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Improvement in light utilization and shoot growth in *Hymenaea stigonocarpa* under high CO_2 concentration attenuates simulated leaf herbivory effects

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ABSTRACT

This study evaluated the photochemical responses of photosystem II and growth of *Hymenaea stigonocarpa* under CO_2-enriched conditions with exposure to simulated herbivory events. After herbivory simulation in two distinct parts of the stem of plants (apex and base), chlorophyll *a* fluorescence, chlorophyll index, growth, extrafloral nectary density, leaf mineral nutrition, and biomass production were evaluated. Plants of *H. stigonocarpa* grown under high [CO_2] after simulated herbivory in the apical part of the stem had higher electron transport rate, effective quantum yield of photosystem II, and chlorophyll contents. However, simulated herbivory in the basal portion of plants grown under high [CO_2] increased plant height, branch and root length, leaf number, leaf area, node number, and leaf expansion rate. In conclusion simulated herbivory at the basal portion and high [CO_2] induce positive responses in *H. stigonocarpa*, leading to the allocation of biomass to vegetative parts related to the capture of resources such as water and light. Apical leaves could compensate for the elimination of part of their leaf blades by increasing their photosynthetic yield. Thus, the increase of [CO_2] attenuated the adverse effects of leaf removal on *H. stigonocarpa* plants by inducing photosynthetic improvement and growth after the loss of leaf tissue.

Keywords: biomass allocation, Cerrado, climate change, leaf damage, leaf development

Introduction

Cerrado, considered a biodiversity hotspot, presents a high degree of endemism, which, however, is threatened. Extensive habitat loss has occurred in this neotropical savanna due to the replacement of native vegetation by agricultural areas (Hughes 2017; Lapola *et al*. 2014) and by the introduction of exotic species, the major threat to its biodiversity and functioning (Klink & Machado 2005; Klink 2013). In addition to the expansion of agricultural frontiers, changes in the climate, such as increased CO_2 concentration ([CO_2]) in the atmosphere, drought intensification, and high temperatures, have threatened this ecosystem. Understanding the vulnerability of Cerrado vegetation to climate change requires the investigation of the effect of high [CO_2] on plant growth and its interactions with biotic and abiotic factors.

Several studies have shown that high [CO_2] results in increased net photosynthesis, the accumulation of non-structural carbohydrates, and decreases of leaf nitrogen content, stomatal conductance, and transpiration (Ainsworth & Rogers 2007; Stiling & Cornelissen 2007; Wang *et al*. 2012; Bunce 2014; Lewis *et al*. 2015; Xu *et al*. 2016). Further, C3

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plants might show increases in leaf production, stem length and diameter (Ainsworth & Long 2005), leaf life span (LLS), and leaf expansion rate (LER; Souza *et al*. 2016). The highest carbon (C) investment in biomass by plants growing under high [CO_2] consequently leads to a decrease in the specific leaf area, diluting the nitrogen (N) concentration in the leaf (Zavala *et al*. 2013; Sá *et al*. 2014). This change in the nutritional quality of leaf provides a basis for the hypothesis of compensatory herbivory (Schädler *et al*. 2007; Zavala *et al*. 2013). In addition, increased leaf area (LA), reduced stomatal conductance, and increased water use efficiency (Ainsworth & Rogers 2007) contribute to lower absorption and thus nutrient dilution in plants growing under high [CO_2]. These changes have implications for both plants and herbivorous species associated with them.

Leaf tissue removal results in the immediate fall of the effective quantum yield (ΦPSII) and potential quantum yield (F_v/F_m) of photosystem II in the region of the damage caused by the insect and a marked reduction in the values of these variables in regions adjacent to foliar injury (Nabity *et al*. 2008). Herbivory might also induce changes in CO_2 assimilation (Aldea *et al*. 2005; Macedo *et al*. 2005) and foliar nutrient concentration (DeLucia *et al*. 2012; Lemoine *et al*. 2017; Peschiutta *et al*. 2018), as well as reduce transpiration and stomatal conductance (Aldea *et al*. 2005; Garcia *et al*. 2011), thereby exerting strong selective pressure on plants. Such predation can reduce plant growth, reproduction, and especially, alter competitive processes that influence the structure and diversity of ecosystems (Coley & Barone 1996; Stiling & Cornelissen 2007; Barton & Hanley 2013; Peschiutta *et al*. 2018).

Loss of nutrients and photosynthetic LA due to herbivory might lead to reduced plant growth (Coley & Barone 1996), especially if the loss of LA includes the removal of apical leaves from the stem. Conversely, herbivory might induce plant growth and physiological adjustment (McNickle & Evans 2018; Zhang *et al*. 2018). Plants can compensate for the loss of tissue in the apical leaves by increasing the net photosynthesis of the remaining leaves or the damaged tissue itself (Retuerto *et al*. 2004; Damascos *et al*. 2005), and minimizing the damage caused by herbivores by investing in growth (Strauss & Agrawal 1999; McNickle & Evans 2018; Zhang *et al*. 2018). As a form of indirect protection, plants are able to attract natural enemies of their herbivores by presenting extrafloral nectaries (EFNs) (Kost & Heil 2005; Röse *et al*. 2006; Schuman & Baldwin 2016; Yamawo & Suzuki 2018). Some plants might increase the number of EFNs in response to leaf damage (Mondor & Addicott 2003). The direction of resource allocation in plants can be explained by the above-and belowground tissue production costs, which might be affected by biotic and abiotic conditions to which plants are exposed (Erb *et al*. 2009; Fürstenberg-Hägg *et al*. 2013); thus, high [CO_2] along with herbivory can alter the patterns of biomass allocation in plants.

Hymenaea stigonocarpa is popularly known as jatobá-do-cerrado. This leguminous species has a homogeneous distribution and occurs in areas of Cerrado *sensu lato* and "Campo Cerrado" (Lee & Langenheim 1975), where it is an endemic species of this domain. It is a late secondary species that occurs in soil with water and nutritional restriction. As a common tree species of the Cerrado vegetation, *Hymenaea stigonocarpa* is abundant and typical in the savanna formations of the Cerrado ecosystems and presents a rich fauna of associated insects that comprises many herbivores and seed predators. In addition, it presents many EFNs and associated ants, making it an interesting model for the studying the influence of increased [CO_2] in both plants and associated organisms.

This study aimed to identify the photochemical responses of PSII, chlorophyll content, foliar nutrients, morphometry, and EFN production in *H. stigonocarpa* growing under ambient or elevated [CO_2] in the atmosphere and subjected to simulated herbivory on the leaves of the basal and apical portions of the stem. High [CO_2] in the atmosphere is considered to reduce the adverse effect of herbivory, since high [CO_2] has been recognized as a factor responsible for the mitigation of the adverse effects of different types of stress (*e.g.*, Oliveira *et al*. 2013; Souza *et al*. 2016; Melo *et al*. 2018). We addressed two questions regarding the consequences of simulated leaf herbivory and high [CO_2] on the morphophysiology of *H. stigonocarpa* plants: 1) could simulated leaf herbivory on distinct stem parts (apical vs basal) result in distinct photochemical and growth responses?; and 2) could a change of the partitioning of biomass between aerial and underground plant structures benefit limiting resource acquisition, such as nutrient absorption?

Plants subjected to simulated herbivory on the leaves of the apical portion of the stem and growing under high [CO_2] should increase ΦPSII of the leaves remaining in this region in order to minimize the damage caused by herbivory. In contrast, simulated herbivory in the leaves of the basal portion of the stem could be compensated by the investment in aerial structures, with greater production of branches and leaves. In order to meet the demand for nutrients necessary for development in high [CO_2] environments, *H. stigonocarpa* plants might be able to invest in mechanisms such as root production and show higher biomass allocation to the roots, improving their nutrient acquisition. Thus, the predicted higher LA in *H. stigonocarpa* growing under high [CO_2] might not result in dilution of the mineral nutrients in the leaves; however, the density of EFNs might be reduced. To our knowledge, this is the first study to analyze the responses of Cerrado plants to simulated herbivory under high [CO_2].

Materials and methods

Study area, plant material, and growth conditions

The experiment, which lasted twenty months, was performed in open-top chambers (OTCs) located at *Campus* Florestal (CAF) of the Federal University of Viçosa, Minas

Gerais, Brazil (19°52'20"S 44°25'12"W). The climate of the region is classified as subtropical, with rainy summers and dry winters; the average values of 30 years of climate records were 1,427 mm of precipitation, a minimum temperature of 13 °C, and a maximum temperature of 28 °C (INMET 2015). CAF is located in a transition area between Atlantic Forest and the Cerrado domain (IBGE 2015).

The soil used for the cultivation of species was typical of the areas of cerrado *sensu stricto* in Minas Gerais, Brazil (Tab. S1 in supplementary material). The soil chemical characteristics were analyzed at the Laboratório de Química Agrícola do Instituto Mineiro de Agricultura (IMA).

Pre-germination treatment and experimental design

Before germination, *Hymenaea stigonocarpa* Mart. Ex Hayne seeds were immersed in an aqueous solution of 2 % sodium hypochlorite for 5 min (Botelho *et al*. 2000), followed by washing in distilled water, drying on paper towels, and breaking integumentary dormancy by using sandpaper (Pereira *et al*. 2011). Subsequently, the seeds were sowed on germitest paper substrates and cultivated for 30 days in a BOD incubator (SL.225; SOLAB, Piracicaba, BR), following the methodology adapted by Botelho *et al*. (2000). After germination in the BOD, the seedlings were transplanted into the soil within the OTCs, where they remained for 30 days to acclimate under ambient [CO_2] of 390 μmol mol^{-1}, after which the treatments of exposure to high [CO_2] started (see Melo *et al*. (2018) for a description of the OTCs). For this, a homogeneous group of 80 seedlings was arranged in OTCs, divided in two treatments with different [CO_2]: 40 seedlings in chambers under high [CO_2] of 1,000 μmol mol^{-1}, and 40 seedlings in chambers under ambient [CO_2] of 390 μmol mol^{-1}. The [CO_2] inside the chambers was monitored daily by using a CO_2 analyzer (Testo 535; Testo, Lenzkirch, Germany). The [CO_2] of 1,000 μmol mol^{-1} was chosen because it is the required concentration for C3 plants to reach CO_2 saturation (Körner 2006). The plants were kept under these conditions for approximately 313 days, during which 15 individuals each were selected from the chambers with high [CO_2] and from those with ambient [CO_2] to begin the experiment with herbivory simulation. Plants grown at high [CO_2] were subdivided into three groups: intact plants (n=5) and plants with leaf tissue removal from the basal (n=5) and apical (n=5) portions of the stem. The plants had an average height of 45 cm, and the lower and upper stem halves were considered as basal and apical portions, respectively. The same procedure was performed for plants growing in ambient [CO_2]. Before herbivory simulation, the area of each leaf in each plant (n=5 plants per treatment) was calculated to remove the parts of the leaf tissue. The equivalent of 50 % of area of the leaves present in the apical or basal portion of the stem was removed over a period of four days (12.5 % per day, Fig. S1 in supplementary material). For foliar tissue removal, carton molds were prepared, according to the area of each leaf.

Chlorophyll content index and chlorophyll a fluorescence

The chlorophyll content index (CCI) of *H. stigonocarpa* was determined using five plants from each treatment. In plants that were not subjected to foliar tissue removal, measurements were performed on intact leaves located in the middle stem region. In plants from which parts of the basal or apical leaves were removed, the measurements were performed on damaged leaves located in the median region of the stem. CCI measurements were performed before the start of the removal of leaf parts and over eleven consecutive days from the beginning of tissue removal. After the eleventh measurement, two others were performed with intervals of three and nine days. The CCI was determined using a clorofiLOG (CFL1030; FALKER, Porto Alegre, BR) chlorophyll meter.

In the same plants that were used to determine the CCI, fluorescence variables of chlorophyll *a* were measured using a light-modulating fluorometer (Mini-PAM; Heinz Walz, Germany). One leaf per plant (n=5 per treatment) located in the medial portion of the stem (same leaves used for CCI measurement) was acclimated to the dark for 30 min by using metal clamps. The clamps were placed in the region close to the damage caused by the simulated herbivory, or in the median portion in the undamaged leaves. Subsequently, leaf tissue was exposed to a weak red light pulse of approximately 1 μmol photons $m^{-2}s^{-1}$ for the determination of basal fluorescence (F0). Next, a pulse of saturating light (12,000 μmol photons $m^{-2}s^{-1}$) with a duration of 0.8 s was used to determine the maximum fluorescence (Fm). The values obtained were used to calculate the potential quantum yield of photosystem II (F_v/F_m; Kitajima & Butler 1975). The same leaf was exposed for 30 s to photosynthetically active radiation at 1,200 μmol $m^{-2}s^{-1}$ and subsequently to a saturating pulse for the determination of the following variables: F (steady state fluorescence, before light-saturating pulse) and Fm' (maximum acclimatized fluorescence to light). The following variables were calculated: effective quantum yield of FSII, (ΦPSII; Genty *et al*. 1989), non-photochemical quenching coefficient (NPQ; Bilger & Björkman 1990), and electron transport rate (ETR; Melis *et al*. 1987).

Foliar nutrient analysis

At 600 days of age (287 days after the simulation of herbivory and 540 days of CO_2 enrichment), a sample of five grams of leaves (n=3 leaves per plant in each treatment located between first (last node) up to fourth node) were collected to determine N, S, P, K, Ca, Mg, Fe, Mn, B, Zn, and Cu. Afterwards, the samples were dried in the oven at 68 °C

for 72 h. N content was determined by titration (Kjeldahl) after sulfur digestion (Jackson 1958). For the determination of boron, the samples were incinerated in an oven at 550 °C for 3 h, and its content was quantified using colorimetry using Azomethine H (Gaines & Mitchell 1979). The other nutrients (P, K, Ca, Mg, S, Cu, Mn and Zn) were determined using nitro-perchloric digestion. The P content was analyzed using colorimetry with the ascorbic acid method, and K was determined using flame photometry (Isaac & Kerber 1971). Atomic absorption spectrophotometry was used for the quantification of Ca, Mg, Cu, Fe, Mn and Zn, and turbidimetry was used for the determination of S.

Leaf area

Before the initiation of simulated herbivory treatments, total plant LA (n=5 individuals per treatment) was determined using a portable leaf area meter (LI-3000C; Li-Cor Inc., Lincoln, Nebraska, USA) in order to obtain the amount of foliar tissue to be removed. Next, the increase of LA in plants was determined by measuring the areas of leaves that emerged weekly (n=5 plants per treatment).

Vegetative morphometry

After the simulated herbivory experiment, the appearance of buds, number of leaves, and length and number of branches (n=5 individuals per treatment) were evaluated weekly for nine months. Leaf development was evaluated by marking 10 buds (n=10 plants per treatment) before leaf emergence to determine leaf expansion interval (LEI, days), leaf expansion rate (LER, $cm^2 days^{-1}$), and leaf life span (LLS, days). The LEI was determined via a weekly record of the length and width of all leaves that emerged after the end of simulated herbivory. When the leaves showed stabilized growth, the final period of LEI was determined. The LER was determined by dividing the LA stabilized by the final period of LEI (LER= LA/LEI). The leaves were marked and monitored weekly until their senescence. New leaves originating on the branches were monitored and considered to have reached senescence when they presented 90 % of chlorosis or foliar abscission.

EFN occurrence and density (EFNs/cm^2) were determined by collecting three leaves from each plant (n=5 plants per treatment), from which LA and the number of nectaries of the basal, median, and apical regions of the leaves were determined (Fig. S2 in supplementary material). EFNs were quantified visually, and the LA was determined using Image J software (Schneider *et al*. 2012).

The crown architecture of plants was analyzed at three times: 200 days after exposure to high [CO_2] (before the removal of foliar tissue), two months after the beginning of the herbivory simulation treatment (373 days of exposure to high [CO_2]), and at the end of the experiment (540 days of exposure to high [CO_2]). Five plants per treatment were used to depict the organization of their branches and leaves (Souza *et al*. 2011). The origin point of each branch and the insertion point of the leaves on the branches and stem were considered as nodes (Souza *et al*. 2011).

Biomass allocation

The biomass allocation measurements (n=5 plants per treatment) were performed when the plants were 600 days old (540 days of exposure to high [CO_2] and 287 days after the simulated herbivory event). The following variables were determined: root length, stem dry mass (SDM), root dry mass (RDM), root volume, number of branches, and diameter and height of stem. The root length and stem height were determined with a millimeter ruler, and stem diameter with a pachymeter. The root volume was obtained by volume displacement of water in a graduated cylinder (Burdett 1979). Stems and roots were dried in an oven (TECNALTE-394/3; Piracicaba, São Paulo, Brazil) at 68 °C until constant weight (Pérez-Harguindeguy *et al*. 2013), and their masses measured using an analytical balance (SHIMADZU-Series BL-320H; Tokyo, Japan).

Statistical analyses

The experiment was performed in a completely randomized block design in a 2×3 factorial scheme with two [CO_2] (390 and 1,000 μmol mol^{-1}) and three levels of simulated herbivory (intact plants, plants with foliar tissue removal in the basal portion of the stem, and plants with foliar tissue removal in the apical portion of the stem), for the following variables: foliar nutrients, SDM, RDM, LEI, LER, LLS, root volume and length, and number of branches, nodes, and leaves. For analyzing EFN distribution, a 2×3×3 factorial scheme was used with two [CO_2], three simulated herbivory levels, and three leaf blade regions (basal, median, and apical). The subdivided plot scheme was used for the variables analyzed over time (chlorophyll *a* fluorescence, chlorophyll index, LA, shoot emergence, EFN density, and node number). The different [CO_2] (390 and 1,000 μmol mol^{-1}) corresponded to the main treatment, whereas the simulated herbivory levels corresponded to the secondary treatment. In both the analyses, we calculated the averages and standard error for all measured variables and used analysis of variance (ANOVA) to test for differences between treatments. The test applied *a posteriori* was the Tukey's test at 5 % significance. R software (CAR Package) was used for all analyses (R Development Core Team 2018).

Results

CCI and Chlorophyll a Fluorescence

Hymenaea stigonocarpa growing under high [CO_2] showed higher Chla, Chlb, and Chltotal, regardless of the treatment

of leaf tissue removal ($p<0.05$, simple effect of CO_2, Fig. S3 in supplementary material). The lowest values of Chla and Chlb were found in plants, in which part of the basal foliar tissue of the stem was removed, grown under high [CO_2] ($p<0.05$, simulated herbivory effects × CO_2).

F0 values decreased in plants grown under high [CO_2] after leaf tissue removal from the apical part of the stem ($p<0.05$, simulated herbivory effects × CO_2; Fig. 1A). However, in plants cultivated in ambient [CO_2], the highest values of F0 were found after leaf tissue removal from the apical part of the stem ($p<0.05$, simulated herbivory effects × CO_2; Fig. 1B). The plants with leaf tissue removal from the apical part of the stem had lower F_v/F_m values when subjected to ambient [CO_2] ($p<0.05$, simulated herbivory effects × CO_2). However, when grown in high [CO_2], even with leaf tissue removal from the apical part of the stem, no decrease in F_v/F_m values was noted ($p<0.05$, simulated herbivory effects × CO_2; Fig. 1C).

Plants exposed to high [CO_2] with part of their apical leaves removed had higher values of ΦPSII and ETR, compared to those in which part of leaves were removed from the basal part of the stem and those of intact plants ($p<0.05$, simulated herbivory effects × CO_2; Fig. 1). The NPQ was not significantly different between plants growing in different [CO_2] ($p> 0.05$); however, leaf tissue removal from the basal and apical parts of the stem increased NPQ values compared to those of intact plants ($p<0.05$, simple simulated herbivory effects; Fig. 2).

Foliar nutrients

The highest leaf contents of Zn, Cu, and S ($p<0.05$, simple effect of CO_2; Tab. 1) were observed in plants growing at high [CO_2]. Leaf tissue removal from the basal and apical portions of the stem increased the uptake of S in plants growing under high [CO_2] ($p<0.05$, simulated herbivory effects × CO_2; Tab. 1). No significant difference was noted for the other foliar nutrients analyzed between treatments ($p>0.05$).

Leaf area

Over time, LA increased in plants under both ambient [CO_2] and high [CO_2] conditions, with values of LA stabilizing from February 2017 ($p<0.05$, months × CO_2 interaction, Fig. S4 in supplementary material). Plants growing under high [CO_2] had higher LA values compared to those of plants growing under ambient [CO_2] ($p<0.05$, simple CO_2 effect; Fig. 3). Leaf tissue removal from the basal and apical parts of the stem resulted in increased LA production in plants growing under high [CO_2], whereas the loss of apical leaf parts in plants grown in ambient [CO_2] decreased LA production ($p<0.05$, simulated herbivory effects × CO_2; Fig. 3).

Vegetative morphometry

From January to early September 2016, no buds emerged in *H. stigonocarpa*. However, at the end of September 2016, *H. stigonocarpa* began to produce buds, showing marked growth ($p<0.05$, simple effect of month, Fig. S5 in supplementary material). Plants grown under high [CO_2] showed more numbers of buds than those grown under ambient [CO_2] ($p<0.05$, simple CO_2 effect). Leaf tissue removal, especially from the basal part of the stem, stimulated the appearance of buds in plants, regardless of the [CO_2] to which they were subjected ($p<0.05$, simulated herbivory effects).

The high [CO_2] had a positive effect on the aerial growth of *H. stigonocarpa* plants (Fig. 4). Plants growing under high [CO_2] showed greater shoot lengths and leaf numbers than those grown in ambient [CO_2] ($p<0.05$, simple CO_2 effect; Fig. 4A, C). Leaf tissue removal from the basal part of the stem resulted in greater lengths of branches and

Table 1. Nutrient contents in leaf tissue of H. stigonocarpa after 540 days of growth under high [CO_2] (1,000 μmol mol^{-1}) or ambient [CO_2] (390 μmol mol^{-1}) followed by simulated herbivory.

Treatments	[CO_2] μmol mol^{-1})	N	P	K	Ca	Mg	S	Cu	Fe	Zn	Mn	B
		(g kg^{-1})						(mg kg^{-1})				
Intact plants	390	12.5 ± 1.4 Aa	1.0 ± 0.1Aa	6.3 ± 0.4Aa	6.4 ± 0.7Aa	1.0 ± 0.1Aa	1.3 ± 0.4Aa	8.15 ± 2.0Ab	99.6 ± 6.6Aa	62.4 ± 7.6Ab	418.1 ± 20.5Aa	13.5 ± 1.6Aa
	1,000	14.8 ± 1.7 Aa	0.8 ±0.04Aa	5.9 ± 0.7Aa	5.1 ± 0.4Aa	0.9 ± 0.1Aa	1.1 ± 0.1Ba	9.51 ± 1.6Aa	112.7 ± 18Aa	65.5 ± 6.4Aa	352.3 ± 43.7Aa	11.6 ± 0.1Aa
Herbivory - basal portion	390	10.6 ± 1.1 Aa	1.1 ± 0.2Aa	6.5 ± 1.0Aa	6.2 ± 0.5Aa	0.9 ± 0.1Aa	1.1 ± 0.2Ab	7.27 ± 1.9Ab	88.8 ± 8.1Aa	70.1 ± 11.7Ab	478.6 ± 57.1Aa	11.1 ± 1.0Aa
	1,000	11.5 ± 0.3 Aa	1.2 ± 0.1Aa	6.2 ± 0.3Aa	10 ± 0.7Aa	1.1 ± 0.1Aa	2.6 ± 0.2Aa	14.3 ± 2.7Aa	97.3 ± 7.0Aa	109.7 ± 4.1Aa	521.6 ± 13.9Aa	9.6 ± 0.3Aa
Herbivory - apical portion	390	12.1 ± 0.9 Aa	0.9 ± 0.1Aa	6.7 ± 0.6Aa	6.7 ± 0.2Aa	1.3 ± 0.1Aa	1.2 ± 0.3Ab	9.25 ± 2.1Ab	107.2 ± 11.1Aa	71.6 ± 4.1Ab	486.6 ± 42.9Aa	10.9 ± 0.2Aa
	1,000	12.7 ± 0.1 Aa	1.2 ± 0.3Aa	6.3 ± 0.5Aa	8.1 ± 1.5Aa	0.9 ± 0.1Aa	3.2 ± 0.6Aa	15.4 ± 2.6Aa	124.3 ± 9.7Aa	87 ± 9.9Aa	529.6 ± 61.7Aa	13.3 ± 2.1Aa

Values represent means ± standard error (n=4). Averages followed by the same uppercase letter indicate comparisons of herbivory levels, and lowercase letters compare differences between distinct [CO_2]. (Tukey's test, α= 0.05). Data are means ± standard error of four replicates.

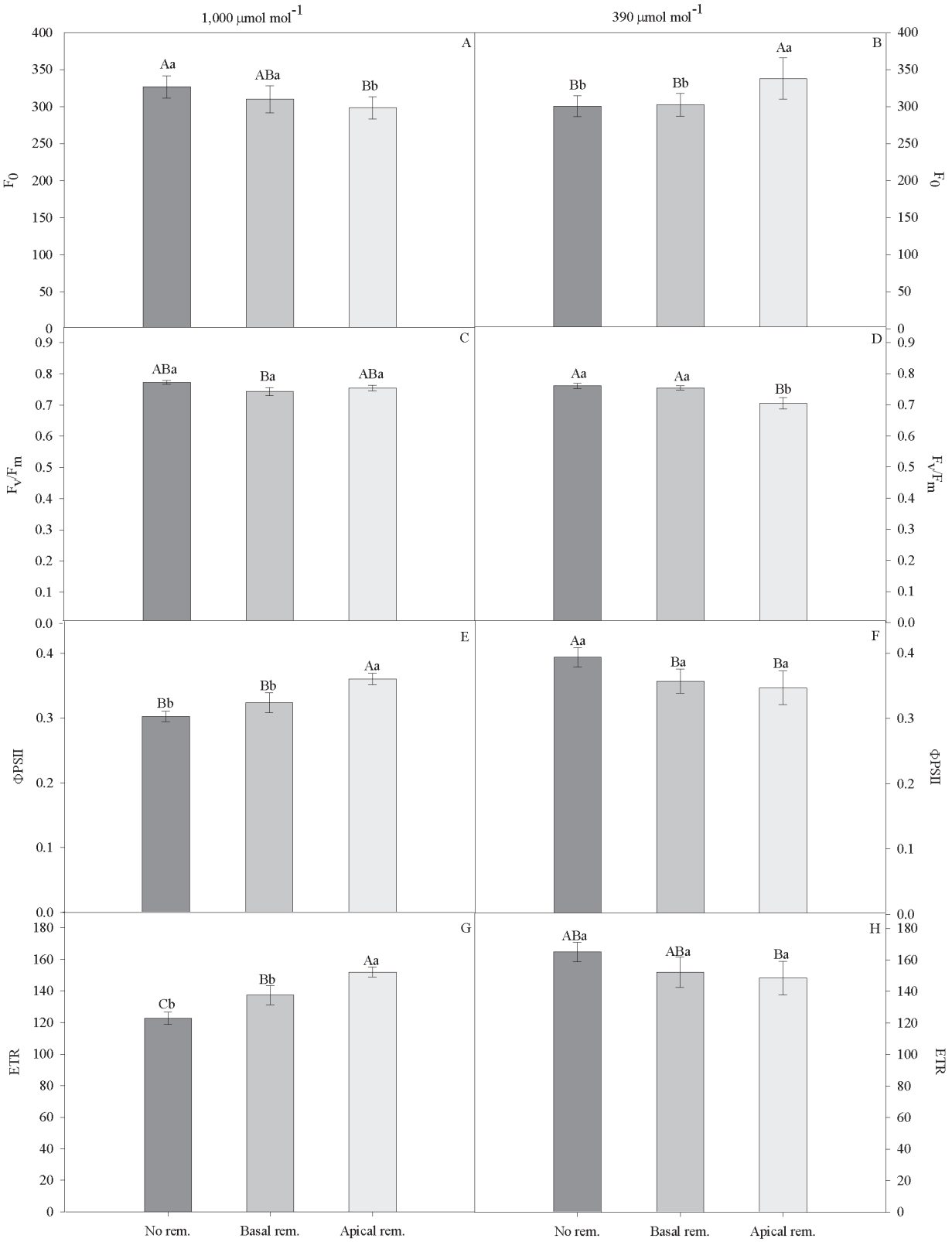

Figure 1. Basal fluorescence (F0, **A-B**), potential quantum yield (F_v/F_m, **C-D**), effective quantum yield of PSII (ΦPSII, **E-F**), and electron transport rate (ETR, **G-H**) in the plants of *H. stigonocarpa* growing under high [CO_2] (1,000 µmol mol^{-1}) or ambient [CO_2] (390 µmol mol^{-1}) subjected to simulated herbivory. No rem.=no removal; Basal rem.=basal removal; Apical rem.=apical removal. In the graphs, the values represent the average of five plants, and the bars indicate means ± standard error. Lowercase letters compare the distinct [CO_2], and uppercase letters compare the levels of herbivory.

number of leaves in *H. stigonocarpa* plants regardless of [CO_2] (p<0.05, simple simulated herbivory effect; Fig. 4B, D). No significant difference was noted in the number of branches in *H. stigonocarpa* plants regardless of [CO_2] or leaf tissue removal (p>0.05; Fig. 4E, F).

Hymenaea stigonocarpa plants maintained under high [CO_2] had higher LEI and LLS than those growing in ambient [CO_2] (p<0.05, simple CO_2 effect; Tab. 2). Leaf tissue removal from the basal or apical portions of the stem did not influence LEI in plants growing under high [CO_2]; however, the LEI decreased in plants with leaf tissue removal (basal and apical) growing under ambient [CO_2] (p<0.05). Simulated herbivory in the apical portion of the stem resulted in increased LER in plants growing under high [CO_2] (p<0.05, simulated herbivory effects × CO_2; Tab. 2).

Hymenaea stigonocarpa plants growing in environments enriched with CO_2 showed decreased EFN density in the leaves (p<0.05, simple effect of CO_2; Fig. 5A). Leaf tissue removal from the basal or apical portions of *H. stigonocarpa* stem did not change the density of EFNs regardless of the [CO_2] treatment to which the plants were subjected. The analysis of the spatial distribution of EFNs showed an asymmetric organization in the leaf blade. Although EFNs were distributed throughout the leaf blade, their density was higher in the basal region than in the apical region, regardless of the leaf tissue removal and [CO_2] to which the plants were subjected (p<0.05, simple effect of part of leaf blade; Fig. 5B).

Individuals growing under high [CO_2] had a higher node number than those growing under ambient [CO_2] (p<0.05, simple CO_2 effect; Fig. 6A). Regardless of [CO_2], node production was higher in plants in which leaves were removed from the basal portions of the stem (p<0.05, simple simulated herbivory effect; Fig. 6B). The production of nodes in *H. stigonocarpa*, regardless of [CO_2], was concentrated between 200 and 253 days, leading to the development of large number of nodes at the end of this period (p<0.05, effect of the exposure time to CO_2; Fig. 6C).

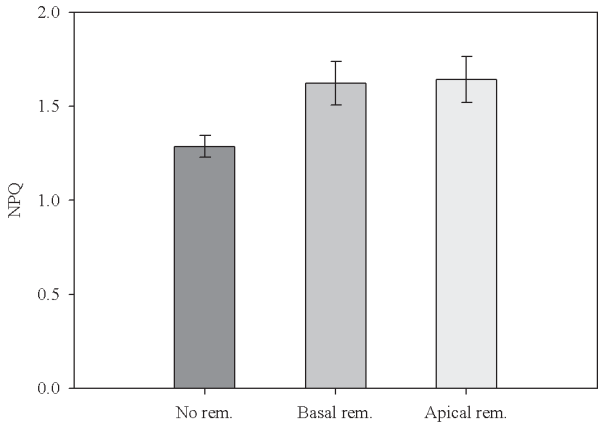

Figure 2. Non-photochemical quenching coefficient of chlorophyll a (NPQ) fluorescence in plants of *H. stigonocarpa* growing under high [CO_2] (1,000 µmol mol^{-1}) or ambient [CO_2] (390 µmol mol^{-1}) subjected to simulated herbivory. No rem.=no removal; Basal rem.=basal removal; Apical rem.=apical removal. In the graph, the values represent the average of ten plants, and the bars indicate means ± standard error. Uppercase letters compare the levels of herbivory.

Biomass allocation

Hymenaea stigonocarpa growing under high [CO_2] showed higher plant height, root length, stem dry mass, stem diameter, and root volume (p<0.05, simple CO_2 effect; Fig. 7A, C, E, G and Fig. S5 in supplementary material). No significant difference was noted between the RDM values for *H. stigonocarpa*, regardless of [CO_2] (p>0.05; Fig. 7G). Plants with leaf tissue removal from the basal part of the stem and cultivated under high [CO_2] showed greater height

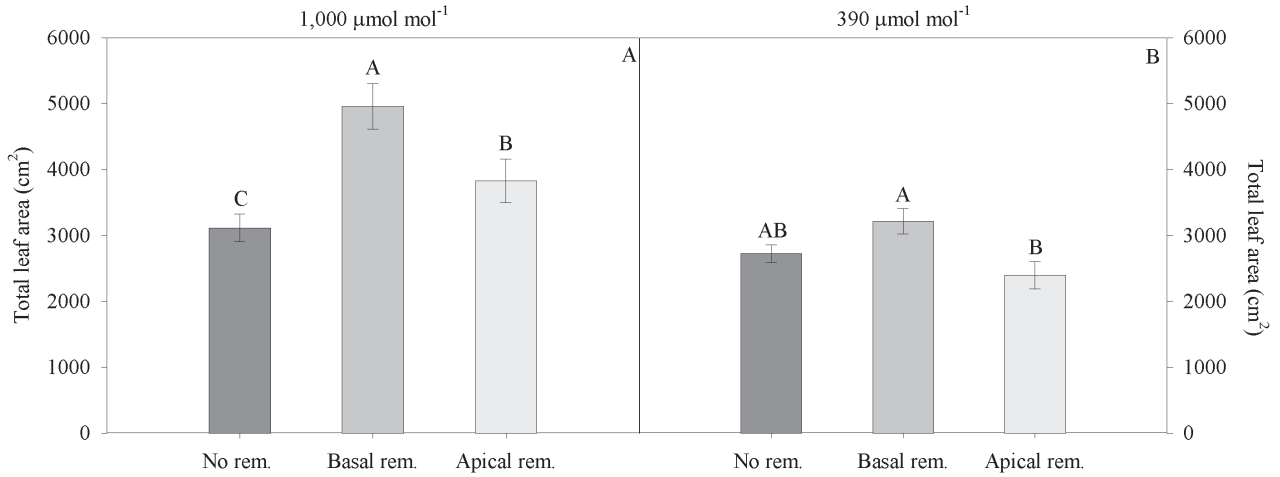

Figure 3. Total leaf area in plants of *H. stigonocarpa* cultivated under high [CO_2] (1,000 µmol mol^{-1}) or ambient [CO_2] (390 µmol mol^{-1}) subjected to simulated herbivory. No rem.=no removal; Basal rem.=basal removal; Apical rem.=apical removal. The values represent the average of five plants, and the bars indicate means ± standard error. Uppercase letters compare the levels of herbivory.

Table 2. Leaf expansion interval (LEI), leaf expansion rate (LER), and leaf life span (LLS) in H. stigonocarpa plants grown under high [CO_2] (1,000 µmol mol^{-1}) or ambient [CO_2] (390 µmol mol^{-1}) subjected to simulated herbivory. Uppercase letters compare the difference between levels of leaf tissue removal, and lowercase letters compare the distinct [CO_2].

	[CO_2] (µmol mol^{-1})	Intact plants	Herbivory-basal portion	Herbivory-apical portion
LEI (days)	390	24.78±3.50Aa	20.72±2.93Bb	21.98±3.10Bb
	1,000	22.54±3.18Ab	24.08±3.40Aa	22.54±3.18Aa
LER (cm^2/day^{-1})	390	5.00±0.27Aa	5.14±0.30Ab	4.88±0.21Aa
	1,000	4.49±0.21Ba	6.02±0.33Aa	5.45±0.41Aa
LLS (days)	390	263.44±6.8Ab	274.7±12.69Ab	276.88±6.56Ab
	1,000	329.5±0.88Aa	312.11±3.06Aa	324±0.01Aa

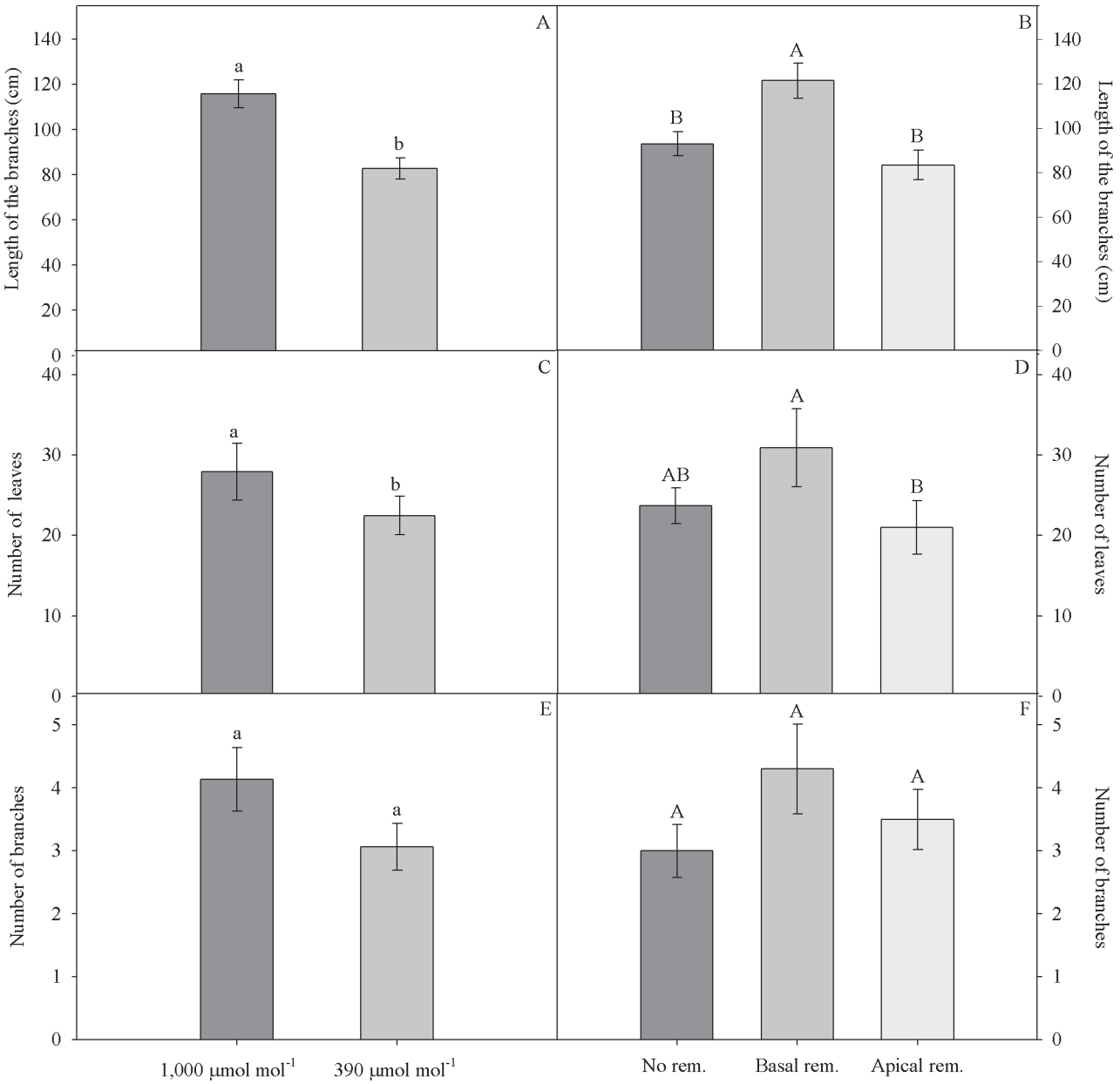

Figure 4. Length of branches (**A-B**), number of leaves (**C-D**), and number of branches (**E-F**) produced by plants of *H. stigonocarpa* cultivated under high [CO_2] (1,000 µmol mol^{-1}) or ambient [CO_2] (390 µmol mol^{-1}) subjected to simulated herbivory. No rem.=no removal; Basal rem.=basal removal; Apical rem.=apical removal. In panels A, C, and E, the values represent the average of fifteen plants; in panels B, D, and F, the values represent the average of ten plants, and the bars indicate means ± the standard error. Uppercase letters compare the levels of herbivory, whereas lowercase letters compare the different [CO_2].

than those growing in ambient [CO_2] ($p<0.05$, simulated herbivory effects × CO_2; Fig.7A). No difference was noted between treatments for leaf tissue removal from the basal or apical parts of the stem for the following variables: root length, SDM, RDM, stem diameter, and root volume ($p>0.05$; Fig. 7D, F, H, Fig. S6 in supplementary material).

Discussion

Simulated herbivory has been intensely utilized to determine likely herbivory effects on plant growth, biomass partitioning, reproduction and morphology (Camargo *et al*. 2015; Pardo *et al*. 2016; Yamawo *et al*. 2018). Besides, simulated herbivory contributes to obtaining physiological responses of plants after damage and the resultant effects of biotic and abiotic interactions (Tscharntke *et al*. 2001; Scott *et al*. 2019).The increase of F0 in the leaves of plants grown in ambient [CO_2] and subjected to simulated herbivory at the basal or apical portion of the stem is possibly related to the existence of damage at the PSII reaction center or to the reduction of energy transfer capacity of excitation from the light collecting system to the reaction center (Baker & Rosenqvist 2004). Thus, the F_v/F_m values were reduced due to the loss of leaf tissue. The results obtained in this study show that foliar injury due to herbivory can also alter the F_v/F_m ratio of Cerrado plants, and thus the efficiency of the conversion of light to chemical energy. However, a high [CO_2] can interfere positively, maintaining the F_v/F_mvalues equal to that in plants without leaf tissue removal, reducing F0, and attenuating the effect of herbivory on *H. stigonocarpa* plants.

Photosynthesis can be promoted to balance herbivory, because of the ability of plants to compensate for the loss of

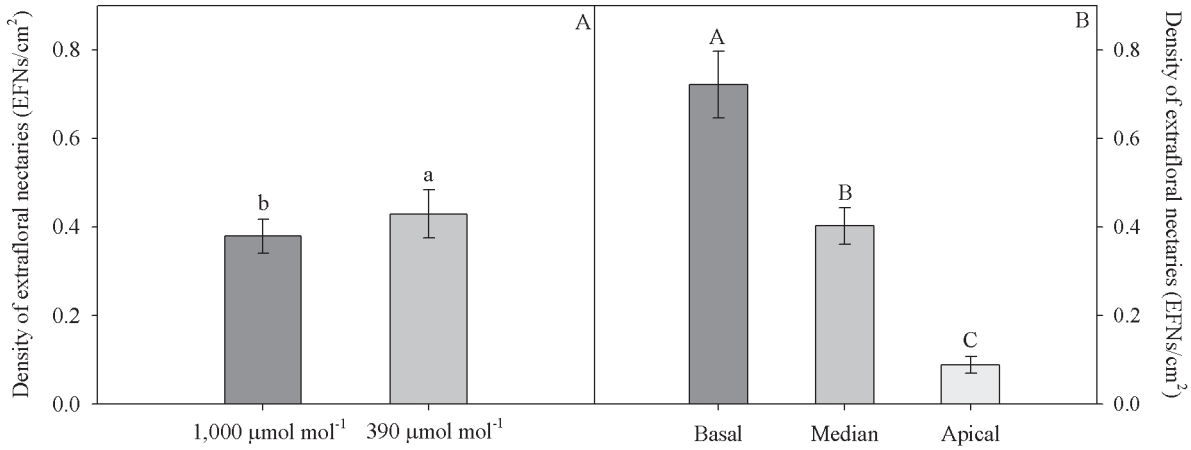

Figure 5. Density of extrafloral nectaries (EFNs/cm^2) in plants of *H. stigonocarpa* grown under high [CO_2] (1,000 µmol mol^{-1}) or ambient [CO_2] (390 µmol mol^{-1}) subjected to simulated herbivory. No rem.=no removal; Basal rem.=basal removal; Apical rem.=apical removal. In panels A-B, the values represent the average of thirty plants, and the bars indicate means ± standard error. The lowercase letters compare the differences between the distinct [CO_2], and the uppercase letters compare the regions (basal, median, and apical) in the leaves.

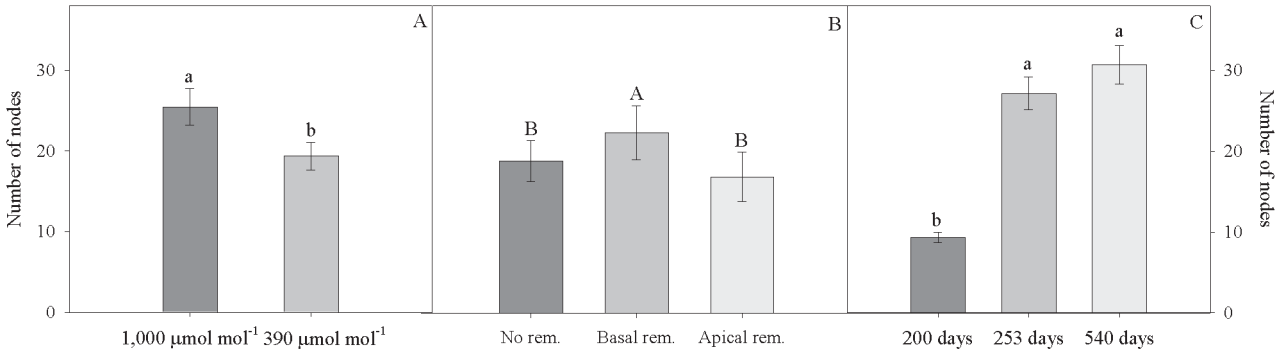

Figure 6. A-C. Number of nodes in plants of *H. stigonocarpa* grown under high [CO_2] (1,000 µmol mol^{-1}) or ambient [CO_2] (390 µmol mol^{-1}) and subjected to simulated herbivory. The number of nodes was evaluated before (200 days of exposure to CO_2), during (253 days of exposure to CO_2), and after (540 days of exposure to CO_2) the simulated herbivory. No rem.=no removal; Basal rem.=basal removal; Apical rem.=apical removal. **A**. Average of fifteen plants. **B**. Average of ten plants. **C**. Average of thirty plants. (A-C) Bars indicate means ± standard error. Lowercase letters compare the different [CO_2] and the period of exposure to high [CO_2], whereas uppercase letters compare the levels of herbivory.

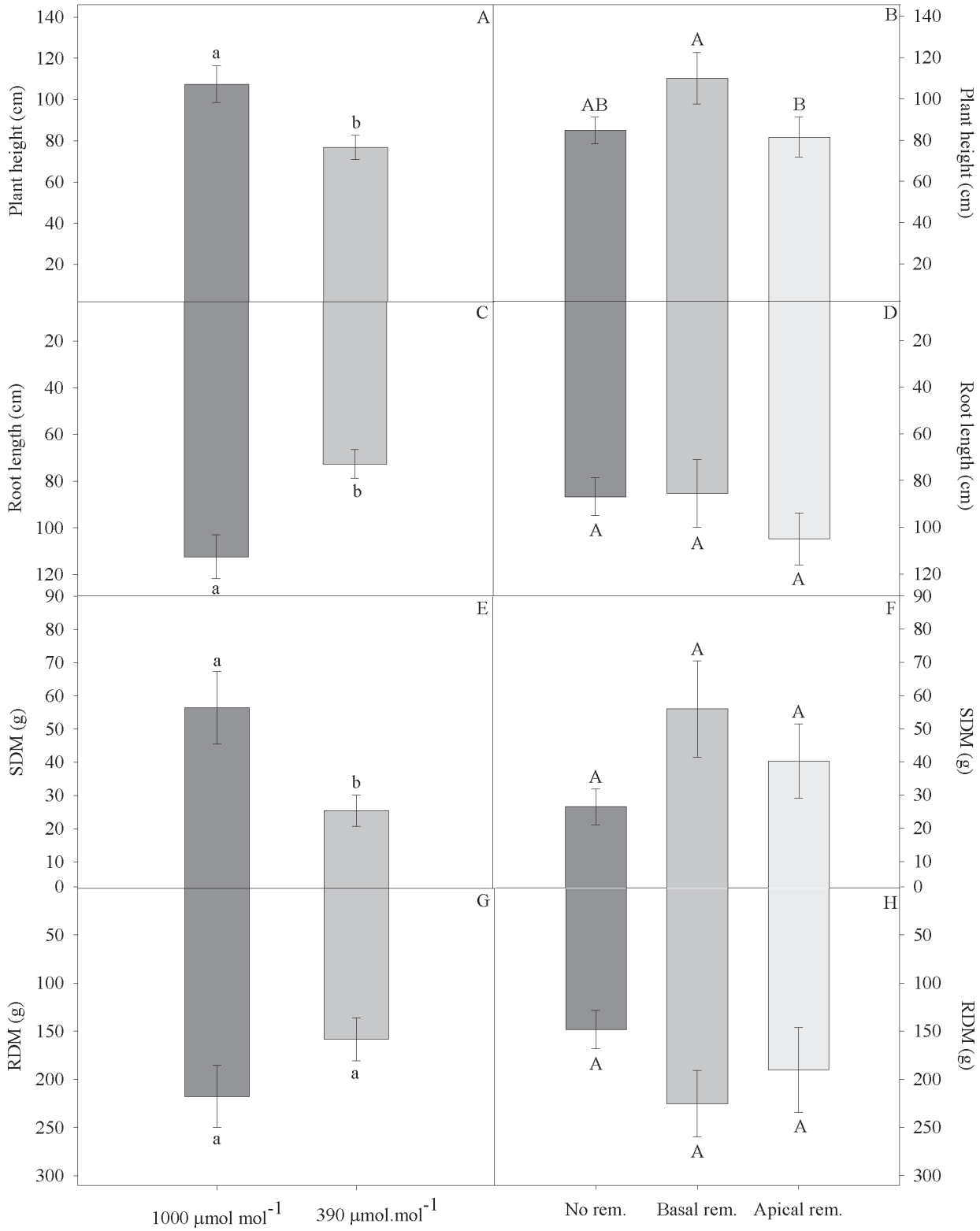

Figure 7. Plant height (**A-B**), root length (**C-D**), stem dry mass (SDM, **E-F**), and root dry mass (RDM, **G-H**) in *H. stigonocarpa* plants grown under high [CO_2] (1,000 µmol mol^{-1}) or ambient [CO_2] (390 µmol mol^{-1}) subjected to simulated herbivory. No rem.=no removal; Basal rem.=basal removal; Apical rem.=apical removal. In panels A, C, E, and G the values represent the average of fifteen plants; in panels B, D, F, and H the values represent the average of ten plants. Bars indicate means ± standard error. Uppercase letters compare the levels of herbivory, whereas lowercase letters compare the different [CO_2].

LA, increasing the photosynthetic activity in the remaining tissue or in the injured tissue (Damascos *et al*. 2005; Zhou *et al*. 2015). A better photochemical performance of PSII was found in *H. stigonocarpa* plants growing under high [CO_2] that had part of their apical leaves removed as a function of ETR and ΦPSII values. A similar increase in the efficiency of light's conversion to chemical energy has been reported for other plant species growing under ambient [CO_2] and exposed to biotic stress caused by insects of different functional groups (Gutsche *et al.* 2009; Halitschke *et al.* 2011). The increase in light conversion observed for the chlorophyll *a* fluorescence parameters in *H. stigonocarpa* growing under high [CO_2] shows its resilience and ability to act against damage caused by simulated herbivory. The simulated herbivory in the apical leaves, which contributes more effectively to the carbon gain of plants (Kitajima *et al*. 2002), induced a compensatory response to reestablish photosynthesis. Conversely, the damage caused to the basal leaves reduces only the potential quantum yield (Chabot & Hicks 1982), and thus is less harmful to the development of plants.

A meta-analysis by Loladze (2014) involving several plant species showed that exposure to high [CO_2] reduces leaf nutrient concentration. The concentration of nutrients analyzed in this study was either equal or greater in the leaves of plants grown under high [CO_2] that appeared after simulated herbivory events, indicating a better absorption and allocation of these nutrients in the leaves of those plants that grew in environments enriched with CO_2. The increase of the nutrient uptake capacity of *H. stigonocarpa* plants growing in environments enriched with CO_2 might be associated with the formation of fine and long roots, evidenced by the greater root length and volume. Under high [CO_2], plants can develop more intensive strategies of soil exploitation, producing smaller ramification angles and a higher density of fine roots (Sá *et al*. 2014; Beidler *et al*. 2015). This adjustment in the root system has many advantages to plants in a future of climate change with a tendency for the dry period to increase (Iversen 2010; Sá *et al.* 2014). Despite the increase in LA in *H. stigonocarpa* subjected to high [CO_2] treatment, no effect of nutrient dilution was noted on the leaves after herbivory events. Thus, despite the expected increase in LA for plants growing in enriched environments, *H. stigonocarpa* was able to absorb more nutrients from the soil and maintain the same concentration of these minerals in the leaves.

Hymenaea stigonocarpa growing under high [CO_2] showed higher bud production because of the better storage capacity of this species in environments enriched with CO_2. Bud production at the end of the dry period, when photosynthesis is lower in Cerrado plants, is expensive. Therefore, the resources required to produce buds need to be acquired from reserves accumulated in the previous growing season. The higher biomass storage capacity associated with plants growing in environments enriched with CO_2 (Ainsworth & Long 2005) might contribute to the higher bud production, which would increase the production of branches and leaves, making the crown more complex. Although high [CO_2] did not remarkably increase the number of branches, an increase in branch length and node and leaf number was observed, especially in plants in which part of the leaf tissue from the basal portion of the stem was removed. This lack of correlation between the number of branches and leaves, previously reported by Souza *et al*. (2016) for *H. stigonocarpa*, differs from that predicted by Ward & Strain (1999). The predominant lateral branching investment in *H. stigonocarpa* might be related to the absence of competition for light under the conditions in which they grew. The increase in height and length of branches observed in *H. stigonocarpa* plants exposed to high [CO_2] did not increase the distance between the nodes, but only the number of nodes. The increase in the number of nodes in *H. stigonocarpa* growing under high [CO_2] is proportional to the higher growth of branches and LA produced, allowing the plants to retain the mass flow moving through the crown (Souza *et al*. 2011).

The higher allocation of biomass directed to aerial structures might be beneficial for the reestablishment of the species after herbivory events (Bond & Midgley 2003), or even fire, environmental filters considerably common in the Cerrado (Bueno *et al*. 2018). However, recovery after the disturbance varies according to the intensity of the damage, since the growth of plants is positively related to the remaining LA. The removal of foliar tissue from the basal portion of *H. stigonocarpa* had less impact on the growth of the species. The basal leaves, unlike the apical ones, contribute less to the total photosynthetic yield of plants (Kitajima *et al*. 2002); however, they can act as important nutrient reservoirs in plants that occur in poor soils, such as in the cerrado *sensu stricto*. Conversely, leaf tissue removal from the apical portion of the stem resulted in lower growth of *H. stigonocarpa*. Thus, the severity of herbivory's effect on plants can be regulated by their ability to compensate for the loss of aerial biomass (Strauss & Agrawal 1999; Schimmel *et al.* 2017; Peterson *et al.* 2017; Zhang *et al.* 2018). The compensatory response exhibited by *H. stigonocarpa* could reduce the severity of the effects of leaf tissue loss from the basal part of the stem in plants under high [CO_2]. However, further experimental studies to test these hypotheses are needed.

The highest leaf longevity found in *H. stigonocarpa* growing under high [CO_2] might be an integral part of resource capture and maintenance strategies (Reich *et al.* 1991; Munné-Bosch & Alegre 2004). Retaining leaves longer in plants ensures more active photosynthetic area for longer periods. In addition, mature leaves act as reservoirs of low-mobility nutrients (Helmisaari 1992).

In *H. stigonocarpa* growing under ambient [CO_2], Paiva & Machado (2006) observed a decrease in the density of EFNs from the basal region to the apical region of the leaves.

The highest density of EFNs in the basal leaf blade region is a valuable protection strategy based on ant recruitment (Delgado *et al*. 2011). The herbivorous attack to the basal leaf blade might decrease the defense ability of plants, because all aspects of leaf development would be impaired. Therefore, in the basal portion of leaves, the cost of herbivory reaches the highest value for plants. Damage in this region might lead to foliar abscission, and any investment in photosynthetic machinery would be lost (Delgado *et al*. 2011). In our study, we found a reduction of EFN density in the leaves of plants growing under high [CO_2]. This reduction was due to the increase in LA. However, the low density of EFNs can be compensated by the rapid expansion of leaves of plants growing under high [CO_2].

The simulated herbivory has some limitations because it cannot sufficiently mimic the damage caused by herbivores in plant tissue. Plants can activate specific responses by recognizing physical and chemical signals left by herbivores during feeding and oviposition. In addition, the amount of tissue removed per day and the frequency of such removals by insects are also difficult to predict. However, simulated herbivory is a very useful method, even with its limitations, since it helps in understanding the effects of herbivory in a simplified way. This study contributes to the understanding of the strategies developed by *H. stigonocarpa* in relation to likely new climatic conditions such as high atmospheric [CO_2]. It is important to emphasize that more realistic future scenarios of [CO_2] levels are between 600 and 800 ppm. However, as leaf photosynthesis in C3 plants only saturates at about 1000 ppm of atmospheric [CO_2], our study is important to test C3 plant response under a saturating CO_2 condition. Verifying the isolated effect of high [CO_2] and simulated herbivory, as well as the interaction of these two factors, on the development of an endemic Cerrado species was possible. In a future where the effect of temperature and [CO_2] on herbivore development is uncertain and variable, predicting how plants will be able to cope with the possible increase in herbivory pressure is important. In this study, we suggest that *H. stigonocarpa* could benefit from the greater availability of CO_2 in the atmosphere against herbivory events. However, along with high [CO_2] more events of drought and increased air temperatures are expected in natural ecosystems, like Cerrado. In previous studies with cerrado species, Souza *et al.* (2016) pointed out that woody plants growing under elevated [CO_2] and soil water deficit showed improved growth. Also, Souza *et al.* (2019) showed an increase in water use efficiency in *Lafoensia pacari* juvelines under elevated [CO_2] and soil water deficit. Therefore, we expected that even with synergism between high [CO_2] and drought events, cerrado plants would be benefited in the future. However, it is important to point out that elevated temperatures could impaired the potential beneficial responses that elevated [CO_2] could generate in cerrado plant species. *Hymenaea stigonocarpa* growing under high [CO_2] directs the allocation of resources to the aerial and underground structures and can respond rapidly after the loss of leaf tissue. Considering the parameters evaluated, the effect of simulated herbivory on *H. stigonocarpa* might be attenuated when it grows under high [CO_2].

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