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Floral activity of *Chrysomya putoria* Wiedemann, 1830 (Diptera: Calliphoridae) enhances the pollination and fruit set rate of *Ricinus communis* L., 1753 (Euphorbiaceae)

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Experiments were conducted to study the foraging behaviour of blowfly species, *Chrysomya putoria* (Diptera: Calliphoridae) on *Ricinus communis* (Euphorbiaceae) in Sudano-Sahelian area of Cameroon in 2017 and 2018. To do so, castor bean plants were flagged and fly foraging parameters were recorded. The effects of blowfly activity on pollination and fruiting rate of *R. communis* were estimated by comparing unrestricted and restricted racemes. *Chrysomya putoria* accounted for 82.33% of the total number of flower visits among the 15 anthophilous insects recorded. Pollen and nectar foraging occurred throughout the day, with a major peak at 8-9 am. The density of foragers was about 300 individuals per plant. The mean duration of a flower visit for male and female flowers was significantly different and resulted in a foraging speed of 3-5 flowers/minute which increases cross-pollination of *R. communis*. The floral activity of *C. putoria* improved the fruiting rate of castor bean by about 11% and conversely, this plant appeared as an important food source for the conservation of its main flower-visitor. Castor bean and the blowfly species seem to have a mutualistic relationship.

Key words: Blowfly, castor bean, cross-pollination, geitonogamy, insect-assist anemophily, yield.

INTRODUCTION

Ricinus communis, commonly called castor bean or castor oil tree, is a plant species belonging to the family Euphorbiaceae. This plant species is native to India and grows up to about 6 m in height (Rana et al., 2012). The castor bean has several ethnobotanical uses mostly involving its oil and crude extracts. Ricin, which is castor bean oil, is used as an important ingredient in cosmetics, paints, fuel for lamps, high-speed lubricant, pharmaceutical and insecticidal formulations (Copley et al., 2005). Currently, *R. communis* is cultivated especially for the production of biodiesel (Sailaja et al., 2008). Also, the plant is known to have several properties such as laxative, antioxidant, analgesic, antitumor, antinociceptic, antiasthmatic, antidiabetic, antimicrobial, larvicidal and adult emergence inhibition of mosquitoes (Kensa and Yasmi, 2011).

Ricinus communis is a monoecious plant species and the flowers are arranged in terminal racemes (Rizzardo et al., 2012). Male flowers develop in the basal and median portions of the raceme, covering approximately two-thirds of the inflorescence, while female flowers are located in the apical third (Rizzardo et al., 2012). Male flowers only produce pollen while female flowers produce nectar (Douka and Tchuenguem, 2014), both of which are easily accessible for flower visiting insects. Outcrossing by wind is the main breeding system in *R*. *communis*; but selfing by geitonogamy also occurred (Rizzardo et al., 2012).

The family Calliphoridae (Diptera) includes blowflies, carrion flies, bluebottles and/or green bottle flies. This family of flies belongs to the order Diptera with about 1,525 identified species in 97 genera over the world (Pape et al., 2011). The genus *Chrysomya* Robineau-Desvoidy, 1830 comprises 36 species and is represented by over a dozen species in the Afrotropical region (Bharti, 2019). This cosmopolitan genus has proven importance in medicine, veterinary and forensic sciences (Prins, 1982). Adults are typically metallic coloured with thick setae on the meron and a plumose antennal arista (Marshall et al., 2011, 2017). Many species are widely distributed in the world (Byrd and Butler, 1997).

Chrysomya putoria Wiedemann, 1830 also known as the tropical African latrine blowfly (Laurence, 1988), is a native species to Africa but has spread to the Americas (Baumgartner and Greenberg, 1984). Adults are about 8-10 mm long, have a metallic green body colour with occasionally a dark blue or bronze metallic tinge (Amorim and Ribeiro, 2001). The face of *C. putoria* is generally dark in colour (Lutz et al., 2018), wings are translucent, and the posterior edge of the abdominal tergites is striped with black bands (Irish et al., 2014). Both sexes have a black frons; females are dichoptic and males are holoptic (Abdalla and Mohamed, 2014). This species has conspicuous dusting on the dorsal side of the thorax, allowing it to be distinguished from many other closely related species such as *C*. *albiceps* Wiedemann, 1819, *C*. *chloropyga* Wiedemann, 1818 and *C*. *megacephala* Fabricius, 1794 (Rognes and Paterson, 2005; Bharti, 2019).

Chrysomya putoria is closely associated with human settlements, thus has significant health risks (Baumgartner and Greenberg, 1984). Adults may act as a mechanical vector of different pathogens for humans through their association with decomposing organic matter. Also, *C*. *putoria* has a forensic significance and can be used as an indicator of postmortem interval, or the time elapsed since death (Baumgartner, 1993; Amorim and Ribeiro, 2001). Adults may deposit their eggs on wounds and cause myiasis on domestic and farm animals, and humans (Abdalla and Mohamed, 2014). Nevertheless, blowflies are generally recognized in the literature as important flower-visiting insects of a variety of plant species. They collect pollen and nectar thereby promoting pollination, which implies that these flies may play an important role in agriculture as pollinator (Li et al., 2014). This research work aimed at proving the important role of *C*. *putoria* on the pollination and fruiting of *R*. *communis* in order to improve the knowledge and implication of this blowfly in plant reproduction and productivity.

MATERIALS AND METHODS

Study site

Field experiments were carried out in Makabaye (Maroua, Far-North Region, Cameroon), near the Mayo Tsanaga Bridge (N 10°34.346'; E 14°16.835'; 420 m a.s.l.) inside a public place of about 3000 m^2, where several castor bean trees grew naturally. Both Experiments were conducted during the flowering period of castor bean, from February to August in 2017 and 2018. Makabaye is a neighborhood of Maroua (Far-North region of Cameroon) which belongs to the Sudano-Sahelian zone where the prevailing climate has two irregular seasons namely the dry season (November to May) and rainy season (June to October). The annual rainfall varies from 400 to 1200 mm and the mean annual temperature varies from 29 to 36°C (Morin, 2000).

Experimental design

Ten castor bean plants were selected at random from the 3000 m^2 plot. Observations on male and female flowers were made twice a week (Wednesday and Sunday) between 06:00 am and 07:00 pm (local time). The number of insect morphospecies visiting *R*. *communis* flowers was counted at each day on the selected plants during 5 to 10 min at seven time periods: 06:00-07:00 am, 08:00-09:00 am, 10:00-11:00 am, 12:00-1:00 pm, 02:00-03:00 pm, 04:00-05:00 pm and 06:00-07:00 pm. We assigned a recognition code to each insect morphospecies recorded. Since some specimens could have been observed more than once, counts on flower visiting insects were expressed as number of visits.

Sampling, preservation and identification of flower visiting insects

At least two individuals per morphospecies were captured through a hand net. Collected specimens were preserved in 90% ethanol.

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Table 1. Abundance of different insect recorded on flowers of *Ricinus communis* in 2017 and 2018.

Order	Family	Species	2017		2018		Total	
			n_i	%	m_i	%	$n_i + m_i$	%
Diptera	Calliphoridae	*Chrysomya putoria*	1874	79.2	1924	85.63	3798	82.33
	Muscidae	*Musca domestica*	103	4.4	96	4.27	199	4.31
	Drosophilidae	*Drosophila melanogaster*	78	3.3	34	1.51	112	2.43
	Asilidae	1 sp.	34	1.44	27	1.20	61	1.32
	Rhiniidae	*Rhyncomya pruinosa*	14	0.6	11	0.49	25	0.54
Total			**2103**	**88.89**	**2092**	**93.10**	**4195**	**90.94**
Hymenoptera	Formicidae	*Camponotus flavomarginatus*	97	4.1	23	1.02	120	2.6
		Polyrachis sp.	12	0.51	16	0.71	28	0.61
Total			**109**	**4.61**	**39**	**1.73**	**148**	**3.21**
Hymenoptera	Vespidae	*Synagris cornuta*	47	1.98	52	2.31	99	2.15
	Apidae	*Apis mellifera*	-	-	6	0.27	6	0.13
Total			**156**	**6.59**	**97**	**4.31**	**253**	**5.48**
Hemiptera	Lygaeidae	*Spilostethus pandurus*	19	0.8	27	1.20	46	0.99
	Coreidae	*Anoplocnemis curvipes*	16	0.68	6	0.27	22	0.48
	Reduviidae	*Reduvius* sp.	13	0.55	2	0.09	15	0.33
Total			**48**	**2.03**	**35**	**1.56**	**83**	**1.8**
Dictyoptera	Mantidae	*Mantis religiosa*	27	1.14	15	0.67	42	0.91
Lepidoptera	Acraeidae	*Acraea acerata*	13	0.55	8	0.36	21	0.46
Coleoptera	Chrysomelidae	*Podagrixina decolorata*	19	0.8	-	-	19	0.41
Total			**2366**	**100**	**2247**	**100**	**4613**	**100**

n_i = number of visits in 2017; m_i = number of visits in 2018; sp. = unidentified species.
Source: Author Survey

Specimens were sorted to family, pinned, dried and kept in boxes. Identification up to the genus level was made using keys of Marshall et al. (2011, 2017) and a stereomicroscope (Olympus SZX7). The terminalia of male and female blowflies were dissected using the methodology of Rognes (2002) and identified to species level using Rognes and Paterson (2005), Irish et al. (2014) and Lutz et al. (2018) and the reference entomological collection of the University of Alicante, Spain. Voucher specimens were preserved at the Department of Biological Sciences, Faculty of Science, University of Maroua, Cameroon.

Foraging parameters of the blowfly species

The following activity parameters of the blowfly were studied: the relative abundance, daily rhythm of activity; whether male (pollen) or female (nectar) flowers were visited on each raceme; mean length of visits per individual insect on both male and female flowers using a stopwatch; density of flies/plant using direct count on racemes; mean number of flowers visited per minute or foraging speed. At each time interval, two values of temperature and humidity were registered with a hygro-thermometer of the brand HT-9227, in order to evaluate the influence of abiotic parameters on the daily activity of the fly species. We also recorded the different fly visit interruptions on racemes, the reasons of interruptions and the animal responsible, in order to study the influence of other flower visiting organisms on the foraging activity of the blowfly species.

Evaluation of the repercussion of the floral activity of the blowfly on castor bean yields

For estimating the impact of the blowfly floral activity on the fruit production of castor bean, 60 racemes of 20 other plants were randomly flagged and split into two groups or treatments of 30 racemes each. One group of racemes (group A) were freely exposed to flower visiting insects while racemes of the other group (group B) were prevented from flower visiting insects with gauze bags. Mature fruits grown from each treatment were harvested. A comparison on the fruiting rate between both treatments was made.

Data analysis

The *t*-test of Student was applied between treatments for comparing their mean values. The Chi-square test X^2 was used for the comparison between the fruiting rates from both treatments. Pearson correlations were established to appreciate the influence of abiotic parameters (temperature and humidity) on the daily activity of the blowfly species. The fruiting rate was determined by the proportion number of actual fruits formed/number of female flowers in unbagged (T_A) and bagged (T_B) racemes. The fructification rate (T_{fr}) due to the influence of blowfly on the production of castor bean was deduced by this equation: (T_{fr}) = $\{[(T_A\text{-}T_B)/\ T_A] \times 100\}$ where T_A and T_B are the fruit set rate in treatments A and B respectively (Tchuenguem et al., 2004). All

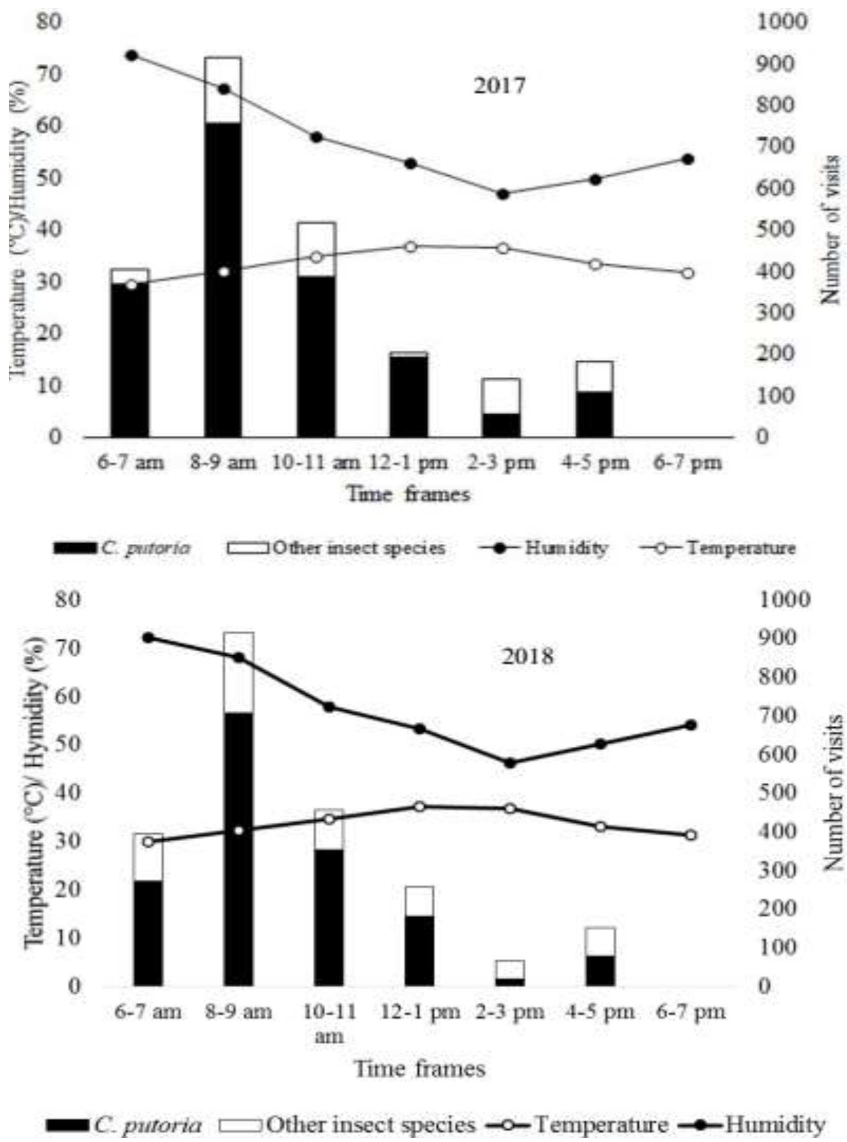

Figure 1. Daily distribution of insect visits on *Ricinus communis* flowers as a function of time frames, variation of ambient temperature and humidity in 2017 and 2018.
Source: Author Survey

analysis were conducted using SPSS 20.0 software.

RESULTS

Table 1 shows the diversity and centesimal frequency of the flower-vising insects of castor bean. Overall, 4613 visits of 15 foragers belonging to six orders and 14 families were recorded. During both years, the relative abundance of Diptera was higher (90.94%) than the combined abundance of the others, which were ranked in increasing order as followed: Coleoptera (0.42%), Lepidoptera (0.45%), Dictyoptera (0.91%), Hemiptera (1.8%) and Hymenoptera (5.48%). The family Calliphoridae, represented only by *C. putoria*, was by far the most abundant with 82.33% of the visits recorded. The other families were Muscidae (4.31%), Formicidae (3.21%), Drosophilidae (2.43%), Vespidae (2.15%), Asilidae (1.32%), Lygaeidae (0.99%), Mantidae (0.91%), Rhiniidae (0.54%), Coreidae (0.48%), Acraeidae (0.46%), Chrysomelidae (0.41%), Reduviidae (0.33%) and Apidae (0.13%). Honey bees were occasional visitors with 0.13% of total visits. The total number of anthophilous insect visits was not different year to year.

C. putoria foraged on castor bean flowers throughout the day, with a major peak in the morning between 08:00 and 09:00 am in both 2017 and 2018 (Figure 1). Visits were significantly more numerous (X^2 = 63.20; df = 1; P < 0.001 in 2017 and X^2 = 42.80; df = 1; P < 0.001 in 2018) for nectar gathering than for pollen harvesting (Figure 2).

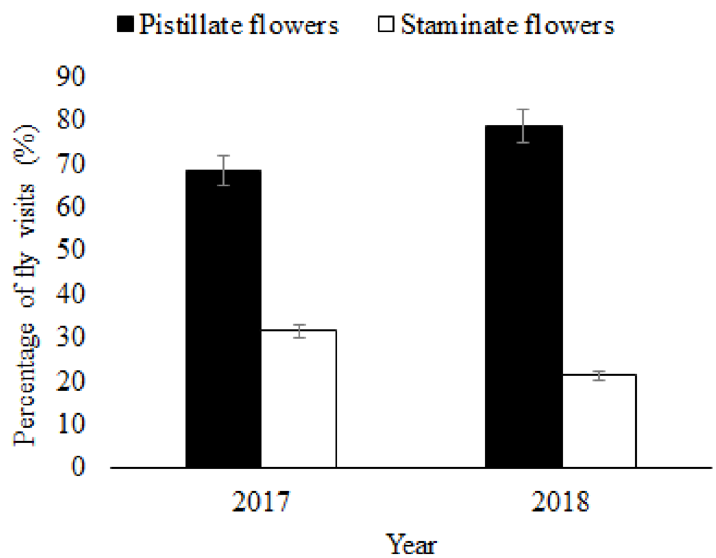

Figure 2. Choice of visitation of either sexes of castor bean flowers by *Chrysomya putoria.*
Source: Author Survey

Figure 3. *Chrysomya putoria* individuals foraging on castor bean.
Source: Author Survey

The average duration of *C. putoria* visit was significantly lower on staminate or male flowers (5.27 ± 1.67 seconds) than on pistillate or female flowers (8.73 ± 1.84 seconds) in 2017 (t = 8.21, df = 198; P < 0.001) and were 6.13 ± 2.14 seconds and 10.27 ± 2.47 seconds, respectively (t = 11.13, df = 198; P < 0.001) in 2018. The mean foraging speed varied from 3.1 ± 0.87 flowers/minute in 2017 to 3.73 ± 1.04 in 2018. Several *C. putoria* individuals were seen simultaneously foraging on the same raceme of a given plant (Figure 3); thus, *C. putoria* density was higher yearly and varied from 293 ± 83 individuals/plant in 2017 to 337 ± 79 individuals/plant in 2018.

The correlation between the daily distribution visits of *C. putoria* and the mean temperature was not significant (r = - 0.31; df = 5; P > 0.05); meanwhile, the correlation between the daily distribution visits of *C. putoria* and relative humidity was significant (r = 0.81; df = 5; P < 0.05). Similar values of the correlations were found

Table 2. Interactions between *Chrysomya putoria* and other flower-visitors.

Type of interruption	Interrupter	Interrupted visits	
		Number of visits	Percentage of visits (%)
Predation	Lizards	28	14
	Chameleons	3	1.5
	Praying mantis	23	11.5
	Robber flies	24	10.5
	Spiders	22	8.5
	Assassin bugs	7	7.5
Collision	Blowflies	93	46.5

Source: Author Survey

Table 3. Fruit yields of *Ricinus communis* as function of treatments in 2017 and 2018.

Treatments	Plants	Racemes	Female flowers		Fruits formed		Fruit set rate (%)	
			2017	2018	2017	2018	2017	2018
Treatment A	10	30	1754	1861	1208	1354	68.87	72.75
Treatment B	10	30	1567	1958	956	1278	61.00	65.27

Source: Author Survey

between the daily variation of the *C*. *putoria* visits and temperature (r = - 0.28; df = 5; P > 0.05), and humidity (r = 0.84; df = 5; P < 0.05) in 2018.

Table 2 shows a list of species that were reported interrupting flower visits by flies. The most common interruption type was matting activity in the blowfly own kind which occurred in the field experiment and accounted for 45.6% of studied visits (n = 200 visits). Furthermore, *R*. *communis* racemes were a prolific site of hunting for several fly predators such as invertebrates (praying mantis, robber flies, spiders and assassin bugs) and vertebrates (lizards and chameleon). These predators were also responsible for the disruption of floral visits of several *C*. *putoria* individuals, lizards being the most active with 14% of total visits studied (n = 200) follows by praying mantis (11.5%), robber flies (10.5%), spiders (8.5%), assassin bugs (7.5%) and chameleons (1.5%).

Fruit set rates of *R. communis* in 2017 and 2018 are given in Table 3. The fruiting rate in unbagged racemes was higher (68.87% in 2017 and 72.75% in 2018) than in bagged racemes (61 and 65.27%, respectively) resulting in an increase of fruit weight of 11.42% in 2017 and 10.28% in 2018 due to the floral activity of *C*. *putoria* on *R*. *communis*.

DISCUSSION

Our study highlights the importance of flies (Diptera) as pollinators of castor bean. Flies have been mentioned as pollinators or regular visitors of thousands of species of flowering plants (Green, 1973; Nye and Anderson, 1974; Kaufmann, 1975; Winder, 1977, 1978; Anderson et al., 1982; Currah and Ockendon, 1984; Jiron and Hedstrom 1985; Kumar et al., 1985; Heard et al., 1990; Catling and Spicer 1995; Jarlan et al., 1997a, b; Dzhambazov and Teneva, 2000; Saeed et al., 2008; Ssymank et al., 2008; Rader et al., 2009). Our two years' observations in Makabaye showed that *C*. *putoria* was the most common insect found foraging on flowers of castor bean. Other *Chrysomya* species were reported as frequent flower visitors, this is the case of *C*. *rufifacies* Macquart, 1843 that is also known as a pollinator of several plant species: *Terminalia bellirica* (Gaertn.), Roxb 1798 (Combretaceae) in India (Anoosha et al., 2018); *Mangifera indica,* L. 1753 (Anacardiaceae) in Japan (Alqarni et al., 2017); *Buchanania lanza,* Hamilton 1798 (Anacardiaceae) in Thaïland (Moophayak and Meeinkuirt, 2017); and *Bridelia stipularis* (L.), Blume 1826 and *Cleistanthus sumatranus* (Miq.), Müll.Arg. 1866 (Phyllanthaceae) in China (Li et al., 2014).

Comparison of other studies on *R*. *communis* shows that the floral entomofauna diversity is variable according to the biomes and/or the biogeographical area of the research. Previous results of Douka and Tchuenguem (2014) mentioned that in the locality of Mayel-Ibbé (10°62'N; 14°33'E; 400 m), another neighborhood of Maroua, *Musca domestica* Linnaeus, 1758 (Diptera: Muscidae) was the most abundant flower-visiting species

(11 species recorded) of *R*. *communis* with 53.04% in 2010. According to the authors, an unidentified species of Calliphoridae was poorly represented with a relative abundance of 0.6% during the same year. At a given site, a particular fly may be the predominant pollinator of a plant species, effecting high percentages of yields, whereas in another study site, that same species is absent from the array of flower visitors (Mesler et al., 1980). Our findings also revealed a scarcity of honey bees and wild bees on flowers of *R. communis*. These results are consistent with the previous findings of Douka and Tchuenguem (2014). According to Eudmar et al. (2011), pollen grains of *R*. *communis* are toxic to honey bees, although in Brazil honey bees were reported as the main foragers and pollinators of castor bean pollen and nectar (Rizzardo et al., 2012).

The higher visit abundance of *C*. *putoria* on *R*. *communis* flowers in the mornings rather than in the afternoons is likely related to the availability and/or depletion of floral ressources. Castor bean flowers are less rewarding in the afternoon (e.g. wilted and closed) because of too high temperatures. Additionally, higher humidity values in the mornings seem to be favorable for fly activity, according to field observation and can justify the prominence of *C*. *putoria* during this time of the day.

Selfing by geitonogamy occurs predominantly on the isolated flowers of bagged racemes. It is known in the literature that the male flowers of *R*. *communis* exhibit particular phenology when they reach maturity. From their opening, the anthers have the ability to explode and pollen is thrown in the air inside the gauze bag and may reach some female flowers located at the uppermost part of the raceme, close to the male flowers (Bianchini and Pacini, 1996). Fruit sets observed in bagged racemes were due to this proper nature of castor bean anthers which probably enables pollination and fruiting in the corresponding treatment.

During its floral activity, individuals of *C*. *putoria* could move from male to female flowers. They could, therefore, be able to transfer the pollen produced by the anthers of the male flowers to the stigmas of the female flowers. This aspect of so-called direct cross-pollination seems efficient by *C*. *putoria* on *R*. *communis* flowers in the morning. According to our observations, between 06:00 am and 09:00 am, the anthers were turgid and pollen grains were still not being dispersed greatly by the wind. At this time frame, *C*. *putoria* could transport pollen attached to his body from flower to flower and thus enable direct or active pollination to be done. Indeed, the ability of flies to carry and transport pollen grains has already been demonstrated (Larson et al., 2001; Moophayak and Meeinkuirt, 2017).

From about 10:00 am till the evening, with increasing ambient temperature and decreasing relative humidity, dry anthers and windborne pollen grains like these are more easily dispersed by the wind (Eudmar et al., 2011). During its activity on male flowers of a given raceme, foragers helped in boosting the release of airborne pollen. This foraging behaviour surely increased geitonogamy rates and contribute to raising fruit set rate of *R*. *communis*. Indeed, *C*. *putoria* individuals that collect pollen from castor beans commonly shake up staminate flowers, scrabbling and trigging anthers and thereby improve significantly air pollen flow that increased the drop of pollen grains mainly on female flowers of the same *R*. *communis* plant. Actually, the castor bean floral arrangement favors pollen dispersion for cross-pollination (Ramprasad and Bandopadhyay, 2010). This behavior was also observed in the pollination of *Zea mays* (Poaceae) by honey bees and wild bees in Nkolbisson, a neighborhood of Yaounde the capital of Cameroon (Tchuenguem et al., 2002). In this previous case, the action of *C*. *putoria* in the pollination of *R*. *communis* was mainly indirect. Thus, the blowfly species could be considered mainly as an insect-assist anemophily on the pollination of castor bean. The importance of the insect-assist anemophily was also documented on *Zea mays* in Cameroon (Tchuenguem et al., 2002) and on *Lasiacis ligulata* (Panicoideae) in Brazil (Dorea et al., 2018). Overall, *R*. *communis* appears to be an ambophilic plant species which is both entomophilous and anemophilous.

The difference in the fructification rate of about 11% observed between treatments A and B is related to the presence of pollinating flies. This increase in the fruiting rate, although modest, is of important gain for *R*. *communis* production on a hectare scale and boosting the annual quantity of seed production. Previous studies on anemophilic insect-assisted pollination have also shown modest values in yields increase. In Brazil, the fruit set rates in the open pollination treatment with the introduction of *A*. *mellifera* colonies were 94 and 79% in the treatment made by bagged raceme with muslin bag (Rizzardo et al., 2012); the deduced input of *A*. *mellifera* in this study was about 16%. In Yaounde (Cameroon) the influence of indirect pollination by honey bee *A*. *mellifera* on the increase in grain yields of *Zea mays* (Poaceae) was about 21% (Tchuenguem et al., 2002).

Conclusion

The present study shows that in Makabaye (Far-North Region, Cameroon), the blowfly species *C*. *putoria* seems to be the main flower visitor of castor bean. This fly is the most frequent and abundant anthophilous insect on *R*. *communis*, being present all day-round and during the whole flowering period. The activity of the fly on castor bean flowers contributed in the pollination of this plant, with important influence on the wind-assist geitonogamy. In turn, the increase in pollination activity improved the fruiting rate of *R*. *communis* by about 11%. Apart from its pollinating behaviour, this blowfly needs pollen and nectar in its diet as source of proteins and sugars that enables individuals to reproduce well and perpetuate their species.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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