

Evaluation of food selection of fire ant *Solenopsis saevissima* (Smith) (Hymenoptera: Formicidae) in resources with and without immature stages of *Chrysomya albiceps* (Wiedemann, 1819) (Diptera: Calliphoridae)

Avaliação da seleção alimentar da formiga *Solenopsis saevissima* (Smith) (Hymenoptera: Formicidae) em recursos com e sem estágios imaturos de *Chrysomya albiceps* (Wiedemann, 1819) (Diptera: Calliphoridae)

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ABSTRACT

Ants of the genus *Solenopsis* Westwood, 1840 play a relevant role in forensic entomology studies due to the changes they can cause in cadaveric fauna. This study aims to evaluate the food selection behavior of *Solenopsis saevissima* (Smith, 1855) (Hymenoptera: Formicidae) ants against the offered combinations of decaying meat with *Chrysomya albiceps* (Wiedemann, 1819) eggs x decaying meat without eggs and decaying meat with *C. albiceps* larvae x decaying meat without larvae. Ant flow did not vary significantly when different diets were compared ($p > 0.05$). However, the correlation between ant flow and temperature showed a higher number of ant activity during periods of higher temperature ($p < 0.05$), reinforcing the relationship of maintenance of metabolism of these individuals and environmental temperature. There was no food selection by the fire ants in the tests performed ($p > 0.05$), but we could observe a positive correlation between flow and temperature ($p < 0.05$). Besides the lack of selection observed in this study corroborates the omnivorous role of species of the genus *Solenopsis*.

Keywords: Ant flow, Blowfly, Decaying meat, Forensic Entomology, Temperature.

RESUMO

Formigas do gênero *Solenopsis* Westwood, 1840 desempenham um papel relevante nos estudos de entomologia forense devido às alterações que podem causar na fauna cadavérica. Este estudo tem como objetivo avaliar o comportamento de seleção alimentar de formigas *Solenopsis saevissima* (Smith, 1855) (Hymenoptera: Formicidae) perante as combinações oferecidas de carne em decomposição com ovos de *Chrysomya albiceps* (Wiedemann, 1819) x carne em decomposição sem ovos e carne em decomposição com larvas de *C. albiceps* x carne em decomposição sem larvas. O fluxo de formigas não variou significativamente quando diferentes dietas foram comparadas ($p > 0,05$). Porém, a correlação entre o fluxo das formigas e a temperatura mostrou um maior número de atividade das formigas nos períodos de maior temperatura ($p < 0,05$), reforçando a relação de manutenção do metabolismo desses indivíduos e a temperatura ambiente. Não houve seleção de alimentos pelas formigas lava-pés nos testes realizados ($p > 0,05$), mas pudemos observar uma correlação positiva entre fluxo e temperatura ($p < 0,05$). Além disso, a falta de seleção observada neste estudo, corrobora o papel onívoro de espécies do gênero *Solenopsis*.

Palavras-chave: Carne em decomposição, Entomologia Forense, Fluxo de formigas, Mosca varejeira, Temperatura.

1 INTRODUCTION

The study of carcass-associated insects is of decisive importance in elucidating the nutrient cycle of a given ecosystem and the biological diversity related to the processes involved. When an organism dies, the process of its deterioration begins. This event is facilitated by the action of decomposing individuals that allow the organic matter present there to be incorporated into the substrate (GOMES & VON ZUBEN, 2006; OLIVEIRA-COSTA & URURAHY-RODRIGUES, 2013).

In Brazil, research in forensic entomology has been carried out for over 100 years. However, studies that deal with the ecology of necrophagous insects, especially ants, since they are complex, expensive, and have some delay in obtaining results, are still incipient (PUJOL-LUZ et al., 2008; CELINO, 2014).

Currently, the interest of the scientific community for the order Diptera (which contains blowflies), has been aroused by studies of Forensic Entomology. This order is present in all geographic regions of the world and has about 153,000 described species and approximately 160 families (THOMPSON, 2008; CARVALHO et al., 2012). Diptera eggs, larvae, pupae, and adults may provide important data for the calculation of the postmortem interval (PMI) estimate, as well as possible movement of the corpus, mode or cause of the disease that lead to death, location and identification of suspects and victims, among others (THYSSEN & GRELLA, 2011).

The great diversity of blowflies acting on carcasses occurs due to factors such as their presence in several biomes with their idiosyncrasies within the Brazilian territory, besides the

different climates between the regions and the size of the territory (CARVALHO et al., 2000; ROSA et al., 2009; BARBOSA et al., 2010; BIAVATI et al., 2010; DOS ANJOS et al., 2010; KOSMANN et al., 2011; ROSA et al., 2011; BARROS-SOUZA et al., 2012; BEUTER et al., 2012; FARIA et al., 2013; ALVES et al., 2014; VASCONCELOS et al., 2014) .

The Calliphoridae family makes it possible to estimate the postmortem interval of human cadavers by means of their larvae and therefore has great relevance for forensic studies. Within Calliphoridae, the species *Chrysomya albiceps* (Wiedemann, 1819) stands out due to its specialization in laying eggs only on organic material of animal origin (ESTRADA et al., 2009).

Together with the Diptera Linnaeus, 1758 and Coleoptera, the Hymenoptera order may play a leading role in forensic entomology studies (OLIVEIRA-COSTA & URURAHY-RODRIGUES, 2013; MENDONÇA et al., 2019). Despite being little studied this group is still an object of interest in several researches related to the cadaveric decomposition processes, both in Brazil (CARVALHO et al., 2000; MORETTI & RIBEIRO, 2006; MORETTI et al., 2008; FONSECA et al., 2015; MACIEL et al., 2015) as

in other countries (EARLY & GOFF, 1986; VELÁSQUEZ, 2008; BONACCI et al., 2011; PRADO E CASTRO et al., 2014).

The family Formicidae is of interest in the forensic context since its individuals relate to the cadaveric ecosystem through significant ecological interactions, such as their eating habits, ranging from predation to necrophagy. Through these relationships, these individuals can alter the decomposition process, increasing or decreasing the time of occurrence of this process due to its influence on the postmortem interval (PMI) estimation (CELINO, 2014).

Fire ants, as they are popularly known, belong to the genus *Solenopsis* Westwood, 1840 that contains about 195 species described that are distributed throughout the Americas (TRAGER, 1991; FISHER & BOLTON, 2016). Individuals of this group are present essentially in anthropized environments and this proximity to man ends up generating disturbance of the colonies and, consequently, sting accidents in several places of the urban environment (BUENO et al., 2017).

Solenopsis has an omnivorous and opportunistic eating habit and can prey on vertebrates and invertebrates (VINSON, 1986; VINSON & GREENBERG, 1986). They are also found in carcasses and, due to their predatory behavior, reduce the number of eggs and larvae of other species that occupy carcasses, directly interfering in the decomposition process (STOKER et al., 1995; MACIEL et al., 2015).

The species *Solenopsis saevissima* (Smith, 1855) plays a relevant role in public health due to the injury caused by its sting that could result in localized burn of even severe allergic reaction

(DREES et al., 2013). These fire ants can also be vectors of pathogenic microorganisms, and introduce infectious agents through their stings (FUNASA, 2001; PINTO et al., 2007).

Although they are relevant in forensic entomology studies, the interference caused by fire ants in the processes that occur in a corpse is not well known, but there are already studies that find alterations in the cadaveric fauna caused by the presence of these ants (PEREIRA et al., 2017; MENDONÇA et al., 2019).

Investigating *Solenopsis* role in decomposition may help to clarify practical issues related to forensic entomology and criminalistic, expanding the information on the participation of these ants in decomposition processes. Thus, this study aims to evaluate the food selection of fire ant *S. saevissima*, given different resources with and without scavengers, verifying if this selection varies with the type of resource, local temperature, and humidity variations.

2 MATERIAL AND METHODS

2.1 PLACE AND PERIOD OF STUDY

The study was conducted at the Laboratory of Behavioral Ecology and Bioacoustics (LABEC), located at the Advanced Laboratory of Zoology (LAZ) of the Universidade Federal de Juiz de Fora. The city of Juiz de Fora has a warm subtropical climate (Cwa), with a rainy season (October to April) and a dry season (May to September), according to the Köppen classification (DE SÁ JÚNIOR et al., 2012).

2.2 MAINTENANCE OF COLONIES IN THE LABORATORY

Six colonies of fire ants *S. saevissima*, obtained in the city of Juiz de Fora, were collected and kept in circular 30 cm diameter plastic containers with damp cotton. The colonies were established by the method proposed by Bueno (2017) as follows:

The entire external mound was collected with a shovel and the same amount was excavated in depth. The collected contents were transferred to a 5L Teflon-lined plastic bucket (Fig 1a.) to prevent ants from escaping. In the laboratory, water was slowly dripped into a drum (20 to 40 drops per minute) (Fig 1b.). As the water filled the container and flooded the earth, the ants separated from the earth carrying the brood and forming a heap on the surface (Fig 1c. and Fig 2 b.). A wire was placed so that the ants could move from the flooded environment to the new wetland container that made it possible to them to build the new colony. After the new colony was established, it was placed on a shelf covered with cellophane to prevent light from entering, since they are underground ants (Fig 2a.).

Fig 1 - a. Material collected in the field and placed in a blue plastic drum. b: Adaptation of a drip separation system. c: Fire ants (*Solenopsis saevissima*) colony separated from land floating on the water surface.

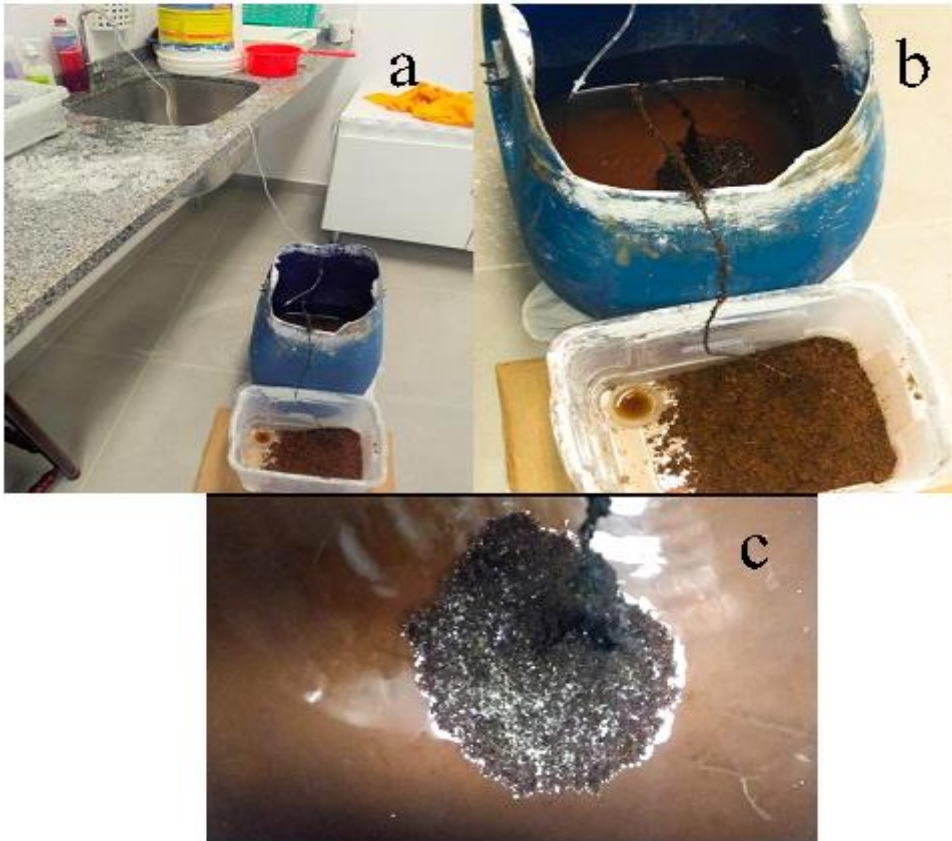
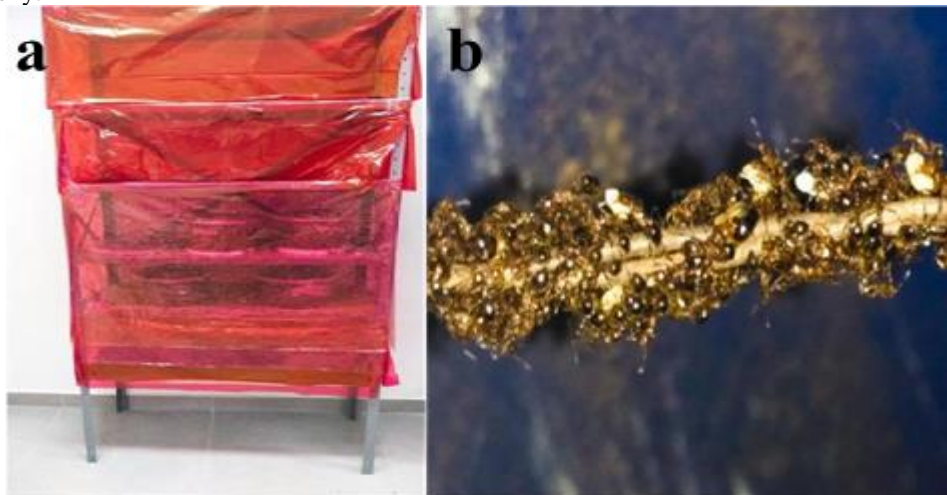


Fig 2. – a. Place of conditioning of the colonies. b. *Solenopsis saevissima* ants carrying their offspring from flooded site to the new colony.



Study of food selection: Diptera eggs and larvae: Criteria for choosing Chrysomya albiceps

The choice of *C. albiceps* is due to its great forensic importance in Brazil and because it fits the criteria proposed by Silva et al. (2017) for model insect use in forensic entomology studies:

- The species must be associated with forensic cases and decomposing bodies;
- It must have wide geographical distribution, be common and abundant;
- Its brood and maintenance in the laboratory should be simple.

Origin, breeding, and maintenance of C. albiceps colonies

Adult dipterans were collected using traps described by Mello et al. (2007). These traps contained inside chicken liver (200 g) maintained, before use, for 72 h in a climate chamber ($27 \pm 2^\circ \text{C}$ and $80 \pm 10\%$ relative humidity [RH]) so that it could start the process of decomposition and become more attractive. The traps were set on the campus of the Universidade Federal de Juiz de Fora (UFJF) ($S21^\circ 46.452'$; $O043^\circ 22.099'$) and inspected daily for adult collection. They were later transported to the UFJF Parasite Arthropod Laboratory where they were identified using dichotomous keys (KOSMANN et al., 2013; GRELLA et al., 2015) with a stereoscopic microscope. After identification, the adults were transferred to plastic entomological cages (35 x 35 x 35 cm) coated with a sheer mesh on the sides. In one of them, there was a plastic extension that served as the opening (15 x 9.4 cm) to facilitate handling inside the cages that were kept in a climate chamber ($27 \pm 2^\circ \text{C}$, $80 \pm 10\%$ RH and 12 h of photophase).

Adult flies were supplied with water and food consisting of a mixture of crystal sugar and water (1: 2 v / v). At the beginning of colony establishment and for three consecutive days animal protein (milk powder and water, 1: 2 v / v) was provided only once to stimulate oogenesis. Water, food, and protein were placed in individual Petri dishes (6 x 1.5 cm) filled with cotton.

For oviposition, polypropylene cylindrical pots (7.5 x 5 cm) containing laying medium (200 g of pork liver) previously decomposed for 72 h in a climate chamber ($27 \pm 2^\circ \text{C}$ and $80 \pm 10\%$ RH) was used. Food and water from adult flies were provided *ad libitum* and changed three times a week to avoid contamination; The decomposed mixture used as oviposition medium was offered according to the need to obtain the immature stages. After oviposition, the pots were placed in larger plastic containers (15 x 9.4 cm) with their interior lined with sawdust so that the mature larvae (third instar) left the rearing medium and started the pupation process (Fig 3 a, b and c); These pots were closed with a lid adapted with voil fabric and then placed in a climate chamber ($27 \pm 2^\circ \text{C}$ and $80 \pm 10\%$ RH).

After the larval development time, the sawdust was sieved and the mature larvae and pupae were separated to maintain the colonies.

The other *C. albiceps* generations were reared following the same methodology and the eggs (F1) and larvae (F2) from them were used in the *in vitro* experiments.

Experiment Setup

The immature stages used in the selection experiment collected from *C. albiceps* cages identified and provided by the Universidade Federal de Juiz de Fora (UFJF) Laboratory of Arthropod Parasites contained only F1 and F2 generations of flies were used to avoid possible interference caused by crossbreeding in the execution of the experiments. The egg mass was collected, placed in a petri dish containing deionized water and 10 eggs were selected in magnifying glass with a brush and placed in one of the 20g pots with meat located at the end of the Y-tube. Another part of the egg mass was maintained in pots with decaying meat for de development of the larvae stages. After the appearance of the larvae, 10 of them were collected and also placed in the pots with decaying pork meat located at the end of the Y-tube. For these experiments were used L1, L2 and L3 instars (the value of 10g per larva was doubled so that the food was not a limiting factor as the larvae may exhibit cannibal behavior)

The Y- tube was placed in contact with the fire ant colony and at its two ends, the treatments were offered containing 20g of meat each (Fig 4.). Observations were made for 30 min and used the counter to count the ants as they passed to either side of the tube for three periods of day 9 am, 1pm and 5pm. In each of the 6 colonies, 4 repetitions were performed for each treatment. A total of 6 colonies were used, 3 for the experiments of the Eggs / Without Eggs combination and 3 for the Larvae / Without Larvae. In each colony 12 repetitions of each treatment were performed, totaling 72 repetitions.

Fig 3. – a. Meat with egg mass from *Chrysomya albiceps* after being removed from the cage. b. *C. albiceps* egg mass in a petri dish with deionized water for selection of the 20 eggs. c. Egg mass under a stereomicroscope for separation.



Food Selection Experiment with Egg and Larva

For this study four treatments were used:

1. Decaying pork meat with egg (E)
2. Decaying pork meat without eggs (W/E)
3. Decaying pork meat with larvae (L)
4. Decaying pork meat without larvae (W/L)

As in the previous experiment, the tube Y-tube was positioned close to the colony and at each end, one of the following treatment combinations was placed: E x W/E and L x W/L to verify the ant food selection.

Fig 4 – Set up a scheme for the food selection experiment where a treatment option was placed in each pot at the end of the Y-tube.



Local Temperature and Monitoring

Prior to the start of each experiment, local temperature and humidity were monitored by thermohygrometer measurement for subsequent correlations with the results.

Data analysis

Data were abnormally distributed ($p < 0.05$) according to the Shapiro-Wilk test and the Mann-Whitney U test was used to analyze the data collected in the food selection study tests. The program used in the analysis was the freeware Past3, version 1.0. For ants flow analysis at different temperatures, the Pearson Correlation was performed using the freeware program BioStat 5.0 (Ayres et al., 2017).

3 RESULTS AND DISCUSSION

In the experiments, the ants spent the time mostly antennating the Y tube and the meat. After the pioneers exploited the resource, they returned to the colony and began mass-gathering of workers. In general, the workers not only eat the meat but also interacted with the eggs and the fly larvae when they were present. Fire ants have been observed foraging and even preying on immature

flies, reaffirming their behavioral flexibility and showing *S. saevissima* foraging as omnivorous and a predator.

In practice, there are several reports that demonstrate that *Solenopsis* try to monopolize the resource by covering the carcass. What appears to be linked to the size of the resource but has not been observed in our study (MACIEL et al., 2015; MENDONÇA et al., 2019).

In the present study there was no significant difference in ant flow for the combination of treatments with eggs and without eggs treatments ($U = 623.5$ $P = 0.786$). The result of ant flow at different times of the day was higher in the morning and lower in the afternoon as shown in table 1.

Table 1 - Results of the mean flow of fire ants (*Solenopsis saevissima*) in the Y tube connected to the fire ant colony to different meat treatments at the three different times of the day, being E- Meat with eggs; W /E - Meat without eggs.

Treatment	Flow at 9am	Flow at 1pm	Flow at 5pm
E	106.41 ± 137.88 (0- 474)a	45.75± 76.15 (0-265)a	51.58 ± 112.14 (0-400)a
W/E	105.83 ± 128.05 (0-421)a	51.41 ± 80.95 (0-288)a	68.33 ± 135.99 (0-477)a

*Values followed by equal letters in columns show no significant difference ($p > 0.05$).

Ant flow for meat with larvae X meat without larvae treatments also did not vary ($U = 577.5$ $p = 0.428$), and ant flow slightly varied at different times of the day, with average variation represented in Table 2.

Table 2 - Results of the average flow of fire ants (*Solenopsis saevissima*) in the Y tube connected to the fire ant colony to meat with larvae and meat without larvae treatments at the three different times of the day tested, being L- meat with Larvae; W / L - Meat without larvae.

Treatment	Flow at 9am	Flow at 1pm	Flow at 5p.m
L	33.75±57.09 (0-183)a	41.16 ± 62.53 (0-198)a	34.66 ± 73.11 (3-263)a
W/L	36± 54.56 (0-178)a	32.25 ± 34.68 (0-95)a	19.25 ± 15.37 (0-42)a

*Values followed by equal letters in columns show no significant difference ($p > 0.05$).

The lack of selection presented in the results reflects the already known and reported omnivorous habit for these ants (VINSON & GREENBERG, 1986). As in Glunn et al. (1981) in a food preference experiment with *S. invicta* Buren (1972) colonies for three types of liquid foods (sugar, rat serum, and oil), they reinforced the finding that the most striking feature of food preference in the tests performed was the heterogeneity of preference patterns.

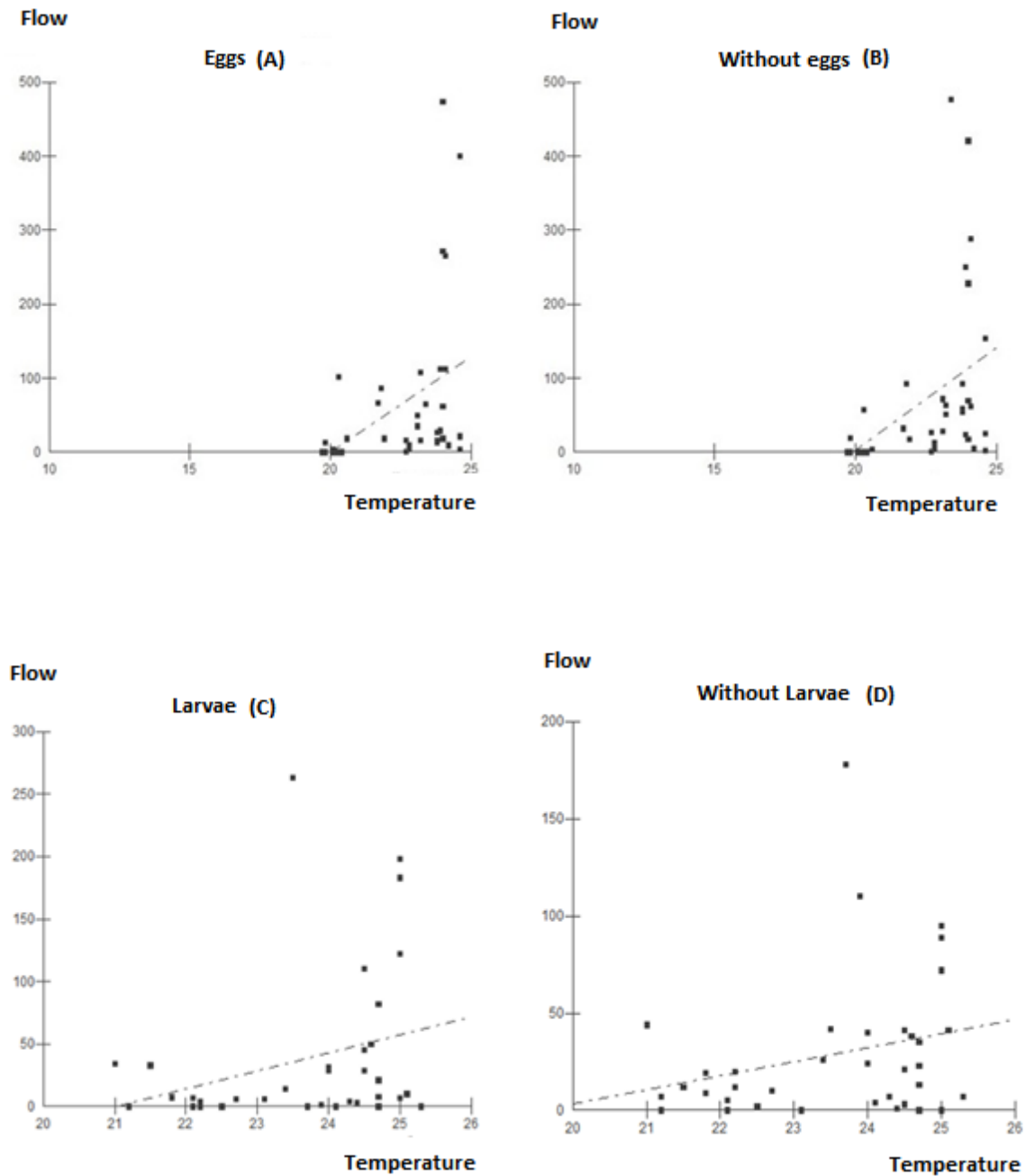
The humidity at the site varied around $63.09\% \pm 9.42$ (47-78) in all experiments but, even with that variation this factor did not seem to interfere in the behavior of the fire ants as was also stated in Potts *et al*, 1984 were, in an experiment testing fire ants humidity preferences, found them to appeared indifferent or unable to detect changes in the relative humidity. It is also known that those ants employ several techniques, to regulate humidity within their nests (HÖLLDOBLER & WILSON, 1990), and that capability is a relevant part of its capacity to adapt in extreme environments.

In the Experiments With Eggs X Without Eggs, the local morning shift temperature averaged $22.4\text{ }^{\circ}\text{C} \pm 1.53$ (19.8-24). Already in the afternoon, the temperature was about $22.75^{\circ}\text{C} \pm 1.65$ (19.8-24.2) at the beginning and $22.75^{\circ}\text{C} \pm 1.65$ (19.8-24.2) at the end of the afternoon.

In experiments with larvae X without larvae, the local temperature averaged 23.22 ± 1.56 (21-25.3) in the morning, early in the afternoon $23.79\text{ }^{\circ}\text{C} \pm 1.32$ (21.5-25) and in the late afternoon, the average temperature at the location was $23.59^{\circ}\text{C} \pm 1.15$ (21.8-25.1).

The correlation between flow and temperature differed significantly for egg experiments ($r_s = 0.053$; $p = 0.0009$), without eggs ($r_s = 0.55504$; $p = 0.0005$) and with larvae experiments ($r_s = 0.3323$; $p = 0.0476$). For the experiment without larvae there was no significant difference ($r_s = 0.2376$; $p = 0.1628$) (Fig 5.).

Fig 5. - Linear scatterplot showing the correlation between ants (*Solenopsis saevissima*) flow in the Y tube connected to the fire ant colony and local temperature during the selection experiment with egg (A), eggless (B), with larvae (C) and without larvae (D) treatments.



Regarding temperature-related analyzes, our experiment found that there is a correlation between ant flow and temperature.

The fact that ant flow, in general, was higher during higher temperatures may be explained by the fact that as most colony growth occurs during the warmer times of the year (MARKIN et al., 1973), whereas, during this period, the collected protein feeds are given preferably to the developing brood. Also, higher temperatures stimulate foraging, as can also be seen in many other insect groups,

such as social wasps, for example (SORENSEN et al., 1981; VINSON & GREENBERG, 1986; LIMA & PREZOTO, 2003).

Cokendolpher and Francke (1985), when comparing the temperature preferences of 4 species of the fire ant *Solenopsis aurea* (Wheeler, 1906), *Solenopsis geminata* (Fabricius, 1804), *Solenopsis invicta* (Buren, 1972) and *Solenopsis xyloni* (McCook, 1880), observed their ability to react quickly to temperature gradients.

Stein et al. (1990) in a study to determine the preference for high carbohydrate or protein bait foraging fire ants and whether these preferences change during the year found that at lower temperatures (average 17°C) *Solenopsis* were more recruited for carbohydrate bait, while at higher temperatures (average 25°C) were found higher numbers of ants in protein bait, showing the influence of temperature in food selection processes of the studied species. This may explain the lack of clear preference in this study, as the temperature variation in the experiments was small.

In an experiment by Hooper and Rust (1997) with *Solenopsis xyloni* (McCook, 1879), using combinations of lyophilized and roasted foods, they found that at temperatures around 32°C lyophilized baits were more attractive than roasts, also finding changes in selection in different temperatures.

Porter and Tschinkel (1985), in a study of thermal fire ants preferences in situations of food abundance and limitation, found that the metabolic growth curve of *Solenopsis invicta* without food deprivation was strongly sloping towards warmer temperatures, with maximum growth occurring near 32°C. In food deprivation, the growth curve was inclined to lower temperatures, with the maximum size occurring around 25°C. Food-limited colonies increase at lower temperatures due to reduced metabolic costs for workers. A strategy that increases the longevity of workers directly associated with brood care.

The absence of food selection of fire ants by the different resources offered, presents itself as a relevant result of this study. This result is the key to understanding the participation of fire ants in the decomposition process, since their flexibility allows them to adapt to any situation, taking the best advantage of the resource and being very successful in their foraging activities in the decomposing resource. In addition, this result also shows the importance of a generalist and opportunistic predator / decomposer in the decomposition process, and reflects the success of these fire ants in occupying altered environments.

4 CONCLUSION

Our results show that ants did not select from the different resources offered. However, ant flow changes significantly as the temperature rises or falls. This paper demonstrates once again the behavioral flexibility of *S. saevissima* ants and their adaptation to environmental factors, which favors their establishment and distribution around the world and makes them an increasingly necessary and relevant object of study.

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