Diel Patterns of Activity for Insect Pollinators of Two Oil Palm Species (Arecales: Arecaceae)

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Abstract

The pollination of two oil palm species, Elaeis guineensis Jacquin and Elaeis oleifera Cortés (Arecales: Arecaceae), depends on a mutualistic relation with insects, which use male inflorescences as a brood site, and visits female inflorescences lured by the emitted odor, which is similar to that of males. Although the activity of visiting the inflorescences by these insects is critical for the adequate natural pollination of the host plant, their activity is poorly documented. In the present study, we determine the diel activity of two specialized pollinator weevils (Coleoptera: Curculionidae) on inflorescences of their respective host-palm: Elaeidobius kamerunicus Faust specialized on E. guineensis, and Grasidius hybridus O’Brien and Beserra specialized on E. oleifera. The average timing of activity was studied by using passive interception traps. Then the pattern and the duration were refined by using aspiration trapping within the active period for each insect species at the male and female inflorescences. All the experiments were conducted in an Ecuadorian oil palm plantation, located close to Amazonian forest. El. kamerunicus and G. hybridus were found to be the pollinators of E. guineensis and E. oleifera, respectively. The two species differed in their diel pattern of activity: E. kamerunicus was active in the morning and G. hybridus during a short period at dusk. For both palm species, insect visits were synchronous on both male and female inflorescences. The synchronicity is discussed as a strategy to maintain the relation mutualistic between partners. These findings increase our understanding of the oil palm pollination system.

Key words: Elaeidobius kamerunicus, Elaeis guineensis, Elaeis oleifera, Grasidius hybridus, pollination

Insect behavior is structured by a succession of activities through time, e.g., seeking food or shelter, mating, oviposition, and resting, among other activities. The time and duration insect pollinators spend foraging on flowers may be affected by intrinsic and extrinsic factors (Willmer 1983, Herrera 1990, Willmer and Stone 2004, Gottlieb et al. 2005). Insect pollinators are generally active fliers, and therefore, the combination of intrinsic factors related to pollinator metabolism (e.g., thermoregulation abilities) and extrinsic factors related to the environment (e.g., ambient temperature or light intensity) constrain insect pollinators to visit flowers at a specific time, termed the “daily activity window” (Stone et al. 1999, Herrera 1990).

The genus Elaeis contains two species of oil palm, the African oil palm Elaeis guineensis Jacquin and the American oil palm E. oleifera (Kunth) Cortés. Both oil palm species are monoeccious; each individual of these palms produces unisexual male and female inflorescences (Adam et al. 2011), which are produced in alternate cycles. As a result, oil palm reproduction requires cross-pollination by insects (Syed 1979). The most important insect pollinators of oil palms are beetles in the families Curculionidae and Nitidulidae (Coleoptera). These pollinators have a mutualistic relation with the host plant; the postanthesis male inflorescences serve as brood sites, and the female inflorescences are pollinated by deceit, as explained below in the following paragraph.

The underlying pollination process is mediated by the release of inflorescence odor. In E. guineensis, female inflorescences emit an anise-like fragrance similar to the fragrance emitted by the male inflorescences at anthesis to attract insects (Opute 1975, Lajis et al. 1985, Husseini et al. 1989). Insect pollination is critical for an economically sustainable oil palm industry because it increases fruit set and consequently oil yields (Genty et al. 1986, Corley 2009). The oil palm industry is dependent on the artificial reinforcement of pollination, using human-assisted pollination or the introduction of insects in areas in which they are absent (Syed et al. 1982). Thus, the study of the pollination system of Elaeis and the interactions with
insect pollinators of these palms may help improve pollination in oil palm plantations. The biology of the pollinators of oil palm species, particularly the timing of visits to inflorescences, is not well understood. To date, most of the published studies on insect activity on oil palm inflorescences have focused on the derelomine weevils of the genus Elaeidobius (Coleoptera: Curculionidae), including Elaeidobius kamerunicus Faust and El. subvittatus Faust, and on a nitidulid beetle Mystrops costaricensis Gillogly (Coleoptera: Nitidulidae). El. kamerunicus and El. subvittatus are both reportedly morning visitors on inflorescences of E. guineensis in Africa, their country of origin (Desmier de Chenon 1981, Mariau et al. 1991) and in regions where these species were artificially introduced, such as Asia (Tandon et al. 2001, Yue et al. 2015) and South America (Mariau and Genty 1988, Chinchilla-López and Richardson 1990, Prada et al. 1998, Moura et al. 2010). El. subvittatus also showed morning activity on E. oleifera inflorescences in South America (Genty et al. 1986, Prada et al. 1998). However, a short second period of activity at the end of the day was observed for E. kamerunicus in West Africa (Mariau et al. 1991) and on Hainan Island, China (Yue et al. 2015). Conversely, M. costaricensis is principally a crepuscular visitor of inflorescences of both E. oleifera and E. guineensis in Central America and on the Pacific coast of South America, with sometimes a period of minimal and short early-morning activity depending on the geographic locality (Genty et al. 1986, Mariau and Genty 1988, Chinchilla-López and Richardson 1990).

These insect pollinators were not present in the Amazonian area of Ecuador (Mariau and Genty 1988) before the successful introduction of E. kamerunicus in oil palm plantations in 1984 (Mariau and Genty 1988). An additional, recently described and very poorly known derelomine weevil, Grasidius hybirdus O’Brien and Beserra (Coleoptera: Curculionidae), was collected in a natural population of E. oleifera in Taisha (Pastaza Province, Ecuador), and introduced in the oil palm plantations of Amazonian Ecuador in 2003 (G. Couturier, unpublished data, P.M. Beserra, unpublished data). The presence of E. kamerunicus and G. hybirdus together in the same oil palm plantation with their respective host plants (many plots of cultivated E. guineensis and some plots of E. oleifera from either Ecuadorian or Brazilian origins) provides the opportunity to study the diel pattern of visits of these insect species to inflorescences, in addition to determine the specificity of interactions with the host plants. We used two complementary trapping techniques: the first to identify the average period of activity and the second to precise the dynamic of visit within these periods for the two insect species. We addressed the following questions: What is the timing of pollination activity for the two insect species E. kamerunicus and G. hybirdus, with a special concern for G. hybirdus for which nothing is known? Are these insect species specialists on their respective host palm inflorescences?

Materials and Methods

Site Location

This study was conducted at the Palmeras del Ecuador (PDE) oil palm plantation (S-0.268325, W -76.552265) located in the north-east of the Amazonian part of Ecuador. Among the 8,650 ha of the plantation, 33% are planted with the introduced African palm E. guineensis, <1% with the native palm E. oleifera, which originated either in Taisha (Amazonian Ecuador) or in Coari (Amazonian Brazil), and 65% with interspecific hybrids of E. guineensis × E. oleifera (Torres Alarcón 2013).

Plant Materials

Four experimental plots of approximately 0.7 ha were used that contained 100 palms of each species. Two plots of E. guineensis, both planted in 2008, and a plot of E. oleifera Taisha planted in 2002 were used for both interception and aspiration trapping. An additional plot of E. oleifera Coari planted in 2011 was also used for aspiration trapping.

Trapping Methods

The period of activity was defined as the interval of time in which insects take off or land on the inflorescences. We used two complementary trapping methods to capture insects and measure their period of activity: 1) passive trapping by using interception traps on inflorescences and 2) active trapping of insects visiting caged inflorescences. For both trapping methods, both the selected male and female inflorescences inside each oil palm plot were at the physiological stage close to the onset of anthesis. Insect sampling and trapping were conducted continuously throughout anthesis and then stopped one day after the end of anthesis, i.e., when all flowers were withered.

The passive interception trapping was designed followed Beaudoin-Ollivier et al. (2017). The trap was a transparent, rectangular Plexiglas plate (180-mm length, 145-mm width, 5-mm thickness, 0.026 m² surface). At each end, strings through holes (3-mm diameter) were used to hang the plate over the palm inflorescence. Each plate was positioned approximately 15 cm above either the male or female inflorescence at an approximately 45° incline relative to the horizontal. Each plate was covered on both sides with a removable, transparent plastic sheet coated with transparent, odorless glue. The interception trapping experiment was conducted for 2 weeks from March to April 2014. At the beginning of each week of the experiment, all male and female inflorescences beginning anthesis inside a plot were equipped with a trap. The sticky sheets were checked and renewed three times a day throughout anthesis after the following periods of time: 1) the first period of trapping was from 9:00 p.m. to 10:00 a.m., which included 9 h of night + 4 h of morning the next day, 2) the second period was from 10:00 a.m. to 4:00 p.m., with 6 h of daytime, and 3) the third period of trapping was from 4:00 p.m. to 9:00 p.m., which included 3 h of daytime + 2 h at night. Each removed sheet was wrapped with transparent plastic kitchen wrap before identification and counting of insects.

The active aspiration trapping was designed to precisely characterize the activity period of pollinators. Insects were captured throughout the period during which insect diel activity was the highest, as indicated in the interception trapping experiment. The aspiration trapping method consisted of covering a palm inflorescence with a cubic cage (500-mm height, 300-mm length, and 250-mm width) wrapped with a white mosquito net (mesh size 0.5 mm). All the visiting insects were collected either hourly (E. guineensis) or at 20-min intervals (E. oleifera Taisha and Coari) using a mouth aspirator. The collected insects were stored in vials with alcohol before identification and counting. Trapping lasted from 7:00 a.m. to 2:00 p.m. for E. guineensis and from 5:00 p.m. to 7:00 p.m. for E. oleifera. The aspiration trapping experiment was conducted during March and December 2015. Because of changes in sunrise and sunset times over the year (between 5:48 a.m. and 6:18 a.m. and between 5:54 p.m. to 6:24 p.m., for sunrise and sunset, respectively), we used min/h after sunrise and min/h before and after sunset rather than clock time. The precise clock times of sunrise and sunset for each day were obtained from http://www.timeanddate.com.
Insect Identification
Depending on the trapping method, the insects were identified and counted either on the sticky sheets protected with transparent plastic kitchen wrap or directly from vials. A stereomicroscope was used for identifications (45× magnification, BSZ-405, Boeco, Hamburg, Germany). El. kamerunicus were identified according to Ripoll et al. (unpublished data). G. hybridus were identified by reference to O’Brien et al. (2004).

Statistical Analyses
Data were analyzed using the R 3.1.2 statistical software (R Development Core Team 2015). A nonparametric Friedman’s test was performed on both the interception and the aspiration trapping data. The number of insects captured according to the sex of inflorescence was compared between each time interval. When the Friedman test was significant for the interception trapping data, a post hoc analysis based on the Wilcoxon rank test was used to make pairwise comparisons between time periods. Pairwise comparisons were also performed for aspiration trapping data to identify differences of insect captures between each interval of time. Seven female and five male inflorescences and five female and seven male inflorescences, for E. guineensis and E. oleifera Taisha, respectively, were monitoring with interception traps. Nine female and 5 male inflorescences, 9 female and 5 male inflorescences, and 11 female inflorescences, for E. guineensis, E. oleifera Taisha and E. oleifera Coari, respectively, were monitored by aspiration trapping.

Results
Diel Pattern of Pollinator Insect Activity at Inflorescences of E. guineensis and E. oleifera Using Interception Trapping
All 2,109 E. kamerunicus captured with interception trapping were on E. guineensis, whereas all 12,178 G. hybridus captured were on E. oleifera. The majority of E. kamerunicus and G. hybridus, 93.6 and 96.3%, respectively, were trapped on the male inflorescences of their respective host plant, with fewer insects trapped on female inflorescences (Fig. 1A and 1B). Few other species were captured during the interception trapping, except flies belonging to Sphaeroceridae and Sciaridae. No pollen was observed on their body, suggesting no role in oil palm pollination. Few stingless bees (Hymenoptera, Meliponidae) were observed visiting male inflorescences of E. oleifera Taisha at the beginning of the aspiration trapping, and some individuals of Forficulidae (Dermaptera) were observed, but not trapped.

Among the five E. guineensis male inflorescences sampled, all E. kamerunicus were captured during the two first periods of trapping (Q = 9.1; P < 0.05), with no catches recorded between 4:00 p.m. and 9:00 p.m. (Fig. 1A). The captures of E. kamerunicus peaked during the second period (10:00 a.m. to 4:00 p.m.) with 71% of the 1,973 individuals captured in total; however, the average number of insects captured on male inflorescences were not significantly different between the first and the second periods of trapping (W = 4; P = 0.21). Among the 136 E. kamerunicus captured on the seven E. guineensis female inflorescences sampled, 90% were captured during the second trapping period (10:00 a.m. to 4:00 p.m.; Fig. 1A); however, this low number of insects captured on the female inflorescences resulted in marginally significant differences between the three periods of trapping (Q = 5.6; P = 0.06).

For E. oleifera Taisha, the average number of G. hybridus caught on the seven male inflorescences and the five female inflorescences varied significantly between the three periods of trapping (Q = 11.2; P < 0.01 and Q = 10; P < 0.01, for male and female inflorescences, respectively). All the 456 insects captured on female inflorescences and 95% of the 11,722 insects captured on male inflorescences were during the third period (4:00 p.m. to 9:00 p.m.) (Fig. 1B).

Precise Pattern of the Activity of Pollinating Insects at Inflorescences Using Aspiration Trapping
A total of 34,688 E. kamerunicus were captured on E. guineensis during the aspiration trapping, with 6,674 and 28,014 insects on five female and nine male inflorescences, respectively. The activity period of E. kamerunicus on male inflorescences started 2 to 3 h after sunrise and ended between 7 and 8 h after sunrise, resulting in approximately 6 h of activity. The insect activity observed on female inflorescences was shorter than that on male inflorescences, ranging from 3 h up to 7 h after sunrise, representing 4 hours of activity. The average number of insects captured on inflorescences varied significantly between the different 1-h intervals of trapping (Q = 37; P < 0.01 and Q = 27.4; P < 0.01, for male and female inflorescences, respectively). Post hoc comparisons performed on inflorescences of each sex revealed no differences of insect captures between intervals ranging from 3 to 7 h after sunrise. On the contrary, insect captures in intervals at each extremities of the activity range (from 1 to 3 h after sunrise and from 7 to 9 h after sunrise) were significantly
different of the previously cited period, except the two intervals ranging from 6 to 8 h presenting no differences. The insect visits occurred in the same range of time on inflorescences of both sexes, peaking between the third and the sixth h after sunrise (83% of total insects) for male inflorescences and between the fourth and the seventh hours after sunrise (87% of total insects) for female inflorescences (Fig. 2A). More insects were trapped on male inflorescences although mean number of captures were not significantly different between the inflorescences of the two sexes ($t = 1.99; P = 0.07$).

Among the 29,476 $G. hybridus$ captured on $E. oleifera$ Taisha, 12,908 and 16,568 insects were on 10 female and 9 male inflorescences, respectively. All 7,536 $G. hybridus$ captured on $E. oleifera$ Coari were on 11 female inflorescences because no male inflorescences were found at the time of the study (Fig. 2B). The average number of $G. hybridus$ individuals captured on inflorescences varied significantly between each 20-min interval of trapping for $E. oleifera$ Taisha male ($Q = 39.7; P < 0.01$) and female inflorescences ($Q = 43.3; P < 0.01$; Fig. 2B) and for $E. oleifera$ Coari female inflorescences ($Q = 46.2; P < 0.01$; Fig. 2B). Precise timing of $G. hybridus$ activity was similar on both $E. oleifera$ Taisha and $E. oleifera$ Coari, with visits concentrated in a 1-h interval between 20 min before and 40 min after sunset (Fig. 2B). The visits in this interval represented 92.8 and 94.8% of total catches on male and female $E. oleifera$ Taisha inflorescences, respectively, and 92.4% on $E. oleifera$ Coari female inflorescences. The culmination of insect activity was perfectly synchronized on the two sexes of inflorescence of $E. oleifera$ Taisha, with activity peaking on both precisely within the 0- to 20-min interval after sunset when 67 and 60% of catches on male and male inflorescences were recorded, respectively. Pairwise comparisons revealed that the average number of $G. hybridus$ captured during this interval was significantly different from all other interval of trapping, for male and female sex of $E. oleifera$ Taisha and for female sex of $E. oleifera$ Coari. Insects were always trapped in lower numbers on female inflorescences in all time intervals; however, differences of mean captures between the inflorescence sexes of $E. oleifera$ Taisha were only marginally significant ($t = 2.19; P < 0.05$).

### Discussion

In this study, a unimodal pattern of flight activity was observed for the two derelomine weevil species $E. kamerunicus$ and $G. hybridus$; notably, the diel pattern of activity differed between the two insect pollinator species depending on both the timing and the duration of the visiting period on the inflorescences of their respective host palms, $E. guineensis$ and $E. oleifera$. Whereas $E. kamerunicus$ had an extended diurnal activity period ranging from 2 to 8 h after sunrise, $G. hybridus$ had a short active period of flight that peaked precisely at dusk, ranging between 20 min before and 40 min after sunset. Consequently, $G. hybridus$ is a crepuscular pollinator, and $E. kamerunicus$ a diurnal pollinator.

To our knowledge, this is the first report of the precise flight activity of $G. hybridus$. $G. hybridus$ was introduced in the PDE plantation in 2003 from some spikelets of male inflorescences collected from a natural population of $E. oleifera$ in Taisha in the Amazonian part of Ecuador (C. L., personal communication, Beserra 2003, unpublished data). Given this species is also found on inflorescences of $E. oleifera$ in various Amazonian localities in Brazil (O’Brien et al. 2004), $G. hybridus$ and $E. oleifera$ likely coevolved in the Amazonian distribution area of $E. oleifera$. In this study, the diel pattern of activity for $G. hybridus$ is similar regardless of the origin of $E. oleifera$ (Taisha or Coari) in the PDE plantation, i.e., the visiting periods on the inflorescences of both palm types, are characterized by the same unimodal pattern, the same duration and take place at dusk. Interestingly, the nitidulid beetle Mystrops costaricensis, which pollinate $E. oleifera$ palms in the Pacific coast of Colombia and Ecuador and throughout Central America (Gentay et al. 1986, Mariau and Genty 1988) share the same ecological niche that $G. hybridus$, whereas these species are geographically isolated by the Andes. Both species behave like crepuscular insects, making this behavior consistent for the pollinators of $E. oleifera$. Further studies on the complex of $E. oleifera$ palms pollinators will be of interest as well as the study of the floral scent emission. The result on the insect behavior addressed the question on the occurrence of hypothetic rhythm of the release of the chemical signal that attracts the insect. The result of the coevolution could be an optimal adjustment between the release and the perception of the chemical signal.

According to our trapping data and frequent observations conducted during the day and at night, the activity pattern of $E. kamerunicus$ in the PDE plantation is proven to be exclusively unimodal and occurring the morning. These findings are consistent with $E. kamerunicus$ activity already reported in their African native area (Desmier de Chenon 1981), and in South America (Chinchilla-López and Richardson 1990, Mariau et al. 1991, Prada et al. 1998, Moura et al. 2010) and Asia (Tandon et al. 2001, Yue et al 2015) where the insect was introduced. The short, additional activity described by Mariau et al. (1991) in Colombia and by Yue et al.
It would be interesting to study the activity of *E. kamerunicus* in different oil palm plantations with contrasting climatic factors to identify which are potentially responsible for shaping the activity of this insect.

*E. kamerunicus* and *G. hybridus* were highly specific to their respective native host palm species. Although the two species of weevil were both in the PDE oil palm plantation and continually exposed to *E. guineensis* and *E. oleifera* growing in artificial sympatry, we never trapped or observed individuals of these insect species cross-attracted by the inflorescences of the other palm species. The synchronization between the timing of anthesis and the activity period of a pollinator insect likely increases plant reproduction (Herrera 1990). In both palm species, the visits of the respective pollinator insects are synchronized with the timing of anthesis for male and female inflorescences. The crepuscular visits of *G. hybridus* occur precisely when flowers on *E. oleifera* (Taisha or Coari) inflorescences of both sexes are functional (pollen emission for male flowers or pollen receptivity for females; T. A., unpublished data). Male and female flowers of *E. guineensis* become functional between 8:00 a.m. and 10:00 a.m. (Tandon et al. 2001), which matched the onset of the *E. kamerunicus* activity period. The insect activity is adjusted to the flower physiology for optimal pollination. Although *E. oleifera* and *E. guineensis* can artificially hybridize (Corley 2009), a temporal specialization between mutualistic plant–insect partners could act as a reproductive barrier and lead to reproductive isolation of the two oil palm species in areas in which they are artificially sympatric, such as in the oil palm plantation of this study.

Both oil palm species and their specific pollinators are implicated in a mutualistic interaction. As other nursery pollination system in which insects develop at the expense of the floral structure of the host-plant they pollinate (Dufay and Anstett 2003), *E. kamerunicus* and *G. hybridus* use male inflorescences of their respective oil palm species as a feeding site, consuming pollen released by freshly opened flowers, and as an oviposition site for larval development. Thousands *E. kamerunicus* can be found in a single *E. guineensis* male inflorescences in anthesis (Syed and Salleh 1988, Chinchilla-López et al. 1990), and *G. hybridus* individuals can reach between 30,000 to 50,000 individuals in *E. oleifera* Taisha male inflorescences (Auffray T., personal observation). Consequently, intraspecific competition for food and brood site may occur, and lead to the departure of insects looking for new sites. As seen previously, the synchronicity between male inflorescence anthesis and insect activity is crucial for partner encounter. Although never demonstrated, female inflorescences are unrewarding, and insects likely visit them by mistake. By synchronizing the anthesis with the timing at which male inflorescences and insects interact, the efficiency of the cheating by female inflorescences could be increased. Thus, the perfect synchronicity between the functional phase of both sexes of palms and the activity pattern of insects could make the interaction beneficial for all partners, allowing the persistence of the mutualistic relation. However, pollinators could have innate preference for the honest male sex; other plant traits, such as similarity of the scents emitted by male and female inflorescences, can added to the activity synchronicity and impede insects to discriminate between sexes (Dufay 2010).

We demonstrated a highly specific relation between the insect pollinators and their native palm species. The temporal specialization between insect activity and inflorescence anthesis ensure reproductive isolation among palms. The knowledge on the biology of pollinating insects could have important implication in the management strategy of pollination, e.g., the artificial release of these insects at the period of receptivity of female inflorescences of the host plant, or the improving of the efficiency of the assisted pollination by pollen intake on female inflorescences at the time of insect pollinator activity.

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