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Transport rate of capsules to leaf-cutting ants *Atta sexdens*

Taxa de transportes de cápsulas para formigas cortadeiras *Atta sexdens*

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ABSTRACT

The search for new management alternatives in pest control is a constant in agriculture. In the case of leaf-cutting ants, the most widespread control is that which makes use of granulated bait with synthetic chemical active ingredient, whose attractiveness comes from the citrus pulp that composes it. Using the same attraction, a capsule was created, inspired by seeds transported by forage workers. Bipartite gelatin capsules were adapted and filled with salt (NaCl), for a total of 20 capsules for each mini colony in three groups of five colonies that received each one capsules with one of three different masses (0.5g, 1g, or 2g). In addition to weight, were also compared citrus pulp and the pheromone from the venom gland extract (VGE) of forage workers were compared in the relation to transport rate too. After statistical analyses, concluded that the highest transport rate was for the capsules, which was empty (internal), only with the attractive citrus pulp on the external surface (0.5g).

Keywords: *Atta sexdens*, alternative control, pheromone, citrus pulp, NaCl.

RESUMO

A busca por novas alternativas de manejo no controle de pragas é uma constante na agricultura. No caso das formigas cortadeiras, o controle mais difundido é aquele que faz uso de isca granulada com princípio ativo químico sintético, cuja atratividade vem da polpa cítrica que a compõe. Utilizando o mesmo atrativo, foi criada uma cápsula, inspirada em sementes transportadas por operárias forrageiras. Cápsulas de gelatina bipartidas foram adaptadas e preenchidas com sal (NaCl), para um total de 20 cápsulas oferecidas na arena de alimentação simultaneamente por 24h. Para cada minicolônia, divididas em três grupos de cinco colônias que receberam cada uma cápsulas com uma das três massas diferentes (0,5g, 1g ou 2g). Além do peso, também foi comparada a polpa cítrica e o feromônio do extrato da glândula de veneno (EGV) das operárias forrageiras, também em relação à taxa de transporte. Após análises estatísticas, concluiu-se que a maior taxa de transporte foi para as cápsulas, que se encontravam vazias (internas), apenas com a atrativa polpa cítrica na superfície externa (0,5g).

Palavras-chave: *Atta sexdens*, controle alternativo, feromônio, polpa cítrica, NaCl.

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INTRODUCTION

Currently the control of leaf-cutting ants is done in general with the attractive granulated bait, composed of citrus pulp and whose active ingredient is sulfluramid, as the most practical, economical and safe according to records (Britto et al., 2016). There are several commercial brands, we still have the baitwith fipronil as an active ingredient, as mentioned for *Atta texana* (Schowalter and Ring, 2017), with questioned use in permanent preservation areas.

Since the Brazilian colonization, several methodologies of controls have been proposed (Mariconi, 1970), citing thermobulization and chemical powder as the most common, but with a proven impact on the environment and the worker (Della Lucia, 2011), until we reach the current scenario, where society's demand for more conscious agriculture, resulted in a less impacting control on the agroecosystem, in this sense the granulated bait with synthetic chemical, already mentioned, was developed, which replaced the one that used dodecachlor as an active principle (Atta-Kill, 2011; Britto et al., 2016).

There is a plant extract bait (*Tephrosia candida*) (Bioisca- COCAPEC) wich circulates on the national market, also considered for being the less toxic ofthe mode of action (rotenoid-type saponin flavones) and recognized by IBD Certifications (Travaglini et al., 2017).

Regarding microbial control, fungi are the main candidates due to the time that has been studied, but entomopathogenic microorganisms encounter limitations in field tests (Lopez and Orduz, 2003; Loreto and Hughes, 2016). Entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* are efficient under controlled conditions (temperature and humidity), reaching high mortality of leaf-cutting ants in laboratory tests, with averages ranging between 24 and 48 hours to reduce 50% of the population exposed to conidia (Castilho et al., 2010; Dornelas et al., 2017).

Still in microbial control, used to decline the main food reserve of leaf-cutting ants, and generate the *Leucoagaricus gongylophorus* inibition, are cited the antagonists *Trichoderma* spp. and *Escovopsis* spp. (Rodrigues et al., 2005; Lopez and Orduz, 2003), because this mycophagous insect whose mutualism with a fungus was essential for evolutionary success (Branstetter et al., 2017). The strategy also applied when secondary metabolites from bacterias are used (Chacon-Orozco et al., 2018; Uribe-Londoño et al., 2019), lines that are still under study.

In this context, the aim of this work was to demonstrate the use of encapsulated baits for foraging (with focus on the transport rate) of leaf-cutting ants containing sodium chloride (NaCl) with three different groups of mass, covered for citrus pulp or natural pheromone from exocrine glands (Tatagiba-Araujo et al., 2012). With this, it is expected to generate stress capable of disorganizing the colony of this social insect in its establishment phase (first year).

MATERIAL AND METHOD

In this test, it was programmed the observation of behavioral acts related to the transport, incorporation, and capsule rejection, which was documented with a NIKON Coolpix L800. Attractiveness tests were performed to contrast the citrus pulp and pheromone transport rate, in this case, the baits were divided into three groups with different masses (0.5, 1, and 2g) to analysis (Figure 1), adjusted with commercial refined table salt (NaCl) introduced inside the capsules. To execute our methodology, we used:

Mini colonies of *A. sexdens*, collected in the field at three months aft er the nuptial flight and kept in the Laboratório de InsetosSociais-Praga (LISP), São Paulo State University "Julio de Mesquita Filho" (UNESP), in the city of Botucatu-SP, in acrylic pots containing three divisions (foraging arena, symbiotic fungus garden and garbage chamber), connected by transparent tubes

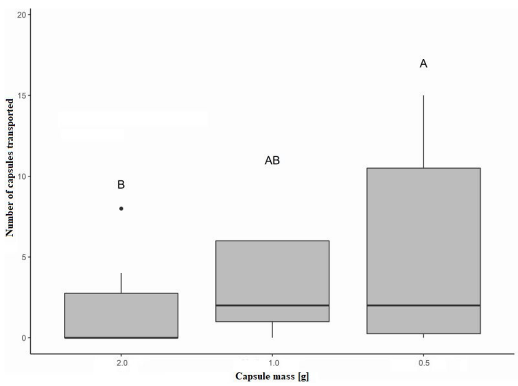

Figure 1. Transport/mass.
The means followed by different letters in the Graph indicate a significant difference between them, at the level of 5% probability by the Tukey test.

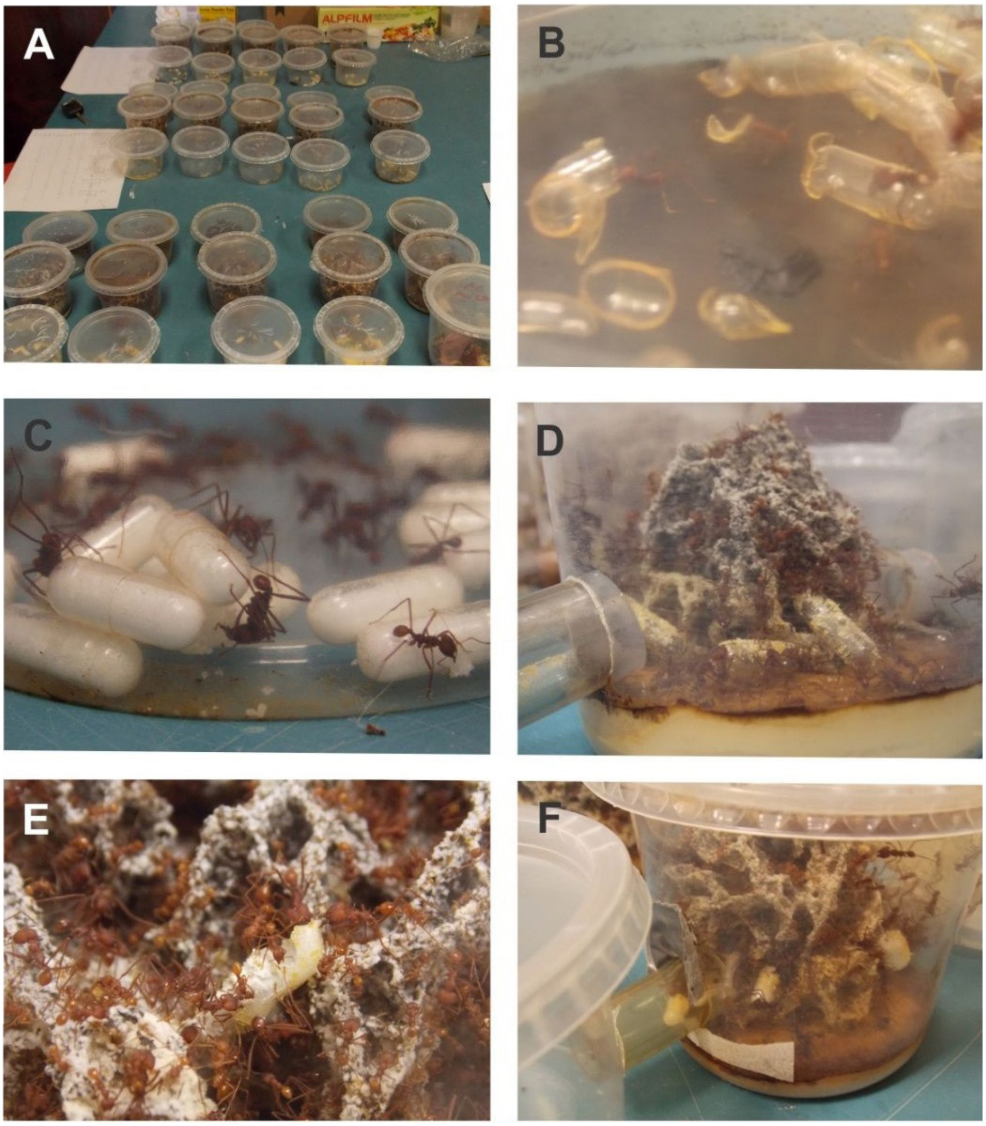

Figure 2. (A) Repetitions with groups of mini colonies containing an avarege of 250ml of symbiotic fungus; (B) Capsules being cut in the foraging arena; (C) Salt capsule sprayed with VEG; (D) Empty attached capsules distributed over the fungus garden; (E) Capsule filled with salt being processed and incorporated into the fungus garden and (F) Capsule with salt waiting to be dragged in the connector.

(Figure 2A), fed daily with leaves of *Acalypha wilkesiana* or crushed corn grain, according to the humidity inside the colony (Forti et al., 1994). Colonies used in this test whose fungus volume was similar to 250ml and complete 1 year in the laboratory.

Ripe oranges were peeled in order to remove the essential oils from the peel and later squeezed, removing the juice from the fruit, leaving the albedo, that is, only the bagasse was used, these went to the greenhouse and went through dehydration at 50°C (for 12h), after being dried, they were crushed in a mill reaching an aspect of flour, the crushed material was packaged and taken to the freezer so that the aroma was preserved (Perri et al., 2017).

For impregnation of the dried and crushed citrus pulp (attractive), it was spread on a tray where the capsules were shaking with a spray of distilled water and molasses (1: 1), in order to increase the adhesion of the attractant in the capsules, which were placed to dry in the fresh air for 12 hours, in the laboratory, the smaller particles of the citrus pulp adhered to the micro-droplets and fused into the wall of these gelatinous capsules, making them look rough.

For the extraction of the venom gland extract (VGE), the largest foragers (except the maximum workers or soldiers) were collected from *A. sexdens* Mirmecology Unit, of the Entomology and Phytopathology Laboratory of the Center for Agricultural Sciences and Technology, of the State University of the North Fluminense "Darcy Ribeiro" (UENF), in the city of Campos dos Goytacazes - RJ. The gaster of the largest foragers was separated and the venom gland was removed, with the help of forceps, surgical scissors, and a stereoscopic microscope with 50-fold magnification. The glands were washed with acetone and placed in a flask containing 1 ml of Pentane, then macerated with a glass stick. Extracts were prepared with 10 glands/mL. Gland concentrations were established based on results obtained from *Atta sexdens* by Gazal et al. (2009) and through the performance of pre-tests. The extracts were kept in a freezer at 10 ºC until used.

Popular bipartite gelatin hard capsules (A4), easily found in handling pharmacies, were kept in a closed container, with dry and crushed citrus pulp, to impregnate the citrus aroma (attractive) in one of the repetitions as mentioned previously and the other to be compared, it was odorized with the help of a trigger, taken from a spray commonly used to spray water on aerial plants, whose spray of 2mL of pheromone from the venom glands extract (VGE) was carried out directly bottle supplied by UENF, at the time that the 20 capsules were placed in each colony.

Regardingthe attractiveness test, it was delineated with repetitions of five colonies for each treatment group (each mass 0.5g, 1g, or 2g). Twenty capsules were offered per colony in the feeding arena of each colony. It was made an additional treatment only with pentane sprayed on the empty capsules, to prove the non-interference in the attractive (VGE). The same pattern has now been replicated with citrus pulp breaded in identical capsules. It was also observed for five hours and the next day (after 24h), thus confirming the transportation rate.

After offering attractive encapsulated baits, continued registering after 24 hours the total number of capsules carried in each colony and for a week there were obsorvedchanges in the colony's garbage and in the fungus garden. The trash chamber was cleaned, allowing to check what was taken to that compartment during this period.

It was used the T student test to compare the transport rates, in addition, the R studio program was used to generate a factorial graph between the massesshowing the standard significance (α=0.05).

RESULT AND DISCUSSION

In the treatment with citrus, the result closest to the expected in this test, the mass, and the transport rate were inversely proportional, with the lightest capsule being the most transported in the same time interval (Figure 1, Table 1), only the number of capsules not being greater due to the limited space inside the device, a fact confirmed by the accumulation of capsules in the ducts that connected the two chambers of the device.

According to Stefanelli et al. (2018) loading represents an important factor in the formulation of ant killer baits and research must be carried out with new compounds of better attractiveness and less insect repellency, so that in this way, the control remains effective. According to Figure 1, it is possible to observe that the loading rate of the number of capsules was higher in compartments with a mass of 0.5 g, the same researcher also points out that the mass and diameter of the pellets are also very relevant factors in the acceptance of the baits toxic. In this way, it was possible to assess that the particle mass influenced the loading rate, corroborating the hypothesis that the mass of charge interferes with transport and probably with the display of specific behavioral acts. Capsules with a 1.0 g loading mass showed an intermediate condition in the number of capsule transport, probably because the level of demand / effort of the task performed was not as simple as loading the 0.5 g capsules, the increase in the mass to be loaded increased the level of transport difficulty. Finally, the worst transport conditions were observed in the capsules with the highest mass (2.0 g), thus a very expressive mass resulted in low efficiency in the transport rate due to the high degree of difficulty of the task performed.

A means comparison test (Tukey Test) was carried out, which is based on the DMS (Minimum Significant Difference) at a 5% significance level to assess the attractiveness of the two substrates used (Pheromone and Citrus Pulp). The DSM value obtained was 1.97 and the

Table 1. Attractiveness of substrates.

Treatment	Average*
Pheromone	0.46 b
Citrus	6.00 a

*The means followed by different letters indicate a significant difference between them, at the level of 5% probability by the Tukey test.

standard error was 0.67. The means of the Pheromone and Citruspulp treatments showed a significant difference according to Table 1.

The lightest capsules were the most transported to the attractive citrus pulp (Figure 2D), comparingeach group of five mini colonies, being 47, 26 and 17, number respectively empty to (0.5g), with half mass (1g) and full (2g). There was great unrest among workers in the food arena, with 50 of them in a given colony, but little transport in the VGE (Figure 2C) assessment, even the empty ones (0.5g) were only 3 capsules transported, the half-load capsules (1g) were 4 and of (2g) none.

The colony with the highest transport rate 15 out of 20 reached 75% of the transported capsules. There were 26 capsules of 1g carried in all between the five colonies with attractive citrus pulp contrasted four of the same capsules of 1g with attractive VGE, that is, more than six times more for the same period of 24h, for these two groups a bi-cauldal distribution, taking into account the uneven variance of the samples, which resulted in a student T-test: (p=0.002933).

As for treatment with Pentano, few workers were observed in the feeding arena, including two colonies with the behavior of cutting the capsule in the arena and transporting the fragments to the symbiotic fungus garden, but not completely, adding up to 4 capsules transported in this waybetween the 5 mini colonies (Figure 2B).

Some capsules are waiting to be processed in the colony's connection tubes (Figure 2F) and the heavier ones suffered an attempt to cut in the foraging arena (Figure 2C), the greater the degree of difficulty in transport the more workers were recruited. Only a fragment of the fungus was observed in the garbage chamber, there was no capsules rejection in the garbage.

There was variation between plots since the rate of foraging varies from colony to the colony (Endringer et al., 2012), but among the attractive, it is evident the highest transport of breaded capsules with citrus pulp, a model already reported in (Travaglini et al., 2017), the rough surface allows the best fit of the jaws to carry out the transport by the worker in addition to the already confirmed attractiveness of the citrus pulp (Nagamoto et al., 2011).

Despite the great agitation due to the pheromone-based attractiveness, its transport was impaired due to the smooth surface of the gelatin capsules, different from what was observed by Tatagiba-Araujo et al. (2012) who made sesame and pheromone-based baits, obtaining a higher rate of loading, with the addition of VGE. For the control treatment using only pentane, such agitation was not repeated as in the treatments with VGE, which proves the importance of pheromone for the communication of the workers regarding the attractiveness of the substrate (Viana-Bailez and Endringer, 2016).

The synergy of the pulp and pheromone must be studied, as it has been confirmed, proving its active residual for 72 hours (Tatagiba-Araujo et al., 2012).

The most observed behavior was the analysis of the food, at least one worker was constantly found in the foraging arena manipulating the offered substrate, that is, licking the capsule with citrus pulp both in the arena and in the distribution of these on the fungus garden, after transport, the act of incorporation was not observed in the first five hours of the experiment, that is, the capsules were whole and closed, however, it was recorded after 24h differing from studies with vegetal tissue and granulated bait (Silva et al., 2015; Catalani et al., 2020).

Other studies demonstrate the susceptibility of the symbiont fungus to different natural compounds or bioinsecticides (Chacon-Orozco et al., 2018), which leads us to believe that focusing on field application technology will be the next step in this study, with the advantage of the stability of the salt compared a biological agent (Travaglini et al., 2017).

After a week of the test, the colonies that incorporated the salt in the fungus garden (Figure 2E), had an opaque hue on the symbiotic fungus garden visibly dehydrated, the dehydration effect led to a slight reduction in the volume of the fungus, even after returning to the laboratory creation, with controlled humidity and temperature and receive the due care of the maintenance technician (Forti et al., 1994). We cannot state categorically but visually comparing with colonies that did not go for the test there was a regression of the symbiotic fungus.

CONCLUSION

This study demonstrates the well-known attractiveness of citrus pulp over other products and showed how to transport salt inside the fungus garden (by capsules), we tried indicates a potential alternative control although inconclusive where synthetic chemicals cannot be used to incipient colonies.

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