponding figures were 47.17, 15.95 and 30.86%, respectively. Cumulative data for the two seasons showed fruiting rate of 61.84%, mean number of seeds per fruit of 13.91%, and percentage of normal seeds of 36.57%, as effected by insect flower visitors.

Pollination efficiency of *Xylocopa olivacea* on *Bixa orellana*

During pollen collection from flowers, individuals of *X. olivacea* were always in contact with the stigma and the anthers (Tables 3 and 4). Thus, this carpenter bee highly increased the pollination of *B. orellana* flowers. A comparison of the fruiting rate (Table 2) showed that the differences observed were highly significant between treatments 2 and 5 ($\chi^2=171.26; P<0.001$) and treatments 4 and 5 ($\chi^2=27.87; P<0.001$). The fruiting rate of flowers exclusively visited by *X. olivacea* (treatment 5) was significantly higher than that of flowers protected during their flowering period (treatments 2 and 4), respectively.

A comparison of the mean number of seeds per fruit (Table 2) showed that the difference observed was highly significant between treatments 2 and 5 ($t=6.81; P<0.001$) and not significant between treatments 4 and 5 ($t=0.44; P>0.05$). The mean number of seeds per fruit of flowers bagged and visited exclusively by *X. olivacea* (treatment 5), was significantly higher than those of bagged flowers (treatments 2 and 4).

A comparison of the percentage of normal seeds (Table 2)
showed that the differences observed were highly significant between treatments 2 and 5 ($\chi^2=262.02; df=1; P<0.001$) and treatments 4 and 5 ($\chi^2=176.07; df=1; P<0.001$). The percentage of normal seeds of flowers exclusively visited by X. olivacea (treatment 5) was significantly higher than that of flowers bagged during their opening period (treatment 4).

The contributions of X. olivacea on the fruiting rate, the number of seeds per fruit and the normal seeds via a single flower visit in 2010 were 35.83, 1.26 and 72.49%, respectively.

**DISCUSSION**

Our results showed that B. orellana has a mixed reproduction mode with the predominance of autogamy over outcross. The predominance of autogamy was reported by Rocha and Polatto (2017) in Brazil.

*Xylocopa olivacea* was the third abundant floral visitor of *B. orellana* during the observation period. Carpenter bee is known as an insect flower visitor of this plant species in Brazil, in the Glória de Dourados-MS and in the Metropolitan region of Fortaleza in particular (Mesquita, 2008; Rocha and Polatto, 2017). The significant difference between the percentage visits of *X. olivacea* within the studied years, could be explained by the presence of several nests of *X. olivacea* near the experimental plot in 2010 when compared to that of 2011.

The peak of *X. olivacea* activity on *B. orellana* flowers was recorded between 7.00 am and 8.00 am, which correlated with the period of highest availability of pollen on this Bixaceae. The abundance of *X. olivacea* individuals per 1000 flowers and the positive and highly significant correlation between the number of *B. orellana* flowers in bloom to the number of *X. olivacea* visit indicates the high attractiveness of pollen with respect to this carpenter bee. During the flowering period of *B. orellana*, *X. olivacea* intensively and regularly harvested pollen. This could be attributed to the needs of individual carpenter bees during the flowering period of the Bixaceae. The disruptions of visits by other insects reduced the duration of certain *X. olivacea* visits. Similar observations were made for the same carpenter bee foraging on flowers of *Phaseolus vulgaris* (Kingha et al., 2012) and *Vigna unguiculata* (Kengni et al., 2015) in Ngaoundéré.

*Xylocopa olivacea* individuals collected pollen with their legs, hairs, thorax, abdomen and mouth accessories as they moved from one plant to another during their foraging activity. This carpenter bee could thus allow self pollination and cross-pollination (Mensah and Kudom, 2011; Kingha et al., 2012). Similar observations have been made for *X. olivacea* foraging on flowers of *Luffa aegyptiaca* (Mensah and Kudom, 2011).

The weight of *X. olivacea* played a positive role in the self pollination: when collecting pollen, *X. olivacea* shakes flowers; this movement could facilitate the release of pollen by anthers, for optimal deposition on the stigma.

Results of the present study confirm those carried out by Mainkte et al. (2019) in Sarh and Kingha et al. (2012) in Ngaoundéré on *Phaseolus vulgaris*.

The higher productivity of fruits in unlimited visits when compared with bagged flowers showed that insect visits were effective in increasing cross and/or self-pollination. The higher productivity of flowers exposed to visits by *X. olivacea* as compared to those under limited visits by all kinds of visitors, shows that this carpenter bee is an important pollinator of *B. orellana* and thus can be targeted for the managed pollination of this plant. Our results confirmed those carried out by Mesquita (2008) and Rocha and Polatto (2017) in Brazil who revealed that *B. orellana* flowers set little fruits in the absence of insect pollinators.

The positive and significant contribution of *X. olivacea* to the yields through its pollination efficiency is in agreement with similar findings in Ghana on *Luffa aegyptiaca* (Mensah and Kudom, 2011).

**CONCLUSION**

From our observations, *B. orellana* is a plant species that highly benefits from pollination by insects among which *X. olivacea* is of great importance. A comparison of the fruit and seed set of unprotected flowers to those of flowers visited exclusively by *X. olivacea*, emphasized the value of this bee in increasing fruiting rate, the number of seeds per fruit and the percentage of normal seeds of *B. orellana*. The installation and/or conservation of *X. olivacea* nests at the vicinity of lipstick fields is recommended to Cameroonian farmers to increase fruit and seed productions.

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**Conflict of interests**

The authors declare that they have no conflicting interests.

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