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Biocontrol and Circadian Rhythms of Medfly larvae emergence and *Monomorium subopacum* ant foraging along the fruiting season in the Argan forest (Morocco)

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ABSTRACT

To illuminate biocontrol traits between the Medfly pest and its predator *Monomorium subopacum* ant, we assessed the daily rhythm of ant foraging and Medfly larva emergence from host fruits in the argan forest. *M. subopacum* foraging activity and *Ceratitis capitata* larvae emergence were monitored in Essaouira argan forest during the fruiting season. Behavioral activities were quantified, and the circadian rhythms of the two species were determined. *M. subopacum* diel foraging was exhibited in a circadian bimodal rhythm with a principal morning peak and a secondary crepuscular one. Larvae's emergence showed unimodal rhythmicity with a single morning peak with 86.42% of daily emergences. The studied behaviors of the two species showed a clear synchronization during their principal activity peaks, which suggests the importance of circadian timing in the temporal partitioning, and chance of encounters between the two species. Besides their adaptive and fundamental ecological values, the understanding of biological rhythms that govern the time of behavioral interactions and predict encounter schedules between pests and their natural enemies may provide a novel efficient tool in sustainable pest management.

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Biological rhythm; pest management; Argan forest; Mediterranean fruit fly; *Monomorium subopacum* ant

Introduction

The widespread use of pesticides in plant protection and pest control inflicts increasing damage to human and ecosystem health. However, in recent decades, a large consensus has been established for reducing the harmful effects of pesticides. With a prodigious understanding of physiological and behavioral mechanisms of pests and their natural enemies and the improvement in analytical techniques underlying species interactions, circadian timing can provide a powerful tool for behavioral manipulations and their applications in bio-control and management approaches of pests in agricultural and forestry systems (Koukkari and Sothern 2006; Saunders et al. 2002; Foster and Harris 1997).

The circadian rhythmicity in living systems appears to be an essential tool in regulating and coordinating physiological and behavioral traits with environmental fluctuations (day/night and associated cycles) (Saunders et al. 2002). Circadian rhythms control deeply a wide variety of behavioral and physiological activities in species and may operate either in individual or population behaviors (Winfree 1967; Sharma 2003; Yerushalmi and Green 2009) and represent a key component in community interactions and ecosystem services (Richards 2002; Wiens, Rotenberry, and Van Horne 1986). In insects, circadian biological rhythms control diel cycles of activity and resting periods (Petersen et al. 1988), reproduction (Sakai and Ishida 2001; Howlader and Sharma 2006), adult, pupa, and larvae emergence (Bertossa et al. 2010; Allemand, and David 1976; Pittendrigh 1954) in addition to other complex behaviors of living organisms. These timekeeping systems control the species interactions and manage their various behavioral abilities to anticipate divers changes in the external environment (Aschoff 1960). The identification of daily temporary organizations may clarify our knowledge of evolution and community functioning and provide essential data to understand the control of pests and management of their natural enemy associations. For instance, it can provide more details on the schedule and possibility of encounters

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between sympatric species in their natural habitat. Synchronous events (causing a good phenological coincidence) are intuitively more likely to influence the establishment of predator-prey associations and appear as an essential condition in the success of the classical pest-control approaches by natural enemies. Indeed, like the spatial overlap, temporal synchronization is fundamental to characterize the phenology of species, the vulnerable time of target species, and the most appropriate time to act for the management of harmful species and populations. The effectiveness of pest control by natural enemies depends essentially on the ability of species to adjust their rhythm of activity with the availability of their hosts. In various biological systems, circadian rhythms control population dynamics at the social and individual levels (Daan 1981). Chambers (1977) and Vinson (1981) have shown the importance of biological rhythms as a significant factor affecting the population structure (spatial and genetic structure). Temporal niche partitioning was mainly involved in limiting competitive interactions between sympatric species (Krittika et al. 2019; Pianka 1978; Schoener 1974), in control of predator-prey associations, and pollinators of various plants (Curio 1976; Daan 1981). For instance, in *Dacustryoni* fruit fly (Diptera: Tephritidae), the circadian rhythmicity of encounters between partners shows significant importance in the success of sexual activity and reproduction (Hawking 1973; Theron 1984). The significance of causal elements of behavioral rhythmicity in insects has been the subject of considerable investigations mainly in diversity and predator-prey systems (Kenagy 1973; Fenn and Mac Donald 1995; Alanara, Burns, and Metcalfe 2001). However, its functional role in pest management and ecological services is rarely examined, probably because of the difficulty of chronobiological studies that require continuous field monitoring of specific behaviors.

The Mediterranean fruit fly, *Ceratitiscapitata* (Wied.) (Diptera: Tephritidae), is one of the world's most destructive fruits and vegetable pests. The arganeraie is the largest natural reservoir known for the proliferation and dissemination of this harmful pest (Sacantanis 1957). *Monomorium subopacum* (Smith 1858), (Hymenoptera: Formicidae) is the most dominant ant species in the argan forest (El Keroumi et al. 2012) and is known as the major predator of *C. capitata* larva below argan trees (El Keroumi et al. 2010).

To understand the organization timing in pests and their natural enemies, the study of the circadian rhythmicity is of primary interest to illuminate the ecological traits that adjusted the complex interactions and in particular the stimuli and the functioning of biocontrol between sympatric species. In the current study, our emphasis interests the quantification and the characterization of temporal patterns that organize the *M. Subopacum* foraging and the medfly larval emergence (larva eclosion from fruits) behaviors below argan trees. Secondly, the present study may shed light on the encounters schedule and determine the possible synchronization and temporal overlap between the two antagonist species. Outcomes could provide baseline data to improve the understanding of the temporal partitioning in *C. capitata* larvae emergence, the foraging activity of its predator *M. Subopacum* and their possible applications in behavior manipulations to guide bio-control strategies and sustainable techniques of pests' management and protection of valued resources and agricultural systems.

Material and methods

The monitoring of the temporary organization of the ant foraging and *Ceratitis* larvae emergence activities were carried out under field conditions below selected argan trees in Boutazarte site within the Essaouiraargan forest. We conducted field experiments during three episodes throughout the fruiting season on June 02, 28, and July 22.

Study area

The Boutazarte site in the littoral band of Essaouira arganeraie is almost 10 km from the Atlantic Ocean and approximately 20 km south of Essaouira city (31.37 N, −9.73 W). The arganeraie in this area is qualified as arganeraie forest, which presents an open canopy with 3 to 7 m tall trees growing on poor, clay, relatively compact, locally stony, and not tilled soil. In this region, the climate is of a Mediterranean type with a long, hot, and dry summer, and relatively mild and rainy winter with average annual precipitations of 300 mm (El Keroumi et al. 2012).

Data collection

M. subopacum foraging activity rhythm

M. subopacum foraging activity was monitored for 2 consecutive days during 3 survey episodes throughout the argan fruiting season on june 02, june 28 and july 22. For substantial sampling, the survey concerned three chosen colonies of ants nesting below three argan trees. This monitoring period coincides with the argan fruit ripening season, marked by the abundance of *C. capitata* larvae below trees. After their emergence from fruit, larvae fall on the soil to find suitable pupation sites in the ground. In each studied nest, ant activity was quantified by computing forager ants leaving or entering a quarter of a 100 cm diameter circle around the nest entrance. At every one-hour interval, we counted ant foragers for five minutes using a hand counter for 48 hours continously. For substantial tests to plot the circadian rhythm of active ants, we have calculated the averages of activity parameters from data of 18 ant foraging tests (3 (ant colonies) x 3 (monitoring periods) x 2 (consecutive days)). Nocturnal observations were carried out using a flashlight that was covered with a clear red filter to reduce the disturbance of forager ants' activity.

Ceratite larvae emergence rhythm

We conducted monitoring of mature larvae emergence from argan fruit below the same trees in the same episodes and field conditions as in the *M. Subopacum* foraging survey. To visualize the diel schedule of larval emergence from argan fruit, mature fruits that have been exposed naturally to Medfly attack were collected locally from argan trees. Three mesh baskets with 2 kg of fruits equipped with holes sufficiently large to allow only the passage of the larvae that left the argan fruits were placed below chosen trees. Each basket was balanced in a large plastic bowl, with almost one centimeter of sand used in the bottom to catch the falling larvae and prevent them from jumping outside the bowl. At every hour, the sand was sieved in each replication, and the collected larvae were counted and eliminated. Larva emergences were counted in each bowl by counting larvae that fall from fruits every hour for two consecutive days (48 h). For samples representativity, data used to plot the circadian rhythm of *Ceratitis* larvae emergence from argan fruit was collected from 18 monitored tests (3 mesh baskets below trees (replications) x 3 monitoring episodes x 2 consecutive days). Ambient temperatures below trees were recorded every hour during the larval emergence survey periods.

In living organisms, light-dark (L/D) and temperature cycles are the key factors in the entrainment of circadian rhythms. An overview of the local environmental situation regarding these two factors (temperature and photoperiod) was assessed during the study period to examine their eventual effect on larvae emergence and ant foraging behavior. During the survey episodes, we recorded ambient temperatures below host trees every hour by an electronic thermometer (with a Stainless-Steel Probe for High-Temp). Informations on daily photoperiode were obtained in the Boutazarte region during our monotoring episodes by using sunrise and sunset times which are often available on the website: https://www.timeanddate.com/sun/@31.379104,-9.734573 (consulted on 2 October 2022). All times are local time for the studied site of Boutazarte and dates are based on the Gregorian calendar.

Data analysis

M. subopacum foraging activity rhythm

From raw data, the average number of active ants along the day was calculated per hour in a studied nest at the 3 defined monitoring episodes. The daily foraging activity curves show a similar rhythmic pattern in the three nests. This allows plotting the daily curve of average activity per hour for the three studied nests. The average curve of activity during the day was obtained by calculating the hourly arithmetic mean of ants in activity in all monitored nests in the 3 sampling episodes to highlight the temporal organization of the circadian foraging rhythm of *M. subopacum* ant.To characterize circadian rhythm patterns of ant foraging, the properties of distribution and the intensity of ants' activity (which refers to the number of outing ants from the nest to forage per unit of time) and a resting period of workers in the *M. subopacum* nests, the average rate of hourly foraging activity during 24 h was plotted. Using this plot, prospective peak acrophase (circadian amplitude) and median activity hours can be identified. This curve was represented by relating the number of active foragers to the total number of active ants every hour throughout the day in all nests of the study. With ant foraging rhythm, ambient temperature and natural photophase (mean time between the sunrise and sunset during fieldwork days) were shown in the same graphic in Figure 4.

Rhythm of Medfly larva emergence fromargan fruit

The diel rhythm of Ceratite larval emergence from fruit was plotted based on the average of larva emergences expressed by the mean rate of larvae exit recorded below argan trees in all replications during the 3 monitoring episodes of the study. Quantitative aspects of rhythmic activity were illustrated by plotting the hourly average rate of the emergence curve for 24 h.

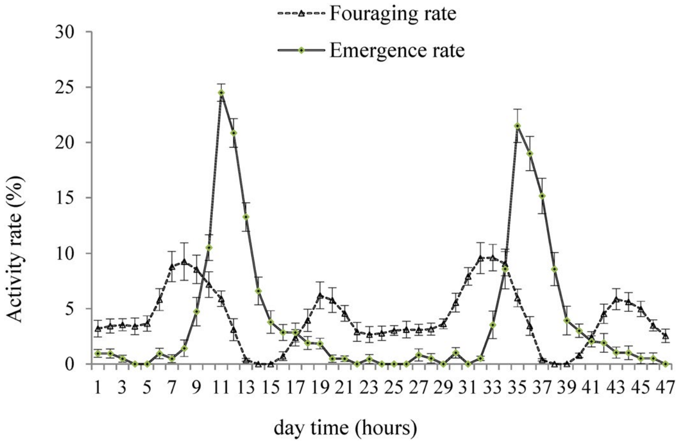

Figure 1. *Monomorium subopacum* fouraging activity and Medfly larva emergence rhthms across 48 hours below argan trees under filed conditions during the fruiting season at the Boutazarte site of Essaouira aragn forest. The two species behaviors show a consistent daily rhythmic pattern.

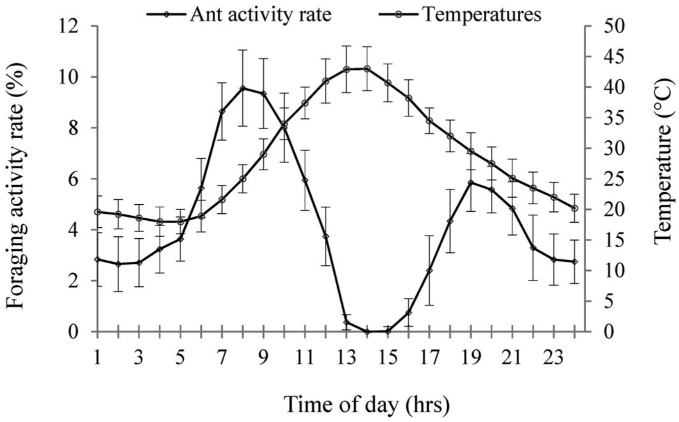

Figure 2. Circadian rhythm of *Monomorium subopacum* foraging under ambient temperatues, and natural lighting in field conditions below argan trees of Essauira argan forest.

Circadian rhythm comparison of M. subopacum foraging and Medfly larva emergence from argan fruit

To compare the daily activity of the two behaviors, circadian rhythms of Medfly larva emergence and ant foraging were presented in the same graph with the filed temperatures and photoperiod intervals. The comparison of circadian activity may highlight the temporal organization of encounters between the two antagonist species in conditions of temperatures and photoperiod intervals below the argan trees as principal entrainment factors that impact behavioral daily rhythms.

The Pearson correlation test was used to highlight the strength of the association between rhythms of *M. subopacum* foraging and *C. capitata* larvae emergence from argan fruits. Circadian rhythm comparison concerns principally the morning peaks of the two species activity. Statistical traitement was performed using SPSS Version 25 (IBM). We considered p value < 0.05 statistically significant.

The data values (species activity rates, temperatures, sunset and sunrise times) used to plot and compare circadian rhythms of the studied species were expressed as means of perfected replications in the monitoring episodes (± Standard Deviation (SD)).

Results

Under field conditions, under argan trees during the fruiting season, the foraging of ants of the species M. subopacum and the emergence activity of medfly larvae show a constant daily rhythmic trend over two consecutive days. (48 h) (Figure 1). This constant rhythmic pattern per day suggests the circadian rhythmicity of the studied behaviors and supports the examination of the chronobiological characteristics of these rhythms and the specific resulting interactions.

Circadian rhythm of M. subopacum foraging activity

The *M. subopacum* workers were active mainly during the day-time period with a bimodal rhythm (two peaks) (Figure 2). The morning peak is the

Table 1. Sunrise and sunset times during the field monitoring episodes in Boutazarte site. Times are local time for the study site and dates are based on the Gregorian calendar.

Date	Sunrise time	Sunset time
2-juin	6:33:36 a.m.	8:38:08 p.m.
3-juin	6:33:24 a.m.	8:38:40 p.m.
28-juin	6:36:07 a.m.	8:46:24 p.m.
29-juin	6:36:20 a.m.	8:46:24 p.m.
22-juily	6:48:31 a.m.	8:40:08 p.m.
23-juily	6:49:08 a.m.	8:39:34 p.m.
Mean	6:39:31 ± 7 m 19 s	20:41:33 ± 3 m 49 s

Data were obtained from the following website: https://www.timeanddate.com/sun/@31.379104,-9.734573 (23 July 2022).

main peak that onset very early in the morning, between 3 and 4 a.m., with 2.5 h before sunrise which occurred locally at 6:39:31 (± 7m19s) a.m. (Table 1) and continues until noon. In this peak over half of the total diel activity was recorded (52.11 ± 7.12%). The peak of ants' activity reached its acrophase between 8 and 9 a.m. with 9.55 ± 2.63% of total diel activity and ambient temperature that have not exceeded 25 °C. During the period of this peack (from 4 a.m. to noon), the average temperature recorded below argan trees was 26 ± 1.7 °C). Over the hottest period at midday, when the temperatures reatched between 25 to 43 degrees Celsius, the ant foraging activity was negatively and strongly correlated with the ambient temperatures ($r = -0.7512$; $p < 0.0001$). The secondary peak of activity occurred from early evening to dusk (between 5 and 10 p.m.) with almost 1.5 h after sunset which occurred locally at 8:41:33 (± 3 m 49 s) p.m. (Table 1) with a foraging rate of 23.70 ± 4.81% of the total diel activity. The acrophase of this peak was situated between 7 and 8 p.m. with an activity rate of 5.66 ± 2.04% of the total daily foraging. The average ambient temperature noted below argan trees in this phase was 34 ± 1.5 °C. Ant activity foraging recorded was almost nil (1.10% of diel activity) between midday and 4 p.m., indeed, during this period the average temperature registered was 43.5 ± 1.7 °C. During the night period, between 9 p.m. and 4 a.m. of the next day, *M. subopacum* showed a resting phase with an average activity rate of 2.58 ± 1.13% of daily foraging activity. The hour between 9 and 10 a.m. is the median hour of ant workers foraging (the hour of the day which marks the accomplishment of 50% of the total daily ants' activity.

Circadian rhythm of Medfly larvae emergence from argan fruit

Figure 3 shows the curve of the diel rhythm of larvae emergence from fruits below argan trees during an experimental period, under field conditions. The results show that larvae emergence occurs in a stable circadian rhythm according to a uni-modal rhythm with a single peak, which started progressively from 4 to 11 a.m. During this peak, most daily larvae emergences occurred (86.42 ± 9.28%). During the rest of the nycthemeron (from noon to 4 a.m. of the next day), only 13.58 ± 2.50% of emergence occurred. The acrophase of Medfly larvae emergence occurs between 6 and 7 a.m. with an activity rate of 25.34 ± 3.82% of the total diel larvae emergence. In Medfly larva emergence, the median hour, which refers to the hour of the day when 50% of daily emergence was performed, was between 6 and 7 a.m.

The larvae emergence in the morning conditions, with a temperature of 20 ± 1.2 °C at 7 a.m. Then, the emergence decreases and tends toward zero at noon, when the temperature reaches its maximum (42.9 ± 2.4 °C). The larva that does not emerge during the morning peak postpones their emergence until the same morning period of the next day. As the same as with ant foraging activity, we noted also that the mature larva emergence begins very early in the morning at 2.5 h previously of sunrise that occurred at 6:39:31 a.m. (±7:19 s) (Table 1).

Circadian rhythm comparison of M. subopacum foraging activity and ceratite larvae emergence from argan fruit (Figure 4)

Figure 4 shows that the circadian emergence of Medfly larvae from the argan fruit was initiated since 3 a.m. and reached its peak between 6 and 7 a.m. However, the ant workers started foraging around 4 a.m. and reached their peak of activity between 8 and 9 a.m. The larva emergence was started an hour early before the ants' exit from their nests and reached their acrophase two hours before the acrophase of ants foraging activity. During the morning peak of the two circadian rhythms, the studied species showed clear circadian rhythmicity and a substantial temporal overlap marked by maximal rates of total activity registered in the day-time; 86.42% and 52.11% for larva emergences and ants foraging respectively (Figure 3). During this period, the hourly activity of ant workers was highly and positively correlated with the larva emergences from argan fruits. In fact, the Pearson correlation coefficient for ants foraging and larva emergence is 0.71, which is significant ($p < 0.001$ for a two-tailed test) with strength relationship ($r = 0.72$, $p < 0.0001$). After their inactivity phase between noon and 4 p.m., ants exhibited a secondary foraging peak less important than the morning one, while during this same period

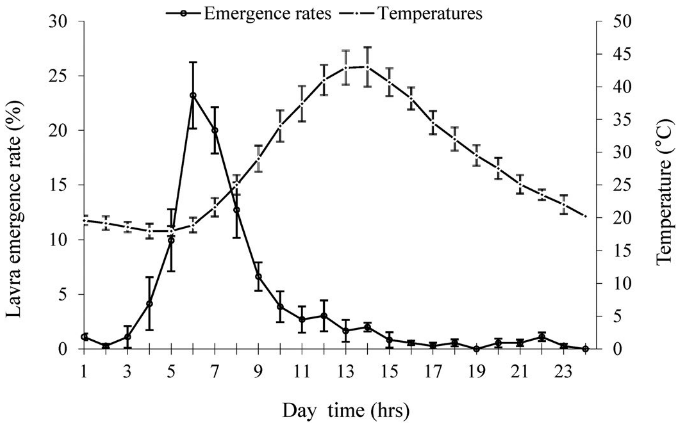

Figure 3. Circadian rhythm of *Ceratitis capitata* larvae emergence from argan fruit with ambient temperatures and natural lighting cycles under field conditions.

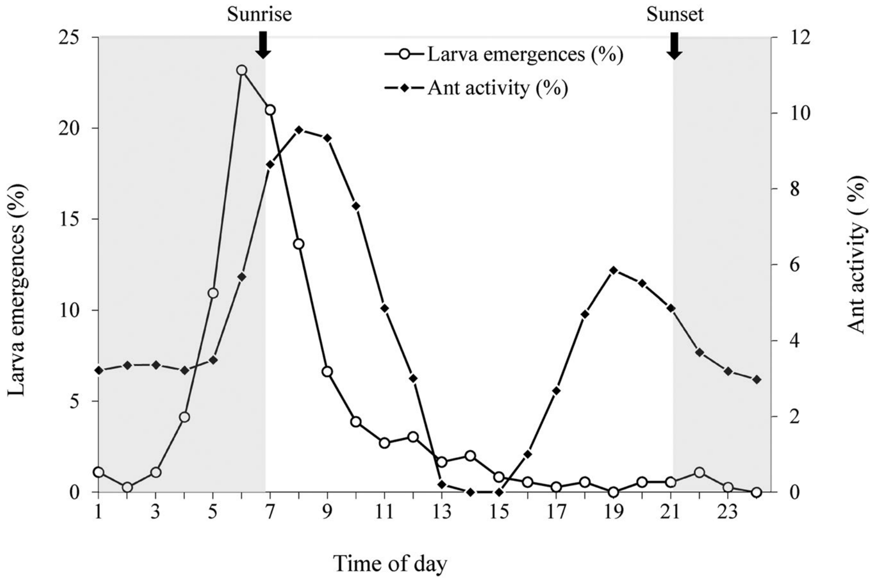

Figure 4. Circadian rhythms of *M. subopacum* foraging activity and the *C. capitata* larva emergence from argan fruit. Gray boxes correspond to night period and clair background represents the day period. The sunrise (6:39:31 ± 7 m 19 s) and sunset (20:41:33 ± 3 m.

of the day, larval emergence from host fruit was almost nil. During the night phase, both the larvae emergence and ant foraging activity were reduced. For the two studied behaviors, we noted that ant foraging activity and larvae emergence onset very early in the morning, with almost 3.5 h before sunrise for larva emergence and 2.5 h for ant foraging.

Discussion

For a better understanding of the complex interactions that govern the services provided by the biota associations in natural ecosystems particularly in pest biocontrol, the study of circadian rhythms of the target pest and their natural enemies is of capital importance. Circadian rhythms carefully adjusted, anticipate and adapt physiology and behavior of organisms to divers' regular environmental changes (Takahashi et al. 2008). In recent decades, little effort have been made to assess the role and functioning of circadian rhythms in biological pest control. This is probably because of the complex nature of behavioral phenomena and chronobiological studies, which require imminent and continuous field monitoring in a wide-ranging of parameters and sympatric species. In the argan forest, for the first time, we have quantified the *C. capitata* larvae emergence from argan fruits and the *M. subopacum* diel foraging continuously over 24 h.

Our results show that larva emergence and activity foraging of ant workers were exhibited according to daily consistent trend (Figure 1). For both species, the average day of larvae emergence and ant foraging show a strong circadian rhythmicity (Figure 3). Both species show extremely synchronous peaks during the morning picks of the two behaviors, indeed, the majority of emergences (86% of total daily emergences) occurred in parallel with the main peak of ant fouraging activity. This attitude approves a regular opportunity of the encounter between the two species and confirms one of the successful aspects in the ecological (El Leroumi et al. 2012), and biocontrol relationships already reported between the two antagonist species in the argan forest (El Keroumi et al. 2010)

Circadian rhythm of M. subopacum foraging activity

The circadian foraging activity of *M. subopacum* workers is exclusively diurnal with a bimodal activity rhythm. The foraging rhythm showed two peaks of unequal importance: an important morning peak (52.11% of total ant activity) between 4 a.m. and noon, and a second, less important peak with a reduced amplitude (23.70% of total daily foraging of ant workers between 4 and 9 p.m.

For ants, this result is consistent with previous investigations suggesting that ants were characterized by a different diel foraging activity that shifts from nocturnal to diurnal circadian rhythms. Some species are active day and night, others during either the whole or a limited part of the night or day-time, from uni to multi-modal rhythms (McCuskey and Soong 1979; Hölldobler and Wilson 1990). In general, the temporal organization in behavior can be viewed as an adaptive response to the combined action of endogenous nature and exogenous stimuli that were entrained by biotic (quality and availability of mating or feeding resources, natural enemies, competition…) and abiotic factors (temperature, light/dark cycles, and humidity…), that may vary on circadian and on seasonal scales (Pawson and Petersen 1990; Orivel and Dejean 2002; Kronfeld-Schor and Dayan 2003; Philpott et al. 2004; Cogni and Oliveira 2004a). Especially in social insects, biological cycles of activity are known by their high plasticity that represents a key characteristic adjusting foraging schedule with environmental events to optimize species fitness. Indeed, the fitness of an organism is optimized if its temporal dynamics facilitate its access to essential resources. Such plasticity represents an important adaptive strategy of predators that may respond by adjusting the foraging activity to the phenology patterns of their prey on a diel and seasonal scale. In fact, depending on seasonal fluctuations (biotic and abiotic environmental conditions such temperature and photoperiod …) and food availability, ants can adjust the schedules of their foraging habits (North 1987, 1993). For instance, many wood ant species, are generally diurnal, but depending on the seasonal climatic fluctuations, when meteorological conditions change, a large nocturnal activity can be perceived (De Bruyn and Kruk 1972; Rosengren 1977a, 1977b; North 1987, 1993).

During *the M. subopacum* activity time over the two foraging peaks, ambient temperatures recorded in the field under the argan trees do not exceed 34 ± 1,5 °C. However, the *M. subopacum* showed decreased activity with almost zero foraging between noon and 4 p.m. in this period of the day, ambient temperatures in the field reach their maximum of 43.5 ± 1,7 °C. This resting phase or mid-day siesta observed in worker ants can be linked to higher temperatures recorded below argan trees, which generally, exceed 40 °C during the summer season. In the Mediterranean ecosystems, it has been shown that the temperature represented one of the most important factors that influence ant foraging schedules (Azcárate, Kovacs, and Peco 2007), the individual survival, species behaviors and population structure (Cerdá, Retana, and Cros 1998; Lighton and Turner 2004; Maysov and Kipyatkov 2009).

Circadian rhythm of C. capitata larva emergence

The rhythm of *C. capitata* larvae emergence is uni-modal with a morning peak. During the peak phase, almost all daily emergences were performed. Outside of this period, larvae emergences were almost nil. Mature larvae that do not have a chance to emerge during the morning phase of the day should wait for the same period of the next day. This noted type of rhythmic emergence pattern corresponds to the same behavior described in the case of many other dipteran species. For instance *Anastrepha spp.* (Tephritidae), which parasitize the *Psidiumguajava* L., (Myrtales: Myrtaceae), Aluja et al. (2005), found that the larvae emergence occurred in preference between 4 a.m. and 8 a.m., while, larvae exited from host fruit; *Citrus aurantium* L., (Sapindales: Rutaceae) according to rhythmic pattern between 4 a.m. and noon. This typical attitude that characterizes tephritid larval emergence from host fruits with larvae massive emergence during the morning phase appears to be a stable behavioral attitude characterizing diel activity in many Dipterians (Denlinger and Zdarek 1993; Tanaka et al. 2013). In Tephritid species, a few studies have attempted to determine factors that initiate the massive emergence of mature larvae from host fruits during the morning time of the day. But, it has been recognized that a combination of exogenous and endogenous factors constitute stimuli that organize the diel tephritid larvae emergence schedule. Indeed, temperature, high relative humidity and first-morning sun rays fluctuations in addition to the physiological endogenous cues might be the most important factors that control the temporal dynamic of both adult and larvae emergences in Dipterians (Tanaka et al. 2013). This outcome is also in concordance with results highlighted in *Anastrepha spp*. (Tephritidae) larvae emergence from *Citrus aurantium* host fruits in Mexican groves (Aluja et al. 2005). Moreover in fruit-flies, the stimuli of endogenous nature that persist in the absence of training factors could be with significant effect in circadian control of larvae emergence from host fruits (Saunders 1982). It is unlikely that the temporal organization of larvae emergence is the consequence of the egg-laying rhythm flies mothers, given the variability of larval development time. Indeed, the absence of emergence during most of the day, suggests that the larvae exit from argan fruit was possible only for a few hours of the day. This all or nothing called "gating event" by Pittendrigh (1966) has been also described in many other insect species (Saunders 1982). The larval emergences occur during a short period of the day; the insects have only a few hours in the morning to exit from host fruits, if they don't realize it exactly at this specific period, they have to wait until the same phase of the next day to leave the fruit.

Comparison of M. subopacum and C. capitata larvae emergence from argan fruits

During ant foraging and larvae emergence peaks, the Pearson correlation coefficient is 0.71, which is significant with strength relationship between the two bahaviours. The circadian rhythms comparison of the two species showed a clear synchronous peak of activity during the fruiting season (Figure 3). The larvae emergence rhythm reaches its peak between 6 and 7 a.m., and the *M. subopacum* workers arrive at the foraging peak between 8 and 9 a.m. with an observed temporal shift of two hours (Figure 3). In fact, some authors suggest that in the prey/predator system, temporal shift in activity peaks of circadian bahaviours is predation-induced and represents a common mechanism of coexistence and encounter among species (Stiling 1999; Alanara, Burns, and Metcalfe 2001). In our case, this temporal shift among the studied behavioral peaks seems to be apparent because, after their exit from the host fruits, the emerged larvae spend more additional time to find a suitable pupation site. This additional time constitutes the needed period for foraging ants to synchronize their peak of presence with that of newly emerged larvae below host trees.

Generally, in Tephritides newly emerged larvae, the time needed to find a suitable site for pupation depends on environmental factors, such as climate, soil physical properties (temperature, pH, texture…) Aluja et al. 2005), pressure or risk of predation… (Fernandes et al. 2012). For instance, in the Mexican grove, Thomas (1995) suggested that the newly emerged larvae of *Anastepha spp* fly from the host fruit need over 1 h before finding an appropriate site to pupate. With the evidence suggesting the importance of the soil conditions below host trees at pupation time of newly emerged larva. The dominance of clay soil relatively compact, dried and not tilled below the chosen trees in our experimentation could further increase the time needed to find a suitable pupation site by Medfly larvae.

The insight around the synchronization of the timing of the studied behavior among the two species show the importance of temporal niches as a principal trait of the prey/predator association that explores the schedule of the encounter between the *M. subopacum* and the *C. capitata* populations below host trees in the argan forest ecosystem.

On the other hand, Medfly larvae show a massive emergence from host fruit synchronous with the peak of *M. subopacum* foraging below the argan trees. Such behavior may provide opportunities to larva for limiting predation risk by ants. The newly emerged larva those are vulnerable to predators, left argan fruits massively for a few hours during the morning peak and stop emerging until the same period of the following day. The exit from host fruits massively for a short period can be advantageous for larva because it reduces the impact of predators which has a limited capacity to catch the high number of larva that emerge from fruits that fall on the ground or that are still on the host tree. The predation risk caused by ants is definitely higher if the availability of larvae preys below trees is more moderate over an extended period of time. We may interpret this pattern as a response by Medfly larva to minimize predation risk. In fact, this approach reminds the anti-predator behavior known in fruit flies and suggesting that newly emerged larvae searching for pupation sites may even prevent and avoid predators, parasitoides, and other abiotic mortality factors (heat shock, humidity ….) (Bressan-Nascimento 2001; Wang and Messing 2004; Fernandes et al. 2012; Aluja et al. 2005).

In the argan forest system, during the fruiting season, diel foraging of *M. subopacum* and medfly larvae emergence from fruits are exhibited according to circadian timing. The rhythm of ants foraging is bimodal with an important morning peak and a secondary crepuscular one. The circadian rhythm of

Medfly larva emergence from host fruits is unimodal with a morning peak synchronous with a principal peak of *M. subopacum* foraging.

This feature of temporal partitioning revealed in the tow studied behaviors highlights the importance of biological rhythms in adjusting encounters and by consequent the bio-control of *C. capitata* by *M. subopacum* ants in the argan forest. The characterization of the circadian rhythm of predator ants and medfly larva emergence from host fruit may provide an important benefit in the determination of appropriate day-time periods to target pests more effectively and to optimize their control to protect agricultural and valued resources. Advances in our understanding in mechanisms that governs circadian bahaviours and selective events that affect activity patterns of pest species and their natural enemies may provide a powerful tool in pest-management. A good proficiency in this issue could guide behavioral traits through manipulative experiments among sympatric species and interactive associations of animals (pest/natural enemy, prey/predator…) to enhance or limit encounters and divers interactions. In addition to this practical benefit, the focus on the circadian timing provides a fundamental and theoretical basis for understanding the role of circadian timing and their adaptive value in the ecological communities. In addition, after the fruiting season of the argan tree, a study on the circadian rhythms of ant foraging will be more important to show the impact on the flexibility and the possible adaptations which can affect the rhythm of *M. subopacum* diel foraging after the disappearance of host larvae emerging from argan fruits.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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