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PLANT-ANIMAL INTERACTIONS - ORIGINAL PAPER

Attack frequency and the tolerance to herbivory of Neotropical savanna trees

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Abstract Tolerance is the ability of a plant to regrow or reproduce following damage. While experimental studies typically measure tolerance in response to the intensity of herbivory (i.e., the amount of leaf tissue removed in one attack), the impact of how many times plants are attacked during a growing season (i.e., the frequency of damage) is virtually unexplored. Using experimental defoliations that mimicked patterns of attack by leaf-cutter ants (*Atta* spp.), we examined how the frequency of herbivory influenced plant tolerance traits in six tree species in Brazil's Cerrado. For 2 years we quantified how monthly and quarterly damage influenced individual survivorship, relative growth rate, plant architecture, flowering, and foliar chemistry. We found that the content of leaf nitrogen (N) increased among clipped individuals of most species, suggesting that *Atta* influences the allocation of resources in damaged plants. Furthermore, our clipping treatments affected tree architecture in ways thought to promote tolerance. However, none of our focal species exhibited a compensatory increase in growth (increment in trunk diameter) in response to herbivory as relative growth rates were significantly lower in clipped than in unclipped individuals. In addition, the probability of survival was much lower for clipped plants, and lower for plants clipped monthly than those clipped quarterly. For plants that did survive, simulated herbivory dramatically reduced the probability of flowering. Our results were similar across a phylogenetically distinct suite of species, suggesting a potential extendability of these findings to other plant species in this system.

Keywords Artificial defoliation · *Atta laevigata* · Cerrado · Chronic herbivores · Leaf-cutter ants

Introduction

Herbivory can reduce plant growth, reproduction, and survivorship (Boege and Marquis 2005). As a result, plants employ multiple strategies to defend themselves against herbivore attacks (Strauss and Agrawal 1999; Van Der Meijden et al. 1988). Perhaps the most extensively studied of these strategies—resistance—comprises any plant traits that reduce the preference or performance of herbivores in an attempt to avoid attacks. In contrast, tolerance is the degree to which a plant can maintain the same level of growth and reproduction following damage (Simms and Triplett 1994; Strauss and Agrawal 1999). This strategy is inversely related to the degree to which fitness is reduced by herbivores (Mauricio 2000). Though not a strict alternative to plant resistance (Strauss and Agrawal 1999), recent findings support the notion that tolerance is an evolutionarily older and a more stable form of defense (Espinosa and Fornoni 2006).

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Plant tolerance to herbivory depends on several intrinsic plant characteristics that influence the ability to regrow. Previous research has extensively focused on traits such as plant size (Boege and Marquis 2005), photosynthetic rates (Meyer 1998), storage capacity (Van Der Putten 2003), and growth rate (Boege 2005; Meyer 1998). However, traits that influence plant architecture, such as branching structure (Fornara and Du Toit 2007) and meristem availability (Fay and Throop 2005), can also influence tolerance (Stowe et al. 2000; Strauss and Agrawal 1999). How plants prioritize resource allocation to intrinsic traits that influence tolerance can change in the face of herbivory (Boege 2005), often in unexpected ways (e.g., Fornara and Du Toit 2007; Reich et al. 1999). Quantifying the responses of both architectural and regrowth traits is necessary to better elucidate mechanisms underlying plant tolerance to herbivores.

In addition to intrinsic plant traits, abiotic conditions (e.g., water or nutrient availability) can also influence plant tolerance (Lau et al. 2008; Strauss and Agrawal 1999). For instance, nutrient availability can be negatively associated with tolerance, although this may be true primarily in sites with greater nutrient availability (Irwin and Aarssen 1996). Biotic factors, such as the abundance and identity of herbivores or the intensity, frequency, or seasonal timing of their foraging, can also influence tolerance. For example, the amount of simulated deer browsing and its timing was found to influence the regrowth responses of willow (*Salix*): moderate clipping induced compensatory growth, and severe clipping resulted in undercompensation (Guillet and Bergstrom 2006), although the effect varied with the remaining length of the growing season (Guillet and Bergstrom 2006). Other studies have found evidence for overcompensation following herbivory. In experiments with the grassland biennial *Gentianella campestris* (Gentianaceae), Lennartsson et al. (1997) found that plants that had 50% of their leaf biomass and stem nodes clipped 4 weeks before the peak of flowering overcompensated by producing approximately 40–100% more fruits than unclipped controls. Thus, the intensity of herbivory appears to play a key role in mediating plant responses to damage.

Despite this knowledge, our general understanding of how herbivore foraging influences plant tolerance remains limited by several factors. First, most studies to date have been conducted with a single species (but see Del-Val and Crawley 2005), which has greatly limited our ability to generalize regarding plant tolerance and its role in influencing plant communities (e.g., Fornara and Du Toit 2007; Huhta et al. 2000; Knight 2003). Second, tolerance is usually studied in response to the intensity of herbivory—usually defined as the amount of total biomass removed in a defoliation event (Guillet and Bergstrom 2006; Huhta et al. 2000; Knight 2003)—and most studies typically remove biomass in a single defoliation event (e.g., Gadd et al. 2001; Guillet and Bergstrom 2006; Hodar et al. 2008; Huhta et al. 2000; Knight 2003). However, plants can be attacked by herbivores multiple times over the course of a growing season or, in the case of perennial plants, over the course of multiple years. Nevertheless, most studies are short-term in duration—days to months—even when tolerance in perennials is studied. Furthermore, the frequency of damage, i.e., the number of times plants are attacked by herbivores during a growing season, is an important, albeit virtually ignored means of describing herbivore intensity in experimental studies of tolerance (but see Del-Val and Crawley 2005). Indeed, the one study of which we are aware that simultaneously evaluated the effects of clipping intensity and frequency on patterns of tolerance concluded that "the detrimental impact of repeated herbivory may not be directly extrapolated from results obtained from single defoliation experiments" (Del-Val and Crawley 2005, p. 1015).

Leaf-cutter ants of the genus *Atta* are one of the most dominant herbivores in the Neotropics, both numerically and in terms of total biomass consumption (Wirth et al. 2003). Recent estimates suggest that, in some sites, leaf-cutter ants consume 13–17% of the net foliar production per annum, a proportion comparable to that of some vertebrate browsers in Africa savannas (Costa et al. 2008). This biomass is typically removed at regular intervals; in some cases, this defoliation is partial (Vasconcelos 1997), while in other cases plants can be completely defoliated (Mundim 2009; Wirth et al. 2003). Given this intensity and pattern of herbivory, and *Atta*'s ancient evolutionary history (Bacci et al. 2009), one might expect that herbivory by leaf-cutter ants would have led to the evolution of increased tolerance and the "browsing-lawn" phenomenon, in which chronic browsing maintains trees in a highly productive hedged state (Fornara and Du Toit 2007). However, despite the considerable research on plant resistance against leaf-cutter ants (e.g., Howard 1988; Hubbell et al. 1984) and observations of plants resprouting after defoliation, to the best of our knowledge there have been no studies elucidating plant tolerance to *Atta* herbivory. In the study reported here, we investigated how the frequency of attack by herbivores influences plant tolerance traits using experimental defoliations that mimic the natural patterns of attack by *Atta laevigata* on six common Neotropical savanna tree species. Over the course of 2 years we quantified how the frequency of simulated ant attacks influenced plant architecture, individual survivorship, relative growth rate, flowering, and foliar chemistry.

Materials and methods

Study site and species

Our study was conducted at the Estação Ecológica do Panga (19°10′S, 48°23′W), a 404-ha reserve 30 km south of Uberlândia, Minas Gerais, Brazil. The mean annual temperature is 22°C and mean annual precipitation is 1,650 mm, with 92% of the rainfall occurring during the October–April rainy season (Oliveira-Filho and Ratter 2002). The reserve includes most vegetation types characteristic of the Brazilian Cerrado (Cardoso et al. 2009), a South American biome whose 2 million km^2 make it second only to Amazonia in total land area. In the Cerrado, savannas of very variable structure (collectively known as *cerrado sensu lato*) are the dominant vegetation physiognomy on the well-drained interfluves, whereas forests occur along the water courses or on areas with richer soils (Oliveira-Filho and Ratter 2002).

Atta laevigata is the most common leaf-cutter ant in the savannas of South America, attaining densities of >4 nests ha^{-1} in some locations (Costa et al. 2008). Similarly to other *Atta* species, these ants use fresh plant material as substrates for the growth of a symbiotic fungus, which is their food source (Hölldobler and Wilson 1990). To maintain the fungal gardens, they remove leaves from trees throughout the year (Hölldobler and Wilson 1990; Wirth et al. 2003). Although considered a generalist forager that exploits many plant species, *A. laevigata* usually focuses on a preferred species subset and rapidly defoliates palatable trees (Vasconcelos 1997). In our sites, individual trees from preferred species are attacked by *A. laevigata* as often as monthly, and attacks usually result in complete defoliation of the crown (Mundim 2009). Evidence of *A. laevigata* activity is conspicuous—they clip the leaf at the petiole, leaving characteristic semi-circular marks on the cut leaves underneath the host plant [Vasconcelos and Cherrett 1997; Electronic Supplementary Material (ESM) 1].

Our work was conducted with individuals of six tree species: *Cardiopetalum callophylum* (Annonaceae), *Coussarea hydrangeaefolia* (Rubiaceae), *Guapira graciliflora* (Nyctaginaceae), *Maprounea guianensis* (Euphorbiaceae), *Vochysia tucanorum* (Vochysiaceae), and *Xylopia aromatica* (Annonaceae) (ESM 2; referred to hereafter by their generic names). These species are widespread in the savannas of the Cerrado biome, reproduce annually (Mundim 2009), and are preferentially harvested by *A. laevigata* (Mundim et al. 2009).

Defoliation experiment

In November 2006 we established a 400-m-long trail in a portion of Estação Ecológica do Panga covered by a savanna with a dense tree cover locally known as "*cerrado denso*" (see Oliveira-Filho and Ratter 2002). Average tree height in this physiognomy is 3–4 m (maximum 6–8 m), with a canopy cover of approximately 60% (Costa et al. 2008). The focal plants were all located in the understory adjacent to a trail. Within 3 m of either side of this trail we marked all individuals of the six focal tree species that met the following size criteria: 0.5–2.5 m in height and with a trunk diameter of at least 0.65 cm at 15 cm above the soil surface. Individuals of this size are capable of flowering (see "Results"), with the exception of *Xylopia* and *Vochysia*, which only reproduce at much larger diameters (F. Mundim, personal observation). Our trail was deliberately established in an area where there was no sign of current leaf-cutter ant activity, as it would have otherwise been very difficult to perform our experiment with controlled levels of defoliation. This, however, does not mean that our focal trees had never experienced attacks by leaf-cutter ants. Because at our study area nest relocations are common, local nest densities vary strongly from year to year (E. H. M. Vieira-Neto, unpublished data).

Marked plants were assigned to one of the three experimental treatments—monthly clipping, clipping every 3 months (i.e., quarterly), and unclipped controls—by selecting three individuals with approximately the same diameter and randomly allocating individuals to one of the treatments ($n = 15$ individuals per treatment). This ensured that the mean size and range of the individuals allocated to each treatment were nearly identical (Table 1). The only exceptions to this design were *Vochysia* and *Xylopia*, for which no individuals were allocated to the quarterly clipping treatment due to low density of these species in our study site.

Clipping plants monthly simulates the highest frequency of attack by *A. laevigata* observed in our field sites, quarterly clipping simulates the average frequency of attack, and the unclipped controls simulate plants that are never or seldom attacked (Mundim 2009). When individuals of our focal species are attacked by *A. laevigata*, they are almost always completely defoliated (Mundim 2009); therefore, we completely defoliated trees when applying our clipping treatments. We clipped leaves at the petioles with a scissor (which simulates leaf-cutter activity; Vasconcelos and Cherrett 1997), after which we marked all clipped twigs. A new and unique marking was used after each experimental defoliation event; trees in the control treatment were similarly marked at the beginning of the experiment. This experiment was conducted from December 2006 to August 2008.

For 2 consecutive years following our first defoliation, we quantified how the frequency of clipping influenced individual survivorship, relative growth rate, foliar nitrogen content, architecture (i.e., branching pattern), and flowering. The presence of flowers was noted in biweekly visits to plants during each species' reproductive period (see ESM 2); we conducted these surveys in two consecutive flowering seasons. Note that these surveys were only conducted with *Cardiopetalum*, *Coussarea*, *Guapira*, and *Maprounea*, since for *Vochysia* and *Xylopia* all individuals were below the size threshold for reproduction.

Table 1 Initial basal diameter of individuals from six species of Cerrado trees at the beginning of the simulated herbivory treatments

Tree species	Initial diameter (cm)		
	Unclipped	Clipped quarterly	Clipped monthly
Cardiopetalum callophyllum	1.54 ± 0.64	1.55 ± 0.64	1.56 ± 0.63
Coussarea hydrangeaefolia	2.82 ± 0.99	2.86 ± 1.02	2.84 ± 0.96
Guapira graciliflora	2.21 ± 0.97	2.19 ± 0.94	2.20 ± 0.94
Maprounea guianensis	1.40 ± 0.59	1.37 ± 0.57	1.38 ± 0.60
Vochysia tucanorum	1.27 ± 0.50	–	1.27 ± 0.50
Xylopia aromatica	2.06 ± 0.82	–	2.06 ± 0.82

Data are presented as the mean ± standard deviation (SD)

For all species, each defoliation frequency treatment initially consisted of 15 individual plants. For each species there was no significant difference in the initial basal diameter of trees in the different treatments ($P \geq 0.993$ for all comparisons)

The growth rate of plants was calculated by measuring the initial diameter (D_0) of each individual prior to applying experimental treatments; the measurement was made 15 cm above the ground and the location of the measurement marked with non-toxic colored glue to ensure subsequent measures were made at the same location. Subsequent measurements were made 9 (D_9) and 20 (D_{20}) months after the first initial measurement. The relative growth rate (RGR) was then calculated after 9 months ($\ln D_9 – \ln D_0/T_9–T_0$) and after 20 months ($\ln D_{20}–\ln D_0/T_{20}–T_0$).

The sequence of branch bifurcations that repeats itself at the end of branches strongly influences total crown volume and hence the ability to capture light. We compared the branching patterns of plants in different treatments by determining the number of branch tips (BT) beyond the markings made at each artificial defoliation. These counts were made 9 months after the start of the experiment because after this time interval, the markings began to disappear and many trees had died.

The influence of attack frequency on leaf nutrient concentrations was evaluated by measuring the nitrogen (N) content in leaves from trees species in each experimental treatment (this analysis was not conducted with *Vochysia* or *Xylopia* because there were only two experimental categories for these species). Leaves were sampled for analysis 9 months after the start of the experiment, which corresponds to the period of leaf flushing (ESM 2). We conducted these analyses using five fully expanded leaves per sapling to minimize the effect of leaf removal on our control and quarterly herbivory treatments. Because leaf-chemistry can change with leaf age (Hodar et al. 2008), all leaves were approximately 1 month old. Prior to the chemical analyses, leaves were washed with distilled water, dried at 60°C for 3 days, and finely milled. The samples were digested in nitric–perchloric acid solution, and total N was measured using the Kjeldahl procedure (Embrapa 1997). All analyses were performed at the Universidade Federal de Uberlândia's Soils and Foliar Analysis Lab in Uberlândia, Brazil.

Statistical analyses

Survival analysis was used to compare the survivorship of plants in the control and experimental treatments. For each species, we compared the probability of survival and the time to mortality of the three treatments. The Fisher's exact test was used to compare the proportion of reproductive individuals (i.e., those that flowered at some point during the reproductive season) between the three defoliation categories. Tests were conducted separately for each reproductive period and species.

For some species, many of the individuals in the monthly clipping treatment died before the third measurement, resulting in null variance in growth rates. Consequently, we were unable to use a repeated measures analysis of variance to compare the relative growth rates of plants subjected to the different defoliation treatments. We therefore used a Kruskal–Wallis test to test for differences in RGR during the first interval and a Mann–Whitney test for comparing the RGR in the second time interval. Each species was analyzed separately. Nemenyi's test (Zar 1999) was used for a posteriori multiple comparisons. To test if the number of BT differed between experimental categories, we used ANOVA followed by a Tukey's HSD post-hoc test. The number of BT was log-transformed (base 10) to meet the assumptions of parametric statistics; data for each species were again analyzed separately. Finally, analysis of variance (ANOVA) was used to test for differences in N content between the three experimental categories. All analyses were conducted using Systat v. 12 (Systat 2007).

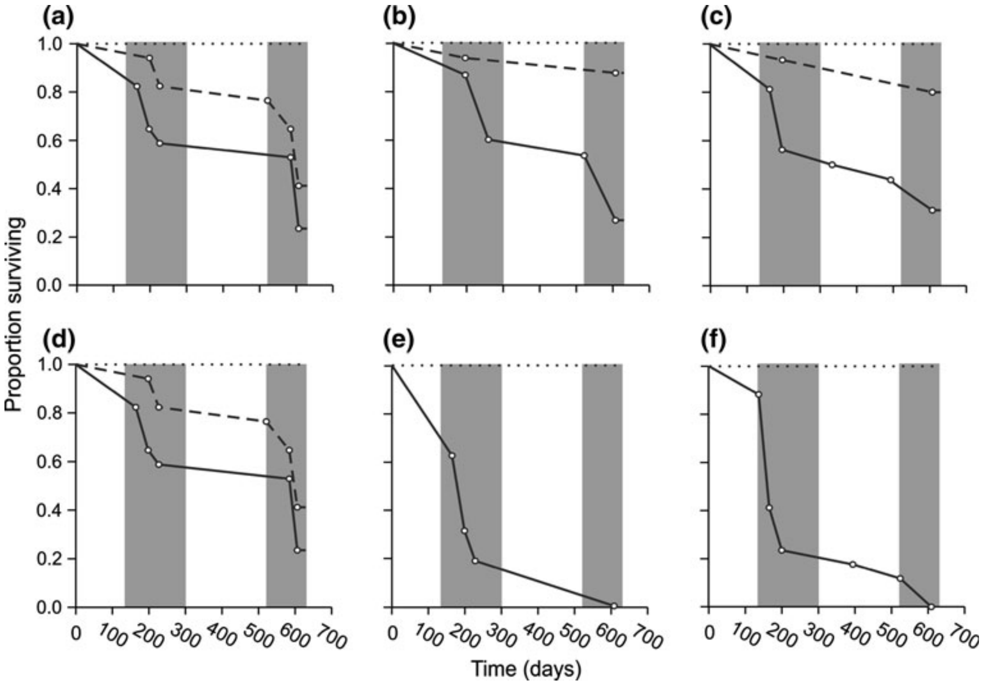

Fig. 1 Survival of six Cerrado tree species exposed to three simulated herbivory treatments over the course of the experiment (629 days). **a** *Cardiopetalum callophylum*, **b** *Coussarea hydrangeaefolia*, **c** *Guapira graciliflora*, **d** *Maprounea guianensis*, **e** *Vochysia tucanorum*, **f** *Xylopia aromatica*. Note that two species—*V. tucanorum* and *X. aromatica*—only had one clipping treatment (monthly). *Open circles* Census dates when mortality was recorded, *gray areas* May–September dry seasons. *Lines* represent the unclipped (*dotted*), clipped quarterly (*dashed*), and clipped monthly (*solid*) treatments

Results

No individuals in the control treatment had died by the end of the study. For all species, however, simulated herbivory significantly reduced the survival of trees (survival analyses $P < 0.001$ for all species; Fig. 1). Mortality was higher for plants clipped monthly than for those clipped quarterly (Fig. 1). Within each treatment mortality also varied among species. For plants clipped monthly, mortality tended to be higher for *Vochysia* and *Xylopia* than for the remaining species (100% in *Vochysia* and *Xylopia* vs. 73–87% for the remaining species). A similar pattern was observed for plants clipped quarterly, with *Cardiopetalum* and *Maprounea* showing a higher mortality (60% for both species) than *Coussarea* and *Guapira* (13 and 27%, respectively). Plant survival was not associated with initial plant size (i.e., trunk diameter) for any of the species (survival analyses $P \geq 0.12$ for all species). However, the higher survival of *Coussarea* and *Guapira* clipped quarterly may partially be a result of their size, since the initial mean diameter of these species was greater than that of *Cardiopetalum* and *Maprounea* (Table 1). Most of the tree mortality occurred during the dry seasons ($n = 100$ of 103 deaths; Fig. 1).

Clipping had dramatic effects on the likelihood of flowering (Fig. 2). In the first 9-month season, an average of 65% of the unclipped trees flowered versus only 45.8% of the trees subjected to the clipping treatments. Nevertheless, clipping only reduced flowering significantly in *Guapira* (Fisher's test $P < 0.001$; $P \geq 0.235$ for all others; Fig. 2). However, 20 months after the start of the experiment only 8.6% of the clipped trees flowered versus 76.6% of the control trees. This difference was significant for all species (Fisher's test $P < 0.001$ for all species).

The clipping treatment also had a strong and negative effect on relative diameter growth rates (Table 2). Nine months after the start of the experiment, the RGR was approximately 1.7- to 40-fold greater in unclipped trees than in clipped ones ($P < 0.01$ in all cases; ESM 3). Trees clipped monthly had a lower growth rate than those clipped quarterly (Table 2; ESM 3). Differences in growth rates between clipped and unclipped trees were still significant 20 months after the experiment was initiated (range 0.00–0.14 vs. 0.05–0.41 mm/month, respectively; Table 2).

Plant architecture was significantly affected by the clipping treatment. For all species, trees clipped monthly had five- to 15-fold more BT than unclipped trees (ANOVA $P \leq 0.007$ for all species; ESM 4; Fig. 3). For five of the six species, the number of BT in trees clipped quarterly did not differ from that in trees clipped monthly (range 36–854 vs. 60–900, respectively; Fig. 3). The exception to this was *Cardiopetalum*, for which the number of BT in trees clipped quarterly was lower than that in trees clipped monthly and not significantly different from that in trees left unclipped (Fig. 3).

For *Coussarea* and *Maprounea*, the N content in clipped plants was 1.4- and 1.2-fold greater than that in unclipped plants (ESM 5; Fig. 4). In contrast, there was no difference between treatments in *Cardiopetalum* and *Guapira* (ESM 5; Fig. 4).

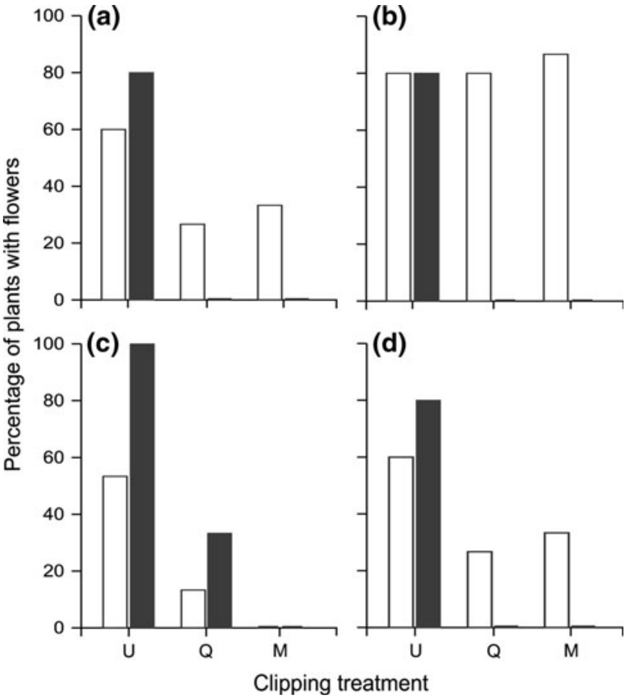

Fig. 2 Percentage of plants that flowered in two seasons after three simulated herbivory treatments: unclipped (*U*), clipped quarterly (*Q*), and clipped monthly (*M*). Seasons were nine (*open bars*) and 20 months (*black bars*), respectively, after the treatments were initiated. **a** *Cardiopetalum callophylum*, **b** *Coussarea hydrangeaefolia*, **c** *Guapira graciliflora*, **d** *Maprounea guianensis*

Discussion

Understanding the mechanisms underlying plant tolerance to herbivory is a major theme in the study of plant–herbivore interactions (Fornara and Du Toit 2007; Guillet and Bergstrom 2006; Huhta et al. 2000; Nunez-Farfan et al. 2007; Strauss and Agrawal 1999). Although it is widely accepted that plant tolerance can be strongly influenced by the intensity of herbivory, the way in which repeated attacks by herbivores influence tolerance has been largely overlooked. Overall, we found little evidence that our focal tree species are tolerant to repeated and consecutive attacks by leaf-cutter ants. Although our clipping treatments affected tree species architecture in ways thought to promote tolerance (i.e., increased tree branching), none of our focal species exhibited the expected compensatory increase in growth. Clipped individuals also had lower relative growth rates than unclipped controls. Finally, the chances of survival and flowering were much lower for clipped than for unclipped plants, particularly for plants that were clipped repeatedly over several months. That our results were similar across a phylogenetically distinct suite of species suggests that this difference is potentially a general characteristic of the plant species in this system.

All control plants survived the entire 21 months of the experiment. In contrast, experimental clipping significantly decreased individual survivorship in all six tree species, although the magnitude of the decline varied as a function of species identity and the frequency of experimental herbivory. In *Coussarea* and *Guapira*, for example, over 80% of plants clipped quarterly survived the experiment. In contrast, by the end of our trials approximately 70% of plants clipped monthly had died. Similar rates of mortality were observed in *Cardiopetalum* and *Maprounea*, although the difference between the two clipping treatments was far less pronounced (<20%). It is notable that in all cases mortality was highest during the dry season (Fig. 1), with *Vochysia* and *Xylopia* having particularly pronounced mortality in the first dry season. This finding suggests that the effects of herbivory can be strongly influenced by abiotic conditions, especially limited water availability. Dry season water stress can be severe in the Cerrado and can have effects at the individual (e.g., plant water status, transpiration, and stomatal conductance), population (e.g., survival, seedling recruitment), and community (e.g., diversity) levels (Oliveira-Filho and Ratter 2002). Our results suggest that in addition to these responses frequent herbivory might act in synergy with this stress to further increase the risk of plant mortality.

For plants that did survive, simulated herbivory dramatically reduced the probability of flowering. Damaged plants tended to flower less in the first reproductive period than undamaged plants, although this difference was only significant for one species (*Guapira*; Fig. 2). In the second flowering season, however, none of the plants clipped monthly flowered, and *Guapira* was the only species that had any plants flowering in the quarterly clipping treatment. Herbivory has previously been shown to influence various components of reproduction, including the probability of flowering and fruit set (Marquis 1984). Our results suggest that the reserves needed to flower are rapidly depleted by leaf-cutter ant herbivory and that even intermediate levels of herbivory frequency can dramatically reduce plant fitness.

In other studies, tolerant plants have been shown to compensate for leaf loss with increased growth (Stowe et al. 2000; Strauss and Agrawal 1999). We did not measure the number of leaves produced following herbivory (given the large number of plants examined in our study), and plants may indeed have compensated for damage by producing more leaves. However, clipped plants had a much higher number of BT than unclipped controls, although the intensity of this effect varied by species (Fig. 3). Increased branching can promote tolerance by increasing the surface area for photosynthesis, thereby enhancing resource accumulation (Fornara and Du Toit 2007; Strauss and Agrawal 1999). Since our study species

Table 2 Monthly relative growth rate (RGR) of six Cerrado tree species submitted to different frequencies of simulated herbivory

Tree species	After 9 months			After 20 months	
	Unclipped	Clipped quarterly	Clipped monthly	Unclipped	Clipped quarterly
C. callophyllum	0.25 (0.15–0.57) a	0.04 (0.00–0.25) b	0.00 (0.00–0.00) c	0.19 (0.11–0.41) a	0.04 (0.00–0.11) b
C. hydrangeaefolia	0.29 (0.14–0.40) a	0.10 (0.00–0.25) b	0.00 (0.00–0.42) c	0.19 (0.12–0.29) a	0.05 (0.00–0.14) b
G. graciliflora	0.25 (0.05–0.59) a	0.07 (0.00–0.25) b	0.00 (0.00–0.07) c	0.17 (0.05–0.35) a	0.03 (0.00–0.13) b
M. guianensis	0.17 (0.00–0.45) a	0.09 (0.00–0.25) b	0.00 (0.00–0.07) c	0.11 (0.07–0.35) a	0.04 (0.00–0.14) b
V. tucanorum	0.40 (0.27–0.57) a	–	0.00 (0.00–0.17) c	–	–
X. aromatica	0.25 (0.00–0.54) a	–	0.00 (−0.45 to 0.15) c	–	–

Relative growth rate (RGR) is defined as the median change in trunk diameter in millimeters per month

The first measurement was made 9 months after the first clipping; there were $n = 15$ individuals for each species × treatment combination. The second measurement was made 20 months after the first clipping; there were $n = 15$ unclipped plants per species, but of the plants clipped quarterly only $n = 6$–13 plants survived. No plants clipped monthly were measured after 20 months because almost none of the plants had survived

Values followed by different lower-case letters represent significant differences between treatments (Nemenyi's test $P < 0.05$). Minimum and maximum growth values are given in parenthesis

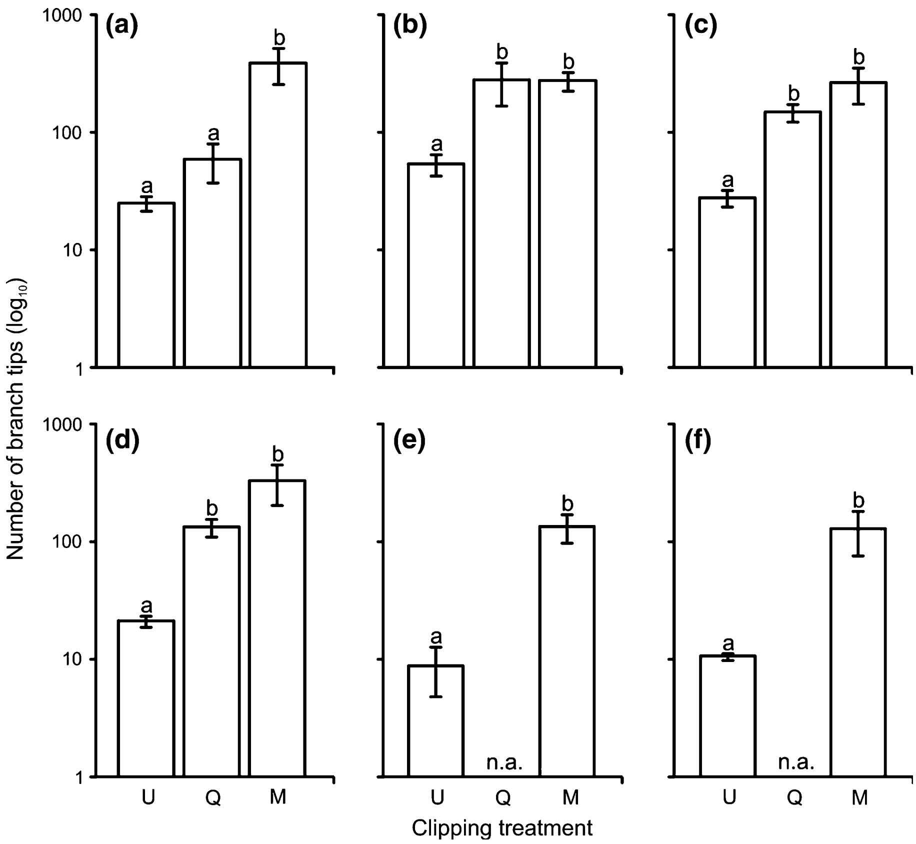

Fig. 3 Number of branch tips (*BT*) produced by six Cerrado tree species 9 months after the initiation of three simulated herbivory treatments: unclipped (*U*), clipped quarterly (*Q*), and clipped monthly (*M*). **a** *Cardiopetalum callophylum*, **b** *Coussarea hydrangeaefolia*, **c** *Guapira graciliflora*, **d** *Maprounea guianensis*, **e** *Vochysia tucanorum*, **f** *Xylopia aromatica*. *Bars* Mean number of BT [± standard error (SE)] (note log scale). *Different letters* represent significant differences between the treatments for each species (Tukey's test $P < 0.05$). *n.a.* Data not available

are long lived, compensation through increased branching may only be expressed at a time scale longer than the one we have evaluated. However, we feel that this hypothesis is unlikely given that frequent herbivory over many years is most likely to cause plant death.

Results from previous studies with herbaceous species indicate that the concentration of leaf N generally increases in response to herbivory (Koricheva 1999). This appears to be true for Cerrado trees as well—in two of the four species there was a significant increase in leaf N content when the plants were clipped (Fig. 4). Although the mechanisms underlying these variable responses in plant chemistry are unclear, they could be related to the very low concentration of soil N in the Cerrado (Bustamante et al. 2006) and to

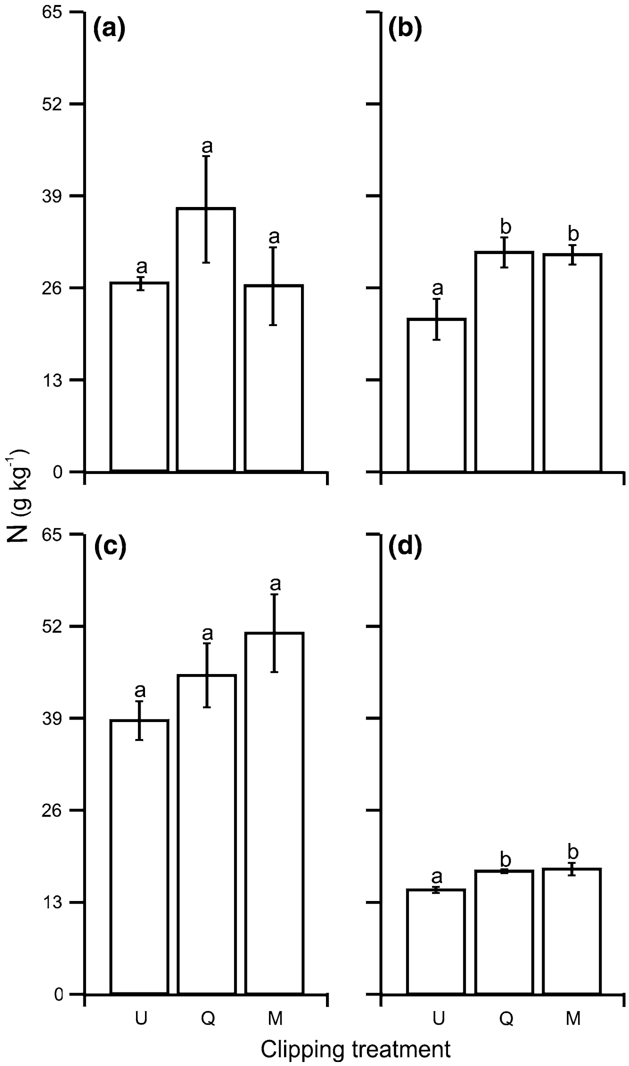

Fig. 4 Leaf nitrogen (*N*) content (g kg^{-1}) of four Cerrado tree species exposed to three simulated herbivory treatments: unclipped (*U*), clipped quarterly (*Q*), and clipped monthly (*M*). **a** *Cardiopetalum callophylum*, **b** *Coussarea hydrangeaefolia*, **c** *Guapira graciliflora*, **d** *Maprounea guianensis*. *Bars* are mean N content (±SE) in recently emerged (approximately 1-month-old) leaves collected 9 months after the initiation of the treatments. *Different letters* represent significant differences between the treatments for each species (Tukey's test $P < 0.05$)

differences in how species store or allocate these elements (Goetz and Prince 1999).

Although plant responses to the intensity of herbivory have been well documented (e.g., Guillet and Bergstrom 2006; Hodar et al. 2008; Huhta et al. 2003), it is clear from the results of our experiments that the frequency of damage can also exert a strong influence on plant responses (see also Del-Val and Crawley 2005). Nevertheless, disentangling these two effects experimentally can be quite challenging, in part because they can be positively correlated (see Del-Val and Crawley 2005). However, we do not believe that in our study plants clipped monthly necessarily had more leaf biomass removed over the course of the experiment than those clipped quarterly. Indeed, we believe the opposite is true in our system—because our focal species needed a few weeks to begin the production of new leaves following damage, we never observed fully recovered tree crowns prior to the application of the next monthly clipping treatment (Mundim 2009). In contrast, leaves on the plants clipped quarterly were often fully expanded in time for the next round of experimental damage. Thus, it is likely that over the course of our experiment we removed much more leaf biomass from plants clipped quarterly than from those clipped monthly. This conjecture is supported by the results of other studies, which have found that plant biomass is negatively correlated with the number of defoliation events (Del-Val and Crawley 2005; Rinella and Hileman 2009). Furthermore, plants can produce significantly smaller or lighter leaves when damaged or defoliated in the previous year (Candolfi-Vasconcelos and Koblet 1990; Ruohomaki et al. 1997). We suggest an important next step is to independently manipulate biomass removal and the frequency of damage when investigating tolerance, as these different metrics of herbivore activity can influence plant tolerance traits in contrasting or complementary ways (Del-Val and Crawley 2005).

While large mammalian browsers and grazers are a speciose and abundant group in African savannas (Fa and Purvis 1997), such herbivores have been almost entirely absent in the Neotropics since the post-Pleistocene period (Webb 1978). Consequently, herbivory is thought to have a negligible effect on the dynamics of Cerrado vegetation (Gardner 2006). However, this conclusion ignores the extraordinary diversity of insect herbivores in the biome (Marquis et al. 2002) and the high abundance and activity of leaf-cutter ants in many areas (Costa et al. 2008). Given *Atta*'s ancient evolutionary history (Bacci et al. 2009), one might expect that, as has been observed among plants of the African savanna subject to chronic browsing by ungulates (Fornara and Du Toit 2007), herbivory by leaf-cutter ants would have led to the evolution of tolerance among its host-plants. It may be that the much higher intensity of attack by leaf-cutter ants—almost all leaves are removed from plants—may be too much for plants to overcome when browsing is too frequent. Furthermore, the consequences of leaf-cutter herbivory are magnified in the dry season, during which their activity continues unabated.

In African savannas, the many species of mammalian herbivores often attack a small subset of available plant species (Codron et al. 2007; Gadd et al. 2001) and do so regularly both within and across seasons (Fritz et al. 1996). This high pressure on fewer plants may drive the evolution of tolerance in this subset of species. In contrast, it appears the opposite might be the case in Neotropical savannas.

Because the diversity and abundance of large mammalian herbivores in the Cerrado is low (Webb 1978), most herbivory is by invertebrates (Marquis et al. 2002). Invertebrate herbivores—especially leaf-cutter ants—have very dynamic plant preferences influenced by local plant diversity and abundance, season, history of habitat use, and colony location (Hubbell et al. 1984; Vasconcelos 1997; Wirth et al. 2003). Consequently, the intensity and frequency of damage are high but diffuse, possibly resulting in lower selective pressure for tolerance in the Cerrado than in other savannas.

The role herbivores play in shaping plant population and community dynamics has been the subject of sometimes contentious debate since the seminal work of Hairston et al. (1960). Although our study was not designed to help resolve this issue, our results nevertheless suggest that leaf-cutter ants can indeed influence both plant demography and diversity in the Cerrado. Increased mortality, reduced growth, and reduced flowering can all contribute to lower population growth rates (Bruna and Oli 2005). In concert with the reduced seedling abundance resulting from seed predation and seedling herbivory by *Atta* and other ant species (Noleto 2010; Vasconcelos and Cherrett 1997), this may lead to a shift in community composition towards species not preferred by leaf-cutter ants. Such shifts have previously been noted in lowland tropical forests (Rao et al. 2001). Given that *Atta* colony density is approximately threefold higher in the Cerrado (Costa et al. 2008) and that the abundance of leaf-cutter ants increase dramatically in disturbed areas (Vasconcelos and Cherrett 1995; Vasconcelos et al. 2006), it is likely that ongoing anthropogenic alterations of the Cerrado (Klink and Machado 2005) that lead to increased *Atta* abundance are likely to exacerbate demographic shifts resulting from herbivory by leaf-cutter ants.

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