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Raquel Mendonça Daniel

Fire ants: where do they live and what do they eat?

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Dissertação apresentada ao Programa de Pós-Graduação em Ciências Biológicas: Comportamento e Biologia Animal, área de concentração em Comportamento Animal, da Faculdade de Ciências Biológicas da Universidade Federal de Juiz de Fora como requisito parcial para obtenção do título de Mestre em Ciências Biológicas.

Prof. Dr. Fábio Prezoto - Orientador

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“A Ciência não é só compatível com a espiritualidade; é uma profunda fonte de espiritualidade.”

Carl Sagan.

RESUMO

As formigas são insetos sociais que constroem ninhos sofisticados que funcionam de maneira complexa e podem ser constituídos por até centenas de câmaras internas utilizadas para o cuidado com a prole. A espécie *Solenopsis saevissima* (Smith, 1855) (Hymenoptera: Formicidae) desempenha um papel relevante na saúde pública, por conta de reações à sua ferroada e sua ação como vetores de agentes infecciosos pela ferroada. No presente estudo, objetivamos uma revisão recente global de todos os trabalhos que contém registros dos sítios de nidificação de *Solenopsis* nos últimos anos. Além disso, essas formigas são relevantes na entomologia forense, pelas interferências que causam no processo de decomposição. Outro objetivo deste trabalho foi descrever uma avaliação do comportamento de seleção alimentar de *S. saevissima* frente a diferentes estímulos alimentares em decomposição e com presença de ovos e larvas de califorídeos da espécie *Chrysomya albiceps* (Wiedemann, 1819) visando desvendar avanços científicos, analisar as informações e apontar áreas incipientes de pesquisa em estudos nessa área, permitindo dessa forma avançar nos conhecimentos sobre o comportamento dos indivíduos desse gênero e suas particularidades. A revisão bibliográfica foi feita nas plataformas Web of Science e Scopus de todos os trabalhos publicados do ano de de 1945 até o mês de Janeiro de 2020. Recuperamos informações dos artigos encontrados e triados sobre: ano do estudo, local do registro, espécies encontradas e substrato utilizado para nidificação. Encontramos 43 artigos que contém registros dos sítios de nidificação de formigas do gênero *Solenopsis.* As publicações foram veiculadas em 24 periódicos científicos e no total, registramos 10 espécies nos estudos encontrados. As formigas preferiram nidificar nos ambientes naturais e as principais áreas de desenvolvimento dos estudos encontradas foram Estados Unidos, Brasil e Argentina respectivamente, além disso, a espécie mais relatada nos trabalhos foi *Solenopsis invicta* Buren (1972). Já no experimento de seleção, as observações foram feitas de Março de 2018 a Outubro de 2019. O trabalho analisou o possível comportamento de seleção de formigas lava-pés frente as combinações de carne em decomposição com ovos de *C. albiceps* x carne em decomposição sem ovos e carne em decomposição com larvas de *C. albiceps* larva x carne em decomposição sem larvas. Não houve seleção alimentar pelas formigas de fogo nos testes realizados ($p<0,05$), Mas pudemos observar uma correlação entre fluxo e temperatura. Nossos resultados reforçaram a relação entre manutenção metabólica e temperatura nesses indivíduos além de seu hábito onívoro.

Palavras- chave: *Solenopsis saevissima*, decomposição cadavérica, local de nidificação, temperatura.

ABSTRACT

Ants are social insects that build sophisticated nests that work in complex ways and can consist of up to hundreds of inner chambers used for offspring care. *Solenopsis saevissima* (Smith, 1855) (Hymenoptera: Formicidae) plays a relevant role in public health because of reactions to its sting and its action as vectors of infectious agents by stinging. In the present study, we aimed at a global recent review of all works that contain records of *Solenopsis* nesting sites in the last years. In addition, these ants are relevant in forensic entomology because of the interference they cause in the decomposition process and another aim of this study was to describe an evaluation of *S. saevissima* feeding selection behavior against different food stimuli: decomposing meat with and without eggs and larvae of the Califoridae species *Chrysomya albiceps* (Wiedemann, 1819) aiming to unveil scientific advances, analyze the information and point out incipient areas of research in studies in this area, thus allowing to advance the knowledge about the behavior of individuals of this genus and their particularities. The literature review was performed on the Web of Science and Scopus platforms of all works published since 1945 up to January 2020. We retrieved information from the articles found and sorted about: year of study, place of registration, species found and substrate used for nesting. We found 43 articles containing records of the nesting sites of *Solenopsis* ants. The articles were published in 24 scientific journals and in total we registered 10 species in the studies found. Ants preferred to nest in natural environments and the main areas of development of the studies found were the United States, Brazil and Argentina respectively, in addition, the species most reported in the works was *Solenopsis invicta* Buren (1972). In the selection experiment, observations were made from March 2018 to October 2019. The work analyzed the possible selection behavior of fire ants against decaying meat combinations with *C. albiceps* eggs x decaying meat without eggs and decaying meat with *C. albiceps* larvae x decaying meat without larvae. There was no food selection by the fire ants in the tests performed ($p < 0.05$), but we could observe a correlation between flow and temperature. Our results reinforced the relationship between metabolic maintenance and temperature in these individuals beyond their omnivorous habit.
Keywords: cadaveric decomposition, nesting site, *Solenopsis saevissima* temperature.

SUMÁRIO

1. WHERE DO THE FIRE ANTS LIVE: A RECENT REVIEW

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Abstract

Ants are social insects responsible for building sophisticated and complex-functioning nests, which can contain from one to hundreds of chambers for the breeding and development of their offspring. Fire ants, ants of the genus *Solenopsis*, contain about 300 species worldwide that are often found in urban environments where they are invasive. Their nests comprise mounds of loose earth with a system of interconnected chambers built in open areas with a high incidence of sunlight helping to regulate nest temperature and allowing colony growth. In the present study, we aimed at a global review of all the works that contain records of the *Solenopsis* nesting sites in recent years, aiming to unveil scientific advances, analyze their information and point out incipient areas of research in studies in this topic, thus allowing advances in knowledge about the behavior of individuals of this genus and their particularities. To obtain the desired records, we performed advanced searches on the Web of Science and Scopus platforms of all works published since 1945 until January of 2020. We retrieved information from articles found and screened about: year of study, place of registration, species found and substrate used. Based on the records found, we created a map of the geographic distribution of the publications and a table with the nesting sites and species found in the selected studies. We found 145 articles published in scientific journals. Of this total, we screened 43 which contain records of the nesting sites of *Solenopsis* ants. The articles were published in 24 scientific journals and total, we registered 10 species in the studies found. Ants preferred to nest in natural environments and the main areas of development of the studies found were the United States, Brazil and Argentina respectively, in addition, the species most reported in the works was *Solenopsis invicta* Buren (1972). This work unveils advances for science and presents an interesting scientific model to be explored, emphasizing an academic contribution with problems faced by the population and the ecological, economic and medical importance of the object of study.
Keywords: Nesting behavior, nest, *Solenopsis*.

1.1. INTRODUCTION

Social insect nests are considered highly sophisticated constructions among animals. Nests are made up of a self-organizing system, and large-scale processes are created through various local interactions among the individuals who live there, without the need for managers to organize and monitor the progress of work (FRANKS & DENEUBOURG, 1997; THERAULAZ et al., 1998). Ants, in the construction of their nests, which can contain from one to hundreds of chambers, collectively create groups of eggs, larvae, and pupae(WILSON, 1971; HÖLLDOBLER & WILSON, 1990; BRIAN, 2012). As the number of individuals within a colony increases, it becomes more complex and the number of new chambers created also increases(MARKIN et al., 1973; HÖLLDOBLER & WILSON, 1990).

The ants of the genus *Solenopsis* Westwood, 1840, popularly known as fire ants, contains about 300 species worldwide, which are often found in urban environments were they are invasive (DE ULLOA, 2003; DREES, 2006; TSCHINKEL, 2006; PACHECO & VASCONCELOS, 2007) and proliferate exponentially due to their adaptive capacity, resource exploitation and nesting in altered locations, gaining a pest status. Their nests comprise mounds of loose soil with a system of interconnected chambers built in open areas with a high incidence of sunlight. The colonies can reach in five to six years, sizes that shelter about 200.000 workers (TSCHINKEL, 1988). These mounds also have the function of capturing the sunlight helping in the regulation of the nest temperature and allowing the growth of the offspring(LOFGREN et al., 1975; VINSON, 1986; HÖLLDOBLER & WILSON, 1990; BUENO & CAMPOS-FARINHA, 1999; PENICK & TSCHINKEL, 2008).

Besides the biological and economic aspects, it is worth mentioning the ecological role of *Solenopsis* ants species in a general context. In the world scenario of massive extinction of species portrayed by the new UN report of 2019, quantifying the alarming number of one million endangered species according to the Intergovernmental Platform for Scientific Policy on Biodiversity and Ecosystem Services (IPBES), insects now take over as the protagonists, as the interactions between plants and animals and ants gain importance (CHRISTIANINI et al., 2014; MELLO, 2014).

In addition to their villainous role, the fire ants also act as secondary dispersers carrying seeds, which aids in the propagation and conservation of plant species such as *Alternanthera sessilis*, *Amaranthus spinosus*, *Amaranthus viridis* of the Amaranthaceae family; *Ageratum houstonianum*, *Artemisia capillaris* of the Asteraceae group; *Brassica campestris* belonging to the family Brassicaceae and the Cyperaceae *Cyperus compressu*s and *Kyllinga brevifolia*, besides being more resistant to human aggression to the environment which highlights the considerable need to study the habits and habitats of these individuals (SILVA et al., 2009; LAI et al., 2018).

Fire ants, because they nest intensively in anthropogenic environments and are near humans and their buildings, play a prominent role in public health, as their sting can cause injuries ranging from local burning sensation or even severe allergic reactions (DREES, 2006). Also, these ants can act as mechanical vectors of pathogens and microorganisms, and introduce infectious agents by the sting (FUNASA, 2001; PINTO et al., 2007). In the economic context, these individuals cause losses when they invade plantations and / or agricultural systems(GUSMAO et al., 2010; FERNANDES, 2016; BRASIL, 2019).

Ants of the genus *Solenopsis* are omnivorous and have mass recruitment of their workers against some source of resources. They preferentially feed on insects and other small vertebrates and are also found feeding on carcasses or eggs deposited by other scavengers in decaying meat (MACIEL et al., 2015; MACIEL et al., 2016; MENDONÇA et al., 2019). The main source of energy is obtained from sugary liquids in adults and proteins in the larval phase (WILLIAMS et al., 1980; PORTER & TSCHINKEL, 1985; CALABI & PORTER, 1989).

Colony growth is rapid, as queens can produce about 500,000 workers in the first year of life (TSCHINKEL & PORTER, 1988). Temperature is a determining factor in the viability and growth of colonies since the metabolic rates of fire ants depend on it (PORTER & TSCHINKEL, 1993). Also, the levels of air humidity, rainfall and temperature are directly proportional to the foundation and growth of colonies (PORTER & TSCHINKEL, 1993; DREES, 1994).

This study aims to perform a global review of the articles that recorded the nesting sites of *Solenopsis*, grouping these records, and unraveling advances for science in the studies in this area, advancing knowledge about the behavior of the individuals of that genus and its idiosyncrasies.

1.1 MATERIAL AND METHODS

For this study, we adapted the methodology of Castro *et al.* (2015) conducting a systematic review that allowed us to compile studies that contain records of *Solenopsis* ants nesting sites around the world. To obtain the desired records we performed advanced searches on the Web of Science and Scopus platforms. All articles published since 1945 until January

2020 with the following keywords were selected: Web of Science: TS = (nest * AND behav * AND place OR site AND area) AND TS = (*solenopsis* OR "fireant"); Scopus: TITLE-ABS-KEY (nest * AND behav * AND place OR site AND area AND *solenopsis* OR "fireant"). To organize the records we use the Endnote X9 tool.

We retrieved the following information from the articles found and sorted: year of study, place of registration, species found and substrate reported as a nesting site. For the substrate types, using the adapted methodology from Zeringota et al, 2014 we created the following categories: Natural Environments (NE), which include nests in the soil, grass, sawdust; Altered Environments (AE), consisting of highways, urban gardens, pavements and sand; and Agrosystems (A), which include pastures and eucalyptus plantations. The journals found were classified according to the Biodiversity Qualis (2013-2016) in extracts A (publications A1 and A2) and extracts B (publications B1 and B2). Based on the records found we created a map of the geographic distribution of the publications and a table containing the nesting sites and species found in the selected studies.

1.2 RESULTS AND DISCUSSION

We found 145 articles published in scientific journals, eight on the Scopus platform and the other 137 on the advanced Web of Science search. Of this total, 43 contained records of the nesting sites of *Solenopsis* ants. The first two articles that record *Solenopsis* nests date from 1992 and both are from the same author (Cox et al., 1992 a;b). The third article was published as early as the following year (Porter, 1993) with a two-year unpublished period until the publication of Macom and Porter (1996). From 1996, the publications became more regular, reaching their peak in 2003 and 2004 with six and five publications, respectively. Most articles (65%) were published in the last 13 years (from 2004 to 2017), showing the recent concern to study these individuals and better understand their lifestyle and how they relate to the environment and man (Table 1).

Table 1 - Table of the data found in the selected articles about the nesting sites of Fire Ants. NE - Natural Environments; AE - Altered Environments; A – Agrosystems

Author /Year	Publishing Journal	Study Location (Country / State)	Species Found	Nesting Site

Cox, G. W., et al. (1992a)	Revista Chilena De Historia Natural	Argentina	*S.richteri*	NE
Cox, G. W., et al. (1992b)	Environmental Entomology	Argentina	*S.richteri*	A
Porter, S. D. (1993)	Journal of Economic Entomology	United States	*S.invicta*	AE
Macom, T. E. and S. D. Porter (1996)	Annals of the Entomological Society of America	United States (Florida)	*S.invicta*	A
Shoemaker, D. D., et al. (1996)	Evolution	United States (Mississipi)	*S.invicta/S.richteri*	AE
Briano, J. A., et al. (1997)	Environmental Entomology	South America	*S.daguerrei*	AE
Calcaterra, L. A., et al. (1999)	Environmental Entomology	Argentina	*S.daguerrei*	NE
Morrison, L. W., et al. (1999)	Annals of the Entomological Society of America	United States (Texas)	*S.geminata*	NE
Russell, S. A., et al. (2001).	Environmental Entomology	United States (Texas)	*S.invicta*	AE
Briano, J. A. and D. F. Williams (2002).	Environmental Entomology	Argentina	*S.richteri* *S.invicta/S.macdonaghi*	AE
Adams, E. S. (2003)	Behavioral Ecology	United States (Florida)	*S.invicta*	NE

Cook, J. L. (2003).	Biodiversity and Conservation	United States (Texas)	*S.invicta*	NE
Hood, W. M., et al. (2003).	Journal of Agricultural and Urban Entomology	United States (Carolina do Sul)	*S.invicta*	NE
Morrison, L. W. and S. D. Porter (2003)	Environmental Entomology	United States (Florida)	*S.invicta*	NE
Stuntz, S., et al. (2003).	Basic and Applied Ecology	Panama	*S. zeteki*	NE
Graham, J. H., et al. (2004).	Journal of Insect Science	United States (Fort Benning)	*S.invicta*	AE
Morrison, L. W. (2004).	Annals of the Entomological Society of America	United States (Texas)	*S.invicta S.texana*	AE
Ness, J. H. (2004)	Oecologia	Europe (Georgia)	*S.invicta*	NE
Weeks Jr, R. D., et al. (2004)	Environmental Entomology	United States (Texas)	*S.invicta*	NE
Weeks, R. D., et al. (2004).	Annals of the Entomological Society of America	United States (Texas)	*S.invicta*	NE
Wetterer, J. K., et al. (2004	Sociobiology	Europe (Portugal)	*Solenopsis sp*	NE

Diehl, E., et al. (2005)	Revista Brasileira De Entomologia	Brazil (Rio grande do Sul)	*S.invicta sp*	NE
Aubuchon, M. D., et al. (2006)	Journal of Economic Entomology	United States (Alabama)	*S.invicta*	NE
MacGown, J. A. and R. L. Brown (2006)	Southeastern Naturalist	United States (Mississipi)	*S.invicta, S.richteri S. molesta*	NE
LeBrun, E. G., et al. (2007)	Ecology	Argentina	*S.invicta*	NE
Milks, M. L., et al. (2007)	Insectes Sociaux	United States (Louisiana)	*S.invicta*	NE
Graham, J. H., et al. (2008)	Southeastern Naturalist	United States (Fort Benning)	*S.invicta*	NE
Penick, C. A. and W. R. Tschinkel (2008)	Insectes Sociaux	United States (Florida)	*S.invicta*	NE
Vogt, J. T., et al. (2008)	Environmental Entomology	United States	*Solenopsis sp*	NE
Andersen, A. N. and A. Brault (2010)	Biodiversity and Conservation	Austrália (Darwin)	*Solenopsis sp*	NE
Braga, D. L., et al. (2010)	Neotropical Entomology	Brazil (Bahia)	*Solenopsis sp*	A
Tucker, J. W., et al. (2010)	Southeastern Naturalist	United States (Florida)	*S.invicta*	NE

Wilkie, K. T. R., et al. (2010)	Plos One	Amazonia (Ecuador)	*Solenopsis sp*	NE
Conover, R. R., et al. (2011)	Agriculture Ecosystems & Environment	United States (Mississipi)	*S.invicta*	NE
Wetterer, J. K. (2011)	Myrmecological News	Novo México	*S.geminata*	NE
McNaught, M. K., et al. (2014)	Journal of Economic Entomology	Austrália (Brisbane)	*S. invicta* *S. geminata*	A
Wetterer, J. K., et al. (2014)	Florida Entomologist	Trinidad	*S.invicta*	NE
Zeringota, V., et al. (2014)	Florida Entomologist	Brazil (Minas Gerais)	*S.saevissima*	A
Costa-Milanez, C. B., et al. (2015)	Sociobiology	Brazil (Minas Gerais)	*S.invicta*	NE
Dejean, A., et al. (2015)	Biological Conservation	French Guiana	*S.saevissima*	NE
Stuhler, J. D. and J. L. Orrock (2016)	Biological Invasions	United States (South Carolina)	*S. invicta S.geminata*	NE
de Souza-Campana, D. R., et al. (2017)	Tropical Conservation Science	Brazil (São Paulo)	*Solenopsis sp.2*	NE
Silva, E. F., et al. (2017)	Sociobiology	Brazil (Maranhão)	*S. substituta*	NE

The publications analyzed reported 53 records of *Solenopsis* ant nests of 10 species nesting in various locations. Based on the distribution by country of origin of the studies, we observed that the largest number of publications by country was registered in the United States (46%), followed by Brazil (14%) and Argentina (12%). This can be explained by the fact that this ant is exotic in the region of the Arctic and causes several inconveniences to the public health of these places as accidents with stings (BUENO & CAMPOS-FARINHA, 1999; FUNASA, 2001; CAMPOS-FARINHA et al., 2002), infestations (TRAGER, J. C., 1991; CALLCOTT & COLLINS, 1996; PHILLIPS et al., 1996), and impacts on local agriculture and fauna(THORVILSON et al., 1992 ; GOTELLI & ARNETT, 2000; TABER, 2000; WOJCIK et al., 2001; LARD et al., 2002; ALLEN et al., 2004; MARTIN et al., 2011; LENOIR et al., 2016) (Table 1).

The articles were published in 24 scientific journals, being Environmental Entomology (n = 8), Annals of the Entomological Society of America (n = 4) Journal of Economic Entomology (n = 3), Sociobiology (n = 3) and Southeastern Naturalist (n = 3), the most frequent ones. The journals found in general have different natures, having several focuses, as in the studies of entomology and insect interaction with biological, chemical and physical factors (n = 2); research in natural history, biology, ecology and evolution of organisms (n = 10); empirical and theoretical biology (n = 1); conservation (n = 1); insect and other arthropod studies (n = 5); relationship between agrosystems and natural environment (n = 1); myrmecology and biological invasion (n = 2) (Table 1).

The number of publications in journals classified as extract A (47%) also indicates the relevance of research in this area and the need to intensify studies of this genus in places where species are native (Fig. 1).

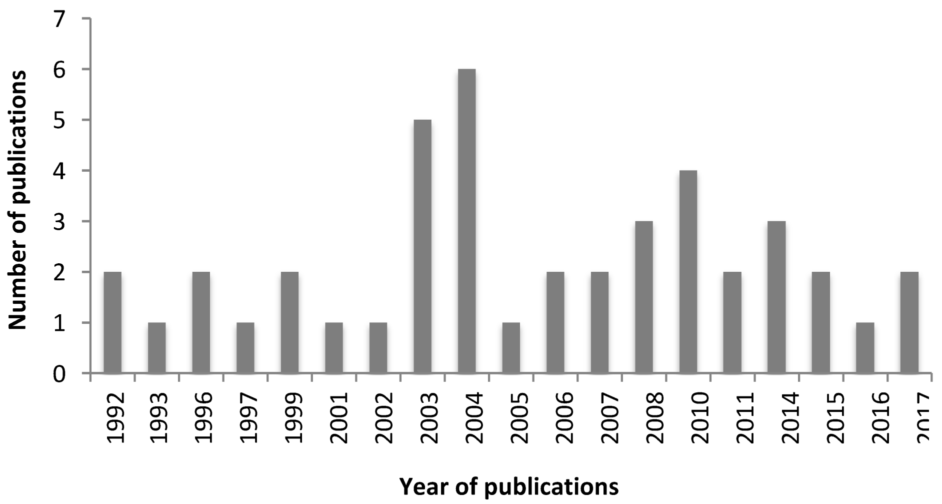


Figure 1 - Number of publications with *Solenopsis* nesting site records over the years.

In total, 10 species were recorded in the studies. *Solenopsis invicta* Buren, 1972 (52%) was the species with the highest number of nest records in studies around the world, followed by *S. richteri* Forel, 1909 (9%) and *S. geminata* Fabricius, 1804 (7%). This difference corroborates the fact that *S. invicta* is the dominant invasive species in the United States, which seeks effective ways to control it by investing heavily in research in the area. Another relevant point is the number of species studied compared with the number of species described. Currently, the genus *Solenopsis* contains about 300 ant species, which means that the number of species recorded in studies worldwide in recent years is only about 5% of all existing species, showing the need for more comprehensive studies (TSCHINKEL, 2006) (Fig. 2).

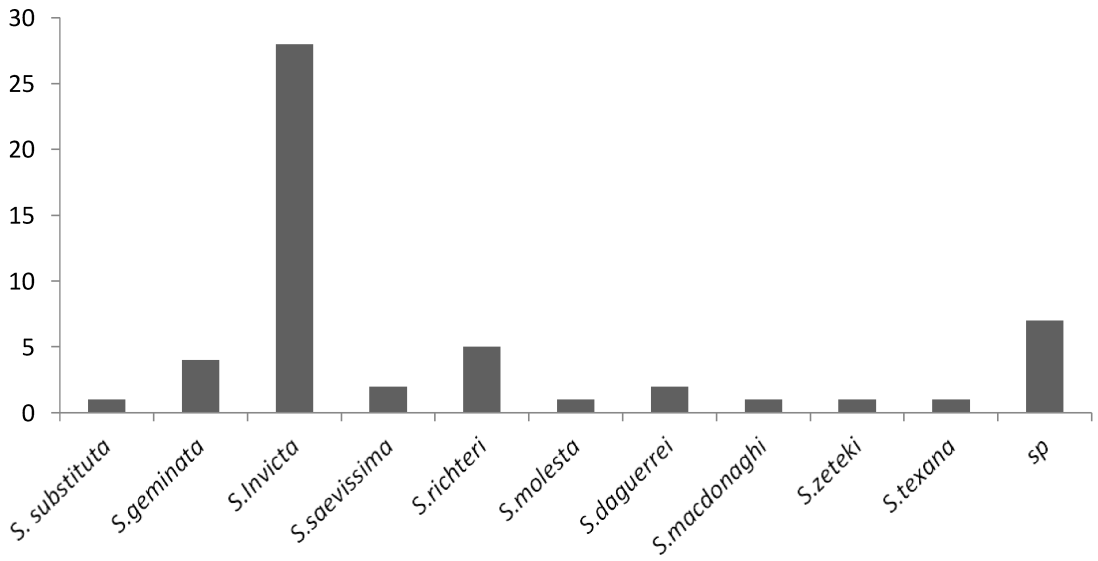
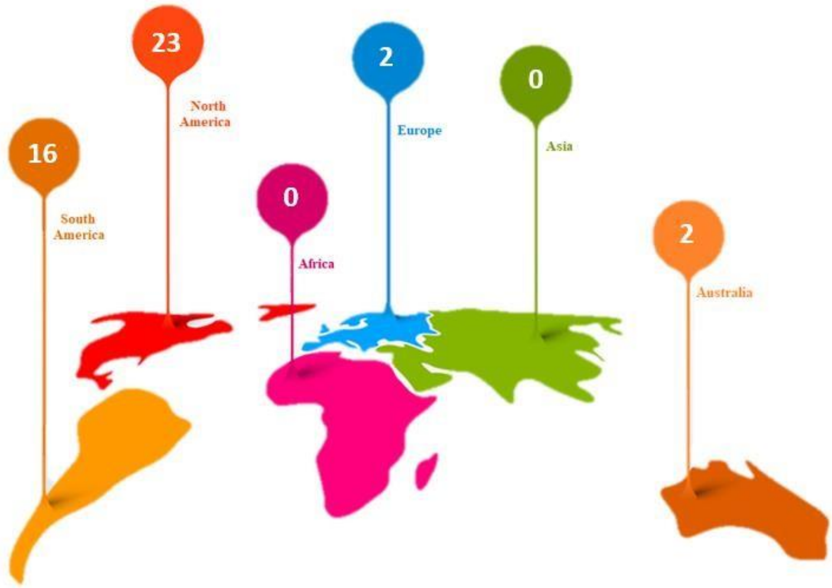


Figure 2 - Different species of *Solenopsis* recorded nesting in the analyzed articles up to the year of 2020.

The distribution of the records by geographic areas emphasizes the worldwide distribution of the genus found in the South and North America, Asia, and Europe regions. The invasion of these ants and the challenges faced in their control in countries such as the United States justify the concentration of most studies in the North America, where these ants are exotic, followed by the South America region where the species is native(TSCHINKEL, 1988; LARD et al., 2002) (Fig. 3).

The record of only two studies in the Europe and Autralia regions and their absence in the Africa and Asia regions can be explained by the fact that probably in these places these ants have not been found or were recently discovered. Also, it is possible that these ants are present but have not yet reached pest status. In Australia, for example, it is believed that before it was discovered in 2001, the species remained undetected for years (GOTELLI & ARNETT, 2000; VANDERWOUDE et al., 2004; HENSHAW et al., 2005; MURAKAMI, 2018) (Fig. 3).



Fonte: https://stock.adobe.com/br/templates/world-map-infographic-with-descriptive-continent-elements-layout-1/171798997
Figure 3 - Distribution of the number of publications on nesting of *Solenopsis* ants by geographic regions.

Another interesting feature observed is the way these fire ants spread around the world. According to the literature, it is possible to observe that anthropogenic transport is an important dispersal agent of these fire ants that acted in the cases cited in this study. In North America, in the mid-1930s it is believed that ants arrived via a ground ballast of ships trading between South America and Mobile, Alabama, and only 20 years later were recognized as a large-scale plague. In Australia, where it was found in 2001 in Brisbane, ants are also suspected of arriving by sea cargo about 3 years before they were discovered. Finally, in Japan, in 2017, fire ants were found on May 29, 2017, carried by a container from China (MINISTRY OF THE ENVIRONMENT OF JAPAN 2017; VINSON, 1986; GOTELLI & ARNETT, 2000; TABER, 2000; VANDERWOUDE et al., 2004; HENSHAW et al., 2005; MURAKAMI, 2018).

Although location sites vary, as the *Solenopsis* genus is known for its ability to adapt and exploit resources, the largest number of records have been made in natural environments. However, it is noteworthy the ease and success that these ants have in occupying altered environments, causing damage such as sting accidents, plantation infestation and transport of pathogens in hospital environments (BUENO & CAMPOS-FARINHA, 1999; BUENO &

BUENO, 2007; SCHÜLLER, 2008; MARTIN et al., 2011; ZERINGÓTA et al., 2014; DEJEAN et al., 2015) (Fig.4).

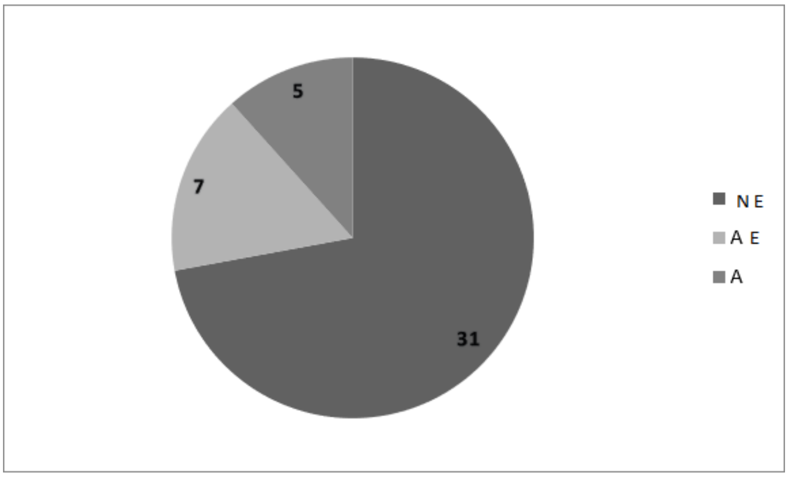


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Figure 4 - Number of records of *Solenopsis* ant nests recorded in articles analyzed up to the year of 2020 in different types of nesting substrates being NE (natural environment); AE (Altered environment) and A (agrosystems)

1.3 CONCLUSION

In general, the nesting behavior of *Solenopsis* is not the main topic in studies with ants of the genus. The construction and maintenance of nests is suppressed by other main objectives of study, being little explored and appearing in secondary form in the articles being reported only superficially, which shows the need for more studies focused directly on the nesting process of these ants, since, understanding which substrates they use most often, which ones adapt most easily, and what benefits they find in each one of them, directly influences the success of their control and in turn helps to mitigate potential damage caused by these fire ants (CAMPOS-FARINHA et al., 2002; ALMEIDA et al., 2007) The most studied specie was *Solenopsis invicta* due to the problems that those ants are causing in United States, and the main nesting siteis the natural one.

Although it is a native species of some countries, which shows the necessity and possibility of exploring this topic in a more objective and detailed way in its place of origin, the studies of the nesting habits of this genus are still incipient in a global context.

This work presents advances for science, showing the need to study more specifically the places where fire ants built their nest more easily, including places where studies are still incipient or nonexistent, such as the Palearctic, Australian, Ethiopian and Eastern regions, seeking to better understand how these ants relate to the environment they occupy, and with this information, draw more efficient ways of management and control of their populations, and greater understanding of their biology and behavior. Also, this paper presents an interesting scientific model to be explored, highlighting a broad topic for the contribution of the academic community with problems faced by the population.

2. 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)

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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 describes the evaluation of the food selection behavior of *Solenopsis saevissima* (Smith,1855) (Hymenoptera: Formicidae) ants against different stimuli. The observations were made from March 2018 to October 2019. The work analyzed the possible food selection behavior of 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. However, the correlation between ant flow and temperature showed a higher number of ant activity during periods of higher temperature, 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 correlation between flow and temperature. 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.

2.1 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, 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 national 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 (Smith, 1986) 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, J., 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.

2.2 MATERIAL AND METHODS

2.2.1 Place and Period of Study

The study was conducted at the Laboratory of Behavioral Ecology and Bioacoustics (LABEC), located at the LAZ (Advanced Laboratory of Zoology) 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).

2.2.2. 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 (Figure 1A) to prevent ants from escaping. In the laboratory, water was slowly dripped into a drum (20 to 40 drops per minute) (Figure 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 (Figure 1C and Figure 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 (Figure 2A)

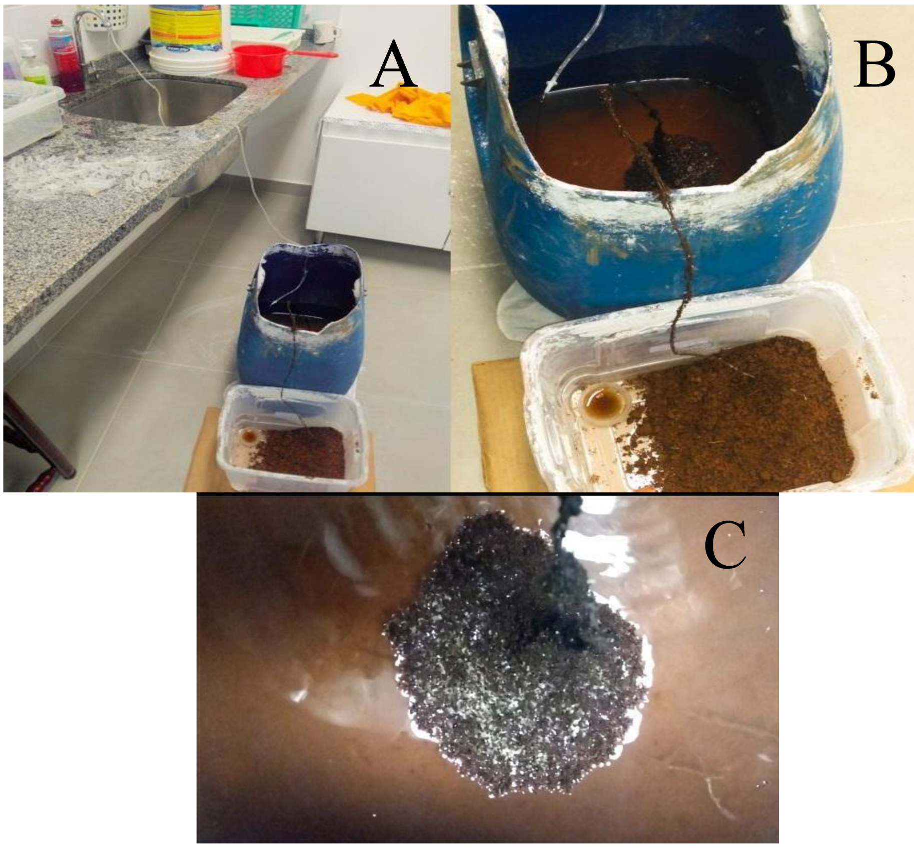


Figure 1 - A. Material collected in the field and placed in a blue plastic drum. B: Adaptation of a drip separation system. C: Fire ants (*Solenopsis saevissima*) colony separated from land floating on the water surface (adapted from: Bueno, 2017).

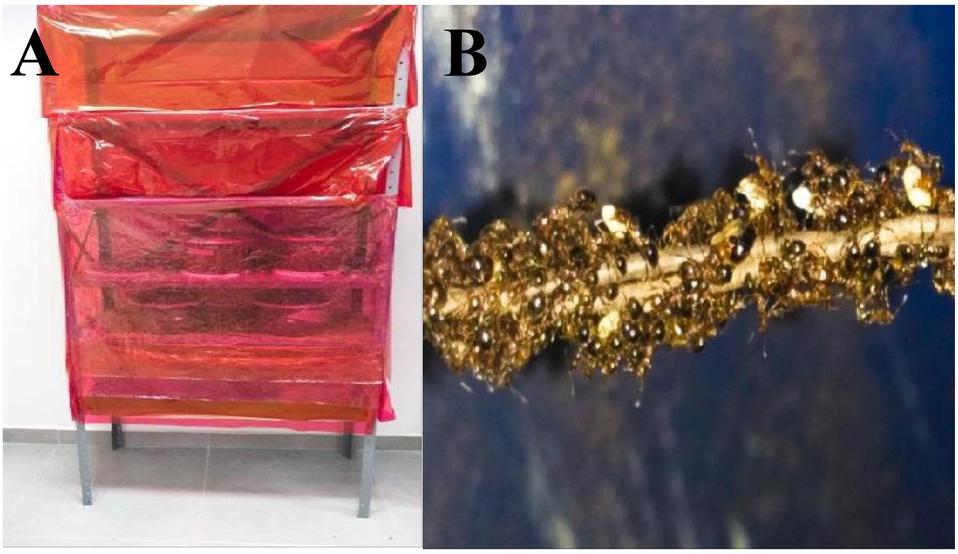


Figure 2 – A. Place of conditioning of the colonies. B. *Solenopsis saevissima* ants carrying their offspring from flooded site to the new colony.

2.2.3 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:

1. The species must be associated with forensic cases and decomposing bodies;

2. It must have wide geographical distribution, be common and abundant;

3. Its brood and maintenance in the laboratory should be simple.

2.2.4 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 ± 2 ° C and 80 ± 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 ° 46.452 ': O043 ° 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 ± 2 ° C, 80 ± 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 ± 2 ° C and 80

± 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 (Figure 3 A, B and C) ; These pots were closed with a lid adapted with voil fabric and then placed in a climate chamber (27 ± 2 ° C and 80 ± 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.

2.2.5 Maintenance of different *C. albiceps* stages

The immature stages used in the selection experiment collected from *C. albiceps* (Wiedemann, 1819) 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 (Figure 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 9am; 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 144 repetitions.

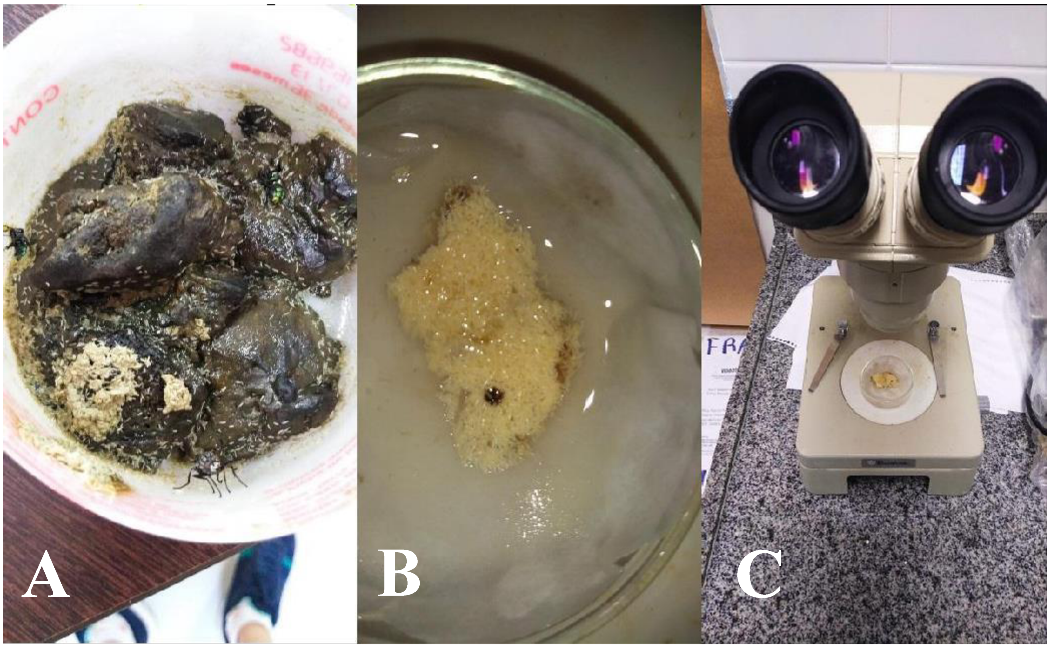


Figure 3 – A. Meat with egg mass from *Chrysomya albiceps* after being removed from the cage. B. *C. albiceps* egg mass in a petri dish with deionized water for selection of the 20 eggs. C. Egg mass under a stereomicroscope for separation.

2.2.6 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.

Figure 4 – A. 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.

2.2.7 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.

2.2.8 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).

2.3 RESULTS AND DISCUSSION

In the experiments, the ants spend 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 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.

Treatament	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

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 (0198)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

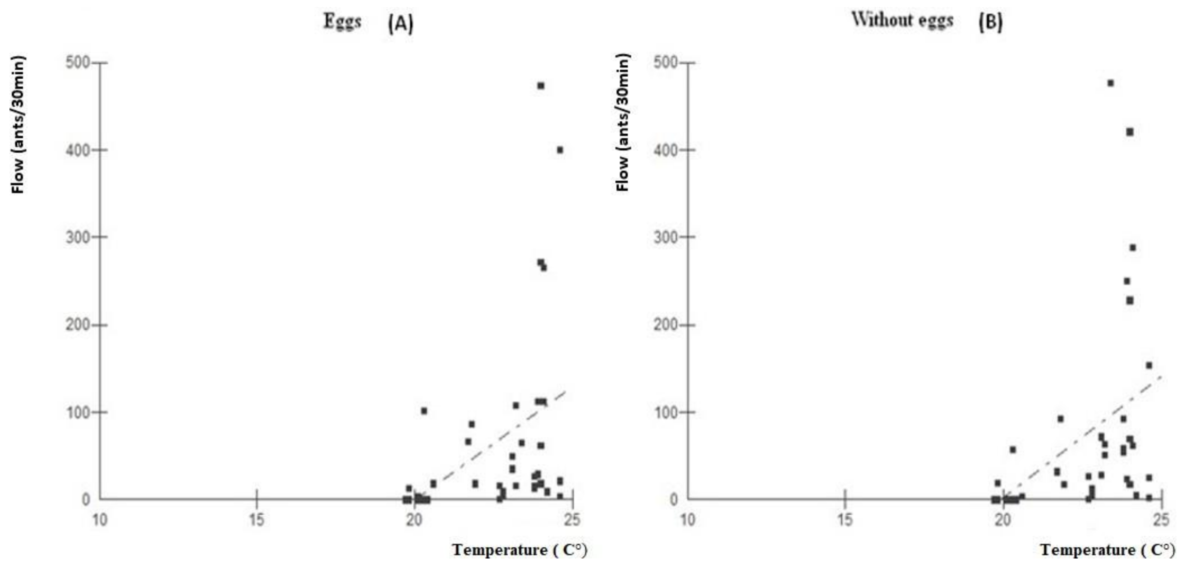
The lack of selection presented in the results reflects the already known and reported omnivorous habit for these ants (VINSON and GREENBERG, 1986). As in Glunn et al. (1981) in a food preference experiment with *S. invicta* Buren (1972) colonies for three types of liquid foods (sugar, rat serum, and 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% ± 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 a 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 and Wilson, 1990), and that capability is a relevant part of its capacity to adapt in extreme enviroments.

In the Experiments With Eggs X Without Eggs, the local morning shift temperature averaged 22.4 °C ± 1.53 (19.8-24). Already in the afternoon, the temperature was about 22.75ºC ± 1.65 (19.8-24.2) at the beginning and 22.75ºC ± 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 °C ± 1.32 (21.5-25) and in the late afternoon, the average temperature at the location was 23.59ºC ± 1.15 (21.8-25.1).

The correlation between flow and temperature differed significantly for egg experiments (*rs* = 0.053; *p* = 0.0009), without eggs (r*s* = 0.55504; *p* = 0.0005) and with larvae experiments (*rs* = 0. 3323; *p* = 0.0476). For the experiment without larvae there was no significant difference (*rs* = 0.2376; *p* =0.1628) (Figure 5).



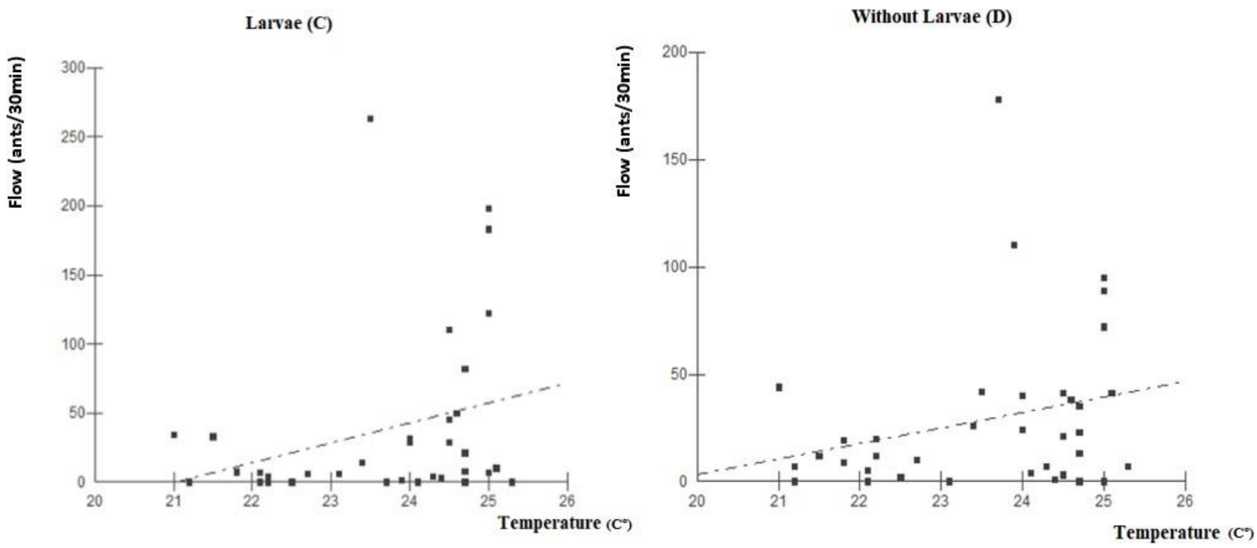


Figure 5 - Linear scatterplot showing the correlation between ants (*Solenopsis saevissima*) flow in the Y tube connected to the fire ant colony and local temperature during the selection experiment with egg (A), eggless (B), with larvae (C) and without larvae (D) treatments.

Regarding temperature-related analyzes, our experiment found that there is a correlation between ant flow and temperature. It is noteworthy that the ant flow in the morning in the egg and the eggless experiment was almost double the flow in the early afternoon time wich can be explained by the predisposition to foraging due to the need for food, intensifying the activity of ants in the early morning hours. In contrast to eggs, the ant flow in the larva experiment remained unchanged at different times of the day.

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 (SORENŞEN et al., 1981; VINSON and GREENBERG, 1986; LIMA and 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 (average25ºC)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 32ºC 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 32ºC. In food deprivation, the growth curve was inclined to lower temperatures, with the maximum size occurring around 25ºC. 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.

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.

3. FINAL CONSIDERATIONS

This study contributed considerably to the advancement of knowledge about the behavior of individuals of the genus *Solenopsis*, the fire ants, and their distribution and establishment around the world, as well as highlighting the necrophagous behavior and role played in cadaveric decomposition of *Solenopsis saevissima* (Smith, 1855) (Hymenoptera: Formicidae) when placed in contact with the offspring of another species of scavenger *Chrysomya albiceps* (Wiedemann, 1819), in view of the still incipient studies addressing nesting habits of this species and their necrophagus behavior, although it directly interferes with the decomposition process and is considered an urban pest species of medical and economic relevance. Fire ants have been found nesting in various parts of the world, which highlights its cosmopolitan distribution and ease of adaptation, making it an increasingly interesting object of study in the area of urban pest studies. In addition, the experiments of its necrophagous habit emphasized its omnivorous alimentary characteristic, besides its direct interference in decomposition processes. Knowing better the idiosyncrasies of individuals of the genus *Solenopsis*, as well as their peculiarities of distribution will certainly contribute to more research in the area, developing better control options and greater knowledge about the interference of these ants in various natural processes.

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