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Ecophysiological aspects of *Tachigali vulgaris* seedlings using different sources and doses of nitrogen

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Abstract

This study aimed to evaluate the effects of sources and increasing doses of nitrogen on growth and gas exchange in plants of *Tachigali vulgaris*. The experiment was conducted in a greenhouse in the period from April to July 2015. The experimental design was randomized blocks in a factorial scheme (4 x 5), corresponding to 4 sources and 5 doses with four replications. The seedlings were grown in pots containing 3 dm^3 of soil. Nitrogen sources such as urea [$(NH_2)_2CO$], ammonium nitrate (NH4NO3), ammonium sulfate [(NH4) 2SO4] and calcium nitrate [Ca (NO3)2] were tested in five doses of 0, 75, 150, 225 and 300 mg/dm^3. They were applied as a solution in four portions, 25, 50, 75 and 100 days after transplanting. The lower growth of cultivated plants may be associated with $N\text{-}NH_4^+$ and lower photosynthetic activity, especially due to negative action of this ion on stomatal conductance. The occurrence of this ion resulted in lower growth and biomass production. Based on the results, for the production of tachi-branco seedlings (*Tachigali vulgaris*) dose of 150 mg dm^{-3} of N applied in installments, 25, 50, 75 and 100 days, by applying ammonium nitrate is recommended.

Keywords: mineral fertilization, forest species, gas exchange.
Abbreviations: A_phothosynthetic rate; Ci_internal concentration of CO_2; E_transpiration; EWU_efficiency of water use; GS_stomatal conductance; N_nitrogen; Φc_instantaneous efficiency of carboxylation.

Introduction

The Tachi-branco (*Tachigali vulgaris* L.G. Silva & H.C. Lima), Leguminosae Caesalpinioideae is a species that brings together promising features for energy plantations in the Brazilian Amazon and neighboring regions (Carpanezzi et al., 1983). Although the available information is scarce, its wood is considered of good features for firewood and charcoal (Ducke 1949, Le Cointe 1947). Adult trees of this species are not large, ranging from 20 to 30m of total height and 70 to 100 cm in diameter at breast height (DBH) (Carpanezzi et al., 1983).

Fertilization is one of several factors that influence crop production. Among the fertilizers being currently used for plants, nitrogen is one of the most complicated to be handled, due to its high demand in the stages of vegetative and reproductive development and also losses due to leaching.

Nitrogen (N) is an essential element to plant metabolism and its deficiency is one of the most common limitations to plant development (Pallardys, 2008). According to Raven et al. (2007), nitrogen is the main component of amino acids, proteins, nucleic acids, phytochrome, chlorophylls and coenzymes. The choice of plants for N source may vary between selective and consequent physiological adaptations pressures (Terce-Laforgue et al., 2004). The lack of this element is considered a deleterious factor in any ecosystem, as they cause drastic reductions in biomass production processes (Silva et al., 2011; Taiz and Zeiger, 2013).

The supply of nitrogen to plants is generally done using fertilizers such as urea, ammonium sulfate or nitrates (calcium, potassium and ammonium). Among the commercial sources of nitrogen Urea is prominent due to its lower cost per N unit, high solubility and compatibility for use in combination with other fertilizers. However, it is quite susceptible to loss by volatilization and has acidifying effect to the substrate. Another commercial source of nitrogen, ammonium sulphate, practically shows no loss by volatilization (<1%) after application (Anjos and Tedesco, 1976) but causes a rapid drop in pH of the soil, inhibiting nitrification (Strong et al., 1976), enduring longer than N in the ammonium form subject to less leaching. Finally, ammonium nitrate is between the nitrogen sources having high cost per unit of N, which is not typically used for fertilization (Fráguas et al., 2003). The calcium nitrate [Ca $(NO_3)_2$], is widely used in Europe as a fertilizer. The difference is the amount of calcium that promotes the maintenance of a desirable pH to the soil. Despite the importance of nitrogen, according to Davies (2000), there are few studies in plants involving this chemical element, especially when we consider native species of the Amazon.

Given the above, this study aimed to evaluate the effects of applying sources and increasing doses of nitrogen on growth and gas exchange in *Tachigali vulgaris* seedlings.

Results

Rate of photosynthesis

A quadratic effect of the doses applied for sources of urea and ammonium sulphate was observed, while no significant response was found to fit the regression model ($p > 0.05$) for ammonium nitrate and calcium nitrate (Figure 1). Application of urea and ammonium sulphate caused maximum efficiency for photosynthesis rates, corresponding to 94.0 (5.30 μmol CO_2 m^{-2} s^{-1}) and 236.7 mg dm^{-3} (5.11 μmol CO_2 m^{-2} s^{-1}) N, respectively. Regarding the main effect of the sources, the average of A did not differ statistically by Tukey test ($p > 0.05$).

Stomatal conductance

For stomatal conductance (*gs*), the model that best fit the data was the 2nd degree polynomial only for ammonium sulfate (Figure 2), showing a quadratic behavior of the gas in terms of N rates. The equation of the fitted model estimated maximum efficiency value for gas at a dose of 225 mg dm^{-3} of N (0.1277 μmol H_2O m^{-2} s^{-1}). The N sources from urea, ammonium nitrate and calcium nitrate showed no significant response on the gas values for setting the regression model ($p > 0.05$). The applied nitrogen sources did not significantly affect stomatal conductance ($p > 0.05$).

Internal concentration of CO_2

Doses for maximum efficiency were 205.28 (191.13 μmol CO_2 m^{-2} s^{-1}), 192.39 (231.79 μmol CO_2 m^{-2} s^{-1}) and 249 mg dm^{-3} (210.67 μmol CO_2 m^{-2} s^{-1}) of ammonium sulfate, ammonium nitrate and calcium nitrate, respectively (Figure 3). For urea source, the maximum value of C was observed at the higher doses used in the experiment. Regarding the main effect of N sources, all showed up statistically equal, by Tukey test ($p > 0.05$).

Transpiration

There was a quadratic effect in doses of sources ammonium sulphate and calcium nitrate (Figure 4). The fit of the regression model for the N levels of the sources urea and ammonium nitrate had no significant effect on E ($p > 0.05$). As described above, the reduction in stomatal conductance, and making the fall of CO_2 assimilation also affects perspiration, since both are diffusive processes which lead to a reduction in the production of assimilates. Regarding the main effect of N sources, all showed up statistically equal by Tukey test ($p > 0.05$).

Efficiency of water use (EWU)

As directly dependent variable values for A and E, there was a significant effect ($p \leq 0.05$) for the interaction between factors (F x D) (Table 2), yielding a quadratic effect on EWU with increasing N rates for all tested sources (Figure 5). However, for this relationship, only the urea showed the behavior of similar regression curve to its curves A and E. The other sources showed concavity turned up, unlike what was found for photosynthesis and transpiration adjustment of their respective sources.

Instantaneous efficiency of carboxylation

Significant effect for the N levels of urea and calcium nitrate was observed, while for the N levels of ammonium sulfate and ammonium nitrate no significance was noticed ($p > 0.05$). For all sources, fitting a regression model was possible such as a quadratic effect on Φc in response to increasing doses of N (Figure 6). Evaluation of main sources of N showed that all means are statistically equal ($p > 0.05$).

Discussion

Nascimento et al. (2014) found reduction in photosynthesis, transpiration, stomatal conductance, instantaneous efficiency of carboxylation, water use efficiency and lower values for the accumulation of dry matter in plants of *Hymenaea courbaril* L., when they were subjected to deficiency of nitrogen supply. The negative influence of nitrogen deficit on photosynthetic rate has been observed for several species and might be explained by the fact that the application of this nutrient determines the formation of compounds related to photosynthesis such as electron carriers and Rubisco (Evans, 1989). Additionally, optimal concentrations of nitrogen may be required to maintain carboxilative rubisco activity (Cruz et al., 2008). Plants grown with an inefficient supply of N may have a significant increase in starch concentration in chloroplasts Cruz et al. (2004). This accumulation is usually accompanied by damage to the thylakoids (Bondada and Syvertsen, 2005), which also helps to explain the reduction of photosynthetic rate under these conditions. Another factor linked to reduced photosynthetic activity can be explained by the commitment of the CO^2 absorption through the stomata in response to stress on the photosynthetic metabolism, such as nutritional disorders (Endres et al., 2010).

The *Manihot esculenta* Crantz. plants grown in high doses of NH_4^+ showed a reduction in photosynthetic rate and opening of stomata. According to the same author, the plants

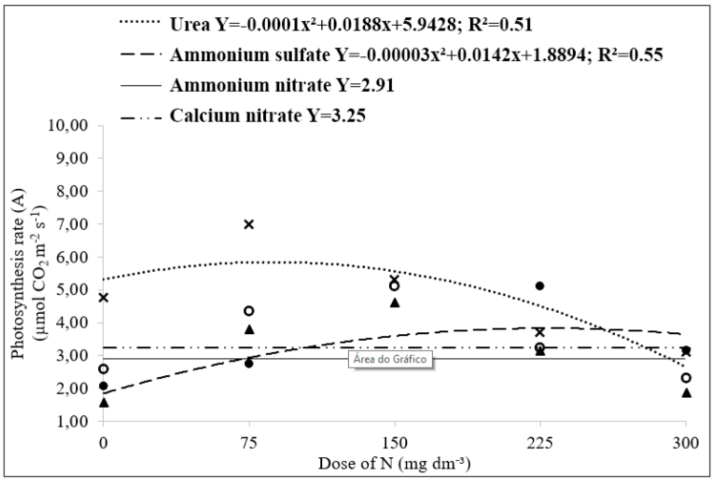

Fig 1. Photosynthetic rate (A) of *T. vulgaris* seedlings in response to nitrogen under four tested sources and doses at the end of the experiment ($p \leq 0.05$).

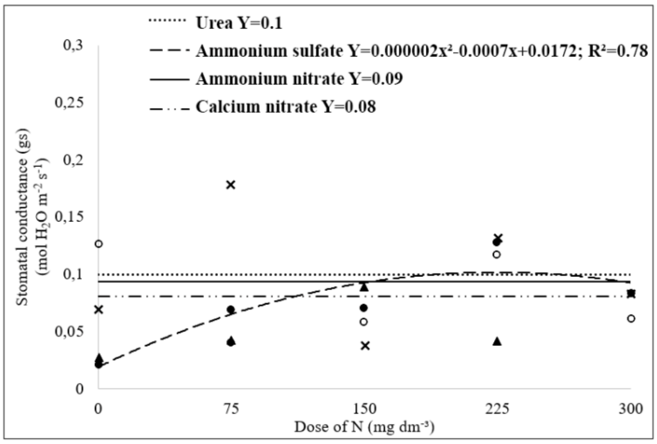

Fig 1. Stomatal conductance (gs) of *T. vulgaris* seedlings in response to four tested sources and doses of nitrogen at the end of experiment ($p \leq 0.05$).

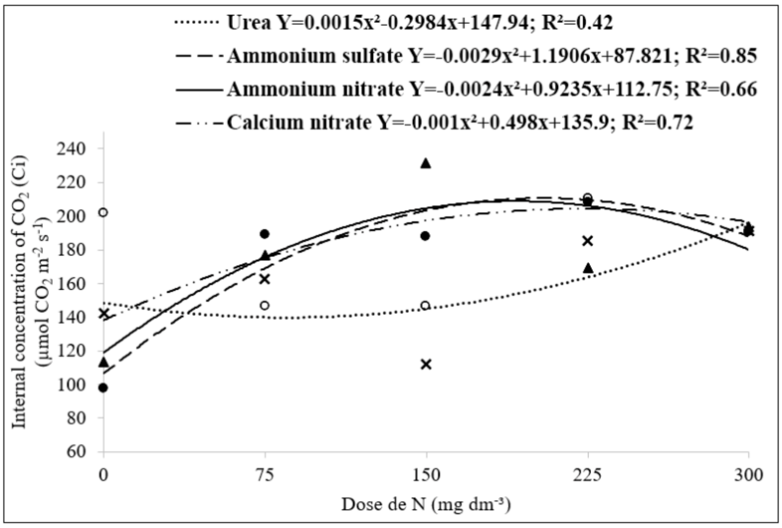

Fig 2. Internal CO_2 concentration (Ci) of *T. vulgaris* seedlings in response to four tested sources and doses of nitrogen at the end of experiment ($p \leq 0.05$).

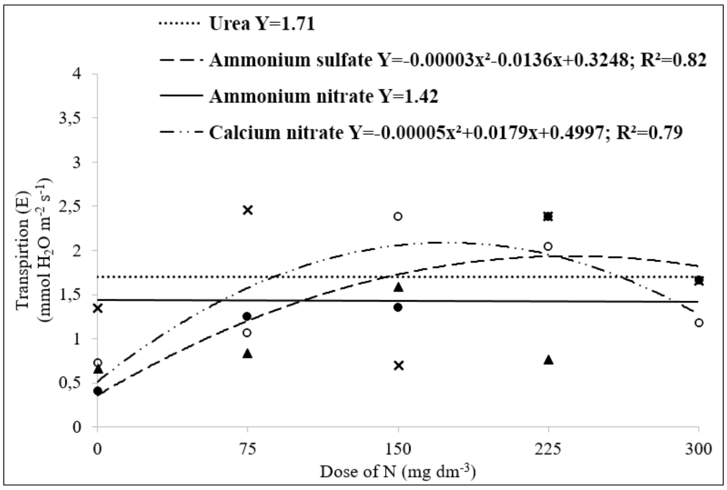

Fig 3. Transpiration (E) of *T. vulgaris* seedlings in response to four sources and doses of nitrogen at the end of the experiment ($p \leq 0.05$).

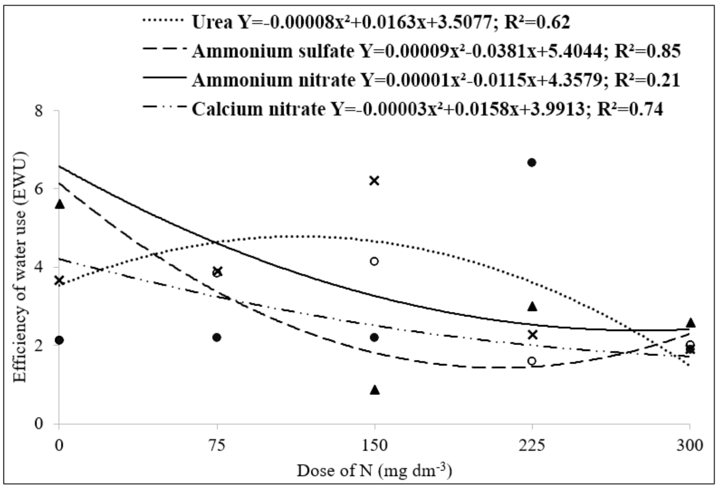

Fig 4. Efficiency of water use (*EWU*) of *T. vulgaris* seedlings to four tested sources and doses of nitrogen at the end of the experiment ($p \leq 0.05$).

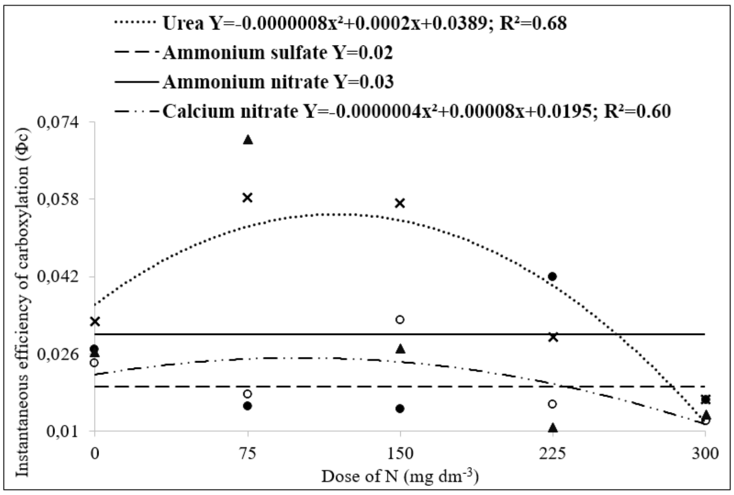

Fig 5. Instantaneous efficiency of carboxylation (Φc) of *T. vulgaris* seedlings in response to four tested sources and doses of nitrogen at the end of the experiment ($p \leq 0.05$).

fertilized with only NO_3 or NO_3 with NH_4^+ showed no differences in their conductance. Melo et al. (2009) also observed a reduction of gas conduction in *Musa spp.* using the urea doses as a source of N. The reason NH_4^+ ions reduce stomatal conductance is not fully understood yet and may be related to several factors occurring at the same time (Lopes and Araus 2006). The NH_4^+ ion concentration reduces the leaf many essential nutrients such as potassium Britto and Kronzucker (2002), which is important for the normal functioning of the stomata (Taiz and Zeiger, 2013). The stomatal closure can also be a result of the lower leaf water potential that these plants may present. The plants grown exclusively under NH_4^+ can reduce the hydraulic conductivity of the roots, thus reducing the transport of water to meet the demand of shoot (Adler, 1996). Additionally, the increase in abscisic acid levels as a consequence of the ammonia fertilizer may also have contributed to the observed stomatal closure (Peuke et al., 1998). The result of the stomata closing mechanism is the reduction of CO_2 availability, which leads to changes of the appropriate balance and transport electrons, carbon metabolism and consumption of ATP and NADPH, which together, make the photosystem II inefficient, compromising production photosynthates (Tezara et al., 2005).

This behavior may indicate that the limitation to the entry of this compound may be the determinant for the reduction of photosynthetic rate of plants grown with higher doses of N. This statement can be found in the relationship between photosynthesis and stomatal conductance (Figures 1 and 2), and photosynthesis and internal CO_2 concentration (Figures 1 and 3). The values of photosynthesis and stomatal conductance of sunflower (Lasa et al., 2000; Lopes et al., 2004) has also been observed as a function of increasing doses of N with the ammonia source. In young plants of *Hevea brasiliensis* Mull., the larger Ci values were found in control (no-fertilization) treatment, followed by fertilized with nitrate (NO_3) and ammonium (NH_4^+) (Carneiro et al., 2015). Nascimento et al. (2014), found no significant difference to the internal concentration of CO_2 in *Hymenaea courbaril* L. seedlings grown in the presence or absence of N at the end of the experiment. However, it might be considered as the most limiting macronutrient for the development of seedlings species, at vegetative stage.

During the gas exchange, the carbon dioxide absorption from the external environment promotes water loss and reduction of this loss also restricts the entry of CO_2 (Shimazaki et al., 2007). This interdependence by the relationship between photosynthesis and transpiration indicates the efficiency of water use (EWU), where the observed values relate the amount of fixed carbon plant per unit of water evapotranspirated (Jaimez et al., 2005). This process is influenced by weather condition; however; the appropriate nutritional supply tends to promote higher photosynthetic rates, resulting in higher yields (Melo et al., 2009).

Ferreira et al. (2012) evaluated young plants of *Bertholletia excelsa* Humb. & Bonpl. subjected to green manure, found EWU values close to 3.5 µmol CO_2 $mmol^{-1}$ H_2O, which is smaller than that found in this study for *T. vulgaris.* Working on banana leaves, Melo et al. (2009), found the maximum value of EWU 4.09 (µmol CO_2 $mmol^{-1}$ H_2O) with the dose of 884 kg ha^{-1} of N. The stomatal closing mechanism to restrict water loss by perspiration can be considered an adaptive strategy used by different species to limit water loss and to exceed the water deficit, as verified for woody species *Tabebuia aurea* (Silva et al., 2003) and *Minquartia guianensis* Aubl. (Liberato et al., 2006) subjected to water deficit. Nascimento et al. (2014), studied the suppression of macronutrients in *Hymenaea courbaril* L. seedlings and found better EWU values in plants nourished with N compared to the omission of this nutrient.

The photosynthetic activity can be affected by non-stomatal factors, notably the factors that have biochemical origin (Farquhar and Sharkey 1982). This aspect can be studied by considering the instantaneous carboxylation efficiency (Φc), which is given by the relation between photosynthesis and intracellular concentration of CO_2 (Zhang et al., 2001). In a ecophysiological context, it is very important how the rate of photosynthesis and stomatal conductance are related because these two physiological variables vary proportionally, enabling the internal CO_2 concentration and the efficiency of water use remain at appropriate levels. The optimization of gas exchange and controlling the carbon dioxide absorption are essential in the formation of compounds resulting from photosynthesis. Melo et al. (2009) also observed a reduction in the instantaneous carboxylation efficiency with increasing nitrogen levels in Musa spp. The reduction of Φc values may have occurred due to excessive supply of N, causing increase in the oxygenase activity of Rubisco enzyme at the expense of carboxylase, causing lower rate of carbon assimilation (Larcher, 2006).

Materials and Methods

Cultivation conditions

The experiment was conducted in a greenhouse of the Institute of Agricultural Sciences at the Federal Rural University of Amazonia in Belém, Pará, in the period from April to July 2015. Each day during the experimental period, air temperature measurements and relative humidity (with a thermohygrometer) inside the greenhouse were taken and showed mean values of 29.6 ± 1.02 ° C and 73.7 ± 3%, respectively.

Plant material

The tachi-branco seeds (*Tachigali vulgaris*) were obtained from the seeds of forest sector of Embrapa Amazônia Oriental, harvested headquarters located in the municipality of Paragominas-PA (2 ° 59 '51 "S, 47 ° 21' 13" W) germinated in seedbeds, with washed sand as a substrate.

Measurements of gas exchange

Point measurements of gas exchange were conducted at the end of the experiment, the periods between 9 and 11 hours, fully expanded leaves and fully ripe with a gas analyzer infrared (Infrared gas analizer - IRGA), Li-6400 model (Li-cor, Biosciences Inc., Nebraska, USA). The photosynthetically active radiation, atmospheric CO_2 inside the leaf chamber and the temperature of the chamber block were kept constant during measurements with values of 1000 µmol Photons m^{-2} s^{-1}, 380 to 400 µmol CO_2 m^{-2} s^{-1} and 26 to 27ºC, respectively. The following variables were calculated: net photosynthetic rate per unit leaf area (*A*, µmol CO_2 m^{-2} s^{-1}), stomatal conductance to water vapor (*gs*, mol H_2O m^{-2} s^{-1}), transpiration rate (E, mmol H_2O m^{-2} s^{-1}) and internal concentration of CO_2 (Ci, µmol CO_2 m^{-2} s^{-1}). The following values of *A, E, gs* and *Ci* the following ratios were calculated: $EWU = {}^{A}/_{E}$ e $\Phi c = {}^{A}/_{Ci}$ (Zhang et al., 2001), where IT means efficiency of water use and Φc instantaneous carboxylation efficiency.

Experimental design and statistical

The experiment constitutes a randomized block design (RBD) in factorial scheme (4 x 5), corresponding to 4 sources of nitrogen and 5 doses with four replicates. Each experimental unit consisted of a vase containing 3 dm^3 of soil. The nitrogen sources were urea $[CO(NH_2)_2]$, ammonium sulfate $[(NH_4)_2SO_4]$, ammonium nitrate (NH_4NO_3) and calcium nitrate $[Ca\ (NO_3)_2]$, applied in five doses of 0, 75 , 150, 225 and 300 mg dm^{-3} N in the form of solution in four equal portions at 25, 50, 75 and 100 days after transplanting to pots.

The data were first analyzed by analysis of variance followed by means for testing (Tukey test). It was used to compare the main effect of N sources and by regression analysis, using the Minitab 14® software to associate the effect of doses. In the choice of regression equations, the significance of the coefficients and the adjusted coefficient of determination (R²) was considered. The significance level used for all analyzes was 5%.

Conclusion

The lower growth of cultivated plants was exclusively occurred due to existence of N-NH_4^+, which was the case of the ammonium sulfate fertilizer. It could be attributed to the lower photosynthetic activity of these plants, because of the negative action of this ion on stomatal conductance, which reflected a lower growth and biomass production. Based on the results, for the production of tachi-branco seedlings (*Tachigali vulgaris*) we recommend dose of 150 mg dm^{-3} of N applied in installments, 25, 50, 75 and 100 days with fertilizer nitrate ammonium.

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