

Do the nutrition and physiology of eucalyptus seedlings respond to silicon (Si) supply?

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Abstract

In Brazil, *Eucalyptus* plantations are found, generally in soils with naturally low chemical fertility. Silicon (Si) can improve photosynthesis, decrease plant transpiration and increase water use efficiency (WUE). This study aimed to evaluate the effect of Si supply at five rates (0, 0.25, 0.50, 0.75 and 1.00 mmol L⁻¹) for growing *Eucalyptus* clone IPB8 seedlings (*E. urophylla* × *E. grandis*) in Clark's nutrient solution in a greenhouse. Plant growth, nutritional status, gas exchange, leaf water potential (Ψ_w), leaf area (LA), inclination angle of leaves and stomatal density (SD) were measured. There was no significant response in *Eucalyptus* seedling growth due to Si application, which was related to the absence of benefits provided by Si in plant nutritional status and physiology. The efficiency of assimilation (EA), efficiency of translocation (ET) and efficiency of utilization (EU) did not show significant results either. The low ET indicates that Si was highly accumulated in the root (75.4 % in relation to total Si absorbed by plants), which may also have contributed to the lack of the benefits expected. Despite that, Si application promoted higher rates of photosynthesis when compared to plants with 0 mmol Si L⁻¹, increasing the total dry matter production by up to 28 % at an Si rate 0.50 mmol L⁻¹, which also provided a trend of higher growth, EU, LA and SLA, showing it to be the best rate for this *Eucalyptus* species.

Keywords: beneficial element; forest nutrition; growth; nutrient solution; plant physiology.

Abbreviations: EA_efficiency of assimilation; ET_efficiency of translocation; EU_efficiency of utilization; LA_leaf area; SLA_specific leaf area; Ψ_w _leaf water potential; Si_silicon; SD_stomatal density; WUE_water use efficiency.

Introduction

Eucalyptus, the most widely planted forest species in Brazil, is susceptible to water stress which, together with an absence of fertile soil for its growth, represents the main limiting factor impairing its persistence and productivity (Gonçalves, 2010; IBÁ, 2016). Growth and wood yield responses in *Eucalyptus* plantations, in tropical conditions, to fertilization with N, P and K nutrients are documented in the literature (Melo et al., 2016). Si is considered a beneficial element, non-essential for plants (Ma and Yamaji, 2006), being the second most abundant element in the earth's crust (Marschner, 2012). Si may increase the quality and productivity of plantations and is absorbed as silicic acid (H₄SiO₄). Si has beneficial effects on many species of plants, especially under conditions of abiotic (salinity, metal toxicity, nutrient imbalance) and biotic stresses (lodging, drought, radiation, high temperature, freezing) (Crusciol et al., 2009). It is deposited in epidermal cell walls as amorphous silica (SiO₂.nH₂O) after water evaporation (Korndörfer et al., 2004a), interfering directly in transpiration rates, as it reduces the plant's water consumption, makes more erect leaves and provides higher photosynthetic rates once leaf erectness affects light interception (Pulz et al., 2008; Marschner, 2012; Campoe et al., 2013). Since leaf angle defines the proportion of radiation that penetrates the tree canopy, the leaf architecture also affects transpiration (Whitehead and Beadle, 2004).

Si also reduces the incidence of fungi and bacteria, for two reasons. The first is that Si promotes the formation of a physical barrier deposited beneath the cuticle, hampering hyphae growth (Datnoff et al., 1997). The second reason is that soluble Si acts as a modulator of host resistance to pathogens, activating some defense mechanisms (Ma and Yamaji, 2006). SiO₂ combines with water molecules (2H₂O), forming H₄SiO₄ which can be considered as a "water deposit" (Carvalho et al., 2003), being important during periods of water stress by providing water to the plants, since water is the most limiting factor for plant growth in tropical regions (Stape et al., 2004).

Plant species differ in their ability to uptake and accumulate Si; according to the SiO₂ percentage in shoot dry matter, it is possible to classify plants as: Si accumulators (100 to 150 g SiO₂ kg⁻¹), intermediary (10 to 50 g SiO₂ kg⁻¹) and non-accumulators (< 5 g SiO₂ kg⁻¹) (Ma and Takahashi, 2002; Marschner, 2012). This percentage is affected by the nutritional efficiency in plants, defined as the EA, ET and EU (Clark, 1983). EA is defined as the capacity for nutrient acquisition in low availability conditions which optimizes fertilization recovery, especially in the initial stages of plant development (Barros and Novais, 1990). ET refers to the transportation of nutrients to photosynthetically active sites of the plant, being influenced by the nutritional status of root cells and the transpiration rate (Abichequer and Bohnen,

1998). EU is the ability to use nutrients in biomass synthesis, influencing its productivity or the economy of nutrient export (Clarkson and Hanson, 1980).

In Gramineae, like rice (Zanão et al., 2010), corn (Goussain et al., 2002), *Brachiaria* (Sarto et al., 2016) and sugar cane (Camargo et al., 2014), the positive responses to Si application are well documented. In these Si accumulator species the element increases the photosynthetic rate and water use efficiency, and enhances the resistance of plants to plagues, diseases and water stress tolerance. Despite the literature being replete with positive plant growth responses to Si application (Epstein, 1999), there is a lack of information related to Si nutrition in tree species. According to Carvalho et al. (2003), even in non-accumulators, a positive response in plant growth and total dry matter production was observed in *Eucalyptus* with Si supply. However, little effort has been dedicated to analyze this element's behavior in *Eucalyptus* (Duarte and Coelho, 2011), indicating the need for more information to better understand *Eucalyptus* responses to Si application, during its early growth.

Considering the above, it is expected that Si supply would lead *Eucalyptus* seedlings to have higher yield and quality, by increasing their photosynthetic rates, water use efficiency and leaf angle, among others. Therefore, this study aimed to verify the *Eucalyptus* response to Si supply and its benefits on the growth, gas exchange, nutritional efficiency and organs of higher accumulation in the plant (root or leaf), as well as related factors.

Results

Plant growth (height and diameter)

From 28 days at the application rate of 0.50 mmol Si L⁻¹, the seedlings started to differentiate from the other treatments, both in height and diameter, keeping ahead of others until the experiment end (Figure 1a and b). In contrast, the application rates of 0.75 and 0 mmol L⁻¹ showed the smallest height and diameter of plants, causing a reduction of 17 and 14 %, respectively.

Root, shoot and total dry matter production and root/shoot ratio

The Si application did not influence dry matter production of *Eucalyptus* seedlings ($p > 0.05$). Nevertheless, the application rate of 0.50 mmol Si L⁻¹ provided a trend of higher root, shoot and total dry matter production (Figure 2a, b and c) with a 28 % increase in total dry matter compared to the control (0 mmol Si L⁻¹). The root/shoot ratio was not affected by the Si application (Figure 2d), although 1 mmol Si L⁻¹ presented a trend of lower root/shoot ratio.

Si concentration and accumulation and EA, ET and EU

The effects of Si application rates on *Eucalyptus* seedlings were significant only for Si concentration in roots and leaves, according to the linear model (Figure 3a). The increase in Si concentration (roots and leaves) in treatments with higher Si rates indicates that: (i) the Si treatment rates were well applied and (ii) the plants were able to uptake this element by the roots and carry it to the leaves, but in a small amount, since there was large Si accumulation in roots (Figure 3b). EA did not show significant differences; however, a trend of linear growth with higher Si rates was noted (Figure 3c). A trend of positive response to Si application in accumulation

(Figure 3b) and EA could be verified, when compared to 0 mmol Si L⁻¹. No significant results were found for ET ($p > 0.05$), since the application rate of 0 mmol Si L⁻¹ had an average value higher than the others (Figure 3d). EU did not show significant values either ($p > 0.05$). The lowest numerical EU value (Figure 3e) was shown at the higher rate (1 mmol Si L⁻¹), which indicates the low efficiency in using this element to synthesize biomass, even with greater EA (Figure 3c) and ET (Figure 3d).

Gas exchange (A, C_i/C_a, g_s, E, WUE) and Ψ_w

The effect of Si application in nutrient solution on gas exchange of *Eucalyptus* seedlings was not significant (Figure 4). The addition of Si promoted higher values of photosynthesis (A) (Figure 4a) when compared to its omission. Despite that, this variable presented an average value of 13 μmol m² s⁻¹ and a trend of higher photosynthetic rates at intermediary Si rates. The leaf internal and external CO₂ concentration ratio, C_i/C_a, did not show significant regression (Figure 4b). The reduction of stomatal conductance (g_s) and transpiration (E) (Figure 4c and d) was not significantly affected by Si application in the present study. The WUE (Figure 4e) was not significant either, with the higher rate (1 mmol Si L⁻¹) showing a lower numerical value.

Neither did Si application change the Ψ_w of *Eucalyptus* seedlings in nutrient solution ($p > 0.05$), either at dawn (5 am) or at noon (12 pm) (Figure 4f). The highest and lowest Ψ_w values found in the coldest and hottest periods (5 am and 12 pm) showed average values of -0.23 and -1.32 MPa, respectively.

LA, SLA, inclination angle of leaves and SD

Si supply did not affect ($p > 0.05$) the LA and SLA of *Eucalyptus* seedlings (Figure 5a and b). Despite the lack of statistical significance, it was verified that the rate of 0.5 mmol Si L⁻¹ showed the highest mean values for both variables, while higher rates (0.75 and 1.00 mmol Si L⁻¹) provided lower average values. The inclination angle of leaves (Figure 5c) and SD (Figure 5d) were not affected by Si rates. It was noted that there was a small difference among Si rates and angle of inclination, with a mean value of 73°. In addition, the 0.50 mmol Si L⁻¹ rate showed a trend of lower SD, even when compared to the 0 mmol Si L⁻¹ rate.

Discussion

The rate of 0.5 mmol Si L⁻¹ provided the greatest average height, whereas both the absence and the highest rates of Si were detrimental to seedling growth in nutrient solution. Higher Si rates may precipitate the other nutrients in the solution, as observed by Bognola et al. (2011). The positive effects seen at lower rates of Si application might have been caused by morphological and physiological changes in the plants, providing higher photosynthetic and lower transpiration rates, as observed by Marschner (2012). Silva and Coelho (2010), who investigated the effects of lime and silicate application in a *Eucalyptus* clone (*E. urophylla* × *E. grandis*) in a field experiment, verified that both sources promoted plant growth in terms of height and diameter at breast height.

As this study was realized in nutrient solution, the short cultivation time of treatments (60 days) could also have limited the difference in growth provided by the Si supply.

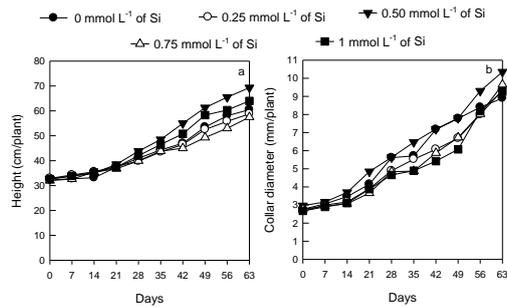


Fig 1. Weekly growth in height (a) and collar diameter (b) of *Eucalyptus* seedlings submitted to Si application rates in nutrient solution.

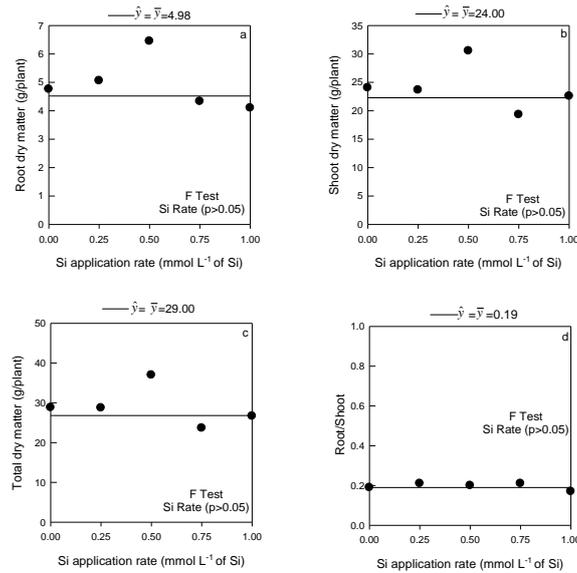


Fig 2. Root (a), shoot (b) and total dry matter production (c) and root/shoot dry matter production (d) of *Eucalyptus* seedlings submitted to Si application rates in nutrient solution.

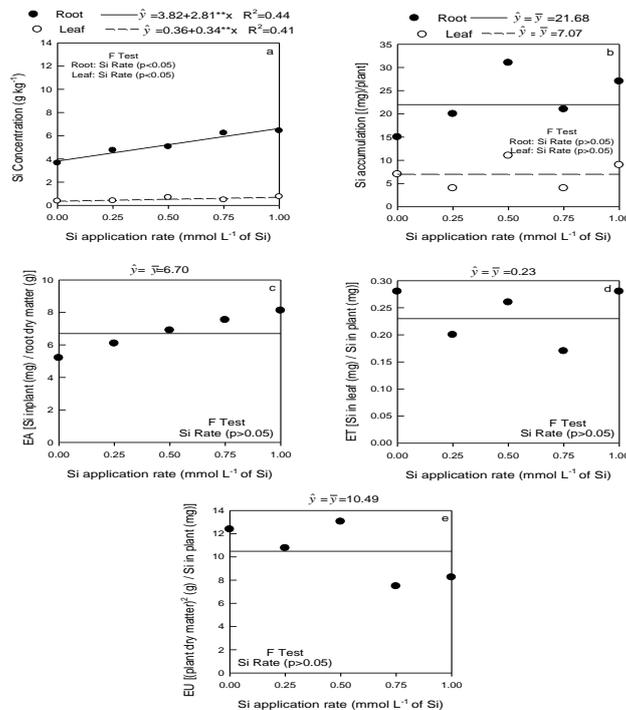


Fig 3. Si concentration (a) and accumulation (b) in roots and leaves, efficiency of Si assimilation –EA (c), of translocation –ET (d), and of utilization –EU (e) of *Eucalyptus* seedlings submitted to Si application rates in nutrient solution. ** represents significance at 1 % by F test ($p < 0.05$).

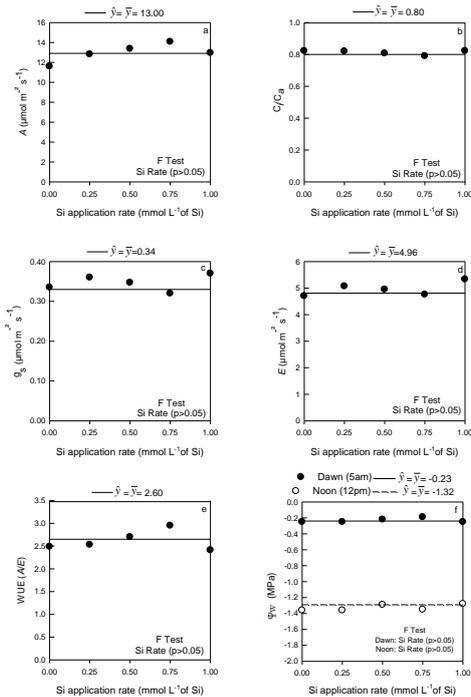


Fig 4. Gas exchange of *Eucalyptus* seedlings submitted to Si application rates in nutrient solution: photosynthesis – A (a), leaf internal (C_i) and external (C_a) CO₂ concentration ratio – C_i/C_a (b), stomatal conductance – g_s (c), transpiration – E (d), water use efficiency – WUE (e) and leaf water potential – Ψ_w (f).

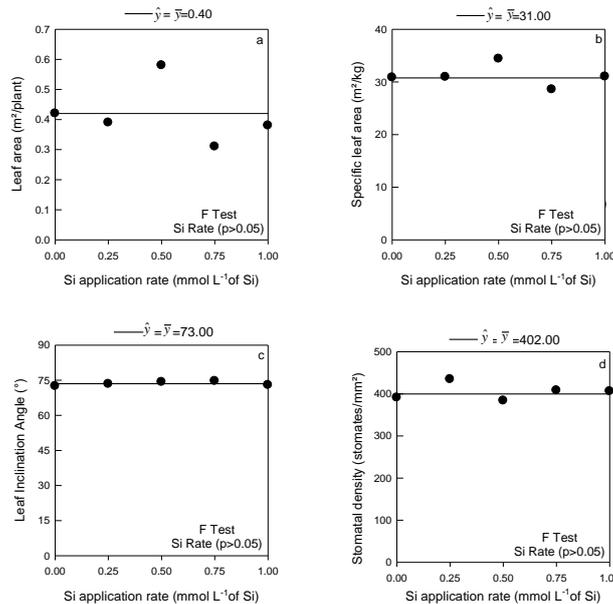


Fig 5. Leaf area – LA (a), specific leaf area – SLA (b), leaf inclination angle (c) and stomatal density – SD (stomates/mm²) (d) of *Eucalyptus* seedlings submitted to Si application rates in nutrient solution.

We can presume that Si application could even induce indirect positive responses, since this element in soil can provide effects like reducing toxic metal levels (Crusciol et al., 2009) and increasing the availability of P (Carvalho et al., 2000), which is not seen in nutrient solution. Higher Si rates (0.75 and 1.00 mmol L⁻¹) promoted the lowest mean values for all compartments of dry matter production, as also observed for height and collar diameter (Figure 1a and b). Perhaps the greater rise in nutrient solution pH provided by higher Si rates could have hindered the growth of seedlings, since this species is adapted to acidic soil conditions

(Gonçalves, 2010). The same decline in growth due to pH increase was observed by Rocha et al. (2008) in lime application and by Bologna et al. (2011) with CaSiO₃ and K₂SiO₃ application in *Eucalyptus* seedlings. Despite the nutrient solution pH having been adjusted just once after Si application in the treatments, the small difference in time between the Si application and pH adjustment could have occasioned nutrient precipitation, since pH increased concomitantly with higher rates (pH = 9.0 in 1 mmol Si L⁻¹). A possible explanation for the root/shoot ratio results, which express the balance between the assimilatory system and its

redistribution to other organs, is that membrane damage may have occurred in roots at high pH, which allowed the loss of nutrients previously absorbed by the plants (Malik, 2002) as well as providing a smaller root area for nutrient absorption. A similar higher Si accumulation in roots was also seen by Carvalho et al. (2003) in *Eucalyptus* seedlings. With the present data, 7.07 mg Si per plant in leaves (Figure 3b) amounts to 15.17 mg SiO₂ per plant. Considering shoot dry matter production (Figure 2b), the average value of leaf only dry matter was 13.08 g per plant; thus, plants accumulated 1.14 g SiO₂ kg⁻¹ in shoot dry matter. Hence, according to the values obtained, this *Eucalyptus* clone can be classified as an Si non-accumulator, according to Marschner (2012). This reinforces the results found by Carvalho et al. (2003) who verified that *E. grandis* is not an Si accumulator, with 3.4 g SiO₂ kg⁻¹, and by Accioly et al. (2009), in which *Eucalyptus* plants had accumulated 2.2 to 3.4 g SiO₂ kg⁻¹.

Variations in nutritional efficiency among species of *Eucalyptus* (Santana et al., 2002) and clones of the same species (Pinto et al., 2011) are well documented, being criteria for superior genotype selection. In this study, a possible hypothesis for the absence of a positive response in nutritional efficiency is plant age, as adult plants may be more efficient in uptake and translocation of this element (Pinto et al., 2011). Carvalho et al. (2003) observed that with advancing plant age, treatment with higher silicate rates showed greater Si translocation. The distribution of Si in the shoot is controlled by transpiration, accumulating in older tissues, because this element is not mobile within the plants (Ma and Yamaji, 2006). The responses in EU may not be due to metabolic contribution by Si application; this could be proved by the efficiency values obtained without Si supply, since they exceeded even the EU values observed with Si application, as was also observed by Carvalho et al. (2003). Another assumption is the method used to supply Si, since foliar fertilization could possibly provide different results, since it allows assimilation by plants with low efficiency in absorbing a specific element. Greater responses to foliar Si application, especially in non-accumulator plants, have been verified (Marschner, 2012).

Lewis et al. (2011), studying variations among *Eucalyptus* species, found a similar *A* value to this study (13 μmol m² s⁻¹). Another benefit of Si application is the increase in the chlorophyll concentration of leaves (Epstein, 2001; Curvêlo et al., 2013). With this, we can presume that the 0.75 mmol Si L⁻¹ rate provided a trend of higher photosynthetic rates, due to better leaf architecture such as higher leaf angles and chlorophyll concentration.

As expected, there was an inverse relation between *C_i/C_a* (Figure 4b) and *A* (Figure 4a) (Concenço et al., 2007). Considering a constant *C_a*, when *A* increases, the *C_i/C_a* ratio decreases. This indicates that *C_i* is fixed, increasing photosynthetic rates, resulting in higher biomass. The average value of 0.8 was also seen by Mattiello et al. (2009) with different clones of *Eucalyptus* in nutrient solution.

According to Dayanandan et al. (1983), silica deposits in the cell walls of xylem vessels form a membrane in epidermal tissue, preventing vessel compression in water stress conditions and protecting plants against excessive loss of water by transpiration. Perhaps the low leaf Si accumulation may be the explanation for the absence of significant responses in *g_s* (Figure 4c) and *E* (Figure 4d).

Since Si application may cause leaves to be more erect, increasing light capture and *A*, WUE can be directly affected by the supply of this element (Ma and Yamaji, 2008). However, increasing *E* or *g_s* per unit LA without increasing *A* results in a lower WUE (Forrester et al., 2012), which can be

seen at the 1 mmol Si L⁻¹ rate, which showed not only a trend of lower values for WUE (Figure 4e) but also for total dry matter (Figure 2c) and EU (Figure 3e). Such results indicate that less productive plants are also less efficient in their use resources (Forrester et al., 2012; Otto et al., 2014).

Ψ_w has been used to understand the leaf mechanism of dehydration avoidance; when the leaf water concentration decays, guard cells lose turgor, resulting in stomatal closure (Levitt, 1980). Facing the higher temperature at noon, which can be considered a biotic stress, it was expected that Si application would reduce the impact of increasing radiation (Crusciol et al., 2009), reducing transpiration rates (Pulz et al., 2008) and Ψ_w values in order to avoid leaf dehydration by excessive water loss.

Larger trees show high light use and/or light use efficiency; this combination may lead to an increase in the growth of trees (Binkley et al., 2013). Thus, we observed a strong link between the values for growth (Figure 1), total dry matter production (Figure 2c), LA and SLA (Figure 5a and b), as a consequence of greater surface area for absorbing sunlight. Pinto et al. (2009), evaluating the effect of excess zinc in the nutrient solution in *Eucalyptus* seedlings, observed a significantly higher LA with Si addition. SLA is an ecophysiological parameter influencing leaf physiology, photosynthesis and whole plant carbon gain, the reduction of which is an important mechanism for reducing water loss from the canopy and which may also affect carbon assimilation (Schulze et al., 2006; Nouvellon, 2010).

The leaf inclination angle is a structural property related to light distribution in the tree canopy, thus affecting plant photosynthetic rates (Leihner and Ortiz, 1978; Sassenrath-Cole, 1995). An average value of 73° was observed, similar to the range found in mature plants of *E. maculate* and *E. globulus* by Anderson (1981) and James and Bell (2000), respectively. According to these authors, *Eucalyptus* has a leaf angle of about 60° to 80°, from the vertical to the horizontal, serving as an adjustment mechanism for reducing the incidence of heat under high radiation at peak times.

The SD variation related to Si rate application could be due to environmental factors, being affected by light, temperature, water status, position of a leaf on the crown and intra-leaf position. According to Pearce et al. (2006), larger stomata with lower densities could be disadvantageous in high irradiance areas, because they would tend to close slowly. We can suppose that at the 0.5 mmol Si L⁻¹ rate, plants had a lower mean SD, and may have had higher stomatal diameter, which was not negative, since this rate also showed the highest total dry matter production (Figure 2c) and LA (Figure 5a). Therefore, stomata were more efficient in CO₂ assimilation, as a small number of stomata produced higher dry matter levels.

Materials and Methods

Experimental procedure and design

The experiment was carried out in a greenhouse at the Nuclear Energy Center in Agriculture (CENA-USP) in Piracicaba, SP, Brazil, from February to April 2015. Air temperature in the greenhouse was recorded daily and reached mean values of 31.30 °C. Plants of the *Eucalyptus* clone IPB8 (*E. urophylla* × *E. grandis*) were cultivated for 30 days, which represented the acclimatization stage in the nutrient solution. The seedlings arrived in plastic tubes, the substrate was removed from roots by washing with tap water and then they were transplanted to a collective tray (10 L) with Clark's nutrient solution (1975). The nutrient solution

was kept under permanent aeration and exchanged weekly, concomitant with a gradual increase of the original solution's concentration (25, 50, 75 and 100 %). After acclimatization, homogeneous seedlings were selected, according to height and collar diameter, and transferred to individual plastic pots (3 L), where treatments were applied for 60 days.

The treatments consisted of five Si application rates (0, 0.25, 0.50, 0.75 and 1.00 mmol Si L⁻¹), as potassium silicate (K₂SiO₃), arranged in randomized blocks with four replications, making 20 experimental units with one seedling each. The nutrient solution pH of different treatments was adjusted to 5.5, during its exchange, by adding HCl or NaOH (0.1 mol L⁻¹). In treatments with the highest Si application rates, the pH reached high values (9.0) before its correction.

Measured variables

The growth in height and collar diameter was measured weekly with a graduated ruler (in cm) and a digital pachymeter (in mm), respectively. Before harvesting, gas exchange was evaluated in the youngest fully expanded leaf of each seedling in the morning (7 to 11 am) with an infrared gas analyzer (IRGA, Li-6400xt, Licor) at environmental humidity and temperature and constant radiation (1.000 μmol m⁻² s⁻¹). Photosynthesis (*A*), the relation between internal and external CO₂ concentration (*C_i* and *C_a*, respectively), stomatal conductance (*g_s*), and transpiration (*E*) were evaluated. With these data, the WUE level in the leaf was calculated by dividing values of *A* by *E*. The leaf water potential (Ψ_w) was measured in the same type of leaves assessed for gas exchange, using a Scholander pressure chamber at dawn (5 am) and at noon (12 pm) (Turner, 1981). The inclination angle 20 leaves per plant was measured with a protractor, randomly selected from all regions and sides of the canopy (Campoe et al., 2013). Four leaves were collected in the middle region of the canopy (one on each side) per plant, for SD evaluation (stomates per mm²) on the abaxial leaf surface by printing on a glass slide. The sampled foliar region was pressed for 10 s against a drop of superglue on the glass slide. Then, using an optical microscope with 10× objective magnification and a digital camera attached, three random fields of 0.116 mm² each were photographed. Stomatal counting was done using the ImageJ program with 150× zoom, and finally the average SD was calculated (Segatto et al., 2004).

After 90 days of cultivation in the nutrient solution, the seedlings, which had their leaves sprayed with deionized water to remove dust, were harvested and their leaves, stems, branches and roots were separated. All leaves, including those previously used for SD analysis, were kept refrigerated to avoid dehydration until delivery to the laboratory for determination of LA, which was obtained with a leaf area integrator (LI-3100). Later, the leaves, stems, branches and roots were oven-dried (65 °C) until constant weight was achieved. Afterwards, each part was weighed to obtain its dry matter. The roots and leaves were ground (Willey mill) and sent for chemical analysis at the Federal University of Uberlândia to evaluate the Si concentration (Korndörfer, 2004b).

With Si concentration values and the dry matter of each part, Si accumulation was calculated [accumulation = concentration (g/kg) × dry matter (g)] in roots and leaves. It was also possible to calculate the nutritional efficiency of plants, through Si quantities EA, ET, and EU, suggested by Swiader et al. (1994), Li et al. (1991) and Siddiqui and Glass (1981), respectively, according to equations 1, 2 and 3. Since the Si concentrations were only determined in roots and

leaves, to calculate the nutrient efficiency the amount of Si in the leaves was considered as the amount of Si in the shoots. The amount of Si in the plant was considered as the sum of the Si amount in the roots and shoots. To calculate the amount of SiO₂ in the shoots, leaf Si accumulation and leaf dry matter production were considered, that is, shoots without stems and branches.

$$(1)EA: \frac{\text{Si in plant (mg)}}{\text{dry matter of roots (g)}}$$

$$(2)ET: \frac{\text{Si in shoots (mg)}}{\text{Si in plant (mg)}}$$

$$(3)EU: \frac{(\text{plant dry matter})^2 \text{ (g)}}{\text{Si in plant (mg)}}$$

Statistical analysis

The data were submitted to analysis of variance (F test) to test the effects of Si on measured variables, and when significant ($p < 0.05$) were evaluated by regression models by the SAS statistical package (SAS, 2004). Linear, quadratic and square root regression models were fitted to describe the relations between Si supply and response variables, and the significant model ($p < 0.05$) with the highest determination coefficient (R^2) was selected. The graphics were done by SigmaPlot software.

Conclusion

Since Si accumulated mostly in the roots and had low leaf concentrations, the expected benefits in plant physiology, such as gas exchange and SD, did not occur significantly. However, the application rate of 0.50 mmol Si L⁻¹ provided a trend of higher growth, dry matter production, EU, LA and SLA, showing it to be the best rate for this *Eucalyptus* hybrid.

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