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Article

Growth of *Vernonia ferruginea* Seedlings Submitted to Thermal Stress

*Crescimento de Plântulas de **Vernonia ferruginea** Submetidas a Estresse Térmico*

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ABSTRACT - Stress caused by extreme temperatures is one of the main elements that limit the geographical distribution and the seasonal growth of several plants, causing a severe delay in their development, reducing the photosynthetic rate and signaling the synthesis of defense compounds. Considering the current environmental changes and the damages that these changes can cause in plant physiology and growth, the objective of this work was to understand the interactions between temperature, physiology and growth, and to characterize the impact of temperature changes on the initial development of *Vernonia ferruginea* Less. Seedlings of this species were conditioned in germination chambers at previously adjusted constant temperatures (10, 20, 25, 30, and 35 °C) for sixty days, in order to evaluate physiological and growth parameters. The optimum temperature range for the initial growth of *V. ferruginea* is between 25 and 30 °C. The stress caused by sub-optimal and over-optimal temperatures affected cell homeostasis and caused a delay in the growth and development of seedlings. In stressful situations, growth inhibition and the activation of response mechanisms were observed for the adaptation and maintenance of cellular homeostasis through the accumulation of the proline osmoprotectant and soluble carbohydrates. Additionally, plants presented a normal development within a wide temperature range, despite the development delay, the change in gas exchanges and the synthesis of substances related to the defense system.

Keywords: biomass allocation, leaf area, gas exchanges.

*RESUMO - O estresse causado por temperaturas extremas configura-se como um dos principais elementos que limitam a distribuição geográfica e o crescimento sazonal de diversas plantas, provocando severo atraso no seu desenvolvimento, reduzindo a taxa fotossintética e sinalizando a síntese de compostos de defesa. Diante das mudanças ambientais em curso e dos danos que essas mudanças podem causar na fisiologia e no crescimento das plantas, o objetivo deste trabalho foi compreender as interações entre temperatura, fisiologia e crescimento, bem como caracterizar o impacto que a alteração da temperatura exerce sobre o desenvolvimento inicial de **Vernonia ferruginea** Less. Para determinação de parâmetros fisiológicos e de crescimento, plântulas dessa espécie foram acondicionadas em câmaras de germinação com as temperaturas constantes dos tratamentos previamente ajustadas (10, 20, 25, 30 e 35 °C) por 60 dias. A faixa ótima de temperatura para crescimento inicial de **V. ferruginea** está entre 25 e 30 °C. O estresse causado pelas temperaturas subótimas e supraótimas afetou a homeostase celular e provocou atraso no crescimento e desenvolvimento das plântulas. Em situações estressantes, observou-se a inibição do crescimento e a*

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ativação de mecanismos de resposta para a adaptação e manutenção da homeostase celular mediante o acúmulo do osmoprotetor prolina, e carboidratos solúveis. Adicionalmente, as plantas apresentaram desenvolvimento normal dentro de uma ampla faixa de temperatura, apesar do atraso no desenvolvimento, da alteração nas trocas gasosas e na síntese de substâncias relacionadas ao sistema de defesa.

Palavras-chave: alocação de biomassa, área foliar, trocas gasosas.

INTRODUCTION

Plants, as sessile organisms, are often exposed to adverse conditions such as water restriction, soil salinity, heavy metal toxicity, nutritional deficiency or toxicity, variations of ultraviolet radiation, as well as high and low temperatures (Silva et al. 2015).

When a species is exposed to stress factors, specific receptors first perceive the signals coming from the environment and, upon activation, initiate a series of signals that transfer information between cells and, in many cases, trigger transcription factors to induce the expression of specific gene sites (Lima et al., 2016).

Stress promoted by sub-optimal and over-optimal temperatures modifies cellular homeostasis, slows plant growth and development, reduces productivity, and sometimes causes plant death (Krasensky and Jonak, 2012).

Climatic factors, such as excessively low or high temperatures, are significant sources of abiotic stress, which act by potentiating or limiting the development of a species, as well as altering its photosynthetic capacity, biomass accumulation and even the senescence rate (Larcher, 2006; Silva et al., 2015).

Photosynthesis is recognized as one of the most sensitive processes to temperature changes. Thus, understanding the physiological mechanisms on which the response to the temperature of photosynthesis and the events associated with acclimatization are based is of extreme importance for both agriculture and the environment (Yamori et al., 2014).

A higher air temperature can directly affect the metabolism of young plants and seedlings, modifying the speed of chemical reactions and the activity of the enzymes that participate in these processes, as well as increasing soil temperature, which can compromise the development of plants (Costa et al., 2015).

Soil temperature has a significant effect on the growth and development of many species, since the metabolic functions of plants can be altered by the nutrients available in the soil and the rate of water absorption (Carneiro et al., 2014).

Low air temperatures or stress caused by cold affect the growth, development, reproduction and distribution of species by reducing their metabolic rate, since low temperatures cause damages to the photosynthetic apparatus and to the chlorophyll molecule (Rodrigues et al. 2014).

At the vegetative phase, cell growth and expansion are directly linked to the moisture content in the cell. The reduction of the ambient temperature can promote the reduction of the water potential gradient between the intracellular space and the atmosphere, limiting water absorption by the root system and, as a result, providing the closure of the stomata and the reduction of transpiration, photosynthetic rate and growth (Lima et al., 2016).

In addition to changes in plant growth and development, stressful conditions can often coordinate or change the rate of metabolite production, since metabolites consist of a chemical interface between plants and the surrounding environment; therefore, their synthesis is usually influenced by environmental conditions (Gobbo-Neto and Lopes, 2007).

The species popularly known as ironweed (*Vernonia ferruginea* Less.), belonging to the Asteraceae family, is considered one of the main pasture weeds. Despite its potential use in the regeneration of disturbed fragments or degraded areas and the economic damages caused by the reduction of pasture capacity throughout the Brazilian territory, there are currently few studies on its development. Ironweed is a plant that originates in the cerrado, it grows naturally in

environments with a markedly seasonal climate and it is potentially subject to growth and development under the threat of extreme weather events.

Thus, the objective of this work was to understand the interactions between temperature, physiology and growth, as well as to characterize the impact that temperature changes have on the initial development of *V. ferruginea*.

MATERIAL AND METHODS

Seedling production

Achenes of *V. ferruginea* were collected in a natural regeneration area, at the geographical coordinates of 21°14' S e 48°16' W, at 560 m of altitude. These seeds were placed to germinate in a container with a 7 L capacity, filled with soil and sand in the ratio of 3:1 (v/v). After filling the pots, a composite sample of the substrate (soil-sand) was collected for routine chemical and physical characterization (Table 1), according to Embrapa's soil analysis procedures (1997).

Table 1 - Physical and chemical properties of the soil

Soil parameter									
pH	MO	P_{resin}	K^+	Ca^{2+}	Mg^{2+}	$H+Al^{3+}$	SB	T	V
($CaCl_2$)	(g dm^{-3})	(mg dm^{-3})	($mmol_c$ dm^{-3})						(%)
6.2	15.0	96.0	5.7	54.0	23.0	10.0	82.7	92.7	89.0
Clay		Silt		Fine sand		Coarse sand		Texture	
379		101		187		333		Clayey	

Sum of Bases (SB) = $K^+ + Ca^{2+} + Mg^{2+}$; Total acidity at pH 7 = $H+Al^{3+}$; Total cation exchange capacity at pH 7 (T) = SB + $H+Al^{3+}$; Base saturation (V) = (100 * SB) / T. Size of soil solid phase components: Clay: <0.002 mm or 2 µ; Silt: 0.02 to 0.002 mm; Fine sand: 0.2 to 0.02 mm; Coarse sand: 2 to 0.2 mm.

Thermal stress

When *V. ferruginea* seedlings reached, on an average, 1.2 cm in height, 7.8 cm in root length, two leaves and 0.17 cm in stalk diameter, they were transferred to a 0.5 L capacity pot, filled with substrates whose properties are described in Table 1.

Seedlings were conditioned in germination chambers (GC) for 60 days, having their constant treatment temperatures previously adjusted (10, 15, 20, 25, 30, and 35 °C), under a light intensity of 110 µmol m^{-2} s^{-1} and a photoperiod of 12 hours.

The experimental design adopted for the experiment was a randomized block design with six treatments in six replications.

Growth analysis

At the end of the stress period, the number of leaves was counted through a direct count of fully expanded leaves per plant; the stem of each plant was collected, being cut at the height of the neck, with the help of a cutter. Plants were fractioned in shoot and roots. After that, it was possible to determine: shoot and root length; neck diameter, with the help of a 0.05 mm accuracy precision caliper; dry matter, placing plants, separated into root, stalk and leaves, in a forced air circulation greenhouse at 40 °C for 120 hours; fluorescence, using a portable fluorometer (PEA - Plant Efficiency Analyzer, Hansatech); and the chlorophyll content of the third fully expanded leaf, with the help of the Falker chlorophyllometer, clorofiLOG model.

Gas exchange

Readings about gas exchange, photosynthetic rate (A), transpiration rate (E), stomatal conductance (gs), and internal carbon ratio and reference carbon (Ci/Cref) were obtained using

an Infrared Gas Analyzer (IRGA - Infra Red Gas Analyzer) (LI-6400; LICOR®, Inc., Lincoln, NE, USA). Measurements were taken on leaves developed at the end of the experimental period, between 8 and 10 a.m., using the temperature and humidity of each treatment and an artificial light source of 1,000 µmol photons m^{-1} s^{-1}.

Cell membrane disruption

For the analysis of the cell membrane disruption, six leaf disks with an area of 113 mm^2 each were collected, which were washed with distilled water and placed on Petri dishes containing 24 mL of distilled water. The plates were sealed with parafilm and maintained at 25 °C for 90 minutes. After this period, the initial electrical conductivity of the medium was measured (ci) (Digital database conductivity meter C708 PLUS Complete). Plates were then subjected to the temperature of 80 °C for 90 minutes and the electrical conductivity was measured again (cf). The quantification of the electrolyte leakage percentage was obtained by adapting the model: (ci/cf) x 100 (Campos and Thi, 1997).

Quantification of soluble carbohydrates

The quantification of soluble carbohydrates was carried out by macerating 350 mg of plant material in 80% ethanol. The macerated material was centrifuged at 3,000 rpm for ten minutes, and the supernatant was transferred to a beaker; the residue was washed with an ethanolic solution and centrifuged two more times. The supernatants were combined, and the volume was added with an 80% ethanolic solution up to 25 mL. Subsequently, they were depigmented with chloroform and evaporated on a heating plate at 60 °C. After evaporation, 3 mL of distilled water were added. From this solution, it was possible to quantify the total soluble carbohydrate contents, which were determined through reactions with anthrone, according to Clegg (1956), reading the samples in a spectrophotometer at 620 nm.

Free proline dosage

The quantification of the free proline content of leaves and roots was performed according to the methodology of Bates et al. (1973), in which 500 mg of lyophilized samples of plant tissue were submitted to reactions with sulfosalicylic acid, glacial acetic acid, ninhydrin acid and toluene. Absorbance readings were made with a spectrophotometer at 520 nm. Proline contents were calculated as mmol of proline/g dry matter (µmol g^{-1} DM).

Statistical analysis

The results of the evaluated parameters were submitted to analysis of variance, and the means were compared by Tukey's test at 5% of probability; data were represented by box-plot graphs.

RESULTS AND DISCUSSION

The presence, absence or even abundance of a species in a given area is conditioned by the action of extreme climatic factors. Air temperature stands out among the determining factors that can limit the geographic distribution of a species, as well as its establishment and survival capacity in different environments.

One of the ways to characterize the development of a plant is by counting the number of leaves of the main stem, since this parameter influences the photosynthetic process and, therefore, the physiology of the plant (Streck et al., 2007).

Plants of *V. ferruginea*, when submitted to treatments with different air temperatures (10, 15, 20, 25, 30, and 35 °C) presented a larger number of leaves in environments with a temperature of 25 °C; this did not present significant differences only for the treatment with a temperature of 30 °C (Figure 1A).

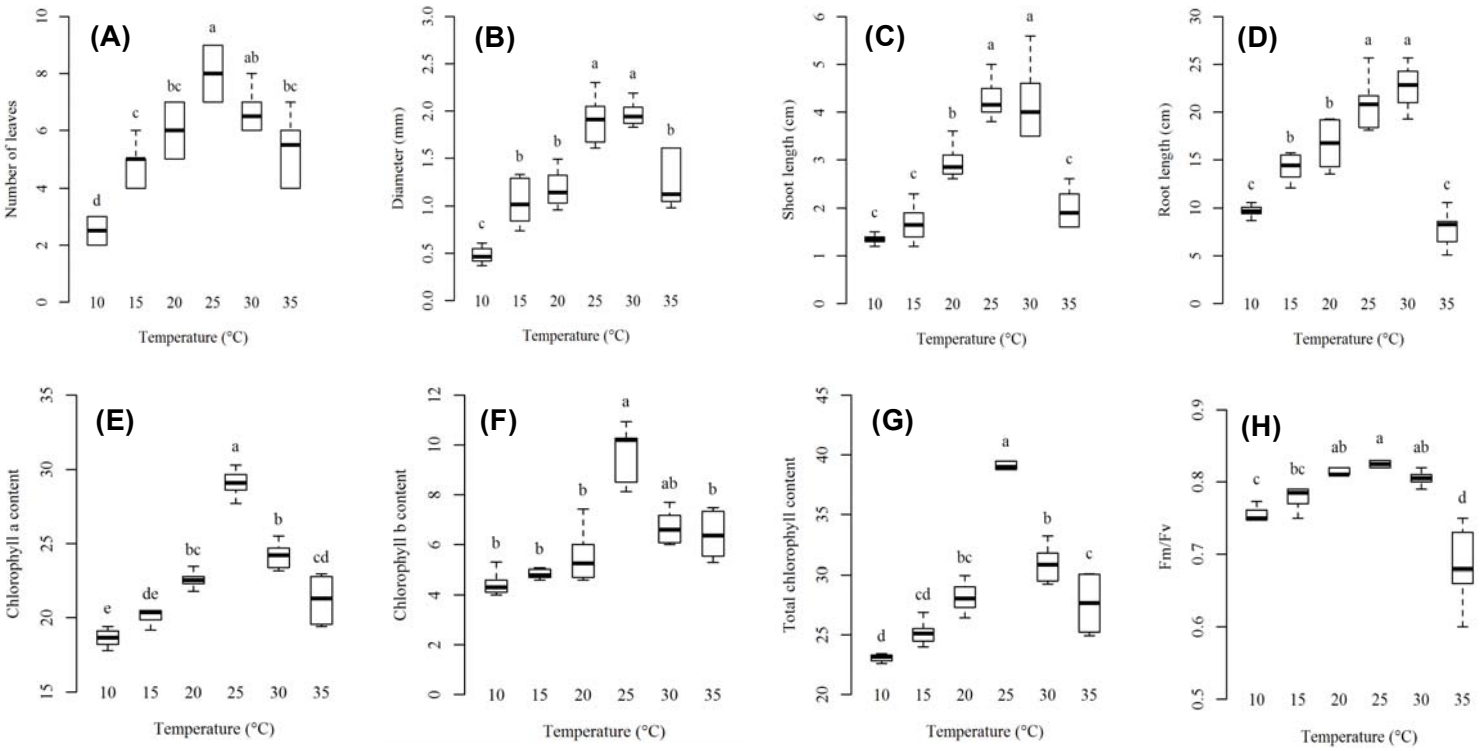

Figure 1 - Number of leaves (A), stalk diameter (B), shoot (C) and root (D) length, chlorophyll a (E), b (F), total (G) chlorophyll content, and quantum efficiency of photosystem II (H) of *V. ferruginea* submitted to different air temperatures (15, 20, 25, 30, and 35 °C).

The leaf number of a plant is related to the definition of the development phase of several species (Schons et al., 2007), and, when compared, they can indicate the most favorable growth conditions. This parameter is also related to the evolution of the leaf area (Schons et al., 2007). In turn, the leaf area is associated with the interception of solar radiation, photosynthesis and good plant development (Schons et al., 2007).

The leaf number variable indicates that this accession is more adapted to air temperatures ranging between 20 and 30 °C. It was also observed that the lowest tested temperature (10 °C) provided the least number of leaves, differing significantly from the other treatments. In the photosynthetic process, CO_2 fixation and reduction occurs slowly at low temperatures, increasing with heating, until reaching the optimum temperature (Costa et al., 2015).

The highest neck diameter values were obtained from seedlings submitted to temperatures of 20 and 30 °C (Figure 1B). These values show greater growth than the other treatments, since a larger diameter reveals greater partitioning of the shoot photoassimilated compounds (Scalon et al., 2001).

At temperatures above the optimum one, the carboxylation of ribulose 1,5-bisphosphate (RuBP) decreases considerably, due to the temperature increase, and the O:CO_2 ratio is modified; the solubility of CO_2 decreases in relation to that of O_2 and, thus, photorespiration is favored, reducing the photosynthetic yield. There is also an unbalance promotion in the input and output of CO_2 by plants (Costa et al., 2015).

The rate at which maximum air temperature limits the development of a species is directly related to its sensitivity to heat and to the intensity of an increased respiration according to the temperature (Lima et al., 2010; Costa et al., 2010).

The increase of the stalk diameter is related to the exchange activity stimulated by the carbohydrates produced in the photosynthesis and the hormones translocated from the apical regions; this can be used as a good evaluation parameter for liquid assimilation (Braun et al., 2007).

Knowing the ability of a species to withstand air temperature variations is important, since each phenological stage is influenced by this temperature gradient (Duarte et al., 2011).

Comparing the mean values of shoot and root length, it was verified that the highest values were also obtained in seedlings submitted to treatments of 25 and 30 °C (Figure 1C, D). Thermal stress is one of the primordial factors that restrict the development and adaptation of some species (Silva et al., 2000), and it may limit them to certain regions according to the temperature. An increase in air temperature promotes the reduction of the metabolic activity and the increased respiration of many species, directly affecting their growth and development (Costa et al., 2015).

Similarly to what was observed through the processes triggered by stress with high temperatures, low temperatures can also produce changes in metabolism; however, according to Lima et al. (2016), responses to low temperatures are different from responses to high temperatures.

Low temperatures limit the growth of some species, due to the obstruction of the expression of their genetic potential, the inhibition of metabolic reactions and, indirectly, the restriction of water absorption and cell dehydration, induced by the low temperature (Perboni et al., 2015) .

Plants submitted to low temperatures can also present an increase in compatible solutes; changes in permeability or even loss of plasma membrane integrity; antioxidant substance increases in various plant organs; and a reduction of the conductivity of the xylem and the moisture content in leaves (Navarrete-Campos et al., 2013, Mora and Serra, 2014, Yang et al., 2015).

Membranes are sensitive to the exposure to low temperatures, as they present lipids with a high percentage of saturated fatty acid chains, which tend to solidify, making the membranes less fluid; in addition, protein components may not function normally. This damage to the chloroplast membrane leads to the reduction of photosynthesis. The primary phytochemical reactions of photosynthesis, such as the electron transport along the thylakoid membrane, are impaired by the loss of membrane fluidity resulting from the exposure to cold (Larcher, 2006). Different components of the photosynthetic process are affected when the plant is exposed to a low temperature, such as the inhibition of chlorophyll biosynthesis and the damage to the chloroplast electron transport chain. Soluble enzymes from the stroma of the photosynthesis biochemical phase can also be affected by low temperatures.

Data about chlorophyll a, chlorophyll b and total chlorophyll content indicate that seedlings submitted to a temperature of 25 °C presented the best development (Figure 1E, F, G). Chlorophyll is involved in the photosynthetic efficiency of plants, and therefore with growth and adaptability to different environmental conditions (Jesus and Marenco, 2008). Chlorophyll content analysis is also used to investigate the nutritional status of several species in relation to N content, due to the significant correlation between green intensity and chlorophyll content with the accumulation of N in the leaf (Pôrto et al. , 2014).

Plants submitted to treatments with temperatures of 10, 15, and 35 °C had the lowest values of total chlorophyll, differing from the treatment at 25 °C. Low values represent a poor chlorophyll content, which may limit the development of a species, since chlorophyll is bound to the photosynthetic processes and its deficiency may restrict development and mass accumulation (Vieira et al., 2014).

As for the photosystem II quantum efficiency of *V. ferruginea* seedlings, treatments at temperatures of 10 °C, 15 °C and 35 °C provided a significant reduction compared to the 25 °C treatment (Figure 1H), indicating that at that temperatures photosynthesis inhibition occurred, since the reduction of the ratio between variable fluorescence (Fv) and maximum fluorescence (Fm) is a reliable indicator of photoinhibition (Janda et al., 1994). The reduction of this variable in response to stress indicates the loss of photochemical efficiency (Wang et al., 2013).

Photosystem II is very sensitive to thermal variations, since the electron transport chain can be affected by temperature changes (Haque et al., 2014). High temperatures can cause the photoinhibition of the photosynthesis. The intensity of this physiological process, defined by the slow and reversible reduction of photosynthesis as a consequence of the exposure to high temperatures, can be estimated by the reduction in the quantum efficiency of photosystem II (Fv/Fm ratio) (Dias and Marenco, 2007).

The distribution of dry matter to the different organs of the plant (root, stalk and leaves), as in the growth variables, varied according to the temperature (Figure 2). Plants showed significant

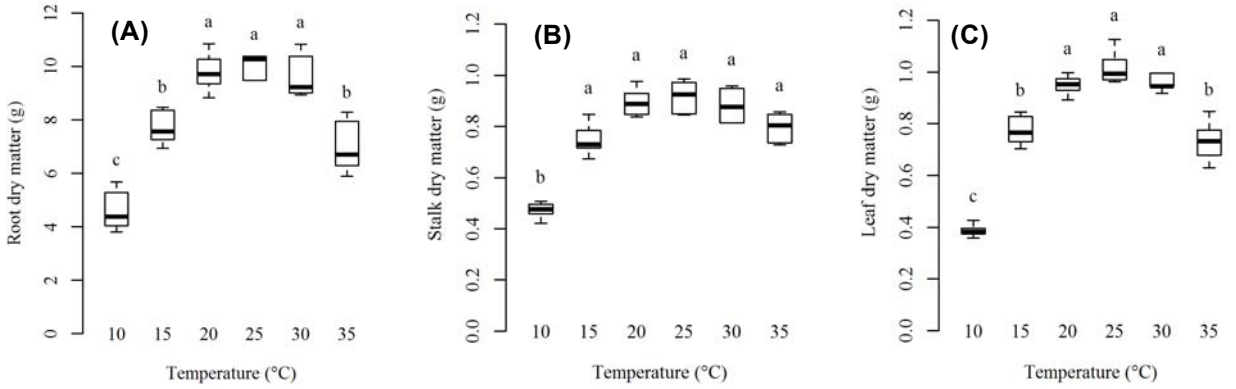

Figure 2 - Root dry matter (A), stalk dry matter (B), leaf dry matter (C) of *V. ferruginea* submitted to different air temperatures (10, 15, 20, 25, 30, and 35 °C).

differences in the dry matter production of roots and leaves, but for the stalk, a significant difference was observed only at the temperature of 10 °C. The proportion of dry matter directed to both roots and shoot increased in plants that developed between the temperatures of 20 and 30 °C.

The lowest temperatures had a negative influence on all analyzed variables. Low temperatures are among the environmental factors that significantly alter plant growth, as they affect photosynthesis, water and nutrient absorption, metabolite production and release, among other processes (Airaki et al., 2012).

At the temperature of 10 °C, seedling growth was reduced and/or discontinued. The available metabolic energy is lower under low temperature conditions, restricting the absorption of water and nutrients. Thus, biosynthesis processes occur at lower intensities, and assimilation is reduced, which may lead to a decreased plant development capacity (Larcher, 2006).

All the parameters evaluated under thermal stress were affected at the highest temperatures. This may have occurred because relatively high temperatures may damage the photosynthetic apparatus due to changes in the thylakoid membrane and in the physical-chemical properties, as well as changes in the functional organization of these cellular structures (Dias and Marenco, 2007).

The highest temperature (35 °C) also provided significant reductions in seedling growth, since the mean of variables such as shoot and root length showed a reduction of 53.5% and 65.1%, respectively, compared to the treatments that presented the greatest growth. Several physiological damages can be observed at high temperatures, such as leaf abscission and senescence, root and shoot development inhibition, changes in plant architecture, net assimilation rate reduction and, consequently, biomass accumulation reduction (Bita and Gerats, 2013).

As for the gas exchange variables (liquid photosynthesis, stomatal conductance, transpiration and ratio between internal and external carbon), significant differences were observed among the treatments (Figure 3).

The photosynthetic rate (A) was affected by the variation of the air temperature, since the highest photosynthetic activity occurred at 25 °C, reaching a minimum value at 10 °C (Figure 3A). Although cell stretching, the main component of plant growth, is among the processes that are most sensitive to high temperature stress, Jajoo and Allakhverdiev (2017) show that photosynthetic disorders stand out as the first sign of a stress status due to heating.

The reduction of the photosynthetic rate at high temperatures occurs mainly because of the sensitivity of the thylakoid membrane, with a reduction of chlorophyll production due to the closure of the stomata and with the consequent reduction of CO_2 assimilation, and to photochemical damages (Bita and Gerats, 2013).

Changes in air temperature affected stomatal conductance (gs); the lowest values were found in the treatments with the highest temperatures (Figure 3B).

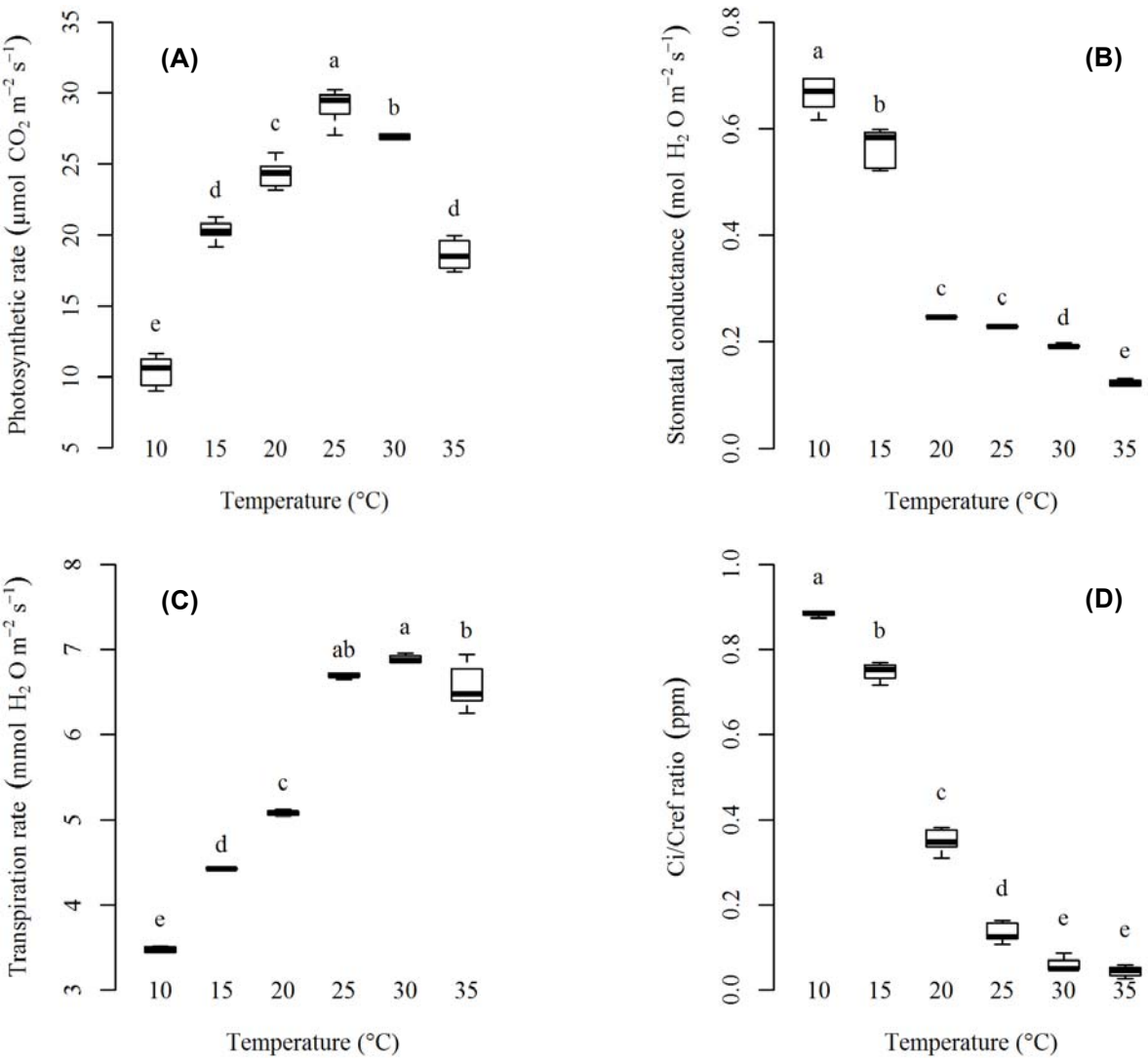

Figure 3 - Photosynthetic rate (A), stomatal conductance (B), transpiration rate (C) and ratio between internal and external carbon (Ci/Cref) (D) of *Vernonia ferruginea* plants submitted to different air temperatures (10, 15, 20, 25, 30, and 35 °C).

Under stressful conditions, the stomatal movement constitutes an important means of plant defense (Taiz and Zeiger, 2017). However, stomatal opening, unlike what was observed under water stress conditions, is slightly affected by high temperatures. A higher temperature effect occurs on the chloroplast ultrastructure, activating senescence and the action of proteolytic and lipolytic enzymes (Bita and Gerats, 2013).

The temperature increase significantly affected the transpiration rate (E) of *V. ferruginea*, and seedlings submitted to the treatment at 10 °C showed a lower value, approximately 3.4 mmol H_2O m^{-2} s^{-1}, differing from the other treatments (Figure 3C).

High temperatures may have a negative effect on the temperature of leaves, increasing exponentially the evaporative demand and providing high transpiration rates and low water potentials (Hall, 2001).

The ratio between internal carbon and reference carbon (Ci/Cref) showed significant differences among the treatments; it was higher in individuals submitted to higher temperatures (Figure 3D).

Increasing the temperature caused an increased permeability of the membrane, allowing greater electrolyte leakage from the cells into the medium (Figure 4). A temperature increase may promote the denaturation of membrane proteins, affecting the activity of enzymes that are important for the preservation of its integrity, such as peroxidase, catalase and superoxide

dismutase antioxidants, and it can also cause membrane lipid peroxidation (Hasanuzzaman et al. 2013).

Temperature also altered the amount of soluble carbohydrates; high temperatures provided higher leaf and root contents of *V. ferruginea* seedlings (Figure 5A, B). Research suggests that high amounts of carbohydrates in plants represent an important physiological trait of thermal stress tolerance (Bita and Gerats, 2013).

Another manifestation of the stress state of an individual, regardless of the nature of the stress agent, is the appearance of osmotically active substances, such as proline.

The proline content of *V. ferruginea* samples ranged from 20.8 to 42.7 µmol g^{-1} DM in the leaves and 7.2 to 13.2 µmol g^{-1} DM in the roots, showing a 104.8% increase in leaves, while in the roots the increase was 83.1% (Table 2).

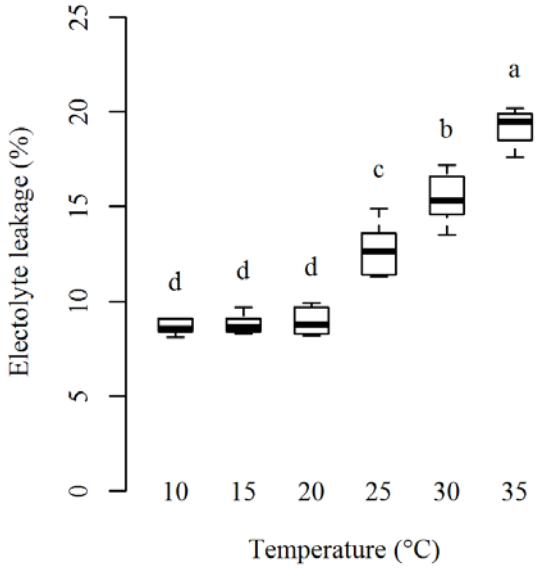

Figure 4 - Electrolyte leakage from leaves of *Vernonia ferruginea* plants submitted to different air temperatures (10, 15, 20, 25, 30, and 35 °C).

The highest proline content was found, both in leaves and roots, in treatments with extreme temperature values. The high proline content found in *V. ferruginea* seedlings under thermal stress suggests that this amino acid has acted as an osmoregulator or a signal, capable of activating multiple responses that are part the acclimatization process (Silva et al., 2015).

The analysis of the results on proline levels still shows a certain relation between proline levels and the stress intensity imposed on the seedling.

The proline accumulation tendency in plants under stress has been commonly reported in literature. Many studies have related proline accumulation to the induction of osmotic adjustment with low and high temperature stress protective effects, to prevent protein denaturation, to preserve the structure of enzymes, to protect membranes from the deleterious effects caused by reactive oxygen species (ROS) and to inhibit protein aggregation (Silva et al., 2015).

Another evaluated factor was the survival of plants submitted to thermal stress. In the treatment at 40 °C, 100% of plants were dead by the 14th day of treatment, and, thus, this treatment

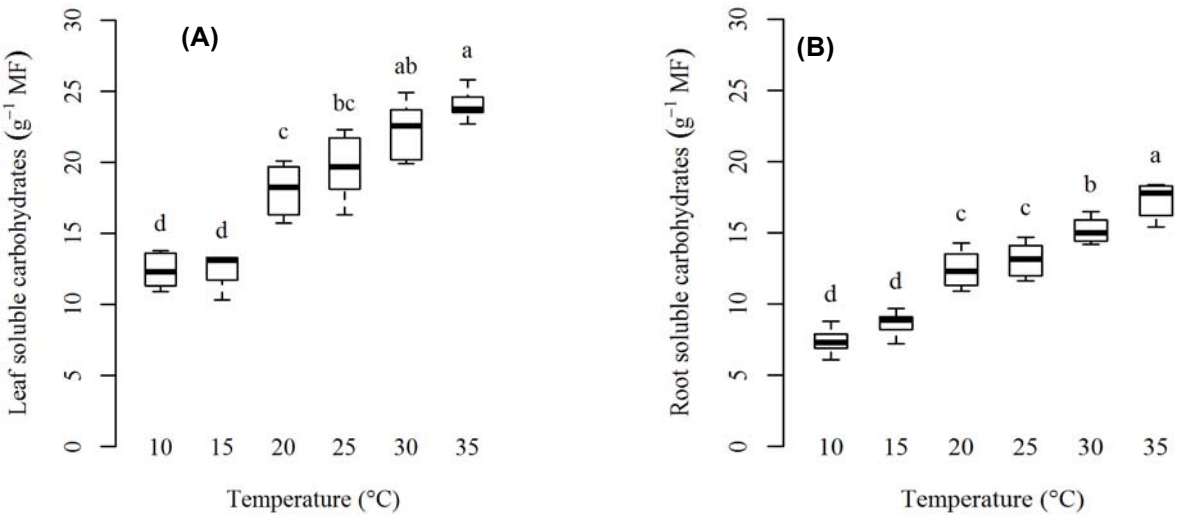

Figure 5 - Total soluble carbohydrate contents of leaves (A) and roots (B) of *Vernonia ferruginea* plants submitted to different air temperatures (10, 15, 20, 25, 30, and 35 °C).

was discarded from further evaluations; whereas the other treatments, even if with different developments, allowed 100% survival.

Thermal stress promotes changes and responses to functional levels in the plant organism, which may become irreversible or compromise its development. This can be verified in the evaluated parameters and in the plants that were submitted to temperatures of 40 °C and did not survive.

Table 2 - Proline content of leaves and roots of *Vernonia ferruginea* plants submitted to different air temperatures (10, 15, 20, 25, 30, and 35 °C)

Temperature	Leaf proline	Root proline
(°C)	(µmol g^{-1} DM)	
10	42.7 A	13.2 a
15	32.8 B	10.4 b
20	27.2 C	7.8 c
25	20.8 D	7.2 c
30	22.1 D	7.3 c
35	33.2 B	11.0 b
F	111.5 **	58.2 **
VC (%)	6.4	8.2
LSD	3.3	1.4

** Significant at 1% probability; Means followed by the same letters do not differ by Tukey's test (p>0.05).

The development of *Vernonia ferruginea* seedlings tends to be greater in a narrower temperature range, 25 °C to 30 °C; however, the species showed a better development over a wide temperature range: 10 °C to 35 °C. During their evolution, some plants have developed processes that help them keep growing and developing despite factors that hinder their homeostasis. These processes are related to genetic diversity and defense mechanisms, modulated by modifications during stressful periods, in order to maintain their photosynthetic performance, as well as optimizing it in different situations (Timperio et al., 2008).

Sometimes, however, this is not possible, since depending on stress, duration and/or stage of plant development, it can be lethal, as the species may not find time to defend itself from these disturbances (Fischer et al., 2011). A limiting temperature for the development and growth of plants is a decisive factor in the distribution of species in different environments, since plants do not have mobility and, when they are under unfavorable conditions, their capacity to support and/or acclimate to the temperature variation of the environment is essential for their survival (Yamori et al., 2014).

Many species adopt a phenomenon known as a shift in the optimal temperature of photosynthesis, through which the plant increases its photosynthetic efficiency at its new growth temperature when changes in temperature occur, whether seasonal changes or unexpected extreme fluctuations (Yamori et al., 2014).

The air temperature variation during the initial development of *V. ferruginea* affects both the growth and physiological parameters, and also the synthesis of compounds such as proline.

The greatest growth in height, stalk diameter, number of emitted leaves and total dry matter was verified in plants submitted to the temperature of 25 °C, and the optimal temperature range for the initial growth of *V. ferruginea* is between 25 and 30 °C.

The stress caused by sub-optimal and over-optimal temperatures affected the cellular homeostasis and caused a delay in the growth and development of seedlings; it also promoted the activation of response mechanisms for the adaptation and maintenance of cellular homeostasis, through the accumulation of the proline osmoprotectant and soluble carbohydrates. However, the species developed at a large range of constant temperatures, confirming that it shows a considerable ability to adjust its photosynthetic characteristics to grow at a wide temperature range.

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REFERENCES

Airaki M, Leterrier M, Mateos RM, Valderrama R, Chaki M, Barroso JB et al. Metabolism of reactive oxygen species and reactive nitrogen species in pepper (*Capsicum annuum* L.) plants under low temperature stress. Plant Cell Environ. 2012;35(2):281-95.

Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant Soil. 1973;39(1):205-7.

Bita CE, Gerats T. Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. Front Plant Sci. 2013;4(273):1-18.

Braun H, Zonta JH, Zonta JSS, Reis EF. Produção de mudas de café 'Conilon' propagadas vegetativamente em diferentes níveis de sombreamento. IDESIA. 2007;25(3):85-91.

Campos PS, Thi ATP. Effect of abscisic acid pretreatment on membrane leakage and lipid composition of *Vigna unguiculata* leaf discs subject to osmotic stress. Plant Sci. 1997;130:11-18.

Carneiro RG, Moura MAL, Silva VPR, Silva Junior RS, Andrade AMD, Santos AB. Variabilidade da temperatura do solo em função da liteira em fragmento remanescente de Mata Atlântica. Rev Bras Eng Agric Amb. 2014;18(1):99-108.

Clegg KM. The application of the anthrone reagent to the estimation of starch in cereals. J Sci Food Agric. 1956;7(1):40-4.

Costa DCC, Pinheiro GS, Oliveira GM, Santos ATN, Santana VS, Angelotti F et al. Emergência e crescimento inicial de plântulas de cebola sob o aumento da temperatura e concentração de CO_2. Rev Bras Geogr Física. 2015;8:471-83.

Dias DP, Marenco RA. Fotossíntese e fotoinibição em mogno e acariquara em função da luminosidade e temperatura foliar. Pesq Agropec Bras. 2007;42(3):305-11.

Duarte LA, Schöffel ER, Mendez MEG, Schallenberger E. Alterações na temperatura do ar mediante telas nas laterais de ambientes protegidos cultivados com tomateiro. Rev Bras Eng Agríc Amb. 2011;15(1), 14:8-153.

Empresa Brasileira de Pesquisa Agropecuária – Embrapa. Manual de métodos de análises de solo. 2ª. ed. Rio de Janeiro: Centro Nacional de Pesquisa de Solos; 1997. 212p.

Fischer I, Camus-Kulanddaivelu L, Alla F, Stephan W. Adaptation to drought in two wild tomato species: the evolution of the *Asr* gene family. New Phytol. 2011:190:1032-44.

Gobbo-Neto L, Lopes N.P. Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. Quím Nova. 2007;30(2):374-81.

Hall AE. Crop responses to environment. Boca Raton: CRC Press; 2001.

Haque MS, Kjaer KH, Rosenqvist E, Sharma DK, Ottosen CO. Heat stress and recovery of photosystem II efficiency in wheat (*Triticum aestivum* L.) cultivars acclimated to different growth temperatures. Environ Exp Bot. 2014;99:139-50.

Hasanuzzaman M, Nahar K, Alam MM, Roychowdhury R, Fujita M. Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. Int J Mol Sci. 2013;14(5):9643-84.

Jajoo A, Allakhverdiev SI. High-temperature stress in plant: Consequences and strategies for protecting photosynthetic machinery. In: Shabala S editor. Plant stress physiology. Boston: CABI; 2017. p.138-54.

Janda T, Szalai G, Kissimon J, Páldi E, Marton C, Szigeti C. Role of irradiance in the chilling injury of young maize plants studied by chlorophyll fluorescence induction measurement. Photosynthetica. 1994;30:293-9.

Jesus SV, Marenco RA. O SPAD-502 como alternativa para a determinação dos teores de clorofila em espécies frutíferas. Acta Amaz. 2008;38(4):815-8.

Krasensky J, Jonak C. Drought salt, and temperature stress-induced metabolic rearrangement and regulatory networks. J Exp Bot. 2012;63(4):1593-608.

Larcher W. Ecofisiologia vegetal. São Carlos: Rima; 2006.

Lima ASF, Cantarelli MMC, Gonçalves AN. Relação entre cálcio e temperatura em explantes de *Eucalyptus grandis in vitro*. Nucleus. 2016;13(1):123-30.

Lima CSM, Gonçalves MA, Tomaz ZF, Rufato AR, Fachinello JC. Sistemas de tutoramento e épocas de transplante de *physalis*. Cienc Rural. 2010;40(12):2472-9.

Mora F, Serra N. Bayesian estimation of genetic parameters for growth, stem straightness, and survival in *Eucalyptus globulus* on an Andean Foothill site. Tree Genet Genomes. 2014;10(3):711-9.

Navarrete-Campos D, Bravo L, Rubilar R, Emhart V, Sanhueza R. Drought effects on water use efficiency, freezing tolerance and survival of *Eucalyptus globulus* and *Eucalyptus globulus* × nitens cuttings. New For. 2013;44(1):119-34.

Perboni AT, Martinazzo EG, Silva DM, Bacarin MA. Baixas temperaturas sobre a fluorescência da clorofila a em plantas de diferentes híbridos de canola. Cienc Rural. 2015;45(2):215-22.

Pôrto ML, Puiatti M, Fontes PCR, Cecon PR, Alves JC. Índice SPAD para o diagnóstico do estado de nitrogênio na cultura do pepino japonês em ambiente protegido. Hort Bras. 2014;32(3):292-6.

Rodrigues HCS, Bevilacqua CB, Bahry CA, Monzon DLR, Viana TP, Zimmer PD et al. Aplicação de índice de estresse para a tolerância ao frio no desenvolvimento inicial de cultivares de arroz. Científica. .2014;42(3):258-64.

Scalon SPQ, Scalon Filho H, Rigoni MR, Veraldo F. Germinação e crescimento de mudas de pitangueira (*Eugenia uniflora* L.) sob condições de sombreamento. Rev Bras Frut. 2001;23(3):652-5.

Schons A, Streck NA, Kraulich B, Pinheiro DG, Zanon AJ. Emissão de folhas e início de acumulação de amido em raízes de uma variedade de mandioca em função da época de plantio. Cienc Rural. 2007;37(6):1586-92.

Silva ACTF, Leite IC, Braz LT. Avaliação da viabilidade do pólen como possível indicativo de tolerância a altas temperaturas em genótipos de tomateiro. Rev Bras Fisiol Veg. 2000;12(2):156-65.

Silva J, Saccini VAV, Santos DMM. Estresse térmico no acúmulo de prolina livre de plântulas de guandu oriundas de sementes tratadas com poliaminas. Semina: Cienc Agr. 2015;36(1):103-22.

Streck NA, Michelon S, Rosa HT, Walter LC, Bosco LC, Paula GM. et al. Filocrono de genótipos de arroz irrigado em função de época de semeadura. Cienc Rural. 2007;37(2):323-9.

Taiz L, Zeiger E. Fisiologia vegetal. 6ª. ed. Porto Alegre: Artmed; 2017.

Timperio AM, Egidia MG, Zolla L. Proteomics applied on plant abiotic stresses: Role of heat shock proteins (HSP). J Proteomics. 2008;71:391-41.

Vieira GHS, Mantovani EC, Sediyama GC, Delazari FT. Indicadores morfo-fisiológicos do estresse hídrico para a cultura da cana-de-açúcar em função de lâminas de irrigação. Biosci J. 2014;30(1):65-75.

Wang P, Sun X, Li C, Wei Z, Liang D, Ma F. Long-term exogenous application of melatonin delays drought-induced leaf senescence in appleq. J Pineal Res. 2013;54:292-302.

Yamori W, Hikosaka K, Way DA. Temperature response of photosynthesis in C3, C4, and CAM plants: temperature acclimation and temperature adaptation. Photosy Res. 2014;119(1):101-17.

Yang Y, Jia Z, Chen F, Sang Z, Ma L. Comparative analysis of natural cold acclimation and deacclimation of two *Magnolia* species with different winter hardiness. Acta Physiol Plant. 2015;37(129):1-11.