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Alternative Control of the Leaf-Cutting Ant *Atta bisphaerica* Forel (Hymenoptera: Formicidae) Via Homeopathic Baits

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Abstract

Leaf-cutting ants are pests that afflict diverse crops, and are most efficiently controlled by chemical methods that are widely utilized. Other methods have been investigated aiming to efficiently control these insects while reducing the environmental impact of applying such chemical products. Therefore, an assay was conducted to evaluate the efficiency of baits, formulated homeopathically, in nests of the leaf-cutting ant *Atta bisphaerica*, in the field. Thirty (30) colonies were chosen and divided into 10 repetitions for each of the following treatments: control (without baits), standard (8 g/m^2 of loose soil of baits based on sulfluramid 0.3%) and homeopathic (60 g/m^2 of loose soil of homeopathic baits parceled into 20g/m^2 doses applied on 3 consecutive days). At 24 hours after bait application on active foraging trails of colonies, the evaluation of of the following parameters was initiated: transport and devolution of the baits, foraging and mortality. The completed assay demonstrated that the transport of baits was greater in the standard (80%) than in the homeopathic treatment (50%). On the other hand, the devolution of baits was significantly higher in the homeopathic treatment (15%) versus the conventional, where devolution/rejection did not occur. Colony mortality was 20% under the homeopathic treatment, differing statistically from the 80% value produced by the standard treatment. Thus, the homeopathic treatment is not demonstrated to be efficient at controlling leaf-cutting ants, suggesting the need for new studies with different methodologies.

Introduction

The insects of the family Formicidae are known for their highly organized colonies that consist of millions of individuals. In terrestrial ecosystems, ants may constitute 15 to 20% of the total animal biomass (Schultz, 2000). The leaf-cutting ants belong to the tribe Attini, are present throughout tropical portions of the Americas and are the greatest consumers of vegetal mass in Brazil when compared with other insects and even with mammals. When they carry the leaves under the soil, the large quantity of organic material that is made available becomes a source of carbon and other nutrients for other organisms. These insects play an important role in ecosystems, but, despite the benefits they confer, they become pests when in proximity to agriculture and need to be controlled (Santos, 2010).

The environmental impact of anthropogenic activity directly affects terrestrial ecosystems, such as, for example, the application of agrochemicals. Before the physicochemical dispersion or biotransformation of insecticides, they can act as an important element in the disruption of trophic interactions in terrestrial ecology (Tiepo et al., 2010). Large quantities of insecticides are used for combating pests, but the introduction of xenobiotic molecules in the environment can damage the equilibrium of the ecosystem because synthetic insecticides may present high toxicity and, generally, low biodegradability, which can lead to a persistent toxic action (Tiepo et al., 2010). A large amount of chemical pesticides is utilized to meet commercial demands of industry and consumers, but given the growing public concern about the presence of their residues in water supplies or food, many producers are coming to adopt production systems that are purely organic or more ecologically integrated (Boff et al., 2008).

In order to reduce the harm caused by leaf-cutting ants, humans have pursued diverse forms of control, ranging from homemade methods to the use of advanced techniques (Souza

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et al., 2011). The economic and environmental aspects have led businesses to improve the operational yield of the chemical control techniques employed (baits and thermal nebulization), and to experiment with new technologies and new toxic active ingredients (Boaretto & Forti, 1997). During the long-term evolution in the methods of controlling leaf-cutting ants, one of the great concerns has been to reconcile the conflict among the three principles of efficiency, economy and safety (Cantarelli et al., 2005). As a consequence of the unfavorable aspects presented by granulated baits (deterioration of the environment, elimination of natural enemies and emergence of resistance), research lines have been generated to discover products with greater specificity and smaller environmental impact (Hebling et al., 2000).

In developed countries, intensive agriculture has not only augmented agricultural productivity, but also, due to its high dependence on great quantities of nonrenewable energy and raw materials, frequently has resulted in soil degradation, environmental pollution and damage to wildlife. For this reason, recent years have seen growing interest in agricultural methods that are both economically and environmentally sound (Betti et al., 2009). Among these, the agrohomeopathy has raised the interest of researchers. Its potential benefits are significant because homeopathic preparations, on account of their extremely high dilution, are relatively inexpensive, have little or no ecological side effect and appear to be, when considered jointly, inoffensive (Elmaz et al., 2004; Lotter, 2003).

All of these attributes make homeopathy optimally adjusted to the holistic approach of organic agriculture, and above all, biodynamic, in which the plants and their interactions with the environment are treated as a unified living organism (Carpenter-Boggs et al., 2000; Heimler et al., 2009). Furthermore, the new approach of applying homeopathic principles can also add to the improvement of nutritional properties (for example the levels of components that induce physiological benefits to human health) (Fonseca et al., 2006) and physiological and qualitative characteristics of plants, in addition to their resistance to stress factors of both biotic (insects and pathogens) and abiotic in nature (physical and chemical damage) (Betti et al., 2009).

In recent years, increasing levels of resistance to insecticides and concerns about insecticide residues in agricultural products have stimulated a growing demand for products cultivated under new strategies of control, raising, in this context, the question of whether homeopathic preparations are able to control pest species (Wiss et al., 2010).

Material and Methods

The assay was conducted on a cattle ranch located in Presidente Prudente, Sao Paulo state, Brazil, in the period from August to October of 2011. The experimental area was constituted by a pasture composed of *Brachiaria decumbens* and some spots of *Paspalum notatum*, with the presence of colonies of the leaf-cutting ants *Atta bisphaerica* and *Atta capiguara*. From the experimental area 30 adult colonies of *Atta bisphaerica* were selected and identified with numbered wooden stakes one meter in height. After this procedure, the area measurements of loose soil were estimated for each colony, with the aid of a measuring tape, namely, the greatest length and greatest width. The quantity of baits to be applied to each colony was calculated from its apparent external area.

The following treatments were applied: T1 – control (0 g of baits/m^2 of loose soil), T2 - standard (8 g of commercial bait based on 0.3% of sulfluramid per m^2 of loose soil) and T3 – homeopathic (baits formulated homeopathically and applied at the dose of 60g/m^2 of loose soil). Treatment T3 was formulated in a private commercial laboratory and the chosen dose had been recommended by the manufacturer, being that the total applied in each colony was parceled into 3 equal portions of 20g/m2 of loose soil, applied for the next 3 days. Each treatment was composed of 10 repetitions, with each repetition constituting one colony.

Prior to application in the field, the baits were weighed in the Laboratory of Entomology at Western Paulista University, according to the measures obtained in the field for each colony, with the total quantity of each repetition being individualized in a plastic sack identified and duly stored until their use. In the field, before application of the treatments, for each repetition one active foraging trail near its area of loose soil was selected to ensure the immediate transport of granules and to avoid competition with workers of other colonies. The parameters evaluated were: transport and devolution of the baits, foraging, presence of recent loose soil, intoxication of individuals and mortality, according to established methodology as a protocol for experiments of this nature (Zanuncio et al., 1997; Nagamoto et al., 1999; Ramos et al., 1999; Zanuncio et al., 2002; Forti et al., 2003; Zanetti et al., 2004).

The transport and devolution of baits were evaluated 24h, 48h and 72h after application, and attributed ratings of 0, 1, 2, 3 or 4, to represent the approximate respective equivalents of 0%, 25%, 50%, 75% and 100% of "pellets" transported and returned by the ants. The transport was evaluated by observing the trails were the baits had been applied, to verify the remaining quantity, while the devolution was evaluated by observing the mound of loose soil of each colony, in order to visualize and quantify the granules deposited on the ground, in relation to the quantity effectively applied.

At 7, 15, 30, 60 and 90 days after installation of the experiment in the field, the remaining parameters were evaluated, verifying the presence or absence of the following: a) foraging – presence of individuals foraging on trails around the nests or on their loose-soil mound, b) loose soil – presence of granules of loose dirt on the mounds of nests, indicating recent activity of excavation, and c) intoxication: presence of young individuals (with clear tegument) on the loose-soil mound, signaling intoxication inside the nests, since under normal conditions, such workers are engaged only with tasks internal to the colonies. These parameters were used to supply data for evaluating the death (or absence thereof) among the colonies.

In the final evaluation, at 90 days, as a function of the established criteria, the presence or absence of mortality was attributed to the colonies, considering dead those that did not show any signal of recent cutting or transporting of leaves and excavation of soil, with their mound of compacted loose soil and their foraging trails inactive.

From the obtained data the mean percentages were calculated, and the results transformed into arcsine √x + 0.5/100, and then submitted to analysis of variance, comparing the means by the test of Tukey at a 5% probability level, in an entirely randomized model of experimental design.

Results

The transport of baits by the ants to the interior of the nests produced an 80% value under the standard treatment versus 50% via the homeopathic approach, differing statistically. The control treatment was represented by the value 0 on account of not having received baits (Fig. 1-A).

The devolution of the pellets transported by ants occurred only in homeopathic treatment, at the rate of 15%, differing from the control and standard treatments, which did not present devolution (Fig. 1-B).

The treatments differed as to efficiency, evaluated through the mortality of the colonies, being null in the control treatment, as expected, 20% in the homeopathic treatment and 80% in the conventional treatment (Fig .1-C).

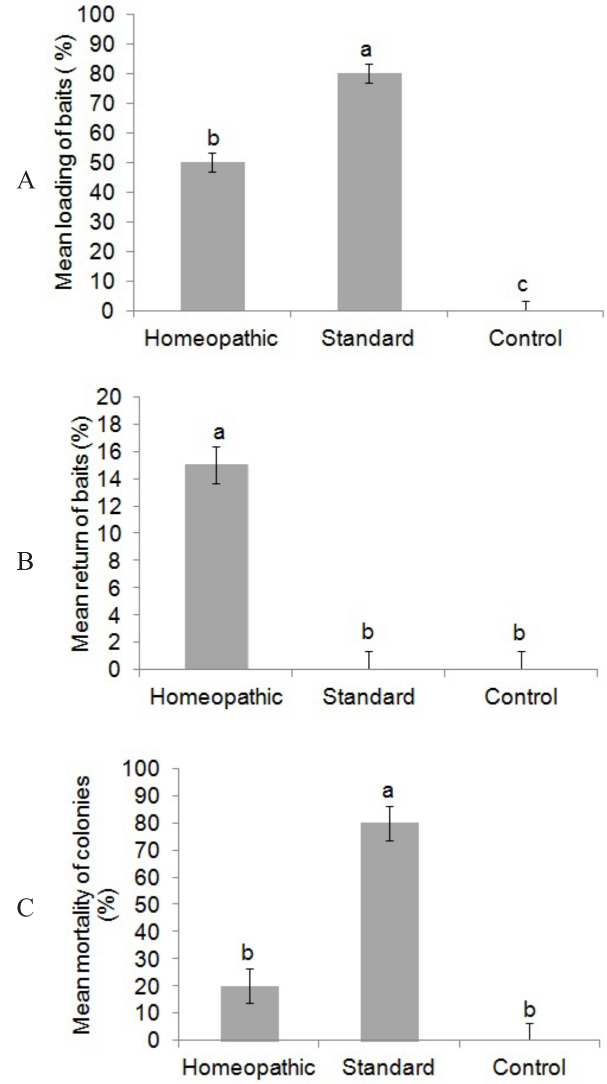

Figure 1. Transport of baits by workers (A), Devolution of baits by workers (B) and, Mortality of *Atta bisphaerica* colonies (C), 90 days after application of treatments, in the field. Presidente Prudente, SP, Brazil, 2011 (Mean percentage values ± mean standard error, obs.: means followed by the same letter do not differ from each other for $P < 0.05$ by the test of Tukey).

Discussion

The 80% bait transport rate under the conventional treatment had been expected since prior experiments utilizing products formulated with the same active ingredient at the same concentration also obtained a high pellet transport rate in the field (Zanuncio et al., 1997; Zanetti et al., 2004). The transport of baits is one of the parameters that define treatment efficiency since the product can only act and express its potential if it is found attractive initially, accepted after investigation and then effectively transported by workers. The baits formulated based on homeopathy were not shown to be sufficiently attractive to the workers, as reflected in the unsatisfactory pellet transport value obtained (50%),which may have negatively influenced the efficiency of the treatment. The cause of the low rate of transport for homeopathic baits was not investigated, and thus it could not be affirmed whether it is a function of homeopathic formulation or of some other random factor such as the unknown quality of citric pulp utilized in the manufacturing of baits.

The devolution of baits, as measured by the quantity of granules deposited on the mound of loose soil, was not observed in the standard treatment, a recurrent fact in field and laboratory assays that similarly evaluated sulfluramid-based baits (Zanuncio et al., 1997; Zanetti et al., 2004). In a contrary and significant manner, a portion (15%) of the homeopathic baits transported were returned by workers onto the loose-soil mound soon after transport, thus demonstrating that, for some reason, they were rejected during the process of post-selecting foraged material, which occurs in the interior of the colonies. Camargo et al. (2003) observed the occurrence of a post-selection process in colonies of *Acromyrmex subterraneus brunneus* when the colonies were offered, in the laboratory, different inert materials such as plastic, polystyrene and clay, which were rapidly differentiated and not selected by the workers for cultivation of the symbiotic fungus. The authors reported that the post-selection of foraged material constitutes strong evidence of the cognitive abilities of workers and of the colony as a whole. Devolution is an important parameter to be evaluated in experiments of this nature since it demonstrates the acceptance or rejection of a product by the ants. Thus, it becomes evident that the lethality of the active ingredient to leaf-cutting ants is necessary but not sufficient since it may be prematurely perceived and rejected by the workers when formulated into baits, a scenario that would compromise the efficiency of the product, which may have occurred in the present assay.

The efficiency of treatments, represented by the mortality of the colonies, was significantly superior in the standard treatment. The high mortality rate of colonies that received application of sulfluramid baits had been expected since there are reports in the literature of innumerable studies of the same nature that presented similar results (Zanuncio et al., 1997; Zanuncio et al., 2002; Zanetti et al., 2004). Sulfluramid is included among the active ingredients currently used for controlling leaf-cutting ants jointly with chlorpyrifos, given that chlorpyrifos is more toxic to mammals, aquatic organisms, fish and bees than sulfluramid (Tiepo et al., 2010).

The effect of the homeopathic treatment on the colonies was inconsistent, a coherent finding if considered a function of low transport of the baits presented and of the devolution rate, despite the low mortality observed. Geisel et al. (2012) found that homeopathic preparations of adults and *Acromyrmex* fungus reduced the activity of ant foraging trails in the field, from the sixth day of application (spraying), extending this effect until 20 days after last application. These results do not agree with those obtained in this test, but it is hard to compare them because the implementation and evaluation methodologies are completely different, with different goals, since in the Geisel et al. (2012) study it was not evaluated the mortality of colonies, the main focus of this study.

Similarly, in an assay conducted on potato plants sprayed with diverse homeopathic preparations, in order to verify the incidence of diseases (*Phytophthora infestans* and *Alternaria solani*) and pests (*Diabrotica speciosa*), Boof et al. (2008) found no significant difference between the homeopathic products tested and the control (water), for all parameters evaluated. In another study, aiming to evaluate the effect of homeopathic preparations on the aphid *Dysaphis plantaginea* in apple seedlings, Wyss et al. (2010) reported no significant difference between homeopathic treatments and the control, by evaluating the quantity of individuals in the seedlings, the damage to the leaves and the fresh weight of the plants.

In an assay performed by Tiepo et al. (2010) to assess a natural formicide considered a "green pesticide," the findings were promising. The product is a mixture of caffeine and common fatty acids, with citric pulp and apple as attractive agents. When its effects were observed on microbiological soil activity and the biomass of worms and plants, short-term toxicity was not detected. The authors reported that the formicide did not present toxic action on the experimental organisms on account of its natural composition, but that when the leaf-cutting ants ingested this product, despite not dying immediately, manifested behavioral disturbances. Field observations, according to the authors, demonstrate a disruption in the social structure of the colony, which leads to the subsequent death of the individuals, considering that the communication constitutes the basis of the ant social structure. The term "green pesticide" should be understood as a natural or synthetic pesticide produced according to the principles of "green chemistry", acting specifically and effectively on the precise target without deleterious or dangerous effects on non-target components of the ecosystem (Tiepo et al., 2010). It is expected that the toxicity of this new and necessary class of "green pesticides" would be low or nonexistent (Kahkonen & Nordstrom, 2008).

The data obtained herein do not justify a recommendation to control leaf-cutting ants by homeopathic baits. However, it is important to emphasize that, to the best of our knowledge, the literature contains no other scientific data on studies of this nature, a scenario that does not permit us to accept or reject definitively the hypothesis that homeopathy is a viable alternative for controlling these insects. In a review study on the use of homeopathic preparations in agriculture, to control pests and pathogens, Betti et al. (2009) concluded that most of the works published and consulted do not provide sufficient information to enable a clear interpretation, in particular, due to the statistical analysis being inadequate or entirely absent, the number of repetitions being unspecified and, frequently, the experimental methodology being poor. Even so, the authors concluded that the studies evaluated can serve as a starting point for future experiments that are more comprehensive and controlled. Similarly, the results presented in the present assay do not permit us to resolve the question of whether homeopathy can control leaf-cutting ants, but rather call for initiation or expansion of this research line for greater investigation, conducted within scientific premises necessary to achieve a complete understanding of the subject.

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