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Review

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Review

Control of Amazonian Leaf-Cutting Ants (Hymenoptera: Formicidae): A Multi-criteria Analysis

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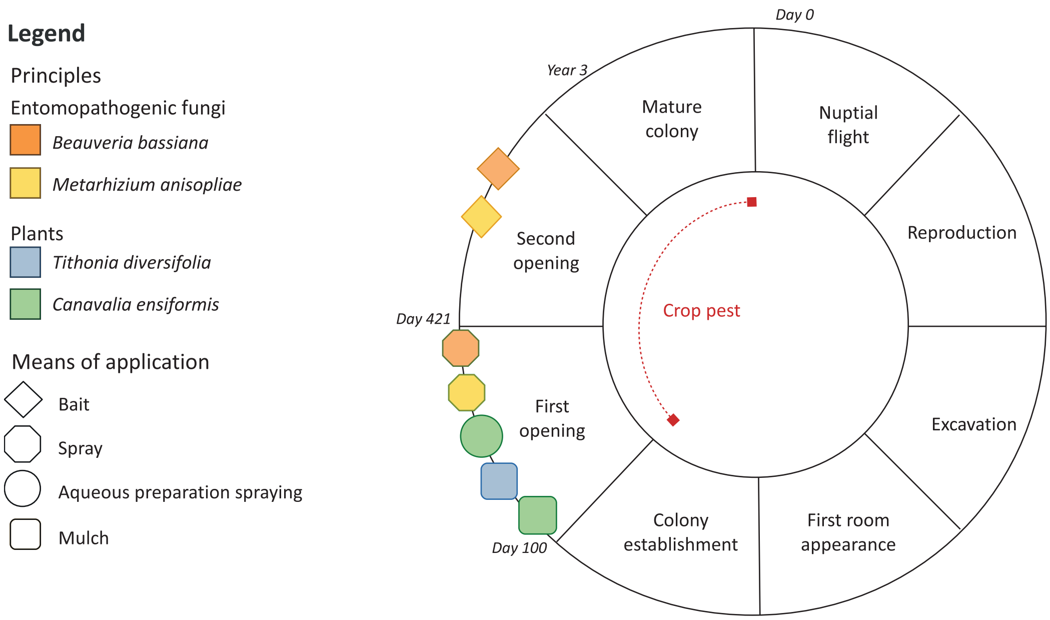
Abstract

Leaf-cutting ants (Hymenoptera: Formicidae) are one of the main pests found in the Americas and they cause global economic losses worth several billions of dollars. While pesticides have been the most widely used control method, new management alternatives in a context of agroecological transition are now being considered. This study focuses on the leaf-cutting ants species found in the pan-Amazon region. As part of efforts to improve management of these pests, this multi-criteria analysis of control strategies covers a total of 691 experiments collected from 153 studies, and the control were evaluated as a function of their management efficacy, environmental and human health impacts, and their ease of application. Chemical control methods were effective but posed a danger to human health and the environment, whereas mechanical methods and integrated management were more sustainable but not always very effective. Some of the biocontrol methods were evaluated as effective and safe for the environment and human health, including the use of entomopathogenic fungi *Beauveria bassiana* (Bals.-Criv) Vuill. (Hypocreales: Cordycipitaceae) and *Metarhizium anisopliae* (Metschn.) Sorokïn (Hypocreales: Clavicipitaceae) in the form of bait or sprayed in the nest, or the application of plant mulch in the nest using *Tithonia diversifolia* (Hemsley) A. Gray (Asterales: Asteraceae) or *Canavalia ensiformis* L. DC. (Fabales: Fabaceae). Because of variations in the efficacy data between laboratory and field tests, we are in favor of evaluating these control methods during field studies with different leaf-cutting ant species and under different environmental conditions. These methods should adopt experimental arrangements that are appropriate for local socioeconomic conditions adapted for farmers.

Graphical Abstract



Life cycle of a leaf-cutting ant colony

Key words: pest management, leaf-cutting ants, biocontrol, Amazonia

Leaf-cutting ants (tribe *Attini*, genera *Acromyrmex* and *Atta*) are one of the main polyphagous insect pests in Latin America (Cramer 1967), where at least 48 species (Schowalter and Ring 2017) inhabit a broad range of habitats from Argentina to Texas (Weber 1972, Vasconcelos and Fowler 1990). Although there are dissimilarities in the biology, ecology, and geographical range size of leaf-cutting ants species (Littledyke and Cherrett 1975, Farji-Brener and Ruggiero 1994, Mikheyev et al. 2007), the common point between all species is their dependence on fresh plant leaves and other plant material (Boulogne et al. 2014). Leaf-cutting ants cause global economic losses worth several billions of dollars in the Americas (Della Lucia et al. 2014).

'Leaf cutting' means that the ants live in symbiosis with the fungus *Leucoagaricus gongylophorus* (Möller) Singer (Agaricales: Basidiomycota; Silva et al. 2006). Several authors have suggested that this symbiotic association, and more specifically the ant–fungus synergism and the unique adaptations of ants that result from these interactions (Mueller et al. 2018) provide an explanation for the ecological success of leaf-cutting ants (Vasconcelos and Fowler 1990). Each colony is created by a founder queen using a fungal pellet from her natal colony (Mueller et al. 2010). As some leaf-cutting ant species are endemic, geographically restricted (Fowler et al. 1989), and vital to their ecosystems as ecosystem engineer (Montoya-Lerma et al. 2012), forest fragmentation favors certain pest species such as *Atta sexdens rubropilosa* Forel (Hymenoptera: Formicidae) at the expense of endemic forest species (Fowler 1995, Barrera et al. 2015). The pest species tend to nest in anthropized, fragmented, or disturbed environments (Silva et al. 2013, Siqueira et al. 2017). The colony grows rapidly for 421 d, reaching maturity at 3 yr (de Britto et al. 2016). The cultivated fungus supplies essential nutrients to the ants by converting plant polysaccharides into nutrients that they can easily assimilate (Gomes de Siqueira et al. 1998). Leaf palatability depends on various factors such as secondary metabolites and water content (Folgarait et al. 1996), nutrient content, leaf age, and light availability (Nichols-Orians 1992). Leaf-cutting ants usually favor young leaves because of their greater nutrient asset and their greater ease to cut (Nichols-Orians, 1992). The nutrients converted by the fungus represent an important share of the worker diet, along with plant sap (Littledyke and Cherrett 1976), and the only food for the larvae and brood (Weber 1966). In return, the generalist workers and gardeners take care of the fungus and ensure good sanitary conditions in the nest, limiting the entry of pathogens (Little et al. 2006). The presence of filamentous actinobacteria producing anti-fungal secretions in the tegument of some species (Mattoso et al. 2011), and the production of antibiotics in the infrabuccal pocket of the workers, contribute to preserving the fungus from external contamination and eradicating competition (Little et al. 2006). The major workers harvest fresh plant material (e.g., leaves, flowers, and fruits) to create a substrate that will enable the fungus to grow (Weber 1972). The plant material used differs as a function of the three types of ant species (Nagamoto et al. 2009, Khadempour et al. 2020): 1) those only eating broadleaf plants, 2) those specialized in grassy weed harvest (Camargo et al. 2006), and 3) those that use both materials as a substrate (Camargo et al. 2006). They tend to prefer young leaves (Vasconcelos and Fowler 1990) so target new plants that are softer than mature plants, contain fewer secondary metabolites and are of better nutritional quality (Della Lucia et al. 2014), although leaf-cutters preferentially harvest crops with a boosted production of young tissues. Preferences may change, depending on the colony (Littledyke and Cherrett 1975) and individuals within the same colony (Roces 2002).

It has been reported that leaf-cutting ants may attack 47 agricultural and horticultural plants and 13 forage species (Cherrett and Peregrine 1976). The leaf-cutting ants will favor the plants treated with fertilizers. These treatments promote higher foliar nitrogen and phosphorus concentrations that favor the symbiotic fungi (Montoya-Lerma et al. 2012). While most of the leaf-cutting ant species are harmless (Montoya-Lerma et al. 2012), the species *Atta cephalotes* Linnaeus (Hymenoptera: Formicidae), *Atta sexdens* Linnaeus (Hymenoptera: Formicidae), and *Acromyrmex octospinosus* Reich (Hymenoptera: Formicidae) can be considered as primary pests (Fernandez and al. 2015) in the pan-Amazonian region (Table 1). The defoliation due to the leaf-cutting ants may result in the killing of the plant. In Trinidad, a study estimated that *Acromyrmex octospinosus* defoliation activity caused the deaths of 6–17% of the trees in cacao or citrus orchards (Boulogne et al. 2014). Despite the tremendous financial losses due to the leaf-cutting ants, the literature provides few data about economic and agricultural losses. These losses are difficult to estimate due to the wide range of damages caused (Boulogne et al. 2014).

Foraging tactics, preferences, and changes to behavior (Camargo et al. 2006), colony behavior and reactions to control efforts may change depending on the genus (i.e., Atta and Acromyrmex) and species (de Britto et al. 2016). Thus, the control method of the leaf-cutting ants pest depends on the genus and species considered. Because of the important economic losses these ants can cause, scientists and farmers have developed chemical, biocontrol, mechanical, and integrated pest control methods. Some of these methods seek to limit their impact on economic activities by protecting the crops or confusing the worker ants. Other methods are designed to destroy or weaken colonies by targeting the workers, the queen, or the symbiotic fungus. These methods can be implemented at different times and places during the ant life cycle.

A variety of control methods have been tested in Latin America, but the data remain scanty due to language differences, i.e., English, Spanish, Portuguese, and scientific articles are little used to disseminate the results. Given the importance of the damage caused in this region, and the stakes involved in developing alternative methods that are compatible with the specifications of agroecological transition, it is now urgent to produce a synthesis of the innovative methods designed in the pan-Amazon Region, in order to identify solutions that could be used by producers.

To achieve this, various methods of research synthesis are available, such as narrative review, vote counting, combining probabilities, meta-analysis (Koricheva et al. 2013), and multi-criteria analysis. Multi-criteria analysis is a method that accounts of a set of objectives and criteria that can be conflicting, multi-dimensional, incommensurable, and incomparable (Basbas and Makridakis 2007). It is a useful tool that can summarize the accumulated knowledge in a particular field and can be used to support complex decision-making situations with multiple objectives (Saarikoski et al. 2016). Multi-Criteria Decision Analysis (MCDA) has been widely used in the scientific field as a tool for evaluating options in decisions involving multiple often conflicting criteria, predefined constraints, as well as stakeholders. This is the case of decision-making in managing plant pests such as leaf-cutting ants,

Table 1. Geographical repartition of the main leaf-cutting ant species in the pan-Amazonian region and main crops attacked

Leaf-cutting ant species	Geographical distribution in the pan-Amazonian region	Crops preference	Impact as pest	References
Atta cephalotes Linnaeus	Brazil, Bolivia, Colombia, Ecuador, French Guiana, Guyana, Peru, Surinam, Venezuela	Coca, citrus, coffee, cotton, maize, cassava, eucalyptus, forages, coconut, cucurbits, sweet potato, mango, avocado, sugarcane	Very wide	Montoya-Lerma et al. (2012), Zanuncio et al. (1996), Rodríguez et al. (2015), CABI (2020), Bezděčková et al (2015)
Atta sexdens Linnaeus	Bolivia, Brazil, Colombia, French Guiana, Venezuela	Cassava, eucalyptus, *Pinus* sp., cashew, citrus, coffee, mango, rice, coconut, cucurbits, rubber, sugarcane	Wide	Montoya-Lerma et al. (2012), Remillet (1988), CABI (2020)
Atta laevigata F. Smith	Brazil, Colombia, Venezuela	Eucalyptus, *Pinus* sp., cashew	Wide	Montoya-Lerma et al. (2012)
Atta bisphaerica Forel	Brazil	Gramineous, sugar cane	Narrow	Montoya-Lerma et al. (2012)
Acromyrmex balzani Emery	Brazil, Colombia	Eucalyptus	Narrow	Montoya-Lerma et al. (2012)
Acromyrmex coronatus Fabricius	Brazil, Colombia, Ecuador	Herbaceous plants	Wide	Montoya-Lerma et al. (2012)
Acromyrmex landolti Forel	Bolivia, Brazil, Colombia, French Guiana, Guyana, Venezuela	*Pinus* sp., grasses	Wide	Montoya-Lerma et al. (2012), Santos-Silva et al. (2016), Remillet (1988)
Acromyrmex octospinosus Reich	Brazil, Colombia, French Guiana, Guyana, Surinam, Venezuela	Vegetables, fruits, cacao, citrus	Wide	Montoya-Lerma et al. (2012), Remillet (1988), Mann (1916), Pérez-Sánchez et al. (2014), Boulogne et al (2014)

that considers economic, environmental, and social dimensions of the control methods.

The objective of this study was, therefore, to perform a multi-criteria analysis based on several independent studies focused on the same issue (Gates 2002), which was the control methods used for leaf-cutting ants in the pan-Amazon Region—i.e., Brazil, Bolivia, Colombia, Ecuador, Peru, Venezuela, Suriname, Guyana, and French Guiana. The main purpose was to highlight the most effective means of control, thus ensuring less impact on human health and the environment.

Materials and Methods

Data Collection

A prospective search of the literature was carried out using Google scholar and using particular keywords: 'leaf-cutting ants', 'leaf-cutter ants', 'fourmis manioc', 'formigas cortadeiras', and 'hormigas cortadoras' (quotation marks indicate that the term was used in its entirety). This use of different languages—i.e., French, Portuguese, and Spanish, as well as English—was essential to gathering information from the different countries impacted by leaf-cutting ants in the pan-Amazonian Region. This approach did not taken into account specific websites, such as websites of agricultural institutions. The data were collected during the last quarter of 2019 by scanning the work done by several universities in South America. The first step consisted in gathering original articles, conference papers, book chapters, working papers, reports in institutional series, and theses dealing with this topic. The control methods studied could be chemical, mechanical, integrated, or biocontrol methods, acting at different places and times over the colony life cycle (Fig. 1).

The second step mainly focused on selecting relevant articles from peer-reviewed scientific publications and conference papers. While the peer-reviewed studies primarily concerned laboratory experimentation, the master's and Ph.D. reports we found covered various aspects of evaluating control methods for leaf-cutting ants, so we have included them if they were of good scientific quality, so as not to lose valuable information that had not necessarily been published in scientific journals. Overall, we selected 112 studies from peer-reviewed journals, 22 master's reports, 11 Ph.D. theses, and 3 conference papers.

Appropriate studies for the multi-criteria analysis were chosen from the database by thoroughly screening the title, abstracts, keywords, and full-text. The studies selected fulfilled the following criteria: 1) they investigated a control method for leaf-cutting ants; 2) the leaf-cutting ants species concerned are present in the pan-Amazonian region; 3) the study described at least the efficacy of the method, and 4) the study involved original experimentation and not just a review of other studies.

In several cases, the same article could present various observations or approaches; for instance, an evaluation of the toxicity of different plant extracts on several leaf-cutting ants species. All the observations complying with our criteria were included in the multi-criteria analysis, so the number of observations used in the multi-criteria analysis (i.e., 692 observations) was larger than the number of publications selected (i.e., 145 articles).

For each observation, the management method and substances employed were listed. For biocontrol methods, the principal type—i.e., plant, fungus, mineral, organic molecule of natural origin—and the family were identified. As a function of the different spatial scales, complexity and location of the selected observations, we

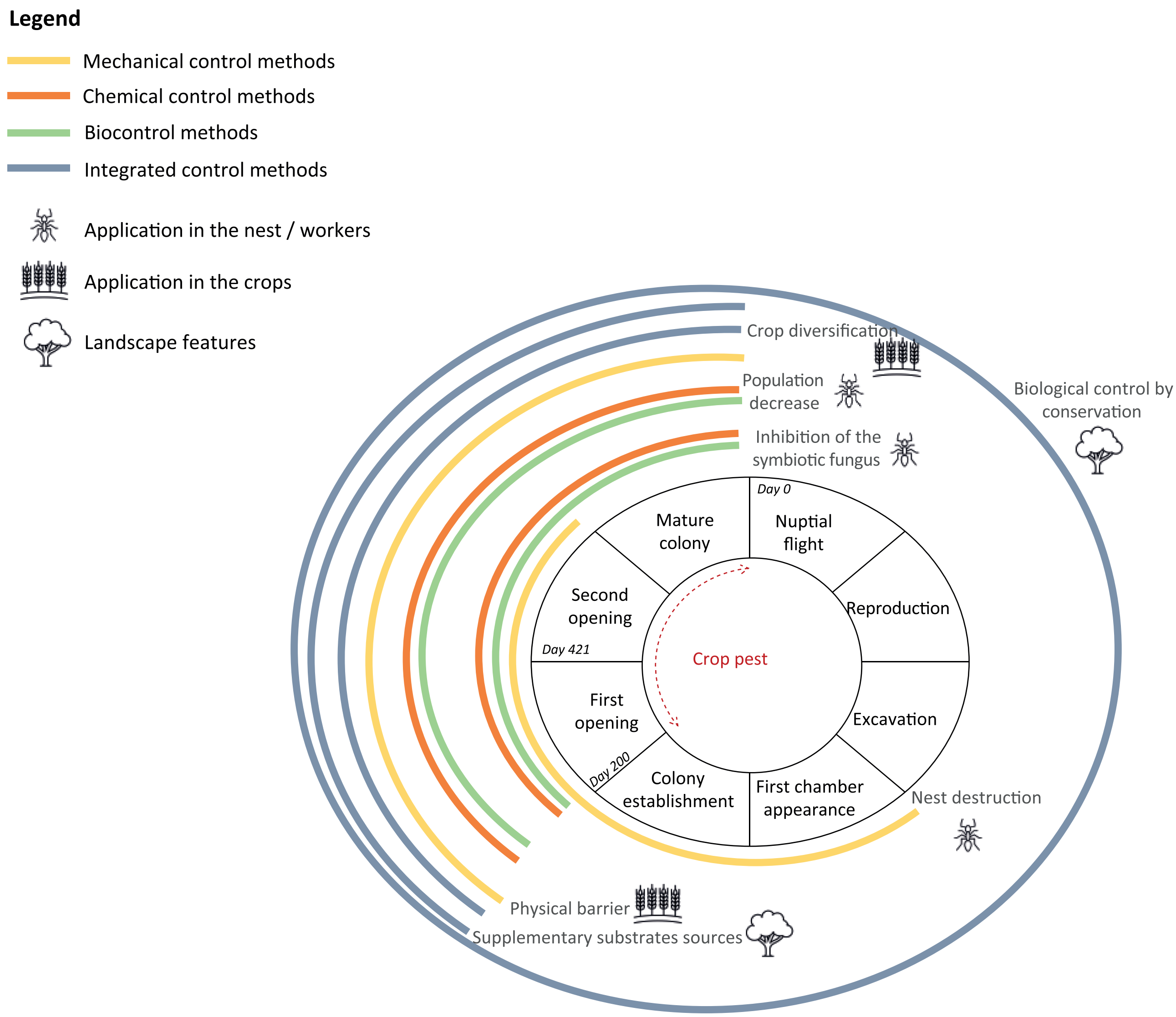


Fig. 1. Type of Control methods used according to nest maturity.

described the factors influencing the study specifications—i.e., publication category, country, region, experimental environment, and the experimental details—i.e., concentration of substance used and type of application, to take account of the heterogeneity of the situations and any potential bias that might affect the relevancy of the explanations.

The studies thus selected were then analyzed using the different criteria, as shown in Fig. 2.

The criteria and scales used to analyze the control methods were based on those specified in ANSES studies (French Agency for Food, Environmental and Occupational Health Safety).

The process used to assess the efficacy of methods involving the control of workers (i.e., ability to control the fungus or colony) in terms of reducing the damage caused by leaf-cutting ants, differed depending on the study. In order to compare results expressed in different ways, we created a value scale from 0 to 4:

0: noneffective observations;
1: studies of potential interest, but insufficient efficacy at present (e.g., natural parasitoid without biocontrol development);
2: significantly effective methods but producing poor results (control or damage limitation less than 50%);
3: significantly effective methods (i.e., control or damage limitation higher than 50% but dependent on different conditions, such as successful control only achieved at high concentrations);
4: methods already significantly effective; i.e., control or damage limitation higher than 50%.

During a second phase, an expert review of the bibliography was made to evaluate the environmental and human health impacts of the different elements evaluated. An appropriate scale was also created, from 1 to 4:

1: elements highly toxic to the environment or human health;
2: elements moderately toxic to the environment or human health;
3: elements weakly toxic to the environment or human health;
4: can be considered as safe to the environment or human health.

A scale was also created regarding the operational readiness of control methods:

1: laboratory testing;
2: ongoing development of the method;
3: method currently available.

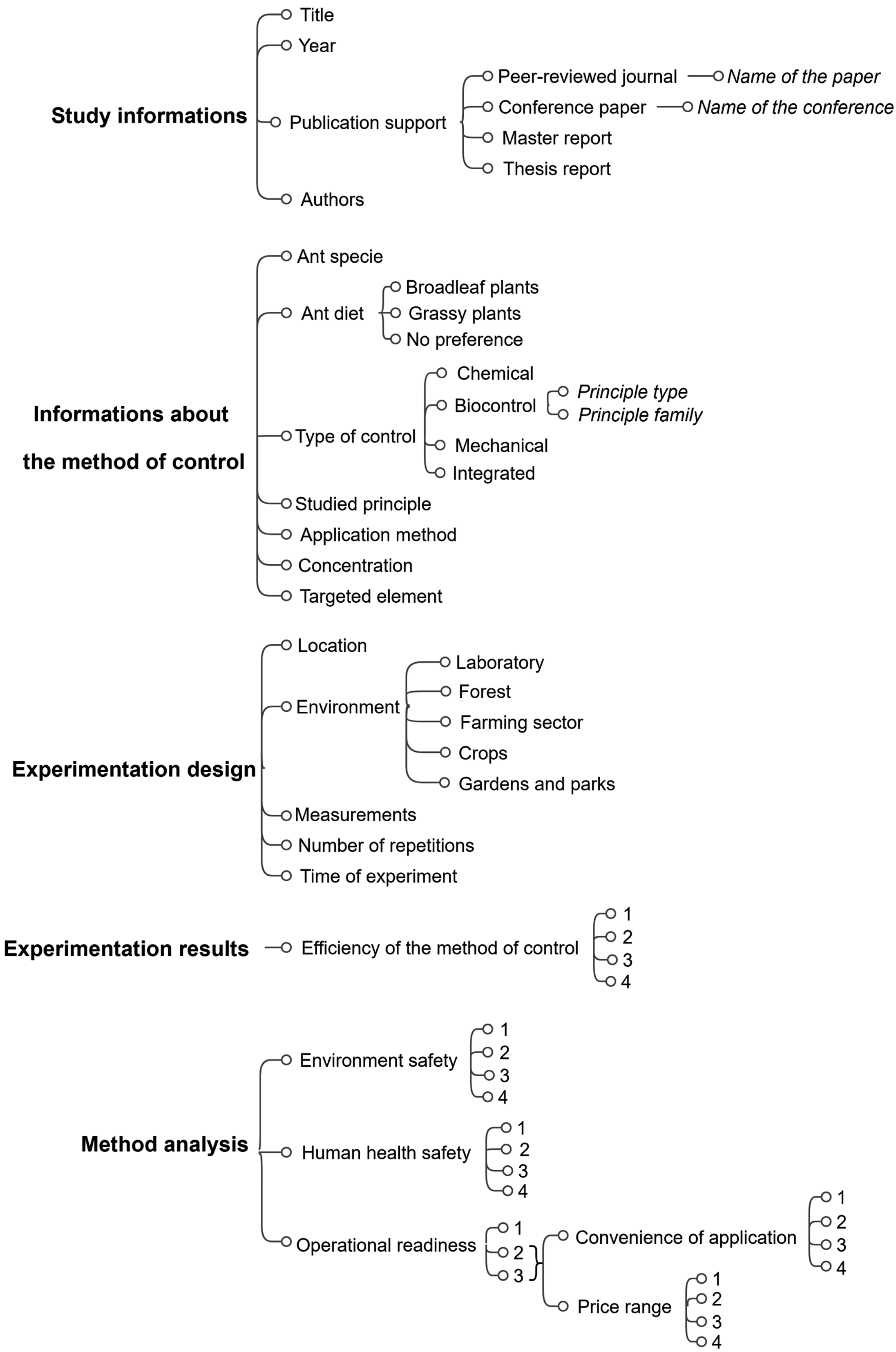


Fig. 2. Criteria used to evaluate the control methods tested in the different studies.

Table 2. Sample size for each variable of the multi-criteria analysis

	Efficacy	Environmental impact	Human health impact	Operational readiness	Convenience of application	Price
PCA	691	689	689	691	135	79
Beauveria bassiana	Against colonies: 4 Against *L. gongylophorus*: 3 Against workers: 3	10	10	10	10	10
Metharizium anisopliae	Against colonies: 4 Against *L. gongylophorus*: 3 Against workers: 11	18	18	18	18	18
Tithonia diversifolia	Against colonies: 1 Against *L. gongylophorus*: 1 Against workers: 1	3	3	3	3	3
Canavalia ensiformis	Against colonies: 3 Against *L. gongylophorus*: 1 Against workers: 2	6	6	6	6	6

For methods that had been developed, the convenience of their application was evaluated on a scale from 1 to 4:

1: complex application;
2: moderately difficult application;
3: simple application;
4: very convenient application.

The prices of the different control methods available were also evaluated on a scale from 1 to 4, taking account of the purchasing power of an average farmer in French Guiana:

1: expensive—i.e., more than €100 per nest;
2: moderately priced—i.e., €50 to €100 per nest;
3: inexpensive—i.e., less than €50 per nest;
4: negligible cost.

The different criteria were then entered in an Excel spreadsheet (Microsoft Pack Office 2017).

Statistical Analysis

As a first step, we ensured that the studies had used independent datasets; i.e., they were performed by different research teams from different organizations in different environments, in order to prevent any bias in the dataset.

We performed the multi-criteria assessment of the type of control, control efficacy, environmental safety, human health safety, operational readiness, convenience and price ranges using Principal Component Analysis (PCA) with the FactoMineR and factoextra packages. The inputs used for the PCA were the semiquantitative ranks described above. The criteria were gathered as centered and normalized variables in a PCA as a precaution, the order of magnitude being the same than for noncentered variables, with information on the control method as qualitative variables, and experimental results and analysis of the method as quantitative variables. The PCA was carried out to illustrate the relationship between the criteria. The individual scatterplot was drawn with the FactoMineR package version 2.3, with levels of the concentration ellipses set as 0.95.

In order to precise the most promising control methods for leaf-cutting ants, various univariate tests have been performed to compare the efficacy of the control methods, the environment and human health safety, then the operational readiness of the different control methods. We also compared the price and convenience of application for interesting methods with available data (Table 2). The average and effect size of each outcome variable was evaluated for the different methods according to the scores defined above. The rankings were compared using the Kruskall–Wallis test and the Mann–Whitney post-hoc test. We considered the differences to be significant at a 5% threshold. The data were compared using the median values—i.e., IQR.

For significantly effective control methods ranking 3 or above, the impacts on human health and the environment were based on the literature and European standards, available via the website at <https://ec.europa.eu>. For the effective control methods with low impacts, we noted the means of application has been developed and its convenience.

Only the most relevant data in the fully analyzed dataset concerning methods with good efficacy, a low impact on the environment and human health are described in greater detail in this article.

Statistical analysis was performed using Rstudio version 3.6.1. software from the R Foundation for Statistical Computing, 2016.

Results

Principal Component Analysis

The ‘Applicator safety’ and ‘Environmental safety’ variables are clearly shown within the correlation circle ($\cos^2 = 0.861$; $\cos^2 = 0.826$, respectively) and explain 79.03% of the first dimension (Fig. 3). The first dimension describes the safety of the method, with the safest method on the right and the most toxic methods on the left, which explains 35.6% of individual distribution. The orientation of the ‘Efficacy’ and ‘Operationality’ variables, which are poorly displayed in the circle ($\cos^2 = 0.204$; $\cos^2 = 0.212$, respectively) explains 19.48% of the first dimension, and appears to indicate that the most effective methods with market applications might be toxic to human health and the environment.

The ‘Price’ and ‘Convenience’ variables are well displayed within the correlation circle ($\cos^2 = 0.670$; $\cos^2 = 0.512$, respectively) and explain 92.67% of the second dimension (Fig. 3). The second dimension describes how easy it would be for farmers to adopt the method, with affordable methods at the top and methods practically unusable for farmers at the bottom of the circle, which explains 21.3% of the individual distribution.

The eigenvalues of the first (2.14) and second (1.28) dimensions are higher than 1, unlike the eigenvalues of the other dimensions.

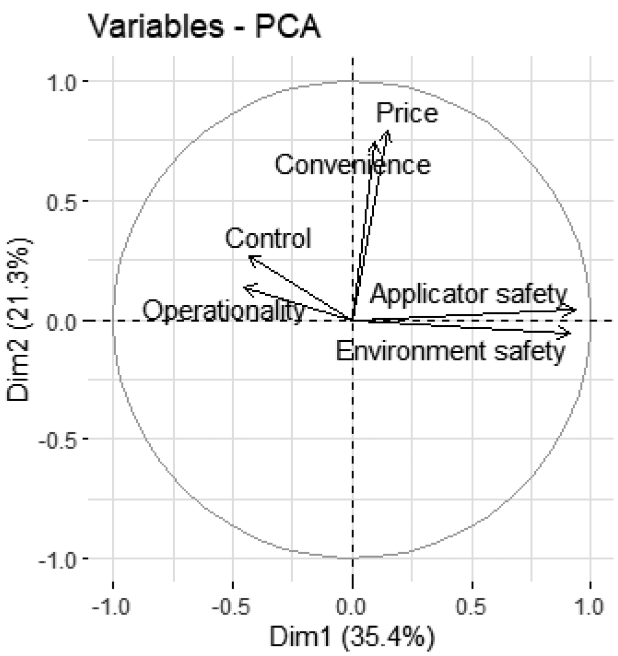


Fig. 3. Correlation circle of variables.

Only the first and second dimensions were therefore selected to explain the individual distributions according to the Kaiser criterion (i.e., selection of principal components with eigenvalues >1).

According to the correlation circle for the variables, the first axis of the individual scatterplot of the PCA defines the safety of the management method, and the second axis explains the ease of adoption of the method by farmers (Fig. 4).

The effective methods and developed products are mostly chemical—i.e., Sulfuramid, Fipronil, Mirex—but these are not sustainable because they are harmful to human health and the environment (Fig. 4). While farmers can easily adopt some of these developed products, some methods are still undergoing laboratory testing or require special equipment, thus hampering their use. Mechanical and integrated methods are sustainable but not very effective in the management of leaf-cutting ants (Fig. 4). Mechanical methods may involve the use of large pieces of equipment such as excavators which can be costly for farmers, or may only require manual labor, which is affordable for most but time-consuming. Most of the integrated methods could not be evaluated in terms of their price or convenience of application. Biocontrol methods include a broad range of methods with differing efficacy, impacts on the environment and human health and ease of adoption by farmers.

Encouraging results were seen with some of the biocontrol methods involving entomopathogenic fungi and plant extracts, as well as a low risk to human health and/or the environment (Fig. 5).

Entomopathogenic Fungi

Use of the *Beauveria bassiana* (Bals.-Criv.) Vuill. (Hypocreales: Cordycipitaceae) fungus caused damage to colonies (IQR = 4) of *Acromyrmex* sp. (Páiz and Granados 2013) and *Atta sexdens rubropilosa* (Canali 2017). *Beauveria bassiana* was toxic to the symbiotic fungus *Leucoagaricus gongylophorus* (IQR = 4) and *Atta sexdens rubropilosa* (Santos et al. 2007, Castilho et al. 2010, Canali 2017), *Atta sexdens sexdens* (Loureiro and Monteiro 2005), *Atta* sp., and *Acromyrmex* sp. (Bezerra 2018) worker ants (IQR = 4). The use of *Beauveria bassiana* with other entomopathogenic fungi such as *Aspergillus nomius* Kurtzman, B. W. Horn and Hesselt. (Eurotiale: Trichocomaceae), *Isaria farinosa* (Holmsk.) Fr. (Hypocreales: Cordycipitaceae) or *Trichoderma harzianum* Rifai. (Hypocreales: Hypocreaceae) was less effective in controlling colonies (IQR = 2.5; IQR = 2; IQR = 2, respectively, than using *Beauveria bassiana* alone, except for the combination of *Beauveria bassiana* and *Metarhizium anisopliae* (Metschn.) Sorokīn (Hypocreales: Clavicipitaceae), which effectively controlled *Acromyrmex landolti fracticornis* colonies (IQR = 4) (Amarilla Salinas and Arias Ruiz Díaz 2011). The *Metarhizium anisopliae* (Metschn.) Sorokīn fungus used alone displayed satisfactory efficacy in controlling colonies (IQR = 3) with good control of *Atta cephalotes* colonies (López Arismendy and Orduz Peralta 2002). *Metarhizium anisopliae* displayed satisfactory efficacy in controlling the symbiont (IQR = 4). It provided an effective control of *Atta sexdens rubropilosa* (Jaccoud et al. 1999, Santos et al. 2007, Castilho et al. 2010, Barcoto et al. 2017, Canali 2017), *Atta cephalotes* (Varón Devia 2006), and *Atta sexdens sexdens* (Loureiro and Monteiro 2005, Barbosa and de Sousa 2012) workers (IQR = 4). *Metarhizium anisopliae* caused high mortality among *Atta sexdens* worker ants when cyclosporine was added (IQR = 4) (Dornelas et al. 2017). The combination of *Metarhizium anisopliae* with *Trichoderma viride* Pers. (Hypocreales: Hypocreaceae) enabled the effective control of *Atta cephalotes* colonies (IQR = 4) (López Arismendy and Orduz Peralta 2002), whereas the use of *Trichoderma viride* alone did not achieve that (IQR = 1) and displayed weak toxicity on the symbiont (IQR = 1) and workers (IQR = 0).

The most effective methods to control field colonies were the commercial bait Bibisav-2 using *Beauveria bassiana* at a concentration of 100 g/m^2 for *Acromyrmex octospinosus* colonies (IQR = 4) (Pérez Álvarez and Trujillo González 2002); artisanal bait made from *Metarhizium anisopliae* powder at a concentration of 10^9 conidia/ml for *Atta sexdens rubropilosa* colonies (IQR = 4) (Travaglini 2017); spraying a solution of 200 g/3 liter *Beauveria bassiana* (IQR = 4) in Acromyrmex sp. nests (Páiz and Granados 2013). Spraying a mixture of *Beauveria bassiana* and *Trichoderma harzianum* at a concentration of 266 g/4 liter was also effective in controlling Atta sp. field colonies (IQR = 4) (Páiz and Granados 2013), but had no effect on workers (IQR = 0). The use of conidia powder in the nest with a mixture of *Beauveria bassiana* and *Aspergillus nomius* or *Isaria farinosa* at 10^9 conidia/ml had a weak effect on colonies (IQR = 2). The use of artisanal bait made from *Metarhizium anisopliae* powder was ineffective at a concentration of 10^9 conidia/g (IQR = 0).

According to the literature and to European legislation, *Beauveria bassiana* and *Metarhizium anisopliae* have been evaluated as being safe for the environment and human health.

Plant Extracts

The use of certain plant extracts such as *Tithonia diversifolia* (Hemsley) A. Gray (Asterales: Asteraceae) and *Canavalia ensiformis* L. DC. (Fabales: Fabaceae) has also been shown to be effective in controlling the activity of leaf-cutting ants.

Tithonia diversifolia extracts also exert fungicidal effects against the symbiotic fungus (IQR = 4) but are only weakly toxic on worker ants (IQR = 2). Using a 10–30 kg mulch of *Tithonia diversifolia* of stems and leaves in nest sizes inferior to 35 m^2, enabled the effective management of *Atta cephalotes* colonies in the field (IQR = 4) (Rodríguez et al. 2015); as use of this plant is not considered to constitute a plant health product, it is considered to be safe to the environment and operator health.

Canavalia ensiformis extracts were shown to be toxic to the symbiont (IQR = 4) but produced mixed results regarding worker

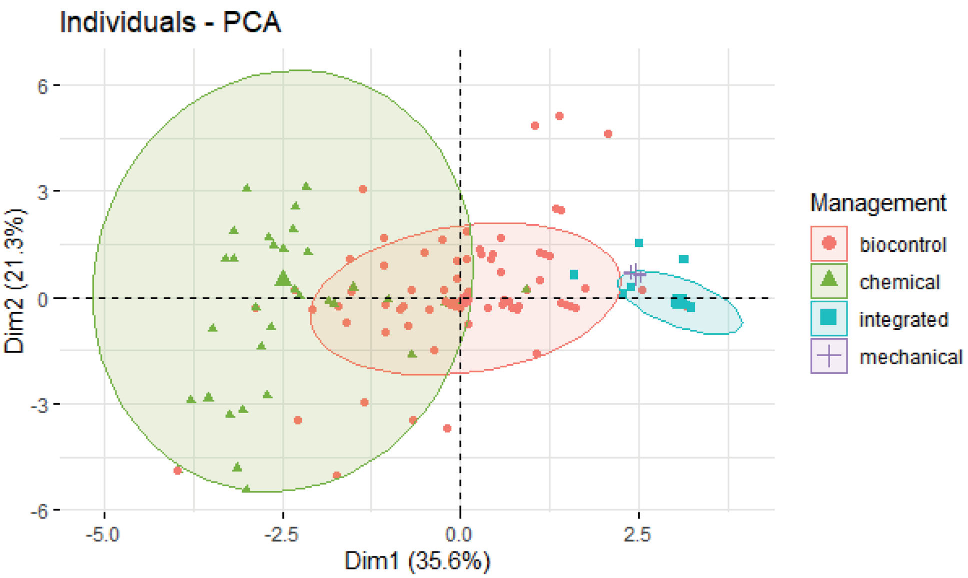


Fig. 4. Individual scatterplot of the PCA.

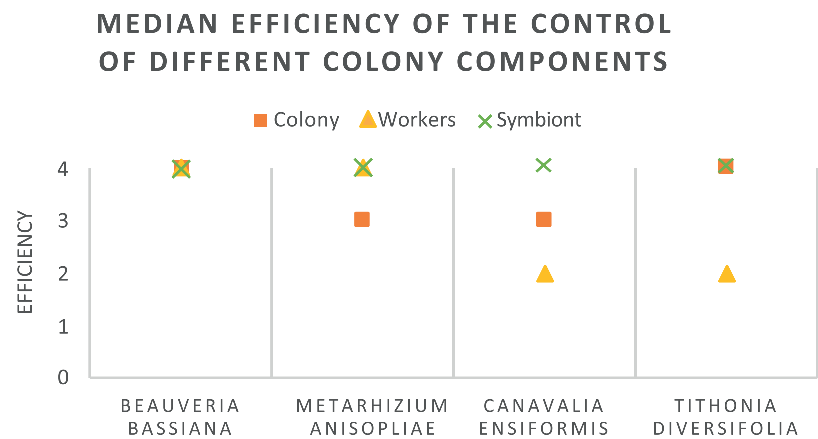


Fig. 5. Median efficacy of the control of different colony components using entomopathogenic fungi and plant extracts.

control (IQR = 2). These extracts were effective in *Atta* sp. controlling colonies (IQR = 3) (Sánchez 2005), whereas their use combined with *Cymbopogon citratus* (DC.) Stapf (Poales: Poaceae) extracts (IQR = 2) or *Gallesia integrifolia* (Spreng.) Harms (Caryophyllales: Phytolaccaceae) extracts (IQR = 2) were little effective. *Canavalia ensiformis* leaves used as a mulch in leaf-cutting ant nests was effective (IQR = 4) in controlling *Atta* sp. field colonies at doses of 7–9 kg per nest for nest of 30 m^2 (Sánchez 2005); an aqueous solution of *Canavalia ensiformis* at a concentration of 1-kg plant material/liter resulted in the good control of *Atta* sp. field colonies (IQR = 3) (Sánchez 2005). Placing macerated seeds in the nests was not effective in controlling colonies (IQR = 0). As for *Tithonia diversifolia*, the application of mulch is not deemed to constitute a plant health product and can be considered as safe for the environment and human health. According to the literature and to European legislation, using an aqueous solution made from *Canavalia ensiformis* plant material has been evaluated as having a low risk for the environment and operator health.

Figure 6 shows the effective methods described above using entomopathogenic fungi or plants, and the times when they should be used as a function of the life cycle of a leaf-cutting ant colony.

Discussion

The management of leaf-cutting ants involves various control methods using chemical products, biological, mechanical, or integrated methods. However, few of these methods combine efficacy with safety for farmers and the environment and their simple adoption. Our analysis of these different methods allowed us to highlight certain methods that combined these requirements.

Leaf-cutting ant management using entomopathogenic fungi has been evaluated during various studies. Using fungi from the genus *Beauveria* and *Metarhizium anisopliae*, which are used as biocontrol agents for various arthropod pests (Goettel et al. 2005) produced some encouraging results regarding the control of leaf-cutting ants in the laboratory. These fungi are toxic to these ants because they produce hydrolytic enzymes that degrade the insect cuticle, such as the subtilisin-like serine protease produced by *Metarhizium anisopliae* (Abdelghany 2015). The *Beauveria* genus produces oosporin, a mycotoxin that disturbs the redox reaction and inhibits the activity of ATPase (Pinto et al. 2012). Entomopathogenic fungi can also develop mechanisms such as causing alterations to cell walls or producing immunomodulatory substances or toxins to overcome the immune system of the host insect (Cova et al. 2009). *Metarhizium*

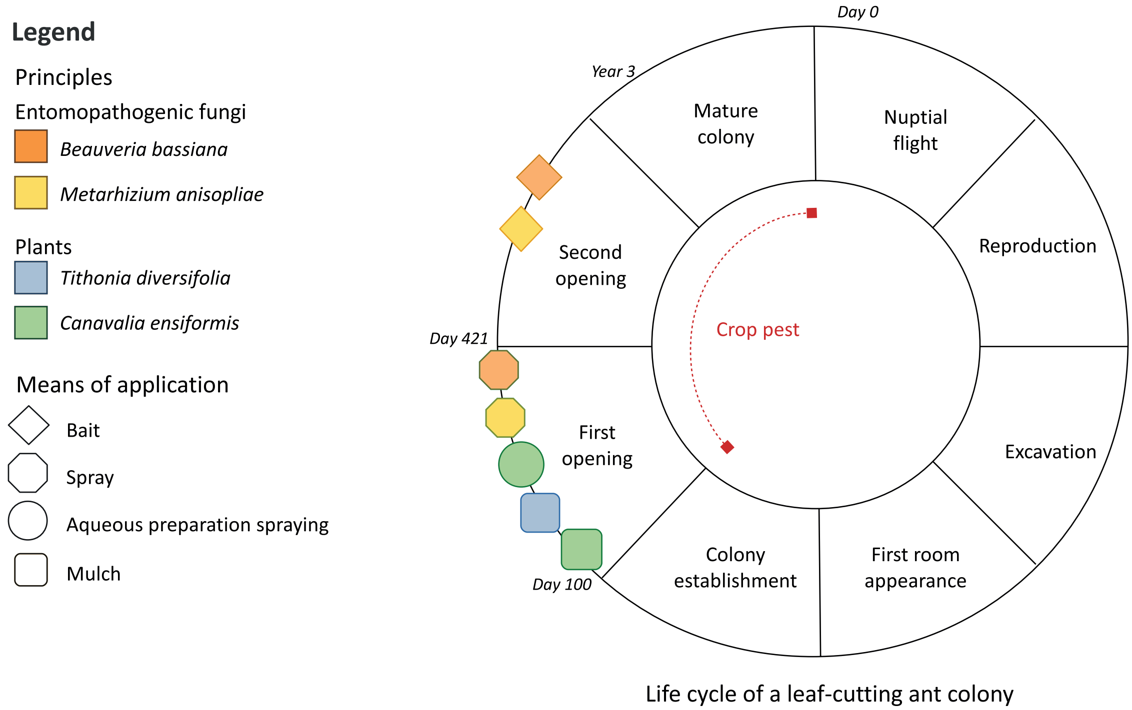


Fig. 6. Effective biocontrol methods for leaf-cutting ants as a function of the life cycle of the colony.

anisopliae can produce a protease that inhibits the adhesion activity and phagocytosis of plasmocytes in the infected insects (Silva 2002). Insects contaminated by *Beauveria* sp. or *Metarhizium* sp. display reduced feeding behavior, starting 1–4 d after contamination and lasting until they die (Abdelghany 2015).

The efficacy of control by *Beauveria bassiana* and *Metarhizium anisopliae* can differ between strains and isolates because of inter- and intraspecies genetic variability of entomopathogenic fungus (Oliveira et al. 2004, Castilho et al. 2010). Sporulation capacity and fungal pathogenicity against leaf-cutting ants depend on the species and isolate of the fungus used (Castilho et al. 2010). The effective control of leaf-cutting ant colonies also depends on the ant species (Castilho et al. 2010), the natural resistance of leaf-cutting ants (Alves 1998), colony size (Leclerc and Detrain 2018), and environmental conditions (Agostini et al. 2015). Leaf-cutting ants defend themselves against infection through behaviors such as grooming—i.e., the act of cleaning themselves and other ants of alien spores—or weeding—i.e., removing contaminated parts of the fungus garden (Currie and Stuart 2001)—and remove dead and sick ants from the colonies (Cardoso 2010). Because of the highly complex and evolved behavior of leaf-cutting ants, the use of moderately virulent isolates could be more effective than that of highly virulent isolates, so as not to stimulate a rapid defense reaction by the ants (Castilho et al. 2010).

Effective laboratory tests are not always successful when transferred to the control of colonies in the field (Castilho et al. 2010). Although laboratory conditions are closed off from field conditions, the stress generated by manipulating the workers and a different colony organization (e.g., the presence of only one cast and absence of a queen) may favor worker mortality (Castilho et al. 2010; Ribeiro et al. 2012). Because of this laboratory stress and the queen's ability to replace workers in the event of deaths, field colonies are more resilient than colonies without a queen tested under laboratory conditions. Field workers adapt their behavior towards the contaminant, changing their activities and using different entrance holes to avoid the hazard (Silva and Diehl-Fleig 1988, Diehl-Fleig and Lucchese 1991); leaf-cutting ants may even relocate their nest in extreme cases (Machado et al. 1988), while they cannot do this under laboratory conditions.

To overcome these problems and achieve the effective control of field colonies, it might be useful to increase the doses, like López and Orduz (2003) who obtained high mortality rates with a bait containing a mix of *Metarhizium anisopliae* and *Trichoderma viride* (1×10^9 conidia/g; 20 g/m^2) and applying the treatment three times instead of once. Spraying with an aqueous solution containing entomopathogenic fungi has also resulted in good field colony management, but the volume of the solution needs to be adapted to the size of the colony. Nevertheless, spraying is less convenient for an applicator than bait, and requires specific equipment. Both *Beauveria bassiana* and *Metarhizium anisopliae* have been evaluated as being safe for the environment and human health, but only some strains have been approved by European regulations (available on the EU Pesticide database website: https://ec.europa.eu.). In case of environmental contamination, a high risk for honeybees has been identified with the application of *Beauveria bassiana* strain 147, but the risk for nontarget arthropods could not be demonstrated, due to the lack of available information (European Food Safety Authority 2015). *Metarhizium anisopliae* shows a low-risk to nontarget arthropod compared with chemicals application. A data gap was identified for the long-term impact of *Metarhizium anisopliae* to the nontarget arthropods, due to the persistency of the fungus in soil and the wide range of hosts (European Food Safety Authority 2012).

This multi-criteria analysis also showed that the use of plants and plant extracts also produced some interesting control results. *Tithonia diversifolia* and *Canavalia ensiformis* displayed toxic effects against *Leucoagaricus gongylophorus*. This toxicity could be explained by the presence of secondary metabolites in the tissue of the plant, such as flavonoids (Mullenax 1979, Chagas-Paula et al. 2012) or a mix of fatty acids (Monteiro et al. 1998). The direct effect of plant substrates in the fungus garden may be limited under field conditions, as gardeners can detect the fungus garden response to the incorporation of plant substrates, and reject those harmful to the garden (Barcoto et al. 2017). *Tithonia diversifolia* and *Canavalia ensiformis* have also displayed insecticidal effects against workers. Secondary metabolites such as glycosides, alkaloids, phenols, or essential oils may explain the toxicity of these plants against insects, as these compounds also act as inhibitors of growth and reproduction as well as insect repellents and deterrents (Jacobson 1989, Saxena 1989). Secondary compounds may also be toxic to the microorganisms involved in ant protection and the decomposition of plant biomass (Rodríguez et al. 2015).

A mulch of *Tithonia diversifolia* and *Canavalia ensiformis* enabled the effective control of field colonies. The incorporation of these plants into soils may indirectly harm the fungus garden, as their decomposition may induce a rise in the pH, as was observed for *Tithonia diversifolia* by Ikerra et al. (2006), or it could affect the fungal symbiont (Loeck et al. 2004). In response to this attack, workers may build new chambers and tunnels to relocate the fungus garden, thus reducing external activity (Rodríguez et al. 2015). The use of a mulch to cover openings may also affect ventilation of the nest (Rodríguez et al. 2015). The use of plant mulch to control leaf-cutting ants nest has been evaluated as safe for the environment and applicator health, and can be used under European regulations. Mulch applications are simple to implement and represent an inexpensive method of control.

Tithonia diversifolia is widely spread around the world and can be found in Brazil, Colombia, Ecuador, French Guiana, and Venezuela (CABI 2020). This plant is considered as invasive in various countries but has been naturalized in the northern regions of Brazil (i.e., Amazonas, Para), in Colombia, French Guiana, and Venezuela (CABI 2020). *Tithonia diversifolia* can be found in various ecosystems including agricultural lands, disturbed areas, and roadsides (CABI 2020). Hence, *Tithonia diversifolia* can be easily found by farmers and used as mulch. *Canavalia ensiformis* has been introduced in various countries of South America and can be found in Brazil, Colombia, Ecuador, French Guiana, Guyana, Peru, Surinam, and Venezuela (CABI 2020). This plant can be found in agricultural land, disturbed areas, natural forests and shrublands (CABI 2020). Farmers from the pan-amazonian may have access to *Canavalia ensiformis* for mulch use.

This multi-criteria analysis highlighted some management methods to control leaf-cutting ants that are effective and safe for human health and the environment. Because of the considerable variability of the results that can occurs for the different control methods, dependent on the genetic diversity of the entomopathogenic fungi or plants used, environmental conditions, the species of leaf-cutting ants and the characteristics of different colonies, more field studies on the application of plant mulch and entomopathogenic fungi to control leaf-cutting ants now need to be performed in order to clarify the factors underlying their success. Leaf-cutting ants are able to adapt their behavior to threats and disturbances (Norman et al. 2017); we therefore encourage a combination of control methods so as to limit their opportunities for adaptation. Biocontrol methods could be used to limit ant activity, alongside mechanical (e.g., physical barriers) and integrated (e.g., crop diversification) management methods to protect crops. Future experiments should be conducted using designs managed in collaboration with local farmers, in order to whether particular methods are suited to the cultural and socioeconomic conditions of the region, thus facilitating their adoption by farmers.

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References Cited

Abdelghany, T. M. 2015. Entomopathogenic fungus and their role in biological control. OMICS Group eBooks, Foster City, CA.

Agostini, T. T., L. T. Agostini, R. T. Duarte, H. X. L. Volpe, C. Salas, and R. A. Polanczyk. 2015. Eficiência de fungos entomopatogênicos para o controle de *Sitophilus oryzae* L. (Coleoptera: Curculionidae) em condições de laboratório. Comun Sci. 06: 90–96.

Alves, S. B. 1998. Controle microbiano de insetos, 2nd edn. FEALQ, Piracicaba, Brazil.

Amarilla Salinas, E., and O. E. Arias Ruiz Díaz. 2011. Control de la hormiga cortadora 'Akeke' Acromyrmex landolti con hongos entomopátogenos. Investig. Agrar. 13: 27–32.

Barbosa, S. E. M., and D. J. de Sousa. 2012. Fungos filamentosos associados às formigas cortadeiras do gênero Atta fabricius e estudo da ação de fungos entomopatogênicos. *In* 8° Seminário de Iniciação científica UFT, V. 4, 11–14 December 2012, Universidade Federal do Tocantins, Palmas, Brazil.

Barcoto, M. O., F. Pedrosa, O. C. Bueno, and A. Rodrigues. 2017. Pathogenic nature of Syncephalastrum in Atta sexdens rubropilosa fungus gardens. Pest Manag. Sci. 73: 999–1009.

Barrera, C. A., L. M. Buffa, and G. Valladares. 2015. Do leaf-cutting ants benefit from forest fragmentation? Insights from community and species-specific responses in a fragmented dry forest. Insect Conserv. Divers. 8: 456–463.

Basbas, S., and C. M. Makridakis. 2007. A review of the contribution of multi-criteria analysis to the evaluation process of transportation projects. Int. J. Sus. Dev. Plann. 2: 387–407.

Bezděčková, K., P. Bezděčka, and I. Machar. 2015. A checklist of the ants (Hymenoptera: Formicidae) of Peru. Zootaxa. 4020: 101–133.

Bezerra, N. S. 2018. Eficiência de fungos entomopatogênicos sobre formigas cortadeiras (Hymenoptera: Formicidae). M.S. thesis, Universidade Federal da Paraíba, João Pessoa.

Boulogne I., H. Ozier-Lafontaine, and G. Loranger-Merciris. 2014. Leaf-cutting ants, biology and control. In Lichtfouse E. (eds). Sustain. Agric. Rev. 13: 1–17.

CABI. 2020. Invasive species compendium. CAB International, Wallingford, United Kingdom.

Camargo, R. S., L. C. Forti, J. F. S. Lopes, and N. S. Nagamoto. 2006. Studies of leaf-cutting ants, *Acromyrmex* spp. (Formicidae, Attini): behavior, reproduction and control. Res. Dev. Entomol. 5: 1–21.

Canali, M. C. 2017. Prospecção de fungos entomopatogênicos para o controle biológico de formigas cortadeiras. M.S. thesis, Universidade Estadual Paulista, Rio Claro, Brazil.

Cardoso, S. R. S. 2010. Morfogênese de ninhos iniciais de *Atta* spp. (Hymenoptera: formicidae), mortalidade em condições naturais e avaliação da ação de fungos entomopatogênicos. Ph.D. dissertation, Universidade Estadual Paulista, Botucatu, Brazil.

Castilho, A. M. C., M. E. Fraga, E. L. Aguiar-Menezes, and C. A. R. Rosa. 2010. Seleção de isolados de Metarhizium anisopliae e Beauveria bassiana patrogênicos a soldados de Atta bisphaerica e Atta sexdens rubropilosa em condições de laboratório. Cienc Rural. 40: 1243–1249.

Chagas-Paula, D. A., R. B. Oliveira, B. A. Rocha, and F. B. Da Costa. 2012. Ethnobotany, chemistry, and biological activities of the genus *Tithonia* (Asteraceae). Chem. Biodivers. 9: 210–235.

Cherrett, J. M., and D. J. Peregrine. 1976. A review of the status of leaf-cutting ants and their control. Ann. Appl. Biol. 84: 124–128.

Cova, L. J., J. V. Scorza, D. E. García, L. M. Cañizález, C. C. Guedez, M. Maffey, and M. G. Medina. 2009. Patogenicidad in vitro de *Beauveria* *brongniartii* (Sacc.) Petch en *Musca domestica* (L.) como posible estrategia de control. Zootec Trop. 27: 113–120.

Cramer, H. H. 1967. Plant protection and world crop production. Leverkusen, Bayer, Germany.

Currie, C. R., and A. E. Stuart. 2001. Weeding and grooming of pathogens in agriculture by ants. Proc. Biol. Sci. 268: 1033–1039.

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

Della Lucia, T. M., L. C. Gandra, and R. N. Guedes. 2014. Managing leaf-cutting ants: peculiarities, trends and challenges. Pest Manag. Sci. 70: 14–23.

Diehl-Fleig, E., and M. E. Lucchese. 1991. Reações comportamentais de operárias de Acromyrmex striatus (Hymenoptera, Formicidae) na presença de fungos entomopatogênicos. Rev. Bras. Entomol. 35: 101–107.

Dornelas, A. S. P., R. A. Sarmento, M. Pedro-Neto, D. G. da Silva, G. R. dos Santos, M. O. do Nascimento, C. A. Oliveira, and D. J. de Souza. 2017. Susceptibility of Atta sexdens worker ants treated with the immunosuppressant Sandimmun Neoral to Metarhizium anisopliae. Pesq. Agropec. Bras. 52: 133–136.

European Food Safety Authority. 2012. Conclusion on the peer review of the pesticide risk assessment of the active substance *Metarhizium anisopliae* var. *anisopliae* BIPESCO 5/F52. EFSA J. 10: 2498.

European Food Safety Authority. 2015. Peer review of the pesticide risk assessment of the active substance *Beauveria bassiana* strain 147. EFSA J. 13: 4261.

Farji-Brener, A. G., and A. Rgguiero. 1994. Leaf-cutting ants (Atta and Acromyrmex) inhabiting Argentina: patterns in species richness and geographical range sizes. J. Biogeogr. 21: 391–399.

Fernandez, F., V. Castro-Huertas, F. Serna. 2015. Hormigas cortadoras de hojas de Colombia: Acromyrmex & Atta (Hymenoptera: Formicidae). Fauna de Colombia. Monografia N 5. Universidad Nacional de Colombia, Bogotá, Colombia.

Folgarait, P., L. A. Dyer, R. J. Marquis, and E. Braker. 1996. Leaf-cutting ant preferences for five native tropical plantation tree species growing under different light conditions. Entomol. Exp. Appl. 80: 521–530.

Fowler, H. G. 1995. The population status of the endangered Brazilian endemic leaf-cutting ant *Atta robusta* (Hymenoptera: Formicidae). Biol. Conserv. 74: 147–150.

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

Gates S. 2002. Review of methodology of quantitative reviews using meta-analysis in ecology. J. Anim. Ecol. 71: 547–557.

Goettel, M. S., J. Eilenberg, and T. Glare. 2005. Entomopathogenic fungi and their role in regulation of insect populations, pp. 361–406. *In* L. I. Gilbert and S. S. Gill (eds.), Comprehensive molecular insect science. Elsevier, Boston, MA.

Gomes de Siqueira, C., M. Bacci, Jr., F. C. Pagnocca, O. A. Bueno, and M. J. A. Hebling. 1998. Metabolism of plant polysaccharides by *Leucoagaricus gongylophorus*, the symbiotic fungus of the ant *Atta sexdens* L. Appl. Environ. Microbiol. 64: 4820–4822.

Ikerra, S., E. Semu, and J. Mrema. 2006. Combining *T. diversifolia* and *minjingu* phosphate rock for improvement of P availability and maize grain yields on a chromic acrisol in Morogoro, Tanzania. Nutr. Cycl. Agroecosyst. 76: 249–260.

Jaccoud, D., W. Hughes, and C. Jackson. 1999. The epizootiology of a Metarhizium infection in mini-nests of the leaf-cutting ant *Atta sexdens rubropilosa*. Entomol. Exp. Appl. 93: 51–61.

Jacobson, M. 1989. Botanical pesticides: present and future, pp. 1–9. *In* J. T. Arnason, B. J. R. Philogene and P. Morand, (eds.), Insecticides of plant origin. American Chemical Society, Washington, DC.

Khadempour, L., H. Fan, K. Keefover-Ring, C. Carlos, N. S. Nagamoto, M. A. Dam, M. T. Pupo, C. R. Currie. 2020. Metagenomics reveals diet-specific specialization of bacterial communities in fungus gardens of grass- and dicot-cutter ants. Front. Microbiol. 11: 22–27.

Koricheva, J., J. Gurevitch, and K. Mengersen. 2013. Handbook of meta-analysis in ecology and evolution. Princeton University Press, Oxfordshire, United Kingdom.

Leclerc, J., and C. Detrain. 2018. Impact of colony size on survival and sanitary strategies in fungus-infected ant colonies. Behav. Ecol. Sociobiol. 72: 1–10.

Little, A. E. F., T. Murakami, U. G. Mueller, and C. R. Currie. 2006. Defending against parasites: fungus-growing ants combine specialized behaviours and microbial symbionts to protect their fungus garden. Biol. Lett. 2: 12–16.

Littledyke, M., and J. M. Cherrett. 1975. Variability in the selection of substrate by the leaf-cutting ants *Atta cephalotes* (L.) and *Acromyrmex octospinosus* (Reich) (Formicidae, Attini). Bull Entomol Res. 65: 33–47.

Littledyke, M., and J. M. Cherrett. 1976. Direct ingestion of plant sap from cut leaves by the leaf-cutting ants *Atta cephalotes* (L.) and *Acromyrmex octospinosus* (Reich) (Formicidae, Attini). Bull. Entomol. Res. 66: 205–217.

Loeck, A. E., C. R. Pierobom, L. Guerra, and A. P. Afonso. 2004. Growth of symbiont fungi of some higher ants in mineral medium. Cienc. Rural. 34: 79–83.

López, E., and S. Orduz. 2003. *Metarhizium anisopliae* and *Trichoderma viride* for control of nests of the fungus-growing ant, *Atta cephalotes*. Biol. Cont. 27: 194–200.

López Arismendy, E., and S. Orduz Peralta. 2002. *Metarhizium anisopliae* y *Trichoderma viride* controlan colonias de *Atta cephalotes* en campo mejor que un insecticida químico. Rev Colomb. Biotecnol. 4: 71–78.

Loureiro, E. S., and A. C. Monteiro. 2005. Patogenicidade de isolados de três fungos entomopatogênicos a soldados de *Atta sexdens sexdens* (Linnaeus, 1758) (Hymenoptera: Formicidae). Rev. Árvore 29: 553–561.

Machado, V., E. Diehl-Fleig, M. E. Silva, and M. E. Lucchese. 1988. Reações observados em colônias de algumas espécies de Acromyrmex (Hymenoptera - Formicidae) quando inoculados com fungos entomopatogênicos. Cienc. Cult. 40: 1106–1108.

Mann, W. M. 1916. The ants of Brazil. Bull. Mus. Comp. Zool. 60: 399–490.

Mattoso, T. C., D. D. Moreira, and R. I. Samuels. 2011. Symbiotic bacteria on the cuticle of the leaf-cutting ant *Acromyrmex subterraneus subterraneus* protect workers from attack by entomopathogenic fungi. Biol. Lett. 8: 461–464.

Mikheyev, A. S., U. G. Mueller, and J. J. Boomsma. 2007. Population genetic signatures of diffuse co-evolution between leaf-cutting ants and their cultivar fungi. Mol. Ecol. 16: 209–216.

Monteiro, M. R., V. L. V. Torkomian, F. C. Pagnocca, P. C. Vieira, J. B. Fernandes, M. F. D. G. F. Da Silva, O. C. Bueno, and M. J. A. Hebling. 1998. Activity of extracts and fatty acids of *Canavalia ensiformis* (Leguminosae) against the symbiotic fungus of the leaf-cutting Atta sexdens. An. Acad. Bras. Cienc. 70: 733–736.

Montoya-Lerma, J., C. Giraldo-Echeverri, I. armbrecht, A. Farji-Brener, and Z. Calle. 2012. Leaf-cutting ants revisited: towards rational management and control. Int. J. Pest. Manage. 58: 225–247.

Mueller, U. G., J. J. Scott, H. D. Ishak, M. Cooper, and A. Rodrigues. 2010. Monoculture of leafcutter ant gardens. PLoS One. 5: e12668.

Mueller, U. G., M. R. Kardish, H. D. Ishak, A. M. Wright, S. E. Solomon, S. M. Bruschi, A. L. Carlson, and M. Bacci, Jr. 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.

Mullenax, C. H. 1979. The use of jackbean (*Canavalia ensiformis*) as a biological control for leaf-cutting ants (*Atta* spp). Biotropica. 11: 313–314.

Nagamoto, N., A. Carlos, S. Moreira, S. Verza, G. Hirose, and L. Forti. 2009. Differentiation in selection of dicots and grasses by the leaf-cutter ants *Atta laevigata* and Atta sexdens rubropilosa. Sociobiology. 54: 127–138.

Nichols-Orians, C. 1992. The acceptability of young and mature leaves to leaf-cutter ants varies with light environment. Biotropica. 24: 211–214.

Norman, V. C., T. Pamminger, and W. O. H. Hughes. 2017. The effects of disturbance threat on leaf-cutting ant colonies: a laboratory study. Insectes Soc. 64: 75–85.

Oliveira, R. C., P. M. O. J. Neves, and L. F. A. Alves. 2004. Seleção de fungos entomopatogênicos para o controle de *Oligonychus yothersi* (McGregor) (Acari: Tetranychidae), na cultura da erva-Mate (Ilex paraguariensis St. Hill.). Neotrop. Entomol. 33: 347–351.

Páiz, D. J. M., and J. J. V. Granados. 2013. Manejo de pobliaciones de zompopos con Beauveria bassiana y Trichoderma harzanium en la finca La Sonrisa des Pinto, comunidad Troilo. M.S. thesis, Universidad Nacional Autònoma de Nicaragua, León, Spain.

Pérez Álvarez, R. P., and Z. G. Trujillo González, G. Zoila. 2002. Combate de *Acromyrmex octospinosus* (Reich) (Hymenoptera: Formicidae); con el cebo micoinsecticida Bibisav-2. Fitosanidad. 6: 41–44.

Pérez-Sánchez, A. J., J. E. Lattke, and M. A. Riera-Valera. 2014. The Myrmecofauna (Hymenoptera: Formicidae) of the Macanao Semi-arid Peninsula in Venezuela: an altitudinal variation glance. J. Biodivers. Biopros. Dev. 1: 116.

Pinto, A. P. F., A. Batista Filhor, J. E. M. Almeida, and I. M. Wenzel. 2012. Patogenicidade de Beauveria bassiana ao psilídeo Diaphorina citri e compatibilidade do fungo com produtos fitossanitários. Pesqi. Agropecu. Bras. 47: 1673–1680.

Remillet, M. 1988. Catalogue des insectes ravageurs des cultures en Guyane française. ORSTOM, Paris, France.

Ribeiro, M. M., K. D. Amaral, V. E. Seide, B. M. Souza, T. Della Lucia, M. C. M. Kasuya, and D. J. De Souza. 2012. Diversity of fungi associated with *Atta bisphaerica* (Hymenoptera: Formicidae): the activity of *Aspergillus ochraceus* and *Beauveria bassiana*. Psyche. 2012: 1–6.

Roces, F. 2002. Individual complexity and self-organization in foraging by leaf-cutting ants. Biol. Bull. 202: 306–313.

Rodríguez, J., J. Montoya-Lerma, and Z. Calle. 2015. Effect of *Tithonia diversifolia* mulch on *Atta cephalotes* (Hymenoptera: Formicidae) nests. J Insect Sci. 15: 32.

Saarikoski, H., D. N. Barton, J. Mustajoki, H. Keune, E. Gomez-Baggethun, and J. Langemeyer. 2016. Multi-criteria decision analysis (MCDA) in ecosystem service valuation. In M. Potschin, and K. Jax (eds.), OpenNESS Ecosystem Services Reference Book. European Centre for Nature Conservation, Tilburg, the Netherlands.

Sánchez, V. A. A. 2005. Aplicación de très insecticidas naturales en el control del tujo (Atta spp.) en el cultivo de cacao en la region de alto beni. Master thesis, Universidad Mayor de San Andrés, La Paz, Bolivia.

Santos, A. V., B. L. de Oliveira, and R. I. Samuels. 2007. Selection of entomopathogenic fungi for use in combination with sub-lethal doses of imidacloprid: perspectives for the control of the leaf-cutting ant Atta sexdens rubropilosa Forel (Hymenoptera: Formicidae). Mycopathologia. 163: 233–240.

Santos-Silva, L., R. E. Vicente, and R. M. Feitosa. 2016. Ant species (Hymenoptera, Formicidae) of forest fragments and urban areas in a Meridional Amazonian landscape. Check List 12: 1885.

Saxena, R. C. 1989. Insecticides from nim, pp. 110–129. *In* J. T. Arnason, B. J. R. Philogene, and P. Morand (eds.), Insecticides of plant origin. American Chemical Society, Washington, DC.

Schowalter, T. D., and D. R. Ring. 2017. Biology and management of the texas leafcutting ant (Hymenoptera: Formicidae). J. Inter. Pest. Manag. 8: 1–8.

Silva, C. C. A. 2002. Aspectos do sistema imunológico dos insetos. Biotecnolog Cienc Desenvol. 2: 68–72.

Silva, M. E., and E. Diehl-Fleig. 1988. Avaliação de diferentes linhagens de fungos entomopatogênicos para controle da formiga *Atta sexdens piriventris* (Santschi, 1919) (Hymenoptera: Formicidae). An. Soc. Entomol. Bras. 17: 263–269.

Silva, A., M. Bacci, Jr., F. C. Pagnocca, O. C. Bueno, and M. J. Hebling. 2006. Starch metabolism in *Leucoagaricus gongylophorus*, the symbiotic fungus of leaf-cutting ants. Microbiol. Res. 161: 299–303.

Silva, P. S. D., A. G. D. Bieber, T. A. Knoch, M. Tabarelli, I. R. Leal, and R. Wirth. 2013. Foraging in highly dynamic environments: leaf-cutting ants adjust foraging trail networks to pioneer plant availability. Entomol. Exp. Appl. 147: 110–119.

Siqueira, F., J. Ribeiro Neto, M. Tabarelli, A. Andersen, R. Wirth, and I. Leal. 2017. Leaf-cutting ant populations profit from human disturbances in tropical dry forest in Brazil. J. Trop. Ecol. 33: 1–8.

Travaglini, R. V. 2017. Bases para o contrôle microbiano de formigas cortadeiras. Ph.D. dissertation, Universidade Estadual Paulista, Botucatu, Brazil.

Varón Devia, E. H. 2006. Distribution and foraging by the leaf-cutting ant Atta cephalotes L. in coffee plantations with different types of management and landscape contexts and alternatives to insecticides for its control. Ph.D. dissertation, University of Idaho, Moscow, ID.

Vasconcelos, H. L., and H. G. Fowler. 1990. Foraging and fungal substrate selection by leaf-cutting ants, pp. 411–419. *In* R. K. Vander Meer, K. Jaffe, and A. Cedeno (eds). Applied myrmecology: a world perspective. Westview Press, Boulder, CO.

Weber, N. A. 1966. Fungus-growing ants. Science. 153: 587–604.

Weber, N. A. 1972. Gardening ants, the attines. American Philosophical Society, Philadelphia, PA.

Zanuncio, J. C., J. B. Torres, W. L. Gasperazzo, and T. V. Zanuncio. 1996. Aferição de dosagens de iscas granuladas para controle de Atta laevigata (F. Smith) pelo número de olheiros ativos. Rev. Árvore. 20: 241–246.