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DIFFERENCES IN WATER DEFICIT ADAPTATION DURING EARLY GROWTH OF BRAZILIAN DRY FOREST CAATINGA TREES

SUMMARY

Previous studies have shown that adaptation to drought in Brazilian Caatinga trees may involve different physiological and morphological strategies. Particular climatic and soil characteristics occur along this dry ecosystem leading to non-homogeneous water availability. Therefore, in certain niches, some species predominate over others, indicating that best performance of a species is not widespread. We studied changes in initial growth, which is the most critical factor during the life of a plant, of two common species of Caatinga (*Erythrina velutina* Willd. and *Enterolobium contortisiliquum* (Vell.) Morong) with variable occurrence through the ecosystem. Young plants were subjected to three water regimes: 450 (control), 225 (moderate stress) and 112.5 mm (severe stress) of water spread over 40 days, which represent years with heavy, moderate and scarce rainfall, respectively. Analyses were performed at 20 and 40 days after the initiation of treatments. When compared to the control group, treatments with water restriction reduced the growth of shoots and roots, the number of leaves and leaflets, the total biomass and the leaf area more in *E. velutina* than in *E. contortisiliquum*. Taking into account the adaptation of both species in Caatinga, we present evidence of different drought tolerance strategies. The ecological importance of early changes in the growth of species is discussed in the paper.

Keywords: Arid environment, *Enterolobium contortisiliquum* (Vell.) Morong, *Erythrina velutina* Willd., initial growth, water stress.

INTRODUCTION

Caatinga is the predominant biome in the semiarid region of north-eastern Brazil. Most of its area presents average annual rainfall lower than 1,000 mm (Menezes *et al.* 2012). Nonetheless, some very dry years often occur, with the last example being 2012 (Santos *et al.* 2014). Due to variable soil depth, which is most often low (Menezes *et al.* 2013), and local climate characteristics, the water availability is concentrated in some months but non-homogeneous over Caatinga. Environmental degradation in many areas has been observed particularly due to slash-and-burn systems of agriculture, overgrazing and intensive fuel wood extraction and also due to soil erosion processes (Menezes *et al.* 2012).

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Water stress is one of the major limiting factors for plant growth and development (Shane *et al.* 2010). In Caatinga, the availability of water affects the abundance of species in a population (Andrade *et al.* 2009). In such conditions the plants must balance strategies to maintain growth and to survive (Claeys and Inzé 2013). On the other hand, moderate drought can increase root growth (Baluska *et al.* 2010) and the net accumulation of solutes per cell (Krasensky and Jonak 2012), which ensures the exploration of soil layers that still have available water. Studies of the ecosystem have led us to suggest that these mechanisms are present but with variable intensities over Caatinga species.

The initial growth is generally critical for most plants species (Rathcke and Lacey 1985). *Erythrina velutina* Willd., popularly known as mulungu, and *Enterolobium contortisiliquum* (Vell.) Morong, popularly known as tamboril or orelha de nego, both belong to the Fabaceae family and occur naturally in Caatinga. In certain niches, one species is abundant over the other, but this is not reported in other niches, suggesting different adaptation strategies to drought between species. In a greenhouse experiment, we aimed to study changes in the initial growth of the two species under different water regimes, which could simulate differences in water supply over growth seasons and/or local niches. We also studied the morphological characteristics of leaves, which may be related to drought adaptation strategies. This paper contributes to the understanding of water adaptation strategies of Caatinga trees.

MATERIALS AND METHODS

The study was carried out in a greenhouse in the Unidade Acadêmica Especializada em Ciências Agrárias (UAECIA) of Universidade Federal do Rio Grande do Norte located in the district of Jundiá, Macaíba-RN, Brazil. During the experiment the average temperature in the greenhouse was 25.9°C.

For both species, these seeds were removed from fruits immediately after collection and stored in glass containers. The sowing was conducted in polyethylene pots of 10 x 15 cm (diameter x height) with soil from UAECIA. Analysis of soil fertility was performed by Empresa de Pesquisa Agropecuária do Rio Grande do Norte, Brazil (EMPARN) (Table 1). Each pot received three seeds sown at a depth of 0.5 cm. The pots were watered daily in the evening with distilled water to the field capacity. Twenty-one days after sowing, thinning was carried out, leaving one plant per pot.

The pots containing one plant each were divided into three groups, each subjected to a water regime as follows: 450 (control), 225 (moderate stress) and 112.5 mm (severe stress) distributed over 40 days. The water regimes tested simulate years with good rain, as well as dry and very dry, in East Seridó of Rio Grande do Norte State, according to EMPARN (2016) historical data. The pots were randomly distributed in the greenhouse. At this stage, irrigation occurred at intervals of two days. To calculate the daily amount of water to be applied to each pot, the surface area and total amount of water to be applied divided by the number of days of the experiment were taken into account. The water volumes applied were measured using a graduated provete.

Table 1 – Analysis of fertility of the soil used in the experiment

pH in water (1:25)	5.53
Calcium (cmol _c /dm ³)	1.46
Magnesium (cmol _c /dm ³)	0.64
Aluminum (cmol _c /dm ³)	0.00
Hydrogen+Aluminum (cmol _c /dm ³)	1.33
Phosphorus (mg/dm ³)	14
Potassium (mg/dm ³)	83
Sodium (mg/dm ³)	11

Five plants per treatment were collected 20 and 40 days after the beginning of the treatments (DAT). The plants were divided into leaves, roots and shoots (stems + petioles). Plant parts, along with a graduated scale for reference, were photographed with a digital camera. The images were analysed with Image J software, version 1.47v, developed by the National Institutes of Health, Bethesda, MD (public domain: <http://rsb.info.nih.gov/ij/>). The area, perimeter, length and width of leaflets were then determined. These data were then used for the determination of leaf and leaflet area, specific leaf area (SLA; leaf area divided by leaf dry mass), leaflet index (LI; length divided by width) and, as an index of the degree of margin dissection independent of leaf size, the perimeter²/area (Sack *et al.* 2003) was calculated. As the analyses were destructive, we obtained the number of required plants plus 40% to ensure uniformity during collections.

To determine the dry mass of plant parts, they were dried in an oven at 70°C until a constant weight was achieved. Partition analyses were performed: leaf area fraction (LAF; leaf area divided by the total plant dry mass) and biomass fractions (dry mass of plant parts divided by the total dry mass) of root (RMF), stem (SMF) and leaf (LMF).

The experiment was carried out with a 2×3 factorial design (two species × three water regimes), completely randomised with five replications. After assessing the homogeneity of variances and normality, according to Fligner–Killeen and Shapiro–Wilk tests, respectively, the data were subjected to analysis of variance and the treatments were compared by the least significant difference obtained by the Student's *t*-test at 5% (Crawley 2012). To analyse the data and obtain figures, the software R (R Development Core Team 2016) for Linux, version 3.2.2 was used.

RESULTS AND DISCUSSION

The water restriction caused greater effects in *E. velutina* (Figure 1). In *E. contortisiliquum*, the shoot length was not significantly affected by treatments, but at the end of the experiment, there was a slight trend of inhibition due to water restriction (Figure 1A). In *E. velutina*, however, the growth of shoots was affected in moderate and severe water stress conditions at 20 and 40 DAT (Figure 1B). In *E. contortisiliquum*, there was no effect of water stress on the

number of leaves and leaflets until 20 DAT (Figure 1C and E), but a trend of reduced leaf area under severe water stress (Figure 1G) was observed, suggesting smaller leaves and leaflets (see Figure 3). At 40 DAT, the severe water stress had induced a reduction in the number of leaves (significant; $p < 0.05$) and a trend to reduce the number of leaflets and leaf area in this species, but moderate water stress caused only small changes (Figure 1C, E and G). The reduction in the number of leaves and leaflets from 20 to 40 DAT under severe water deficit indicates senescence, which was verified. In *E. velutina*, until 20 DAT, only under severe water stress, there was inhibition of the number of leaves, leaflets and leaf area (Figure 1D, F and H). However, at 40 DAT, under moderate stress, there was a reduction in these parameters (Figure 1D, F and H), indicating leaf senescence, as described above in *E. contortisiliquum*. Under severe stress, acclimation must have occurred earlier in this species because there was a tendency towards an increase in the number of leaves, leaflets and consequently leaf area throughout the experiment, but at a much lower rate than in the control plants (Figure 1D, F and H). At 20 DAT, no significant effect of water restriction on the accumulation of total biomass was seen in either species. However, at 40 DAT, there was inhibition under severe stress in *E. contortisiliquum* and under the two water regimes tested in *E. velutina* (Figure 1I and J).

The LAF was not significantly affected by treatments on *E. contortisiliquum* throughout the experiment; however, a slight trend to increase under water restriction was shown (Figure 2A). In *E. velutina* there were also no significant changes (Figure 2B). In relation to biomass partitioning, there was a trend towards increasing LMF in *E. contortisiliquum* under water stress; this increase was significant at 40 DAT (Figure 2C). In *E. velutina*, however, no changes were found under water restriction (Figure 2D). In *E. contortisiliquum*, minor but not significant variations in SMF and RMF were observed on the two collection dates (Figure 2E and G). In *E. velutina*, however, effects that indicate an interaction between the stress restrictions tested and the investment of plants on stems or roots were observed at 20 DAT. Under moderate stress, there was greater investment in roots rather than stems, while under severe water stress, the plants invested more in the stems instead of roots (Figure 2F and H). Interestingly, this effect did not persist at 40 DAT (Figure 2F and H).

The drought stresses tested had little or no effect on the size of leaves and leaflets and SLA in *E. contortisiliquum* (Figure 3A, C and E). In *E. velutina*, however, we verified significant reductions in the size of both leaves and leaflets in the treatments under water restriction at 40 DAT (Figure 3B and D), but no effect occurred in SLA for the same time period (Figure 3F). Despite this effect in *E. velutina*, there was no effect on leaf format parameters, indicated by the absence of change in LI and dissection index (DI) (Figure 3H and J). In *E. contortisiliquum* also, no significant effects of water stress on leaf format were observed. The only significant effect was the reduction in DI at 40 DAT in stressed plants (Figure 3G and I).

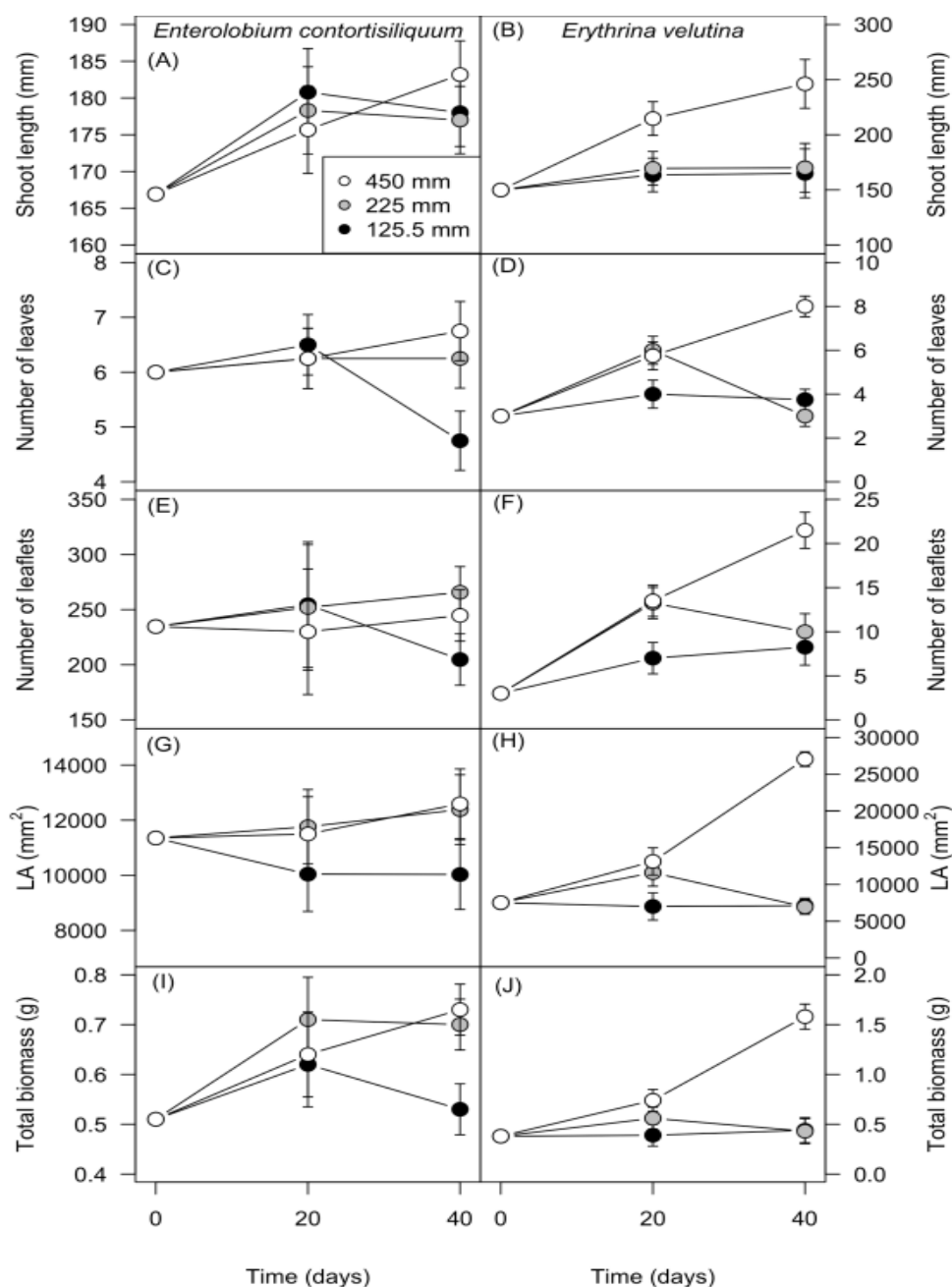


Figure 1 – Shoot length (A, B), number of leaves (C, D), number of leaflets (E, F), leaf area (G, H) and total biomass (I, J) in young plants *E. contortisiliquum* (Vell.) Morong. and *E. velutina* Willd., at three water regimes: 450 mm (control group), 225 mm (moderate stress) and 112.5 mm (severe stress) distributed over 40 days. The error bars represent the least significant difference by Student's *t*-test at 5%.

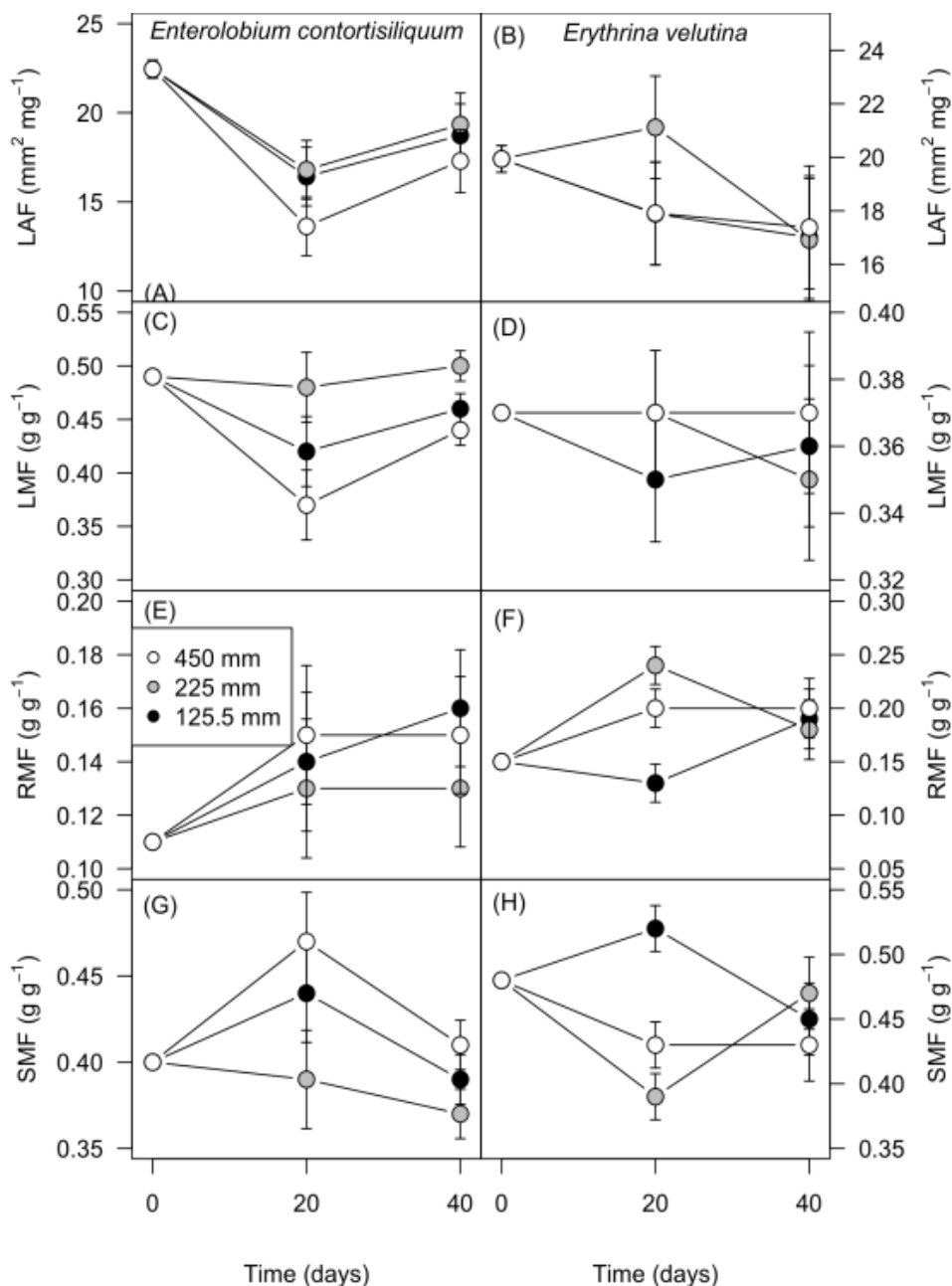


Figure 2 – LAF (A, B), LMF (C, D) RMF (E, F) and SMF (G, H) in young plants *E. contortisiliquum* (Vell.) Morong and *E. Willd.*, at three water regimes: 450 mm (control group), 225 mm (moderate stress) and 112.5 mm (severe stress) distributed over 40 days. The error bars represent the least significant difference by Student's *t*-test at 5 %.

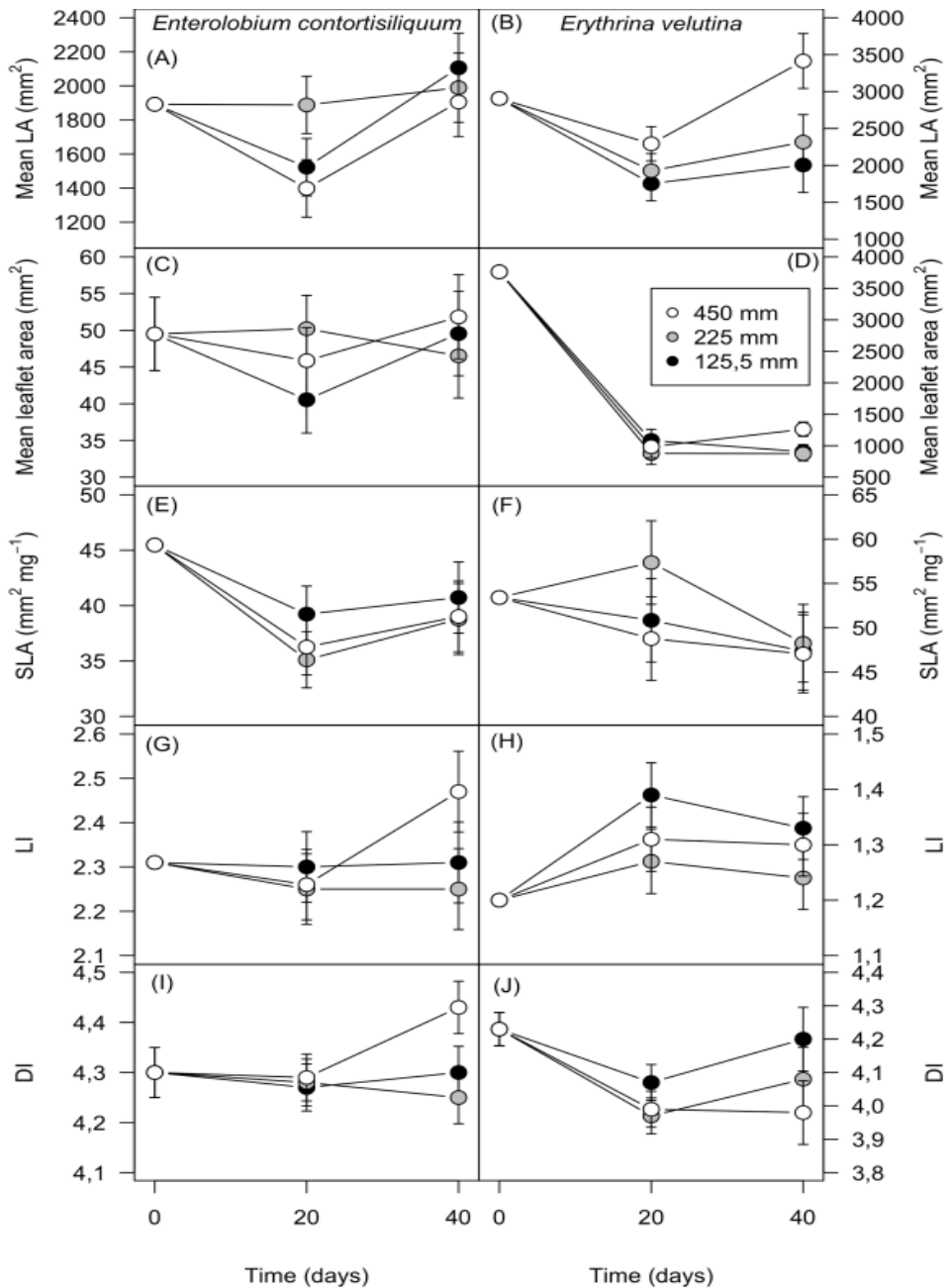


Figure 3 – Mean leaf area (A, B), mean leaflets area (C, D) specific leaf area (E, F), leaflet index (G, H) and dissection index (I, J) in young plants *E. contortisiliquum* (Vell.) Morong and *E. velutina* Willd., at 20 and 40 DAT with three water slides: 450 mm (control group), 225 mm (moderate stress) and 112.5 mm (severe stress). The error bars represent the least significant difference by Student's *t*-test at 5%.

Studies of several species have demonstrated the inhibitory effect of water stress on plant growth (Claeys and Inzé 2013). Generally, a reduction in biomass accumulation due to water restriction is observed (Magalhães Filho *et al.* 2008, Suassuna *et al.* 2012). Morphological and physiological changes occur to avoid the negative effects of water stress (Machado *et al.* 2009), leading us to hypothesise that photosynthesis should have been less inhibited in *E. contortisiliquum* in our study. In a field study with *Auxem maoncocalyx*, *Caesalpinia ferrea*, *Caesalpinia pyramidalis*, *Calliandra spinosa*, *Tabebuia aurea* and *Mimosa caesalpiniiifolia* (Dombroski *et al.* 2011), species which occur in Caatinga, it was found that even in the dry season, only the latter species showed significant inhibition of stomatal conductance and thus photosynthesis. *M. caesalpiniiifolia* is a species that produces leaves very early in the rainy season and which loses them faster than other species in the dry season (Maia, 2004), indicating a differential survival strategy. Our results lead us to consider that *E. contortisiliquum* should present strategies for dealing with drought that are similar to the majority of species studied by Dombroski *et al.* (2011); that is, this species must invest in mechanisms to maintain high water status in tissues for the maintenance of gas exchange. *E. velutina*, on the other hand, should have a strategy similar to *M. caesalpiniiifolia*: faster growth or inhibition of growth, depending on the availability of water.

In *M. caesalpiniiifolia*, *Prosopis juliflora* and *T. aurea* plants under water stress, reduction but not complete inhibition of the production of new leaves were observed (Silva and Nogueira 2003). In the same study, the authors verified leaf abscission in *E. contortisiliquum*, as reported in both species in our experiment. The availability of water in the environment determines the leaf area of a plant (Baerenfaller *et al.* 2012, Massonnet *et al.* 2015), inducing larger areas in humid and smaller in arid environments. This feature is an important defence mechanism against excessive water loss; reduced leaf area is xeromorphic characteristic among the many that are identified in plants under water shortage conditions (Villagra and Cavagnaro 2006). A large leaf area can be advantageous to plants for the production of biomass, but in conditions of water stress, this may be disadvantageous because it increases the surface transpiration, leading to the rapid depletion of ground water. Taking into account that no major changes in leaf form characteristics were found in either species, the inhibition of leaf and leaflet size in *E. velutina* supports our hypothesis that water restriction tolerance in this species should be related to the inhibition of growth.

In *E. contortisiliquum*, we may parallel the small effect of water restriction on biomass partitioning with the small growth inhibition. Thus, this should be a species that maintains higher water status under restriction but not investing more in roots or stems. In *E. velutina*, variation in investment preference in roots or stems, under moderate and severe water restrictions, respectively, may indicate that different parts of the plants suffered the effects of water restriction differently, or that the partition of biomass is a tolerance strategy in this species. Greater biomass investment in roots under water restriction is well documented

in the literature (Baluska *et al.* 2010, Praxedes *et al.* 2010, Xu *et al.* 2013), which must be a strategy to explore a larger volume of soil. However, based on our study, we cannot yet indicate a controlled change in growth in response to water deficit.

In most of the studies with water restriction, the authors state that less inhibition of growth is correlated with stress tolerance. Therefore, it should be said that *E.velutina* suffered most as a result of our experimental conditions. However, both species studied here are well adapted to the dry climate of Caatinga. Thus, the restriction in growth should be a strategy for living with drought as important as the strategies aimed at maintaining growth.

CONCLUSION

Generally the growth of *E.velutina* was more inhibited by water stress than in *E.contortisiliquum*. Inhibition of growth in adverse situations must be one important strategy for dealing with drought in Caatinga.

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REFERENCES

- Andrade, W.M., Lima, E.A., Rodal, M.J.N., Encarnaç o, C.R.F. & Pimentel, R.M.M. 2009. Influ ncia da precipita o na abund ncia de popula es de plantas da Caatinga. *Revista de Geografia*, 26:161-184.
- Baluska, F., Mancuso, S., Volkmann, D. & Barlow P.W. 2010. Root apex transition zone: a signaling-response nexus in the root. *Trends in Plant Science*, 15:402-408.
- Baerenfaller, K., Massonnet, C., Walsh, S., Baginsky, S., Buhlmann, P., Hennig, L., Hirsch-Hoffmann, M., Howell, K.A., Kahlau, S., Radziejowski, A., Russenberger, D., Rutishauser, D., Small, I., Stekhoven, D., Sulpice, R., Svozil, J., Wuyts, N., Stitt, M., Hilson, P., Granier, C. & Grissem, W. 2012. Systems-based analysis of Arabidopsis leaf growth reveals adaptation to water deficit. *Molecular Systems Biology*, 8:1-18.
- Claeys, H. & Inz , D. 2013. The agony of choice: How plants balance growth and survival under water-limiting conditions. *Plant Physiology*, 162:1768-1779.
- Crawley, M.J. 2012. *The R Book*. John Wiley & Sons Ltd, West Sussex, UK. 1076 pp.
- Dombroski, J.L.D., Praxedes, S.C., Freitas, R.M.O. & Pontes, F.M. 2011. Water relations of Caatinga trees in the dry season. *South African Journal of Botany*, 77:430-434.
- EMPARN, Empresa de Pesquisa Agropecu ria do Rio Grande do Norte. 2016. *Monitoramento Pluviom trico*. <http://186.250.20.84/monitoramento/> (accessed November 13, 2016).
- Krasensky, J. & Jonak, C. 2012. Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. *Journal of Experimental Botany*, 63: 1593-1608.
- Machado, R.S., Ribeiro, R.V., Marchiori, P.E.R., Machado, D.F.S.P., Machado, E.C. & Landell, M.G.A. 2009. Respostas biom tricas e fisiol gicas ao d ficit h drico em cana-de-a u car, em diferentes fases fenol gicas. *Pesquisa Agropecu ria Brasileira*, 44: 1575-1582.

- Magalhães Filho, J.R., Amaral, L.R., Machado, D.F.S.P., Medina, C.L. & Machado, E.C. 2008. Deficiência hídrica, trocas gasosas e crescimento de raízes em laranja 'Valência' sobre dois tipos de porta-enxerto. *Bragantia*, 67: 75-72.
- Maia, G.N. 2004. *Caatinga: árvores e arbustos e suas utilidades*. Leitura e Arte, São Paulo, Brazil. 413 pp.
- Menezes, J.A.L., Santos, T.E.M., Montenegro, A.A.A. & Silva, J.R.L. 2013. Comportamento temporal da umidade do solo sob Caatinga e solo descoberto na Bacia Experimental do Jatobá, Pernambuco. *Water Resources and Irrigation Management*, 2: 45-51.
- Menezes, R.S.C., Sampaio, E.V.S.B., Giongo, V. & Pérez-Marin, A.M. 2012. Biogeochemical cycling in terrestrial ecosystems of the Caatinga Biome. *Brazilian Journal of Biology*, 72: 643-653.
- Massonnet, C., Dauzat, M., Bédiée, A., Vile, D. & Granier, C. 2015. Individual leaf area of early flowering Arabidopsis genotypes is more affected by drought than late flowering ones: A multi-scale analysis in 35 genetically modified lines. *American Journal of Plant Sciences*, 6: 955-971.
- Praxedes, S.C., Lacerda, C.F., DaMatta, F.M., Prisco, J.T. & Gomes-Filho, E. 2010. Salt tolerance is associated with differences in ion accumulation, biomass allocation and photosynthesis in cowpea cultivars. *Journal of Agronomy and Crop Science*, 196: 193-204.
- R Core Development Team. 2016. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. <http://www.R-project.org/> (accessed November 13, 2016).
- Rathcke, B. & Lacey, E. 1985. Phenological patterns of terrestrial plants. *Annual Review of Ecology and Systematics*, 16: 179-214.
- Sack, L., Cowan, P.D., Aikumar, N.J. & Olbrook, N.M.H. 2003. The 'hydrology' of leaves: co-ordination of structure and function in temperate woody species. *Plant, Cell & Environment*, 26: 1343-1356.
- Santos, M.G., Oliveira, M.T., Figueiredo, K.V., Falcão, H.M., Arruda, E.C.P., Almeida-Cortez, J., Sampaio, E.V.S.B., Ometto, J.P.H.B., Menezes, R.S.C., Oliveira, A.F.M., Pompelli, M.F. & Antonino, A.C.D. 2014. Caatinga, the Brazilian dry tropical forest: can it tolerate climate changes? *Theoretical and Experimental Plant Physiology*, 26: 83-99.
- Shane, M.W., McCully, M.E., Canny, M.J., Pate, J.S., Huang, C., Ngo, H. & Lambers, H. 2010. Seasonal water relations of *Lyginiabarbata* (Southern rush) in relation to root xylem development and summer dormancy of root apices. *New Phytologist*, 185: 1025-1037.
- Silva, E.C. & Nogueira, R.J.M. 2003. Crescimento de quatro espécies lenhosas cultivadas sob estresse hídrico em casa-de-vegetação. *Revista Ceres*, 50: 203-217.
- Suassuna, J.F., Fernandes, P.D., Nascimento, R., Oliveira, A.C.M., Brito, K.S.A. & Melo, A.S. 2012. Produção de fitomassa em genótipos de citros submetidos a estresse hídrico na formação do porta-enxerto. *Revista Brasileira de Engenharia Agrícola e Ambiental*, 16: 1305-1313.
- Villagra, P.E. & Cavagnaro, J.B. 2006. Water stress effects on the seedling growth of *Prosopis argentina* and *Prosopis alpataco*. *Journal of Arid Environments*, 64: 390-400.
- Xu, W., Jia, L., Shi, W., Liang, J., Zhou, F., Li, Q. & Zhang, J. 2013. Abscisic acid accumulation modulates auxin transport in the root tip to enhance proton secretion for maintaining root growth under moderate water stress. *New Phytologist*, 197: 139-150.