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## Original article

# Why are there few seedlings beneath the myrmecophyte *Triplaris americana*?

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## ABSTRACT

We compared the relative importance of chemical allelopathy, pruning behaviour of resident ants and other non-related agents to ant-plant mutualism for seedling establishment beneath *Triplaris americana* L. (Polygonaceae), a myrmecophyte plant. We also included a preliminary analysis of effects of fragmentation on these ecological processes. Seeds and seedlings of *Theobroma cacao* L. (Sterculiaceae) were used as the target species in all experiments. Leaf-tissue extracts of the myrmecophyte plant did not inhibit germination of cacao seeds. Resident *Pseudomyrmex triplarinus* Weddell (Pseudomyrmecinae) ants did not remove seeds under the canopy of their host plants. The main seed consumer was the leaf-cutting ant *Atta sexdens* L. (Myrmicinae). Leaves of cacao seedlings were partially or totally pruned by *Pseudomyrmex* ants mainly in forest fragments studied. We offer evidence pointing to the possibility that the absence of seedlings beneath *Triplaris* may result from effects of both ant species. We discuss the benefits of pruning behaviour for the resident ant colony and the effects of ant-ant interactions on seedling establishment beneath this ant-plant system.

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## 1. Introduction

Pruning behavior by resident ants is frequently reported from myrmecophyte systems (Heil et al., 2001; Federle et al., 2002; Bruna et al., 2004; Frederickson et al., 2005), such as *Triplaris americana* L. (Polygonaceae), a common tree of the Neotropical region (Davidson et al., 1988). Leaf-pruning by resident ants can limit potentially dangerous invasion by leaf-foraging ant species, most of which use chemical defences and can be behaviourally dominant to resident ants (Davidson et al., 1988; Hölldobler and Wilson, 1990). As suggested by Janzen (1969) creation of basal clearings may also enhance plant (and ant colony) fitness by

precluding growth of neighbouring plants. Thus, pruning behaviour could produce similar effects to allelopathy because resident ants could eliminate competitors of their host plants (biological allelopathy, Janzen, 1969). However, allelopathy in the strict sense inhibition of the survival or/and establishment of adjacent plants by chemical compounds found in leaves, flowers or fruits (Whittaker and Feeny, 1971) could also occur. In fact, aqueous extracts of *T. americana* leaves experimentally reduced the growth of wheat (*Triticum aestivum* L.) roots by 16% (Mongelli et al., 1997). Nevertheless, the allelopathic effects of *T. americana* tissues as well as the intensity and magnitude of pruning behaviour by resident ants are little known.

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Other organisms not involved in the ant-plant mutualism may also affect recruitment processes beneath myrmecophytic plants. Among these, leaf-cutting ants, widespread throughout the Neotropics are highly efficient in their seed-foraging behaviour, which gives them an advantage over other ground-foraging granivorous ants. These ants transport seeds to their nest to form and maintain their “fungus gardens” and they can create understory gaps by covering the forest floor with large quantities of excavated soil, clearing the area of understory vegetation and modifying the availability of resources (Hölldobler and Wilson, 1990; Farji-Brener and Illes, 2000; Farji-Brener and Medina, 2000; Lorini, 2000; Woods, 2002). Their impact on seed survival and dynamics of seedling establishment of many plant species is largely known; nevertheless, their effects beneath myrmecophytic plants remain unknown.

As suggested for other plant-animal relationships (Aizen and Feinsinger, 1994; Murcia, 1995; Ledezma, 1999; Lorini, 2000; Rico, 2000), forest fragmentation could be modifying ant-plant interactions. Rapid transformation of tropical forests into grazing pastures and agricultural fields reduces continuous forests in small and isolated forest fragments, which present abiotic and biotic conditions different than those in continuous forest (Murcia, 1995). It is unknown whether ant-plant interactions such as the pruning behaviour by resident ants or seed predation by leaf-cutting ants can be modified by this kind of landscape transformation.

The goal of the present study was to explain the low seedling establishment that occurs under a myrmecophytic plant species, *T. americana* associated with the ant *P. triplarinus*. We compare the relative importance for seedling establishment of chemical allelopathy (leaf-tissue aqueous extract), resident ants (pruning behaviour) and other mortality agents not related to this ant-plant mutualism. We also investigate whether habitat fragmentation could alter these interactions. Specifically, we attempt to answer the following four questions: (1) Can aqueous extracts of *T. americana* leaves reduce seed germination of a test species? (2) Do resident ants transport and/or reduce seed viability under host plants? (3) What is the effect of pruning behaviour by resident ants on seedling survival of a test species? (4) How does forest fragmentation affect these patterns?

## 2. Study area

The study was carried out at the Beni Biological Station Biosphere Reserve (EBB), Bolivia (14°30′–14°50′S, 66°00′–66°40′W). This Reserve covers ca. 135,000 ha. Average temperature is 26 °C with 1927 mm of annual average rainfall. Vegetation is a mosaic of different forest types alternating with bodies of water and humid savannas. The forest displays emergent trees (30–50 m in height), a lower arboreal stratum (approximately 20 m high), and a shrub stratum (about 5 m). The most common tree species are *Astrocaryum murumuru* (Arecaceae), *Pseudolmedia laevis* (Moraceae), *Inga edulis* (Mimosaceae), *Celtis schippii* (Ulmaceae) and *Viola sebifera* (Myristicaceae). Forest fragments scattered through the savanna occur in different phases of regeneration. The forest fragments have a canopy height of 20–25 m, with *Acacia lorentensis* (Mimosaceae), *Attalea*

*phalerata* (Arecaceae), *Eugenia patricii* (Myrtaceae), *Machaerium hirtum* (Fabaceae), *A. murumuru* and *Triplaris americana* (Poligonaceae) as the most frequent trees (Simonetti et al., 2001). We chose two areas of continuous forest (Campo Mono and Marimonos) and two forest fragments (Mid and Taita A). Continuous forest zones are separated by 8 km. The fragments are small (<5 ha) and separated by 1.2 km.

## 3. The plant and resident ant

*Triplaris americana* (Polygonaceae) is a common dioecious myrmecophytic tree among the most frequent tree species at study site and exhibits scarce recruitment of both con- and heterospecific seedlings under its canopy (Simonetti et al., 2001). The abundance of heterospecific seedlings within 2 m of the canopy of *T. americana* is only half that found more than 2 m from the canopy (J.A. Simonetti unpublished data). Like other species, *T. americana* grows in forest fragments and in continuous forest, and in both kinds of sites recruitment of conspecific seedlings under its canopy is also relatively low. In forest fragments, about 3.3% of all seedlings grow under a parental *T. americana* tree, whereas in the continuous forest no conspecific seedlings exist growing under the canopy of these trees (Simonetti et al., 2001), suggesting that the occupation rates of *Triplaris* trees by *Pseudomyrmex* ants could be different between both habitats.

The zone of low seedling recruitment coincides with the maximum patrolling distance of *Pseudomyrmex triplarinus* Weddell (*Pseudomyrmecinae*), the ant species which commonly colonizes *T. americana* trees (Oliveira et al., 1987). These ants vigorously attack any intruder touching the nest tree. They also attack and remove intruding insects, and are significantly more efficient in these activities than *Crematogaster* spp. that also occupy *Triplaris* (Oliveira et al., 1987; Hölldobler and Wilson, 1990). *Pseudomyrmex triplarinus* ants are wholly dependent on the host plant, for their food, the mutualism is thus highly obligated in at least one direction (Davidson et al., 1988; Hölldobler and Wilson, 1990).

## 4. Methods

We used a combination of observation and field and laboratory manipulative experiments. Allelopathic effects of leaves were analyzed in a laboratory experiment, while the effects of the resident ant and of other mortality agents were evaluated by field experiments. The effect of habitat fragmentation upon these interactions was analysed using a natural experiment, given that fragments were already present in the study zone (Morales, 2000; Simonetti et al., 2001). Seeds and seedlings of *Theobroma cacao* L. (Sterculiaceae) were used as the target species in all experiments. We chose this species because it had mature fruits during the study time (February–May 2003) and because it is native to the study area. Seeds of *T. cacao* are relatively large (<2 cm) and too heavy for transportation by *Pseudomyrmex* ant; therefore, we focused on assessing the effect of pruning by resident ants on the survival of target seedlings.

#### 4.1. Chemical allelopathy

We obtained 450 seeds from 15 fruits of *T. cacao* in order to evaluate the allelopathic effect of *T. americana* leaves upon germination rate of these seeds. We placed the seeds in 90 Petri dishes (5 seeds/Petri dish). Seeds of 60 Petri dishes were watered every two days with 2 cm<sup>3</sup> of an aqueous extract made from fresh leaves of *T. americana*. Crushed leaves were soaked in cold water for 48 h at 6 °C (Mongelli et al., 1997). Seeds in the remaining 30 Petri dishes acted as a control and were watered with 2 cm<sup>3</sup> of water. To evaluate whether fragmentation influenced the allelopathic effect of *T. americana* leaves on seed germination rates, we obtained extracts from leaves of trees growing in continuous forest and from trees from forest fragments. Out of the 60 Petri dishes, 30 were watered with solutions from the continuous forest and the other 30 with the extract from forest fragments. We evaluated seed germination every two days recording the number of seeds germinated in each Petri dish. A seed was considered to have germinated if it exhibited an emerged radicle at least 2 mm long.

#### 4.2. Ant–seed interactions

In order to assess whether defensive activities of resident ants may reduce seed survival under host plant canopy, we recorded seed removal rate near and far from *T. americana* trees. In two sites of continuous forest and two forest fragments, we selected 10 *T. americana* trees and 10 trees of another species (among them *P. laevis*, *A. phalerata*, *E. patricii* and *I. edulis*), located 15–20 m from the focal *T. americana* tree. Under the canopy of each tree (<2 m radius) four experimental treatments were placed radially: three of them restricted seed removal by vertebrates and/or ants, while the fourth treatment was a control site allowing free access to all granivores.

Vertebrate exclosures were circular (35 cm diameter), 30–35 cm high, built with galvanized wire mesh with hexagonal openings of approximately 2.5 cm. Ant exclosures were built with sticky traps. Vertebrate and ant exclosures were combined with a sticky trap which, covered with the galvanized wire mesh, was staked to the ground. The distance between experimental treatments was <1.5 m. In each of them we deposited ten seeds of *T. cacao* and evaluated seed removal daily for one week. A seed was considered removed if it was not found within 30–35 cm of stakes. Seeds were set at 8:00–8:30 h and checked at 30-min intervals over a period of 3–4 h during which the behaviour of *Pseudomyrmex* ants was recorded.

#### 4.3. Ant–seedling interactions

To determine whether the pruning behaviour of resident ants may reduce seedling survival beneath host plants, we recorded survival of *T. cacao* seedlings and the pruning rate by resident ants near and far from *T. americana* trees. Beneath the same trees that we selected to record seed removal and after a month of finishing these field experiments, we prepared four experimental treatments: three of them allowed seedling pruning by vertebrates and/or ants, while the fourth treatment was a control one allowing free access to both kinds of animals. Into each treatment we planted two nursery-grown

*T. cacao* seedlings about a month old, 15–20 cm high with three to five leaves. The *T. cacao* seedlings used in the experiment were planted in the field without their nursery pots.

Vertebrate exclosures were the same as those used to evaluate the seed removal. To exclude ants a 3 × 3 cm piece of pressed cardboard covered with a thick layer of a sticky trap was placed around the stem, which was daily reinforced. Combined exclusion of vertebrates and ants was obtained by adding a metallic grid (2.5-cm mesh size). Each day we recorded the number of leaves pruned, the total number of leaves on the seedlings and the number of surviving seedlings during a seven-day period. A leaf was considered pruned when it was separated from the main stem of the seedling through a cut in the petiole.

#### 4.4. Statistical analysis

Differences in germination, seed removal and leaf pruning were evaluated using Multivariate Analysis of Variance (MANOVA) and Newman–Keuls *a posteriori* tests. We used the MANOVA test instead of repeated-measures analysis of variance because its use is highly recommended when  $N - M > k + 9$ , where  $N$  = number of subjects observed,  $M$  = number of levels from factor design, and  $k$  = number of observations (Scheiner, 1993; von Ende, 1993). The dependent variables were the number of seeds removed and the proportion of pruned leaves. The independent variables were distance to *T. americana* trees (near and far), exclosure type (ants and/or vertebrates and control) and fragmentation (continuous forest/fragments). We performed arcsine transformations on the proportions of pruned leaves to normalize treatment variances for the statistical analysis. Due to low replication in the evaluation of the effect of habitat fragmentation ( $n = 2$ ), we considered the *T. americana* trees as independent units; therefore, pseudoreplication is included in the interpretation of results (Hurlbert, 1984). Differences in seedling survival as a function of habitat type (forest fragments and continuous forest) and distance to *T. americana* trees (near and far) were evaluated through a Kaplan–Meier survival analysis test. The package Statistica (version 6.0) was used for all analyses (StatSoft Inc., 2001).

## 5. Results

### 5.1. Chemical allelopathy

Aqueous extracts of *T. americana* leaves did not inhibit the germination of *T. cacao* seeds (Wilk's  $\Lambda = 0.91$ ;  $df = 10$ ;  $P = 0.68$ ). A total of 98% of the seeds watered with aqueous extracts of *T. americana* leaves (either from continuous forest:  $4.9 \pm 0.3$  seeds/Petri dish [mean  $\pm$  SE] or forest fragments:  $4.9 \pm 0.3$ ) germinated after ten days and 99.3% of the seeds watered with plain water germinated by the tenth day of experimentation ( $4.9 \pm 0.2$ ).

### 5.2. Ant–seed interactions

Overall, seed removal was independent of the distance to a *T. americana* tree (Wilk's  $\Lambda = 0.98$ ;  $df = 7$ ;  $P = 0.63$ ). After

seven days, the number of seeds removed under the canopies of *T. americana* trees ( $3.6 \pm 0.3$ ) (seed mean  $\pm$  SE,  $n = 160$ ) did not differ from the number of seeds removed away from the trees ( $3.5 \pm 0.3$ ) (Fig. 1).

Seed removal was dependent on the consumer type (Wilk's  $\Lambda = 0.86$ ;  $df = 21$ ;  $P = 0.001$ ). After seven days, the number of seeds removed was significantly higher in control treatments ( $4.7 \pm 0.5$ ) (mean  $\pm$  SE,  $n = 80$ ) and vertebrate exclusions ( $4.5 \pm 0.5$ ), than in ant ( $2.9 \pm 0.4$ ) and combined exclusions ( $2.2 \pm 0.4$ ) ( $P < 0.0001$ , Newman-Keuls test). This result shows that the main seed consumers were ants. We observed individuals of the leaf-cutting ant *Atta sexdens* L. (Myrmicinae) carrying partially or completely destroyed *T. cacao* seeds. We captured individuals of *A. sexdens* in 47.5% of ant exclusions (38/80 sticky traps), and 35% of the combined exclusions (28/80 sticky traps). These results suggest that leaf-cutting ants rapidly discovered the *T. cacao* seeds on the forest floor, in contrast to *Pseudomyrmex* ants, individuals of which were not recorded in the exclusions (0/80 sticky traps) and not recorded carrying seeds.

After one week, the number of seeds removed was significantly higher under trees growing in the two forest fragments ( $5.1 \pm 0.4$ ) (mean  $\pm$  SE,  $n = 160$ ) than in continuous forests ( $1.9 \pm 0.3$ ) (Wilk's  $\Lambda = 0.82$ ;  $df = 7$ ;  $P = 0.001$ ), regardless of exclusion type ( $P < 0.002$ , Newman-Keuls test). In both forest fragments, the number of seeds removed in control, vertebrate, ant and combined exclusion treatments was 1.9, 2.0, 4.5 and 6.1 times higher than in continuous forest, respectively. In forest fragments studied, we captured individuals of *A. sexdens* in 62.5% of ant exclusions (25/40 sticky traps), and 47.5% of combined exclusions (19/40 sticky traps), whereas in continuous forests we registered *A. sexdens* in only 32.5% of ant exclusions (13/40 sticky traps), and 22.5% of combined exclusions (9/40 sticky traps). These results show that leaf-cutting ants were more abundant and effective as seed consumers in the two forest fragments than in continuous forests. In contrast, *Pseudomyrmex* ants were not recorded in any of the treatments suggesting its indifference to seed presence near to the host plant or, more likely, its inability to transport the *T. cacao* seeds.

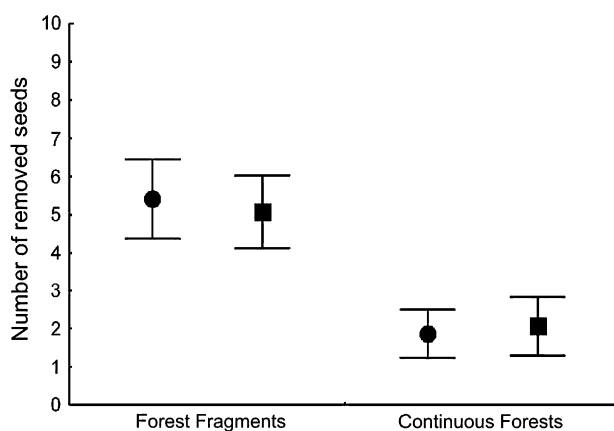


Fig. 1 – Seed removal (mean  $\pm$  SE) of *Theobroma cacao* (Sterculiaceae) near (●) and far (■) from *Triplaris americana* (Polygonaceae) trees growing in two contrasting habitats, Beni Biological Station Biosphere Reserve (EBB), Bolivia.

### 5.3. Ant-seedling interactions

Pruning of seedlings was dependent on the distance from a *T. americana* tree (Wilk's  $\Lambda = 0.83$ ;  $df = 7$ ;  $P = 0.001$ ). After seven days, the proportion of pruned leaves from seedlings was significantly higher under the canopies of *T. americana* trees ( $< 2$  m) ( $41.2 \pm 3.8$ ) (mean (%)  $\pm$  SE,  $n = 152$ ) than away from them ( $> 2$  m) ( $9.8 \pm 2.0$ ) (Fig. 2).

Under the canopy of *T. americana* trees, 74 of 152 seedlings had pruned leaves (48.7%). All leaves were pruned by *P. triplarinus*, which cut seedling leaves at the petiole base (27.1%) or, more frequently, cut the seedling's stems, causing the loss of all leaves (72.9%). The number of ants that we observed patrolling the leaves or fallen seedlings varied from one to seven individuals ( $3.9 \pm 0.4$ ) (mean  $\pm$  SE,  $n = 20$ ). Ants did not use pruned leaves for any purpose. Some ants patrolled the leaves or fallen seedlings for three to four hours (23.6% of total cases). Far from *T. americana* trees, 25 of 152 seedlings had pruned leaves (16.4%). In this case, leaves were cut by *A. sexdens* which cut leaves at the petiole base (71.9%) more often than they cut stems (28.1%).

Pruning of the seedling leaves was independent of exclusion type (Wilk's  $\Lambda = 0.91$ ;  $df = 21$ ;  $P = 0.23$ ). After seven days, no difference was found in the proportion of leaves pruned in control ( $30.1 \pm 4.9$ ) (mean (%)  $\pm$  SE,  $n = 76$ ), vertebrate ( $27.1 \pm 4.8$ ), ant ( $22.7 \pm 4.3$ ), and combined exclusions ( $22.7 \pm 4.5$ ). Enclosures for ants and combined enclosures were unable to completely exclude the ants. Near *T. americana* trees, we recorded *P. triplarinus* individuals inside 45% of ant enclosures (17/38 sticky traps), and 42% of combined enclosures (16/38 sticky traps). Individuals of *A. sexdens* were not recorded in any type of enclosures. Although we found leaf-cutter ants patrolling near target seedlings under attack by resident ants, we did not observe aggressive encounters between both ant species. Far from *T. americana* trees, we did not capture *P. triplarinus* individuals in any of the treatments (0/80 sticky traps), indicating the high dependence of this species on the host plant.

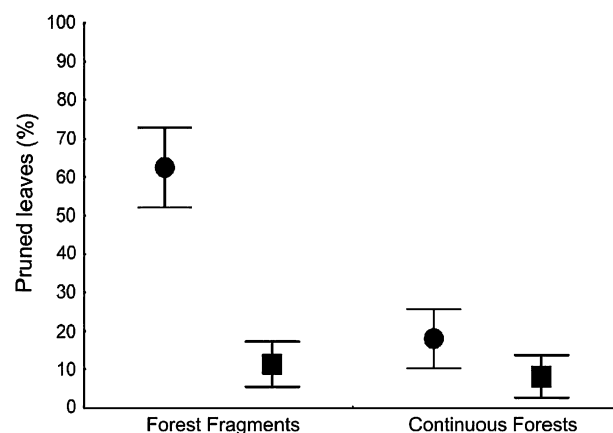


Fig. 2 – Proportion of pruned leaves (mean  $\pm$  SE) from nursery-grown *Theobroma cacao* (Sterculiaceae) seedlings which were planted near (●) and far (■) from *Triplaris americana* (Polygonaceae) trees in two contrasting habitats, Beni Biological Station Biosphere Reserve (EBB), Bolivia.

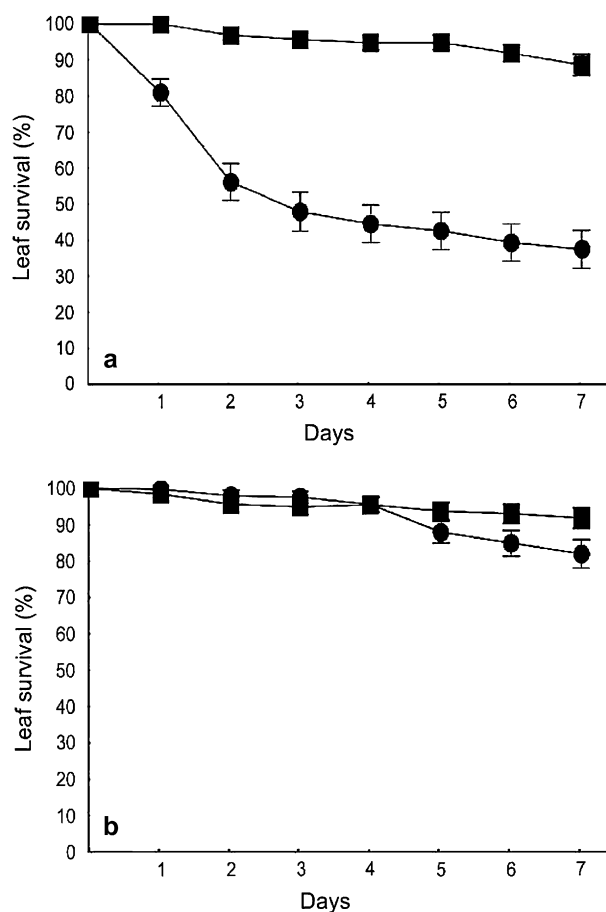
Pruning of leaves was dependent on habitat fragmentation (Wilk's  $\Lambda = 0.79$ ;  $df = 7$ ;  $P = 0.001$ ). After one week, the proportion of leaves pruned was significantly higher in both forest fragments ( $36.9 \pm 3.6$ ) (mean (%)  $\pm$  SE,  $n = 160$ ) than in continuous forests ( $13.1 \pm 2.4$ ,  $n = 144$ ). Likewise, in the forest fragments studied, the proportion of pruned leaves was significantly greater close to *T. americana* trees ( $62.5 \pm 5.2$ ,  $n = 80$ ) than far from them ( $11.4 \pm 2.9$ ) ( $P < 0.0001$ , Newman-Keuls test). Whereas in both zones of continuous forests, we did not find differences in the proportion of pruned leaves near ( $18 \pm 3.9$ ,  $n = 72$ ) and far from *T. americana* trees ( $8.2 \pm 2.7$ ) ( $P = 0.22$ , Newman-Keuls test). A significant interaction was observed between distance and fragmentation, which is accounted for by the low pruning intensity in the continuous forest and its independence of distance in this habitat type (Wilk's  $\Lambda = 0.84$ ;  $df = 7$ ;  $P = 0.001$ ).

In both forest fragments, the proportion of leaves pruned near and far from *T. americana* trees was 3.5 and 1.4 times larger than in continuous forest sites, respectively. Close to *T. americana* trees in forest fragments, we recorded *P. triplarinus* individuals in 60% of ant exclusions (12/20 sticky traps), and 65% of combined exclusions (13/20 sticky traps), while near *T. americana* trees in continuous forest, we recorded *P. triplarinus* individuals in 27.8% of ant exclusions (5/18 sticky traps), and in 16.7% of combined exclusions (3/18 sticky traps). In neither site did we capture resident ants far from *T. americana* trees (0/40 sticky traps to each case).

Survival of *T. cacao* seedlings was significantly higher in both continuous forests (93.7%) than in forest fragments (68.1%) ( $Z = -7.443$ ;  $P < 0.0001$ ). Seedling survival in fragments was significantly higher far from *T. americana* (95%) than near them (41.3%) ( $Z = -9.674$ ;  $P < 0.0001$ ) (Fig. 3a), while in continuous forest sites we did not find any effect of distance from *T. americana* trees on seedling survival ( $Z = -1.162$ ;  $P = 0.091$ ) (Fig. 3b).

## 6. Discussion

Tree recruitment under the canopies of the ant-plant *T. americana* is lower than under the canopies of other trees (Simonetti et al., 2001). This reduced recruitment includes con- and hetero-specific individuals, suggesting the existence of chemical and/or biological agents that would affect both plant groups. Our results showed that aqueous extracts of *T. americana* leaves did not inhibit the seed germination of a target species, *T. cacao*. Therefore, it is unlikely that allelochemicals can explain the rarity of seedling recruitment under this ant-plant species. Future researches could investigate the effects of a range of concentrations of aqueous leaf extracts on seeds of other target species, and to determine whether the roots of *T. americana* may also act as allelopathic agents on other species. These experiments should consider those exotic species, commonly used in assays of allelopathy react differently to potential allelochemicals (Larrea-Alcázar, 2003) and that interpretation of results requires physiological monitoring of the target species (Inderjit and Nielsen, 2003). In addition, new designs of *in situ* experiments on allelopathy could improve our understanding of effects of this ant-plant on seedling recruitment.



**Fig. 3 – Leaves survival (mean  $\pm$  SE) from nursery-grown *Theobroma cacao* (Sterculiaceae) seedlings in relation to time (days), habitat (a: Forest Fragments, b: Continuous Forests) and distance to *Triplaris americana* trees (● Near, ■ Far), Beni Biological Station Biosphere Reserve (EBB), Bolivia.**

However, the scarce recruitment under *T. americana* canopies appears to be a consequence of the defensive activities of the resident ant, *P. triplarinus*. Ants attempt to prevent the invasion of the host tree by other ant species by reducing the access routes for competitive ants, e.g., by cutting leaves and twigs of the canopies of neighbouring trees that reach and overlap the canopy of *T. americana* (Davidson et al., 1988; Heil et al., 2001; Federle et al., 2002; Bruna et al., 2004). *Pseudomyrmex triplarinus* did not affect the survival of seeds of the target species because, in part, of its high nutritional dependence on the host plant (Davidson et al., 1988; Hölldobler and Wilson, 1990) and also because of the large size of seeds of the target species used in this work. This suggests that seed removal by resident ants can not account for rarity of seedling recruitment beneath *T. americana* trees. Nevertheless, due to the high aggressiveness of *P. triplarinus* and its potent venom (Hink et al., 1994), it probably influences the arrival of zoochorous seeds by discouraging animals from approaching *Triplaris* trees.

The leaf-cutting ant *A. sexdens* was the main seed consumer under *T. americana* canopies. This ant species removed seeds

independently from the distance to *Triplaris* because they may attack seeds on larger spatial scales as a result of their large foraging ranges (Lorini, 2000). These leaf-cutting ants continually transported leaves and seeds to their nests to support their fungus gardens (Hölldobler and Wilson, 1990). On the other hand, leaf-cutting ants are not equally distributed within forests in the Beni Biological Station Biosphere Reserve (EBB). Their colonies can be much more common in forest fragments than in continuous forests (Lorini, 2000). The higher seed removal in the forest fragments suggests also greater abundance of *A. sexdens* in these habitats, which could increase their probability of finding seeds both close to *T. americana* trees and far from them. Both forest fragments contain from one to two nests of *A. sexdens* (Lorini, 2000), in which colony size can be from  $10^6$  to  $2.5 \times 10^6$  individuals (Hölldobler and Wilson, 1990). Curiously, together with *A. sexdens*, 24 other species of granivorous ants have been recorded from forest fragments in the EBB (Verhaagh and Røsciszewski, 1994); however, none of them was captured during this study suggesting that their abundance is low or, more likely, that they do not consume large seeds such as those of *T. cacao* (<2 cm).

Presence of target seedlings close to *T. americana* caused the defensive response of resident ants, which partially or totally pruned one or both target seedling placed near each plant. Our data support the hypothesis that pruning behaviour reduced seedling survival, and that defensive activities of *P. triplarinus* can be interpreted as a biological allelopathic agent (*sensu* Janzen, 1969) against neighbour seedlings of the host plant. The pruning behaviour is not a new finding. Davidson et al. (1988) demonstrated that experimental contact between *Triplaris* and surrounding vegetation induced pruning by *Pseudomyrmex*. However, in the current study, we showed that not only the ants prune when there are plants in contact with their host plant, they also prevent any possible contact by pruning at the seedling stage. This suggests that the surroundings of this myrmecophytic plant may be unsafe sites for seedling recruitment. In fact, the incapacity or indifference of *P. triplarinus* ants to transport the target seeds shows that the area under the canopy of their host plants could create microsites with severe seed-seedling conflicts (Schupp, 1995; Jordano and Herrera, 1995; Rey and Alcántara, 2000). Seeds that survived predation by leaf-cutting ants may have found a relatively safe site, but such sites become unsafe when seedlings emerge; thus, seed survival would not assure the survival of future seedlings (Schupp, 1995). As shown by our results, once the seedlings emerge they could be rapidly pruned by resident ant. Even though in other tropical forests the seed cleaning activity (removal of fleshy part) of many leaf-cutting ant species can facilitate seed germination and promote seedling establishment of some woody species (Farji-Brener and Silva, 1996; Leal and Oliveira, 1998; Farji-Brener and Medina, 2000). Our results suggest that at least, for the target species *T. cacao*, individuals of *A. sexdens* can be strong seed predators and, likely, unimportant predators of seedlings underneath *T. americana* trees. Seed predation by *A. sexdens* and seedling pruning by *Pseudomyrmex* shows how two ant species may affect different stages of seedling establishment under canopy of a myrmecophytic plant species.

The elimination of seedlings by resident ants could also act on conspecific seedlings. The absence of conspecific

individuals strongly suggests that the defensive activity of *P. triplarinus* could also affect the recruitment of *T. americana*, creating areas that could not be occupied by any seedlings of any species, including its own. Other mechanisms could explain the absence of conspecific seedling, among them, physiological limitations produced by shade of the host plant canopy, which could affect to these light demanding seedlings (Morawetz et al., 1992). However, it is necessary to evaluate experimentally whether resident ants can act in the same way against seedlings of the host plant. On the other hand, although the number of sites in which we evaluated the effect of fragmentation was low, this is one of the few studies that simultaneously addresses multiple factors influencing plant establishment under a myrmecophyte plant (Frederickson et al., 2005). However, since pseudoreplication is included in the interpretation and data analysis, our explanation on the effects of habitat fragmentation is still far from conclusive. Thus, any interpretation of the outcome and intensity of pruning behaviour is strictly speculative.

In summary, regardless of the precise mechanisms that may increase ant activity in forest fragments, our results suggest that pruning behaviour by resident ants can strongly affect the dynamics of seedling establishment, particularly beneath the host plant's canopy. By cutting vegetation *P. triplarinus* may modify the diversity and abundance of plants recruiting under *Triplaris* trees, as well as the spatial pattern of natural regeneration.

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