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# MORPHOMETRIC STUDIES AND ANALYSES OF GERMINABILITY IN *CENOSTIGMA MACROPHYLLUM* TUL. IN AN URBAN-RURAL GRADIENT IN TERESINA-PI, BRAZIL

## ESTUDO MORFOMÉTRICO E ANÁLISE DA GERMINABILIDADE EM *CENOSTIGMA MACROPHYLLUM* TUL. NO GRADIENTE URBANO-RURAL EM TERESINA, PIAUÍ, BRASIL

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## ABSTRACT

Urbanization can alter environmental factors that affect the morphological and germinative characteristics of seeds. We evaluated the influence of an urban-rural gradient on the morphometrics of the fruits and seeds of *Cenostigma macrophyllum* Tul. (Fabaceae) and on seed germinability in the region near Teresina, Piaui State, Brazil. One hundred fruits were collected in the urban zone and 100 in the rural area, and the lengths, widths, thicknesses, and weights of both the fruits and seeds were measured, as well as the numbers of seeds per fruit. The seeds were then used in greenhouse germination experiments (during two months) to calculate the percentages of emergence (E%) and survival (S%), the emergence rate index (ERI), synchronization index (Z), and Timson's index (T). The results showed significant statistical differences between the two areas in terms of the biometric patterns of the fruits and seeds as well as seed germination, with higher values in the urban zone. As such, urbanization was found to affect the morphologies and germination processes of plant species.

**Keywords:** ecophysiology; semideciduous seasonal forests; caneleiro.

## RESUMO

A urbanização altera os fatores ambientais, que podem afetar as características morfológicas e germinativas das sementes. Dessa forma, objetivou-se avaliar a influência do gradiente urbano-rural sobre a morfometria de frutos e sementes e germinabilidade de sementes de *Cenostigma macrophyllum* Tul. (Fabaceae) na região de Teresina, Piauí. Foram coletados 100 frutos na zona urbana e 100 na rural, sendo mensurados comprimento, largura, espessura, peso dos frutos e sementes e número de sementes por fruto. As sementes foram utilizadas no experimento de germinação em casa de vegetação, durante dois meses, sendo calculadas as porcentagens de emergência e de sobrevivência e mensurados os índices de velocidade de emergência, de sincronização e de Timson. Os resultados indicaram diferenças estatísticas entre as áreas para a biometria dos frutos e sementes e germinação de sementes, observando-se valores maiores para a zona urbana. Desse modo, foi possível concluir que a urbanização afeta a morfologia e o processo de germinação das espécies vegetais.

**Palavras-chave:** ecofisiologia; floresta estacional semidecidual; caneleiro.

# INTRODUCTION

The lifecycles of plants include the critical phase of seed germination, which can directly influence their community structure, species conservation, and forest regeneration (LIU *et al*., 2017; SOUZA; FAGUNDES, 2014). Germination processes vary between and within individuals in many plant species (SEGURA *et al*., 2015) and are influenced by a wide set of factors, including seeds' biological characteristics. The germination rate and seed germination speed of *Artocarpus heterophyllus* L. (Moraceae) in the tropical region of India, for example, were found to increase as a function of seed mass (KHAN, 2004). Other studies (e.g., SOUZA; FAGUNDES, 2014) have shown that seed weight and size are attributes can influence germination processes.

Abiotic factors, such as temperature and water availability, are among the environmental factors known to affect seed germination and seedling establishment (e.g., DOUSSEAU *et al*., 2013; MARAGHNI; GORAI; NEFFATI, 2010), and temperature has been shown as having a strong influence on seed germination in humid and semiarid regions in Brazil (OLIVEIRA *et al*., 2013; ARAÚJO *et al*., 2016; RIBEIRO *et al*., 2015). The northeastern region of that country includes extensive areas of seasonally dry tropical forest ('Caatinga') whose plants are adapted to low rainfall levels (300–1,000 mm/year) (QUEIROZ *et al*., 2017) that affect seed germinability (FERREIRA; MEIADO; SIQUEIRA-FILHO, 2017; MEIADO *et al*., 2010). Investigations have shown that seed germination and seedling establishment can also be influenced by environmental variations provoked by anthropic actions, revealing potential threats to natural ecological succession processes.

Urban-rural gradients are among the anthropic phenomena that considerably alter many abiotic factors in the environment, such as temperature and humidity — which tend to be higher and lower, respectively, in urban areas as compared to rural sites (VALIN JR. *et al*., 2015). Those differences are related to the fact that urban areas have greater heat retention capacities (forming "Urban Heat Islands") and demonstrate low humidity levels due to the greater impermeability of their soils and surfaces (ARGÜESO *et al*., 2014). Those environments present the control of water availability by irrigation, with water availability being among the principal activators of the reproductive phase of plants – mainly in environments with well-defined dry seasons (ZHANG; ZHANG; TIAN, 2012). Those environmental factors observed in urban areas (and others, such as high $CO_2$ levels and high solar radiation) can influence the morphological characteristics of fruits and seeds and, under favorable environmental conditions, it might be expected that their greater seed sizes and masses would positively influence germination and establishment.

The western portion of the Brazilian semiarid region includes an extensive ecotone zone at the intersection of the Amazon Forest, Cerrado (Neotropical savanna), and Caatinga (deciduous, thorny, drylad vegetation) biomes and represents the largest phytoecological domain in the Parnaíba River Basin (PRB) (SOUSA *et al*., 2009). The climatic complexity and habitat heterogeneity found there, allied to the expanding urbanization (especially surrounding the principal urban centers) make the PRB region appropriate for studying the influence of urban-rural gradients on seed germination.

*Cenostigma macrophyllum* Tul. (Fabaceae), popularly known as 'caneleiro', is widely distributed in the PRB (AGUIAR *et al*., 2016). That arboreal (or shrub) species is considered the plant symbol for the capital (Teresina) of Piauí State (Municipal Decree nº 2.407, August 13, 1993). In addition to having pharmaceutical properties (VIANA *et al*., 2013; COELHO *et al*., 2013), "caneleiro" is commonly used in arborization and landscaping projects of urban areas (MACHADO *et al*., 2006). As such, the main objective of the present work was to evaluate the influence of the urban-rural gradient on the morphometrics of the fruits and seeds of *C. macrophyllum* Tul. (Fabaceae) and on seed germinability in the region near Teresina, Piaui State, Brazil.

# MATERIALS AND METHODS

## Study area

The municipality of Teresina (4º47'25" – 5º35'11" S × 42º35'50" – 5º31'58" W) is situated in central-northern Piauí State, Brazil (IBGE, 2017). According to the Köppen classification system, the regional climate is hot and humid (Aw') (JACOMINE *et al*., 1986), with a mean annual rainfall rate of 1393 mm, a mean annual temperature of 27.7ºC, and well-defined dry (June through October) and rainy (November through May) seasons (INMET, 2017).

The region comprises a variety of phyto-ecological units, including semideciduous seasonal forests and Cerrado, and transition zones into forest and Caatinga areas (JACOMINE *et al*., 1986). *C. macrophyllum* is widely distributed throughout the region (QUEIROZ, 2009). The state capital of Teresina still retains some remnant forest areas, as well as many parks and squares with tree cover (MACHADO *et al*., 2006). *Cenostigma macrophyllum* individuals were selected for this study from both urban and rural areas:

1. along a tree-lined but heavily trafficked urban road (5º03'03"S × 42º44'19"W);
2. in a rural area near the BR-343 Highway (5º03'24"S x 42º41'W).

Those "caneleiro" trees occurred in groups, without other nearby tree species.

## Data collection

The experiments described here were undertaken at the Ethnobiology and Plant Ecology Laboratory (LEEV) and in greenhouses at the Federal University of Piauí (UFPI), between January and February/2017. We randomly collected 100 fruits of *C. macrophyllum* from both urban and rural areas (200 total mature fruits) to analyze their morphometrics. The lengths, widths, and thicknesses of the fruits were measured using digital calipers, with an accuracy of 0,01 mm; their masses were determined using a precision analytical balance, with accuracy of 0,0001 g.

One hundred seeds from each area were randomly chosen for morphometric examination (measuring their lengths, widths, and weights as well as the numbers of seeds per fruit) and subsequently used them in germination tests to determine any relationships between their morphometric values and germinability.

The seeds were sown into trays containing topsoil, which were subsequently maintained in a greenhouse and watered daily. Each treatment consisted of four replicates of 25 seeds. Seedling emergence was evaluated daily, adopting a definition of germination as the appearance of the seedling's aerial portion on the substrate surface. The observations on germination continued for two months, ended by the absence of any seedling emergence during 10 consecutive days. We calculated the percentages of emergence (E%) and survival (S%) , obtained by the number of plants that emerged and survived by the number of the seeds used, respectively, the emergence rate index (ERI) was determined by the sum of plants that emerged on the day of the emergency (MAGUIRE, 1962). The synchronization index (Z) to verify the distribution of the relative frequency of germination during the study period, and Timson's index (T), which represents the progressive total of the cumulative percentage of germination recorded at specific intervals (each day of study) or a given period of time, higher values correspond to the better conditions of germination (RANAL; SANTANA, 2006).

## Statistical analyses

Data's normality and homoscedasticity were tested to calculate any differences between the morphometric variables analyzed, using ANOVA and the Tukey *a posteriori* test. The widths did not demonstrate normality, requiring the use of the Mann-Whitney test. The original data of the total weights of the seeds in each fruit did not demonstrate homoscedasticity and were square root transformed. Thickness data was x**1.5 transformed due to the observed high coefficient of variation (CV).

The germination tests were entirely randomized and their data submitted to Generalized Linear Model (GLM) procedures to evaluate any differences between the two areas. Only seeds from the urban area were used to determine if seeds' weights and sizes influenced their germinability, as the germination rates of seeds from the rural area were too low for accurate statistical analyses. GLM was used to compare the weights and sizes of the urban area's germinated and non-germinated seeds, and the Spearman correlation test to examine any relationships between the numbers of days required for germination and seed weights and sizes. All of the analyses were performed using SAS System 9.0 software.

# RESULTS AND DISCUSSION

Evaluations of the *Cenostigma macrophyllum* fruits' morphometric data revealed that the biometric means of the fruits harvested from the urban area were significantly greater than those harvested from the rural area, except for width (Table 1). The fruits from the urban area had: widths between 1.66 and 3.41 cm; thickness between 0.40 and 0.86 cm; lengths between 4.50 and 10.33 cm; weights between 2.90 and 12.85 g. Fruits harvested from the rural area had: widths between 2.09 and 3.77 cm; thicknesses between 0.2 and 0.8 cm; lengths between 2.98 and 10.11 cm; and masses between 2.09 and 9.43 g.

The morphometrics of the seeds were similar to those of the fruits, with their measurements being greater in the urban area when compared to the rural area (Table 1). The variable of width demonstrated the greatest numbers of seeds in the interval between 1.22 and 1.42 cm in the urban area, while the greatest numbers of seeds in the rural area were observed in the interval between 0.82 and 1.22 cm (Figure 1A).

Large differences in seed thicknesses were observed between the two areas. The greatest numbers of seeds collected in the urban area were observed in the width interval between 0.243 and 0.390 cm; the seeds from the rural area were thinner, with the highest numbers within the width intervals from 0.094 to 0.243 cm (Figure 1B). In terms of seed length, most of the seeds collected in the urban area were in the length class 1.56 to 2.02 cm; while seeds from the rural area were largely included between the length interval of 1.33 to 1.79 cm (Figure 1C).

The greatest numbers of seeds collected in the urban area weighed between 0.292 and 0.584 g; while seeds from the rural area were largely included within the weight interval of 0.000 to 0.292 g. The seeds from the urban area were therefore both larger and heavier (Figure 1D), which most likely contributed to their higher germination rates (Table 2).

Each fruit in the urban area contained from one to five seeds, with most having between one and four seeds; fruits from the rural area had from zero to four seeds, with most of them having either one or two seeds (Figure 1E).

**Table 1 – Mean values of the morphometrics of the fruits and seeds of *Cenostigma macrophyllum* Tul. harvested in urban and rural areas in the municipality of Teresina (PI), Brazil.**

| Variables | Urban area | Rural area | p* |
|---|---|---|---|
| *Fruit morphometry* | | | |
| Width (cm) | 2.74 | 2.84 | p < 0.05 |
| Thickness (cm) | 0.65 | 0.45 | p < 0.01 |
| Length (cm) | 7.48 | 7.32 | n.s |
| Fruit weight (cm) | 6.66 | 5.26 | p < 0.01 |
| Number of seeds per fruit | 2.19 | 1.32 | p < 0.01 |
| Weight of seeds per fruit (g) | 1.03 | 0.27 | p < 0.01 |
| *Seed morphometry* | | | |
| Weight (g) | 0.45 | 0.20 | p < 0.01 |
| Width (cm) | 1.27 | 1.06 | p < 0.01 |
| Thickness (cm) | 0.31 | 0.15 | p < 0.01 |
| Lenght (cm) | 1.73 | 1.60 | p < 0.01 |

p*: probability of significance.

*C. macrophyllum* demonstrates phenotypic plasticity (a common capability among forest species), with wide morphometric variations being observed in their fruits and seeds (CRUZ; CARVALHO, 2003) due to endogenous factors, such as genetic variability (KRISHNAN; BORGES, 2018), and exogenous factors

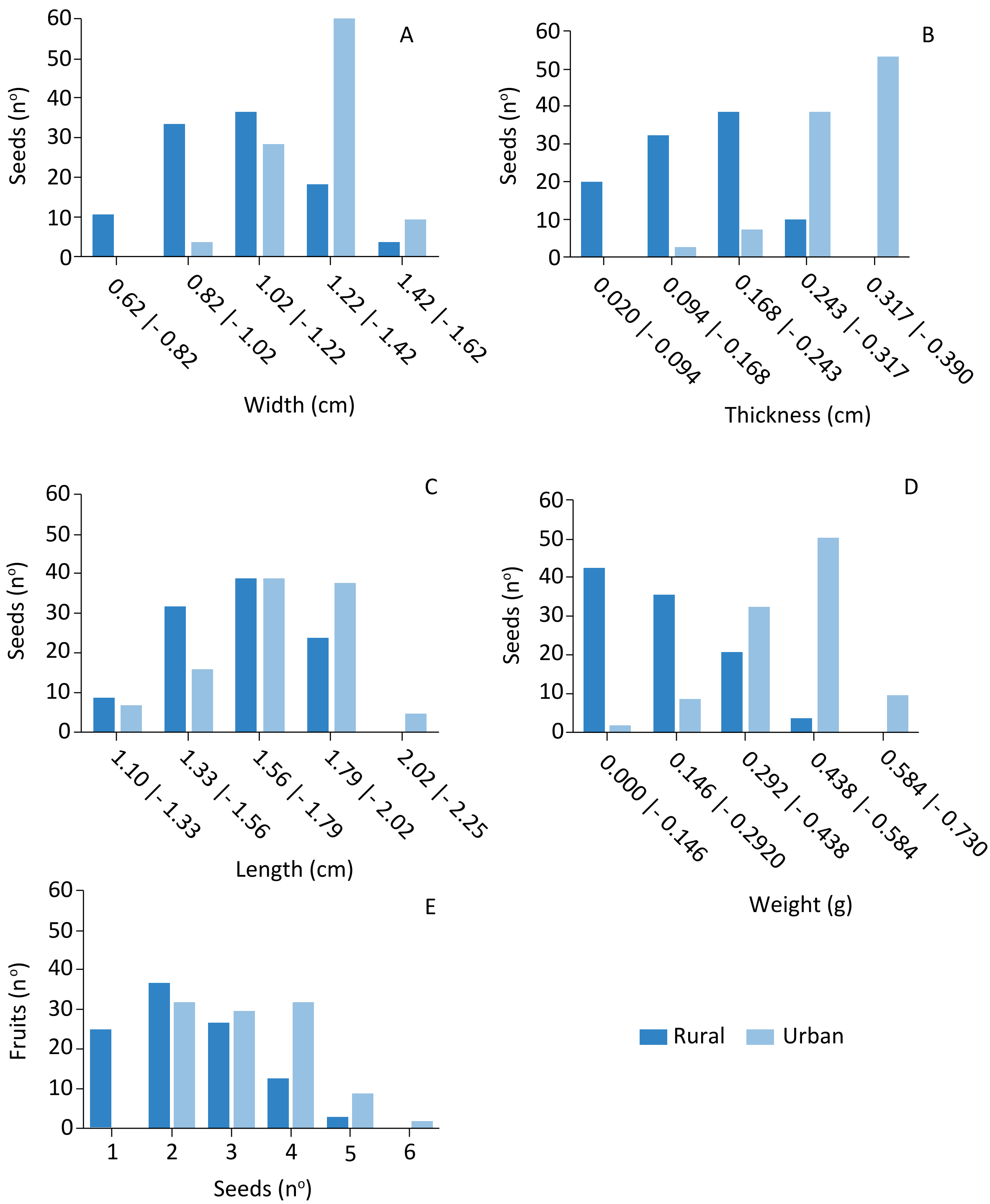


**Figure 1 – Biometrics of the seeds of *Cenostigma macrophyllum* Tul. (A, B, C and D) and the numbers of seeds per fruit (E) harvested in urban and rural areas in the municipality of Teresina (PI), Brazil.**

such as the availability of light, nutrients and water that can influence phenotypic variation (SILVA *et al*., 2012) and cause morphological alterations (MISHRA *et al*., 2014).

Martins *et al*. (2005) examined the influence of seed weight on germination and seedling vigor in *Carica papaya* L. (papaya), and likewise reported that the heaviest seeds demonstrated the highest germination rates. Those results were like ours in the present work, as seeds collected in the urban area were heavier and demonstrated greater germination rates than the lighter seeds produced in rural areas, most likely reflecting the fact that larger seeds have more nutrient reserves and greater advantages in terms of germination (ZHANG *et al*., 2016).

In comparing the sizes and weights of germinated and ungerminated seeds from the urban area, it was observed that germinated seeds were significantly thicker than those that had not germinated; the other morphometric characteristics examined here did not demonstrate any significant differences (Table 3). Only seed thickness was weakly correlated with the timing of germination, with thicker seeds germinating slightly quickly ($R = 0.20$; $p < 0.05$).

These results indicated that their greater thickness was related to greater amounts of stored humidity or larger nutrient reserves. Silva and Carvalho (2008) observed that increases in the sizes of *Clitoria fairchildiana* R.A. Howard. (Fabaceae) seeds were not related to their water contents, supporting the idea that greater thickness is most likely related to their greater nutrient content. The *C. fairchildiana* seeds had all been collected in the same area, and probably had similar water contents proportional to soil humidity. In the present study, the *C. macrophyllum Tul.* (Fabaceae) trees in the urban area were irrigated daily, which would presumably increase seed humidity and, consequently, their thickness.

Our results corroborated the published results relating to seed morphometrics and germination, which have shown that seed sizes and weights influence not only germination but also initial seedling development (BOUCHARDET *et al*., 2015), and that seed biometric measurements are directly related to their physiological quality and germinative viability (OLIVEIRA-BENTO *et al*., 2013).

Seed germination indices in the two areas were significantly different, with greater percentages of plants

**Table 2 – Mean values of the germination of *Cenostigma macrophyllum* Tul. seeds in urban and rural areas in the municipality of Teresina (PI), Brazil.**

| Parameters | Urban area | Rural area | p* |
|---|---|---|---|
| E% | 55.00 | 4.00 | 0.02 |
| S% | 52.00 | 3.00 | 0.02 |
| ERI | 1.53 | 0.10 | 0.02 |
| Z | 2.30 | 0.50 | 0.02 |
| T | 5519.53 | 398.43 | 0.02 |

E%: Emergence percentage; S%: survival percentage; ERI: emergence rate index; Z: synchronization index; T: Timson's index; p*: probability of significance.

**Table 3 – Mean sizes and masses of the germinated and non-germinated seeds of *Cenostigma macrophyllum* Tul. from an urban area in the municipality of Teresina (PI), Brazil.**

| Variables | Germinated seeds | Non-germinated seeds | p* |
|---|---|---|---|
| Weight (g) | 0.47 | 0.43 | 0.14 |
| Width (cm) | 1.27 | 1.26 | 0.98 |
| Thickness (cm) | 0.32 | 0.30 | 0.04 |
| Lenght(cm) | 1.72 | 1.71 | 0.87 |

p*: probability of significance.

emergence and survival among those harvested from the urban area (Table 2). The emergence velocities of seeds harvested from the urban area were significantly higher than those from the rural area, both in terms of the partial numbers of germinated seeds (ERI) and the partial percentages of germination — using Timson's index (T) for more precise comparisons.

Greater ERI values directly influence a species' emergence (E%) and survival percentage (S%), favoring a faster establishment and diminishing the chances seeds infection by pathogenic microorganisms or consumption by predators (FAGUNDES; CAMARGOS; COSTA, 2011) — creating an evident relationship between greater emergence velocity and greater emergence and survival percentages.

Carvalho and Nakagawa (2000) found that vigorous seeds with higher emergence capacities demonstrated high emergence velocities — while the opposite was observed with less vigorous seeds more susceptible to deterioration. Emergence percentage is also directly related to percentage survival, as greater numbers of germinating seeds indicate a greater probability of survival (ZHANG *et al*., 2016).

The synchronization index (Z) (which was greater for seeds harvested in the urban area) is of great importance to successful plant and plant community development as synchronous seed germination will tend to increase synchrony in all subsequent life cycle phases, including reproductive phenophases, with community synchrony being of extreme importance to efficient seed production and dispersal (MORELLATO *et al*., 2016).

The high percentages of successfully germinating seeds collected from the urban area (and the probable relationship with greater water availability in that environment) demonstrates that *C. macrophyllum* seeds have a "hydric-memory". Rito *et al*. (2009) observed that discontinuous cycles of hydration/dehydration favored the germination of *Cereus jamacaru* (Cactaceae) seeds, as a reflection of their hydric-memory, which appeared to preserve characteristics resulting from imbibition throughout the dry period, with germination only occurring when humidity conditions became favorable.

Although the *Cenostigma macrophyllum* seeds used here were not exposed to cycles of hydration/dehydration, it is possible that they had experienced contrasting situations of water availability in their development (due to fluctuations in soil water availability to the mother plant). The hydric-memory of a given species is reflected in its germinative behavior, which, in turn, is influenced by its surrounding environment, so that the urban-rural gradient may be affecting important eco-physiological characteristics that are important to the maintenance of species in their respective ecosystems.

The importance of water availability for seed germination has been discussed in the literature (PELEGRINI *et al*., 2013), and is considered one of the main factors gating their reproductive phase (NEIL *et al*., 2014) and a limiting factor for seed germination and seedling growth (DÜRR *et al*., 2015). Water availability represents one of the principal factors affecting fruit biometrics and the viability of *C. macrophyllum* seeds.

Urbanization results in enormous environmental alteration (ARGÜESO *et al*., 2014) and is almost surely responsible for certain phenotypic variations in plants. The observed morphometric differences between fruits and seeds in the urban-rural gradient suggest that exogenous factors associated with urbanization are directly involved. Those factors can provoke changes in plant phenotypes as they adapt to urban environments, quite possibly making them less fit for establishment in natural environments and reducing their utility for restoration projects (BOTEZELLI; DAVIDE; MALAVASI, 2000).

The different environments in urban and rural areas are known to act directly on the vegetative and reproductive phases of plants (NEIL *et al*., 2014), so that their fruits' morphological characteristics (as well as seed germination) will be affected by abiotic factors acting directly on plant structures.

In addition to the availability of water resources, $CO_2$ assimilation can also alter the vegetative growth of plants and their biomass distributions (XU *et al*., 2014). Seed germination has been found to be strongly affected by atmospheric $CO_2$ levels and can cause allometric modifications of seedling growth (MARTY; BASSIRIRAD, 2014). As higher $CO_2$ concentrations will be found in urban areas, the resulting alterations in plant biomass allocation can result in the production of larger and better-quality fruits (AIDAR et al., 2002), as seen here with *C. macrophyllum* (except for seed width).

## CONCLUSIONS

The fruits and seeds of *Cenostigma macrophyllum* Tul. (Fabaceae) collected in an urban area demonstrated biometric means and amplitudes larger than of those collected in a rural area; the germinabilities of seeds from the urban area were likewise significantly greater. These results indicate that abiotic factors within the urban-rural gradient are acting directly on the growth and development of *C. macrophyllum,* the morphometrics of their fruits and seeds and seed germination.

The data generated in the present study will be important for better understanding the impacts of urbanization on natural ecosystems, and how abiotic factors along a rural/urban gradient directly affect plant growth and reproduction. Additional studies will still be needed; however, that can identify the environmental factors of urbanization that are directly affecting the reproductive fitness of those plants and the consequences of those alterations for the natural environment.

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