Biological Control and Crop Protection

Chemical control of leaf-cutting ants: how do workers disperse toxic bait fragments onto fungus garden?

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ABSTRACT

Leaf-cutting ants are controlled with toxic baits. For the method's greater efficiency, the baits must be distributed and processed by workers during fungus cultivation. To test hypotheses whether the mode of action of the active ingredients, which blocks the sodium channels and interrupts the production of ATP in the mitochondria, interferes with fragment distribution in nests; the dispersion of pellets fragments during fungus cultivation by workers was assessed, spatially referencing the fragment onto the fungus garden. Pellets fragments were randomly distributed, but the amount pellets fragments was influenced by the way that the active ingredient acts in the colony.

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Introduction

Leaf-cutting ants – *Atta* Fabricius, 1805 and *Acromyrmex* Mayr, 1865 (Hymenoptera: Formicidae) – grow their symbiont fungus, *Leucocoprinus gongylophorus* Heim, 1957, with several economically relevant plant species, thus being considered as pests for agriculture, forests and livestock (Fowler et al., 1989; Montoya-Lerma et al., 2012; Mueller et al., 2018). Currently, the most efficient leaf-cutting ant control method is formicide baits for being technically, economically and operationally viable. These baits contain an attractive substrate and an insecticide (Britto et al., 2016).

Sulfluramid is the most used active ingredient due to its characteristics, namely: mode of action (Nagamoto et al., 2007, 2004; Schnellmann and Manning, 1990); non-repellency (Forti et al., 1998; Nagamoto et al., 2004); even dispersion onto colonies (Forti et al., 2007; Pretto et al., 1995; Pretto and Forti, 1993); stability when exposed to the environment (Cameron, 1990); and low toxicity to humans and other organisms (Britto et al., 2016). Sulfluramid, present in ant workers body, is broken and transformed into DESFA (perfluorooctanesulfonamide) and acts in the oxidative phosphorylation process, interrupting ATP production in the

mitochondria and becoming lethal to the insect. Ant workers show typical symptoms of intoxication by sulfluramid, i.e., slow movement and decreased aggressiveness due to their low energy levels, which drop until their metabolism is interrupted, causing their death (Britto et al., 2016; Schnellmann and Manning, 1990).

The mode of action of the active ingredient is any bait used in the control of leaf-cutting ants is important for its success in pest management programs. Ideally, the characteristics of an effective bait includes that the behavior of the foragers is not altered with the action of the insecticide and every bait offered is loaded into the nest before the workers die, increasing the distribution of the bait in the colony (Delabie et al., 2000); and consequently will have greater contamination of workers and control effectiveness. Therefore, without perceiving the presence of the active ingredient, fungus cultivation behaviors are not compromised; and thus, this molecule is efficient in controlling leaf-cutting ants (Della Lucia et al., 2014).

Formicide baits and vegetal substrate are equally processed by workers, and perform behaviors for fungus cultivation (Diniz and Bueno, 2009). In this way, the substrate is carried to the fungus garden, and workers groom it for its asepsis and moistening. Later, they chop the substrate and chew its edges; afterwards, these fragments are inserted into the fungus, and hyphae are inoculated on them (Andrade et al., 2002; Camargo et al., 2017; Garrett et al., 2016; Silva et al., 2015). Such behaviors are performed by garden and generalist workers, since these castes account for the greatest proportion of individuals in the colony (Andrade et al., 2002; Camargo et al., 2017, 2007; Lopes et al., 2004; Wilson, 1980). For

this reason, the substrate incorporation process is important in dispersing the active ingredient, as there is greater participation of these castes (Silva et al., 2015). Thus, the importance of an insecticide that allows the workers to perform all the behaviors involved in fungal cultivation, its spread, and low detectability, should be favored (Forti et al., 2007).

Slow-acting insecticides work for long periods of time in the colony, for this reason, sulfluramid is efficient as it kills workers slowly (Nagamoto, 2003). Due to its action, during processing, which lasts up to 18 h after baits are provided, workers do not perceive the presence of the insecticide, and 70% become contaminated (Forti et al., 2007; Pretto et al., 1995; Pretto and Forti, 1993). After the third or fourth day, workers stop foraging, there is a disarrangement in the colony, while a high worker mortality, as well as great fungus growth without possibility of recovering the mutualist fungus, are observed; the death of all individuals occurs between 16 and 22 days (Pretto and Forti, 1993). Thus, the way that this active ingredient acts in the colonies, sulfluramid-based formicide baits are efficient in controlling leaf-cutting ants (Britto et al., 2016; Cruz et al., 1996; Forti et al., 2003; Zanuncio et al., 1999, 1996), because every bait provided to the colony is processed and incorporated into the fungus.

In insect-pest control, an alternative is to use insecticides that block sodium channels, such as indoxacarb, which is highly efficient in controlling Lepidoptera-pests (Wing et al., 2000, 1998). Indoxacarb, once absorbed or ingested by the insect, interrupts feeding, which may occur within 2–8 hours (Dias, 2006), causing mortality in 48 h (Barr, 2003). Indoxacarb is a neurotoxic substance that, when present in the insect, after bioactivation, results in the formation of metabolite IN-JT333 (N-decarbomethoxyllated, DCJW), which blocks sodium channels; that is, it compromises the nervous function, interrupts feeding, paralyzes and kills the insect (Dias, 2006). For example, in *Solenopsis invicta*, this insecticide causes rapid foraging suppression, within 48 h, and occurring before colony elimination (Barr, 2003); however, indoxacarb has a potential use in leaf-cutting ant control (Stefanelli, 2019).

Every vegetal substrate carried by leaf-cutting ants is distributed into fungus chambers, at different depths, and the same happens with formicide baits (Forti and Silveira Neto, 1989; Moreira and Forti, 1999; Pretto and Forti, 2000). Thus, the distribution of the substrate transported by them relates to bait efficiency (Moreira et al., 2003; Moreira and Forti, 1999). It is known that baits are distributed into fungus chambers (Forti and Silveira Neto, 1989; Moreira et al., 2003; Moreira and Forti, 1999; Pretto and Forti, 2000), with a great participation of workers in processing, and, consequently, contaminating them (Camargo et al., 2017; Forti et al., 2019, 2007); nevertheless, it is not known how dispersion on the fungal cultivar occurs.

In light of the foregoing, we can raise the hypothesis that the mode of action of active ingredients in formicide baits directly affects workers during symbiont fungus cultivation and, consequently, the distribution of formicide bait fragments and worker contamination in the nest. Thus, we ask, "How do workers disperse toxic bait fragments onto the fungus garden?". To answer this question, the dispersion of pellets fragments during symbiont fungus cultivation by workers was assessed, spatially referencing the fragments and, later, applying a geostatistical analysis to access their spatial distribution.

Material and methods

Study species

Nine colonies of *Atta sexdens* used in this study were collected in March 2017, in Botucatu (22° 50′ 48″ S; 48° 26′ 06″ W), SP, and taken to the Laboratory of Social Insects-Pest [*Laboratório de Insetos Sociais-Praga*] (LISP) belonging to the Vegetal Protection Department, located at the College of Agronomical Sciences of the State University of São Paulo (FCA/UNESP), in Botucatu.

The colonies were stored in transparent plastic cylindrical containers (750 ml) with a plaster layer at the bottom (1 cm) to keep the fungus garden moist. Two holes were made on the opposite sides of these containers and, with pieces of a transparent hose, two containers with capacity for 250 ml were connected, one for vegetal substrate provision and one for residue deposition (waste). *Acalypha* sp. plant species was provided as vegetal substrate for fungal culture maintenance. The colonies were exposed to a temperature of 24 ± 2 °C and relative humidity of 70%.

Citric pulp pellets preparation

Pellets were manually prepared from citric pulp. Oranges were peeled and cut into small pieces, which were then dried in oven at 50 °C for 72 h; after drying, the pulp was crushed and stored in freezer until pellets preparation (Verza et al., 2006).

As tracer, a powdered liposoluble fluorescent yellow dye was used (BioQuip®, Rancho Dominguez, CA). The treatments employed were: control (no active ingredient), 0.3% sulfluramid (Britto et al., 2016) and 0.15% indoxacarb. Pellets preparation used citric pulp (65%), carboxymethylcellulose (CMC) (10%), corn oil solution with 5% fluorescent dye (25%), insecticide and distilled water to form a pasty mixture. The mixtures were put inside disposable plastic syringes (3 ml) to form fillets, which were then arranged on aluminum trays and oven dried at 50 °C for 21 h; after drying, they were stored in freezer until use in the experiment (Sousa et al., 2017).

Substrate dispersion onto the fungus

The experiment was run with three treatments and three repeats, totaling nine colonies; each colony had 600 ml of fungus on average. The colonies were not provided with substrate for 24 h before the experiment started. Pellets (0.51 g) with an average size of 0.5 cm in length were provided for each colony (Forti et al., 2012). Twenty-four hours after pellets provision, the colonies were stored in freezer for 24 h for assessment of the dispersion of pellets fragments processed and incorporated into the fungus. The fungus was assessed with the aid of an ultraviolet lamp, in a dark room, taking into consideration the location of incorporated fragments with fluorescent pigments in relation to axes X, Y and Z (the distance between the points was measured in centimeters (cm)); after measuring the distance of the fragment in relation to the axes, it was removed with entomological forceps.

Geostatistical analysis

The most commonly used method for geostatistical analysis is a variogram, which is a graph showing the spatial dependence of an organism where sampling variances of sampling pairs are plotted in relation to the distance between sampling points. These variances can be defined in terms of γ as a function of the separating distance between points (h):

$$\gamma(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} \left[Z_{x_i} - Z_{x_{i+h}} \right]^2$$

where: Z_{x_i} is the sampling value (1 = fungus presence and 0 = fungus absence) observed locally; x_i, $Z_{x_{i+h}}$ is the sampling value observed locally; x_{i+h} and $N(h)$ is the total number of sampling pairs for each of the separating distances (Isaaks and Srivastava,
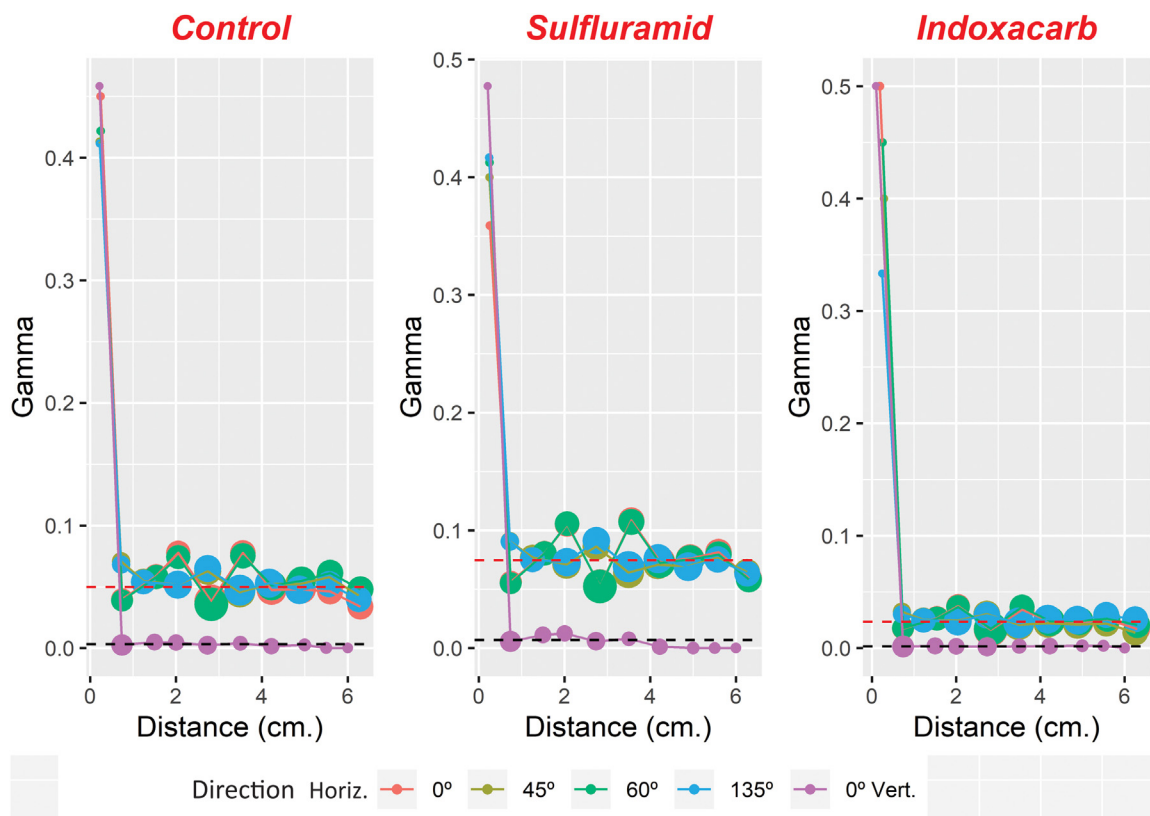
Fig. 1. Variograms columns: control, sulfluramid and indoxacarb.

1989). RGeostats package (Renard et al., 2017) was used together with R 3.4.0 integrated development environment for statistical and graphic calculations (Team, 2017).

Statistical analyses

Data on average number of fragments incorporated by workers in each treatment were compared by the ANOVA and the Tukey multiple comparisons test with 5% significance.

Results

The models derived from our study are presented below in Figure 1 and 3-D graphs:

https://www.youtube.com/watch?v=LzqBVScQITI
https://www.youtube.com/watch?v=N3Psu1WvAKw
https://www.youtube.com/watch?v=c7VPQvElVXk

In Fig. 1, the circles size is proportional to the number of point pairs used to calculate the variances. Because the first point represents only 0.042% of point pairs on average, it appears represented in the graph but was not considered important for model adjustment.

Variances for horizontal distances are greater than nearly null variances for vertical distances in control and sulfluramid treatments. In the treatment with indoxacarb, the variances of both distances are nearly null. The variation magnitude of the horizontal distances indicates a density of baits in the horizontal layers of the fungus and the variation magnitude of the vertical distances indicates density of baits along the depth profile of the fungus. Higher magnitudes indicates lower bait densities and vice versa.

The semivariogram morphology presents three important and interactive structures: the nugget effect (y-intercepts), threshold (x-axis point where sampling variance does not increase

or becomes random) and spatial dependence range (distance to threshold).

These random distribution variograms are linear and have little or no inclination, and the nugget effect is equal to the threshold. The main differences between these variograms are the low coefficient of determination and the high variation found in the random variogram. The determination coefficients ranged from −0.14 to −0.08. The graphs' presentation scale does not allow for viewing this phenomenon that characterizes randomness and is present in the three treatments.

As for mean number of pellets fragments incorporated into the fungus garden by workers, it varied between treatments ($F_{2,4}$ = 26.158, pval < 0.05) (Fig. 2). The multiple comparison test showed significant statistical differences in mean number of fragments incorporated by workers between control (A) and indoxacarb (C) treatments ($q_{3,6}$ = 4.34, D.F. = 2, $p <$ 0.05). A significant statistical difference was observed in mean number of fragments incorporated by workers between treatments with sulfluramid (B) and indoxacarb (C) ($q_{3,6}$ = 4.34, D.F. = 2, $p <$ 0.05). However, no significant statistical differences were observed in mean number of fragments incorporated by workers between control (A) and sulfluramid (B) treatments ($q_{3,6}$ = 4.34, D.F. = 2, $p >$ 0.05) (Fig. 2).

Discussion

In the present study, pellets fragment distribution occurred randomly, that is, it was subject to unpredictable variations resulting from uncontrollable factors related by chance (Underwood, 1997). The distribution is prone to randomness because workers place pellets fragments on the fungus growth region, since fungus grows randomly (Fig. 1). Nevertheless, amount of fragments was influenced by the way that the active ingredient acts in the colony (Fig. 2). Thus, results confirm the hypothesis that the mode of
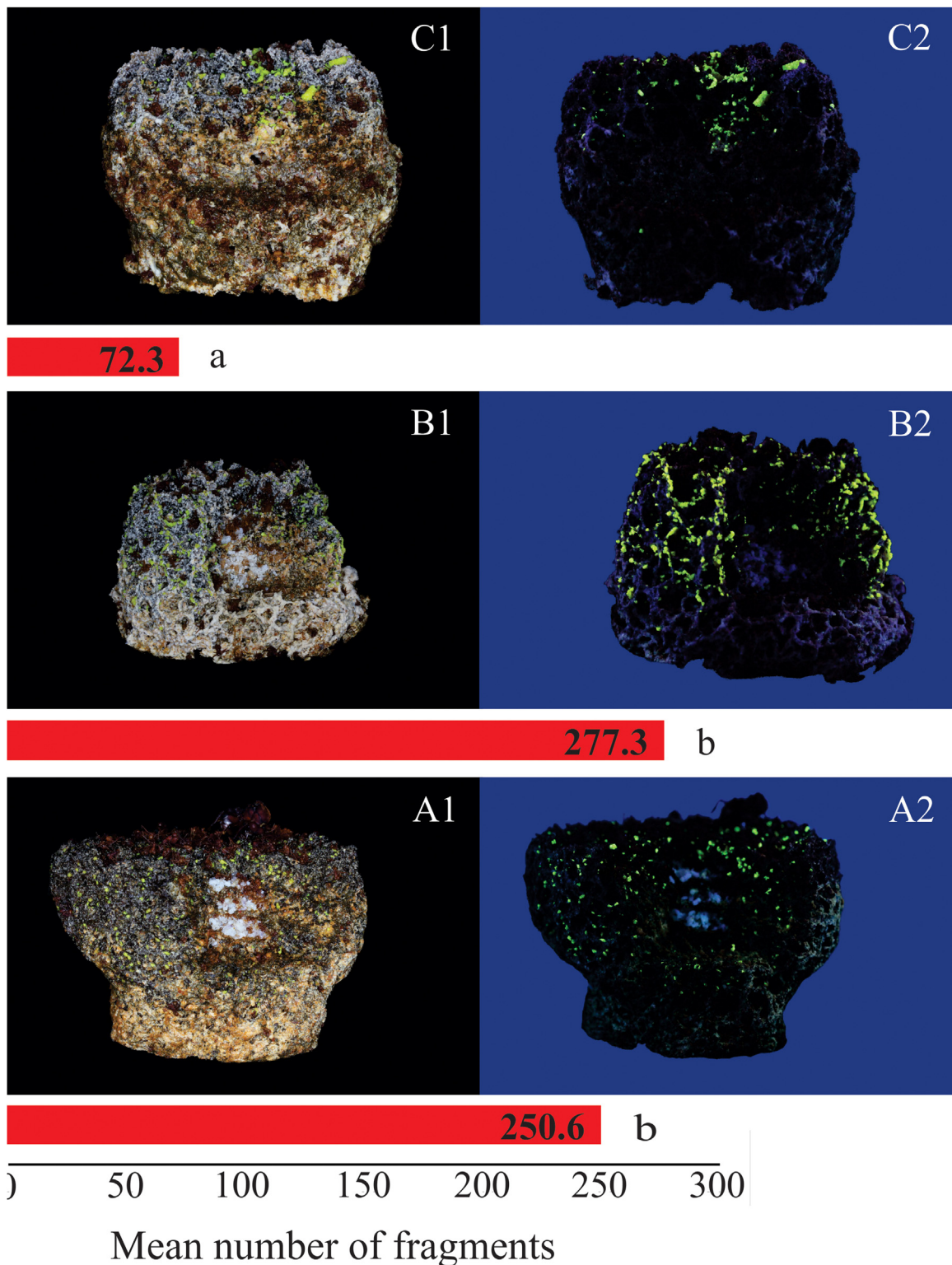
Fig. 2. Pellet fragment distribution onto the fungus garden, with and without active ingredients. A2, B2, C2: fragment distribution with ultraviolet light. A1 and A2: pellets without active ingredient. B1 and B2: pellets with sulfluramid. C1 and C2: pellets with with different action modes. A1, B1, C1: fragment distribution without ultraviolet light indoxacarb. Treatment followed the same letter is not significantly different.

action of active ingredients in formicide baits directly affects workers during fungus cultivation and, consequently, pellets fragments distribution.

The baits are processed by workers that perform a series of behaviors (Diniz and Bueno, 2009). The substrate is carried to the fungus garden, where they lick it until it is moist and simultaneously groom this material; they chop the substrate and chew its edges; these fragments are then incorporated into the fungus and inoculated (Andrade et al., 2002; Camargo et al., 2017; Garrett et al., 2016; Silva et al., 2015). The active ingredient disperses during fungus cultivation, contaminating workers (Forti et al., 2019; Silva et al., 2015). The mode of action of insecticides

can affect bait fragment distribution, because workers are contaminated with insecticide, and thus, affecting behaviors during fungus cultivation (Fig. 2). In sulfluramid and control treatments, distribution was similar; the same did not occur in the treatment with indoxacarb (Fig. 2).

In sulfluramid treatment the pellets were all processed and distributed onto the fungus without the workers perceiving the presence of the active ingredient. Insecticide with action such as sulfluramid, means a greater substrate distribution onto the fungus, allowing for workers to perform all behaviors involved in fungus cultivation until they start to die. However, using an active ingredient such as indoxacarb, prevents workers from completing pellets processing (Fig. 2). This occurs due to the action of the insecticide, which results in worker mortality in a short period of time (Dias, 2006).

Because distribution is affected by the insecticide's mode of action, those with action such as sulfluramid are more efficient in controlling leaf-cutting ants, as they act for longer periods in the colony, while faster ones act on the first days (Nagamoto, 2003). This active ingredient interrupts ATP production in the mitochondria, reducing the organism's energy production and becoming lethal to insects (Schnellmann and Manning, 1990). Workers show typical symptoms of intoxication by sulfluramid, i.e., slow movement and decreased aggressiveness due to their organisms' low energy levels, which drop until their metabolism is interrupted, causing their death (Britto et al., 2016; Schnellmann and Manning, 1990). Therefore, because of the action of sulfluramid, this active ingredient allows for workers to carry the baits, leaving them exposed to the insecticide during fungus cultivation and distributing the fragments (Fig. 2); that is, without perceiving the presence of the active ingredient, fungus cultivation behaviors are not compromised. This confirms the efficiency of this insecticide in controlling leaf-cutting ants (Britto et al., 2016; Cruz et al., 1996; Forti et al., 2003; Zanuncio et al., 1999, 1996).

Indoxacarb is a neurotoxic substance, which blocks sodium channels; that is, it compromises the nervous function, interrupts feeding, paralyzes, which may occur within 2–8 h, causing death (Dias, 2006). However, due to its action, this active ingredient interferes with pellets fragments distribution in the colony, because the workers die during processing (Fig. 2).

With the hypothesis on how bait fragment dispersion occurs and whether it is related to the mode of action of the active ingredient, it is concluded that acting insecticides such as sulfluramid, allows for fragment distribution onto and incorporation into the fungus. Thus, the fragment distribution is essential to the contamination of a higher number of workers.

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Conflict of interests

The authors declare no conflicts of interest.

Author's contribution

GCC, RSC and LCF conceived, designed and performed the experiment; GCC, RSC, CAOM and LCF analyzed the data; GCC, KKAS, RSC, NC, CAOM and LCF wrote the paper.

References

Andrade, A.P.P., Forti, L.C., Moreira, A.A., Boaretto, M.A.C., Ramos, V.M., Matos, C.A.O., 2002. Behavior of *Atta sexdens rubropilosa* (Hymenoptera: formicidae) workers during the preparation of the leaf substrate for symbiont fungus culture. Sociobiology 40, 293–306.

Barr, C.L., 2003. Fire ant mound and foraging suppression by indoxacarb bait. J. Agric. Urban Entomol. 20, 143–150.

Britto, J.S., Forti, L.C., Oliveira, M.A., Zanetti, R., Wilcken, C.F., Zanuncio, J.C., Loeck, A.E., Caldato, N., Nagamoto, N.S., Lemes, P.G., Camargo, R.S., 2016. Use of alternatives to PFOS, its salts and PFOSF for the control of leaf-cutting ants *Atta* and *Acromyrmex*. Int. J. Res. Environ. Stud. 3, 11–92.

Camargo, R.S., Puccini, C., Forti, L.C., Matos, C.A.O., 2017. Behaviors in fungus garden cultivation: routes of contamination of leaf cutting ant workers with fat-soluble tracer dye. Int. J. Agric. Innov. Res. 5, 555–560.

Camargo, R.S., Forti, L.C., Lopes, J.F.S., Andrade, A.P.P., Ottati, A.L.T., 2007. Age polyethism in the leaf-cutting ant *Acromyrmex subterraneus brunneus* Forel, 1911 (Hym., Formicidae). J. Appl. Entomol. 131, 139–145, http://dx.doi.org/10.1111/j.1439-0418.2006.01129.x.

Cameron, R.S., 1990. Potential baits for control of the Texas leaf-cutting ant, *atta texana* (hymenoptera: formicidae). In: Vander Meer, R.K., Jaffe, K., Cedeno, A. (Eds.), Applied Myrmecology: A World Perspective. Westview Press, Boulder, pp. 628–637.

Cruz, A.P., Zanuncio, J.C., Zanetti, R., Gomes, O.S., 1996. Eficiência de iscas formicidas à base de sulfluramida e de clorpirifós no controle de *Atta sexdens sexdens* (Hymenoptera, Formicidae), no trópico úmido. Acta Amaz. 26, 145–150.

Delabie, J.H.C., Della Lucia, T.M.C., Pastre, L., 2000. Protocolo de experimentação para avaliar a atratividade de novas formulações de iscas granuladas utilizadas no controle das formigas cortadeiras *Acromyrmex* spp. E *Atta* spp.(Hymenoptera: formicidae: myrmicinae: attini) no campo. An. da Soc. Entomológica do Bras. 29, 843–848.

Della Lucia, T.M., Gandra, L.C., Guedes, R.N., 2014. Managing leaf-cutting ants: peculiarities, trends and challenges. Pest Manag. Sci. 70, 14–23, http://dx.doi.org/10.1002/ps.3660.

Dias, J.L., 2006. Environmental Fate of Indoxacarb. Department of Pesticide Regulation, Sacramento - CA.

Diniz, E.A., Bueno, O.C., 2009. Substrate preparation behaviors for the cultivation of the symbiotic fungus in leaf-cutting ants of the genus *Atta* (Hymenoptera: formicidae). Sociobiology 53, 651–666.

Forti, L.C., Camargo, R.S., Andrade, A.P.P., Catalani, G.C., Sousa, K.K.A., Silva, A.A.C., Caldato, N., Ramos, V.M., 2019. Contamination route of leaf-cutting worker ants analyzed through a fat-soluble tracer dye in toxic bait. Neotrop. Entomol. 48, 349–355, http://dx.doi.org/10.1007/s13744-018-0652-1.

Forti, L.C., Nagamoto, N.S., Pretto, D.R., 1998. Controle de formigas cortadeiras com isca granulada. In: Berti Filho, E., Mariconi, F.A.M., Fontes, L.R. (Eds.), Simpósio Sobre Formigas Cortadeiras Dos Países Do Mercosul. FEALQ, Piracicaba. , pp. 113–132.

Forti, L.C., Nagamoto, N.S., Ramos, V.M., Andrade, A.P.P., Lopes, J.F.S., Camargo, R.S., Moreira, A.A., Boaretto, M.A.C., 2003. Eficiencia de sulfluramida, fipronil y clorpirifos como sebos en el control de *Atta capiguara* Gonçalves (Hymenoptera: formicidae). Pasturas Trop. 25, 28–35.

Forti, L.C., Pretto, D.R., Nagamoto, N.S., Padovani, C.R., Camargo, R.S., Andrade, A.P.P., 2007. Dispersal of the delayed action insecticide sulfluramid in colonies of the leaf-cutting ant *Atta sexdens rubropilosa* (Hymenoptera: formicidae). Sociobiology 50, 1149–1164.

Forti, L.C., Silva, M.S., Fujihara, R.T., Caldato, N., Garcia, M.G., 2012. Trajectory of water-and fat-soluble dyes in the grass-cutting ant *Atta capiguara*: evaluation of infrabuccal cavity, post-pharyngeal glands and gaster (Hymenoptera, Formicidae). Sociobiology 59, 511–520, http://dx.doi.org/10.13102/sociobiology.v59i2.614.

Forti, L.C., Silveira Neto, S., 1989. Distribuição de substrato em colônia de *Atta capiguara* Gonçalves, 1944 (Hymenoptera, Formicidae). An. da Soc. Entomológica do Bras. 18, 347–356.

Fowler, H.G., Pagani, M.I., Silva, O.A., Forti, L.C., Silva, V.P., Vasconcelos, H.L., 1989. A pest is a pest is a pest? The dilemma of neotropical leaf-cutting ants: keystone taxa of natural ecosystems. Environ. Manage. 13, 671–675.

Garrett, R.W., Carlson, K.A., Goggans, M.S., Nesson, M.H., Shepard, C.A., Schofield, R.M.S., 2016. Leaf processing behaviour in *Atta* leafcutter ants: 90% of leaf cutting takes place inside the nest, and ants select pieces that require less cutting. R. Soc. Open Sci. 3, 150111, http://dx.doi.org/10.1098/rsos.150111.

Isaaks, E.H., Srivastava, R.M., 1989. An Introduction to Applied Geostatistics. Oxford University Press, New York.

Lopes, J.F.S., Forti, L.C., Camargo, R.S., 2004. The influence of the scout upon the decision-making process of recruited workers in three *Acromyrmex* species (Formicidae: attini). Behav. Processes 67, 471–476, http://dx.doi.org/10.1016/j.beproc.2004.08.001.

Montoya-Lerma, J., Giraldo-Echeverri, C., Armbrecht, I., Farji-Brener, A., Calle, Z., 2012. Leaf-cutting ants revisited: towards rational management and control. Int. J. Pest Manag. 58, 225–247, http://dx.doi.org/10.1080/09670874.2012.663946.

Moreira, A.A., Forti, L.C., 1999. Distribuição de substratos nas colônias de Atta laevigata (F. Smith, 1858) (Hymenoptera: formicidae). Sci. Agric. 56, 465–469, http://dx.doi.org/10.1590/S0103-90161999000200029.

Moreira, A.A., Forti, L.C., Boaretto, M.A.C., Andrade, A.P.P., Rossi, M.N., 2003. Substrate distribution in fungus chambers in nests of Atta bisphaerica Forel, 1908 (Hym., Formicidae). J. Appl. Entomol. 127, 96–98, http://dx.doi.org/10.1046/j.1439-0418.2003.00719.x.

Mueller, U.G., Kardish, M.R., Ishak, H.D., Wright, A.M., Solomon, S.E., Bruschi, S.M., Carlson, A.L., Bacci Jr, M., 2018. Phylogenetic patterns of ant–fungus associations indicate that farming strategies, not only a superior fungal cultivar, explain the ecological success of leafcutter ants. Mol. Ecol. 27, 2414–2434, http://dx.doi.org/10.1111/mec.14588.

Nagamoto, N.S., 2003. Estudos Toxicológicos De Princípios Ativos Utilizando Como Modelo Atta sexdens Rubropilosa (Hymenoptera, Formicidae). 234f. Tese (Doutorado Em Agronomia: Proteção De Plantas), Faculdade De Ciências Agronômicas, Universidade Estadual Paulista, Botucatu.

Nagamoto, N.S., Forti, L.C., Andrade, A.P.P., Boaretto, M.A.C., Wilcken, C.F., 2004. Method for the evaluation of insecticidal activity over time in Atta sexdens rubropilosa workers (Hymenoptera: formicidae). Sociobiology 44, 413–432.

Nagamoto, N.S., Forti, L.C., Raetano, C.G., 2007. Evaluation of the adequacy of diflubenzuron and dechlorane in toxic baits for leaf-cutting ants (Hymenoptera: formicidae) based on formicidal activity. J. Pest Sci. 80 (2004), 9–13, http://dx.doi.org/10.1007/s10340-006-0143-8.

Pretto, D.R., Forti, L.C., 2000. Dyed baits distribution in nests of Atta sexdens L. (Hym., Formicidae). J. Appl. Entomol. 124, 37–39, http://dx.doi.org/10.1046/j.1439-0418.2000.00431.x.

Pretto, D.R., Forti, L.C., 1993. Metodologia Para Estudo Da Dispersão De Inseticidas Em Colônias De Atta sexdens Rubropilosa Forel, 1908 (Hymenoptera, Formicidae), in: International Simposium on Pest Ants. 11° Encontro De Mirmecologia, Belo Horizonte - MG., pp. 61.

Pretto, D.R., Forti, L.C., Nagamoto, N.S., 1995. Dispersão de inseticida em colônia de atta sexdens rubropilosa forel, 1908 (hymenoptera, formicidae) no laboratório. In: Brasileiro de Entomologia, Congresso (Ed.), 15° Congresso Brasileiro De Entomologia. Caxambú - MG. , p. 437.

Renard, D., Bez, N., Dsesassis, N., Beucher, H., Ors, F., Freulon, X., 2017. RGeostat: The Geostatistical R Package [V 11.12].

Schnellmann, R.G., Manning, R.O., 1990. Perfluorooctane sulfonamide: a structurally novel uncoupler of oxidative phosphorylation. Biochim. Biophys. Acta - Bioenerg. 1016, 344–348, http://dx.doi.org/10.1016/0005-2728(90)90167-3.

Silva, L.C., Camargo, R.S., Forti, L.C., Matos, C.A.O., Travaglini, R.V., 2015. Do Atta sexdens rubropilosa workers prepare leaves and bait pellets in similar ways to their symbiotic fungus? Sociobiology 62, 484–493, http://dx.doi.org/10.13102/sociobiology.v62i4.772.

Sousa, K.K.A., Camargo, R.S., Forti, L.C., 2017. Communication or Toxicity: What Is the Effect of Cycloheximide on Leaf-Cutting Ant Workers? Insects 8, 126, http://dx.doi.org/10.3390/insects8040126.

Stefanelli, L.E.P., 2019. Estudo Comparativo Dos Inseticidas Sulfluramida E Indoxacarbe Para Atta sexdens Rubropilosa Forel, 1908 (Hymenoptera: Formicidae). 107p. Dissertação (Mestrado Em Agronomia: Proteção De Plantas), Faculdade De Ciências Agronômicas, Universidade Estadual Paulista, Botucatu.

Team, R.C., Vienna, Austria 2017. R: a Language and Environment for Statistical Computing [Internet]., pp. 2014.

Underwood, A.J., 1997. Experiments in Ecology. Cambridge University Press, Cambridge.

Verza, S.S., Forti, L.C., Matos, C.A.O., Garcia, M.G., Nagamoto, N.S., 2006. Attractiveness of citrus pulp and orange albedo extracts to Atta sexdens rubropilosa (Hymenoptera: formicidae). Sociobiology 47, 391–399.

Wilson, E.O., 1980. Caste and division of labour in leaf-cutter ants (Hymenoptera: formicidae: atta). I. The overall pattern in Atta sexdens. Behav. Ecol. Sociobiol. (Print) 7, 143–156.

Wing, K.D., Sacher, M., Kagaya, Y., Tsurubuchi, Y., Mulderig, L., Connair, M., Schnee, M., 2000. Bioactivation and mode of action of the oxadiazine indoxacarb in insects. Crop Prot. 19, 537–545, http://dx.doi.org/10.1016/S0261-2194(00)00070-3.

Wing, K.D., Schnee, M.E., Sacher, M., Connair, M., 1998. A novel oxadiazine insecticide is bioactivated in lepidopteran larvae. Arch. Insect Biochem. Physiol. 37, 91–103, http://dx.doi.org/10.1002/(SICI)1520-6327(1998)37:1<91::AID-ARCH11>3.0.CO;2-5.

Zanuncio, J.C., Cruz, A.P., Santos, D.F., Oliveira, M.A., 1996. Eficiência da isca Mirex-S (sulfluramida 0, 3%) no controle de Atta cephalotes (Hymenoptera: formicidae) em três dosagens. Acta Amaz. 26, 115–120.

Zanuncio, J.C., Zanuncio, T.V., Pereira, J.M.M., Oliveira, H.N., 1999. Controle de Atta laevigata (Hymenoptera: formicidae) com a isca Landrin-F, em área anteriormente coberta com Eucalyptus. Ciência Rural 29, 573–576.