Growth- and leaf-temperature effects on photosynthesis of sweet orange seedlings infected with *Xylella fastidiosa*

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The effects of growth and leaf temperature on photosynthesis were evaluated in sweet orange seedlings (*Citrus sinensis* cv. Pera) infected with *Xylella fastidiosa* (the bacterium that causes citrus variegated chlorosis, CVC). Measurements of leaf gas exchange and chlorophyll *a* fluorescence were taken at leaf temperatures of 25, 30, 35 and 40°C in healthy and infected (without visible symptoms) seedlings submitted to two temperature regimes (25/20 or 35/20°C, day/night), not simultaneously. The CO₂ assimilation rates (*A*) and stomatal conductance (*gₛ*) were higher in healthy plants in both temperature regimes. Values for *A* and *gₛ* of infected and healthy plants were higher in the 35/20°C regime, decreasing with leaf temperature increase. In addition, differences between healthy and infected plants were higher at 35/20°C, while no differences in chlorophyll *a* fluorescence parameters were observed except for potential quantum efficiency of photosystem II, which was higher in infected plants. Low *A* values in infected plants were caused by low *gₛ* and probably by biochemical damage to photosynthesis. The high alternative electron sink of infected plants was another effect of reduced *A*. Both high growth and high leaf temperatures increased differences in *A* between healthy and infected plants. Therefore this feature may be partially responsible for lower growth and/or productivity of CVC-affected plants in regions with high air temperature.

**Keywords**: chlorophyll fluorescence, *Citrus sinensis*, citrus variegated chlorosis, gas exchange, thermoinhibition

Introduction

In international trade the importance of Brazil’s contribution to orange juice and fruit production is well known (FNP, 2002). The state of São Paulo accounts for 86% of Brazilian orange production (FNP, 2002). However, the occurrence and high dissemination of citrus variegated chlorosis (CVC) has become one of the greatest threats to the Brazilian citrus industry (Almeida et al., 2001). CVC, caused by the bacterium *Xylella fastidiosa*, is a vascular disease of sweet orange plants that limits plant production (Rossetti, 1991). Infected plants show chlorotic leaf spots followed by necrosis, leaf wilting and poor quality fruits (Rossetti, 1991).

*Xylella fastidiosa* is also responsible for other diseases that affect economically important plants, such as Pierce’s disease in grapevine, alfalfa dwarf disease, phony peach disease, and leaf scorch of elm, oak, mulberry, almond, sycamore, plum and coffee (Raju & Wells, 1986; Hopkins, 1989; Paradela Filho et al., 1997).

The pathogenicity mechanism of the diseases caused by *X. fastidiosa* is as yet unclear. Dysfunction of the water-conduction system, production of phytotoxins, and imbalance of growth regulators are proposed as pathogenicity mechanisms (Hopkins, 1989). Evidence points to the hypothesis that the pathogenicity mechanism is related to water stress induced by vascular occlusion (Hopkins, 1989; Machado et al., 1994; Habermann et al., 2003a, 2003b; Ribeiro et al., 2003a). However, the low number of occluded xylem vessels and the lack of visible leaf wilting in grapevines with Pierce’s disease suggested that chlorosis and necrosis were caused by a toxin (Goodwin et al., 1988). Ribeiro et al. (2003a, 2003b) and Habermann et al. (2003b) suggested that the photosynthetic mechanism of sweet orange plants infected with *X. fastidiosa* was also affected by biochemical damage.

The presence and development of CVC is related to environmental conditions and plant age. Young sweet orange plants are more sensitive to CVC than mature ones (Machado, 1997), and the disease is also more severe in regions where additional stresses occur, such as high atmospheric demand and/or water deficits (Salva et al., 2004).
Xylella fastidiosa, citrus and photosynthesis

1995). The simultaneous action of these environmental factors and CVC probably increases dysfunctional effects on plant metabolism.

The physiological and biochemical mechanisms that characterize the establishment of CVC are not completely understood, especially under stress conditions. Therefore studies that take into account the relationship between environmental conditions and CVC establishment are helpful for plant disease management programmes. The objective of the present work was to study the effects of growth and leaf temperatures on the photosynthetic apparatus of sweet orange seedlings infected with X. fastidiosa, the hypothesis being that vascular occlusion caused by X. fastidiosa, associated with high growth and leaf temperatures, could increase dysfunction of the photosynthetic process of infected plants without visible symptoms of CVC.

Materials and methods

Plant material

Measurements of leaf gas exchange and chlorophyll a fluorescence were taken in 9-month-old seedlings of sweet orange (Citrus sinensis) cv. Pera, grown in 3 L plastic pots filled with soil mixture (50% soil, 25% sand, 25% cow dung, amended with NPK fertilizer), under greenhouse conditions (maximum and minimum air temperatures of 42 and 18°C, respectively, minimum RH 30%, maximum irradiance =1800 μmol m⁻² s⁻¹, photoperiod between 13-4 and 10-6 h). The cultivar Pera was chosen because it is the most commonly grown sweet orange variety in Brazilian orchards. A nutrient solution was applied according to van Raij et al. (1996) to ensure that no nutrient deficiency could limit plant growth. Irrigation was provided every morning until soil saturation, and weekly pesticide applications prevented any occurrence of insects or additional diseases.

Plant inoculation

Inoculation with X. fastidiosa was according to Almeida et al. (2001). Needle inoculation was performed by probing five times with a no. 0 insect pin through a 2 μL drop of bacterial suspension into three positions on the stem of a seedling. There were two lots, the first with four healthy plants, the second with four infected plants. All plants were analysed by polymerase chain reaction (PCR) (Minsavage et al., 1994) and by isolation and culture in periwinkle–wilt–GelRite solid medium (Hill & Purcell, 1995; Almeida et al., 2001) for detection of bacteria. Infected plants did not show visible symptoms of CVC such as leaf chlorosis, necrosis and wilting, although PCR and isolation results confirmed the presence of X. fastidiosa. Measurements started immediately after the detection of X. fastidiosa, which occurred 7 months after inoculation.

Thermal treatments

Healthy and infected sweet orange seedlings were moved to a plant growth chamber (E-15, Conviron, Winnipeg, Canada) under a 14 h photoperiod, photosynthetic photon flux density (PPFD) of 600 μmol m⁻² s⁻¹ and an air vapour pressure deficit (VPD) of 1·0 kPa. Healthy and infected plants were submitted to temperature regimes of 25/20 or 35/20°C (day/night) for 7 days, not simultaneously. The following measurements were taken on the seventh day in each temperature regime.

Measurements of leaf gas exchange and chlorophyll a fluorescence

Measurements of leaf gas exchange and chlorophyll a fluorescence were taken simultaneously using a modulated fluorometer (FMS1, Hansatech, King’s Lynn, UK) adapted to the gas analyser sensor head of the infrared gas analyser (LI-6400, Licor, Lincoln, NE, USA). Measurements of CO₂ and H₂O vapour fluxes were taken at leaf temperatures of 25, 30, 35 and 40°C in both 25/20 and 35/20°C regimes, for the same mature and fully expanded leaves (=6 months old). CO₂ assimilation (A) and transpiration (E) rates, stomatal conductance (gₛ) and intercellular CO₂ concentration (Cᵢ) were calculated using the LI-6400 data analysis program which uses von Caemmerer & Farquhar’s (1981) general gas-exchange formula. The environmental conditions of the LI-6400 leaf chamber were the same as those of the plant growth chamber E-15. Regardless of temperature, leaf chamber conditions were 600 μmol m⁻² s⁻¹ PPFD and 1·0–1·5 kPa VPD. Vapour pressure deficit was maintained around these values to prevent stomatal closure, using a dew point generator (LI-610, Licor) attached to the LI-6400. As measurements were taken in leaves without visible symptoms of CVC, the effects of reduced photosynthetic active area were excluded.

Maximal (Fₚₚ) and basal (Fₒ) fluorescence yields were measured in dark-adapted (30 min) leaves, whereas steady-state (Fₛ) and maximal (Fₚₚ) fluorescence were sampled in a light-adapted state (van Kooten & Snel, 1990). Variable fluorescence yield was determined in dark-adapted (Fₛ – Fₒ) and in light-adapted (ΔF = Fₚₚ – Fₒ) states. Fₒ is the basal fluorescence yield after photosystem I excitation by far-red light. The parameters calculated were: potential (Fₛ/Fₚₚ) and effective (ΔF/Fₚₚ) quantum efficiency of photosystem II (PSII); photochemical (ϕₗ = [(Fₚₚ – Fₛ)/(Fₚₚ – Fₒ)]) and nonphotochemical (NPQ = [(Fₚₚ – Fₒ)/(Fₚₚ)]) fluorescence quenching; and apparent electron transport rate [ETR = (PPFD × ΔF/Fₚₚ × 0·5 × 0·84)] (Bilger et al., 1995; Maxwell & Johnson, 2000). For the calculation of ETR, 0·5 was used as the fraction of excitation energy distributed to PSII, and 0·84 as the fraction of light absorption. Alternative electron sinks (AES) were calculated as the relation between the effective quantum efficiency of PSII (ΔF/Fₚₚ) and quantum efficiency of CO₂ fixation (ϕCO₂), as AES = (ΔF/Fₚₚ/ϕCO₂) (Edwards & Baker, 1993). ϕCO₂ was calculated as ϕCO₂ = [A/(PPFD × 0·84)], where PPFD is the light reaching the leaf (600 μmol m⁻² s⁻¹) and 0·84 is the fraction of light absorption. Dark respiration was not included in ϕCO₂ calculation because healthy and infected plants do not show differences in this variable (Ribeiro et al., 1994).
Statistical analysis

The experiment was arranged in a split-split-plot design with four replicates (blocks), where the plots were healthy and infected seedlings, the split plots were temperature regimes (25/20 and 35/20°C), and the split-split plots were leaf temperatures (25, 30, 35 and 40°C). All results were subjected to ANOVA procedures, and Tukey’s test at the 0-05 probability level was employed to determine statistical significance between measured parameters.

Results

Effects of temperature on leaf gas exchange

The highest photosynthetic rates were found at 25°C, while minimum values were observed at 40°C for both temperature regimes (25/20 and 35/20°C day/night) (P < 0.05) (Fig. 1a,b). The pattern of CO₂ assimilation (A) in relation to leaf temperature (T_L) was similar for both healthy and infected plants, but A values were always higher in healthy plants (Fig. 1a,b). Mean reduction of A with T_L increase from 25 to 40°C, considering both temperature regimes, was ≈77.8% (from 6.81 to 1.51 µmol m⁻² s⁻¹) for healthy plants and 89.9% (from 5.33 to 0.54 µmol m⁻² s⁻¹) for infected plants. The highest A values of both healthy and infected plants were reached in the 35/20°C regime, showing a mean increase in A of 67.5 and 24.9% for healthy and infected plants, respectively, when the temperature regime was changed from 25/20 to 35/20°C (Table 1). Thus differences between healthy and infected plants were higher in the 35/20°C regime (P < 0.05).

Figure 1 CO₂ assimilation (a,b) and stomatal conductance (c,d) of healthy (solid symbols) and X. fastidiosa-infected (open symbols) plants as functions of leaf temperature under two growth-temperature regimes (day/night): 25/20°C (a,c) and 35/20°C (b,d). Vertical lines, ± SE (n = 4).

Table 1 Mean values of CO₂ assimilation (A), stomatal conductance (gₛ) and alternative electron sinks (AES) in healthy and infected plants at temperature regimes (day/night) of 25/20 and 35/20°C.

<table>
<thead>
<tr>
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<th>A (µmol m⁻² s⁻¹)</th>
<th>gₛ (mol m⁻² s⁻¹)</th>
<th>AES</th>
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<tbody>
<tr>
<td>Plants</td>
<td>25/20°C</td>
<td>35/20°C</td>
<td>25/20°C</td>
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<tr>
<td>Healthy</td>
<td></td>
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<tr>
<td></td>
<td>3.17*</td>
<td>3.31*</td>
<td>0.059**</td>
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<tr>
<td>Infected</td>
<td>2.57*</td>
<td>3.21**</td>
<td>0.037**</td>
</tr>
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</table>

Data are mean values of all measurements taken in each temperature regime (n = 16). *, **, Significant differences between healthy and infected plants at P < 0.05 and P < 0.01, respectively. ***, Non-significant difference between healthy and infected plants.

The highest stomatal conductance (gₛ) values of healthy plants were observed at 25°C (Fig. 1c,d) whereas minimum values were found at 40°C (P < 0.05). The lowest gₛ values in plants infected with X. fastidiosa occurred at T_L ≥35°C in the 25/20°C temperature regime and only at 40°C in the 35/20°C regime (P < 0.05). Increasing T_L from 25 to 40°C in the 35/20°C regime caused a more accentuated decrease in gₛ of healthy plants. When the temperature regime was changed from 25/20 to 35/20°C, mean increases in gₛ were 74.6 and 51.4% in healthy and infected plants, respectively (Table 1). These results showed that healthy plants were more sensitive to growth- and leaf-temperature increases.

As E is directly related to gₛ, similar changes were observed in both parameters. The E values of healthy plants (data not shown) were in accordance with transpiration rates reported for citrus of 1.1–4 mmol m⁻² s⁻¹ (Khairi & Hall, 1976; Machado et al., 1994, 2002).

The mean difference in gₛ between healthy and infected plants at 25/20°C was ≈37.3%, but this difference increased to 45.6% at 35/20°C (Table 1). Therefore differences in gₛ, like those in A, were also more accentuated at 35/20°C. Although gₛ values were reduced in infected plants, nonsignificant differences were found in intercellular CO₂ concentration (Cᵢ) between healthy and infected plants (data not shown).

Effects of temperature on chlorophyll a fluorescence

The potential quantum efficiency of PSII (Fᵥ/Fₘ) was higher at 25°C, decreasing with T_L increase (P < 0.05). Reductions in Fᵥ/Fₘ of healthy and infected plants were more accentuated at leaf temperatures above 35°C (Fig. 2a,b). As observed with gas-exchange measurements, healthy and infected plants showed higher values of Fᵥ/Fₘ at 35/20°C (P < 0.05), but the highest differences of Fᵥ/Fₘ between healthy and infected plants were detected at 25/20°C (Fig. 2a,b). Curiously, Fᵥ/Fₘ of infected plants was higher than that of healthy plants in all cases (P < 0.05). The results indicated that the high Fᵥ/Fₘ in infected plants was caused by changes in the basal (Fₘ) and maximal (Fₘ) fluorescence yields which determined changes in the variable fluorescence yield (Fᵥ) (Fig. 2c,d).
Increased $T_l$ caused decreases in $F_v/F_m$ because of the reduction in $F_m$, mainly in the 25/20°C regime.

Reduction in the effective quantum efficiency of PSII ($\Delta F/F_m'$) occurred at $T_l$ >30°C in the 25/20°C temperature regime ($P < 0.05$). However, $\Delta F'/F_m'$ did not decrease with $T_l$ increase in the 35/20°C regime (Fig. 3a,b). Higher $\Delta F'/F_m'$ values were recorded in the 35/20°C regime ($P < 0.05$), but no significant differences between healthy and infected plants were observed in this regime. Only in the 25/20°C regime did infected plants show higher $\Delta F'/F_m'$ values than healthy plants ($P < 0.05$) (Fig. 3a,b).

The apparent electron transport rate (ETR), as well as photochemical ($q_P$) and nonphotochemical (NPQ) fluorescence quenching, was not apparently affected in plants infected with X. fastidiosa ($P > 0.05$) under either temperature regime. Mean ETR was influenced by temperature regime ($P < 0.05$), showing an increase of 31.1% at 35/20°C compared with the lower temperature regime. With regard to $T_l$ increase, changes from 25 to 40°C caused a reduction in ETR only in the 25/20°C regime (Fig. 3c,d).

High AES activity was observed at high $T_l$, especially in infected plants, in both temperature regimes ($P < 0.05$). The mean AES value of infected plants was 2.79 and 2.56 times higher than that of healthy plants in the 25/20 and 35/20°C regimes, respectively (Table 1). As expected, AES activity was highest at a $T_l$ of 40°C under both temperature regimes, and it was at this $T_l$ that the differences between healthy and infected plants were most evident (Fig. 5).

Discussion
In general, photosynthesis of citrus plants tends to decrease at air temperatures >31°C and VPD >2.0 kPa (Khairi & Hall, 1976). In this study the reductions in $A$ with increasing $T_l$ were probably related to reduction in mesophyll conductance to CO₂ diffusion (Khairi & Hall, 1976) and reduction in carboxylation activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) (Laisk et al.,
also showed low another disease caused by the partial occlusion of xylem vessels (Esau, 1948). In Habermann's work, it was suggested that symptoms were entirely caused by physical obstruction of xylem vessels by the bacterium (Andersen et al., 2003a, 2003b). However, some researchers do not support the hypothesis that symptoms produced by pathogens, acting simultaneously or not. Goodwin et al. (1988) suggested the hypothesis that symptoms produced by the bacterial pathogen were responsible for leaf chlorosis and necrosis, as there was a low number of obstructed xylem vessels and a lack of visible leaf wilting in peaches with phony disease. In accordance, some researchers do not support the hypothesis that symptoms were entirely caused by physical obstruction of xylem vessels by the bacterium (Andersen & French, 1987; Ribeiro et al., 2003a, 2003b). However, the influence of such toxins on the photosynthetic mechanism is still unknown.

Concerning temperature effects, decreases in photosynthesis can be associated with impairments in biochemical reactions as well as in photochemical ones. Reductions in $F_v/F_m$ and $F_m$ with $T_i$ increase indicate injury to the photosynthetic mechanism, named thermoinhibition (Bilger et al., 1995; Laisk et al., 1998). Changes in $F_v/F_m$ result from alterations in the efficiency of nonphotochemical quenching (Maxwell & Johnson, 2000), which are indicated by NPQ values. Moreover, low $F_m$ values resulting from $T_i$ increase cause $F_v$ decrease, which is related to low quantum yield of PSII (Krause & Weis, 1984; Laisk et al., 1998). In addition, simultaneous increase in NPQ and reduction in $A$ with $T_i$ increase suggest a protective mechanism related to the xanthophyll cycle, which is often used as an indicator of the dissipation of excess radiant energy as heat in the PSII antenna complexes (Demmig-Adams & Adams, 1992). The present results show that low $F_v$ was probably the main cause of high $F_v/F_m$ of infected plants. $F_v$ is independent from photochemical reactions (Krause & Weis, 1984) and is a measure for the initial energy distribution to PSII and for the efficiency of the excitation trap at $P_{max}$ (Schreiber & Bilger, 1987). If an increase of $F_v$ under inhibitory conditions is related to the reduction of the acceptor side.

Figure 5 Alternative electron sinks (AES) of healthy (solid symbols) and X. fastidiosa-infected (open symbols) plants as functions of leaf temperature under two growth-temperature regimes (day/night): 25/20°C (a) and 35/20°C (b). Vertical lines, ± SE ($n = 4$).

According to Laisk et al. (1998), reduction in $A$ with increasing $T_i$ is caused by increase of respiratory and photosynthetic activities resulting from higher oxygenase activity of RuBisCO.

In citrus plants the optimum temperature for $g_s$ is $30^\circ$C (Khairi & Hall, 1976), but the present results indicated maximum $g_s$ at $25^\circ$C, which was closer to the temperature of maximum $A$ reported in some studies (Khairi & Hall, 1976; Medina et al., 1999). Healthy plants showed a more marked $g_s$ response than infected plants to $T_i$ increase in the $35/20^\circ$C temperature regime. In accordance with this, Habermann et al. (2003a) did not observe changes in $A$ and $g_s$ of infected plants when VPD increased from 1-2 to 2-5 kPa.

According to Esau (1948), gum deposition in xylem vessels of grapevines with Pierce’s disease occurs before the development of external symptoms. Therefore it is reasonable to assume that one of the most probable causes of reduction in $A$ of infected plants without visible symptoms is water restriction promoted by the blockage of xylem vessels by gum and tylosis (Esau, 1948). Accordingly, infected sweet orange plants without visible CVC symptoms showed reductions of 56% in daily sap flow (Oliveira et al., 2000).

It has been reported that low $g_s$ of infected plants is caused by low leaf water content (Machado et al., 1994; Habermann et al., 2003a, 2003b), probably resulting from the partial occlusion of xylem vessels (Esau, 1948). In another disease caused by X. fastidiosa, infected almond also showed low $g_s$ values (Goodwin et al., 1988). As a general mechanism, reduced shoot hydration causes reduction in water vapour loss to the atmosphere (transpiration) by stomatal closure (Nobel, 1999). This affects $A$ by reducing the amount of CO$_2$ substrate. At first sight, the present results did not show reduced substrate for photosynthetic activity (data not shown), but the calculation of $C_i$ could be unreliable when CO$_2$ and H$_2$O vapour fluxes were low, as happened in infected plants. High $C_i$ values could be attributed to the occurrence of patchy stomatal closure and the importance of cuticular transpiration as stomata close. These factors could cause an overestimation of $C_i$, masking any stomatal effect on the reduction of $A$ (Cornic, 2000).
of PSII (Laisk et al., 1998), the results of the present study suggest that lower $F_v$ in infected plants than in healthy ones could be related to the more oxidized or less reduced state of the acceptor side of PSII in infected plants. Additionally, the high $F_v/F_m$ values of infected plants could be attained by a hardening of PSII complexes caused by water stress, as the blockage of xylem vessels causes shoot water deficit (Habermann et al., 2003a; Machado et al., 1994). It has been suggested that water stress could strengthen lipid–PSII protein interaction through changes in the lipid composition of the thylakoid membranes in plants under water stress (Havaux, 1992).

Thylakoid membrane properties can also change with alteration in plant growth temperature, presumably by composition adjustment (Schreiber & Berry, 1977). According to Berry & Björkman (1980), an increase in growth temperature can render the photosynthetic apparatus more tolerant to heat stress, which is supported by the high values of $F_v/F_m$, Δ$F'/F'_m$ and ETR at 35/20°C in the present study. This increase in thermotolerance could be caused by changes in thylakoid membrane lipid composition (Pearcy, 1978). This thermotolerance could be attained after 7 days at 35/20°C as measurements of $F_v/F_m$, Δ$F'/F'_m$ and ETR were taken in the same leaf tissue.

Under natural conditions, simultaneous decrease in $A$ and increase in $q_P$ indicate that molecular oxygen is acting as an efficient electron acceptor, reoxidizing the plastoquinone pool and maintaining high $q_P$ (Schreiber & Bilger, 1987). The results of the present study showed that, although photochemical reactions were active, $A$ values of infected plants were still lower than those of healthy ones. In fact, the proportion of electrons driven to alternative none pool and maintaining high $q_P$ (Schreiber & Bilger, 1987). The results of the present study showed that, although photochemical reactions were active, $A$ values of infected plants were still lower than those of healthy ones. In fact, the proportion of electrons driven to alternative pathways is related to the capacity of protective processes such as antioxidant action in active oxygen species. Photosynthesis and the reduction of molecular oxygen (part of the Mehler reaction) are the main AES, representing at first sight some energy waste and oxidative stress, respectively. However, photosynthesis and the reduction of molecular oxygen can be mechanisms to avoid photoinhibition and consequent photodestruction, dissipating excess ATP and NADPH (Kozaki & Takeba, 1996; Osmond et al., 1997). Increase in AES with $T_i$ increase was another effect of low $A$ in plants infected with X. fastidiosa, caused by low $g$, values and probably by biochemical injuries to the photosynthetic mechanism, as proposed by Habermann et al. (2003b) and Ribeiro et al. (2003a, 2003b). We suggest that the infected plants did not show significant differences in Δ$F'/F'_m$, ETR, $q_P$ and NPQ under photorespiratory conditions because the high AES dissipated the excess energy, protecting the photosynthetic apparatus.

As the highest values of $A$ were observed in the 35/20°C regime ($P < 0.05$), it can be suggested that this environmental condition is more suitable for plant growth, stimulating plant photosynthesis. This was confirmed by the higher values of $A$ and $g$, and the higher photochemical capacity of healthy plants in the 35/20°C regime. The maximal growth rate of citrus occurs at around 30°C (Reuther, 1977) and the highest values of $A$ are found in periods of active growth, characterized by high air temperatures (Machado et al., 2002). As differences between healthy and infected plants became more evident at 35/20°C ($P < 0.05$), it is suggested that the differences between healthy and infected plants are greater in environments where plants can express their physiological potential.

The pathogenicity of X. fastidiosa, considered an occasional and opportunistic pathogen, is aggravated by the occurrence of additional stresses (Hopkins, 1989). It has been proposed that water stress, high temperature, injuries to the root system and overproduction of fruit can be favourable to CVC development (Hopkins, 1989). In Brazil, the high CVC severity in the north and west of the state of São Paulo, where the incidence of CVC is around 17% higher than in the central and southern regions together (Fundecitrus, 2002), is associated with water deficit and high atmospheric demand (Salva et al., 1995). Thus, apart from water deficit and high VPD (Machado et al., 1994), high growth and leaf temperatures also increase differences in $A$ between healthy and infected plants without visible symptoms, which may be partially responsible for lower growth and/or productivity of CVC-affected plants.

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