

## Gas exchange and photosynthetic light response curves in nematode-infected tomato plants treated with *Thuja occidentalis*

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### Abstract

The use of homeopathic formulations on plants may cause changes in plant metabolism and result in disease control by inducing resistance. This work aimed to study the physiological responses of tomato plants infected with nematode (*Meloidogyne incognita*) and treated with highly diluted preparations of *Thuja occidentalis*, a homeopathic remedy. The different treatments included *T. occidentalis* 6CH, 24CH, and 50CH (Centesimal Hahnemanian), a water control (with nematode and without treatment), and an absolute control (no treatment and no nematode). The assay was conducted in a greenhouse using a randomized block design with four replications. Gas exchange parameters were measured at specific times before and after nematode inoculation. Photosynthetic rate, stomatal conductance, transpiration, leaf internal CO<sub>2</sub> concentration, and leaf temperature were measured at various photosynthetic photon flux densities. No significant difference was found in the different gas exchange specific measure parameters between the treatments. Untreated infected plants showed an increase in net photosynthesis and in the carboxylation capacity, as shown by the light response curve. Treatment with *T. occidentalis* 24CH inhibited the increase in CO<sub>2</sub> fixation in tomato plants inoculated with *M. incognita*, leading to a similar behavior as healthy plants, independently of the photon density.

**Keywords:** alternative control, homeopathy, photosynthetic photon flux density, root-knot nematode, *Solanum lycopersicum* L.

### Introduction

Homeopathy is a system of alternative medicine that uses highly diluted substances in treatment protocols, and the use of homeopathic substances in agriculture has been increasingly growing, especially in the field of plant protection (Toledo et al., 2011). According to the viewpoint of homeopathy, illness is the consequence of loss of homeostasis, and although many studies have been performed in this field, little is known about the physiological effects of homeopathic substances in treated plants (Bonato, 2007).

Plants infected with the nematode *Meloidogyne* spp. exhibit formation of galls (swellings) on the roots, resulting in hyperplasia and hypertrophy of cells around the nematode feeding site. As a consequence, the vascular elements are interrupted and deformed, preventing the normal translocation of water and nutrients to the canopy plant and interfering with plant growth (Castagnone-Sereno et al., 2013). Furthermore, when infecting a host plant, the nematode rapidly breaks physiological routes involving most of the major processes, such as respiration, photosynthesis, nutrient translocation, and hormone and water balance (Karssen and Moens, 2006).

Some studies have focused on controlling nematode infections in tomato plants (*Solanum lycopersicum* L.) using highly diluted substances (Sukul et al., 2013; Swarowsky et al., 2014; Mioranza et al., 2017), which may activate plant defense mechanisms and induce resistance. For instance, Mioranza et al. (2017) studied the use of *Thuja occidentalis* in the control of *M. incognita* in tomato plants, and observed that the dynamization of 100CH can reduce the number of nematodes, while the 24CH and 200CH improved the growth parameters of plants and increased the activity of some plant defense related enzymes, such as peroxidase and phenylalanine ammonia-lyase. Messchimdt (2013) found that plants infected with *M. javanica* have increase in photosynthesis until the 60 days after inoculation and after that, there was a reduction. Sarkar et al. (2017) used ultra-high dilution of *Mercuric chloride* (200CH) in *Vigna unguiculata* (L.) and verified that the treatment promotes improve of photosynthesis. However, there is no study about the leaf gas exchange and their parameters in the interaction plant-nematode-high diluted substances. Therefore, to understanding this interaction, the aim of this study was to evaluate the physiological responses, as gas

exchange, of tomato plants infected with *Meloidogyne incognita* (Kofoid and White) Chitwood, when treated with highly diluted solutions of the homeopathic remedy *Thuya occidentalis*.

## Results

### Gas exchange and infestation level

No significant differences were found in gas exchange parameters between homeopathic and control treatments before inoculation and five, eight, and 13 days after inoculation (ANOVA,  $p \leq 0.05$ ) (data not shown). The same results were observed to nematode infestation level, with no differences among the treatments (Table 1).

### Photosynthetic response curves to treatment with *T. occidentalis* 6CH

To the treatment *T. occidentalis* 6CH no significant relationship was found with PPFD response curves, showing that this treatment did not influence the CO<sub>2</sub> net assimilation rate, which was statistically similar to that of the controls (healthy plants) (Figure 1). According to the exponential curve, the CO<sub>2</sub> saturated net photosynthetic rate occurred at a high PPFD concentration (about 1200  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD).

In relation to the stomatal conductance ( $g_s$ ), the 6CH treatment showed a lower average than the water and absolute controls, regardless of the photon density (Figure 1B). This shows that plants treated with *T. occidentalis* 6CH tended to have stomatal closure (0.27  $\text{mol m}^{-2} \text{s}^{-1}$ ).

The internal CO<sub>2</sub> concentration ( $C_i$ ) showed an inverted exponential behavior curve, decreasing from 0  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD onwards and beginning to stabilize at about 900  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Concentrations between 900 and 1800  $\mu\text{mol m}^{-2} \text{s}^{-1}$  correspond to a period of large stomatal aperture, affecting CO<sub>2</sub> diffusion. The  $C_i$  average of plants treated with *T. occidentalis* 6CH was lower (338.73  $\mu\text{mol CO}_2 \text{ mol}^{-1}$ ) than those of the two controls (350.49 and 352.06  $\mu\text{mol CO}_2 \text{ mol}^{-1}$ ), probably due to a lower stomatal opening rate. Nevertheless, this did not seem to interfere with CO<sub>2</sub> fixation (Figure 1C).

The PPFD response curve for leaf transpiration rate ( $E$ ) showed an exponential behavior, with  $E$  increasing with photon flux density. Plants treated with *T. occidentalis* 6CH had a lower  $E$  average (3.88  $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ ) than the absolute (4.76  $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ ) and water (4.88  $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ ) control treatments (Figure 1D). The lower water loss of plants treated with *T. occidentalis* 6CH may be due to stomatal closure (Figure 1B).

Intrinsic water use efficiency (IWUE) was stable at approximately 450  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (PPFD), which means that stomatal opening occurred at the same time as the increase in CO<sub>2</sub> fixation from 450 to 1800  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (PPFD) (Figure 1F). A significant difference in IWUE was found between treatments. Irrespectively of the PPFD level, plants treated with *T. occidentalis* 6CH had a higher IWUE (23.05) than the water and absolute controls (16.36 and 15.07, respectively). This confirms the stomatal closure resulting from this homeopathic treatment, shown in Figure 1B.

The WUE ratio, i.e. the ratio between liquid CO<sub>2</sub> uptake rate and leaf transpiration, provides the relative value of

H<sub>2</sub>O vapor loss for each CO<sub>2</sub> set (Figure 1E). The WUE ratio followed a hyperbolic model with a maximum at approximately 800  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (PPFD), slightly decreasing after that until 1800  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . This trend matches the observed exponential increase in water loss ( $E$ ; Figure 1D). No significant relationship was found between plant treatments and PPFD in terms of WUE (Figure 1D). However, when averages were compared, water use efficiency was higher in plants treated with *T. occidentalis* 6CH. This is probably due to lower  $g_s$  values in these plants when compared to the control treatments, directly influencing leaf transpiration rate (Figures 1B and 1D).

Average carboxylation capacity ( $AC_i$ ) was not significantly different between treated plants and controls (Figure 1G). The exponential regression model revealed that  $AC_i$  stabilized at about 1400  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (PPFD) and reached a maximum carboxylation capacity of 0.07 ( $AC_i$ ) at 1800  $\mu\text{mol m}^{-2} \text{s}^{-1}$ .

Leaf temperature ( $T_{\text{leaf}}$ ) showed a linear behavior, decreasing from 0 to 1800  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (PPFD) (Figure 1H). This decrease is due to the increase in leaf transpiration rate, which causes cooling of the leaf. Temperature was more stable in the absolute control treatment than in those with an increase in photon flow densities.

### Photosynthetic response curves to treatment with *T. occidentalis* 24CH

The PPFD response curve of the CO<sub>2</sub> liquid uptake rate ( $A$ ) in plants treated with *T. occidentalis* 24CH (Figure 2) was different from that observed in plants which received the 6CH treatment (Figure 1A). There was a significant relationship in terms of  $A$  between treated plants and PPFD. The exponential adjusted model revealed that saturation of the net CO<sub>2</sub> absorption occurred at around 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (PPFD). The light compensation point was lower for healthy plants (16.68  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) than for those treated with *T. occidentalis* 24CH (18.31  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), whereas the water control showed a higher light compensation point (23.89  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ).

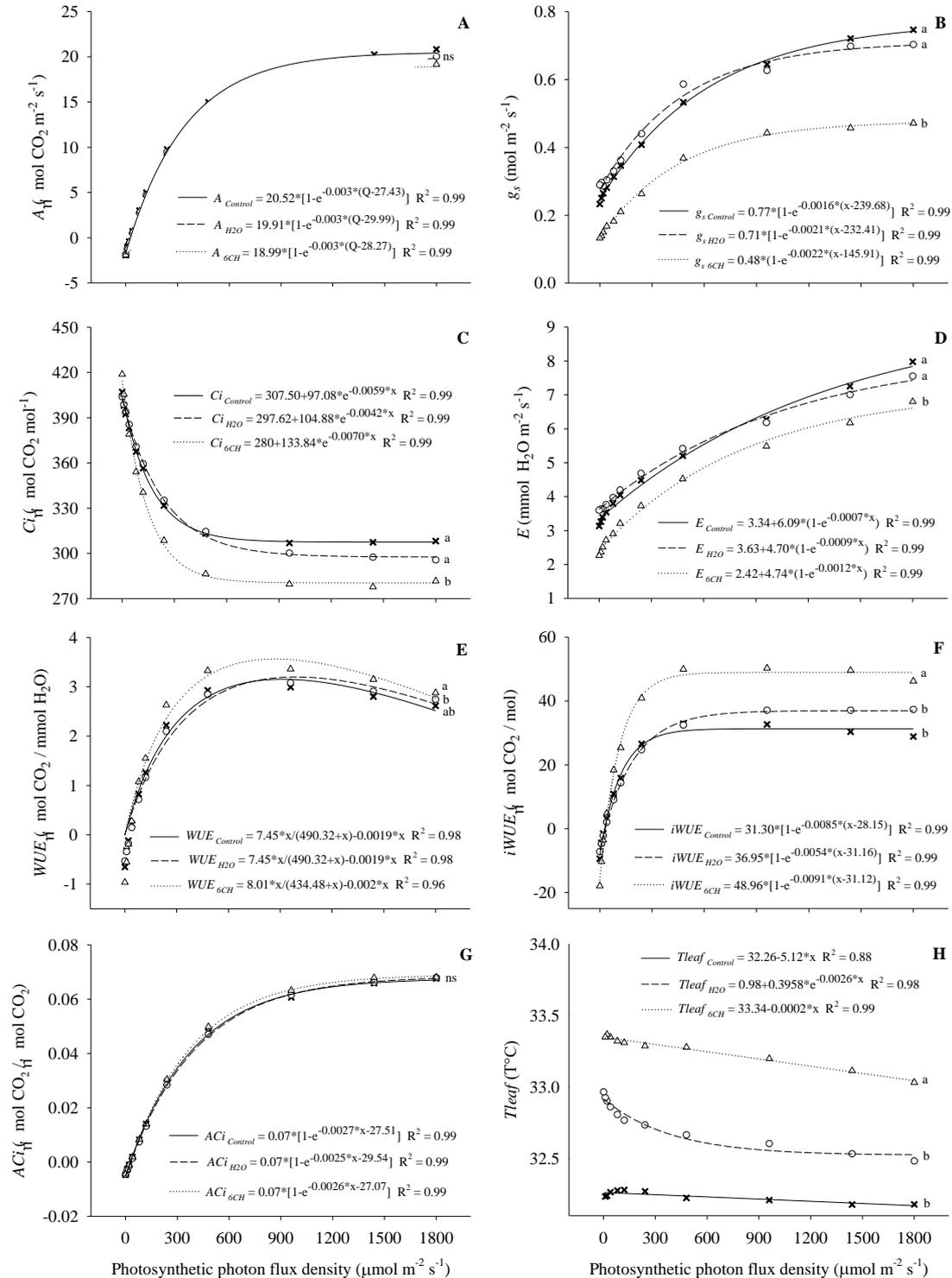
Plants treated with water showed higher photosynthetic rates at 480  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (PPFD) than plants treated with *T. occidentalis* 24CH, but not different from the control. When densities of 960, 1440, and 1800  $\mu\text{mol m}^{-2} \text{s}^{-1}$  photon flux were used, the water control had higher averages than the other treatments (Figure 2A). Regression analysis showed that plants treated with water, *T. occidentalis* 24CH, and untreated not inoculated plants showed maximum CO<sub>2</sub> net uptake rates of 22.09, 18.88, and 18.75  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$ , respectively.

The observed maximum CO<sub>2</sub> net uptake rates were related to stomatal conductance ( $g_s$ ). Although no significant relationship was found between treatments and PPFD in terms of  $g_s$ , the highest average stomatal conductance (0.60  $\text{mol m}^{-2} \text{s}^{-1}$ ) was found in plants treated with water (Figure 2B). Control plants and plants treated with *T. occidentalis* 24CH showed the lowest average (0.38 and 0.35  $\text{mol m}^{-2} \text{s}^{-1}$ ). The  $C_i$  curve showed an inverse exponential behavior, and the responsive averages to the behavior of  $g_s$  variable. Plants treated with water had a higher average internal CO<sub>2</sub> concentration (359.58  $\mu\text{mol CO}_2 \text{ mol}^{-1}$ ) than other plants, probably due to a greater stomatal opening. The PPFD

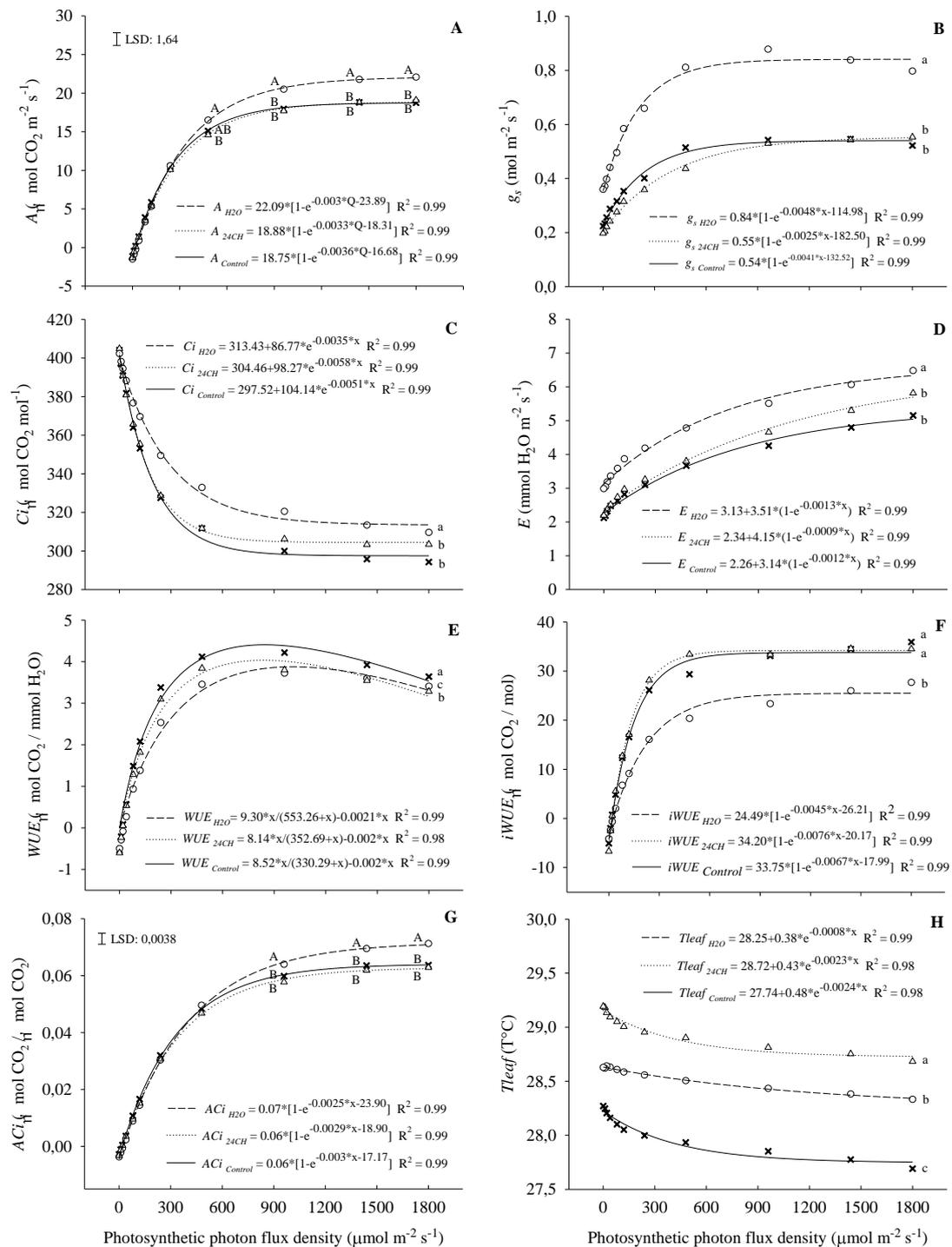
**Table 1.** Infestation level of tomato inoculated with *Meloidogyne incognita* and treated with *Thuya occidentalis*.

Treatments	Infestation level
<i>T. occidentalis</i> 6CH	827 a
<i>T. occidentalis</i> 24CH	1106 a
<i>T. occidentalis</i> 50CH	1001 a
H <sub>2</sub> O	869 a
CV (%)	13.2

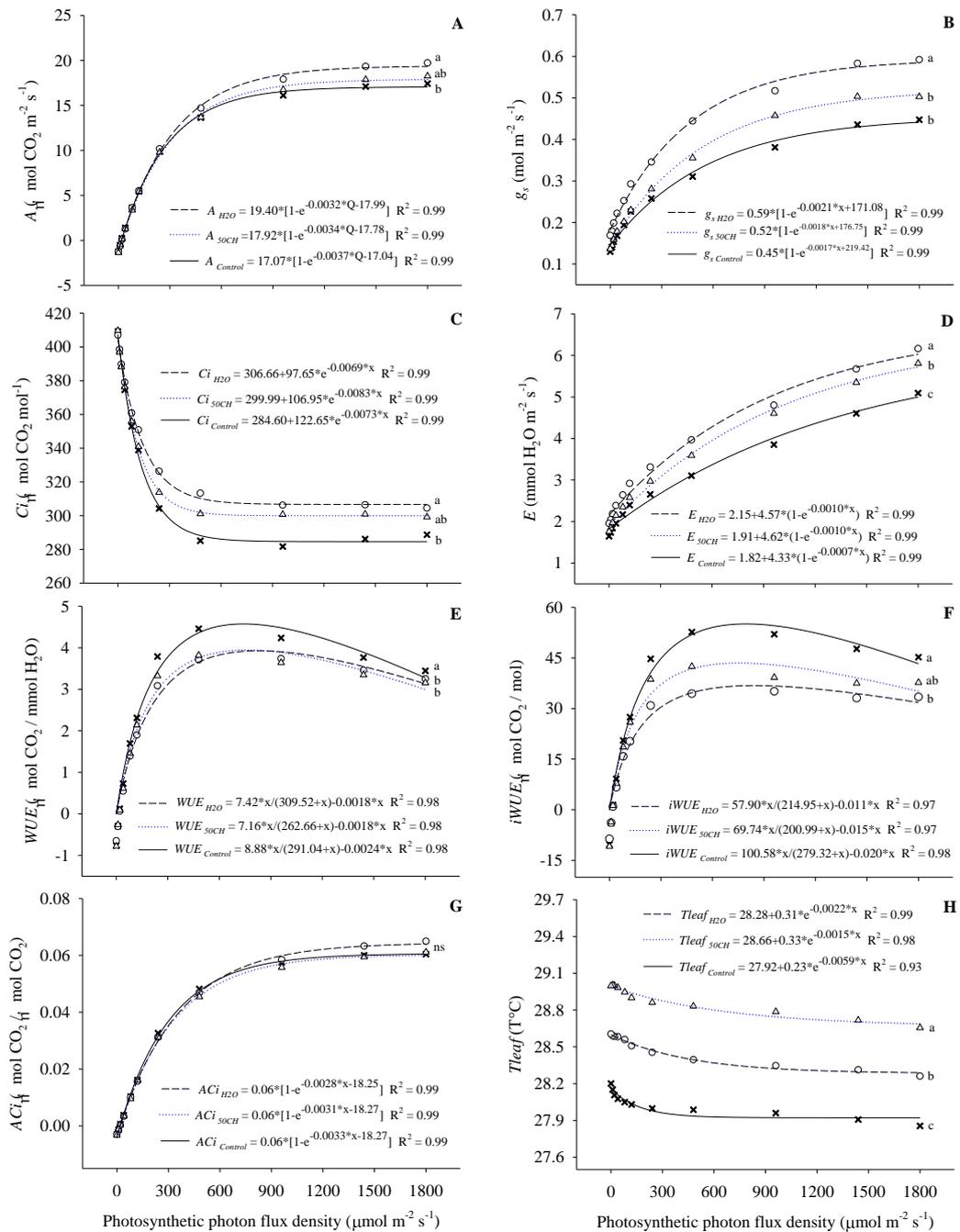
In the column, means followed for the same letter do not differ from each other by Tukey's HSD test ( $P \leq 0.05$ ).



**Fig 1.** CO<sub>2</sub> net assimilation rate (A), stomatal conductance (B), internal CO<sub>2</sub> concentration (C), transpiration rate (D), water use efficiency (E), intrinsic water use efficiency (F) carboxylation capacity (G) and leaf temperature (H) of healthy tomato plants (control), inoculated with *Meloidogyne incognita* and untreated (H<sub>2</sub>O) and inoculated and treated with *Thuya occidentalis* 6CH. At the end of the curved: ns = not significant, a, b or c = Different by Tukey's HSD test ( $p \leq 0.05$ ).



**Fig 2.** CO<sub>2</sub> net assimilation rate (A), stomatal conductance (B), internal CO<sub>2</sub> concentration (C), transpiration rate (D), water use efficiency (E), intrinsic water use efficiency (F) carboxylation capacity (G) and leaf temperature (H) of healthy tomato plants (control), inoculated with *Meloidogyne incognita* and untreated (H<sub>2</sub>O) and inoculated and treated with *Thuya occidentalis* 24CH. At the end of the curved: ns = not significant, a, b or c = Different by Tukey test (p ≤ 0.05). LSD = least significant difference.



**Fig 3.** CO<sub>2</sub> net assimilation rate (A), stomatal conductance (B), internal CO<sub>2</sub> concentration (C), transpiration rate (D), water use efficiency (E), intrinsic water use efficiency (F) carboxylation capacity (G) and leaf temperature (H) of healthy tomato plants (control), inoculated with *Meloidogyne incognita* and untreated (H<sub>2</sub>O) and inoculated and treated with *Thuya occidentalis* 50CH. At the end of the curved: ns = not significant, a, b or c = Different by Tukey test (p ≤ 0.05).

response curve for leaf transpiration rate (E) was also higher in these plants (Figures 2C and 2D). Plants treated with water lost an average of 4.28 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> (E), which was more than the loss in transpiration of plants treated with *T. occidentalis* 24CH and the absolute control (healthy plants) (3.44 and 3.23 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>, respectively). Healthy plants showed a more efficient water use with an average value of 2.10, which was higher than the WUE of plants treated with *T. occidentalis* 24CH (1.87) and with water (1.72) (Figure 2E). This means that, although plants infected with nematodes and treated with water fix more CO<sub>2</sub> than plants receiving other treatments, they lose more

water vapor. Healthy plants, without nematode inoculation, lose less water to CO<sub>2</sub> fixation. These plants limit the photosynthetic activity at lower photon flux densities, between 0 and 300 μmol m<sup>-2</sup> s<sup>-1</sup> (Figure 2A). Although no significant relationship was found between PPF and treatment in terms of WUE, the response curve for leaf transpiration rate in water treated plants increased in all photon flux density levels, reducing the average WUE (Figures 2D and 2E).

The intrinsic water use efficiency (IWUE) achieved stability at about 600 μmol m<sup>-2</sup> s<sup>-1</sup> (PPFD) in absolute control plants and plants treated with *T. occidentalis* 24CH. Infected plants

treated with water only, stabilized at  $900 \mu\text{mol m}^{-2} \text{s}^{-1}$  (PPFD) (Figure 2F). Regression analysis revealed that the maximum IWUE of water control plants was 24.49, with an average of 11.63, which was lower than the average IWUE of plants treated with *T. occidentalis* 24CH (18.07) and with the absolute control (19.93) (maximum values of 34.20 and 33.75, respectively). *T. occidentalis* 24CH prevented negative effects of nematode infection on the stomatal opening behavior, altering the plant's metabolism so that the stomatal behavior remains similar to that of healthy plants. The  $A/C_i$  ratio, which relates photosynthesis ( $A$ ) with the internal concentration of  $\text{CO}_2$  in the mesophyll ( $C_i$ ), shows the *in vivo* estimated carboxylation efficiency of the rubisco enzyme (Figure 2G). A significant relationship between PPFD and treatments was found for photon flux densities of 960, 1440 and  $1800 \mu\text{mol m}^{-2} \text{s}^{-1}$ , whereby infected plants treated with water had higher carboxylation capacity. Despite the fact that the homeopathic treatment with *T. occidentalis* 24CH leads to higher WUE and IWUE averages than the treatment with water, the  $A/C_i$  ratio demonstrates that the ability to fix  $\text{CO}_2$  did not follow the trend of the other ratios (WUE and IWUE). This suggests that, in the present study,  $\text{CO}_2$  fixation in tomato plants was only dependent on the inoculation with *M. incognita*, since the use of *T. occidentalis* 24CH inhibited the effect of the inoculation. Average leaf temperature (Tleaf) was, similarly to the results of the 6CH treatment, higher in plants treated with *T. occidentalis* 24CH ( $28.98^\circ\text{C}$ ) than in water treated plants ( $28.54^\circ\text{C}$ ) and in untreated plants ( $28.03^\circ\text{C}$ ). Nevertheless, no significant relationship was found between plant treatments and light densities (PPFD).

#### **Photosynthetic response curves to treatment with *T. occidentalis* 50CH**

No significant relationship was found between treatments and PPFD in terms of  $A$ ,  $g_s$ ,  $C_i$ ,  $E$ , WUE, IWUE,  $A/C_i$  and Tleaf in plants treated with *T. occidentalis* 50CH. However, a significant difference was found in average values between treatments (Figure 3).

The response curve of the  $\text{CO}_2$  net assimilation rate under different PPFD in plants treated with *T. occidentalis* 50CH, as well as in the two controls, followed the same trend as the curve of *T. occidentalis* 24CH treated plants (Figure 2A). Water treated plants reached a net assimilation rate of  $19.40 \mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$  and a light compensation point of  $17.99 \mu\text{mol m}^{-2} \text{s}^{-1}$ , as revealed by the adjusted exponential model (Figure 3A). The absolute control had a lower rate, with a maximum of  $17.07 \mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$  and a compensation point of  $17.04 \mu\text{mol m}^{-2} \text{s}^{-1}$ , which was not different from the average assimilation rate of nematode infected plants treated with *T. occidentalis* 50CH.

Net photosynthesis, internal  $\text{CO}_2$  concentration, and leaf transpiration rate related directly to stomatal conductance (Figures 3A, 3C, 3D, and 3B). As was seen in the treatments with *T. occidentalis* 6CH and 24CH (except for the results shown in Figure 1A), treatments that showed higher  $g_s$  also showed a greater  $\text{CO}_2$  diffusion ( $C_i$ ), and thus, higher net  $\text{CO}_2$  assimilation ( $A$ ) and leaf transpiration rate ( $E$ ). *T. occidentalis* seems to inhibit the effect of the nematode on the photosynthesis and may lead to a low plant production of photo assimilates, which are used by the nematodes.

The response curves of the WUE and IWUE ratios (Figures 3E and 3F) were similar to those of plants treated with *T. occidentalis* 24CH (Figures 2E and 2F), showing that healthy plants, under different PPFD, have higher water use efficiency and intrinsic water use efficiency.

Although significant differences were found in  $A$  and  $C_i$  between treatments (Tukey's test,  $P \leq 0.05$ ), the  $A/C_i$  ratio did not show significant differences, neither between treatments nor in the interaction between treatments and PPFD levels (Figure 3G). However, regression analysis of the average PPFD within each treatment resulted in an adjusted exponential equation with the same  $A/C_i$  maximum point (0.06) for all three treatments.

Tomato plants treated with *T. occidentalis* 6CH, 24CH, and 50CH always achieved higher average leaf temperature (Tleaf) than plants receiving other treatments (Figures 1H, 2H and 3H). Regression analysis showed that the best model suggested that leaf temperature decreases as photon flow density increases (Figure 3H).

#### **Discussion**

From the exponential curve obtained for  $\text{CO}_2$  net assimilation rate, the saturation of net  $\text{CO}_2$  photosynthesis occurred at higher concentrations of about  $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD, which may be due to limitation of carboxylation reactions (Taiz and Zeiger, 2013). It is possible note that plants treated with *T. occidentalis* 6CH have decreased the stomatal conductance. Silva (2005), by applying *Sulphur* 6CH on soil under the plants *Sphagneticola trilobata* (L.) Pruski, found that  $\text{CO}_2$  assimilation tended to be lower in plants subjected to homeopathic medicine, attributing the effect to a possible pathogenesis that *Sulphur* is capable of causing, since when applied *Appis mellifica*, we obtained results in the increase of  $\text{CO}_2$  assimilation in the control. In this study, plants subjected to the homeopathic treatment of *T. occidentalis* 6CH tended to decrease in  $A$  and  $g_s$ , though this hypothesis cannot be generalized, since the tomato plants in this study showed no apparent aspect of pathogenesis.

The literature reports that when the plant is subjected to stress conditions, they respond by producing compounds that can lead to stomatal closure, such as abscisic acid and reactive oxygen species to induce increasing in  $\text{Ca}^{2+}$  concentration in the cytosol of the kept cells, leading to stomatal closure (Klüsener et al., 2002). This dynamization can be not the ideal, and when it is happens, the homeopathy show negligible results (BONATO; SILVA, 2003). Although the 6CH treatments had lower mean values for  $A$ ,  $g_s$ ,  $C_i$  and  $E$ , the homeopathic treatment presented greater efficiency in water use. Therefore, nematode inoculated plants when treated with *T. occidentalis* 6CH were more efficient at fixing  $\text{CO}_2$   $\mu\text{mol}$  per  $\text{mmol}$  of water by transpiration rate. Plants infected with nematodes and without *T. occidentalis* 6CH had similar WUE rates of healthy plants without inoculation, demonstrating efficacy of homeopathic treatment in relation to this variable. When there is a decrease in stomatal conductance, transpiration rate also decreases, and this decrease is greater than the decrease in net photosynthetic rate (Habermann et al., 2003).

The effect of IWUE also corroborates with the results of WUE, in this way the use of *T. occidentalis* 6CH also shows efficiency in the fixation of  $\text{CO}_2$  by the stomatal opening,

that is, for each mol of water used in the stomatal turmoil there were larger amounts of  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  fixed compared to *Control* and *H2O* treatments. According to Martin et al. (1994), leaf photosynthetic rate and water use efficiency are controlled by stomatal properties and by the anatomical characteristics of the leaves. Since WUE expresses the production of biomass per unit of water, leaf characteristics can be important when plants grow in water-limited environments (Van Den Boogaard et al., 1997).

The diffusion of  $\text{CO}_2$  occurs through the stomatal opening increasing its intercellular concentration, however the greater stomatal opening provided by the increase in photon densities, the higher the net assimilation rate of  $\text{CO}_2$ . In the present study the ratio of increase of *A* and decrease of *C<sub>i</sub>*, were proportional resulting in similar means for *AC<sub>i</sub>*. The instantaneous carboxylation capacity was not influenced by nematode infection in roots of nematode plants.

The loss of water in the form of vapor by the plants through the leaves provoke the foliar cooling, preventing greater losses of photosynthetic efficiency, such as the activity of the rubisco enzyme (Taiz and Zeiger, 2013). Plants inoculated with nematodes and treated with *T. occidentalis* 6CH showed higher leaf temperature means, this behavior is directly related to leaf cooling due to transpiratory rate of *Control* and *H2O* treatments.

In Figure 2A is possible to realize that healthy plants require less  $\mu\text{mol m}^{-2} \text{ s}^{-1}$  of photons to start the photosynthesis process. Habermann et al. (2003) found a smaller light compensation point in healthy citrus plants when compared to plants with CVC (citrus variegated chlorosis), a disease that leads to the obstruction of plants' vascular system. These authors concluded that an increase of irradiance on the light compensation point results in increased photosynthesis, indicating that photosynthesis is limited by the amount of available light and that the PPFD saturation curve is limited by the electron transport rate and the regeneration of the rubisco enzyme.

Apparently, *T. occidentalis* 24CH inhibited the negative effect of nematodes on stomatal conductance, since plants treated with this substance showed a similar behavior to healthy plants. The behavior observed in plants treated with water can be the result of a hormonal imbalance promoted by nematode infection. The stomatal behavior can be affected by both climatic factors such as hormonal factors (Dodd, 2003).

Pathogens are capable of interfering with hormonal changes promoted by the plant as a defense strategy and produce phytohormones as an infection strategy (Robert-Seilaniantz et al., 2007). Root knot nematodes, such as *M. incognita*, are capable of producing conjugates of auxin and cytokinin, which can interfere with the hormonal balance of plant cells (De Meutter et al., 2005).

At the time the nematode reaches the vascular bundles and during the early stages of the nematode-plant interaction, Lohar et al. (2004) found peaks of cytokinin in transgenic plants of *Lotus japonicus* L., although it was unclear whether this increase in cytokinin was due to nematode secretion or to the sensitivity of the host. In addition, Bird and Loveys (1980) found differences in cytokinin content in tomato plants infected with *Meloidogyne javanica* (Treub) Chitwood when compared to control plants, especially in periods of change in developmental stage of the nematode (J2 to J3 and J4).

However, De Meutter et al. (2003) detected the presence of cytokinins in exudates *M. incognita* *in vitro* experiment, zeatin and benzyladenine being the most prominent forms, noting that this hormone may be important in the activation of cell cycle and induction of feeding site. To Farquhar and Sharkey (1982), the increased supply of cytokinins in roots could lead to an increase in photosynthesis, stimulating chlorophyll formation and protein synthesis, and at the same time, promoting stomatal opening. In relation to auxin, there is evidence that this hormone can promote stomatal closure (Dodd, 2003). The cytokines produced in the root are transported through the xylem to the shoot of the plants to regulate their growth (Takey et al., 2001). Photosynthetic pigments present in plants, such as chlorophyll and carotenoids, are essential for photosynthesis, capturing solar radiation and initiating the photochemical step by fixing  $\text{CO}_2$  and producing carbohydrates (Kluge et al., 2015). According to Bird (1974), nematodes can use these growth hormones (cytokine and auxin) for their own purpose and require a certain level in their host plants for their growth and reproduction.

Messchmidt (2013) reported that a peach graft susceptible to *M. javanica* presented the highest rate of net  $\text{CO}_2$  assimilation in the first 60 days after grafting, which was statistically similar to the rate at day 90, 120 and 150, and may be an initial response to nematode infection. The measurements of this study were performed after 14, 15, and 17 days of infection, even without visual symptoms of the disease. These results corroborate with the results of this study, where plants infected with *M. incognita* and not treated showed highest rate of  $\text{CO}_2$  assimilation that plants without nematode or with and treated with 24CH and 50CH (Figure 2C).

Abrão and Mazzafera (2001) observed that cotton plants, Acala (susceptible cultivar), inoculated with 5.000 J2 and eggs of *M. incognita* showed increase in photosynthesis, chlorophyll and sugar content compared with plant no inoculated, and the authors suggest that it can be related with the increased demand for photoassimilates in plants attacked by nematodes. In this work, *T. occidentalis* 24CH inhibited this effect, and the photosynthesis rate of treated plants were statistically similar that plants without nematode. According with Bonato (2007), when the correct homeopathic medicine is applied in the plant that is suffering abiotic or biotic stress, the response will be the reduction of harmful effects and re-establishment of plant health, in biochemical and energy levels.

Other works confirm that the homeopathy is able to promote change in biology of plants, as exposed by Panda et al. (2013), where the potentised homeopathic *Arsenicum album* e *Baryta carbonica* increased in chlorophyll a, chlorophyll b and chlorophyll total. Cotton plants treated with homeopathic drugs suffering influence in their genetic process, as acceleration of the germinative process, shortening cultivation period, increase in yeald and quality of product (Gangar, 2007).

The increase in photosynthesis can be related both to increased stomatal opening, which allows greater  $\text{CO}_2$  diffusion into the intercellular spaces, as may be associated with increased carboxylation efficiency (Machado et al., 2005). Tahery (2012a) found an increase in stomatal conductance in *Hibiscus cannabinus* L. plants infected with *M. incognita* after 60 and 90 days of inoculation.

Higher rates of  $E$  may decrease water use efficiency, this behavior can be observed by the greater stomatal opening in plants with nematodes without homeopathic treatment (24CH). This effect caused higher transpiration rates, decreasing  $WUE$ . According Blum (2009) the water use efficiency is important to indicate the yield under stress conditions. Messchimidt (2013) found higher water use efficiency rates in healthy peach grafts than in peach grafts infected with *M. incognita*. Tahery (2012b) studied the effect of the nematode *M. incognita* in water responses of *H. cannabinus* and found similar results: plants inoculated with nematodes had the lowest  $WUE$  and  $IWUE$  values, when compared to healthy plants after 30, 60 and, 90 days of inoculation. Treatments with 6CH *T. occidentalis* dynamics induced stomatal closure, resulting in lower transpiration rate and higher leaf temperature, as well as 24CH and 50CH treatments.

## Material and Methods

### Treatments

*T. occidentalis* was obtained in a homeopathic pharmacy in the potency of 6CH (according to Hahnemann's centesimal scale) and diluted to 24CH and 50CH. A water control (with nematode and untreated) and an absolute control (without nematode and untreated) were used. This method consisted in, 1 part of homeopathic preparation diluted in 99 parts of 30% ethanol, followed by 100 successions, in accordance with the Brazilian Homeopathic Pharmacopoeia (FHB, 2011).

### Inoculum of *Meloidogyne incognita*

The pure population of this nematode was collected from tomato roots cultivated in green house. The eggs and second stage juveniles (J2) were obtained with Boneti and Ferraz (1981) methodology.

### Experiment deployment

Santa Clara' tomato seeds were sown in polystyrene trays containing commercial substrate to obtain seedlings. After 25 days of sowing, seedlings were transplanted to plastic pots (2 L) containing a sterilized mixture of soil, sand and organic compound (2:2:1). Sterilization was performed by autoclaving at 120 °C (1 atm.) for 1 h.

At the time of transplanting, the root system of the tomato seedlings cultivar 'Santa Clara' was dipped in 0.1% of treatment solution (1 mL of solution diluted in 1 L of distilled water) before seedlings were planted in the pots. Seven days after planting, seedlings were inoculated with *M. incognita* suspension containing 4350 eggs and 700 J2. Subsequently, plant shoots were weekly sprayed with the different treatments. The infestation level (egg mass rate) was evaluated after 40 days of inoculation with nematodes, according to Taylor and Sasser (1978) methodology.

### Gas exchange evaluation

Gas exchange was measured on full sun days, between 9:00 am and 11:00 am, using an infrared gas analyzer (IRGA) portable photosynthetic system (Li-6400XT, LI-COR, Lincoln, Nebraska, USA). Gas exchange measurements were done

prior to nematode inoculation and five, eight, and 13 days after inoculation. Measurements were performed in 6 cm<sup>2</sup> of the second fully expanded leaf that was exposed to sunlight. The following parameters were determined:  $A$  - CO<sub>2</sub> net assimilation rate ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ),  $E$  - transpiration rate ( $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ),  $g_s$  - stomatal conductance ( $\text{mol m}^{-2} \text{ s}^{-1}$ ), and  $C_i$  - internal CO<sub>2</sub> concentration ( $\mu\text{mol CO}_2 \text{ mol}^{-1}$ ). Air temperature within the chamber of the photosynthetic system was 21.63 °C prior to inoculation and 28.56, 25.45, and 29.95 °C after five, eight, and 13 days of inoculation, respectively. Vapor pressure deficit ( $VPD$ ) was, respectively, 1.46, 2.71, 2.22, and 2.36 kPa, whereas relative humidity was, respectively, 38.25, 39.43, 44.71, and 43.08%. In addition, the photosynthetic response curve to light was measured in plants receiving the 6CH, 24CH, 50CH, water control, and absolute control treatments. The response curve was measured 14, 15, and 17 days after inoculation.

The CO<sub>2</sub> liquid uptake rate ( $A$ ) response curve for different photosynthetic photon flux density (PPFD) was carried out using an artificial red light emitting diode (LED) controlled by a sensor located within the leaf chamber. The curve was obtained by varying the PPFD from 1800  $\mu\text{mol m}^{-2} \text{ s}^{-1}$  to zero and measurements started at 400  $\mu\text{mol mol}^{-1}$  of CO<sub>2</sub>. The  $A$  response curve for PPFD was adjusted according to the equation of Prado and Moraes (1997). The other parameters were adjusted with the model that provided the highest correlation coefficient value. The following ratios were also calculated: water use efficiency ( $WUE$ ) ( $A/E$ ,  $\mu\text{mol CO}_2/\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ), carboxylation capacity ( $AC_i$ ) ( $A/C_i$ ,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}/\mu\text{mol CO}_2 \text{ mol}^{-1}$ ), and intrinsic water use efficiency ( $IWUE$ ) ( $A/g_s$ ,  $\mu\text{mol CO}_2/\text{mol m}^{-2} \text{ s}^{-1}$ ) (Zhang et al., 2001).

### Experimental design and data analysis

Experiments were conducted in a greenhouse using a randomized block design with four replications. Averages of egg mass (infestation level) was submitted to Tukey test. Averages of the PPFD response curves were subjected to regression analysis. To analyze the interaction between plant treatment and PPFD response curve, a split analysis was performed in which the different treatments were analyzed within each level of photosynthetic photon flux density. It was used the statistical program SISVAR (Ferreira, 2011).

### Conclusion

Gas exchange measurements at specific time did not show a significant difference between treatments. The most important physiological changes observed in tomato plants infected with *M. incognita* were the increase in net photosynthesis and in the carboxylation capacity, as seen in the response curve to light. Treatment with the homeopathic plant *T. occidentalis* 24CH inhibited the increase in CO<sub>2</sub> fixation in tomato plants inoculated with *M. incognita*, resulting in a behavior similar to that of healthy plants, independent of the photon density.

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