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Development and gas exchange of fig plants submitted to dynamized high dilutions

Desenvolvimento e trocas gasosas de plantas de figo submetidas a altas diluições dinamizadas

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Highlights

Thuya occidentalis improved leaf production rate and leaf retention of fig plants.
Roxo de Valinhos was more responsive to the treatments.
Thuya occidentalis and *Belladonna* enhanced net photosynthesis.

Abstract

The productive potential of crops directly depends on their primary metabolism, for which photosynthetic efficiency is the best indicator. This study aimed to evaluate the effect of dynamized high dilutions on the development and photosynthetic efficiency of fig plants under greenhouse conditions. The treatments were *Belladonna, Thuya occidentalis,* and the nosode of fig leaves with rust at 30 CH (centesimal hahnemannian dilution order). Distilled water was the control treatment. The subplot consisted of fig cultivars Roxo de Valinhos and Branco Rosa Lages. Weekly sprays started 30 days after transplanting and lasted for 5 weeks. Plant height and total number of leaves were evaluated at 0, 37, 44, 51, 58, 65, 86, 100, and 114 days after transplanting. Photosynthetic efficiency was estimated at 5, 15, 30, and 50 days after the first application with an Infra-Red Gas Analyser – IRGA by measuring gas exchange: (a) CO_2 assimilation rate, (b) transpiration rate, (c) stomatal conductance and (c) internal concentration of CO_2 in the leaf. Water use and carboxylation efficiency were also determined. According to the data, the cultivar Roxo de Valinhos was more sensitive to the dynamized high dilutions concerning leaf emission per day and remaining leaves, and it showed a higher response to *Thuya occidentalis* and the nosode. Net photosynthesis increased after cumulative applications on the cultivar Roxo de Valinhos treated with *Thuya* and *Belladonna.* The dynamized high dilutions can influence plant development and

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photosynthesis, and Roxo de Valinhos is highly sensitive to such dilutions.
Key words: Ficus carica. Secondary metabolism. Photosynthesis.

Resumo

O potencial produtivo de cultivos vegetais depende diretamente do seu metabolismo primário, no qual a eficiência fotossintética é o melhor indicador. Este estudo teve como objetivo avaliar o efeito de altas diluições dinamizadas no desenvolvimento e na eficiência fotossintética de plantas de figueira em casa de vegetação. Os tratamentos foram *Belladonna, Thuya occidentalis* e nosódio de folhas de figueira com ferrugem a 30 CH (ordem de diluição centesimal hahnemanniana). Água destilada foi o tratamento controle. A subparcela foi constituída pelas cultivares de figo Roxo de Valinhos e Branco Rosa Lages. As pulverizações semanais começaram 30 dias após o transplante e duraram por cinco semanas. A altura das plantas e o número total de folhas foram avaliados aos 0, 37, 44, 51, 58, 65, 86, 100 e 114 dias após o transplante. A eficiência fotossintética foi estimada aos 5, 15, 30 e 50 dias após a primeira aplicação com Infra Red Gas Analyzer – IRGA medindo as trocas gasosas: (a) taxa de assimilação de CO_2, (b) taxa de transpiração, (c) condutância estomática e (c) concentração interna de CO_2 na folha. A eficiência do uso da água e a eficiência de carboxilação também foram determinadas. De acordo com os dados, a cultivar Roxo de Valinhos foi mais sensível às altas diluições dinamizadas em relação à emissão de folhas por dia e folhas remanescentes, apresentando maior resposta a *Thuya occidentalis* e nosódio. A fotossíntese líquida aumentou após aplicações cumulativas na cultivar Roxo de Valinhos tratada com *Thuya* e *Belladonna.* Altas diluições dinamizadas são capazes de influenciar o desenvolvimento das plantas e a fotossíntese, sendo Roxo de Valinhos altamente sensível às mesmas.
Palavras-chave: *Ficus carica.* Fotossíntese. Metabolismo secundário.

Introduction

The fig *Ficus carica* L. is one of the oldest species of domesticated plants. It is closely associated with cultural and culinary habits in several countries, and it has high socio-economic importance worldwide (Rodrigues et al., 2019a). In Brazil, the production of *F. carica* was 18,227 tons in 2022, in a crop area of 2,130 hectares, mostly located in the Southern and Southeast regions (Instituto Brasileiro de Geografia e Estatística [IBGE], 2023). Brazil is considered a crucial exporter of fresh fig fruits during the international off-season, with annual revenues of US$ 7.92 million in 2021 (Food and Agriculture Organization of the United Nations [FAO], 2023). Commercial fig orchards are cultivated with few clones, and the cv. Roxo de Valinhos is the most representative one in Brazil (Rodrigues et al., 2019b).

Fig production can be compromised by several biotic and abiotic factors. Diseases can affect photosynthesis by reducing the photosynthetic area (Keeley et al., 2022). Climatic conditions, e.g., droughts and excessive rain, can also reduce the photosynthetic efficiency of plants, leading to reduction of yields (Ammar et al., 2020). Breeding fig species to improve tolerance to these factors is a high priority to farmers.

However, there is a major constraint, namely the lack of the exclusive wasp pollinator *Blastophaga psenes,* which does the fertilization of inflorescence to produce viable seeds (Proffit et al., 2020). In addition, the intensive use of external inputs not only increases production costs, but also causes a residual impact on the agroecosystem. To minimize all these issues, non-residual technologies are a requirement from consumers and society at large.

The intensified methods of cultivation have increased the susceptibility of fig orchards to disease infections and pest infestations (Ecker et al., 2018). The primary disease impacting fig production is fig rust, caused by the biotrophic fungus *Cerotelium fici* (Cast.) Arth (Galleti & Rezende, 2016). Mezzalira et al. (2015) report that fruit production losses can reach up to 80%, with affected plants experiencing total defoliation within 30 days, particularly in extended periods of rainfall that favor rust proliferation.

Dynamized high dilutions (DHD) - homeopathies - are non-residual therapies with biological effects of improving crop health, as reported elsewhere (Boff et al., 2021). Plants react to dynamized high dilutions, according to previous agronomic trials. Thus, it is assumed that they actually interfere with the secondary metabolism of plants, for example, their resistance to diseases and pests, and their development (Mioranza et al., 2017). The expression of the productive potential of plants depends on directly to their primary metabolism, and photosynthetic efficiency is the best indicator (Ferraz et al., 2020). Mazón-Suástegui et al. (2020) demonstrated that the dynamized high dilution *Manganum metallicum* 31CH could increase photosynthesis and growth of *Vigna unguiculata.* The plant respiration process, which contributes to biomass accumulation, utilizes a significant portion of the compounds produced in photosynthesis. Therefore, if one could reduce respiration and increase gross photosynthesis, fruit production could gain more efficiency (Kluge et al., 2015).

The use of DHD to treat plants in Brazil is allowed by the organic production legislation, although not restricted to them (Ministéria da Agricultura, Pecuária e Abastecimento [MAPA], 2021). Dynamized high dilutions are embodied into non-residual therapies, and it has the fundamental principle of healing an individual as a unique, integrated, and harmonious being (Teixeira, 2023). This way, dynamized high dilutions are applied to restore the balance not only of the plant itself but also of the surrounding agroecosystem. It is, thus, an integrative therapy. In addition to that, it has the potential to reduce production costs and reduce the farmers' dependence for external input (Boff et al., 2021).

Considering the above-mentioned context, this work aimed to evaluate the effect of dynamized high dilutions on the development and photosynthetic efficiency of fig plants.

Material and Methods

The study was conducted with fig plants grown in pots in the greenhouse of the Experimental Station of Epagri, Lages, SC, Brazil. Experiments were performed with two clones: (a) commercial cv. Roxo de Valinhos and (b) landrace Branco Rosa Lages that is growing by farmers in Lages city, SC, BR.

The fig plant multiplication was made by bevel-cut of 10 cm length herbaceous cuttings. The cuttings were introduced into boxes with sand and vermiculite 1:1 (v/v). Transplanting was performed 60 days later when the main sprout was 20 cm high and had uniform rooting. Each plant was planted in 15-liter polyethylene pots with soil, sand, and Bokashi 1:1:1 (v:v:v) and kept in the greenhouse.

The dynamized high dilutions were selected by repertorization method, using the HomeoPro® software. To this end, the symptoms of the plant infected by the rust *C. fici* were considered, as well as the environmental conditions that favour the occurrence of fig rust. The fragility and weakness of the fig plants were also considered. The final choice was made with the help of the Homeopathic Materia Medica, which indicated *Thuya occidentalis* and *Belladonna* as the most closely to the totally symptomatic of described fig plant (Lathoud, 2010).

The matrix preparations were purchased at 3CH in a competent pharmacy. Afterwards they were prepared to the used potency in the Laboratory of Homeopathy and Plant Health at the Experimental Station of Epagri, Lages, SC, according to the Brazilian Homeopathic Pharmacopoeia (Farmacopeia Homeopática Brasileira, 2011), consisting of sequential dynamizations (dilution+sucussion) from 3CH to 28CH with alcohol 70%, and after this reaching 30CH in distilled water. All the dynamizations were carried out using the centesimal scale of the Hahnemannian method, utilizing multiple amber flasks of 30mL. The biotherapic nosode was prepared by crushing fig leaves with C. fici, also following the Brazilian Homeopathic Pharmacopoeia. The potency 30CH was chosen according previously tests with satisfactory results in the growth of plants, and management of diseases and pests (Oliveira et al., 2021; Zanco, 2022; Pulido et al., 2017).

Two experiments (crop cycle 1 - 2020/21 and crop cycle 2 - 2021) were performed in the same experiment design and conducted in a complete randomized block in split plots, five replications, with the sampling unit consisting of a vase with a plant. The main plot consisted of the preparations *of Thuya occidentalis, Belladonna,* the nosode of rusted fig leaves and distilled water. The subplot was composed of the cv. Roxo de Valinhos and Branco Rosa Lages.

The treatments were applied on a weekly basis with a 5 L hand sprayer (Guarany®), covering the leaf canopy and reaching to the soil near the plant, with 20mL per plant. The total applied volume was full dynamized. The spray applications started 30 days after transplanting and lasted for five weeks.

Plant development

Plant height, number of leaves, and number of remaining leaves on the plant were evaluated at 30, 37, 44, 51, 58, 65, 86, 100, and 114 days after transplanting (DAT). The rate of number of leaves day^{-1} (NLD) was calculated by the formulas NLD = $i\Sigma^{n-1}$ $[(x_i + x_{i+1})]/2\ (t_{i+1} - t_i)$, where "n" is the number of evaluations; $(t_{i+1} - t_i)$ is the time between two evaluations, and $(x_i + x_{i+1})$ is the value of two evaluations. Plant height data was used in equations to calculate the absolute stem growth rate (ASGR), by the formula: ASGR = (L2 - L1)/ (t2 - t1) cm day^{-1}, where L1 is the measure of plant height at time t1, and L2 is plant height at time t2, expressed in cm day^{-1}, according to Araujo et al. (2014).

Photosynthetic efficiency of fig plants

The gas exchange variables were assessed during the period of 8:00 am to 1:00 pm at 5, 15, 30, and 50 days after the first application of the treatments, on fully expanded leaves located at the middle third of the plant. Quintuplicate readings were performed by using equipment with an open photosynthesis system with a CO_2 and water vapor analyser by infrared radiation ("Infra-Red Gas Analyzer – IRGA", model LCpro-SD, ADC BioScientific®). From that it was assessed the concentrations of water vapor and CO_2 that were released (transpiration – water vapor) and the assimilated (CO_2 assimilation) through the leaf stomata.

The gas exchange analyses were: (a) CO_2 assimilation rate (A, µmol CO_2 m^{-2} s^{-1}), (b) transpiration rate (E, mmol water vapor m^{-2} s^{-1}), (c) stomatal conductance (gs, mol m^{-2} s^{-1}) and (d) internal concentration of CO_2 in the leaf (Ci, µmol CO_2 mol^{-1} air). Water use efficiency (WUE, µmol CO_2 (mmol H_2O^{-1}) was determined by the ratio of CO_2 assimilation rate to transpiration rate (A/E). Carboxylation efficiency of the enzyme ribulose 1, 5-diphosphate carboxylase (Rubisco) was determined by the ratio between of CO_2 assimilation rate to the internal concentration of CO_2 in the leaf (A/Ci).

Data analysis

Data underwent analysis of variance, and the means were compared with Tukey's test at 5% error probability, using the R software (RStudio Team, 2020).

Results and Discussion

Development of fig plants

The interaction between homeopathic preparations (main plot) and cultivar (subplot) was not significant for absolute stem growth rate (ASGR). Therefore, the simple effects were analysed. The two cultivars showed no difference in growth rate between them during the evaluation period. Thus, the analyse of both cultivars was separated. The nosode of rust presented higher ASGR in comparison to the others in crop cycle 1. In crop cycle 2, *Belladonna* showed the highest ASGR. The ASGR data about *Thuya occidentalis* was similar between the two cycles, 2020/2021 and 2021. *Thuya occidentalis* showed lower stem growth in comparison to the others, considering the evaluation period. For cycle 2, Branco Rosa Lages plants treated with *Thuya occidentalis* showed lower stem growth than the others during the evaluation period (Table 1).

Table 1
Absolute stem growth rate (cm day^{-1}) of fig plants, during 80 days in the greenhouse, submitted to dynamized high dilutions. Lages, SC, BR

Preparations	Cycle 1			Cycle 2		
	Branco Rosa Lages	Roxo de Valinhos	Mean	Branco Rosa Lages	Roxo de Valinhos	Mean
Thuya occidentalis	4.73ns	4.96ns	4.84 b	2.19 b	3.37ns	2.80 b
Nosode	6.49	6.31	6.40 a	4.23 a	3.10	3.66 ab
Distilled water	5.36	6.83	6.10 ab	3.13 ab	3.03	3.09 ab
Belladonna	4.90	5.83	5.36 ab	4.38 a	4.53	4.47 a
CV	19.09	19.41	13.72	26.19	28.64	30.97

Means followed by the same letter in the column do not differ from each other, for each cycle, according to Tukey's test ($p \leq 0.05$). Cycle 1 - 12/30/2020 to 03/17/2021; Cycle 2 – 08/02/2021 to 10/18/2021.

For the rate of number of leaves per day, the interaction between the cultivars and dynamized high dilutions (DHD) was significant in both crop cycles. In the first cycle, the DHD in the Branco Rosa Lages cultivar did not differ among themselves, contrary to what was found for the Roxo de Valinhos cultivar. For this cultivar, the *Thuya occidentalis* preparation stood out from the others, providing a greater number of leaves per day, followed by the nosode. Plants treated with distilled water showed a low leaf emission capacity when compared to the other treatments (Table 2).

Table 2
Number of leaves per day of fig tree cultivars, for 80 days in the greenhouse, submitted to dynamized high dilutions, in two cultivation cycles

Preparations	Cycle 1		Cycle 2	
	Branco Rosa Lages	Roxo de Valinhos	Branco Rosa Lages	Roxo de Valinhos
Thuya occidentalis	2.50 Aa	3.37 Aa	1.82Aa	2.07Aab
Nosode	1.77 Ba	3.17 Aab	0.39Bb	2.69Aa
Distilled water	1.94 Aa	1.57 Ac	0.89Aab	1.00Ab
Belladonna	1.77 Aa	2.04 Abc	1.11Aab	1.86Aab

Means followed by the same uppercase letter in the row for the same cycle and lowercase in the column for the same cycle do not differ from each other, according to Tukey's test ($p \leq 0.05$). Cycle 1 - 12/30/2020 to 03/17/2021, CV subplot 27.56%; CV plot 30.52%; Cycle 2 – 08/02/2021 to 10/18/2021, CV subplot 30.59%; CV plot 36.53%.

The plants treated with *Thuya occidentalis,* which showed lower stem growth, had the highest rate of number of leaves per day. This behaviour could be due to the fact that stimulating plants with this treatment redirected their growth to the leaves instead of the steam. Low-stature fruiting reduces the demand for harvesting equipment, facilitates the maintenance and increases production (Musacchi et al., 2021). The nosode was the only treatment that responded differently among cultivars: the number of leaves per day in Roxo de Valinhos increased 44% in comparison to Branco Rosa Lages (Table 2).

In the second cycle, *Thuya occidentalis* could increase the stem growth in Branco Rosa Lages in a higher proportion than the other treatments. In the Roxo de Valinhos, the nosode presented the best result in terms of increasing the stem growth of fig plants. Jäger et al. (2011) found also that the nosode of duckweed stimulated the growth rate of the same plant from what was prepared the nosode. In the present study, the nosode was made with leaves of the Roxo de Valinhos cultivar with rust, which may explain this response of the corresponding cultivar rather than that of Branco Rosa Lages. Unlike homeopathy, which complies with the “Law of Similars” and seeks to treat the individual as a whole, the nosodes are target directly to the specific plant and/or disease (Farmacopeia Homeopática Brasileira, 2011).

The number of remaining leaves was analysed separately for each cultivar and each cycle. In the first cycle, during the 80-day period, it was found that certain treatments were better than others on specific evaluation dates (Figure 1a and b). For the Branco Rosa Lages cultivar, the plants showed the same behaviour regardless of the treatments (Figure 1a). For the Roxo de Valinhos cultivar, at 86 days after transplanting (DAT), plants treated with *Thuya occidentalis* showed a higher number of remaining leaves (Figure 1b). At 100 and 114 DAT, the plants treated with *Thuya occidentalis* and nosode preparations had a higher number of remaining leaves (Figure 1b).

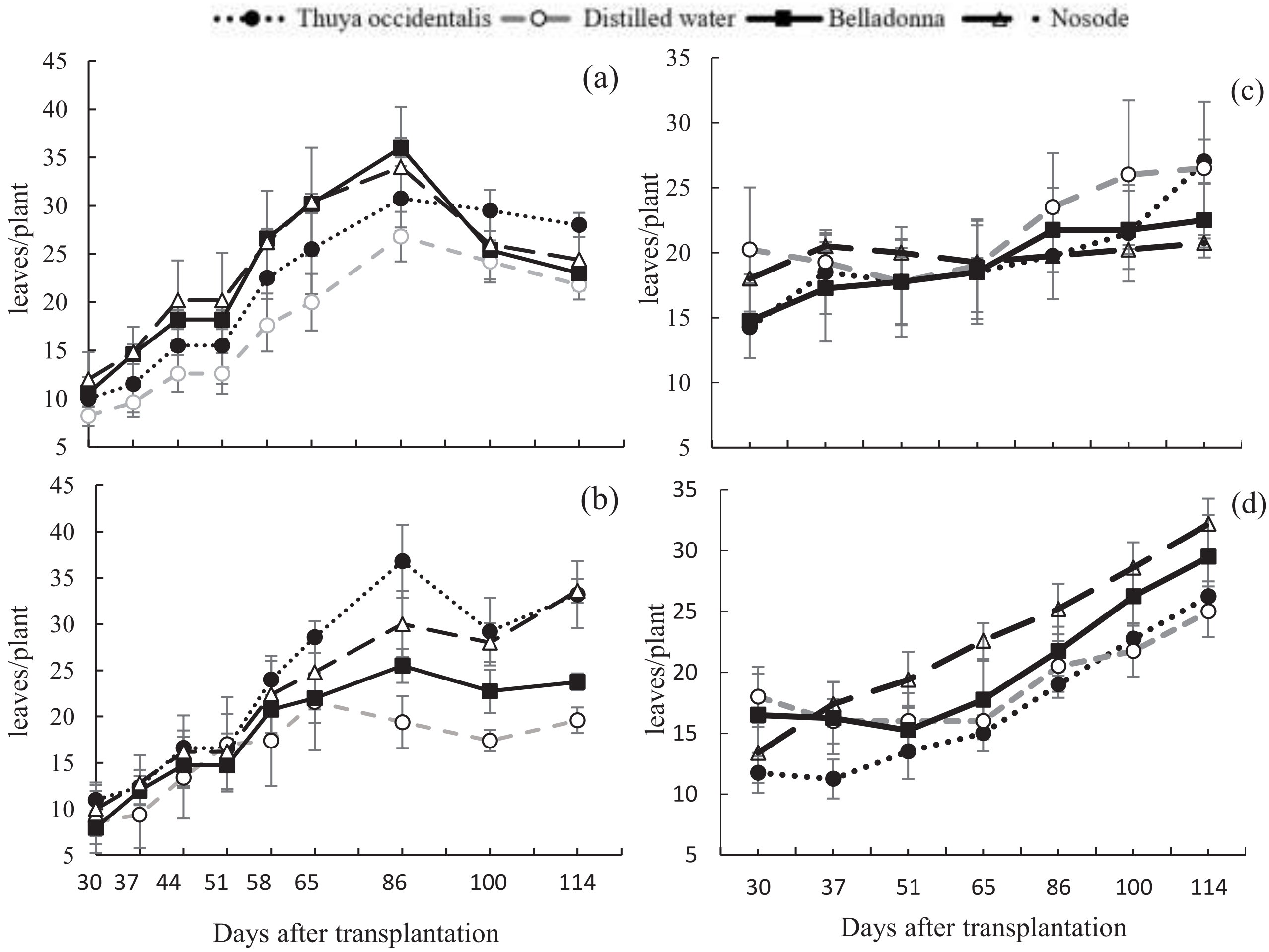

Figure 1. Remaining leaves of fig cultivars Branco Rosa Lages (a and c) and Roxo de Valinhos (b and d) in cycle 1 (a and b) and cycle 2 (c and d), treated with homeopathic preparations.
Bars in each mean correspond to standard error. Means of each evaluation day were compared by Tukey's test (p ≤ 0.05). Cycle 1 - 12/30/2020 to 03/17/2021; Cycle 2 – 08/02/2021 to 10/18/2021.

Thuya occidentalis provided a higher number of leaves per day and it also provided higher leaf retention. Fig plants lose leaves during the cycle, and this abscission can be accelerated by biotic and abiotic factors such as rust, lack of water, excessive heat, etc (Ammar et al., 2020). The greater the retention capacity of these leaves by the plants, the better their productive performance, because leaves are essential for photosynthesis and fruit protection (Ferraz et al., 2020).

In cycle 2, the remaining leaves of Branco Rosa Lages and Roxo de Valinhos did not differ among the treatments during the experiment. This result was probably due to the climatic conditions, when the period of cycle 2 was in spring - 08/02/2021 to 10/18/2021, with mild temperature and low humidity. That may have influenced the behaviour of the plants and the subtle effect of homeopathic preparations. Similarly, the intensity of rust *Cerotelium fici,* a frequent disease in fig plants that causes leaf fall, could be influenced by temperature and humidity (Parthasarathy et al., 2020).

Photosynthetic efficiency of fig plants

Air temperature during the analysis ranged between 16.34 and 23.54 ºC, and daily insolation ranged between 0 and 12.57 h dec (Figure 2). On average, leaf temperature was 0.5 ºC higher than air temperature (data not shown), which is similar to the rates reported by Silva et al. (2010).

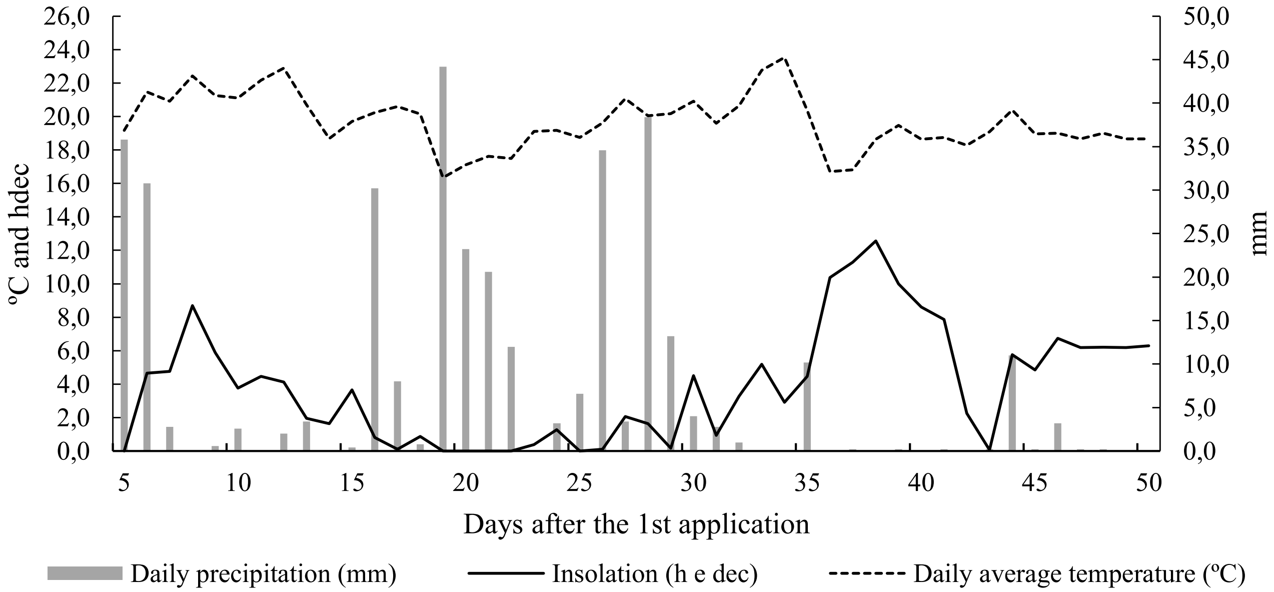

Figure 2. Climatic data from the meteorological station of Epagri, Lages, SC during the evaluation period, from Jan/2021 to Feb/2021.

Statistical analysis was performed for each evaluation date DAA (DAA = days after the first application). For the variable net photosynthesis ("A"), there was an interaction between the cultivar and homeopathic preparation factors at 30 DAA only (2 days after the 5th application) (Figure 3a). In that period, the cultivar Roxo de Valinhos treated by *Thuya occidentalis* and *Belladonna* presented 24.16% higher net photosynthesis than distilled water. For the cultivar Branco Rosa Lages, there was no difference among treatments. Within the *Belladonna* treatment, the cultivar Roxo de Valinhos showed 28.47% higher net photosynthesis than Branco Rosa Lages. At 15 and 50 DAA, *Belladonna* excelled as a treatment, showing a greater residual effect than *Thuya occidentalis.*

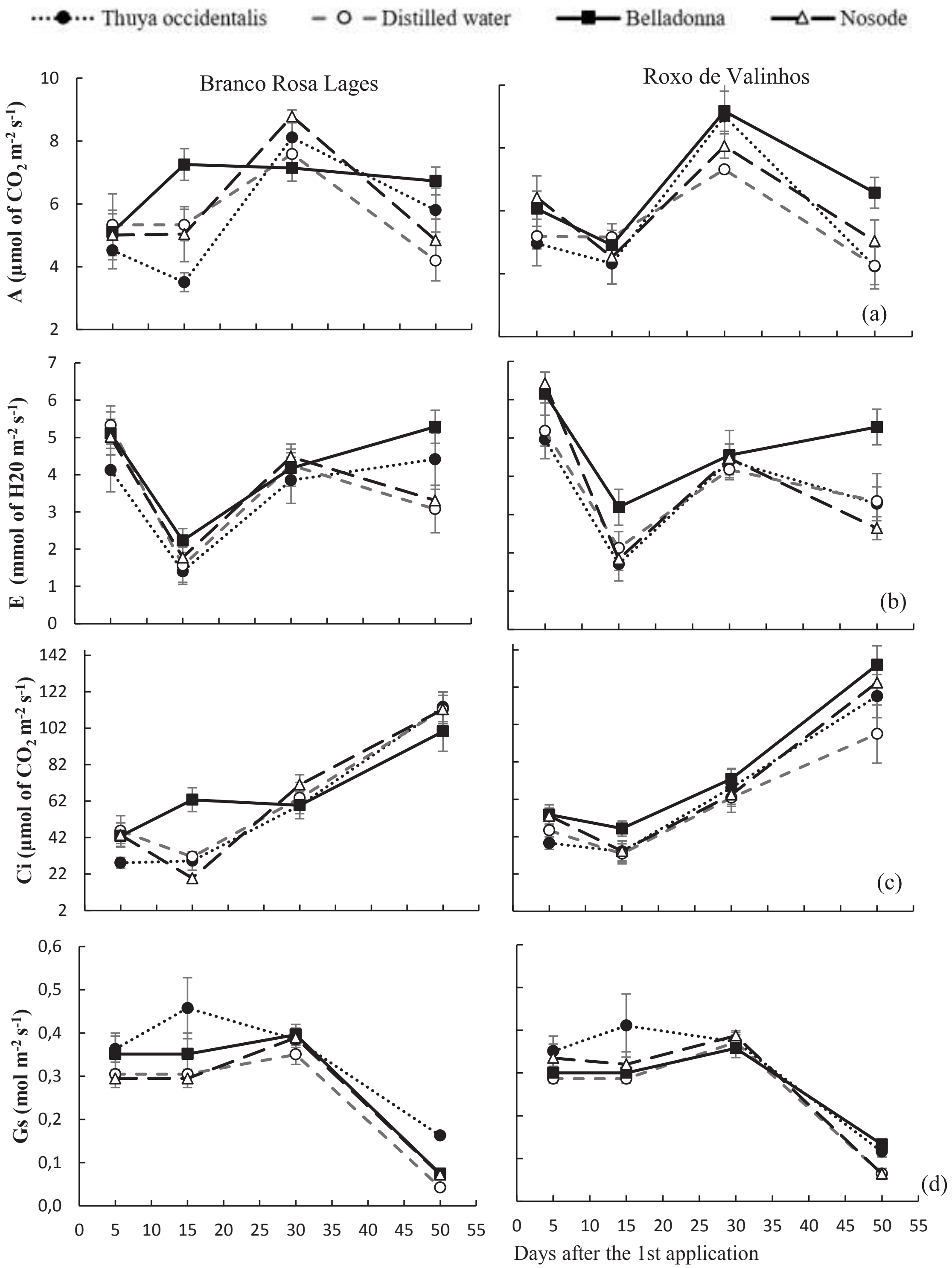

Figure 3. Gas exchanges on different evaluation days expressed by (a) Net photosynthesis, (b) Evapotranspiration, (c) Internal carbon concentration and (d) Stomatal conductance of fig cultivars submitted to dynamized high dilutions.
Treatment applications were performed at 0, 7, 14, 21, 28 days. Bars correspond to standard error. Means compared by Tukey's test ($p \leq 0.05$).

The lack of response at 5 DAA indicated that more time is probably needed to sensitize the plants, and/or the cumulative effect of more applications is required to increase photosynthetic efficiency. Mioranza et al. (2018) found that treatment with *Thuya occidentalis* 24CH reduced CO_2 fixation in tomato plants inoculated with *Meloidogyne incognita,* at 13 days after treatment had been applied. The increase in this parameter could be observed after more applications and/or more time after the treatments. In general, "A" was lower in *Thuya occidentalis* than in *Belladonna.*

The "A" values ranged between 3.5 and 9.17 µmol m^{-2} s^{-1}. These results are considered low if we compare them with those of Silva et al. (2010). These authors reported 12.6 µmol m^{-2} s^{-1} for Roxo de Valinhos fig plants. In the present study, as the plants were grown in greenhouse conditions, there was no direct solar incidence compared with grown in the field. Therefore, lower light absorption can be expected for photosynthesis.

The increase in photosynthetic rates depends, among other factors, on the maximum use of available light, owing to the phenological age of the leaves and cultural treatments and management. In this process, plants assimilate CO_2 from the atmosphere and reduce it to triose phosphate, which can then be used to produce carbohydrates, especially sucrose and starch. All phytomass production and fig architecture formation depend directly on photosynthetic activity (Anthony & Minas, 2021).

For the evapotranspiration variable ("E"), there was no interaction between the cultivars and dynamized high dilutions in any evaluation dates. At 5 DAA, the Roxo de Valinhos cultivar showed higher evapotranspiration than Branco Rosa Lages. That may be due to the architecture of wider leaves and consequent greater area per leaf. A high leaf area for a plant, in addition to allowing high light energy interception, also means a large exposed surface that can provide a high transpiration rate. At 15 DAA (one day after the third application), the cultivar Branco Rosa Lages stood out, responding to the action of the preparations, and increasing its evapotranspiration. At 15 and 50 DAA, the *Belladonna* preparation provided higher evapotranspiration (Figure 3b).

The "E" values ranged between 1.41 and 6.42 mmol of H_20 m^{-2} s^{-1}, which corroborates the results from Silva et al. (2010). Ammar et al. (2020) found that fig plants submitted to water stress presented a transpiration rate between 0.24 and 1.03 mmol of H_20 m^{-2} s^{-1}; however, with full irrigation, it recovered and presented evapotranspiration between 2 and 3 mmol of H_20 m^{-2}. The period of low "A" and "E" values corresponded to the low temperature, shorter insolation time, and higher precipitation rate than on other days (Figure 2). That plant response suggests that dynamized high dilutions improved plant readaptation to the environment.

For the internal carbon concentration variable ("Ci"), there was an interaction between the factors at 15 DAA: *Belladonna* showed an increase of 99% in the Branco Rosa Lages cultivar compared to distilled water. When treated with the nosode, the Roxo de Valinhos cultivar had 34% higher response than distilled water (Figure 3d). The carbon concentration variable is the concentration of CO_2 in the substomatal chamber of leaves (Harrison et al., 2020). The efficiency in the CO_2 assimilation rate is inversely related to net photosynthesis. The higher the CO_2 concentration in the substomatal chamber, the lower the photosynthetic CO_2 assimilation (Campelo et al., 2015). Internal carbon concentration showed negative correlation with "A", suggesting that the drop in "A" is related to partial stomatal closure.

For stomatal conductance ("gs"), there was an interaction between the factors only at 50 DAA. The Branco Rosa Lages cultivar treated with Thuya occidentalis showed the highest values whereas Roxo de Valinhos treated with *Thuya occidentalis* and Belladonna were better than the others. Within *Thuya occidentalis,* the Branco Rosa Lages had the highest stomatal conductance, but for *Belladonna* it was Roxo de Valinhos. Thus, it was found that the different cultivars respond differently to the treatments for the ability of the leaves to perform gas exchanges with the surrounding environment. At 15 DAA (one day after the third application), there was a difference among treatments; *Thuya occidentalis* was better than the others (Figure 3c).

In contrast to the results, Mioranza et al. (2018) showed that tomato plants treated with *Thuya occidentalis* 6CH tended to have stomatal closure (gs) (0.27 mol m^{-2} s^{-1}) and lower Ci 13 days after treatment. Different potencies of dynamized high dilutions can present different effects on the plants in terms of development (Abasolo-Pacheco et al., 2020), disease and pest management (Reis & Ottoni, 2021), secondary metabolism (Deboni et al., 2021), etc.

The relation between CO_2 assimilation "A" and stomatal conductance "gs" can be explained as a function of the high concentration of CO_2 at the carboxylation site when there is an increase in stomatal conductance. CO_2 fixation may also have been restricted by carbohydrate metabolism in stomatal conductance (Campostrini & Yamanishi, 2001). In the present work, "gs" and "A" were inversely proportional to "Ci" (Figure 3c and 3d). In other words, the higher the results of "Ci", the smaller the results of "gs" and "A". The increase in gaseous diffusion resistance – lower gs – may be a limiting factor in the CO_2 assimilation rate (Ferraz et al., 2020).

The variables "A" and "gs" showed decreases after 30 DAA, the day on which the application of treatments ended, and this result was associated with significant increases in "Ci" and "E" (Figure 3). The high internal concentration of carbon "Ci" and low stomatal conductance "gs" starting at 30 DAA (Figure 3c and 3d), suggest that high temperatures and insolation, followed by low temperatures and little rain, induced gradual stomata closure ("gs"), reduced net photosynthesis ("A"), and, thus induced the plant to accumulate CO_2 in the mesophyll ("Ci"). As reported by Harrison et al. (2020), CO_2 diffuses into the substomatal chamber and the air spaces between the mesophyll cells. Diffusion to the chloroplasts begins in the layer of water that moistens the walls of the mesophyll cells and continues through the plasma membrane, the cytosol, and the chloroplast. Each section of this diffusion route imposes resistance to CO_2

diffusion; therefore, the supply of CO_2 for photosynthesis faces several points of resistance (Eckardt et al., 2023). In this way, another reason is that other resistance points could influence all CO_2 transport in the mesophyll to the chloroplasts.

Mlinaric et al. (2017) found that fig leaves showed good primary photochemical efficiency in the morning. However, this behaviour changed at noon, when these leaves showed a certain level of photoinhibition of the photosynthetic apparatus. Stressful conditions are widely recognized to trigger efficient photoprotection and repair mechanisms within the photosynthetic system. The combination of increased irradiance and high temperature is among the most commonly stresses factors under field conditions. These adaptations are observable across various stages of light conversion along the electron transport chain (Zivcak et al., 2014).

In general, the cultivars responded to the gas exchange variables after the 3rd application of the treatments, at 15 DAA. The greatest response to the Roxo de Valinhos cultivar was that of *Belladonna* (Figure 3).

Regarding gas exchange data, water use efficiency and instantaneous carboxylation efficiency were calculated. For water use efficiency, the evaluations at 15 and 50 DAA showed interaction between factors and differences between treatments. On these days, the Branco Rosa Lages cultivar was not responsive, unlike the Roxo de Valinhos cultivar. At 15 DAA, the nosode provided higher WUE results, while *Belladonna* had lower results (Figure 4a).

In the instantaneous carboxylation efficiency variable, which is closely related to Ci and A, there was also a difference at 15 and 50 DAA. At 15 DAA, the cultivar Roxo de Valinhos responded better to *Belladonna.* At 50 DAA, the Branco Rosa Lages cultivar showed the best results with *Belladonna* (Figure 4b). The non-stomatal limitations on photosynthesis are often linked to a decrease in carboxylation efficiency. This decline can be attributed to the accumulation of salts within the mesophyll, leading to changes in the internal concentration of CO_2 (Veloso et al., 2022). According to Silva et al. (2010), water use efficiency presents a similar behaviour to that of the net CO_2 assimilation rate, and it is usually higher in leaves with medium leaf area, around 180cm^2, but it decreases in larger leaves. This behaviour can be seen in the lower results for the Cultivar Branco Rosa Lages, which has a smaller leaf area than Roxo de Valinhos (data not shown).

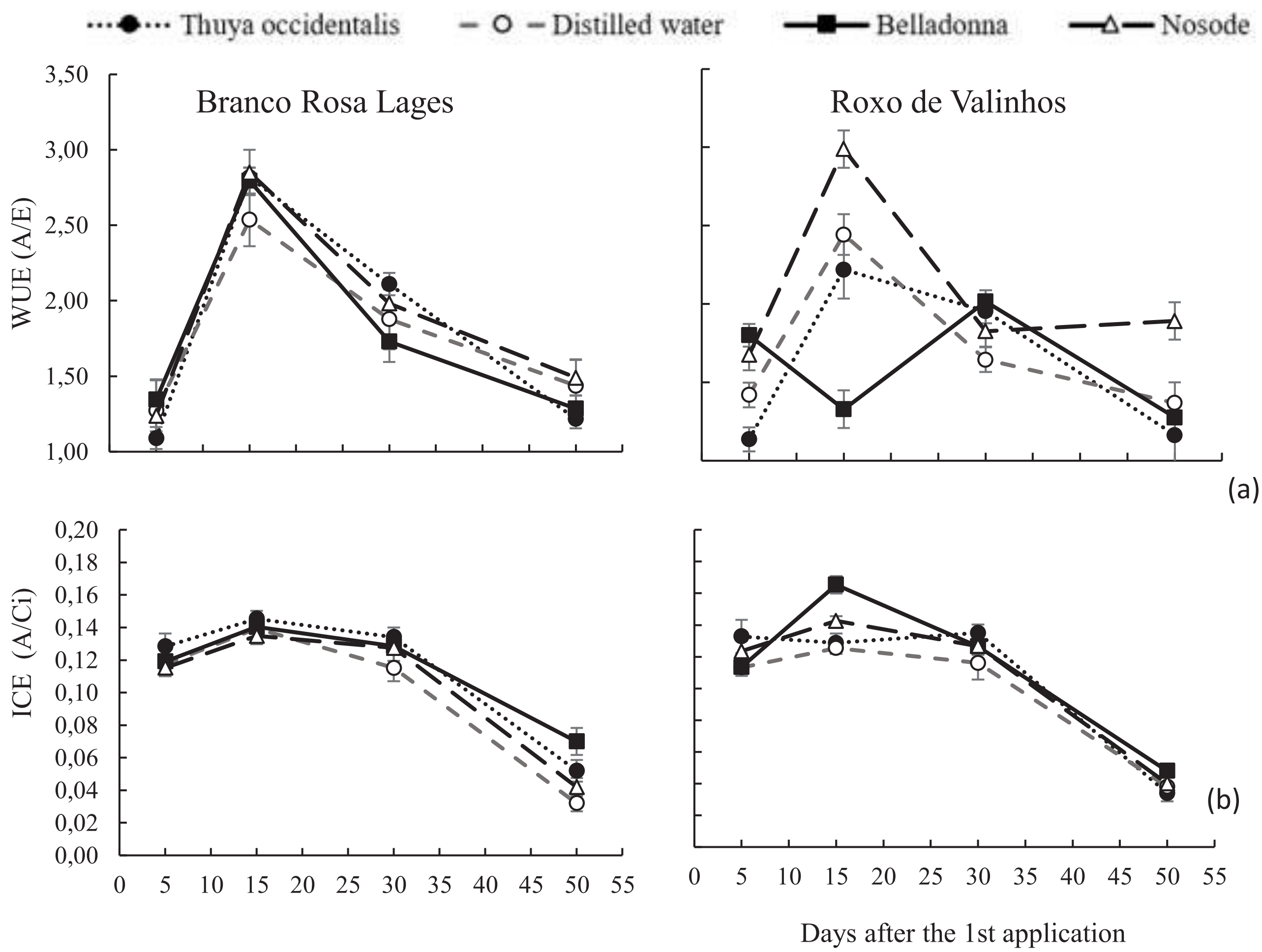

Figure 4. Water use efficiency (a) and Instantaneous carboxylation efficiency (b) for the Branco Rosa Lages and Roxo de Valinhos fig cultivars submitted to dynamized high dilutions, on different evaluation days.
WUE: [(μmol m^{-2} s^{-1}) (mmol H_2O m^{-2} s^{-1})$^{-1}$]; ICE: [(μmol m^{-2} s^{-1}) (μmol mol^{-1})$^{-1}$]. Bars correspond to the standard error. Means compared by Tukey's test ($p \leq 0.05$).

The enzyme ribulose-1,5-bisphosphate carboxylase, RuBisCO, is used in the Calvin cycle to catalyse the first step of carbon fixation. RuBisCO is the most abundant protein in the world and is located within the chloroplast stroma. Carboxylation efficiency is the speed at which fixed CO_2 is processed, and it is limited mainly by the amount, enzymatic activity, and availability of CO_2. There are also other factors that influence carboxylation efficiency, for example, acceptor concentration, temperature, level of hydration of the protoplasm, supply of mineral substances, and levels of plant development and plant activity (Von caemmerer, 2020).

Conclusions

The cultivar Roxo de Valinhos is rather sensitive to the high dynamized preparations and responded to leaf emission and the persistence of leaves on fig plants during the crop cycle. Roxo de Valinhos presents a higher response to *Thuya occidentalis* and to the nosode of fig leaf rust, which suggests it could be a good option to treat that cultivar. *Thuya occidentalis* and *Belladonna* can increase net photosynthesis on the cultivar Roxo de Valinhos after cumulative applications.

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