



## Biological Control and Crop Protection

# Physiological characteristics of citrus plants infested with citrus blackfly



Aliry Magda Santos do Vale Gomes, Fabrício de Oliveira Reis , Raimunda Nonata Santos de Lemos \*, Janaína Marques Mondego, Heder Braun, José Ribamar Gusmão Araujo

Universidade Estadual do Maranhão, Programa de Agroecologia, São Luís, MA, Brazil

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### ABSTRACT

*Aleurocanthus woglumi* (Ashby, 1915) is an important agricultural pest that causes yield losses of 20–80% in citrus plants by removing plant nutrients while feeding and allowing the formation of sooty mold. The objective of this study was to evaluate physiological changes in citrus plants in response to *A. woglumi* infestation under field conditions. The experiment was conducted in a citrus orchard in Paço do Lumiar, Maranhão, Brazil. Thirty-two citrus plants were used, including eight of each of the following varieties: Tahiti lime, Tanjara tangerine, Nissey tangerine, and Ponkan tangerine. Four random plants with *A. woglumi* infestation and four plants free from this pest were selected from each variety. The physiological parameters evaluated were photochemical efficiency and gas exchange. Regarding photochemical efficiency, infested plants presented photoinhibition damage, with a performance index of 4.22. The gas exchange parameters of infested plants changed, with reductions in photosynthetic CO<sub>2</sub> assimilation of 69.7% (Tahiti), 64% (Tanjara), 68.8% (Nissey) and 63.3% (Ponkan). Plants infested with *A. woglumi* also presented physiological changes; their photosynthetic CO<sub>2</sub> assimilation, stomatal conductance, instantaneous transpiration, and performance indexes were affected. The infested citrus plants showed photoinhibition of photosystem II. The photosynthetic CO<sub>2</sub> assimilation decreased approximately 70% in Tahiti lime, Tanjara tangerine, Nissey tangerine, and Ponkan tangerine plants infested with *A. woglumi*.

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## Introduction

Citrus species are some of the most produced and consumed fruits in the world, and citrus is the most important fruit tree group in Brazil due to the nutritive value of its fruits and its socioeconomic importance (Oliveira et al., 2009). The estimated orange production in Brazil in 2017 reached 18.7 million Mg; a large part of this production was exported to several countries as orange juice (IBGE, 2018).

However, citrus producers in Brazil have been challenged by several insect pests, such as the citrus blackfly (*Aleurocanthus woglumi* Ashby, 1915; Hemiptera: Aleyrodidae), which can cause yield losses of 20–80% in citrus plants (Mapa, 2014). This pest is indigenous to southwestern Asia (Correia et al., 2011); it infests more than 300 host plants (Farias et al., 2011) and citrus species are its

preferred hosts, in which it develops large populations (Lopes et al., 2013).

Direct damage is caused by the continuous sucking of *A. woglumi* of the leaf sap, which decreases plant vigor; indirect damage is caused by its sugary excretion, which favors the emergence of a sooty mold (*Capnodium* sp.) that covers the surface of leaves, branches, and fruits, hindering the plant's photosynthesis, respiration, and transpiration (Moraes et al., 2014; Mapa, 2014). Physiological changes caused by these direct and indirect damages slow plant growth and reduce the quality, size, and number of fruits, compromising fruit production and commercial value (Lazzari and Carvalho, 2009).

Physiological parameters such as photosynthetic CO<sub>2</sub> assimilation, transpiration, and stomatal conductance are affected by biotic and abiotic factors, such as light, temperature, water and nutrient availability, pests, and diseases (Taiz and Zeiger, 2013). Thus, the physiology, growth efficiency, and capacity of adaptation of a species to environmental conditions can be determined by studying the interactions of these parameters with each environmental factor.

\* Corresponding author.

E-mail: [rlemos@cca.uema.br](mailto:rlemos@cca.uema.br) (R.N. Lemos).

Plants are continuously exposed to the attack of insect pests that seek nutrients for their growth and development. This process includes several interactions whose effects depend on the species involved and the characteristics of the attacked plant; determining these effects depends on the responses of the plants to these interactions (Gurevitch et al., 2009).

Few studies have evaluated the effect of insect pests on the physiological processes of plants (García-Tejero et al., 2010; Bilgin et al., 2010). Sap-sucking insects (xylem and phloem feeders) cause long-term physiological damage to plants; they suck the sap of the plant, decreasing growth and physiological parameters, such as the photosynthetic CO<sub>2</sub> assimilation, transpiration, and stomatal conductance of plants (Garcia et al., 2010; Velikova et al., 2010). This causes the appearance of chlorosis, which can lead to premature leaf loss and decreased plant productivity (Li et al., 2013). A high photochemical efficiency can increase plant productivity; this relationship is related to the radiation used by chlorophyll (Silva et al., 2014).

In this context, evaluating the effect of environmental factors on the photosynthetic metabolism of plants is needed (Streibet and Govindjee, 2011). Thus, the objective of this study was to evaluate physiological changes in citrus plants in response to *A. woglumi* infestation in citrus orchards.

## Materials and methods

### Location and characterization of the experimental area

The experiment was carried out at the São Judas Tadeu citrus orchard (2°30'4.9"S, 44°04'22.8"W), in Paço do Lumiar, MA, Brazil, from March 2014 to September 2015.

The orchard was composed of Tahiti lime (*Citrus latifolia* Tanaka), Tanjara tangerine (*Citrus reticulata* Blanco), Nissey tangerine (*Citrus reticulata* Blanco var. Nissey), and Ponkan tangerine (*Citrus reticulata* Blanco var. Ponkan) citrus plants. Paço do Lumiar has an Aw, tropical climate, based on the Köppen climate classification, altitude of approximately 4 m, average annual temperature of 28 °C and average annual precipitation of 2200 mm (Labmet-Nugeo, 2015).

The predominant soil in this region is a red-yellow Argissolo (Ultisol) with a sandy-loam to silty-loam texture (Embrapa, 2006). During the experiment, typical cultural practices recommended for citrus plant fertilization, weeding, pruning, and irrigation (micro sprinkler system) were applied.

### Experimental design

The experiment was conducted in a completely randomized design with four replications, using a 4 × 2 factorial arrangement consisting of four citrus varieties (Tahiti lime, Tanjara tangerine, Nissey tangerine, and Ponkan tangerine) and the presence and absence of citrus blackfly. The experimental unit consisted of one plant, totaling 32 experimental units (plants), composed of eight 4-year-old plants of Tahiti lime, eight 4-year-old plants of Tanjara tangerine, eight 10-year-old plants of Nissey tangerine, and eight 10-year-old plants of Ponkan tangerine.

### Experiment implementation and conduction

Four plants of each variety, infested with eggs, nymphs, pupae, and adults of *A. woglumi* and with the presence of sooty mold and four plants with no symptoms of the pest were randomly selected for evaluations of physiological parameters.

Plants free from *A. woglumi* infestation were obtained by using applications of imidacloprid (200 SC) at 15-day intervals at the

rate recommended by the Brazilian Ministry of Livestock and Food Supply (4 g per 100 L of water).

Two evaluations were carried out for each physiological parameter, with 8 readings per treatment. The photosynthetic CO<sub>2</sub> assimilation (*A*), stomatal conductance (*g<sub>s</sub>*), instantaneous transpiration (*E*), and ratio between internal CO<sub>2</sub> concentration in the leaf mesophyll and external CO<sub>2</sub> concentration of the air (*C<sub>i</sub>/C<sub>a</sub>*) were used to determine the gas exchange of the plants. These parameters were evaluated using a portable gas measurement system consisting of an infrared gas analyzer (IRGA, LI-6400®, LI-COR, Lincoln, USA) coupled to an artificial light with blue- and red-light emitting diodes (LED) with an intensity of 1500 μmol m<sup>-2</sup> s<sup>-1</sup>.

Two readings per plant were carried out in the morning (7:00 to 10:00 a.m.) to determine gas exchange, using two fully expanded leaves from the middle third of the plants grown under full sun.

Chlorophyll-*a* fluorescence was measured in fully expanded leaves grown under full sun from the middle third of the plants in the morning (7:00 to 10:00 a.m.), using a pocket plant efficiency analyzer (PEA, Hansatech, UK). The leaf tissues were adapted to the dark for 30 min before evaluation of the F<sub>v</sub>/F<sub>m</sub> ratio (maximum quantum yield of photosystem II) using tweezers that were provided by the PEA manufacturer to open the reaction centers (*Q<sub>a</sub>* oxidized). The fluorescence induction was performed after the adaptation of the leaf to the dark using a single 1 second light-saturating pulse (3500 μmol m<sup>-2</sup> s<sup>-1</sup>), which was applied with three LEDs (650 nm).

A fluorometer was used to evaluate plant vitality, characterized by a performance index (PI), which integrates three other independent parameters: the density of the active reaction centers (RC) per unit of chlorophyll molecules in the antenna system of photosystem II (RC/ABS); the efficiency of the electron that reduces quinone A (*Q<sub>a</sub>*) and enters the electron transport chain (ET/(TR-ET)); and the ratio between the capacity of reduction of *Q<sub>a</sub>* by the absorbed photon and the energy dissipated as heat (F<sub>v</sub>/F<sub>0</sub>).

### Statistical analysis

An exploratory analysis of the data was performed, the data were subjected to analysis of variance (ANOVA), and the means were compared by Tukey's test at 5% probability. Statistical analyses were performed using the SAEG 9.1 program (SAEG, 2007).

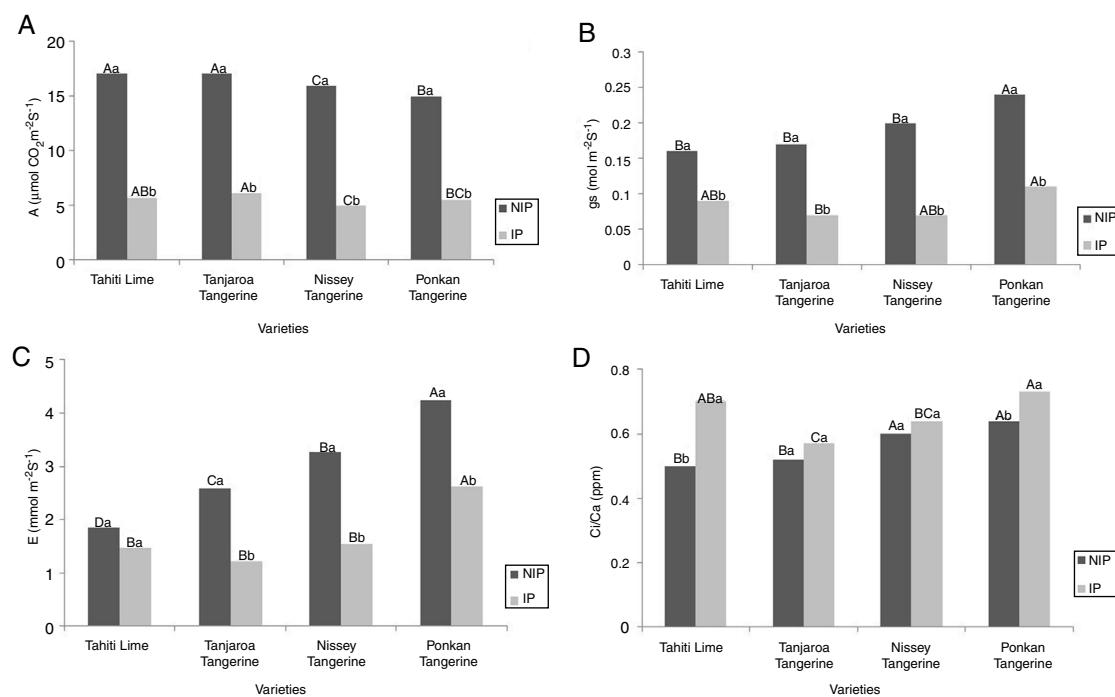
## Results

### Gas exchange of citrus plants

According to the analysis of variance for the gas exchange parameters, the interaction between the factors (citrus blackfly infestation and citrus varieties) had no significant effect (*p* < 0.05) on the photosynthetic CO<sub>2</sub> assimilation, stomatal conductance, instantaneous transpiration, and C<sub>i</sub>/C<sub>a</sub> ratio.

The highest photosynthetic CO<sub>2</sub> assimilation (*A*) (Fig. 1A) was found in noninfested Tahiti (17.06 μmol m<sup>-2</sup> s<sup>-1</sup>) and Tanjara plants (17.04 μmol m<sup>-2</sup> s<sup>-1</sup>). Tanjara plants also presented the highest *A* (6.12 μmol m<sup>-2</sup> s<sup>-1</sup>) among plants infested with *A. woglumi*. The plants subjected to stress caused by *A. woglumi* infestation (Fig. 1A) presented lower mean *A* (5.71 μmol m<sup>-2</sup> s<sup>-1</sup> for Tahiti, 6.12 μmol m<sup>-2</sup> s<sup>-1</sup> for Tanjara, 4.97 μmol m<sup>-2</sup> s<sup>-1</sup> for Nissey, and 5.49 μmol m<sup>-2</sup> s<sup>-1</sup> for Ponkan, representing a reduction of 66.5; 64.0; 68.8, and 63.3%, respectively) when compared to plants without the pest.

Noninfested Ponkan tangerine plants showed higher stomatal conductance (*g<sub>s</sub>*) (0.24 mol m<sup>-2</sup> s<sup>-1</sup>) (Fig. 1B), differing



**Fig. 1.** Photosynthetic CO<sub>2</sub> assimilation (A), stomatal conductance (B), instant transpiration (C), and ratio between internal CO<sub>2</sub> concentration in the leaf mesophyll and external CO<sub>2</sub> concentration of the air (Ci/Ca) (D) of noninfested (NIP) and citrus blackfly infested (IP) citrus plants (Tahiti lime, Tanjaroa tangerine, Nissey tangerine, and Ponkan tangerine), which were grown in field conditions during the rainy season in São Luís MA, Brazil, 2015. Means followed by the same uppercase letter in the varieties and lowercase letter in the treatments (noninfested and infested plants) are similar by Tukey's test at the 5% probability level ( $p < 0.05$ ).

significantly ( $p < 0.05$ ) from the other varieties without *A. woglumi* infestation. Although the  $g_s$  of infested plants were similar ( $p < 0.05$ ), the highest mean  $g_s$  was found in the Ponkan variety ( $0.11 \text{ mol m}^{-2} \text{ s}^{-1}$ ), whereas Tanjaroa plants were the most attacked by *A. woglumi* and presented the lowest mean  $g_s$  ( $0.07 \text{ mol m}^{-2} \text{ s}^{-1}$ ).

The plants subjected to infestation with citrus blackfly (Fig. 1B) were severely affected and had significant decreases ( $p < 0.05$ ) in  $g_s$  when compared to the control treatment, with decreases of 43.7% (Tahiti), 58.8% (Tanjaroa), 65% (Nissey), and 54.1% (Ponkan). Ponkan plants had the highest mean instantaneous transpiration (E) (Fig. 1C) in the control ( $4.25 \text{ mmol m}^{-2} \text{ s}^{-1}$ ) and infested ( $2.63 \text{ mmol m}^{-2} \text{ s}^{-1}$ ) treatments, differing significantly ( $p < 0.05$ ) from the other varieties. Regarding the infested plants, only Tahiti plants ( $1.48 \text{ mmol m}^{-2} \text{ s}^{-1}$ ) had no effects from *A. woglumi* infestation, differing significantly ( $p < 0.05$ ) from the Tahiti, Tanjaroa, and Nissey plants. Infested plants of Tanjaroa, Nissey, and Ponkan had decreases in E values ( $1.22 \text{ mmol m}^{-2} \text{ s}^{-1}$ ,  $1.55 \text{ mmol m}^{-2} \text{ s}^{-1}$ , and  $2.63 \text{ mmol m}^{-2} \text{ s}^{-1}$ , respectively) when compared to control plants.

According to the ratio between the internal CO<sub>2</sub> concentration in the leaf mesophyll and the external CO<sub>2</sub> concentration in the air (Ci/Ca) (Fig. 1D), noninfested Ponkan and Nissey plants differed significantly from the others ( $p < 0.05$ ), presenting the highest Ci/Ca ( $0.64 \text{ ppm}$  and  $0.61 \text{ ppm}$ , respectively). In the treatment with infested plants, Ponkan and Tahiti differed significantly ( $p < 0.05$ ) from the other varieties, presenting the highest mean Ci/Ca ( $0.73 \text{ ppm}$  and  $0.70 \text{ ppm}$ , respectively); the lowest Ci/Ca were found in Tanjaroa plants ( $0.57 \text{ ppm}$ ).

The Ci/Ca of infested Tanjaroa and Nissey plants were similar to those found in the control plants ( $p < 0.05$ ) (Fig. 1D). Infested Tahiti and Ponkan plants had mean Ci/Ca of  $0.70 \text{ ppm}$  and  $0.73 \text{ ppm}$ , respectively, differing significantly ( $p < 0.05$ ) from the control plants.

**Table 1**

Maximum quantum yield of PS II (Fv/Fm) and the performance index (PI) of noninfested and *Aleurocanthus woglumi*-infested citrus plants, which were grown under field conditions during the rainy season in São Luís, MA, Brazil, 2015.

Varieties	Fv/Fm	PI
Tahiti lime	0.78 b	7.80 b
Tanjaroa tangerine	0.79 a	8.08 b
Nissey tangerine	0.78 b	9.50 a
Ponkan tangerine	0.75 c	7.87 b
Citrus blackfly infestation	Fv/Fm	PI
Noninfested plants	0.82 a	12.40 a
Infested plants	0.73 b	4.22 b

Means followed by the same letter in the column are similar by Tukey's test at the 5% probability level ( $p < 0.05$ ).

#### Photochemical efficiency of the citrus plants

According to the analysis of variance of the photochemical efficiency of the citrus plants, the interaction between the factors (citrus blackfly infestation and citrus variety) had no effect ( $p < 0.05$ ) on the maximum quantum yield of photosystem II (Fv/Fm) and the performance index (PI).

The highest Fv/Fm values were found in Tanjaroa plants, with a mean of 0.79, differing from those of Tahiti (0.78), Nissey (0.78) and Ponkan (0.75) plants (Table 1). Ponkan plants presented the lowest Fv/Fm. The mean Fv/Fm of noninfested plants was 0.82, corresponding to their maximum energy efficiency in the photochemical process (Table 1). Possible photoinhibition damage occurred in the reaction centers of photosystem II in plants with the presence of *A. woglumi*, with a mean Fv/Fm of 0.73, due to the damage caused by the presence of the pest and sooty mold.

The Nissey variety had the highest PI (9.50), denoting its better performance, differing significantly ( $p < 0.05$ ) from the Tahiti (7.80), Tanjaroa (8.08), and Ponkan (7.87) varieties (Table 1). The

PIs of plants subjected to the stress caused by the presence of the pest were affected negatively, differing significantly ( $p < 0.05$ ) from those of the noninfested plants, presenting a reduction of 66%.

## Discussion

The highest  $A$  found in noninfested plants (Fig. 1A) confirms the results of Mattos Junior et al. (2005), who determined the maximum rate of photosynthesis in orange (*Citrus sinensis* Linnaeus, 1753) plants under natural conditions – air temperatures of 22 °C to 25 °C, without a soil water deficit.

The limit for  $A$  in citrus plants is 4.0 to 10  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (Machado et al., 2010); an  $A$  lower than 4.0  $\mu\text{mol m}^{-2} \text{s}^{-1}$  characterizes a stress condition in plants. The lower  $A$  of plants infested with *A. woglumi* (Fig. 1A) can be attributed to decreases in  $\text{CO}_2$  availability in the mesophyll and carboxylation sites due to their stomatal limitations (Ribeiro and Machado, 2007), since stomatal conductance was also negatively affected by the citrus blackfly, presenting low values. This result can be connected to the citrus blackfly population dynamics during the rainy season in the region, when *A. woglumi* infestation is lower compared to the dry period (Medeiros et al., 2009). However, the experimental period (rainy season) presented low precipitation and high temperatures, which possibly favored pest activity in the hosts.

Gonda-King et al. (2014) found similar results, with decreases in photosynthetic rates in *Tsuga canadensis* (Linnaeus, 1763) infested by *Adelges tsugae* (Annand, 1928) (Hemiptera: Adelgidae), and reported that the long permanence of sap-sucking insects on the same plant causes physiological impacts due to the high removal of nutrients from the xylem or phloem, decreasing plant photosynthesis and growth. Simpson et al. (2012) found that infestations by *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae) significantly reduced photosynthetic rates and altered the osmotic potential of *Brassica oleracea* (Linnaeus, 1753), presenting symptoms of water deficiency and reduced stomatal conductance and chlorophyll levels.

According to Huang et al. (2013), high infestations of *Phenacoccus solenopsis* (Tinsley, 1898) (Hemiptera: Pseudococcidae) in tomato plants caused disruptions of photosynthetic organelles and reduced the efficiency of the photosynthetic apparatus. Mechanical stimulus and saliva from the pest and the feeding time cause physiological changes around the feeding point. This can reduce the transport of photoassimilates through the phloem and nutrients through the xylem and can reduce photosynthetic rates due to the removal of nutrients (Gonda-King et al., 2014).

This study evaluated direct damage caused by the continuous suction of nutrients from the leaves by nymph and adult *A. woglumi* insects and indirect damage due to the development of sooty mold on the exudates of *A. woglumi* nymphs on the leaves. The dark color of this fungus on the leaves probably decreased the interception of solar radiation (photosynthetic photon flow) by the reaction centers of the photosynthetic apparatus. This can block photosynthesis, decrease almost all photosynthetically active radiation, and cause a decrease of up to 70% of the net photosynthesis (Lopes et al., 2009), restricting the plant's respiration and decreasing leaf nitrogen content (Moraes et al., 2014). Reductions in photosynthesis affect carbohydrate reserves of plants, decreasing the yield and quality of their production.

The  $gS$  values found (Fig. 1B) were within the normal range for citrus plants (0.1 to 0.3  $\text{mmol m}^{-2} \text{s}^{-1}$ ), according to Mattos Junior et al. (2005). Gonda-King et al. (2014) found similar results in *Tsuga canadensis* L. infested by *Adelges tsugae*, with decreases of the stomatal components, and attributed these results to the stress caused by sap-sucking insects, which also affected photosynthesis. Thus,

the greater the limitation of the stomatal aperture, the smaller the amount of  $\text{CO}_2$  to the substomatal chamber and the less raw material for photosynthesis.

The citrus plants exhibited parallel decreases in photosynthesis and stomatal conductance with increasing stress. The high air temperature (28 °C) during the experiment probably positively affected the action of the pest since the photochemical efficiency of photosystem II also decreased.

According to Cerqueira et al. (2004), transpiration intensity varies with internal factors (surface area, distribution, and number of stomata) and external factors (light, air humidity, temperature, and availability of water in the soil). The significant decrease in instantaneous transpiration of the infested plants (Fig. 1C) may indicate an increase in plant leaf temperature, probably due to low stomatal conductance because of the action of the citrus blackfly and the presence of sooty mold. This was a similar trend to that found for  $gS$ , probably due to the stress caused by the pest, because of the partial closure of the stomata, which restricted the output of water vapor and the input of  $\text{CO}_2$  into the cell. However, despite this reduction, the values found for transpiration remained within the range found for orange trees (1.0 to 12  $\text{mmol m}^{-2} \text{s}^{-1}$ ), according to Mattos Junior et al. (2005).

Reduced transpiration in citrus varieties under *A. woglumi* infestation was also found by Moore et al. (2010), with a reduction of 40% of instantaneous transpiration in *Arundo donax* (Linnaeus, 1753) plants infested with *Rhizaspidiotus donaci* (Leonardi, 1920) (Hemiptera: Diaspididae) for 10 weeks when compared to the control.

The highest  $Ci/Ca$  (Fig. 1D) corresponded to the highest internal  $\text{CO}_2$  concentrations found, denoting greater accumulation of  $\text{CO}_2$  inside the mesophyll. However, this increase did not correspond to the highest photosynthetic rates. These results show a possibly nonstomatal negative effect on photosynthesis, which reduced the photosynthetic carbon assimilation, denoting problems in the photosynthetic apparatus, since  $\text{CO}_2$  was not assimilated, causing an increase in intercellular  $\text{CO}_2$  concentrations. This can be explained by the eventual increase in the opening of the stomata, which made possible a greater  $\text{CO}_2$  assimilation.  $Ci$  and  $gS$  usually increase together; thus, stomatal limitations would be the main factor that affected the photosynthetic performance because the greater the stomatal opening, the greater the  $\text{CO}_2$  diffusion to the substomatal chamber (Silva et al., 2015). Higher  $Ci/Ca$  are found when the photosynthetic apparatus remains at efficient levels under disturbances, such as insect pest attack.

Studies have shown that the presence of herbivores feeding on plants results in changes in gas exchange; many of these changes are related to damage to the photosynthetic apparatus (Simpson et al., 2012; Gutsche et al., 2009). Studies on the effect of lesions caused by herbivores on plant physiology may assist in understanding the physiological mechanisms of plant tolerance and resistance to these herbivores.

The  $Fv/Fm$  values found (Table 1) were within the ideal range ( $0.80 \pm 0.05$ ), indicating maximum energy efficiency in the photochemical process (Trovão et al., 2007).  $Fv/Fm$  values lower than 0.75 denote stress conditions and, therefore, a decrease in the quantum yield of the photosystem in the leaves (photoinhibition) (Guo et al., 2006). Therefore, the  $Fv/Fm$  found in plants infested with *A. woglumi* (Table 1) could be even lower as a function of the stress period.

The results found for  $Fv/Fm$  in plants infested with citrus blackfly (Table 1) are similar to those found by Huang et al. (2013) in tomato plants, who reported that the infestation period and the density of *Phenacoccus solenopsis* (Tinsley, 1898) (Hemiptera: Pseudococcidae) feeding on the plants are connected to the damage

in the photosynthetic apparatus, and high infestations of this pest damage photosynthetic organelles, hindering the efficiency of the photosynthetic apparatus. Moreover, [Golan et al. \(2015\)](#) reported that *Coccus hesperidum* (Linnaeus, 1758) (Hemiptera: Coccoidea) significantly affected Fv/Fm rates in *Citrus limon* L. plants. Therefore, the type and severity of stress can affect the physiology of the plant ([Silva et al., 2010](#)).

The high PI found in noninfested plants ([Table 1](#)) denotes their vitality, which was probably favored by the light absorption intensity, the probability of the energy transport following the electron transport chain, and its maximum quantum yield ([Christen et al., 2007](#)). Thus, the lower PI values found in infested citrus plants may indicate photoinhibition and reduction of the efficiency of the photosynthetic process. The plants infested with citrus blackfly probably dissipated less energy in the form of heat and therefore presented more active reaction centers ([Ripley et al., 2004](#)).

## Conclusion

Citrus plants infested with *A. woglumi* (citrus blackfly) insects and presenting sooty mold underwent physiological changes regarding their photosynthetic CO<sub>2</sub> assimilation, stomatal conductance, instantaneous transpiration, and performance indexes. Plants of the Tahiti, Tanjaroa, Nissey, and Ponkan varieties infested with citrus blackfly present photoinhibition of photosystem II. Plants of the Tahiti, Tanjaroa, Nissey, and Ponkan varieties infested with *A. woglumi* present reductions of approximately 70% in photosynthetic CO<sub>2</sub> assimilation.

## Conflicts of interest

The authors declare no conflicts of interest.

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