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Interactions between ants and mealybugs in sugarcane: species and effects on insect pests

Interações entre formigas e cochonilhas em cana-de-açúcar: espécies e efeitos sobre os insetos pragas

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ABSTRACT - The objective of this work was to evaluate ant species interacting with mealybugs in sugarcane plantations, the frequency of occurrence, and the effects of the interaction with ants on mealybug population size. The data was collected in a sugarcane plantation area in Seropedica, Rio de Janeiro, Brazil. The insect species were collected from 70 thoroughly inspected sugarcane plants and the nature and frequency of the interactions were evaluated. The effect of the interaction with ants on mealybug abundance was evaluated by counting the number of adult females of each mealybug species on ten plants isolated and ten plants not isolated from ants. Two mealybug species were collected: *Aclerda takahashii* (Kuwana, 1932) and *Saccharicoccus sacchari* (Cockerell, 1895). Ten and seven ant species were found interacting with *A. takahashii* and *S. sacchari*, respectively, which were distributed among six genera; the most frequent genera were *Crematogaster* and *Camponotus*. Three ant species interacted exclusively with *A. takahashii*, whereas the others interacted with both mealybug species. The abundance of *A. takahashii* females was significantly higher on non-isolated than on isolated plants (Paired t-test; $t = 2.34$; $p = 0.04$). However, no significant difference in *S. sacchari* abundance was found between isolated and non-isolated plants ($t = 0.27$; $p = 0.8$), nor for the two species combined ($t = 1.9$; $p = 0.09$). Thus, ant species that interact with mealybugs in sugarcane plantations cause increases in *A. takahashii* populations which, at a larger scale, can reduce crop yield.

Keywords: *Aclerda takahashii*. Formicidae. Mutualism. *Saccharicoccus sacchari*.

RESUMO - O objetivo desse trabalho foi realizar o levantamento das espécies de formigas que apresentam interações com cochonilhas em plantios de cana-de-açúcar, registrando a frequência de ocorrência e o efeito da interação com as formigas sobre as populações de cochonilhas. A pesquisa foi realizada em plantio de cana-de-açúcar no município de Seropédica, estado do Rio de Janeiro. Os insetos foram coletados em 70 plantas de cana-de-açúcar, observando-se a natureza das interações e a sua frequência. Para avaliar a influência da interação das formigas sobre a abundância das cochonilhas, foi obtido o número de fêmeas adultas de cada espécie de cochonilha em dez plantas isoladas de formigas e dez não isoladas. Foram coletadas duas espécies de cochonilhas: *Aclerda takahashii* (Kuwana, 1932) e *Saccharicoccus sacchari* (Cockerell, 1895). Dez espécies de formigas foram observadas interagindo com *A. takahashii*, e sete espécies com *S. sacchari*, distribuídas em seis gêneros, sendo os mais frequentes *Crematogaster* e *Camponotus*. Três espécies de formigas interagiram somente com *A. takahashii*, as demais interagiram com ambas espécies de cochonilha. A abundância de fêmeas de *A. takahashii* foi significativamente maior nas plantas não isoladas que nas isoladas (t= 2,34; p = 0,04). Não foi observada diferença significativa na abundância de *S. sacchari* entre plantas isoladas e não isoladas (t= 0,27; p = 0,8), e para as duas espécies em conjunto (t= 1,9; p= 0,09). As espécies de formigas que interagem com cochonilhas em cana-de-açúcar provocam o aumento da população de *A. takahashii*, o que em maior escala pode reduzir a produtividade.

Palavras-chave: *Aclerda takahashii*. Formicidae. Mutualismo. *Saccharicoccus sacchari*.

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INTRODUCTION

Brazil has large areas cultivated with sugarcane (*Saccharum* spp. L.; Poaceae) plantations, accounted for approximately 8.4 million hectares planted in the 2019/2020 crop season and an estimated production of 615.98 million Mg of sugarcane, 31.8 million Mg of sugar, and 30.0 billion liters of ethanol (CONAB, 2019). The country stands out in the world production, holding the top position in the ranking; the state of São Paulo accounts for 55% of the planted area in Brazil (INVESTSP, 2020). Rio de Janeiro is another state that stands out in the Southeast region of Brazil due to its increasing sugarcane production, with a harvested area of 21.4 thousand hectares in the 2018/2019 crop season on an estimated area of 30.6 thousand hectares in the 2019/2020 crop season (CONAB, 2019). Sugarcane is one of the most economically important crops in the country and is associated with the constant demand for renewable energy (SILVA; SILVA, 2019).

Insects are among the main organisms causing damage to sugarcane plantations, especially Hemiptera species, which include leafhoppers, mealybugs, and aphids (HOLTZ et al., 2015; ALMEIDA, 2016). These insects cause economic losses in agricultural areas by attacking leaves, branches, stems, fruits, and seeds (OLIVEIRA et al., 2014), sucking sap, and spreading diseases (GUIMARÃES; MICHEREFF FILHO; LIMA, 2019). High population densities of these insects can make the production of agricultural crops unfeasible, as they cause high economic losses to growers (THOMAZINI, 2001). Mealybugs stands out among Hemiptera insects, as they are abundant and cause significant problems for agricultural crops (SOUZA et al., 2001; BENVENGA et al., 2011).

Mealybugs can be found in different plant parts because they can live under leaf sheaths, calyxes, and roots; they can cause direct damage to plants at different development stages by sucking the sap and indirect damage by introducing toxic substances and pathogens into the plants (SANTA-CECÍLIA; REIS; SOUZA, 2002; MONTEIRO, 2019), thus reducing the yields of the attacked plants (FORNAZIER et al., 2017). Furthermore, their sugary liquid excretions favor the development of sooty mold, a fungus that impairs the photosynthetic process of plants and, consequently, the agricultural production (GARCIA MORALES et al., 2016). A total of 103 mealybug species have been associated with sugarcane plantations worldwide (GARCIA MORALES et al., 2016). Eighteen species have been recorded in Brazil, mainly in the Southeast region, where seven species were recorded in the state of Rio de Janeiro (LIMA, 1968; CLAPS; WOLFF; GONZÁLEZ, 1999; CLAPS; WOLFF; GONZÁLEZ, 2001; GARCIA MORALES et al. al., 2016; MONTEIRO et al., 2019).

The population density of some Hemiptera species, including mealybugs, may increase due to mutualistic interactions with ant species (GUINDANI et al., 2017). Ants protect Hemiptera species from natural enemies while feeding on the sugary excretions of these sucking insects (DAANE et al., 2007). However, some ant species prey on Hemiptera insects (DELABIE et al., 2007). Thus, interactions between species of ants and Hemiptera are diverse and complex and the function of ants in these interactions brings both benefits and challenges for agricultural growers.

Interactions between ants and mealybugs are frequently observed in agricultural areas (SOUZA et al., 2007; HUANG; ZHANG, 2016; GUINDANI et al., 2017), but further research is needed to provide more information about the nature, frequency, and the species involved in these interactions in different crops. Furthermore, the consequences of these interactions on mealybugs can vary.

Ant activity can reduce parasitoid populations and increase mealybug populations (DAANE et al., 2007). The myrmecofauna can also increase the population density of phytophagous Hemiptera species without affecting parasitism rates, denoting a potential effect of ants on predators of these Hemiptera insects (CALABUIG; GARCIA-MARÍ; PEKAS, 2014). Additionally, the effectiveness of ant protection provided to mealybugs can vary depending on the ant species (BUCKLEY; GULLAN, 1991); therefore, increases in density of phytophagous Hemiptera through interactions with ants may not always occur (VILELA; DEL -CLARO, 2018).

Therefore, understanding the nature of interactions between mealybugs and ants and the effects of these interactions on the abundance of Hemiptera species in sugarcane plantatations can contribute to identify and describe possible patterns.

Therefore, the objective of this work was to survey ant species that interact with mealybugs in a sugarcane plantation area, recording the frequency of occurrence and the effect of the interaction with ants on mealybug populations. Additionally, the occurrence of parasitoids was evaluated.

MATERIAL AND METHODS

The research was carried out in a 500 m^2 sugarcane (*Saccharum officinarum* L.; Poales: Poaceae) plantation area at the Terraço experimental area of the Brazilian Agricultural Research Corporation (Embrapa Agrobiology), in Seropedica, Rio de Janeiro, Brazil (22°44'59'S, 43°40'01"W). The sugarcane variety CB 47-355 (Mulata Pelada) was planted in January 2018. The sugarcane plants presented, on average, heights of 2.58±0.23 m, stem circumference of 8.56±0.79 cm, and 6.4±1.5 plants per clump. The mean temperature during data collection was 33.5±0.42 °C. The area was cultivated following principles of agroecology, with limited use of synthetic chemical products (EMBRAPA, 2019).

The survey of ecological interactions between ants and mealybugs consisted of a meticulous inspection of 70 sugarcane plants to observe and record the type of interaction occurring. Field collections were performed between November 2018 and March 2019. Seven field expeditions were carried out, with a 15-day interval between them, inspecting 10 plants in each expedition, considering approximately 10 m distance between plants; different plants were inspected in each expedition. The insects were collected from the culms, placed in labeled flasks, stored on 70% alcohol for preservation, and then taken to a laboratory for identification. The ants were dry-mounted and then identifyed at the genus level using the key of Baccaro et al. (2015). The ant specimens were grouped into morphospecies, with identification at the species level based on specific identification keys for the genera and comparisons with previously identified ants from the Costa Lima Entomological Collection (CECL), which are widely used procedures for identification of myrmecofauna in species surveys (AMARAL; VARGAS; ALMEIDA, 2019; ESTRADA et al., 2019). The collected mealybugs, including aspects of parasitism, were identified at the Entomology Laboratory of the Faculty of Agricultural and Veterinary Sciences (FCAV) of the Sao Paulo State University (UNESP), Jaboticabal, São Paulo.

The collected mealybugs were mounted on slides, following the technique of Willink (1996) for subsequente identification of species using the keys of Williams and Willink (1992) and McConnell (1954). Parasitoids that emerged from the collected mealybugs were identified according to De Santis (1964), Noyes (1980, 2000), Noyes and Hayat (1994), Sharkov (1996), and Sharkov and Woolley (1997). Voucher specimens of mealybugs and parasitoids were deposited in the Reference Insect and Mite Collection (CRIA) of the Department of Plant Health at FCAV/UNESP.

The air temperature near the analyzed plant was recorded during field collections using a thermohygrometer. Arthropods natural enemies of mealybugs at the study site were recorded.

Ten sugarcane plants were selected and isolated from ants using an adhesive tape (Neudorff®) placed 20 cm above the ground on the stem of each plant to prevent interactions between ants and mealybugs. Precautions were taken to prevent the access of ants from another plant to the plant with adhesive tape; leaves that would facilitate the movement of ants were removed when necessary. Additionally, a plant at 0.5 to 0.7 meters from each isolated plant was marked with an adhesive tape that did not hinder the movement of ants. The average height of isolated and non-isolated plants was 2.58±0.23 m. The adhesive tapes were installed in November 2018 and remained in place until March 2019, totaling 90 days of isolation. The number of adult female mealybugs was obtained immediately after isolating and marking of plants and after 90 days. Mealybugs on isolated and non-isolated sugarcane plants were collected 90 days after isolation to confirm the species identification. The number of parasitoids that emerged from mealybugs on isolated and non-isolated plants was counted.

The frequencies of occurrence of interactions between ants and mealybugs were calculated and then evaluated using the G Test. The numbers of mealybugs on isolated and non-isolated plants were compared using the paired t-test. Statistical analyses were performed using the software Bioestat 5.3 (AYRES et al., 2007).

RESULTS AND DISCUSSION

The mealybug species found in the evaluated sugarcane plantation were *Aclerda takahashii* (Kuwana, 1932) and *Saccharicoccus sacchari* (Cockerell, 1895). These species are often found in sugarcane plantations and their dispersion is favored by the transportation of sugarcane stalks between fields, which can also cause infestations in other host crops (MONTEIRO; PERONTI; MARTINELLI, 2017; QIN et al., 2017). These two mealybug species are among the main pests of sugarcane, as they can live in different parts of the plant at different phenological stages (OLIVEIRA; OLIVEIRA; MOURA, 2012; FUNDECITRUS, 2017).

Aclerda takahashii is a monophagous mealybug that is often found on a small number of plant species from the family Poaceae (GARCIA et al., 2017). Muniappan (2001) observed small necroses and discoloration in grasses of the species *Vetiveria zizanioides* (L.) Roberty caused by mealybug attack and reported that it can lead to deformities, thus affecting plant development due to the sooty mold resulting from the sugary excretions of *A. takahashii*. According to field evaluations, individuals were found mainly near the leaf sheath, between leaves and stems, which may provide protection from natural predators/enemies and climatic factors.

S. sacchari is commonly known as pink sugarcane mealybug and has been frequently found in sugarcane fields in Brazil (MONTEIRO; PERONTI; MARTINELLI, 2017; QIN et al., 2017; ELROBY, 2018). This species can live under different environmental conditions and is found on sugarcane plants at the different development stages, from infesting roots in the soil after planting the stalks until the plant is ready to be harvested, which makes mealybug control difficult (SARTIAMI et al., 2017; MIRABAL-RODRÍGUEZ, 2018). Rajendra (1974) stated that cultural and biological control of this insect is more economically viable than using insecticides. Few information about direct and indirect damages caused by this pest in Brazil are found in the literature; however, Puttarudriah (1954) reported the death of young shoots and growth delay in sugarcane plants, in addition to decreases in °Brix, which defines the available sucrose contents (GAMAL EL-DEIN et al., 2009; YAKOUB, 2012). Moreover, this insect pest can be a vector of the sugarcane bacilliform virus (ScVb) (AUTREY et al., 1995; VICTORIA et al., 2005).

Ants preying on mealybugs were not found in the evaluated sugarcane plants, but apparently positive interactions between ants and mealybugs were observed, which is consistend with other studies on different crops (DAANE et al., 2007; MARQUES et al., 2018). The behavior of ants interacting with individuals of the two mealybug species denoted a mutualistic interaction, as the ants fed on the sugary excretions of the mealybugs and did not exhibit an aggressive behavior, which is often observed when ant species have mutualistic interactions with Hemiptera species (FREITAS; ROSS, 2015).

Ten ant species interacting with mealybugs were collected; seven of them were found interacting with *S. sacchari* (Table 1). *A. takahashii* mealybugs were found in 45% of the replications and ants interacting with *A. takahashii* were found in 74.1% of the plants where these mealybugs were found. *S. sacchari* was found in 26.7% of the replications, with ants occurring in 87.5% of the plants where these mealybugs were found.

Crematogaster sp.1 was the predominant ant species found interacting with *A. takahashii*, followed by *Camponotus crassus* (Mayr, 1862). The ant species that presented the highest number of interactions with *S. sacchari* were *Crematogaster* sp.4, *C. crassus*, and *Camponotus melanoticus* Emery, 1894. A significant difference was found in proportion of interactions between ant species and mealybug species (G test = 19.41; p = 0.02). *Crematogaster* sp.1 stood for its high frequency of interaction with *A. takahashii* and absence of association with *S. sacchari*. Rodrigues et al. (2010) observed ant species interacting with aphids of the species *Toxoptera citricida* (Kyrkaldy, 1907) (Sternorrhyncha) in tangerine trees within an integrated system of agroecological production and reported the occurrence of ants from the genera *Brachymyrmex*, *Crematogaster*, and *Solenopsis* and the species *Camponotus rufipes* (Fabricius, 1775) and *C. crassus*, which were also found in the present study.

Table 1. Frequency (in percentage) of occurrence of interactions between ant species and mealybugs of the Hemiptera species *Aclerda takahashii* (Kuwana, 1932) and *Saccharicoccus sacchari* (Cockerell, 1895) on sugarcane (*Saccharum officinarum* L.) plants.

Ant species	*Aclerda takahashii* (Kuwana, 1932)	*Saccharicoccus sacchari* (Cockerell, 1895)
Brachymyrmex sp.1	5.0	1.7
Brachymyrmex cordemoyi Forel, 1895	3.3	1.7
Camponotus crassus Mayr, 1862	11.7	8.3
Camponotus melanoticus Emery, 1894	6.7	8.3
Camponotus blandus (Fr. Smith, 1858)	1.7	0.0
Crematogaster sp.1	16.7	0.0
Crematogaster sp.4	1.7	8.3
Solenopsis sp.1	1.7	1.7
Pseudomyrmex laevivertex (Forel, 1906)	1.7	1.7
Wasmannia auropunctata (Roger, 1863)	3.3	0.0

No significant difference was found for mean number of adult females of *A. takahashii* on isolated (3.5±0.5 individuals) and non-isolated sugarcane plants (3.8±0.8 individuals) (paired t-test; t = -0.82; p = 0.43) immediatally after isolating the plants from ants. A similar result was found for *S. sacchari* on isolated (1.0±0.4 individuals) and non-isolated sugarcane plants (1.2±0.3 individuals) (t = -0.51; p = 0.62). No significant difference was found for number of individuals of the two mealybug species on isolated (4.5±0.7 individuals) and non-isolated plants (5.0±0.9 individuals) (t = -0.81; p = 0.44).

The abundance of *A. takahashii* individuals was significantly higher on non-isolated plants than on isolated plants (t = 2.34; p = 0.04) at 90 days of isolation (Figure 1). Thus, the interaction with ants provided benefits for the mealybug populations, increasing the number of *A. takahashii* individuals on sugarcane plants. Other studies have also reported the beneficial effects of interaction with ants on the abundance of phytophagous Hemiptera insects, whose population density increases under presence of ants (GUINDANI et al., 2017; CALABUIG; GARCIA-MARÍ; PEKAS, 2014), which prevents attacks from natural enemies (DAANE et al., 2007). However, no significant difference in abundance of *S. sacchari* was found between isolated and non -isolated plants (t = 0.27; p = 0.8) (Figure 2). A similar result was found for the two mealybug species combined (t = 1.9; p = 0.09) (Figure 3). The effects of the interaction with ants on mealybugs may vary depending on the species involved in the interaction (CALABUIG; GARCIA-MARÍ; PEKAS, 2014; MONTEIRO, 2019).

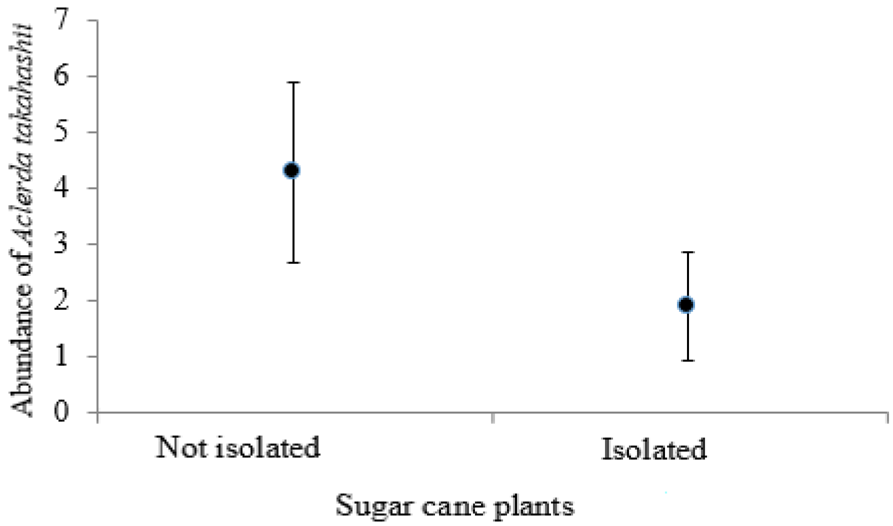

Figure 1. Mean abundance (± standard deviation) of females of *Aclerda takahashii* (Kuwana, 1932) on sugarcane plants (*Saccharum officinarum* L.) isolated and not isolated from plants. Paired t-test: t = 2.34; p = 0.04.

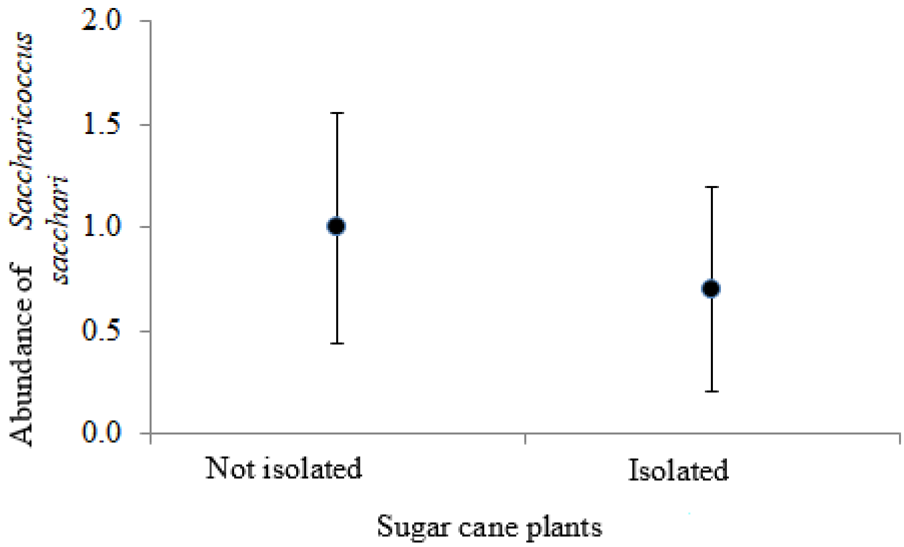

Figure 2. Mean abundance (± standard deviation) of females of *Saccharicoccus sacchari* (Cockerell, 1895) on sugarcane plants (*Saccharum officinarum* L.) isolated and not isolated from plants. Paired t-test: t = 0.27; p = 0.8.

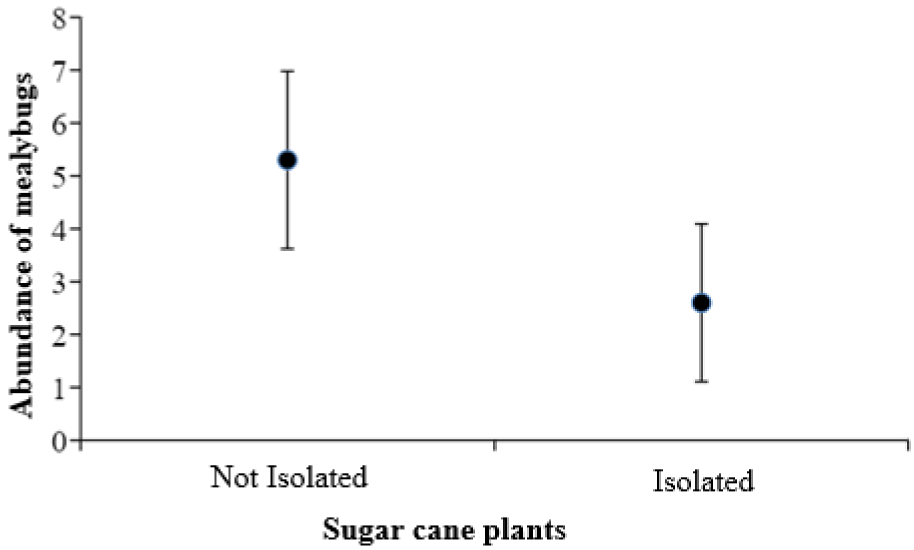

Figure 3. Mean abundance (± standard deviation) of female mealybugs of the species *Aclerda takahashii* (Kuwana, 1932) and *Saccharicoccus sacchari* (Cockerell, 1895) on sugarcane plants (*Saccharum officinarum* L.) isolated and not isolated from plants. Paired t-test: t = 1.9; p = 0.09.

Populations of phytophagous Hemiptera species producing sugary liquid excretions that feed higly aggressive ant species may have lower rates of parasitism when compared to Hemiptera species interacting with less aggressive ants (BUCKLEY; GULLAN, 1991). The occurrence of *Solenopsis* sp.1, *Wasmannia auropunctata* (Roger, 1863), and a high frequency of *Crematogaster* sp.1 interacting with *A. takahashii* may be factors that contributed to the higher abundance of mealybug individuals on sugarcane plants not isolated from ants.

Ants of the genus *Solenopsis* and the species *W. auropunctata* are often reported as aggressive species (ALMEIDA; QUEIROZ.; MAYHÉ-NUNES, 2007; VONSHAK et al., 2009). According to Lutinski et al. (2018), the genera *Crematogaster*, *Solenopsis*, and *Wasmannia* can be included in the guild of epigeic, omnivorous, and dominant ants, which are generalists and aggressive.

Calabuig, Garcia-Marí and Pekas (2014) found a decrease in density of sugary excretion-producing Hemiptera species when isolating them from ant species and concluded that the management of myrmecofauna can contribute to decreases in phytophagous species that are harmful to agricultural crops. Similarly, the results of the present study enable to correlate the decrease found in *A. takahashii* population with a decrease in abundance of ant species, especially aggressive ant species. However, the presence of aggressive ant species can contribute to decreases in the population sizes of other insect pests in crop areas, such as beetles, stink bugs, and Orthoptera species (EUBANKS, 2001; ESTRADA, 2017).

Regarding parasitoid emergence, 37 individuals of *Mariola flava* (Noyes, 1980) (Hymenoptera: Encyrtidae) and 6 individuals of *Mucrencyrtus aclerdae* (DE SANTIS, 1972) (Hymenoptera: Encyrtidae) emerged from *A. takahashii*, but not from *S. sacchari*.

Cruz et al. (2019) have previously identified these parasitoid species as natural enemies of *A. takahashii* in some regions of the state of Sao Paulo. Nineteen parasitoids of mealybugs were collected and recorded during the sampling for evaluating the ecological interactions; eigteen parasitoids were found in mealybugs on plants isolated from ants and six parasitoids were found in mealybugs on non-isolated plants. Therefore, ants can effectively protect from parasitoids, reducing damages to mealybug populations (DAANE et al., 2007).

Predators popularly known as ladybugs (Coleoptera: Coccinellidae) have been identified as a natural enemies of *S. sacchari* (CRUZ et al., 2019). In the present study, these beetles were found on sugarcane plants that had presence of mealybugs; however, they were not analyzed in the laboratory.

Therefore, parasitoids from the family Encyrtidae and predators from the family Coccinellidae are natural enemies of the mealybug species found in the evaluated sugarcane plantation in the present study. These insects may be useful for the management of mealybug species in sugarcane plantations in the state of Rio de Janeiro.

CONCLUSION

The ant species interacting with the two mealybug species (*Aclerda takahashii* and *Saccharicoccus sacchari*) found in the evaluated sugarcane plantation may cause an increase in the population of *A. takahashii*. A positive effect of interaction with ants was not found for *S. sacchari*. The increase found for the abundance of *A. takahashii* individuals is mainly connected to the performance of more aggressive ant species, such as *Wasmannia auropunctata*. Thus, decreases in populations of these ant species can lead to decreases in abundance of *A. takahashii* populations. This is the first report of occurrence of the parasitoid species *Mariola flava* and *Mucrencyrtus aclerdae* in the state of Rio de Janeiro, Brazil.

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