

Behavioral investment in the neotropical fire ant *Solenopsis saevissima* (Smith, 1855) during the critical phase of colony establishment

Orçamento comportamental na formiga neotropical *Solenopsis saevissima* (Smith, 1855) durante a fase crítica de estabelecimento da colônia

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ABSTRACT

Behavioral investment of fire ant queens during the foundation of a new colony may be related to alone foundation (haplometrosis) or a group foundation (pleometrosis). Thus, the objective of the present study was to evaluate, through behavioral records, how *Solenopsis saevissima* queens behave during preemergence until the 3rd post-emergence week, in haplometrosis and pleometrosis, in the initial periods of the colony. For this, an ethogram of queens and workers were elaborated, to later evaluate the behavioral budget in both types of foundations. From a total behavioral budget of 8014 minutes of observation, it can be seen that the queen's behavioral acts change according to the colony cycle period (Pre-Egg Period, Egg Period, Larvae Period, Pupal Period and Post-Emergence Period), and that at first are directed to behaviors of exploration and interaction with the environment, such as walking and antennating the container and the tube. From the appearance of the first eggs, larvae or pupae, the interaction with the offspring becomes more intense. The post-emergency was first hit by haplometrosis

queens and the workers started to actively protect the immature as well as do other activities. Thus, the period of the colony's development cycle is a determining factor in the manifestation of queens' behavior, which shows whether the foundation is alone or in groups.

Keywords: Ethogram Exploration, Foundation, Immature, Parental care.

RESUMO

O investimento comportamental das rainhas de formigas lava-pés durante a fundação de uma nova colônia pode estar relacionado à fundação isolada (haplometrose) ou à fundação em grupo (pleometrose). Assim, o objetivo do presente estudo foi avaliar, por meio de registros comportamentais, como as rainhas de *Solenopsis saevissima* se comportam durante a pré-emergência até a 3ª semana de pós-emergência, na haplometrose e pleometrose, nos períodos iniciais da colônia. Para isso, foi elaborado um etograma de rainhas e operárias, para posteriormente se avaliar o orçamento comportamental em ambos os tipos de fundações. A partir de um orçamento comportamental total de 8.014 minutos de observação, verificou-se que os atos comportamentais das rainhas mudam de acordo com o período do ciclo da colônia (Período Pré-ovo, Período de Ovo, Período Larvar, Período Pupal e Período de Pós-emergência), e que num primeiro momento são direcionados para comportamentos de exploração e interação com ambiente, como caminhar e antenar o recipiente e o tubo. A partir do aparecimento dos primeiros ovos, larvas ou pupas, a interação com a prole torna-se mais intensa. A pós-emergência foi atingida pela primeira vez por rainhas em haplometrose e as operárias passaram a proteger ativamente os imaturos, além de realizar outras atividades. Assim, o período do ciclo de desenvolvimento da colônia é um fator determinante na manifestação do comportamento das rainhas, e pode apresentar diferenças nas fundações isoladas ou em grupos.

Palavras-chave: Etograma, Fundação, Imaturos, Cuidado parental.

1 INTRODUCTION

Fire ant queens *Solenopsis* Westwood, 1840 have different reproductive strategies for starting a new colony, where they either start alone (haplometrosis) or in group (pleometrosis) (Markin et al. 1972; Tschinkel and Howard 1983; Rissing and Pollock 1987; Rissing et al. 1989; Nonacs 1990; Hölldobler and Wilson 1990; Bernasconi and Keller 1996; Deheer and Tschinkel 1998; Rissing et al. 2000; Tschinkel 2013), and there after stay inside a chamber and use their nutritional reserves to raise their offspring until the emergence of the first worker (claustral period) (Hölldobler and Wilson, 1990). Because the pre-emergence phase involves a discrete period, understanding the behavior of fire ant queens at this time in the cycle is still a topic that needs further study.

Although fire ants are an abundant group on the American continent, with native species in South America and invasive species in Central and North America (Tschinkel 1988a; 2013), where they receive pest status (Tschinkel 1993a; Collin and Scheffrahn 2001; Pitts et al. 2018), there are few studies that characterize the behavioral description of queens during the claustral phase, especially in neotropical species such as *Solenopsis saevissima* (Smith, 1855). This is due to the difficulty of observation, which limits the understanding of the behavioral variation of isolated and group queens.

Given the importance of further investigating the behavioral repertoire of *S. saevissima* fire ants queens and workers, in different periods of the colony's biological cycle and in isolated and group foundations, the present study aimed to evaluate in which activities queen and workers invest their time during the establishment of the colony.

2 MATERIAL AND METHODS

The queens were collected from a nupcial flight on October 3, 2017, rainy season according to the Classification Köppen (1970) (Sá Júnior et al. 2012), on the campus of the Universidade Federal de Juiz de Fora (UFJF), Juiz de Fora (21 ° 46'29.49 " S, 43 ° 22 ' 6.26 " W, 800 m asl), state of Minas Gerais, southeastern Brazil. The nupcial flight began around 10 am and the queens were obtained around 4 pm at a distance of approximately 200 meters from the original colony when the already wingless queens (fertilized) were found in the ground searching natural cavities. The collection of the specimens took place by monitoring the flight through an active search method.

The 55 queens, all collected from the same colony, were randomly distributed in transparent 400 ml (9 cm high x 12 cm in diameter) plastic containers. For the formation of haplometrosis groups, 10 queens were maintained individually, and for the pleometrosis groups, 10 containers with two queens and five containers with five queens were assembled. Each container contained moistened cotton and an Eppendorf-type microtube (1.5mL) to provide shelter. Every two days, the food resources, 1 drop of honey diluted in 50% water and 1 gram sardine or 1 gram chicken protein, were replenished and at this time the cotton was also moistened.

Behavioral records of queens and workers were performed until the third post-emergence week. Ethograms were elaborated by the *ad libitum* method, with 10 hours of observation for haplometrosis and pleometrosis, and for the behavioral frequencies, the Animal Focal method was applied (Altmann 1974). The sessions to record the frequencies were made in one minute periods with one-minute intervals, in the morning (from 8h to 12h) and in the afternoon (from 13h to 15h), totaling 8014 minutes of sampling effort, corresponding to 3869 for haplometrosis and 4145 for pleometrosis.

The behavioral budget (%) was obtained from the frequencies of each behavioral act exhibited by queens and workers (organized into categories) (Table 1), in each colony development period, defined below:

- Pre-Egg Period (PEP) period before oviposition until the appearance of the first egg;
- Egg Period (EP): egg period until the appearance of the first larva;

- Larvae Period (LP): the period from the encounter of the first larva until the appearance of the first pupa.
- Pupal Period (PP): the period from the encounter of the first pupa until the appearance of the first worker.
- Post-Emergence Period (PEmP): the period after the emergence of the first worker.

Table 1 - Behavioral categories and description of acts exhibited by fire ants *Solenopsis saevissima*, in haplometrosis and pleometrosis in different periods of colony development, under experimental conditions.

Behavioral categories	Behavioral acts	Description of acts performed
Exploration	Walking in the tube	Move randomly through the tube antennating the surface of the tube
	Walking in the container	Move randomly through the container antennating the surface of the container
	Antennating on the tube	Touch the tube with the antenna
	Antennating on the container	Touch the container with the antenna
	Staying inside the tube	Standing immobile inside the tube
	Staying inside the container	Standing inside the container
	Under the cotton	Standing under the cotton
Care with the offspring	Antennating on the eggs	Touch the eggs with the antenna
	Antennating on the larvae	Touch the larvae with the antenna
	Antennating on the pupa	Touch the pupa with the antenna
	Caring the offspring	Attach the offspring to the jaw and move to another location
	Touch the larva with the jaw	Touch the larva with the jaw
	Touch the pupa with the jaw	Touch the pupa with the jaw
Interaction with adult	Antennating with the queen	Touch the queen with the antenna
	Antennating with the worker	Touch the worker with the antenna
Foraging	In moistened cotton	Touch the jaw on the cotton and stay for a while
	In honey	Touch the jaw on honey and stay for a while
	In protein	Touch the jaw on the protein and stay for a while

The normality of the data was verified with the Shapiro-Wilk test. The Student's t-test was used in the parametric data to verify the statistical difference between the behaviors and the foundation type (haplometrosis and pleometrosis) in the PEP, while the Mann-Whitney was applied to non-parametric data to verify if there is a statistical difference between the behaviors and the type of foundation in the periods EP, LP, PP and PEmP. For all data, a significance level of $p < 0.05$ was considered and the results were analyzed by the freeware software BioEstat version 5.3 (Ayres et al. 2015).

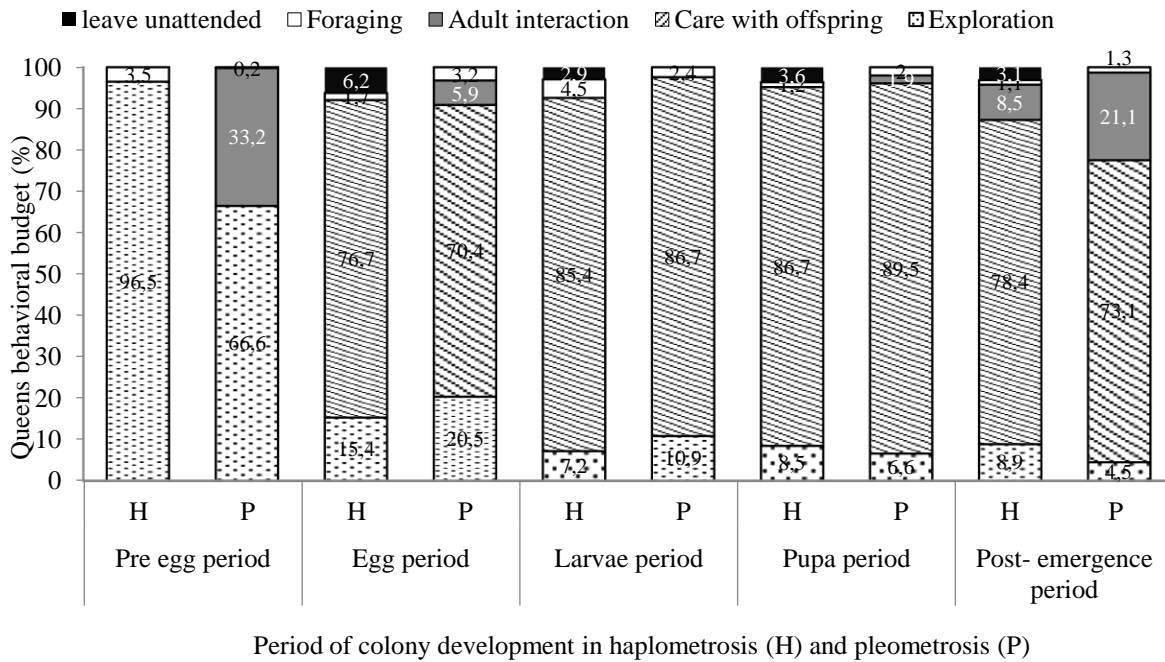
The identification of the species was confirmed by molecular analysis of specimens, performed at the Instituto Biológico (IB) of São Paulo.

3 RESULTS

Among the 18 behavioral acts recorded, queens in haplometrosis and pleometrosis exhibited similar behavioral repertoire, and there was also an equivalence in the percentage of their performance, since there was no statistical difference ($p = 0.2059$) between the time taken to perform the behavioral acts, nor on different colony cycles (Figure 1).

But when analyzing the periods of the colony cycle (PEP, EP, LP, PP and PEmP) there were changes in the queen's behavioral acts, which at first were directed to exploration and interaction with the environment, such as walking and antennating the container and the tube, and then replaced with care with the offspring (Figure 1).

Fig 1: Behavioral budget (%) of fire ant queens *Solenopsis saevissima* in haplometrosis and pleometrosis maintained under experimental conditions during the five developmental periods studied.



In both haplometrosis and pleometrosis, exploratory behaviors were more performed in PEP (96.5% and 66.6%, respectively; $p = 0.2051$). After oviposition, in the EP, the frequency of exploration decreased to 15.4% in haplometrosis and 20.5% in pleometrosis ($p = 0.2222$) and continued to decline in the following periods of development.

With oviposition, behaviors related to offspring care emerged (76.7% in haplometrosis and 70.4% in pleometrosis; $p = 0.4997$). In LP the intensity of interaction with offspring, especially larva, increased to 85.4% in haplometrosis and 86.7% in pleometrosis ($p = 0.4791$) and continued to increase in PP to 86.7% and 89.5%, respectively ($p = 0.4727$). Unattended offspring and adult interaction behaviors were only recorded in isolated queens, the latter only occurring in the PEmP. And all queens ingested water in all five periods of the colony, while only isolated queens ingested honey during PEP.

In pleometrosis, queen-queen interaction (33.2%), through antennation, was the second most performed behavior in PEP and after oviposition the occurrence was reduced (5.9%). In PEP, however, there was an increase in the frequency of interactions (21.1%), due to contacts between worker-queen and worker-worker. Physical contact during adult interaction involved identifying behaviors through antennation.

During all experiment periods, no aggressive interactions were recorded between the queens. However, in two groups with double queens, one was crushed by other queens and removed from the

tube,, and in another container, a queen occupied the tube, where she oviposited and remained caring for the eggs, while the other stayed outside, without ovipositing.

In PEP, isolated queens performed significantly ($p < 0.001$) more behaviors of walking in the container (9.73%), foraging in water (2.54%) and staying under cotton (36.25%), while queens in group were antennating on the tube more often (21.32%) and remained longer standing in the tube (22.76%) (Table 2).

Table 2 - Percentage (%) of acts performed by queens and workers of fire ants *Solenopsis saevissima*, on haplometrosis and pleometrosis and in different periods of colony development, under experimental conditions.

Behavioral acts	HAPLOMETROSIS (%)					PLEOMETROSIS (%)				
	PEP	E P	LP	PP	PEmP	PEP	EP	LP	PP	PEmP
Walk in the tube	8.5	4.5	1.06	3.02	1.51	7.12	3.9	2.89	2.01	1.12
Walk in the container	9.73	1.55	2.12	0.92	-	0.94	3.78	1.29	1.74	-
Antennating on the tube	12.2	5.74	2.18	1.55	1.26	21.32	3.35	2.78	1.13	-
Antennating on the container	15.31	1.01	1.02	1.58	-	-	10.2	1.15	1.76	-
Staying inside the tube	14.5	2.59	1.54	0.51	0.43	22.76	1.21	2.21	0.45	0.48
Under the cotton	36.2	-	-	-	-	14.32	-	-	-	-
Antennating on the egg	-	72.5 7	21.0 9	11.1 5	7.15	-	74.76	20.96	20.3	6.23
Carring the egg	-	4.12	2.29	1.17	1.15	-	2.01	1.21	1.69	1.99
Antenating on the larvae	-	-	32.2 8	21.6	9.36	-	-	31.15 2	20.1	7.34
Touch the jaw on the pupa	-	-	28.1 9	26.1 6	2.5	-	-	28.46 2	27.0	2.91
Antennating on the pupa	-	-	-	17.5 8	7.09	-	-	-	19.4 2	6.51
Touch the jaw on the pupa	-	-	-	-	0.96	-	-	-	7.1	0.66
To go in moistened cotton	2.54	1.71	5.02	1.14	0.37	0.22	3.44	2.21	2.15	0.45
To go in honey	0.92	-	-	-	-	-	-	-	-	-
Antennating with the queen	-	-	-	-	-	33.32	6.53	-	2.11	4.72

Antennating with the worker	-	-	-	-	3.02	-	-	-	-	2.65
Leave offspring unattended	-	6.21	3.21	3.21	1.12	-	-	-	-	-

PEP - Pre-Egg Period; EP - Egg Period; LP - Larvae Period; PP - Pupal Period; PEmP - Post-Emergence Period

In pleometrosis, antennating with other queens (33.32%) was the most performed behavior and sometimes queens could antennate two individuals at the same time. This specific type of antennating behavior was recorded in all interactions between individuals and in the different periods of the pleometrosis colony cycle.

Regarding the choice of oviposition site, isolated queens preferred under cotton (45.64%), followed by inside the tube (37.14%) and in the container out of contact with the cotton (17.22%). Pleometrotic queens oviposited more in the tube (51.23%), followed by oviposition in the container outside the cotton (34.46%) and under the cotton (14.31%).

Haplometrotics queens were the first to oviposit on the third day after the nuptial flight, and the oviposition of pleometrotic queens was recorded on the fourth day. Both interacted (antennating) significantly more with the eggs ($p < 0.0031$) than other activities. Antennating on the tube (5.74%) and carrying eggs in the jaw (4.12%) were more significantly ($p < 0.001$) performed by isolated queens, while walking in the container (3.75%) foraging in water (3.44%) were more significantly performed by group queens. The behavior of leaving eggs unattended was recorded only in haplometrosis (6.21%) while antennating with queens (6.53%) only in pleometrosis.

In LP, the most frequent behaviors ($p < 0.0017$) for haplometrosis and pleometrosis were antennating on larvae (32.28% and 31.15%), eggs (21.09% and 20.96%) and touching the larvae with the jaw (28.19% and 28.46%). When comparing queen behavior and foundation type, there was a statistical difference ($p < 0.001$) only for water foraging (5.02%) and carry offspring in the jaw (2.29%), in the two cases most performed by queens in haplometrosis.

In the PP, haplometrotic queens invested significantly ($p < 0.0021$) more time in interaction with larvae, touching with the jaw (29.16%) and antennating (21.6%), then with pupae (antennating 17.58% and touching with the jaw 10.41%) and with eggs (antennating 11.15%). Group queens also invested more time ($p < 0.0034$) with larvae (touching with the jaw 27.02% and antennating 20.12%), then invested in eggs (antennating 20.3%) and less on pupae (antennating 19.42% and touching with the jaw 7.1%), besides visiting more cotton with water and carrying more offspring.

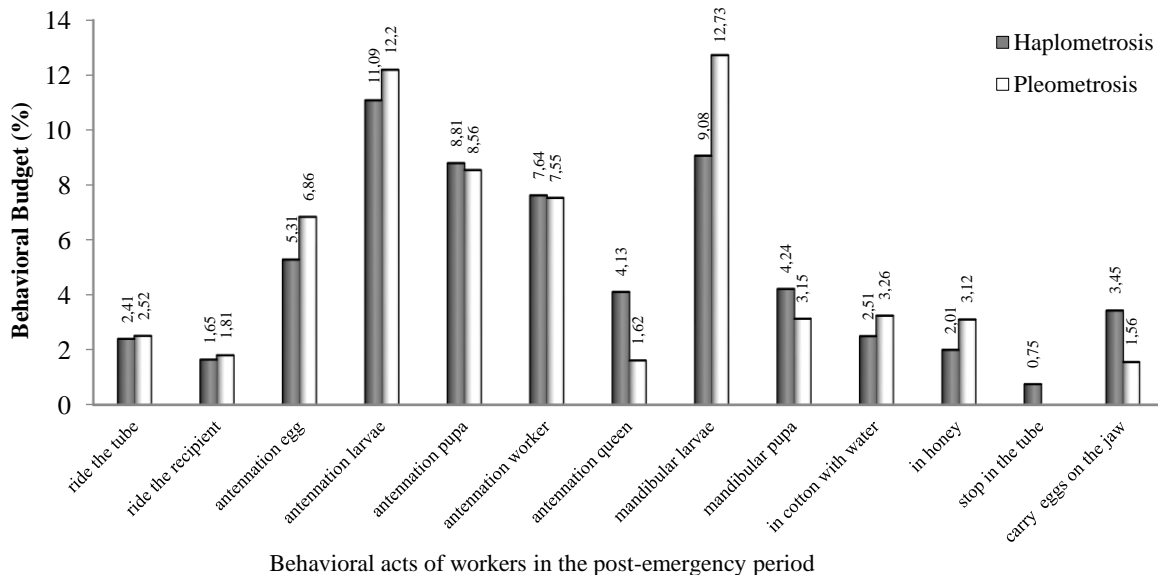
Isolated and group queens invested significantly ($p < 0.0092$) longer time in offspring antennation, respectively, 7.15% and 6.23% on eggs, 9.36% and 7.24% on larvae, 7.09% and 6.51% on pupae and 3.02% and 2.65% with workers.

Post-emergence was first achieved by haplometrotic colonies (after 106 days of the nupcial flight), while colonies containing two founding queens reached post-emergence in 110 days and colonies with five founders in 125 days.

Moving and antennating on the tube behaviors; antennating on the egg, larva, pupa and with the worker; touching pupa with the jaw and leaving offspring unattended were most often performed by queens in haplometrosis ($p < 0.001$). While antennating with another queen, touching larva with the jaw, foraging in cotton with water, standing still in the tube and carrying offspring in the jaw were mostly performed by pleometrotic queens.

In PEP, in both haplometrosis and pleometrosis, there was a reduction in the founders' behavior regarding the care with the offspring, and the first workers started to assume this function (Figure 2). For workers, antennation with co-specifics (offspring and adults) remained as the most performed activity ($p < 0.0038$), whereas those originated from the foundation in haplometrosis, antennate more, respectively, on pupae, other workers and queens, than on eggs and larvae, as recorded for pleometric workers.

Fig 2 Behavioral budget of workers of post-emergence fire ants *Solenopsis saevissima* (Smith, 1855), under experimental conditions.



* Columns with equal letters differ statistically at 5% significance by the Mann-Whitney test.

Workers from pleometrotic touched with the jaw significantly more larvae ($p < 0.0045$), while pupae ($p < 0.0090$) were more touched with the jaw by workers from colonies founded by a queen. Also, they visited honey (2.01% haplometrosis and 3.12% pleometrosis) and water-soaked cotton (2.51% haplometrosis and 3.26% pleometrosis), more than queens.

Generally, female workers (48.58 ± 29.55 in haplometrosis and 50.64 ± 40.82 in pleometrosis) invested significantly more time in colony activities than queens (27.63 ± 28.92 in haplometrosis and 29 ± 24.02 in pleometrosis) ($p < 0.004$).

4 DISCUSSION

In general, isolated or group queens exhibited similar behavioral repertoire in both performed acts and time spent, and the exploratory behaviors presented at the beginning of the foundation, were important as a way of ensuring an adequate nesting site choice, and after oviposition, behaviors related to offspring care were replaced by exploratory ones. Part of a foundation's success is due to the choice of a suitable nesting and egg-laying site, a good deal of time is expected to be spent exploring and interacting with the environment, and the container and tube antenation become essential to recognize the physical space.

Haplometrotic queens have spent more time exploring the environment, interacting and protecting their offspring, and foraging, because as they are alone they must perform all tasks in the early stages of the colony to ensure its success (Tschinkel 2003; 2013). Also, they stay longer under cotton, which may be associated with the choice of oviposition site, as half of them oviposited at this location. The physical contact with cotton combined with the darkening of the area promoted by the cotton may have attracted females in choosing this as the ideal point of protection and comfort, which would be closer to the underground chambers of a natural colony, as is common in the claustral phase (Tschinkel, 1998). Queens in pleometrosis probably because they felt safer, tended to oviposit in the Eppendorf tube, since together they could better watch and protect their offspring.

Queens haplometrotic were more dedicated to the care of their offspring and, consequently, their colonies reached first post-emergence, because, through this care, workers can be hastened to take on the tasks within the colony (Cassill and Tschinkel 1999a), as they actively act to protect immatures.

Interaction between individuals has been shown to be effective through direct physical contact, such as antenation, so queens in pleometrosis are often antenating, and according to Adam and Tschinkel (1995) and Tschinkel (1998) the most critical period of development of a colony is foundation, in the case of multiple queens antenation of co-specifics is an essential interaction for

establishing cooperation and harmony in the colony. Since queens were obtained from the same flight in the present study, this raises the degree of kinship and decreases aggressive interactions.

The oviposition occurred between the 3rd and 4th day after the flight which corroborates data obtained by Souza et al. (2004) for *S. saevissima*. For *S. invicta*, Tschinkel (1993) that reports those five days after the beginning of the foundation more than 40% of the eggs are in more advanced stages of development. The reduction in the behaviors of exploration and interaction with the environment, after oviposition and the increase in the behaviors of care with the offspring, ensures the success of egg development and encourages the queen to maintain this care (Tschinkel, 1993b).

Queens in pleometrosis antennated more on the eggs, as there was more than one queen who took turns performing this behavior. When the containers were manipulated, the queens that had oviposited in the container, both in haplometrosis and pleometrosis, carried the eggs in the jaw and moved them elsewhere as a way of finding a new more protected place. These behaviors show queens' attention regarding the vulnerability of eggs through antennation when they can detect irregularities.

Isolated queens left their offspring at times when they went out to forage or to monitor the environment, unlike in pleometrosis where eggs always remained protected by a queen while another went out to forage or explore.

Regardless of the type of foundation, queens have directed greater larval care effort, which reflects their successful development in the larval stage, considering their need to be nourished by adults (Glunn et al. 1981; Hölldobler and Wilson 1990; Cassill and Tschinkel 1995; 1999b). Besides, Tschinkel (1988b), when feeding *S. invicta* larvae with dyes, found that it was transferred by workers to queens and eggs and according to the author 4th instar larvae may be a digestive and metabolic variety that processes protein for eggs production by the queens.

Unlike what happens during the claustral phase in a natural environment, isolated queens have been observed foraging in honey. It is believed that although they can survive this period using their reserves, the availability of carbohydrates has caused them to seek the resource, probably to compensate for energy expenditure at the time of foundation. And the absence of foraging reinforces the considerations that queens have a nutritional reserve to ensure their survival and the maintenance of the colony in the claustral phase (Hölldobler and Wilson 1990; Cassill and Tschinkel 1995). A study by Tschinkel (1993) shows that *S. invicta* queens in haplometrosis lost 54% of their weight, 73% of fat and 67% of energy and fat percentage decreased from 44% to 33% concerning the amount of progeny produced. This is in agreement with Keller and Passera (1989) who claim that isolated queens need a higher nutrient reserve, and therefore maintain a higher level of fat than grouped queens, and this fat is

consumed between nuptial flight and the emergence of early adults. In the present study, water intake was recorded in all types of foundation and in all periods, occurring immediately after the cotton was moistened. Moisture probably plays a crucial role in maintaining homeostasis in the colony and is therefore much sought after by queens.

Queens who founded in groups seem to invest their reproductive success in quantity, as they oviposit more and form a unique "mass" of eggs, as observed in the present work, and thus all participate in the care of the offspring, to ensure the development of the immature.

In the post-emergence, the workers took over the foraging tasks, while the time of queens in these activities reduced, remaining busy with oviposition. In both haplometrosis and pleometrosis, workers are more frequent with larvae and queens, as they require more specific care with food. It was also observed the foraging of workers in honey and cotton soaked in water, which indicates a need to obtain environmental resources, both to maintain their vital activities and to nourish the larvae. Thus, the workers gradually replace the functions that hitherto had been performed by the queens, whether they are in the care of the offspring, colony inspection or foraging.

Preference for nesting in pre-existing underground chambers as reported by Souza et al. (2004) for the same species can be a relevant factor in choosing the foundation strategy. Thus, in an urban environment, where nesting sites are less available, starting a colony in association with other queens is more advantageous than in isolation, and this is reflected in the success of this species in occupying the urban environment, as noted by Zeringóta et al. (2014) in the same place of this study.

It is important to highlight that many issues that involve the success of fire ants in urban environments need to be studied, including the behavioral aspects that make these ants a kind of interest in forensic studies (Mendonça et al. 2020).

5 CONCLUSION

The acts and behavioral frequencies of *S. saevissima* queens change according to the period of the colony cycle, but not concerning the type of foundation. At the beginning of the foundation, the behaviors are directed towards exploration and interaction with the environment and after the emergence of the first immatures, the interaction with the offspring becomes more intense. With the emergence of the workers, they take care of the offspring and maintenance of the colony. Thus, the period of the developmental cycle is a determining factor in the behavioral manifestation of queens, either in groups or in isolation.

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