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The effect of homeopathic preparations on the activity level of *Acromyrmex* leaf-cutting ants

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ABSTRACT. The effect of homeopathic preparations on the activities of the leaf-cutting ants *Acromyrmex* spp. was studied. A field experiment involving ant nests in six experimental areas was performed using a randomised complete block design. Within each block, every ant nest was considered to represent one repetition. The treatments consisted of the following: *Belladonna* homeopathic preparations of macerated or triturated *Acromyrmex* spp. adults, homeopathic preparations of macerated or triturated ant nest fungus (*Leucoagaricus gongylophorus*) collected from *Acromyrmex laticeps* and *Acromyrmex heyeri* nests, a homeopathy *Belladonna*, and dynamised water. All of the homeopathic treatments were tested at the 30 CH (thirtieth centesimal Hahnemannian) dynamisation. An untreated nest served as the control. The total number of ants from each trail was counted, including both those carrying or not-carrying green plant fragments, immediately before the daily homeopathic applications. All of the tested homeopathic preparations, except for the water, significantly reduced the activity level of *Acromyrmex* spp. The homeopathic preparations of *Belladonna* and the macerated nosodes of *Acromyrmex* spp. reduced the activity level of the ants beginning with the sixth day after the first treatment application; the activity level reduction effect lasted more than 20 days after the last application.

Keywords: foraging, non-residual substance, high dilutions, nosodes.

Atividade de formigas cortadeiras *Acromyrmex* spp. submetidas a preparações homeopáticas

RESUMO. O efeito de preparados homeopáticos sobre as atividades de formigas cortadeiras *Acromyrmex* spp. foi estudado. O experimento foi realizado no campo, utilizando formigueiros distribuídos em seis diferentes áreas. Em cada área cada, formigueiro foi considerado uma repetição. Os tratamentos consistiram de preparados homeopáticos obtidos da tintura-mãe dos triturados e macerados de adultos de *Acromyrmex* spp. e do fungo *Leucoagaricus gongylophorus* de formigueiros de *Acromyrmex laticeps* e *Acromyrmex heyeri*. A homeopatia *Belladonna* e água dinamizada também foram testados. Todos os preparados homeopáticos foram testados na trigésima diluição centesimal hahnemanniana (30 CH). Formigueiros não tratados foram utilizados como testemunha. O número total de formigas que se deslocam em cada carreiro, transportando ou não fragmentos vegetais, foi avaliado imediatamente antes da aplicação dos tratamentos. Os resultados mostraram que todos os preparados homeopáticos testados, exceto agua dinamizada, mostraram significativa redução nas atividades das formigas cortadeiras *Acromyrmex* spp. Os preparados homeopáticos de *Belladonna* e nosódio de *Acromyrmex* spp. reduziram, significativamente, as atividades de formigas *Acromyrmex* a partir do sexto dia de aplicação e o efeito na redução das atividades prolongou-se até 20 dias após a última aplicação.

Palavras-chave: forrageamento, preparados não residuais, altas diluições, nosódios.

Introduction

Leaf-cutting ant species of the *Acromyrmex* and *Atta* genera are the most important herbivores that exist in subtropical regions throughout Latin-American countries (GUSMÃO; LOECK, 2002). While the *Atta* species predominate in lowlands and in hot climates, the *Acromyrmex* species prefer highlands and cool regions. The highlands in Santa Catarina State, in southern Brazil, contain ecosystems with rich plant diversity that are located near agricultural and horticultural areas. To support their colonies, the *Acromyrmex* spp. ants search out and harvest young plant parts, such as sprouts, buds, leaves, and flowers, which they carry back to their nests in order to cultivate a symbiotic fungus, *Leucoagaricus gongylophorus*; this fungus is the main food source for the colony (ROCES, 1990; MUELLER, 2002).

While the intense foraging activity of leaf-cutting ants can damage cropped plants in a short period of time, they play an important role in the ecosystem (GRURZMACHER et al., 2002). They promote changes in soil structure and nutrient content by recycling organic matter that is deposited in the vicinity of their nests (DELABIE et al., 2000). According to Delabie et al. (2000), the complete extermination of leaf-cutting ant colonies is not recommended once these colonies are established as part of the biological structure of the food web. Thus, low densities of leaf-cutting ant colonies are necessary to keep a biological equilibrium among living organisms (DELABIE et al., 2000).

The chemical control of leaf-cutting ants with large quantities of synthetic insecticides is costly, hazardous to the environment, and sometimes ineffective (BUENO et al., 1995; JACCOUD et al., 1999). Those leaf-cutting ants that evade previous chemical applications of synthetic insecticides can develop resistance, thereby reducing the effectiveness of subsequent chemical interventions (LOECK; et al., 2003). In addition, as they perceive human interference in their habitat, the leaf-cutting ant colonies exposed to insecticides are driven to establish new colonies elsewhere (RAMOS et al., 2003).

Ecologically based technologies should be developed in order to maintain the pests at low population levels, thereby improving crop resilience instead of completely eliminating the causative agent (GHINI; BETTIOL, 2000). The use of homeopathic preparations as an alternative method has recently emerged; this approach is designed to manage pests and crop diseases, while restoring the health of the agro-ecosystem. Several works in the field of plant health, soil, and water (ANDRADE et al., 2001) have demonstrated the efficacy of homeopathic preparations in agricultural systems. According to Bonato and Silva (2003), homeopathic preparations not only target a particular pest and disease but also facilitate the re-organisation of the living system as a whole without destroying the food web. The use of homeopathic preparations is a suitable technology that helps to redesign sustainable agricultural systems, which include all of the complex interactions that occur in an agro-ecosystem. The biological advantage of managing leaf-cutting ants, without their eradication, is to reduce the selective pressure on and avoid the revival and multiplication of ant colonies. Bonfim et al. (2010) note that homeopathic preparations of nosodes, obtained from the pest organisms or diseased plants, can provide farmers with a simple and efficient tool to quickly overcome a local problem in a cheap and readily available way.

The most suitable homeopathic preparation for a specific agricultural problem can be chosen by making an analogy with the symptoms described in the Materia Medica, a compendium that describes the therapeutic properties of homeopathic remedies for human beings. Despite the fact that very little is known about the mechanisms of action of homeopathic preparations on agro-ecosystems, their effects can be measured through experimental studies on specific species because living organisms might respond differently among kingdoms (BONATO; SILVA, 2003; BONATO et al., 2009).

The aim of this research was to evaluate the effect of homeopathic preparations on the total activity and foraging activity levels of *Acromyrmex* leaf-cutting ants in the highland plateau of Santa Catarina State in southern Brazil.

Material and methods

Study area

The leaf-cutting ant nests selected for study were situated in the Lages and Campo Belo do Sul district in Santa Catarina State in southern Brazil. The study area was located within 942 and 1058 m above sea level (27° 45' to 28° 02' S, 50° 15' to 50° 27' W). Natural plant grasses, associated with an *Araucaria angustifolia* forest, originally covered the study area. These areas are currently used for agriculture and re-forestation with exotic pine species. The research was carried out during the summer season of 2007 (December to March), and data were collected from 42 *Acromyrmex* ant nests, submitted to seven treatments, including six homeopathic preparations and a control.

Experimental set-up

The Acromyrmex spp. nests were identified by a small mound of debris on the soil surface and confirmed by the presence of four spine pairs on the dorsal thorax of the worker ants. Treatments were randomised in six blocks, represented by ant nests distributed in different areas; each nest represented one repetition. Ant nests were separated by a distance of 25 m to 100 m within the same block. A sample of worker ants was collected and the species was confirmed in the laboratory using the taxonomic key described by Loeck et al. (2003). Each ant nest represented one experimental plot and the data were collected from two primary foraging trails per ant nest; in other words, treatment applications were administered where ant activity was greatest.

Preparation of homeopathic treatments

The treatments included the following: a) ant nosodes prepared from the maceration of adult *Acromyrmex* spp. or from the ant nest fungus, *Leucoagaricus gongylophorus*; b) ant nosodes prepared from trituration of adult *Acromyrmex* spp. or from ant nest fungus; c) a homeopathy *Belladonna* from a pharmacy; d) dynamised water and e) an untreated ant nest used as a control plot. All of the used preparations were created in the Homeopathy and Plant Health Laboratory at the Lages Experimental Station of EPAGRI (Agricultural Research and Extension Service Agency of Santa Catarina State, Brazil), according to the standards of "Farmacopéia Homeopática Brasileira" (BRASIL, 1997). The macerated and triturated ant nosode preparations were obtained from 50 worker ants randomly collected from the primary forage trails. The macerated and triturated symbiotic fungus preparations were obtained from well-established fungus obtained from ant nests. For the trituration method, the lactose (0.27 g) was divided into three portions and triturated in a porcelain mortar with a pestle over the course of an hour, in three-sequential 20-minute periods. The first third of lactose (0.07 g) was then pre-ground and having added 0.03 g of ants (prime meter at 1% of the total). Subsequently, during each 20-minute period, two sequential periods of 10 minutes included 6 minutes of circular movements and 4 minutes of collecting the material from the grail wall back to the centre in vertical movements. At the end of each third 20-minute period, another third of lactose was added to obtain a 1 CH trit. (first centesimal Hahnemannian dilution) of ant or fungus nosode (BRASIL, 1997). Again, by taking one part of the 1 CH trit., adding 99 parts of lactose into a porcelain mortar and grinding it over the course of an hour, as described above, it reached the 2 CH trit. This procedure was repeated until the 3 CH trit. was obtained. After that, each dynamisation level until the 28 CH (twenty-eighth centesimal Hahnemannian) dilution was prepared by taking one part from the previous dynamisation into 99 parts of a 70% alcohol solution, and succussed (sequence of rhythmically vertical 90° angular movements) 100 times using a mechanical dynamiser (Autic®, Mod. Denise 10-20).

A macerated mother nosode tincture was obtained by mixing 1 g of active ants with 45 mL solution of water + alcohol + glycerine (1:1:1), and kept in an amber glass bottle at room temperature for 48h (BRASIL, 1997). This dynamisation protocol was then followed: 0.2 mL of mother tincture was combined with 19.8 mL of alcohol in a 30 mL amber bottle. The bottle was placed in a mechanic dynamiser (Autic®, Mod. Denise 10-20) for 100 sucussions, in order to obtain a 1 CH (first centesimal Hahnemannian) dilution. The same procedure was repeated to obtain a 2 CH dilution and the successive dynamisations until a 28 CH (the stock matrix) dilution was reached. A *Belladonna* tincture of 3 CH was obtained from a pharmacy and prepared until a 28 CH dynamisation, using a 70% alcohol solution, was reached. The homeopathic preparations were elevated to the 30 CH dilution, just before going to the field, using water instead of alcohol. The water treatment was obtained through the same procedure; starting with 100 succussions of pure water until a 30 CH dilution was reached.

Treatment applications

The treatments were applied using a double-blind method, where neither the applicator nor the evaluator knew the identity of the treatment. The treatments were individualised and identified by codes. Each dynamisation of ant and fungus nosodes, *Belladonna* homeopathy, and water were applied with an individualised garden sprayer (Brudden®). Applications consisted of spraying 10 mL of the homeopathic preparation along 0.5 m of the two primary foraging trails, of each of the six ant nests in each treatment. To ensure that minimal disturbance was caused, the application sites were located 1.0 m away from each ant nest. Daily applications were made in the morning, from 7:00 to 9:00, during the first five days and in the afternoon, from 16:00 to 18:00, during the last five days. This schedule was followed for practical reasons.

Evaluations and data analysis

The foraging and total activity of the ants was recorded just before each application. Foraging activity was considered as that of ants moving and carrying green plant fragments. Total activity was recorded when ants were moving with and without green plant fragments. Both activities were estimated separately by counting the number of ants that were passing through a given point along the forage trail, located 1.0 m away from the ant nest, over the course of one minute. These evaluations were conducted just before each daily treatment application, during the 10-day treatment period; the last evaluation was made 20 days after the last application. The first evaluation was made just before the first application, prior to any treatment applications.

Data analysis was done using the sum of ant counts, from the two primary foraging trails of each

ant colony nest. An analysis of variance (ANOVA) was performed to analyse the effect of the treatments and the interaction between application days and homeopathic preparations.

The reduction rates of total activity and foraging activity were calculated from the average daily rates until the day in question, respectively, for each treatment. The size of the ant nests of the different species was compared with contrast analysis, and a T-test was applied.

Results and discussion

The identification of the leaf-cutting ants collected from the studied nests showed the presence of two species, *Acromyrmex laticeps* and *Acromyrmex heyeri*. The *A. laticeps* spp. had an average nest area of 0.56 ± 0.18 m^2 whereas the *A. heyeri* spp. had an average nest area of 0.55 ± 0.15 m^2. No significant difference was observed between the size of the nests using a T-test (n = 52; p = 0.94). There was no significant interaction between the "treatment" and "application time" variables: total activity (p = 0.93) and foraging activity (p = 0.99). However, the total activity was highly correlated with foraging activity for all of the treatments, *Belladonna* (r = 0.62, p < 0.001), *Acromyrmex* triturate nosode (r = 0.93, p < 0.001), *Acromyrmex* macerate nosode (r = 0.94, p < 0.001), *Leucoagaricus* macerate nosode (r = 0.72, p < 0.001), *Leucoagaricus* triturate nosode (r = 0.85, p < 0.001), dynamised water (r = 0.84, p < 0.001) and no application (r = 0.77, p < 0.001).

Homeopathic preparations of triturated and macerated *Acromyrmex* spp. and *Belladonna* steadily reduced the total activity and foraging activity in comparison to the control, from the fifth application onwards (Figures 1 and 2).

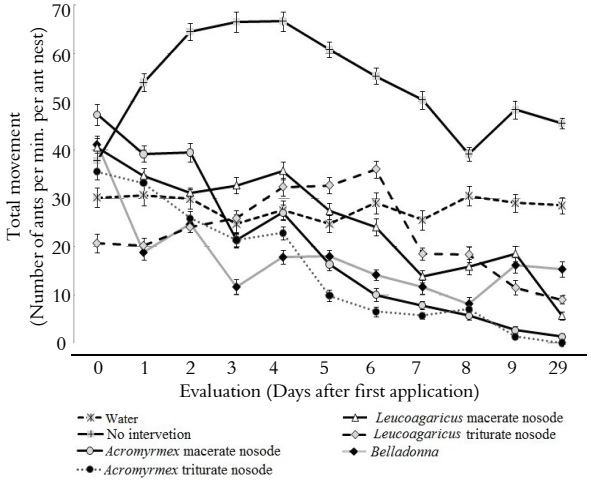

Figure 1. Total activity of *Acromyrmex* spp. submitted to homeopathic preparations at 30 CH (thirtieth centesimal hahnemannian dilution). Santa Catarina State, Brazil, 2007. Data are average from six replications, considering the sum of the two main forage trails in each ant nest. Bars represent the standard error of means.

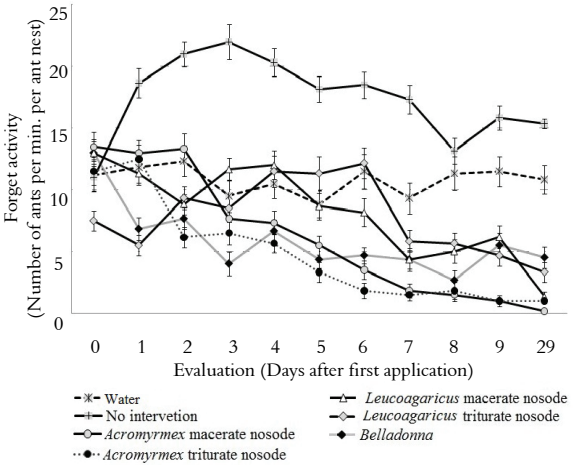

Figure 2. Foraging activity of *Acromyrmex* spp. ants submitted to homeopathic preparations at 30 CH (thirtieth centesimal hahnemannian dilution). Santa Catarina State, Brazil, 2007. Data are average from six replications and bars are standard error of means.

When a nosode of *Acromyrmex* spp. was used, the reduction of ant activity lasted for more than 20 days after the last application.

The daily reduction rate of total activity (Figure 3) and foraging activity (Figure 4) indicate that most of the homeopathic preparations had a positive effect after 5-6 days of applications, except with the *Leucoagaricus* triturate nosode application. The water with a 30 CH dilution had a rate oscillating around 0 (zero), whereas the control plot had an increase in total activity and foraging activity.

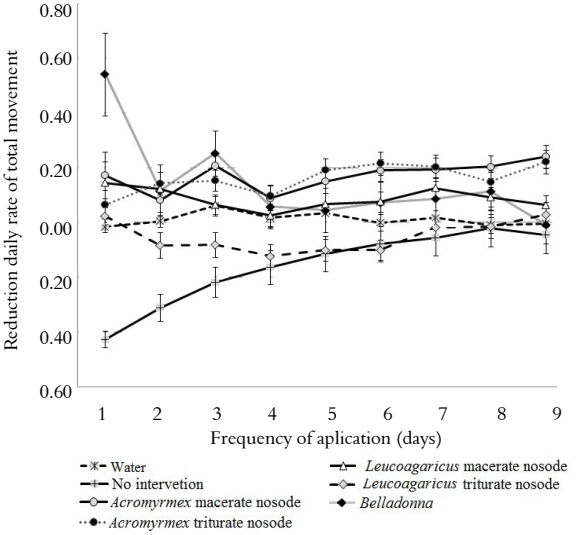

Figure 3. Daily reduction rate of total activity of *Acromyrmex* spp. Data are expressed by the daily mean of such period of time considered from the beginning of application. Lages, Santa Catarina State, Brazil, 2007. Data are average of six replications and bars represent standard error of means.

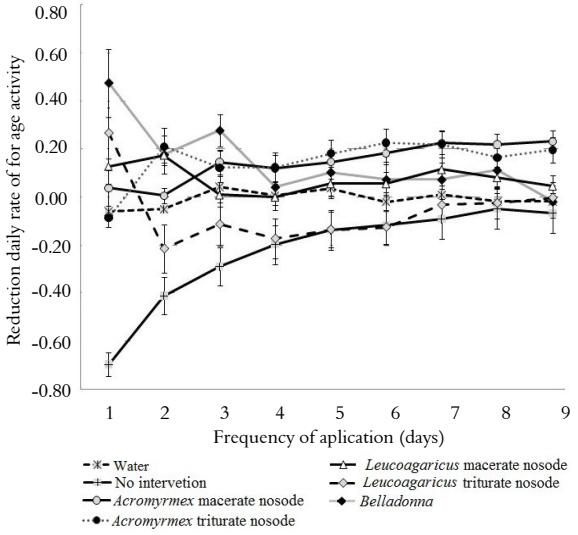

Figure 4. Reduction daily rate of forage activity of *Acromyrmex* spp. Data are expressed by the daily mean of such period of time considered from the beginning of application. Lages, Santa Catarina State, Brazil, 2007. Data are average of six replications and bars are standard error of means.

The results demonstrate that there is high correlation between reduction of foraging activities and reduction on total activities by the all tested homeopathic preparations, suggesting that the homeopathic preparation acts on the behaviour of the entire ant colony.

The species of *Atta* and *Acromyrmex* genera represent the common leaf-cutting ants that are widespread in the Neotropics and which frequently affect crop yields (CORREA et al., 2006). Due to the damage they cause in agriculture, the leaf-cutting ants of the genus *Acromyrmex* has been the target of various means of control, ranging from homemade recipes, to high-tech interventions (ZEH et al., 1999). Nevertheless, such conventional practices, when targeting ant nests, do not solve the problem over the long term. Reducing the activity level of leaf-cutting ants, without causing the colony to collapse, is an important low side-effect strategy. Despite the fact that *Acromyrmex* ants are highly polyphagous and capable of defoliating several native forest species, in addition to various agricultural and horticultural crops (CORREA et al., 2006), they play an important role in nutrient recycling and in the ecosystem's food chain (FARJI-BRENER; MEDINA, 2000).

It has been demonstrated that the use of homeopathic preparations can reduce foraging activity by an average of up to 85%. Homeopathic preparations carry none of the risks to biological diversity that are frequently associated with synthetic pesticides. The reduction of foraging activity, at minimum level to prevent the ant colony from collapsing, allows the farmers to consider the ants as part of their agro-ecosystem and to accept their presence without unnecessary alarm. To maintain biodiversity, it is also important to improve the resilience of the agro-ecosystem as a whole. In a similar way, farmers in Panama have protected plants from *Atta cephalote* invasions, by using the discarded litter, which this leaf-cutting species dumps as a deterrent; yet, the colonies still exist (ZEH et al., 1999). In our study, it was also observed that the daily application with homeopathic preparations over 10 days, showed an influence on the behaviour by immediately reducing leaf-cutting ant activity (loaded or unloaded ants) in the evaluated trials, as well as in other observations (these data are not presented here). It is important to note that the reduction of leaf-cutting ant activity, near the nests and along the primary foraging trails, was maintained for a period of 20 days after the last application of nosodes of macerated and triturated *Acromyrmex* spp. Despite the fact that relatively little work has been carried out on the use and effects of homeopathic preparations on leaf-cutting ants, the results of our study show that the use of homeopathic preparations is an available alternative method for reducing the damage or losses caused by leaf-cutting ants on economically valuable crops. For instance, farmers could use homeopathic preparations to limit the foraging activity of *Acromyrmex* spp. when crops are more susceptible to foraging damage, thus avoiding yield losses. By adopting this alternative method, farmers would be able to familiarize themselves with the leaf-cutting ants in their agro-ecosystems. A period of 25-30 days is sufficient to protect plants from foraging damage to annual or perennial crops caused by leaf-cutting ants. To achieve this, farmers must be able to identify the critical periods during crop cycles when homeopathic applications are needed. Applications should be timed to protect the flowering period of most crops and, if necessary, an additional 10 days of treatment should be applied after the flowering period to ensure an additional 20 days of protection. Nevertheless, this must be tested for different farm conditions and adjusted as necessary (FILHO; DORVAL, 2003).

From the results obtained in this research, we have shown that in order to have a positive effect with homeopathic preparations on the reduction of leaf-cutting ant activity, it is necessary to pay attention to the application frequency. A significant reduction in ant activity was observed after five daily applications of macerated or triturated *Acromyrmex*

nosodes. It could be argued that a single dose of a homeopathic preparation, as sometimes recommended for animal and human homeopathy, will most likely not work to control leaf-cutting ants. However, this hypothesis must be tested with one-application treatments. If the ant nest colony were viewed as a society, it may be argued that homeopathic preparations most likely take some time to have an effect, but once that effect happens, the entire ant colony changes. Other conventional interventions, like the use of synthetic insecticides, require even more application frequency, yet they do not reduce the foraging activity for a long time, as reported by Cantarelli et al. (2005); rather, the management of ants requires the constant use of toxic bait due to the rapid re-colonisation of leaf-cutting ants.

In this research study area, no new ant nests in the vicinity of those that were treated with homeopathic preparations have appeared as of 29 days after the first application. This means that the homeopathic preparations did not perturb the ant nests to a level that caused them to re-colonise elsewhere. Preventing re-colonisation is particularly important for perennial crops, such as those in orchards, because it induces a longer-term effect in the same treated area. Further, organisms that are sensitive to homeopathic treatments become more relaxed and less excitable, which may also reduce their foraging activity.

Homeopathic preparations are used everywhere in the world for the treatment of humans, despite the criticisms and arguments that they only elicit a placebo effect (BONATO et al., 2009). The ineffectiveness of water at 30 CH, and the similar lack of change in the control plot, disproves the placebo effect in our study. In Brazil, there are several examples of successful results in animal production systems, where companion animals are treated only with homeopathic preparations. Nevertheless, this is not common in plant health or plant protection strategies. A few research projects involving homeopathic preparations used on crop systems may be the main cause of the limited use of agro-homeopathy. However, homeopathy as an applied science involves changing our perception about living systems, in the sense that co-existence and mutual aid should be the most important principle in developing a sustainable agricultural system. In this sense, homeopathic preparations applied to agriculture are more feasible to implement under organic crop systems. In other words, agro-ecologists must consider expanding their fields to take on the challenge to improve agro-ecosystems with technologies, such as homeopathic preparations, that have no side effects, are inexpensive, and are somewhat easy to apply.

Conclusion

From this study, the following can be concluded:

- Homeopathic preparations obtained from macerated or triturated *Acromyrmex* spp., sprayed at 30 CH (thirtieth centesimal Hahnemannian) dynamisation, are effective at reducing the movement and foraging activity of *Acromyrmex laticeps* and *A. heyeri*;

- Homeopathic preparations can be used to manage leaf-cutting ants without causing colony collapse or re-colonisation elsewhere;

- Homeopathic preparations can be used for restoring the dynamic equilibrium in agro-ecosystems by targeting a particular point of disturbance, such as the intensive multiplication of leaf-cutting colonies in agro-ecosystems.

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