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Effects of Diafenthiuron in Toxic Baits on Colonies of Leaf-cutting ants (Hymenoptera: Formicidae)

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Abstract

Dianfenthiuron is a pre-insecticide that can be activated by photolysis, and may be a promising formicide. This study evaluated the effect of diafenthiuron after photolysis in colonies of *Atta sexdens rubropilosa* Forel, 1908. The experiment was conducted in a completely randomized design with five treatments and five replications: control (no active ingredient), sulfluramid (standard formicide), diafenthiuron (no exposure to UV), diafenthiuron (2h exposure to UV) and diafenthiuron (6h exposure to UV). Toxic baits were applied at a rate of 0.5 g per colony, and we observed the transport and incorporation of the baits into the colonies. A grading scale was used (0 to 4) to measure the cutting of *Acalypha* L. (Euphorbiaceae) leaves by workers at 2, 7, 14 and 21 days after application (DAA) and we also measured the garden mass (fungus + adult + brood) at 21 DAA in order to check for growth of the fungus culture. Total loading and incorporation occurred one hour after application of the baits. Colonies that received sulfluramid did not transport leaves at 2 DAA. Workers that received baits with D2h showed an average of 20% transport and 55% incorporation of leaves at 21 DAA. The grading scale indicated that treatments D2h and D6h had the lowest averages, 0.80 and 2.00, respectively. The treatments D2h and D6h reduced cutting of leaves and fungus garden mass, but did not kill the colonies of *A. sexdens rubropilosa*.

Introduction

The leaf-cutting ants are considered important pest insects in agricultural and forestry crops by causing economic damage in areas of the Neotropical region, mainly in Brazil (Reis et al., 2010). The main challenge in controlling leaf-cutting ants is the search for insecticides which are less harmful to agricultural ecosystems, but still effective when formulated as toxic granular baits (Forti et al., 2007).

Some insecticides have already been tested for control of leaf-cutting ants, such as chlorpyriphos (Forti et al., 2003), diflubenzuron and dechlorane (Nagamoto et al., 2007), but without success in manufacturing and marketing them as formicide baits. However, diafenthiuron ($C_{23}H_{32}N_2OS$) is presented as a promising formicide; its activity against other groups of insects and mites is very well known (Ishaaya et al., 2007). Moreover, as it affects cellular respiration (Dekeyser, 2005), its mechanism of action in insects is similar to that of sulfluramid, which is the main active ingredient in toxic baits.

Diafenthiuron is a pre-insecticide that, after photolysis, is converted into carbodiimide, becoming a potent insecticide (Ruder & Kayser, 1993). This physical-chemical characteristic can be manipulated in the laboratory. In this context, the present study evaluated the effect of diafenthiuron after photolysis, when supplied in formulation of toxic bait to colonies of *Atta sexdens rubropilosa* Forel, 1908.

Material and Methods

Study site

In 2012, young colonies (8 months old) from the Laboratory of Social Pest Insects, FCA/UNESP, Botucatu, São

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Paulo, Brazil, were maintained under controlled temperature and humidity (24°C ± 2 and 70 ± 5%) in plastic containers with a capacity of 395 mL volume for the fungus chamber and 250 mL for the waste and foraging chambers.

At the fungus chamber, a 1.0 cm plaster layer was placed at the base (maintenance of humidity). Colonies were given leaves of *Acalypha* L. (Euphorbiaceae) as vegetable substrate, which were offered in a natural state or dehydrated, according to humidity conditions.

Preparation and application of treatments

The baits applied to the colonies of *A. sexdens rubropilosa* had the following treatments: Ctl - control (no active ingredient); Sul - sulfluramid (standard formicide - 0.3% of the active ingredient - Mirex-S®); Dne - diafenthiuron (no exposure to ultraviolet light); D2h - diafenthiuron [2h exposure to ultraviolet light (UV)] and; D6h - diafenthiuron [6h exposure to ultraviolet light (UV)]. The use of sulfluramid as formicide is already known (Zanetti et al., 2003; Nagamoto et al., 2004), and so it was used as a standard comparative to diafenthiuron.

Treatments with diafenthiuron contained the following ingredients: vegetable soybean oil (Soya®) (5%), dehydrated citrus pulp (94%), active ingredient (1%) and distilled water. The treatments D2h and D6h were mixed with a solution of acetone + distilled water (3:1) and then exposed to ultraviolet light with a peak wavelength of 253nm (TUV-30W T8, Philips Electronics Brasil®) (adapted and modified from Keum et al., 2002) during the predetermined periods (2h and 6h). Afterwards, they remained in the absence of light for 24 hours for the decomposition into bioactive forms (mainly carbodiimide).

We applied 0.5g baits/colony, the equivalent of 22 pellets of 0.5 cm in length each. The baits were offered in the foraging chamber only once, when it was observed the transport and the incorporation to the colony.

Evaluations and data analysis

A grading scale was used [(grading - cutting % - leaf cutting area cm^2): 0-0-0.00; 1-25-17.38; 2-50-34.76; 3-75-52.14; 4-100-69.52] to evaluate the cutting of leaves of *Acalypha* by workers after the treatment with baits at 2, 7, 14 and 21 days after application (DAA).

This scale was designed based on the average sampling of *Acalypha* leaves supplied daily (1 whole leaf of average size for each colony) after analysis of leaf area by the equipment LI-COR® BIOSCIENCES (LI-3100C AREA METER). The mass of the garden (fungus + adult + brood) was also measured at 21 DAA, to check for growth of the fungus culture.

The experiment was conducted in a completely randomized design with five treatments (bait) and five replications, each plot was represented by a colony. To assess the cutting of *Acalypha* leaves (grading scale), it was used a factorial arrangement (5x4), where the first factor was the group of treatments with baits, and the second, the days after application of baits (DAA), comparing the means by Tukey's test ($P < 0.05$).

Data of transport and incorporation of the leaves were evaluated in percentage. Differences in the growth of the fungus culture (mass) were tested by analysis of variance with comparison of means of treatments with baits by Tukey's test ($P < 0.05$). The assumption of normality was tested by Shapiro-Wilk test ($P < 0.05$). The statistical analysis was performed in the Sisvar 5.3 software (Ferreira, 2011).

Results and Discussion

Total transport and incorporation of baits were observed in all treatments, one hour after application. Although young, the colonies exhibited significant foraging activity throughout the rearing period. However, at 2 DAA, the workers that received sulfluramid no longer transported leaves (Fig 1-A) and showed visible symptoms of poisoning, such as slow motion, stretched and paralyzed hind legs (Nagamoto et al., 2007), reducing or even stopping the execution of the task.

At 7 DAA, only the colonies that received the treatment Ctl continued to transport leaves in a way similar to that observed before the beginning of the experiment. The treatments D2h and D6h have already demonstrated reduced transport of leaves (Fig 1-A). In the treatment Ctl, 100% of the transported leaves were incorporated, and the percentages were higher than 90% in Dne and D6h. On the other hand, at 7 DAA, there was no incorporation in Sul, due to the mortality of workers and in D2h, the average incorporation of leaves was reduced by 55% at 21 DAA (Fig 1-B).

The grading scale for the cutting of leaves of *Acalypha* by foragers ($F = 115.94$; $P = 0.0001$) (Table 1) assigned the highest score to the treatment Ctl, and then to Dne. The treatments D2h and D6h reached intermediate scores because the workers made the cut, but carried few fragments to the fungus chamber. On the other hand, sulfluramid prevented the cutting of leaves by the workers, yielding a score of zero (no cutting). This score refers to the disruption of the activity, because ants stop performing it after contact with the bait containing this active ingredient.

However, the average of the grading scale was obtained regardless of the period of observation of the cutting behavior, because the worker can reduce, maintain or increase this activity when under the effect of active ingredients. Thus, the evaluation of cutting of leaves was based on the significant interaction between treatments (bait) and DAA ($F = 3.39$; $P = 0.0005$) breaking down the treatments within each day and using the grading scale in the period from 7 to 21 DAA (Table 1). Thus, Ctl colonies maintained an average of about 4.00 in all periods analyzed. In Dne, the cutting of leaves decreased over time (3.00) and D2h showed a marked decrease (0.80).

Table 1. Grading scale (0 to 4) for the cutting of *Acalypha* sp. leaves by *Atta sexdens rubropilosa* workers after the treatment with baits at 2, 7, 14 and 21 DAA (days after application).

Treatment	DAA			
	2	7	14	21
Ctl	3.80 ± 0.20 cA	3.80 ± 0.20 dA	3.60 ± 0.24 dA	4.00 ± 0.00 cA
Sul	0.00 ± 0.00 aA	0.00 ± 0.00 aA	0.00 ± 0.00 aA	0.00 ± 0.00 aA
Dne	3.80 ± 0.20 cA	3.40 ± 0.40 cdA	3.40 ± 0.24 cdA	3.00 ± 0.00 bcA
D2h	3.80 ± 0.20 cC	2.40 ± 0.60 bcB	1.60 ± 0.24 bAB	0.80 ± 0.37 aA
D6h	2.04 ± 0.24 bA	2.00 ± 0.32 bA	2.20 ± 0.37 bcA	2.00 ± 0.32 bA

Means, within a column/row, followed by different small/capital letters are different by Tukey test (P<0,05). Treatments: Ctl (control); Sul (sulfluramid); Dne (diafenthiuron no exposure to ultraviolet light), D2h (diafenthiuron with 2h exposure to ultraviolet light); D6h (diafenthiuron with 6h exposure to ultraviolet light). D6h (diafenthiuron with 6h exposure to ultraviolet light). DAA: days after application.

To assess the general development of the colony, we analyzed the growth based on the mass of the fungus chamber = queen, workers and brood (F = 25.75, P = 0.0001) (Table 2). At 21 DAA, the mass of *A. sexdens rubropilosa* was higher in Ctl due to lack of active ingredient, followed by Dne, D2h and D6h. There was no assessment in Sul, given the total mortality of the colony.

Aspects of poisoning of workers (symptoms), presence of dead ants, growth of contaminant yeast (mainly *Escovopsis* Muchovej & Della Lucia, Ascomycetes) and survival of the colony at the end of the study are shown in Table 3. The effect of baits in the colonies of *A. sexdens rubropilosa* was only detected when baits contained the active ingredient sulfluramid.

This result was remarkable in this treatment for all the variables cited, however, the baits applied with Dne, D2h and D6h caused symptoms of poisoning, reduced mobility (slowness) during the first DAA, but somehow ants managed to regain mobility without affecting survival of the colony, only changing the natural state of development. A general analysis of diafenthiuron efficiency as formicide leads to some questions before assessing its actual effect on colonies of *A. sexdens rubropilosa*:

The bait was attractive? When preparing a formicide, the attractiveness can directly affect the efficiency, because it is essential to encourage the contact and ingestion with the active ingredient, thus allowing the contamination by the insecticide.

Table 2. Mass of the fungus of *Atta sexdens rubropilosa* colonies 21 DAA (days after application).

Treatment	Fungus garden + adults + offspring (g)
Ctl	30.38 ± 1.46 c
Dne	26.00 ± 1.22 b
D2h	22.85 ± 0.24 b
D6h	17.35 ± 0.97 a
Coefficient of variation (%)	10,00

Means followed by different letters are significantly different by Tukey test (P<0,05). Treatments: Ctl (control); Dne (diafenthiuron no exposure to ultraviolet light), D2h (diafenthiuron with 2h exposure to ultraviolet light); D6h (diafenthiuron with 6h exposure to ultraviolet light).

However, the transport by the workers was 100% one hour after application of baits, proving the attractiveness of citrus pulp, which has been already recognized (Brugger et al., 2008).

Were the colonies in constant foraging? Another problem is the activity of the colonies prior to experimentation (rearing stage), because according to Delabie et al. (2000), it is important to make sure that nests used in experiments of attractiveness are active. These should have good transport of leaves and large size workers (head width greater than 2 mm). Although some of these steps are determined for experimentation in the field, they must also be taken in the laboratory (Nagamoto et al., 2004), since they can mask the efficiency of promising insecticides.

Was there a simple rejection (selection) of the leaves of Acalypha after application of baits? In Figure 1-A, treatments with diafenthiuron reduced the transport of leaves, reaching only 20% with D2h. In this case, it would hardly be possible, because from the beginning of rearing it has been offered, cut and transported the same plant material, preventing confusion in the selection due to the conditioning of foragers workers and scouts to remain with this odor, making it a characteristic sign of the substrate, as mentioned by Brugger et al. (2008).

Once clarified these questions they may be disregarded, because all colonies transported the baits efficiently and did not discharge them in the waste chamber, or in the foraging chamber, in observations held 24 hours after application.

What is the mechanism of action of diafenthiuron? Sulfluramid is a chemical compound that acts directly on the mitochondria by inhibiting the ATP synthesis (adenosine triphosphate). The de-ethylated metabolite of sulfluramid is produced by the metabolism of cytochrome P450, a potent electron uncoupler of the mitochondrial respiratory chain, interrupting the production of energy. This disruption and subsequent loss of ATP results in inactivity, paralysis and death of the insect exposed to sulfluramid (Heong et al., 2011). Diafenthiuron also has a mechanism of action similar to sulfluramid in insects and mites (Ruder & Kayser, 1993), which could be enhanced when subjected to UV, as it is a pre-insecticide, which needs to turn into a secondary metabolite, such as the carbodiimide, to become a potent insecticide (Kayser & Eilinger, 2001; Dekeyser, 2005).

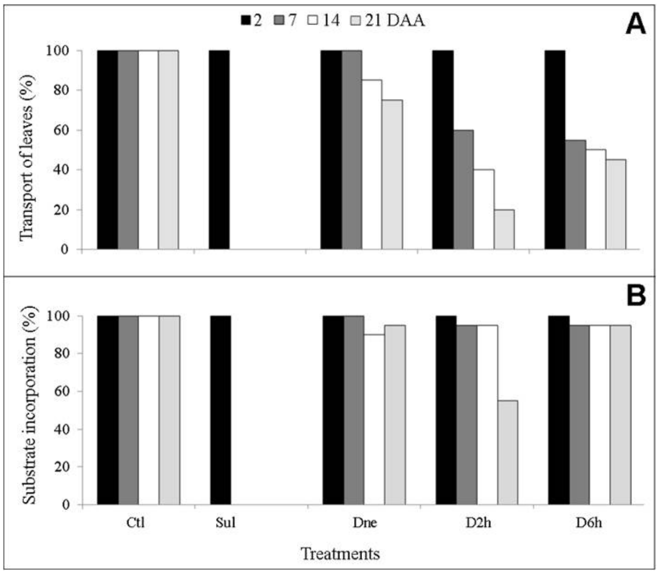

Fig 1. Transport of *Acalypha* leaves (A) and substrate incorporation (B) by *A. sexdens rubropilosa* workers after application of baits. Treatments: Ctl (control); Sul (sulfluramid); Dne (diafenthiuron no exposure to ultraviolet light), D2h (diafenthiuron with 2h exposure to ultraviolet light); D6h (diafenthiuron with 6h exposure to ultraviolet light). DAA (days after application).

Diafenthiuron was initially developed to control pests, such as mites, white flies and aphids (Kayser & Eilinger, 2001). Given the low toxicity to some species of insects, it has been reported as a promising insecticide when applied at recommended doses, and with potential to be used in Integrated Pest Management (IPM), as it is not harmful to beneficial insects, especially the biological control agents of insect pests (Preetha et al., 2009).

Among these, stands out the predator of aphids *Chrysoperla carnea* (Stephens, 1836) (Neuroptera: Chrysopidae), beetles of the family Coccinellidae and *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) wasps. For *C. carnea*, for instance, the application of a high dose of diafenthiuron (3.2 $g.L^{-1}$) caused 53% of adult mortality, and a low dose (0.8 $g.L^{-1}$), only 3%, showing low effect on this species, as the increase in diafenthiuron by up to four times only increased by 50% the adult mortality (Preetha et al., 2009).

Table 3. General state of *Atta sexdens rubropilosa* colonies after application of baits.

Treatment	State of colonies: Intoxication	Dead ants	Contaminant fungus	Dead colonies
Ctl	DNO	NM	DNO	DNO
Sul	1 DAA	TM	3 DAA	5 DAA
Dne	OFDAA	NM	DNO	DNO
D2h	OFDAA	NM	DNO	DNO
D6h	OFDAA	NM	DNO	DNO

Treatments: Ctl (control); Sul (sulfluramid); Dne (diafenthiuron no exposure to ultraviolet light), D2h (diafenthiuron with 2h exposure to ultraviolet light); D6h (diafenthiuron with 6h exposure to ultraviolet light). DNO: do not occurred. DAA: days after application. OFDAA: occurred in the first days after application. NM: natural mortality. TM: total mortality.

In leaf-cutting ants, the low toxicity of diafenthiuron, when ingested, can be explained by two ways: (*i*) due to the possibility of low dose application to the treated colonies, which reduces the effect of the compound, and (*ii*) due to the resistance of workers to the insecticide, which may have presented mechanisms of detoxification in relation to diafenthiuron or its secondary metabolite, although it had affected the execution of activities by workers.

At last, treatments containing D2h and D6h reduced the cutting of leaves and fungus garden mass, but did not kill the colonies of *A. sexdens rubropilosa*. Bueno (2013) also studied the toxicity of active ingredients (diafenthiuron and tolfenpyrad) in *A. sexdens rubropilosa* workers and the results showed significant reduction in the survival of workers when compared with controls of each ingredient, particularly when they were dissolved in soybean oil. However, baits containing the active ingredients were not effective in controlling colonies of *A. sexdens rubropilosa*. Diafenthiuron did not kill the colonies of *A. sexdens rubropilosa* on the doses studied, however it should be tested in other dosages of ultraviolet light and in different formulations or in consortium with other chemicals compounds before being totally discarded as formicide.

After photolysis by UV, diafenthiuron interfered with the cutting and transport of leaves to the colony, but was not efficient against young colonies of *A. sexdens rubropilosa*. The colonies of leaf-cutting ants that received toxic baits with diafenthiuron (6h exposure to UV) developed less mass of the fungus 21 days after application.

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