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Review

An Overview of Integrated Management of Leaf-Cutting Ants (Hymenoptera: Formicidae) in Brazilian Forest Plantations

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Abstract: Brazilian forest producers have developed integrated management programs to increase the effectiveness of the control of leaf-cutting ants of the genera *Atta* and *Acromyrmex*. These measures reduced the costs and quantity of insecticides used in the plantations. Such integrated management programs are based on monitoring the ant nests, as well as the need and timing of the control methods. Chemical control employing baits is the most commonly used method, however, biological, mechanical and cultural control methods, besides plant resistance, can reduce the quantity of chemicals applied in the plantations.

Keywords: *Atta*; *Acromyrmex*; control method; integrated pest management; monitoring

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1. Introduction

Integrated Pest Management (IPM) involves various types of control methods based on economical, ecological and social parameters to keep the pest population below the economic injury level.

Therefore, IPM includes the knowledge of culture and pests, quantification of population density of the pests and their natural enemies, decision-making regarding control and the judicious selection and application of suitable methods to reduce the pest populations. The Brazilian forest producers initiated the development of IPM in the early 1990s, chiefly for leaf-cutting ants that are the main pests of these cultures.

Leaf-cutting ants of the genera *Atta* and *Acromyrmex* are the main pests found in *Pinus* and *Eucalyptus* plantations [1–3]. The *Atta* build nests with hundreds of interconnected underground chambers and trails opening out at the soil surface [4]. Just outside their colonies, the characteristic loose soil that has been removed during chamber construction is visible [5]. Openings on ground surface are called holes, which are located either inside or outside the portion of the nest covered with loose soil. The sprouting of seedlings in native forests can be prevented by high infestation of leaf-cutting ants [6]. A big nest of about 200 m^2 may host a population of about six million ants [7].

Atta and *Acromyrmex* have existed symbiotically with the Basidiomycetes fungus *Leucoagaricus gongylophorus* for over 50 million years [8] and complex interactions are noted between these organisms. Besides, this fungus can help to maintain the architecture of the ant colony [9]. The fungus provides the ants with easily assimilated nutrients from the plants in a highly protected environment and the ants remove the contaminants [10] and secrete antibiotics from their metapleural glands to protect it [11,12].

The symbiotic fungus, which is rich in carbohydrates and proteins, forms the basic food of the leaf-cutting ants [13]. This substrate is the only nutrient source for the ant larvae and the temporary winged castes. However, the queen, on the colony-founding stage, relies entirely on the reserves accumulated on her body to start a new colony. The adult workers ingest mainly plant sap and only 9% of their energy is derived from the fungus [14].

2. Damage by Leaf-Cutting Ants

Leaf-cutting ants, the main pests of Brazilian forest plantations, cut leaves, flowers, buds and twigs which are transported to the interior of their nests [15]. Thus, they cause direct losses, such as seedling death and reduction of tree growth, as well as indirect losses by decreasing the tree resistance to insects and pathogens [16].

Simulating the damage caused by leaf-cutting ants, the artificial defoliation of *Gmelina arborea* and *Pinus caribaea* showed that the latter was the most affected by successive defoliations with a 12% reduction in height increase and a 17.4% drop in diameter growth, as well as mortality of 11.7% [17]. However, reduction in height and diameter growth was higher in *P. taeda* during the first 12 months of plant growth. Defoliation occurring between 12–24 months of age affects only the height [18].

Simulation of 100% winter defoliation of *E. grandis* revealed a reduction in plant diameter and height increase by 78.9% and 60.7%, respectively [19]. A one-time defoliation reduced the total wood volume by 37.9% and three defoliations reduced the total volume by 79.7% [20].

Leaf-cutting ants are more harmful during the first three years of plant age. A complete defoliation reduced the growth in *Eucalyptus grandis* [20], whereas two consecutive ones resulted in tree death [21]. After three years of age, *Eucalyptus* trees can die if they experience three consecutive total defoliations [22].

A single leaf-cutting ant colony per hectare of forest can reduce the annual tree growth by 5% in *Eucalyptus* and by 10% in *Pinus* [23]. These estimates were based on the relationship between the leaves correlating to the fungus mass used as ant food and the quantity of waste produced during the same time period. This represents a 2.1% loss in the annual wood production [24]. However, this method has limitations as it is difficult to ascertain if the leaf-cutting ant nests were totally excavated, and also because of the lack of studies in determining the correct sample size and the deposition of material in the waste chambers [25].

The method used to correlate the volume produced by the plants in places with varying natural densities of ant nests could be employed to assess the crop production under the impact of this pest under natural conditions. Densities exceeding 30 nests of *Atta laevigata* in less than 10-year-old *P. caribaea* plantations can reduce wood productivity by up to 50%, as observed in Venezuela [26].

Each unitary increment in the *Atta* spp. nest area per hectare can reduce wood production from 0.04–0.13 m^3 ha^{-1}, in a eucalyptus plantation and has resulted in an economic injury in areas with 13.4–39.2 m^2 ha^{-1} of ant nests in the Atlantic Forest region, Brazil [27].

The average reduction of wood produced for all the Eucalyptus species was 0.87% for every 2.76 m^2 of ant colony per hectare, in the Brazilian Savannah region. *Corymbia citriodora* showed the greatest reduction (3.26%), followed by *Eucalyptus tereticornis* (1.78%) and *E. camaldulensis* (0.68%), however, no effect was observed in *E. cloeziana* and *E. urophylla*, even for those species having a similar area of nests per hectare. This is indicative of either their higher resistance to attack or more rapid recovery after defoliation [1].

3. Monitoring Nests of the Leaf-Cutting Ants

Brazilian forest producers have implemented monitoring systems to reduce the environmental impacts caused by the indiscriminate use of insecticides and the costs involved in controlling the leaf-cutting ants [28]. Monitoring enables the estimation of the number and size of the nests as well as the particular species of leaf-cutting ants per hectare. These and the other parameters mentioned earlier aid in wise decision-making. The economic aspects of the plantation and the cost-benefit ratio in controlling this pest, determined by the economic injury level (EIL), are also considered [1,29].

Sampling of the leaf-cutting ant nests is done based on the data on the census of the nests in the cultivated areas and computer simulations using the random sampling method [30], transects [3,31,32] and the technique of “the worst focus” [29,33].

Random sampling is the most common method using plots of fixed size (720–1080 m^2) with a width corresponding to two to three lines of plants in the field. One plot is randomly used every three to five hectares, where all the nests of the leaf-cutting ants are identified and divided in size classes. The random sampling technique involves one parcel of 720 m^2 every five hectares, independent of the areas affected by the leaf-cutting ant [34]. This method also evaluates the number of defoliated trees and the degree of defoliation. The density of the leaf-cutting ant nests per size class in these parcels is recorded and processed with software to find the degree of need, time and suitable control type.

Transects vary in length (6–9 m wide), following the row of planting. Transects are used from the third or fifth row of the plantation, with 96–180 m between them [35]. This method consists of 6 m wide transects at every 120 m to estimate the leaf-cutting ant population as described in the Brazilian savannah regions [31].

The technique of "the worst focus" involves the selection of areas with more intense tree defoliation, and recording the nest size and the degree of tree damage and defoliation with a reduction of up to 30% in the areas requiring control [29]. The remaining 70% had only one control measure for leaf-cutting ant done per year compared with the conventional method, in which control measures were conducted every six months. One control was possible every 14.5 months resulting in a 58% cost reduction and less environmental contamination.

Every forest stand needs to be sampled at specific intervals, preferably twice per year during the first 12 months of growth, and annually in forests older than 12 months. Sampling was to be performed based on a sampling plan developed per region to determine the number and size of the leaf-cutting ant nests [36].

The monitoring systems used by the Brazilian forest producers are found to be more beneficial compared to traditional practices of walking the whole area. Monitoring systems not only produce immediate results in leaf-cutting ant control, but provide information on the effects of the cultivated species and the strips of native vegetation on these pests [37], their population dynamics [38] and the impact of these pests on forest production [39]. This in turn helps to assess the degree of economic damage for leaf-cutting ants in the *Eucalyptus* plantations [1].

4. Strategies and Tactics for the Management of Leaf-Cutting Ants

4.1. Chemical Control

Chemical control is the most common method used to control leaf-cutting ants in the forest areas. Initial control should be done between 45 and 60 days before soil preparation or before harvesting. Seedlings are highly susceptible to the ants and complete control of colonies of this pest is necessary in the first year of the plantation. Surveillance should be done in the first month after planting or when the first buds appear. Thereafter, treatment should follow for four months. After one or two years, the forests will reach maintenance stage, and control needs to be done once or twice a year after sampling, preferably during the dry season [40].

A fixed dose per active nest hole of the leaf-cutting ants should be used only after sampling to determine the relationship between the nest area and number of holes. As the nest area increased, the number of active nest holes/m^2 was found to decline [41,42]. Thus, the largest nests would not receive sufficient doses based on nest holes because of their disproportionally small number of holes. Sampling can determine the spatial distribution of the nests because colonies may be more concentrated near the forest edges, allowing for effective control, without needing to control the entire area [43,44].

The most common active ingredients used in the control of leaf-cutting ants are sulfluramid, fipronil, deltamethrin and fenitrothion [45]. Granulated baits, dry powder or fogging are the main formulations, the last involving the use of a fogger machine.

Baits are more practical, economical and operational to control these pests. Baits use an active attractive ingredient like pellets of dehydrated citrus pulp and other organic materials that act as a carrier. The ants transport these pellets into their nests [46,47]. However, pellets should not be applied in rainy days as they dissolve in the water and get wasted. Pellets can be used in nests of any size [40]. Baits kill the leaf-cutting ants slowly; however, they paralyze their cutting activity within a few days [48]. Filamentous fungus, virulent to the ant symbiotic fungus, can show synergistic effect on the sulfluramid [49]. Baits applied in the most active nest holes showed higher efficiency of transport and control. Baits can be applied manually with a recommended dose/m^2 of nest or systematically with a distribution of a specific dose/m^2 of area planted [1], and the application of fixed doses in each active hole [50]. The monitoring-based bait application can reduce the control cost by 80% in forest areas [51].

Fogging is an effective control measure, although the cost of equipment maintenance makes it more expensive compared to granulated baits [52,53]. The active ingredient is mixed with a vehicle (kerosene or diesel) and introduced directly into the nest as a smoke [35]. This method is adequate for plantations during the initial stage, where the nests, especially the bigger ones, can be easily found [40]. In such cases, the nests are killed quickly [54]. The product is best applied directly into the active nest holes with the spearhead of the device being introduced into the openings and waiting for the reflux of the smoke produced by nebulization [40]. Plant extracts such as d-limonene can only be applied by fogging to control the leaf-cutting ants [55].

Dry powders are applied directly into the active nest holes, using dusters. This is a slow process and only recommended for small nests up to 5 m^2 [50] only during the dry season because moist soil is more difficult for the product to penetrate.

4.2. Plant Resistance

Eucalyptus species may be susceptible or resistant to leaf-cutting ants (Table 1). The density of the ant colonies was higher in *E. grandis* plantation (28.84 nests/ha) compared to those of *E. pellita* and *E. tereticornis* (12.84 and 11.07 nests ha^{-1}, respectively) [38]. The resistance of the eucalyptus plants can be related to mechanical, physical and chemical factors, favoring their use in commercial plantations.

Table 1. Leaf-cutting ants' susceptible and resistant *Eucalyptus* species.

Species	Susceptible species	Resistant species	References
Atta sexdens	*E. tereticornis* * *E. cloeziana*	*E. grandis* **, *E. dunni*, *E. pilularis*, *E. propinqua* **, *E. maculata*, *E. deanei*, *E. mesophila*, *E. nova-anglica* **, *E. acmenoides* **, *E. maculata* **, *E. deanei* **, *E. andrewsii* **, *C. citriodora* **	[56–59]
Atta laevigata		*E. cloeziana*, *E. mesophila*, *E. nova-anglica* **, *E. acmenoides* **, *E. maculata* **, *E. grandis* **, *E. deanei* **, *E. andrewsii* **, *E. propinqua* **	[58,60]
Acromyrmex laticeps nigrosetosus	*E. urophylla* * *E. camaldulensis* *	*E. cloeziana*	[59]

* Species is preferred over others; ** species causes adverse effects on the behavior and survival of the ants.

4.3. Mechanical Control

Excavation of the young nests and capturing the ant queens are effective ways of controlling the leaf-cutting ants in smaller areas. Excavation is recommended only during the third and fourth months after the nuptial flight, when the queens are about 20 cm deep in the soil [61].

"Barriers" are one of the oldest control methods used for these ants, but only in small orchards [62]. Plastic tape coated with grease, plastic cylinders and strips of aluminum, plastic or metal are fastened around the trunks [63]. However, constant inspections and repairs are necessary to protect the trees.

4.4. Cultural Control

Leaf-cutting ants are polyphagous using different plant species as substrates for their symbiotic fungus. Therefore, the use of vegetative material far away from their nests reduces their impact on trees. So, crop rotation, alternate cropping and different sowing dates are advised. The phosphate fertilizers reduced the damage by the leaf-cutting ants on the eucalyptus plants by about 35% [64]. Leaf-cutting ants may prefer foraging on plants with drought stress [65].

Plowing can eliminate the leaf-cutting ant nests within four months after the nuptial flight, when the queens are at 20 cm depth under the soil [66,67]. Soil preparation may be not enough to prevent damage to the plant by the leaf-cutting ants [66]. However, the practice of minimum tillage which reduces soil preparation throughout the area and adopted by many forest producers may increase the number of leaf-cutting ant nests [1]. The use of limestone was inefficient in controlling *A. sexdens* [68].

Plants like sesame, grass species [69], castor [70] and sweet potato, combined with the culture, may serve as an alternative food or a trap crop with deleterious or repellent effects on the leaf-cutting ants [67].

Plants can be toxic to leaf-cutting ants and to their symbiotic fungus (Table 2). Insecticides, like β-eudesmol from eucalyptus leaves, may interfere with ant behaviour [71–73]. Sesquiterpene modified the chemical composition of the ant worker cuticles, confusing their recognition within the nest and triggering alarmed and aggressive behavior [73].

The symbiotic fungus acts as a mediator of ant nutrition by hydrolyzing the plant polysaccharides [74]. This fungus produces large quantities of enzymes that the ants feed upon and return to the fungus garden as fecal liquid, which in turn digests the plant tissues [75]. This association is essential for the fungus to draw the nutrients from the plants that the ants carry into their nests [74]. Natural products can be toxic to the symbiotic fungus *Leucoagaricus gongylophorus*, as observed for those of *R. communis* (Euphorbiaceae), *Helietta puberula* (Rutaceae), *Simarouba versicolor* (Simaroubaceae) and *Canavalia ensiformis* (Fabaceae) [76–79]. Baits with plant extracts were effective in the field as a control measure, stopping ant activity between three and 12 days of application [35].

All alternative control methods should be exhaustively tested before being recommended for controlling the leaf-cutting ants [68].

Table 2. Plants that can be toxic to leaf-cutting ants and/or to their fungus and their references (Ref.).

Family	Plant Species	Toxic to	References
Asteraceae	*Tithonia diversifolia*	*Atta cephalotes* workers	[80]
Euphorbiaceae	*Ricinus communis*, *Jatropha curcas*	*Atta sexdens rubropilosa* workers	[81]
Myrtaceae	*Eucalyptus maculata*	*Atta sexdens rubropilosa* workers	[71–73]
Fabaceae	*Hymenaea courbaril*	*Atta sexdens rubropilosa* workers	[82]
Meliaceae	*Cedrela fissilis*	*Atta sexdens rubropilosa* workers and fungus	[83]
Rutaceae	*Raulinoa echinata Coffea* spp.	*Atta sexdens* fungus *Atta sexdens rubropilosa* workers and fungus	[84,85]
Pedaliaceae	*Sesamum indicum*	*Atta sexdens rubropilosa* workers	[86]
Piperaceae	*Piper piresii*	*Atta sexdens rubropilosa* workers and fungus	[87]
Simaroubaceae	*Simarouba versicolor*	*Atta sexdens* workers and fungus	[76]

4.5. Biological Control

The entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* were tested against the leaf-cutting ants [88,89]. Baits containing these pathogens gave 20%–70% efficiency in controlling the *Acromyrmex* species [90,91] and *A. sexdens* [92]. The ENA04 isolate of *M. anisopliae* was found to be most pathogenic to the *A. bisphaerica* soldiers, with a TL_{50} of 1.15 days, with more than 80% mortality during the first three days of application, and high spore production on ant cadavers [93]. The insecticide imidacloprid, at very low concentrations, applied with *B. bassiana*, can change ant behavior and increase its susceptibility to this fungus [94].

The fungus *Paecilomyces farinosus* was also effective against *A. sexdens*, with mortality above 80% during the first four days of inoculation [89]. Entomopathogenic fungi combined with sub-lethal doses of the insecticide imidacloprid caused high mortality in the ants (>64%), indicating that this insecticide increased the susceptibility of the ants to this fungus [53].

The isolates HA58 and HA48 of *Bacillus thuringiensis* caused mortality of *Acromyrmex lundi* at levels of 80% and 100%, respectively [95]. The pathogenicity of the nematodes *Steinernema* and *Heterorhabditis* was increased by the symbiotic bacteria, *Xenorhabdus* and *Photorhabdus*. These nematodes are present in the intestine of their infective juveniles and can penetrate into host tissue [96,97]. The bacterium *Photorhabdus temperate* K122 was highly virulent to *Acromyrmex subterraneus subterraneus* with 90% mortality within 24 hours, reaching 100% mortality within 48 hours [98]. The surveys on pathogenic organisms appear promising, but leaf-cutting ants possess specific mechanisms to defend their colonies. They have developed special behaviors, such as disposal of symbiotic fungus and infected ants in the garbage chambers, and morphological features, such as cuticle sclerotisation forming a protective barrier, infrabucal cavity and anal hairiness to protect themselves against the invading pathogens [11,99,100].

Endophytic fungus on plant leaves can also reduce the foraging and processing efficacy of leaf-cutting ants [101,102] probably because of low volatility compounds released after their wounding [103].

Maintenance and/or manipulation of the environmental conditions may favor the ants' natural enemies, such as increasing the plant diversity between the *Eucalyptus* trees and strips of native vegetation. The main advantage of these areas is to allow the fauna to move between the plantations and native areas thus increasing the diversity and quantity of the natural enemies of the leaf-cutting ants [104]. Native mammals, birds and insects such as beetles and wasps (Vespidae) are the natural predators of the leaf-cutting ants in Brazil. Several diurnal raptors prey alate individuals of leaf-cutting ants [105]. Phorid flies can parasitize leaf-cutting forage workers [106].

The incidence of the leaf-cutting ant nests was 18 times higher in the areas lacking vegetation under the *Eucalyptus* trees than in areas having dense vegetation [107]. The number of new ant nests was 11.5 times lower when vigorous vegetation was allowed to grow under the Eucalyptus trees [108]. The number and the size of the ant colonies increased by 8.2 and 14.2 times, respectively, in areas without vegetation under the eucalyptus trees [109] while these values were 1.7 times in number and 2.8 times in the size of nests in those areas with vegetation growing under the eucalyptus trees. Native vegetation under the *Eucalyptus* plants reduced the density of the leaf-cutting ants by 11% and decreased the control costs by 11% [38].

5. Conclusions

Leaf-cutting ants cause significant damage to *Pinus* and *Eucalyptus* plantations, making it necessary to control them. Brazilian foresters have invested in monitoring systems to reduce costs and environmental impacts. Leaf-cutting ants are mainly controlled using chemicals. Alternative methods are being studied, including biological control (fungi or bacteria and ways to increase their natural enemies, including predators and parasitoids), the use of plant extracts, mechanical control and cultural methods. Selecting an optimal control method, as well as identifying the particular ant species causing the damage, is important, apart from locating and monitoring the areas with critical density of nests. These are the basis for the integrated management of leaf-cutting ants in Brazilian forest plantations.

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Conflicts of Interest

The authors declare no conflict of interest.

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