

Regulator Growth Vegetative and Photosynthetic Parameters in Seedling Fig

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Abstract

*The aim of this work was to study the effect of the plant growth regulator, trinexapac-ethyl, on growth and photosynthesis in *Ficus carica* L. The experimental design was a randomized block, considering a control (no application) and one or two applications of trinexapac-ethyl in seven concentrations in solution: 0; 62,5; 62,5 + 62,5; 125; 125 + 125; 250; 250 + 250 and 500 mg L⁻¹ distributed in four blocks. Were evaluated: plant height, canopy area, number of leaves per branch, branch length, number of internodes, rate of CO₂ assimilation, transpiration rate, stomatal conductance, internal CO₂ concentration, leaf temperature, use water efficiency, carboxylation activity of the enzyme ribulose 1,5-bisphosphate carboxylase, chlorophyll a, chlorophyll b and carotenoids. The use of the plant growth inhibitor applied once in a concentration of 500 mg L⁻¹ trinexapac-ethyl decreased internode and shoot growth fig. Moreover, two applications of 250 and 500 mg L⁻¹, pronounced way affected increasing photosynthesis, and increase the content of pigments (chlorophyll a, chlorophyll b and carotenoids) that participate in the photosynthetic process in plants fig.*

Key Words: *Ficus carica* L., trinexapac-ethyl, photosynthetic pigments, assimilation CO₂.

1. Introduction

The fig tree (*Ficus carica* L.) is one of the oldest fruit species used by humans. The cultivation of the fig tree was reports made since the time of Egyptian, Greek and Roman empires, as well as being described in biblical texts.

The Turkey produces about 26%, which combined with Egypt, Iran, Greece, Algeria and Morocco are responsible for about 70% of total world fruit production (Stover & Aradhya, 2007) other countries such as Brazil has also been highlighted. The subtropical fruit, like the fig tree, highlight the demand for fresh fruit has been growing and diversifying in recent years, especially in regions with areas suitable for the cultivation of these species climate. The *F. carica* species adapts easily to different environments, from arid or semiarid climate, passing through tropical and subtropical to temperate climate with very harsh winters. The climate change is mainly the increase of air temperature since the late 80's, led to profound changes in phenology and as a result, the yield of fruiting plants in various planet regions (Chmielewski, Muller, & Bruns, 2004). The knowledge of the phenology of plants can be great importance in planning, organization and implementation of activities related to agriculture (Ruml & Vulic, 2005). Thus, there is need for the introduction of new technologies in order to add to the handling benefits of the fig tree, making farming increasingly adapted to different environmental conditions. The increase in production capacity of the fig tree allied to scientific advances and t availability of technologies for production sector, such as the use of plant growth regulators can promote, inhibit or modify a given physiological process, such as reduction in plant height.

Plant growth regulators have become an integral part of agriculture, horticulture and modern fruit, especially for being new products part to break dormancy and thinning agents, which appear to be more urgent for use in fruit (Rademacher, 2010). Understanding the environmental factors (water, temperature and light) and their interaction with physiological processes such as photosynthesis rate, transpiration, stomatal conductance and density directly affect the photosynthetic process and are important for the improvement of agricultural practices and optimize photosynthetic carbon assimilation, increase productivity and fruit quality (Restrepo-Díaz, Melgar & Lombardini, 2010). Therefore, the aim of this study was to investigate the effects of trinexapac-ethyl on growth and photosynthesis in seedlings of fig (*Ficus carica* L.).

2. Material and methods

2.1 Environmental characterization and cultural practices

The experiment was conducted in full sun in the Department of Horticulture, belonging to Faculdade de Ciências Agrônomicas – FCA, Campus of Botucatu at Universidade Estadual Paulista – UNESP, Botucatu - Sao Paulo – Brazil, located in the geographical coordinates of 22°55'55" latitude South 48 26'22 " W longitude and height of 810 m. The prevailing climate is warm temperate (mesothermal) with rains in summer and dry in winter (Cwa - Köppen), with average annual temperature of 20,5°C and annual average rainfall of 1,533 mm. The experiment was conducted in the period March-July 2012, the period between the collection of cuttings and planting, transplanting and physiological analysis and biometric plants. Stem cuttings of fig cv. Roxo-de-Valinhos were taken from mother plant and placed in trays of expanded polystyrene, containing 72 cells, filled and covered with carbonized rice husk and the trays were kept in a mist chamber for 60 days.

With help of hand sprayer spraying the cuttings with methyl thiophanate fungicide at a dosage of 120 g 100 L⁻¹ of water for preventive pathogens control was performed. After 60 days, the rooted cuttings were transplanted to pots, which were experimental units consisting of four pots with a capacity to 14 L filled with a substrate composed of soil and humus decomposed cattle in the ratio of 2:1, respectively. Liming was performed 15 days before the transplant and consisted of approximately 5,62 t ha⁻¹ (35 g pot⁻¹) calcined dolomite (75,1%), calculated according to soil chemical analysis and recommendation to raise the base saturation value (V%) the soil in 70%. The planting fertilization was based on initial soil chemical analysis according with recommendations to fig tree, and so, we used 34 g pot⁻¹ of urea, 444,44 g pot⁻¹ of triple superphosphate and 103,44 g pot⁻¹ of potassium chloride as sources of N, P e K, respectively. The fig trees were exposed to sun and irrigated with micro-sprinklers distributed 1 m², with flow 200 L hour⁻¹, the whole system being switched irrigation of 8:00 at 18:00 hours daily, with irrigation every half hour for a period of 60 seconds.

2.2 Trinexapac-ethyl application and Treatments

Proceeded to trinexapac-ethyl application on March 28, 2012 using a backpack sprayer CO₂ with constant adjustment pressure (manometer) in 2,812 kgf cm⁻² and tip of the cone type X³, as the previous test conducted on selected plants in the area and defining the spray volume to 125 mL solution for each plant. The plant growth regulator from the group of growth inhibitors used was trinexapac-ethyl in commercial product form Moddus® containing 25% w/v (250 g L⁻¹) trinexapac-ethyl (chemical name: ethyl ester of 4-(cyclopropyl- α -hydroxy-methylene)-3,5-dioxociclohexano carboxylic acid) Syngenta Crop Protection Inc.

The experimental design was a randomized block, considering a control (no application) and one or two applications of trinexapac-ethyl in seven concentrations in solution form: 0; 62,5; 62,5 + 62,5; 125; 125 + 125; 250; 250 + 250 and 500 mg L⁻¹ active ingredient, distributed in four blocks. The plot consisted of four plants and a plant border on each side of the plot. Treatments were applied in two periods, the first spray was in new branches (shoots) with standardized 26,0 cm long and containing 16 internodes (around 16 axillary buds), while the second spray was performed in the same lines, 45 days after first application.

2.3 Gas evaluations

During the experiment reviews of gas exchange were carried out, besides the content of pigments like chlorophyll (a e b) and carotenoids. Furthermore, at 120 days after treatment application, we evaluated the plant height, canopy area, number of leaves per branch, branch length and number of internodes. The gas exchange was evaluated at 5 days after application (5 DAA) and at 10 days after application (10 DAA) treatments using open photosynthesis system analyzer equipment with CO₂ and water vapor by infrared radiation (Infra Red Gas Analyser – IRGA, model LI-6400, of Li-Cor). The difference between the concentration values of CO₂ and vapor present in the chamber without the water sample and the sample allows the calculation of these measures, thereby obtaining the concentration of CO₂ and released water vapor (transpiration) and assimilated (assimilation CO₂) stomata of leaves.

The gas exchange assessments were performed at the time 9:00 at 10:30 hours sunny days in fully expanded leaves, with no signs of senescence and healthy. The gas exchange evaluations were: assimilation rate CO₂ (A, $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), transpiration rate (E, $\text{mmol water vapor m}^{-2} \text{ s}^{-1}$), stomatal conductance (gs, $\text{mol m}^{-2} \text{ s}^{-1}$) and CO₂ concentration in the intercellular spaces (Ci, $\text{mmol m}^{-2} \text{ s}^{-1}$). These variables were calculated by the analysis program meter data equipment photosynthesis that uses the general equation of gas exchange (VonCaemmerer & Farquhar, 1981). The efficiency of water use (EWU, $\mu\text{mol CO}_2 (\text{mmol H}_2\text{O})^{-1}$) was determined by the relationship between assimilation CO₂ and transpiration rate (A/E) (Berry & Downton, 1982). From the above measured data carboxylation activity of the enzyme ribulose 1,5-bisphosphate carboxylase (Rubisco) by the ratio of assimilation rate was calculated CO₂ and internal concentration of CO₂ in the leaf (A/Ci). The CO₂ concentration reference used during the assessments were present in the environment, 380 $\mu\text{mol CO}_2$, and to homogenize the repeats, the flux density photosynthetic photon (FDPP) generated by a light emitting diode coupled to the photosynthesis chamber was standardized to the ambient brightness in each evaluation period. For all plants were under the same conditions of light was used 1500 $\mu\text{mol m}^{-2} \text{ s}^{-1}$.

2.4 Biochemical analyzes

Biochemical analyzes of the pigments were made 10 days after treatment application (10 DAA) random samples of three leaves collected per plot that were later taken to the laboratory for biochemical analysis to be cut, homogenized and separated into small samples 20 mg fresh frozen and powdered plant material in liquid nitrogen (N₂). Chlorophyll contents a, b and carotenoids were determined biochemically (Sims & Gamon, 2002).

2.5 Growth Evaluation

Height plant (PH) is a biometric measurement performed with aid of the attached tape in centimeters (2,0 m tall) rule. It was considered the plant height, as the distance from ground level (plant lap) to the apex of the apical bud (highest point of the plant). Area canopy (m²) was measured from the perpendicular and parallel to the tree rows (length and width) axes, these measures, averages, which then were transformed into square meters have been obtained to express the estimate of the area of the canopy of the plants in the plot, as follows: Length (cm) x width (cm)/10.000 = Area of the canopy in m². Plant canopy diameter (length) was measured with the aid of the attached tape in centimeters (2,0 m high) rule. Was defined as plant canopy diameter (length), measurement within range of fig tree growing, starting from outer edge of the first sheet to canopy edge outermost sheet from the other side of the canopy. The plant diameter canopy (width) was measured with the aid of the same rule. We considered plant diameter (width), measured between the rows of cultivating fig tree, starting from the outer edge of the first sheet to canopy edge of the outermost sheet from the other side of the canopy. The leaves number per branch (NLB) was obtained by counting total number of fig leaves on selected and marked with colored narrow ribbon branch, starting at the base of the branch to its apex. The length of the branch (LB) was determined with the help of measuring tape graduated in centimeters, considering the branch base to the apical bud tip of the same branch selected and marked with colored narrow ribbon. The internodes number (NI) was obtained by counting the average number of internodes selected and marked with colored narrow ribbon branch, the branch starting at the base up to its apex.

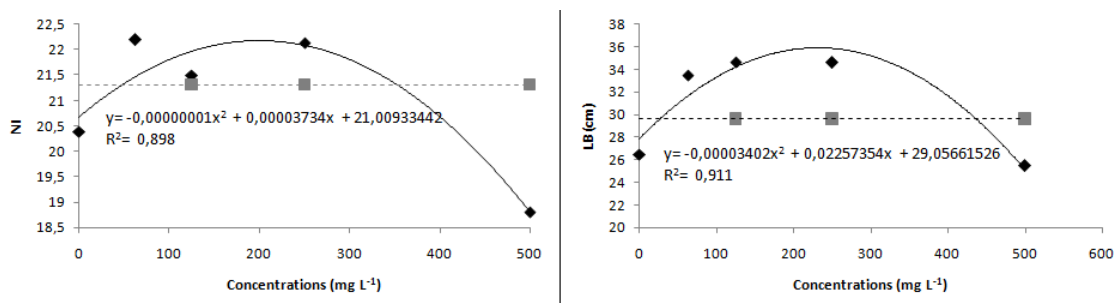
2.6 Experimental design and statistical analysis of the data

The results obtained were subjected to analysis of variance (F test), observing the homogeneity of variances by Cochran and uniformity of treatment by Shapiro-Wilk test. As necessary, respective data analyzed were transformed into square root. Furthermore, we applied the regression analysis of concentrations, using the software of statistical analysis SAS.

3. Results and discussion

The regression study showed significant differences in mean length of branches (LB) and internodes number (NI) in treatments that have undergone an application of trinexapac-ethyl, showing the concentration of 500 mg L⁻¹ lower LB and NI (Figures 1 A e 1 B). The Figures 1 A e B illustrate that increasing the LB and NI resulting from different concentrations application of the growth inhibitory in an application, concentrations of up to 250 mg L⁻¹ there was a reduction of these characteristics. The action to inhibit the branches elongation can reduce excessive shoot growth, facilitating harvesting, besides reducing the plant material amount and therefore reserve substances, including carbohydrates, which would otherwise be discarded during the winter pruning realized plants. This reduction of the length of the branches, in the future, may facilitate the spacing reduction of fig plants and probably increase the density of plants in the area, increasing crop productivity. Apple and pear trees treated with 500 mg L⁻¹ trinexapac-ethyl decreased the average length of the branches (LB), and increase fruit retention (Spinelli, Vannete, & Costa, 2012). Trinexapac-ethyl inhibits active gibberellins formation, resulting in reduction in cell elongation (Taiz & Zeiger, 2010). The elongation of cells during plants development is regulated by light and gibberellins (GAs), but the mechanism of this interaction remains unclear, along with the interaction that explains how plants integrate the light-GA signals to optimize growth and development in response to changes in environment (Lucas et al., 2008).

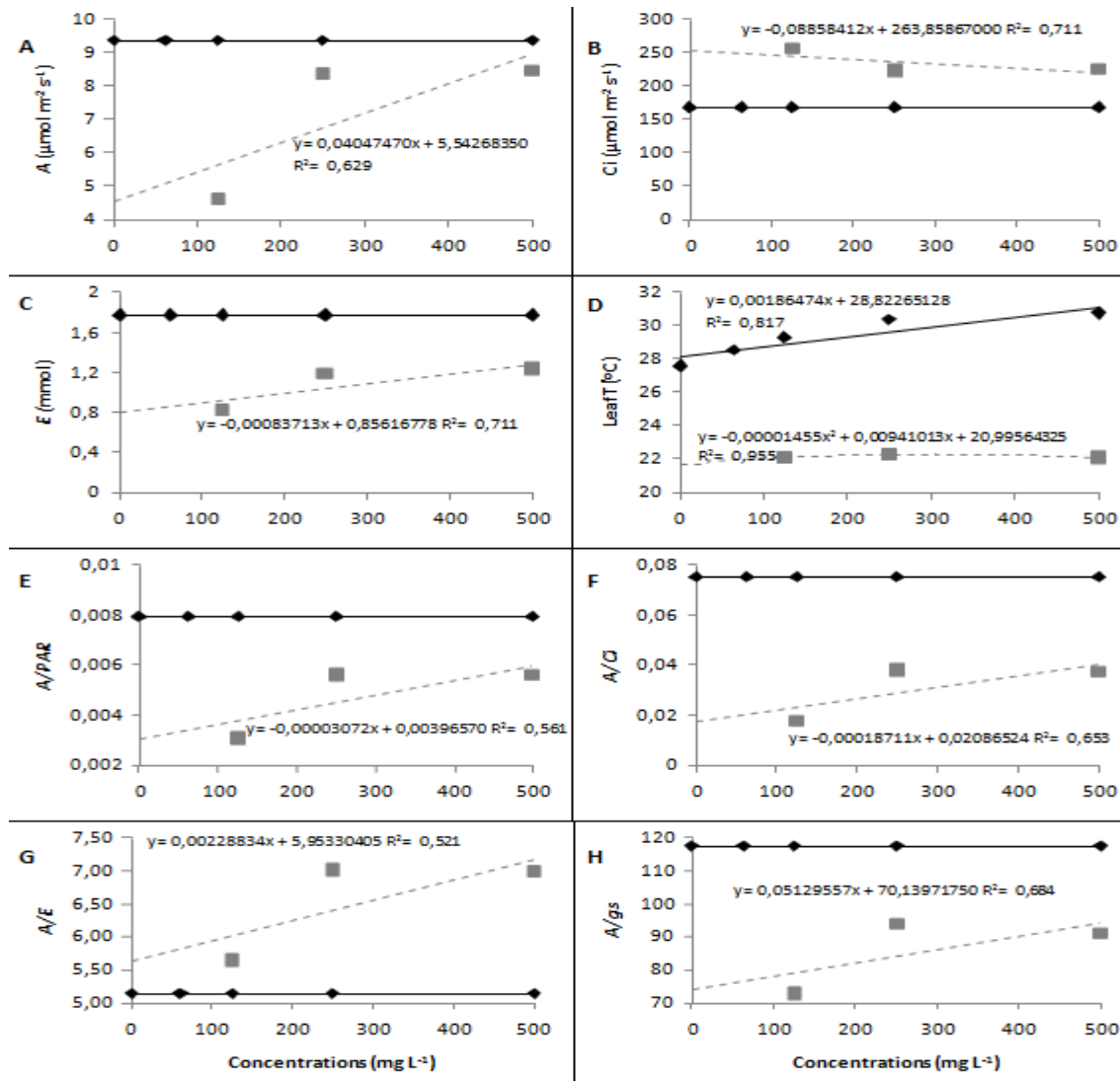
Analyzing data from gas exchange of fig leaves during the experiment with seedlings at 5 DAA growth inhibitor, applied once only, there was no effect of treatment only in leaf temperature (Figure 2 D), whereas at 10 days after application (10 DAA, Figure 3), photo synthetically active radiation was between 600,78 to 1502,29 μmol photons m⁻² s⁻¹. Was no pronounced effect on the rate of assimilation of CO₂ (A), stomata conductance (gs), leaf temperature (LeafT) and assimilation rate CO₂ in relation to photo synthetically active radiation or flux density photosynthetic photon (A/FDPP), efficiency of water use (A/E), instantaneous efficiency of water use (IEWU) and the activity of Rubisco through instantaneous carboxylation efficiency (A/Ci). Already at 5 and 10 DAA growth inhibitor, and two applications both influence the internal concentration of CO₂ (Ci), transpiration (E), leaf temperature (LeafT) and instantaneous water use efficiency (IWUE), Figures 2 and 3. This fact can be justified due to the mode of action of trinexapac-ethyl, which partially inhibits the transport of electrons in the mitochondrion, leading to a decrease in cellular respiration (Heckman et al., 2002).



When evaluated separately, the two applications of trinexapac-ethyl, it was observed at 5 DAA (Figure 2) treatments showed higher assimilation (A), consequently more efficient carboxylation (A/Ci) may be related to improved efficiency of water use (EWU) promoted by increased transpiration (E), decreased in internal concentration of CO₂ e A/FDPP. The photo synthetically active radiation was between 1201,63 to 1801,98 μmol photons m⁻² s⁻¹.

However, it was found proportional increase between the concentrations of trinexapac-ethyl and most of the features listed above, except for the internal concentration of CO₂ (Ci) that with increasing concentrations responded with a reduction (Figure 2 B). At 10 DAA (Figure 3), transpiration rates (E) followed the results of stomatal conductance (gs) and instantaneous water use efficiency (IWUE), consequently, the CO₂ internal concentration.

Transpiration and stomatal conductance (E and g_s , Figures 3 B and C) decreased with increasing concentrations of applied twice, however, the E , LeafT and A/g_s (Figures 3 D, E and H) increased with increasing concentrations, in two applications. In fig plant, the photosynthesis (A) was directly correlated with stomatal conductance (g_s), transpiration (E), leaf area and relative chlorophyll content until full leaf expansion at 30 days after emergence (Gonzalez-rodriguez & Peters, 2010).



The interactions of environmental factors and plant physiology can influence directly in the photosynthetic rates, transpiration, and stomatal conductance and density affect yield and quality of fruits (Restrepo-Díaz, Melgar & Lombardini, 2010). The fig plant, in Botucatu (Brazil), in March 2007, the rate of carbon assimilation ranged from $-0,370 \mu\text{mol m}^{-2} \text{s}^{-1}$ (leaf 1 of the base of the branch, considered low for the plant) to $14,378 \mu\text{mol m}^{-2} \text{s}^{-1}$ (leaf 4 of the branch, characterized as adult) and photo synthetically active radiation ranged from 798 to $1.868 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (causing saturation brightness). In fact it was observed that leaves with leaf area above 60 cm^2 (21 days after anthesis leaf) exhibited assimilation rates CO_2 with positive absorption of photons emitted by chloroplasts for photosynthetic active radiation (Leonel et al., 2010).

Parameters of gas exchange in fig leaves with a year during the spring-autumn (France) observed that the rate of net photosynthesis ranged from 15 to $20 \text{ mmol CO}_2 \text{ d}^{-1} \text{AGM}^{-1}$ and photo synthetically active radiation ranged between 750 and $800 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, range in which occurred the maximum net assimilation CO_2 (Grison-Pigé et al., 2001). Seven cultivars of fig in Athens (Greece) and found that the cv. San Pedro had one of the highest rates of CO_2 cultivars assimilation 14 to $16 \mu\text{mol m}^{-2} \text{s}^{-1}$ in June, August and October 2008, with a large number of fruits per branch, which may be indicative of high productivity (Pisimisi, Vemmos & Mili, 2012).

Receptors radiation with chlorophyll photosynthesis are absorption maxima in the range from blue to red (green wavelength, around 550 nm), as well as accessory pigments such as carotenoids (orange color with absorption in the region of 400 to 500 nm) and xanthophylls with absorption in blue as well as ultraviolet (Taiz & Zeiger, 2010). The pigments involved in photosynthesis (chlorophyll *a*, chlorophyll *b* and carotenoids) found in seedling leaves of fig tree are shown in Figure 4. In general, chlorophyll contents *a* and *b* and carotenoids were higher at all concentrations of trinexapac-ethyl applied once only, except for carotenoids, there was no difference to the witness. In addition, to the chlorophylls *a* and *b* treatment with two applications of trinexapac-ethyl (125 + 125 mg L⁻¹) the results show a positive effect in increasing concentrations, similar to the other treatments. Also, two applications 250 + 250 mg L⁻¹ had a favorable effect on increasing chlorophyll levels *b* (Figure 4).

In general, there was a gradual increase in chlorophyll with increasing inhibitor of growth concentration, both in one application as for two applications (Figure 4). The highest levels of chlorophyll *a* and *b* (Figure 4 A and B) were obtained at a concentration of 500 mg L⁻¹, in one application. With two applications there is an increase in levels proportional to the gradual increase in chlorophyll concentrations. To the levels of carotenoids (CAR, Figure 4C) revealed that the highest levels of concentration 500 mg L⁻¹, both in one as in two applications. Chlorophyll degradation and carotenoid synthesis is enhanced with the application of 400 mg L⁻¹ prohexadione-Ca of a acylcyclohexanediones as trinexapac-ethyl and present results support the hypothesis that there may be an inverse relationship between moderate vigor and color development, for example in citrus peel (Barry, 2010). This increase may be related to gene regulation or activity plastid transcription core (Egea et al., 2010) would act in the conversion of most of chloroplasts to chromoplasts, ones who accumulate large amounts of carotenoids and protein would possess much as chlorophyll biosynthesis of proteins involved in chlorophyll degradation (Barsan et al., 2010).

However, an analysis of tomato plants (*Solanum lycopersicon* L.) dwarfs, gibberellin deficient revealed that levels of carotenoids and chlorophyll were unchanged and the phenotype (normal plants) rescued by applying gibberellic acid (Schie, 2007) demonstrating that the physiological and phenotypic responses, mainly related to carotenoid metabolism and chlorophylls, besides growth may differ according to the species studied.

4. Conclusion

One trinexapac-ethyl application with concentration 500 mg L⁻¹ decreased the growth of branches and internodes fig. The concentrations 250 and 500 mg L⁻¹ applied once or twice increased photosynthesis and increased chlorophyll *a* and *b* contents and carotenoids.

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Figure Legends:

Fig. 1. Number of internodes (NI) and average length of branch (LB, in cm) of fig trees treated with trinexapac-ethyl. 1 ● application 2 ■ applications.

Fig. 2. Rate of assimilation CO₂ (A , $\mu\text{mol m}^{-2} \text{s}^{-1}$), concentration of CO₂ in the intercellular spaces (C_i , $\mu\text{mol m}^{-2} \text{s}^{-1}$), transpiration (E , mmol), leaf temperature (C , LeafT), photosynthetically active radiation (A/PAR), carboxylation efficiency (A/C_i), instantaneous water use efficiency (A/E) and intrinsic water use efficiency (A/g_s) in fig leaves (*Ficus carica* L. cv. Roxo de Valinhos), 5 days after application (5DAA) trinexapac-ethyl in different concentrations.

1 ● application 2 ■ applications.

