



Effects of landscape disturbance on seed germination of *Enterolobium contortisiliquum* (Fabaceae) in Brazilian seasonally tropical dry forest: Are seeds a sensitive biomarker of environmental stress?

Isabela Botelho Cardoso^a, Marina do Vale Beirão^a, Pablo Cuevas-Reyes^b,
Yurixhi Maldonado-López^c, Joan Sebastian Aguilar-Peralta^b, Patrícia de Abreu Moreira^{a,*}

^a Programa de Pós-Graduação em Ecologia de Biomas Tropicais, Departamento de Evolução, Biodiversidade e Meio Ambiente, Universidade Federal de Ouro Preto – UFOP, Ouro Preto, Minas Gerais, Brazil

^b Laboratorio de Ecología de Interacciones Bióticas, Universidad Michoacana de San Nicolás de Hidalgo, Francisco J. Mújica S/N Col. Felicidades del Río, Ciudad Universitaria, Morelia C.P. 58030, Michoacán, Mexico

^c Cátedras CONACYT-Instituto de Investigaciones sobre los Recursos Naturales, Universidad Michoacana de San Nicolás de Hidalgo, Av. San Juanito Itzicuaru S/N, Nueva Esperanza, Morelia C.P. 58030, Michoacán, Mexico

ARTICLE INFO

Keywords:

Seed fluctuating asymmetry
Land-use change
Seed germination
Tropical dry forest

ABSTRACT

Habitat disturbance of native forests is currently the major threat to biodiversity conservation. Plant species can be negatively affected as a result of landscape conversion; thus, it is essential to detect the potential effects of environmental stress on plant performance using reliable bioindicators of quick, cheap diagnosis. Fluctuating asymmetry (FA) describes the degree of the random differences in shape and size among two sides of a bilateral trait of the organisms and reflects the developmental instability of an organism, so we can use it to predict the effects on plant fitness. We evaluated the land-use change stress on seed FA levels, seed germination, and the subsequent seedling development of seed produced by trees that occur in an anthropized and a preserved area of seasonally tropical dry forests (STDF) in Brazil. We sampled 10 ten reproductive trees of *Enterolobium contortisiliquum* of each landscape condition, and then, 100 seeds per each tree were collected. Additionally, 50 seeds were randomly selected for each tree to determine plant fitness. Despite, we found no true FA in the community, the anthropized area had a lower seed nutritional quality and percentage of germination, and higher germination time of seeds produced than in preserved areas. The dry weight of the aerial part of seedlings did not differ between areas. However, root dry weight and total dry weight were higher in seedlings developed from seeds of anthropized than in preserved areas. Our findings show that seeds of *E. contortisiliquum* are not a sensitive biomarker to detect full stress conditions of habitat disturbance. However, we demonstrate that habitat disturbance negatively affects plant fitness, decreasing germination percentage, and nutritional quality, and increasing germination time. This fact has potential consequences on recruitment, establishment and survival of *E. contortisiliquum* in regenerating areas of STDF's in Brazil.

1. Introduction

Ecosystems have been altered as a result of different anthropogenic activities that differ in frequency, duration and intensity (Chazdon, 2003). The most common disturbances are caused by deforestation, tourism, urbanization and the conversion of mature forests to agricultural fields and livestock soils (Chazdon and Guariguata, 2016), that induce changes in both abiotic (i.e., temperature levels, toxic gas

emissions, hydric pollution and soil fertility) and biotic factors such as the structure and composition of natural communities and also plant performance (Aguilar et al., 2006, 2008; Aizen and Feinsinger, 1994; Ashworth and Martí, 2011; Cascante et al., 2002; Cunningham, 2000; Ghazoul, 2005). For example, it has been demonstrated that landscape conversion, as well the reduction and isolation of fragments as a result of human activities, decrease species richness of birds, mammals, insects and plants (Haddad et al., 2015), and affect the patterns of seed

* Corresponding author at: Universidade Federal de Ouro Preto (UFOP), Instituto de Ciências Exatas e Biológicas (ICEB), Programa de Pós-Graduação em Ecologia de Biomas Tropicais, Campus Morro do Cruzeiro, 35400.000 Ouro Preto, Minas Gerais, Brazil.

E-mail address: patricia.moreira@ufop.edu.br (P. de Abreu Moreira).

<https://doi.org/10.1016/j.ecolind.2021.107451>

Received 14 May 2020; Received in revised form 8 January 2021; Accepted 21 January 2021

Available online 13 February 2021

1470-160X/© 2021 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

dispersal, germination and/or recruitment of plants (Kolb, 2008; Rey et al., 2017; Yamashita et al., 2009). Species respond negatively to land-use change (Ashworth and Martí, 2011; Tucker-Lima et al., 2018), so it is a great challenge to identify stress-mediated effects on organisms before changes in fitness itself is evident, and affect their life-history parameters (Clarke, 1985).

The abiotic and biotic changes caused by land cover change represent to plants a set of stressing factors that can lead to morphological, physiological, cellular and chemical adjustments (Cuevas-Reyes et al., 2011a). However, in some cases, plants are incapable of performing these regulatory processes, resulting in developmental instability of different organs, such as leaves and flowers (Cornelissen and Stiling, 2010; Cuevas-Reyes et al., 2011a, 2011b; Santos et al., 2017). Thus, the assessment of developmental instability may be used to detect stressful conditions and predict subsequent changes in fitness (Clarke, 1985).

Developmental instability is the failure of a particular genotype to generate the same phenotype under specific genetic and environmental conditions and is referred to as an indicator of environmental stress (Cuevas-Reyes et al., 2013; Maldonado-López et al., 2019; Møller and Swaddle, 1997). It has been suggested that reproductive success is higher in developmentally stable individuals. Thus, these individuals had a higher growth rate, higher fecundity, and better survival than developmentally unstable individuals (Møller, 1997).

An excellent used tool to measure the developmental instability is fluctuating asymmetry (FA), which describes the degree of the random differences in shape and size among two sides of a bilateral trait of the organisms (Palmer and Strobeck, 1986; Clarke, 1992; Palmer, 1994). Thus, it represents the levels of environmental stress that an individual experiences and which consequently affects its fitness and offspring (Freeman et al., 1993; Galloway, 2001; Mal et al., 2002; Santos et al., 2017; Smith, 2016).

Some studies have shown that increased FA levels are associated with stressful conditions as electromagnetic field emissions, pollution, deficiencies or excess of nutrients, inbreeding, hybridization, the incidence of parasites and herbivores, and agropastoral activities (Freeman et al., 1999; Cornelissen and Stiling, 2010; Cuevas-Reyes et al., 2011a, 2011b; Fernandes et al., 2016; Costa et al., 2017). However, most studies of FA in plants have focused on demonstrating that FA is a sensitive monitor to detect stress in plants using leaf measurements (Cuevas-Reyes et al., 2011a, 2011b, 2013, 2018). There is little information of the possible relationship between FA and plant fitness (but see Andalo et al., 2000; Siikamäki et al., 2002; Botto-Mahan et al., 2004 for herbs studies), even less in seeds and germination success in tropical trees of disturbed landscape in seasonally dry forests.

In the Neotropical region the seasonally tropical dry forests (STDF's) are distributed, across Latin America, including southeast of Brazil and Chaquenián region in Argentina and Paraguay (Pennington et al., 2000), and throughout the Caribbean (Linares-Palomino et al., 2001). Unfortunately, STDF's have been one of the most degraded, altered and transformed ecosystems (Sánchez-Azofeifa et al., 2005, 2009), with a deforestation rate of 12% in Latin America from 1980 and 2000 (Miles et al., 2006). STDF occupy around 268,875 km² in South America, of which, 17% occurs in Brazil, mostly in the northeast and the north of the state of Minas Gerais (Werneck, 2011), and 3.9% is protected (Portillo-Quintero and Sánchez-Azofeifa, 2010). Moreover, conversion of these forests to pastureland and agriculture has increased recently (Espírito-Santo et al., 2009) generating a mosaic of forest fragments with different succession stages, secondary forest cover, agricultural and livestock fields and conserved forests (Chazdon and Guariguata, 2016; Miles et al., 2006; Portillo-Quintero and Sánchez-Azofeifa, 2010) with a potential for biodiversity storage and maintenance ecosystem services (Arroyo-Rodríguez et al., 2015).

In this sense, we determined the development and seeds germination of *Enterolobium contortisiliquum* (Vell.) Morong (Fabaceae, Mimosoid Clade) trees produced in the human-modified area and preserved STDF's landscape. We tested the following hypotheses: i) the yield of produced

seeds by trees from STDF areas will be better than produced seeds by trees from anthropized areas. Hence, seeds from the preserved STDF area will have fewer FA levels, higher weight, faster and higher germination, and better nutritional quality in comparison to seeds from the anthropized area. ii) seedlings of seeds from preserved STDF area will have higher dry weight in comparison to seedlings from the anthropized area. iii) symmetric seeds have a higher germination rate than asymmetric seeds.

2. Materials and methods

2.1. Study area

This study was conducted within the Lapa Grande State Park (LGSP) and surrounding areas in the north of Minas Gerais state, Brazil. The region is characterized by dry winters and rainy summers (Aw climate according to Köppem classification), with a mean temperature between 21 °C and 25 °C. The mean annual rainfall varies between 700 and 1200 mm, distributed in six months (Antunes, 1994). The predominant vegetation is STDFs, characterized by the dominance of deciduous trees (at least 50%) and a high abundance of xerophytic species in the undergrowth. The tree stratum has a height that varies between 15 and 25 m, forming a slightly continuous canopy (Gentry, 1995; Pedralli, 1997; Sánchez-Azofeifa et al., 2005), that is characteristic of sites with high water deficiency and outcrop soils in limestone (Fernandes, 2002). LGSP was created in 2006 in a protected area, considered as preserved in this study owing of the local biodiversity conservation, and the immediately surrounding areas of LGSP was considered as anthropized area due to the historical of human activities such as agricultural and livestock, drifting in an altered matrix with the presence of remnant trees in pastures. Besides, the anthropized area has less canopy cover, more sunlight, less richness, and abundance of species in comparison to areas within the park.

2.2. Study species

Enterolobium contortisiliquum (Vell.) Morong is a dominant tree characteristic of STDF's (Gentry, 1995; Mayle, 2004; Särkinen et al., 2011) of the Fabaceae family (LPWG, 2017). It is a native tree distributed from South America, over the "Pleistocenic Arc" (Prado and Gibbs, 1993, Prado, 2000). Its flowering occurs in a short time (September–November), when tree is leafless, and its fruits ripen between June and July (Backes and Irgang, 2009). Fruits are smooth, bright, indehiscent, and dark brown, while seeds are hard, oval and brown (Barretto and Ferreira, 2011). The development of fruits and seeds occurs during almost a year and their dispersion is during the dry season, just before the next flowering. In this context, ripe fruits and seeds correspond to the pollination of last year. As *E. contortisiliquum* is typical tree of STDF's that occurs in conserved areas inside the LGSP and in disturbed areas outside LGSP, and seeds spend almost a one year developing on the tree, this is a good study model to evaluate the effects of habitat disturbance and its consequences in progeny.

2.3. Sampling design

We randomly selected ten reproductive trees from the anthropized area and other ten of the preserved STDF landscape. We collected 20–25 fruits for each tree and seeds were separated in aborted, pre-dispersal predated and undamaged ones. After that, 100 undamaged seeds were selected of each tree, and then all 2000 seeds were individually identified, weighed with a high precision scale (BEL Photonics LW230i) and photographed with a Canon DS126231 camera.

2.4. Fluctuating asymmetry measurements

We used the seed funiculus as a reference point in order to measure

the longitudinal plane. In each digital image, 11 landmarks and two additional landmarks were placed as reference of size (Fig. 1). Landmarks 1 and 11 correspond to the beginning and end of the longitudinal plane. At the middle of the longitudinal plane, the distances of the right (D) and left (L) sides were measured from exactly middle of it (landmarks 5, 6 and 7). Besides, the distances of D and L sides were measured in the upper (landmarks 2, 3 and 4) and lower (landmarks 8, 9 and 10) middle of each seed. Fluctuating asymmetry (FA) value was obtained by dividing the absolute value of the difference between right and left sides (D-L) by the average distance (D-L/2) (Palmer and Strobeck, 1986). The same measurements were also made at the upper and lower halves from each seed, obtaining three FA values in total for each seed (Fig. 1). These three values were averaged to perform statistical analyzes. All distances were measured with digital image using the software ImageJ (Schneider et al., 2012).

True FA patterns are identified when D-L values have a normal distribution, reflecting deviations in characters with bilateral symmetry (Palmer and Strobeck, 1986). To confirm the FA presence, normality tests (Lilliefors) and *t*-test were performed for all samples. Besides, 30% of seed samples were randomly selected to make repeatability measurements, obtaining new FA values, and then, a simple correlation analysis of these values was made with the first obtained FA values. The correlation was 83%, which guarantees the effectiveness of the measures.

2.5. Seed germination and seedling development

In each landscape condition and for each tree, 50 seeds were randomly selected to determine the germination percentage, being 1000 seeds in total (500 per each landscape condition). Seeds were put in a sodium hypochlorite solution at 2% for 2 min to disinfect them. Subsequently, they were placed in polystyrene foam trays with individualized divisions that contained vermiculite as a substrate. Nystatin at 10% was applied as a fungicide, and then, seeds were placed in a BOD (Biochemical Oxygen Demand) germination chamber (Solab sl-224) with a controlled temperature between 26 and 30 °C and with 12-hours photoperiod. Irrigation was carried out every 48 h with distilled water. The seeds were observed every 24 h during 30 days, those with developed radicle were considered as germinated seeds. Once that cotyledons fell, the seedlings were removed from the tray and stored in a freezer in paper bags. After that, these seedlings were dehydrated at 60 °C for 48 h in an oven to evaluate the dry weight of the shoot (aerial part) and root (underground part), and total dry weight (Souza and Fagundes, 2004).

2.6. Nutritional quality of seeds

To determine the nutritional quality of seeds, we randomly selected 30 seeds for each landscape condition (N = 60). We use three replicas of each group of seeds to carry out the analyzes of nitrogen (N), phosphorus (P), sodium (Na), calcium (Ca), magnesium (Mg), sulfur (S), copper (Cu), iron (Fe), zinc (Zn), manganese (Mn), boron (B) and carbon (C) in the Laboratory of Soil Analysis, Plant Tissue and Fertilizer (Laboratório de Análise de Solo, Tecido Vegetal e Fertilizante/UFV).

The nitroperchloric digestion was conducted. The sample ones were inserted in a drying oven for 72 h at a temperature between 68 and 72 °C. After this, the samples were weighed and ground. The grind was to homogenize the samples. The digestion to determinate the P, Na, Ca, Mg, S, Cu, Fe, Zn, Mn, and B was by nitric-perchloric acid. A 0.5 g of the sampling was used, and to this added 10 mL of nitric acid and perchloric acid (4:1 proportion) in a fume hood. The mixture was heated to 80 °C, and the temperature was increasing to 200 °C. When the extract became crystals, the mixture was suspended from the heat. To these crystals is added deionized water until the volume is 25 mL. The minerals were quantified using inductively coupled plasma optical emission spectrometry (ICP-OES; Perkin Elmer Optima 8300 DV) (Sarruge and Haag, 1974).

For nitrogen digestion, we used the Kjeldahl method with sulphuric digestion. A 0.2 g of the sampling was added to sulphuric acid. Then, the temperature was raised from 120 °C to 350 °C. When the extract becomes a crystal, the temperature is lower. To this crystal is added deionized water until the volume 50 mL is completed. The crystal is added to 25 mL of NaOH (Kjeldahl digestion). In this reaction, NH₃ is released with the water vapor to a 50 mL Erlenmeyer with 20 mL of a boric indicator. The boric indicator provides an H⁺ to recompose the NH₄⁺, which is dosed with a 1 N HCl solution. For carbon analysis, the sample ones were dried at 105 °C, weighed (initial weight), and then they were inserted in a muffle (Linn Elektro Therm) for 1 h at 200 °C. After, the samples remained in the muffle for 1 h at 400 °C, and then, for 3 h at 550 °C. Afterwards, the samples were weighed (final weight) and the difference between the initial and the final weight corresponds to the organic carbon content.

2.7. Statistic analyses

To evaluate the effect of landscape condition (preserved STDF vs. anthropized areas) on the development and germination of *E. contortisiliquum* seeds, seven generalized linear mixed models (GLMM) were performed using the nlme package (Pinheiro et al. 2020). The models considered landscape condition as a independent variable, and FA of seed, seed weight, seed germination percentage, germination time of seeds, shoot, root, and total dry weight were used as response

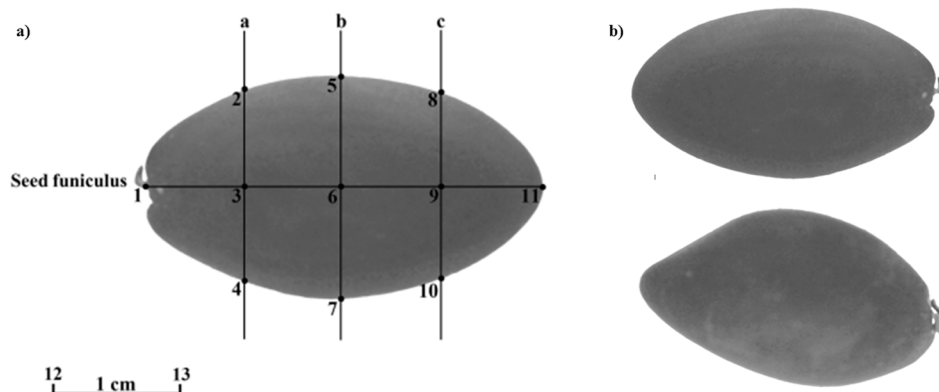


Fig. 1. Digital image of *Enterolobium contortisiliquum* seed showing the 11 morphological landmarks along the seed. Note the seed funiculus as a reference point to measure the longitudinal plane. Two additional landmarks (12 and 13) were added on a reference ruler as scale. Letters represent the measurements used to estimate seed FA (a). Representation of different *E. contortisiliquum* seed shapes (b).

variables (R Core Team, 2017). A pseudoreplication was assumed, considering the tree as a random effect since 100 seeds were selected from the parent tree. All this analysis were performed in R (R Core Team, 2017).

To evaluate the differences in seeds nutritional quality between landscape conditions (preserved and anthropized areas), we performed a principal component analysis (PCA) and obtained a PCA biplot that shows the association of realized replicas for each group of seed with the different nutrients (SAS, 2000; Stokes et al., 2000).

3. Results

In this study we did not find true FA pattern, the distribution of the difference between the right side and the left was not normal (Lilliefors, $p < 0.001$) and the true difference in means of the right side and the left one was not equal to 0 ($t = -1.969$; $p = 0.049$). So, we could not test the asymmetry between the landscape condition or whether asymmetry affects seed germination. Also, we did not find significant differences in the seed weight of *Enterolobium contortisiliquum* seeds between the anthropized and preserved STDF ($F = 2.586$; $P = 0.125$). The average value of seed weight was $0.357 (\pm 0.102)$ g.

The produced seeds of *E. contortisiliquum* by trees located in preserved STDF area germinated more ($25\% \pm 0.43$ SD) than seeds from human-disturbed area ($14\% \pm 0.35$ SD) ($F = 18.0$; $P < 0.0001$) (Fig. 2). Additionally, germination time varied among landscape conditions ($P < 0.0001$) (Fig. 3). Seeds from trees that occur in the preserved STDF area germinated 1.9 times faster than seeds from trees located in the anthropized area. Also, seeds from preserved STDF area germinated on average 25 days (± 8.7 SD), while seeds from the anthropized area in 29 days (± 2.0 SD).

The dry weight of the aerial part of seedlings did not differ between preserved STDF and anthropized area ($F = 3.9$, $P > 0.05$). However, the root dry weight vary among study areas ($F = 46.5$; $P < 0.0001$), as well as total dry weight ($F = 8.5$; $P < 0.004$). Seedlings resulting from the germination of tree seeds located in the anthropized area showed a higher root dry weight ($0.02 \text{ g} \pm 0.006$ SD) in comparison to seedlings from located trees in the preserved STDF area ($0.01 \text{ g} \pm 0.01$) (Fig. 4). The total dry weight was higher in seedlings from the anthropized area ($0.11 \text{ g} \pm 0.03$ SD) than in seedlings from the preserved STDF area ($0.07 \text{ g} \pm 0.02$ SD) (Fig. 5).

According to the PCA, an 80.9% of the variation in the nutritional quality of seeds from anthropized and preserved areas was explained by the two principal components (Fig. 6). The PC1 explained better our results by representing a 58.1% of the variance. Thus, the first PCA axis associated the three replicas of seeds group from anthropized areas with Zn, Fe, Ca, Mn, Mg and B on the right side, and the three replicas of seeds group from preserved area with P, N, Cu, S, K, and C on the left side. The second PCA axis explained two replicas of anthropized area with two replicas of preserved area and B, Cu, and S in the lower side, and one

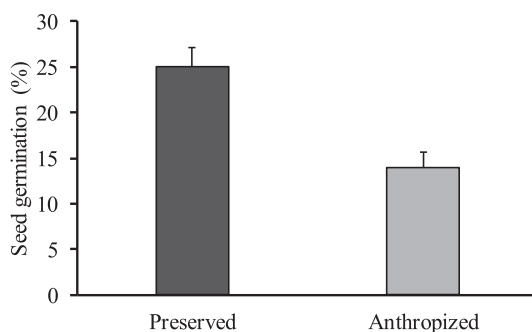


Fig. 2. Differences in seed germination percentage of *Enterolobium contortisiliquum* between preserved and anthropized areas. The bars represent the standard error.

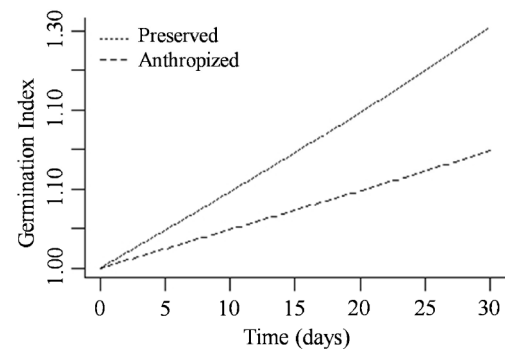


Fig. 3. Germination time of *Enterolobium contortisiliquum* seeds in preserved and anthropized areas.

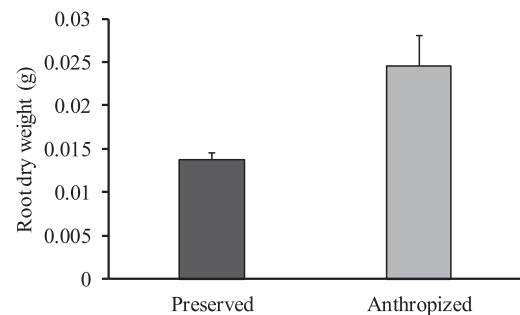


Fig. 4. Root dry weight of seedlings from *Enterolobium contortisiliquum* seeds preserved and anthropized areas.

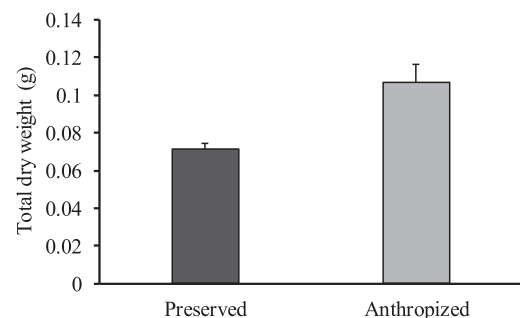


Fig. 5. Comparison of total dry weight of seedlings from *Enterolobium contortisiliquum* between preserved and anthropized areas.

replica of anthropized area with one replica of preserved area and C, K, Mg, Mn, P, N, Ca, Fe, Zn in the upper side.

4. Discussion

Our results of fluctuating asymmetry and seed weight suggest that land-use change did not negatively affect the seed development of *Enterolobium contortisiliquum*. However, STDF conversion into pasture and agriculture had adverse effects on seed germination and germination time. Besides, there is a variation in seedlings as well as the nutritional quality of seeds from distinct landscapes.

Although FA is considered one of the main indicators to evaluate environmental stress in plants (Cuevas-Reyes et al. 2018), our results do not show FA in seeds of *E. contortisiliquum*. Our prediction was based on the idea that the anthropized area is hotter and drier and, consequently, present stressful conditions to plants compared to the preserved area. Environmental conditions of the anthropized area (i.e., high incidence of radiation and extremes of temperature and humidity) may not be

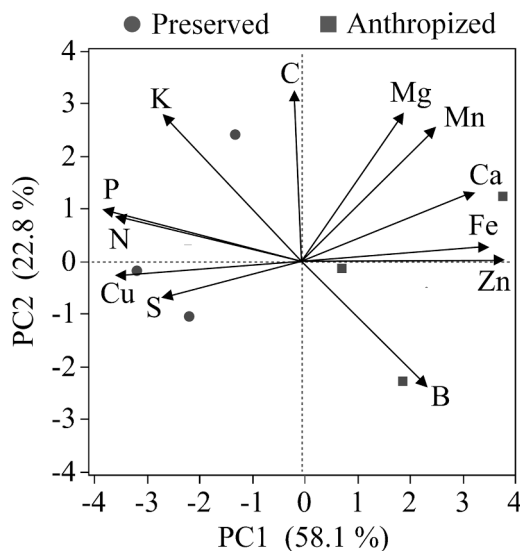


Fig. 6. Results of PCA biplot that shows the variation in seeds nutritional quality between groups of seeds of preserved and anthropized areas, besides of the association of seeds groups with different nutrients. The circles represent the three realized replicas for the seeds group of preserved area and squares the replicas of the seeds group of anthropized area.

stressors for *E. contortisiliquum*. A possible explanation is that STDF's plant species have optimal strategies to cope with drought through better efficiency in the use of water, allowing them to have physiological, anatomical and morphological adjustments at high temperatures and less water availability (Lohbeck et al., 2015; Pineda-García et al., 2013). An alternative explanation is our inappropriate choice of characters to measure FA, and the lack of knowledge in the use of seeds as bio-monitors of stressful conditions for plants (Møller, 1993; Palmer, 1994).

In the seed development phase, the formed products by photosynthesis of parental trees are allocated to seed growing and are used to create new cells, tissues and endosperm (Peske et al., 2012). It is presumable that the formation of photosynthesis products, as well as nutrient transport, were not affected by the differences in the abiotic and biotic conditions between anthropized and preserved STDF areas. This fact could be explained due to the symbiotic association that Fabaceae family has with nitrogen-fixing bacteria in its roots (Barretto and Ferreira, 2011; Lima et al., 2010; Oliveira, 1999), which could contribute to the acquisition and storage of nutrients from the soil to seeds. As a result, *E. contortisiliquum* seeds from anthropized and preserved STDF areas have a similar development. However, several studies have not shown changes in the FA of plants under stressful conditions, such as air pollution, nutrient deficiency or herbivore attack (Bañuelos et al., 2004; Botto-Mahan et al., 2004; Costa et al., 2013; Díaz et al., 2004; Sinclair and Hoffmann, 2003). For example, in flowers of *Iris germanica* (Iridaceae) from urban areas did not show differences in FA levels compared to flowers located in uncontaminated environments (Klisarić et al., 2016).

Although we did not detect the effects of habitat disturbance on fluctuating asymmetry levels in seeds, we detected a direct effect on seed germination. Seeds produced by trees from the preserved STDF area require less time for germination than seeds produced by trees from the anthropized area. Faster germination is essential to avoid seed viability loss due to deterioration and microorganisms attack (Debeaujon et al., 2000; Gharoobi, 2011; Walters, 1998). Besides, the produced seeds by trees from the preserved STDF area had a higher percentage of germination in comparison to produced seeds by trees from the anthropized area. Anthropogenic disturbance can affect the parent tree during seed development, and its effects are more evident after germination during

the initial growth of seedlings (Schmid and Dolt, 1994). This fact is because the environment can act as a filter and directly affect the offspring phenotype through parent supply (Donohue and Schmitt, 1998; Roach and Wulff, 1987). Because both areas have different environmental conditions, trees can support different types of stress, in this sense, anthropized areas have less vegetation cover, and hence, higher water evaporation due to soil exposure to a higher solar radiation and wind intensity (Mass, 1995; Frishkoff et al., 2015; Senior et al., 2017). These suffered tensions by the parental plants during the seed development, influence negatively the germination process (Guterman, 2000). Water deficit and high temperature induce fast deterioration of seeds, changing properties of parent tissue around them, and affecting seed germination (Guterman, 2000). The *E. contortisiliquum* fruit development, and consequently, seeds, occurs over a long time, so that produced seeds by trees from the anthropized area are negatively affected by these factors, which result in a lower percentage of seed germination.

Seed nutritional quality also depends on the availability of resources from the soil in which the parental trees are growing (Rengel and Graham, 1995). Therefore, deficiencies in soil nutrients exert a negative effect on the growth and development of parental trees, generating a reduction in seed nutritional quality and, hence, less vigor and viability (Rengel and Graham, 1995; White and Veneklaas, 2012). Higher the nitrogen availability, seeds germinated faster and are able to absorb water faster, improving more seed vigor (Hara and Toriyama, 1998).

On the other hand, phosphorous and copper content of seed contribute with the initial development, increasing the initial establishment and growth (White and Veneklaas, 2012), while zinc content favors the vegetative growth (Rengel and Graham, 1995). In our study, we found lower content of nitrogen, phosphorous, and copper in seeds from maternal trees located in the anthropized area, which could explain the lower germination percentages and the longer time to germinate registered in this anthropized area in comparison to seeds from preserved STDF area. Besides, the lesser nitrogen content and higher zinc content in seeds from anthropized areas may have contributed to the higher root dry weight. This fact is because when nitrogen content is low, this nutrient is mostly allocated to root development in order to promote higher growth, explore the soil and obtain nutrients (Naegle et al., 2005). Moreover, this higher root dry weight could have been driven at the same time by the higher zinc content that we found in seeds from the anthropized area, which nutrient promotes vegetative growth (Rengel and Graham, 1995).

A general assumption is that individuals more asymmetric have a lower growth rate and fecundity and little survival in comparison with symmetric individuals (Clarke, 1985; Møller, 1997). However, we did not find any support for a negative relationship between FA and fitness components, as we did not detect instability in seed development. As measures of plant fitness, we used seed weight, germination percentage and time, and seedlings vigor, components of fecundity and recruitment, and further plant vigor (Siikamäki et al., 2002). Nonetheless, although we have noticed a negative effect on plant fitness according to landscape condition these harmful consequences of land-use change for *E. contortisiliquum* were not related to FA. So, seeds fluctuating asymmetry cannot be used as an indicator of landscape disturbance in *E. contortisiliquum*. No relationship between FA and fitness was detected at individual level in *Lotus corniculatus* neither in *Echinopartum horridum* (Andalo et al., 2000; Komac and Alados, 2012).

In conclusion, our results show that the fluctuating asymmetry of seeds is not an effective indicator of environmental stress in *Enterolobium contortisiliquum* in anthropized areas. Our findings illustrate that even though seedlings from seeds produced by trees from the anthropized area had higher total dry weight and root dry weight, we detected negative effects on their seed germination and germination time. Furthermore, it has potential consequences on the recruitment, establishment, and survival of *E. contortisiliquum* individuals in regenerating areas of STDF's.

CRedit authorship contribution statement

Isabela Botelho Cardoso: Data curation, Writing - original draft. **Marina Vale Beirão:** Methodology, Formal analysis. **Pablo Cuevas-Reyes:** Formal analysis, Writing - review & editing. **Yurixhi Maldonado-López:** Formal analysis, Writing - review & editing. **Joan Sebastian Aguilar-Peralta:** Formal analysis, Writing - review & editing. **Patrícia Abreu Moreira:** Conceptualization, Methodology, Resources, Visualization, Writing - review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank the Instituto Estadual de Florestas (IEF) for permission to access the study area (permission UC 017/10). We also thank the logistical support provided by Universidade Estadual de Montes Claros (UNIMONTES). We gratefully acknowledge Universidade Federal de Ouro Preto (UFOP) and Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG) for a research scholarship to IB Cardoso, and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the PNPd fellowship of MV Beirão. Pablo Cuevas-Reyes thanks Coordinación de la Investigación Científica for their generous support.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

- Andalo, C., Bazin, A., Shykoff, J.A., 2000. Is there a genetic basis for fluctuating asymmetry and does it predict fitness in the plant *Lotus corniculatus* grown in different environmental conditions? *Int. J. Plant Sci.* 161, 213–220.
- Aguilar, R., Ashworth, L., Galetto, L., Aizen, M.A., 2006. Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. *Ecol. Lett.* 9, 968–980.
- Aguilar, R., Quesada, M., Ashworth, L., Herrerias-Diego, Y., Lobo, J., 2008. Genetic consequences of habitat fragmentation in plant populations: susceptible signals in plant traits and methodological approaches. *Mol. Ecol.* 17, 5177–5188.
- Aizen, M.A., Feinsinger, P., 1994. Habitat fragmentation, native insect pollinators, and feral honey bees in Argentine “Chaco Serrano”. *Ecol. Appl.* 7, 378–392.
- Antunes, F.Z., 1994. Caracterização Climática-Caatinga do Estado de Minas Gerais. *Informe Agropecuário* 17, 15–19.
- Ashworth, L., Martí, M., 2011. Forest fragmentation and seed germination of native species from the Chaco Serrano Forest. *Biotropica* 43, 496–503.
- Arroyo-Rodríguez, V., Andresen, E., Bravo, S.P., Stevenson, P.R., 2015. Seed dispersal by holwer monkeys: current knowledge, conservation implications, and future directions. In: Kowalewski, M., Garber, P.A., Cortés-Ortiz, L., Urbani, B., Youlatos, D. (Eds.), *Howler MONKEYS: Behavior, Ecology, and Conservation*. Springer, New York, pp. 111–139.
- Backes, P., Irgang, B., 2009. *Árvores do Sul: guia de identificação & interesse ecológico: as principais espécies nativas sul-brasileiras*, second ed. Paisagem do Sul, Porto Alegre.
- Bañuelos, M.J., Sierra, M., Obeso, J.R., 2004. Sex, secondary compounds and asymmetry. Effects on plant herbivore interaction in a dioecious shrub. *Acta Oecol.* 25, 151–157.
- Barretto, S.S.B., Ferreira, R.A., 2011. Aspectos morfológicos de frutos, sementes, plântulas e mudas de leguminosae mimosoideae: *Anadenanthera colubrina* (vellozo) Brenan e *Enterolobium contortisiliquum* (vellozo) morong. *Rev. Bras. Sementes* 33, 223–232.
- Botto-Mahan, C., Pohl, N., Medel, R., 2004. Nectar guide fluctuating asymmetry does not relate to female fitness in *Mimulus luteus*. *Plant Ecol.* 174, 347–352.
- Cascante, A., Quesada, M., Lobo, J.A., Fuchs, E.A., 2002. Effects of dry forest fragmentation on the reproductive success and genetic structure of the tree *Samanea saman*. *Conserv. Biol.* 16, 137–147.
- Chazdon, R.L., 2003. Tropical forest recovery: legacies of human impact and natural disturbances. *Perspect. Plant Ecol. Evol. Syst.* 6, 51–71.
- Chazdon, R.L., Guariguata, M.R., 2016. Natural Melbourne regeneration as a tool for large-scale forest restoration in the tropics: prospects and challenges. *Biotropica* 48, 716–730.
- Clarke, G.M., 1985. Relationships between developmental stability and fitness: application for conservation biology. *Conserv. Biol.* 9, 18–24.
- Clarke, G.M., 1992. Fluctuating asymmetry: a technique for measuring developmental stress of genetic and environmental origin. *Acta Zool. Fenn.* 191, 31–35.
- Cornelissen, T., Stiling, P., 2010. Small variations over large scales: fluctuating asymmetry over the range of two oak species. *Int. J. Plant Sci.* 171, 303–309.
- Costa, F.V., Azevedo, I.F.P., Braga, L.L., Perillo, L.N., Neves, F.S., Leite, L.O., Silva, B.L.R., Ribeiro, L.C., Fernandes, G.W., Cuevas-Reyes, P., 2013. Fluctuating asymmetry and herbivory in two ontogenetical stages of *Chamaecrista semaphora* in restored and natural environments. *J. Plant Interact.* 8, 179–186.
- Costa, R.N., Solé, M., Nomura, F., 2017. Agropastoral activities increase fluctuating asymmetry in tadpoles of two neotropical anuran species. *Austral Ecol.* 42, 801–809.
- Cuevas-Reyes, P., Canché-Delgado, A., Maldonado-López, Y., Fernandes, G.W., Oyama, K., González-Rodríguez, A., 2018. Patterns of herbivory and leaf morphology in two Mexican hybrid oak complexes: importance of fluctuating asymmetry as indicator of environmental stress in hybrid plants. *Ecol. Ind.* 90, 164–170.
- Cuevas-Reyes, P., Fernandes, G.W., González-Rodríguez, A., Pimenta, M., 2011a. Effects of generalist and specialist parasitic plants (Loranthaceae) on the fluctuating asymmetry patterns of ruprestrian host plants. *Basic Appl. Ecol.* 12, 449–455.
- Cuevas-Reyes, P., Gilberti, L., González-Rodríguez, A., Fernandes, G.W., 2013. Patterns of herbivory and fluctuating asymmetry in *Solanum lycocarpum* St. Hill (Solanaceae) along an urban gradient in Brazil. *Ecol. Ind.* 24, 557–561.
- Cuevas-Reyes, P., Oyama, K., González-Rodríguez, A., Fernandes, G.W., Mendoza-Cuenca, L., 2011b. Contrasting herbivory patterns and leaf fluctuating asymmetry in *Heliocarpus pallidus* between different habitat types within a Mexican tropical dry forest. *J. Trop. Ecol.* 27, 383–391.
- Cunningham, S.A., 2000. Effects of habitat fragmentation on the reproductive ecology of four plant species in Mallee Woodland. *Conserv. Biol.* 14, 758–768.
- Debeauxon, I., Léon-Kloosterziel, K.M., Koornneef, M., 2000. Influence of the testa on seed dormancy, germination, and longevity in *Arabidopsis*. *Plant Physiol.* 122, 403–414.
- Díaz, M., Pulido, F.J., Møller, A.P., 2004. Herbivore effects on developmental instability and fecundity of holm oaks. *Oecologia* 139, 224–234.
- Donohue, K., Schmitt, J., 1998. Maternal environmental effects: adaptive plasticity? In: Mousseau, T.A., Fox, C.W. (Eds.), *Maternal effects as adaptations*. Oxford University Press, Oxford, pp. 137–158.
- Espírito-Santo, M.M., Sevilha, A.C., Anaya, F., Barbosa, R., Fernandes, G.W., Sánchez-Azofeifa, G.A., Scariot, A., Noronha, S.E., Sampaio, C.A., 2009. Sustainability of tropical dry forests: two case studies in southeastern and central Brazil. *For. Ecol. Manag.* 258, 922–930.
- Fernandes, A., 2002. Biodiversidade da caatinga. In: Araújo, E.L., Moura, A.N., Sampaio, E.S.B., Gestrinari, L.M.S., Carneiro, J.M.T. (Eds.), *Biodiversidade, conservação e uso sustentável da flora do Brasil*. Imprensa Universitária, Recife, UFRPE/SBB, pp. 42–43.
- Fernandes, G.W., Oliveira, S.C.S., Campos, I.R., Barbosa, M., Soares, L.A., Cuevas-Reyes, P., 2016. Leaf fluctuating asymmetry and herbivory of *Tibouchina heteromalla* in restored and natural environments. *Neotrop. Entomol.* 45, 44–49.
- Freeman, D.C., Graham, J.H., Emlen, J.M., 1993. Developmental stability in plants: symmetries, stress and epigenesis. *Genet. 89*, 97–119.
- Freeman, D.C., Graham, J.H., Tracy, M., Emlen, J.M., Alados, C.L., 1999. Developmental instability as a means of assessing stress in plants: a case study using electromagnetic fields and soybeans. *Int. J. Plant Sci.* 160, 157–166.
- Frishkoff, L.O., Hadly, E.A., Daily, G.C., 2015. Thermal niche predicts tolerance to habitat conversion in tropical amphibians and reptiles. *Glob. Chang. Biol.* 21, 3901–3916.
- Galloway, L.F., 2001. The effect of maternal and paternal environments on seed characters in the herbaceous plant *Campanula americana* (Campanulaceae). *Am. J. Bot.* 88, 832–840.
- Gentry, A.H., 1995. Diversity and floristic composition of neotropical dry forests. In: Bullock, S.H., Mooney, H.A., Medida, E. (Eds.), *Seasonally Dry Tropical Forests*. Cambridge University Press, Cambridge, pp. 146–194.
- Gharoobi, B., 2011. Effects of seed size on seedlings characteristics of five barley cultivars Iran. *J. Plant Physiol.* 1, 265–270.
- Ghazoul, J., 2005. Pollen and seed dispersal among dispersed plants. *Biol. Rev.* 80, 413–443.
- Guterman, Y., 2000. Maternal effects on seeds during development. In: Fenner, M. (Ed.), *Seeds: The Ecology of Regeneration in Plant Communities*. CAB International, pp. 59–84.
- Klisarić, N.B., Miljković, D., Avramov, S., Živković, U., Tarasjev, A., 2016. Developmental instability in German Iris flower as a potential biomonitoring method. *Arch. Biol. Sci.* 68, 837–844.
- Kolb, A., 2008. Habitat fragmentation reduces plant fitness by disturbing pollination and modifying response to herbivory. *Biol. Conserv.* 141, 2540–2549.
- Haddad, N.M., Brudvig, L.A., Clobert, J., Davies, K.F., Gonzalez, A., Holt, R.D., Lovejoy, T.E., Sexton, J.O., Austin, M.P., Collins, C.D., Cook, W.M., Damschen, E.I., Ewers, R.M., Foster, B.L., Jenkins, C.N., King, A.J., Laurance, W.F., Levey, D.J., Margules, C.R., Melbourne, B.A., Nicholls, A.O., Orrock, J.L., Song, D., Townshend, J.R., 2015. Habitat fragmentation and its lasting impact on Earth's ecosystems. *Sci. Adv.* 2, e1500052.
- Hara, Y., Toriyama, K., 1998. Seed nitrogen accelerates the rates of germination, emergence, and establishment of rice plants. *Soil Sci. Plant Nutr.* 44, 359–366.
- Komac, B., Alados, C.L., 2012. Fluctuating asymmetry and *Echinospartum horridum* fitness components. *Ecol. Ind.* 18, 252–258.

- Lima, A.L.S., Zanella, F., Casto, L.D.M., 2010. Crescimento de *Hymenaea courbaril* L. var. *stilbocarpa* (Hayne) Lee et Lang. e *Enterolobium contortisiliquum* (Vell.) Morong (Leguminosae) sob diferentes níveis de sombreamento. *Acta Amazônica* 40, 43–48.
- Linares-Palomino, R., Oliveira-Filho, A.T., Pennington, T., 2001. Neotropical Seasonally Dry Forests: diversity, endemism, and biogeography of woody plants. In: Dirzo, R., Young, H.S., Mooney, H.A., Ceballos, G. (Eds.), *Seasonally Dry Tropical Forest: Ecology and Conservation*. Island Press, Washington, pp. 3–21.
- Lohbeck, M., Lebrija-Trejos, E., Martínez-Ramos, M., Meave, J.A., Poorter, L., Bongers, F., 2015. Functional trait strategies of trees in dry and wet tropical forests are similar but differ in their consequences for succession. *PLoS ONE* 10, e0123741.
- LPWG, 2017. A new subfamily classification of the Leguminosae based on a taxonomically comprehensive phylogeny. *Taxon* 66, 44–77.
- Mal, T.K., Uveges, J.L., Turk, K.W., 2002. Fluctuating asymmetry as an ecological indicator of heavy metal stress in *Lythrum salicaria*. *Ecol. Ind.* 1, 189–195.
- Maldonado-López, Y., Vaca-Sánchez, M.S., Canché-Delgado, A., García-Jaín, S.E., González-Rodríguez, A., Cornelissen, T., Cuevas-Reyes, P., 2019. Leaf herbivory and fluctuating asymmetry as indicators of mangrove stress. *Wetlands Ecol. Manage.* 27, 571–580.
- Mass, J.M., 1995. Conservation of tropical dry forest to pasture and agriculture. In: Bullock, S.H., Mooney, H.A., Medida, E. (Eds.), *Seasonally Dry Tropical Forests*. Cambridge University Press, Cambridge, pp. 299–422.
- Mayle, F.E., 2004. Assessment of the Neotropical dry forest refugia hypothesis in the light of palaeoecological data and vegetation model simulations. *J. Quat. Sci.* 19, 713–720.
- Miles, L., Newton, A.C., DeFries, R.S., Ravilious, C., May, I., Blyth, S., Kapos, V., Gordon, J.E., 2006. A global overview of the conservation status of tropical dry forests. *J. Biogeogr.* 33, 491–505.
- Møller, A.P., 1993. Developmental stability, sexual selection, and speciation. *J. Evol. Biol.* 6, 493–509.
- Møller, A.P., 1997. Developmental stability and fitness: a review. *Am. Nat.* 149, 916–932.
- Møller, A.P., Swaddle, J.P., 1997. *Developmental Stability and Evolution*. Oxford University Press, Oxford.
- Naegle, E.R., Burton, J.W., Carter, T.E., Rufty, T.W., 2005. Influence of seed nitrogen content on seedling growth and recovery from nitrogen stress. *Plant Soil* 271, 329–340.
- Oliveira, D.M.T., 1999. Morfo-anatomia do embrião de leguminosas arbóreas nativas. *Rev. Bras. Bot.* 22, 413–427.
- Palmer, A.R., 1994. Fluctuating asymmetry analysis: a primer. In: Markow, T.A. (Ed.), *Developmental Instability: Its Origins and Evolutionary Implications*. Kluwer, Dordrecht, pp. 335–364.
- Palmer, A.R., Strobeck, C., 1986. Fluctuating asymmetry: measurement, analysis, patterns. *Ann. Rev. Ecol. Syst.* 17, 391–421.
- Pedralli, G., 1997. Florestas secas sobre afloramentos de calcário em Minas Gerais: florística e fisionomia. *Bios* 5, 81–88.
- Pennington, R.T., Prado, D.E., Pendry, C.A., 2000. Neotropical seasonally dry forests and Quaternary vegetation changes. *J. Biogeogr.* 27, 261–273.
- Peske, S.T., Lucca-Filho, O.A., Barros, A.C.S.A., 2012. *Sementes: fundamentos científicos e tecnológicos*, second ed. Editora Universitária/UFPel, Pelotas.
- Pineda-García, F., Paz, H., Meinzer, F.C., 2013. Drought resistance in early and late secondary successional species from a tropical dry forest: the interplay between xylem resistance to embolism, sapwood water storage and leaf shedding. *Plant, Cell Environ.* 36, 405–418.
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., 2020. *nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-149*, <URL: <https://CRAN.R-project.org/package=nlme>>.
- Portillo-Quintero, C.A., Sánchez-Azofeifa, G.A., 2010. Extent and conservation of tropical dry forests in the Americas. *Biol. Conserv.* 143, 144–155.
- Prado, D.E., 2000. Seasonally dry forests of tropical South America: from forgotten ecosystems to a new phytogeographic unit. *Edinb. J. Bot.* 57, 437–461.
- Prado, D.E., Gibbs, P.E., 1993. Patterns of species distributions in the dry seasonal forests of South America. *Ann. Mo. Bot. Gard.* 80, 902–927.
- R Core Team. *R: A Language and Environment for Statistical Computing*. <<http://www.R-project.org/>> (2017).
- Rengel, Z., Graham, R.D., 1995. Importance of seed Zn content for wheat growth on Zn-deficient soil. *Plant Soil* 173, 267–274.
- Rey, P.J., Cancio, I., González-Robles, A., Bastida, J.M., Manzaneda, A.J., Valera, F., Salido, T., Alcántara, J.M., 2017. Local-scale and landscape disturbances impact through distinct pathways on the regional variation in seed dispersal by mammals in threatened semiarid habitats. *Perspect. Plant Ecol. Evol. Syst.* 28, 10–18.
- Roach, D.A., Wulff, R.D., 1987. Maternal effects in plants. *Annu. Rev. Ecol. Syst.* 18, 209–235.
- Sánchez-Azofeifa, G.A., Kalácska, M., Quesada, M., Calvo-Alvarado, J.C., Nassar, J.M., Rodriguez, J.P., 2005. Need for integrated research for a sustainable future in tropical dry forests. *Conserv. Biol.* 19, 1–2.
- Sánchez-Azofeifa, G.A., Quesada, M., Cuevas-Reyes, P., Castillo, A., Sánchez-Montoya, G., 2009. Land cover and conservation in the area of influence of the Chamela-Cuixmala Biosphere Reserve, Mexico. *For. Ecol. Manage.* 258, 907–912.
- Santos, J.C., Alves-Silva, E., Cornelissen, T.G., Fernandes, G.W., 2017. Differences in leaf nutrients and developmental instability in relation to induced resistance to a gall midge. *Arthropod Plant Interact.* 11, 163–170.
- Särkinen, T., Igance, J.R.V., Linares-Palomino, R., Simon, M.F., Prado, D.E., 2011. Forgotten forests – issues and prospects in biome mapping using seasonally dry tropical forests as a case study. *BMC Ecol.* 11, 1–15.
- Sarrive, J.R., Haag, H.P., 1974. *Análise química de plantas*, first ed. Escola Superior de Agricultura Luiz de Queiroz, Piracicaba.
- SAS, 2000. *Categorical Data Analysis using the SAS System*. SAS Institute, Cary.
- Schmid, B., Dolt, C., 1994. Effects of maternal and paternal environment and genotype on offspring phenotype in *Solidago altissima* L. *Evolution* 48, 1525–1549.
- Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* 9, 671.
- Senior, R.A., Hill, J.K., del Pliego, P.G., Goode, L.K., Edwards, D.P., 2017. A pantropical analysis of the impacts of forest degradation and conversion on local temperature. *Ecol. Evol.* 7, 7897–7908.
- Siikamäki, P., Lammi, A., Mustajärvi, K., 2002. No relationship between fluctuating asymmetry and fitness in *Lychnis viscaria*. *Evol. Ecol.* 16, 567–577.
- Sinclair, C., Hoffmann, A.A., 2003. Monitoring salt stress in grapevines: are measures of plant trait variability useful? *J. Appl. Ecol.* 40, 928–937.
- Smith, M.C., 2016. Heavy metal contamination increases fluctuating asymmetry in *Rhus glabra* L. (Anacardiaceae). *The Southwest. Nat.* 61, 156–159.
- Souza, M.L., Fagundes, M., 2004. Seed size as key factor in germination and seedling development of *Copaifera langsdorffii* (Fabaceae). *Am. J. Plant Sci.* 5, 2566–2573.
- Stokes, M.E., Davis, C.S., Koch, G.G., 2000. *Categorical Data Analysis using the SAS System*, second ed. SAS Institute, Cary.
- Tucker-Lima, J.M., Caruso, N.M., Clugston, J., Kainer, K.A., 2018. Landscape change alters reproductive phenology and sex expression in *Attalea* palms (Arecaceae) of southwestern Amazonia. *Plant Ecol.* 219, 1225–1245.
- Walters, C., 1998. Understanding the mechanisms and kinetics of seed aging. *Seed Sci. Res.* 8, 223–244.
- Werneck, F.P., 2011. The diversification of eastern South American open vegetation biomes: historical biogeography and perspectives. *Quat. Sci. Rev.* 30, 1620–11248.
- White, P.J., Veneklaas, E., 2012. Nature and nurture: the importance of seed phosphorus content. *Plant Soil* 357, 1–8.
- Yamashita, O.M., Guimarães, S.C., Silva, J.L., Carvalho, M.A.C., Camargo, M.F., 2009. Fatores ambientais sobre a germinação de *Emilia sonchifolia*. *Planta Daninha* 27, 673–681.