# $CO_2$ assimilation, photosynthetic light response curves, and water relations of 'Pêra' sweet orange plants infected with *Xylella fastidiosa*

**Gustavo Habermann[1]*, Eduardo Caruso Machado[2], João Domingos Rodrigues[1] and Camilo Lázaro Medina[3]**

*[1]Departamento de Botânica, Instituto de Biociências, Universidade Estadual Paulista, CP 510, 18618-000, Botucatu, SP, Brasil; [2] Instituto Agronômico de Campinas, Centro de Ecofisiologia e Biofísica, 13001-970, Campinas, SP, Brasil; [3] Centro APTA Citros "Sylvio Moreira", CP 04, 13490-970, Cordeirópolis, SP, Brasil. *Corresponding author: gh@lpnet.com.br.*
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Plants with citrus variegated chlorosis (CVC), a disease caused by the xylem-limited bacteria *Xylella fastidiosa,* have leaves with water deficiency symptoms and are associated with decreases on the net photosynthesis and transpiration rates. Using healthy and CVC-affected 'Pêra' sweet orange plants on 'Rangpur' lime rootstock, the leaf gas exchange variables were measured with an open-gas portable photosynthesis system. All plants were watered and the leaf water potential ($\Psi_w$) was measured by isopiestic thermocouple psychrometric technique. The net photosynthesis (*A*) vs. internal leaf $CO_2$ concentration (*A/Ci* curves) was analyzed. The relative effect of stomatal resistance on photosynthesis (S%) and the estimation of carboxylation efficiency were calculated. The rates of photosynthesis and transpiration, stomatal conductance, and internal leaf $CO_2$ concentration (*Ci*) were also measured while varying the photosynthetic photon flux density (*PPFD*). The S% values were approximately 30 % greater in infected plants when compared to healthy ones. The light compensation point for diseased plants was higher than in the healthy ones, and the saturation light point in plants with CVC was twofold lower. The lower $\Psi_w$ in diseased plants favours the hypothesis of xylem occlusion, which probably caused a lower water supply to the mesophyll, thus decreasing the photosynthesis and transpiration rates. Nevertheless, there was also a reduction in the photosynthetic metabolic activities, represented by lower carboxylation efficiency and photochemical disturbances that were detected in diseased plants.

**Key words:** A/Ci curves, citrus variegated chlorosis, *Citrus sinensis* L. Osbeck, photosynthetic photon flux density, photosynthetic metabolism, stomata.

**Assimilação do $CO_2$, curvas de resposta fotossintética à luz e relações hídricas de plantas da laranja doce 'Pêra' infectadas com *Xylella fastidiosa*:** Plantas com clorose variegada dos citros (CVC), uma doença causada pela bactéria *Xylella fastidiosa,* restrita ao xilema, apresentam folhas com sintomas de deficiência hídrica, associados com diminuição na fotossíntese líquida e na taxa de transpiração. Utilizando-se plantas sadias e com CVC de laranjeira 'Pêra' sobre limoeiro 'Cravo', as variáveis de trocas gasosas foram medidas com sistema portátil aberto de fotossíntese. Todas as plantas foram irrigadas e o potencial da água na folha ($\Psi_w$) medido pela técnica psicrométrica de termopar isopiéstico. Fotossíntese líquida (*A*) *vs*. concentração interna de $CO_2$ na folha (curvas *A/Ci*) foram analisadas. O efeito relativo da resistência estomática sobre a fotossíntese (S%) e a estimativa da eficiência de carboxilação foram calculados. Taxas de fotossíntese e transpiração, condutância estomática e concentração interna de $CO_2$ na folha (*Ci*) foram também medidas quando se variou a densidade de fluxo de fótons fotossinteticamente ativos (*DFFFA*). Os valores de S% foram 30 % maiores nas plantas doentes, comparados às sadias, e seu ponto de compensação de luz também foi maior e o ponto de saturação de luz nas plantas com CVC foi duas vezes menor. O menor $\Psi_w$ nas plantas doentes reforçou a hipótese de oclusão do xilema, que provavelmente causou o menor suprimento de água para o mesofilo, diminuindo as taxas de fotossíntese e de transpiração. Todavia, houve também uma redução nas atividades metabólicas da fotossíntese, representada pela baixa eficiência de carboxilação e distúrbios fotoquímicos detectados nas plantas doentes.

**Palavras-chave:** *Citrus sinensis* (L.) Osbeck, clorose variegada dos citros, curvas A/Ci, densidade do fluxo de fótons fotossintéticos, estômato, metabolismo fotossintético.

## INTRODUCTION

Citrus variegated chlorosis (CVC) has been observed in the States of São Paulo and Minas Gerais, Brazil, since 1987 (Rossetti et al., 1990), and it has been causing a yearly loss of about 100 million U.S. dollars in São Paulo State alone (Laranjeira, 1997). Symptoms include conspicuous variegations on older leaves, presenting chlorotic areas on the upper side with corresponding light brown lesions, and a gum-like material on the lower side. Affected fruits become smaller, hardened, with higher sugar content and have no commercial value (Rossetti and De Negri, 1990; Rossetti et al., 1990; Laranjeira and Palazzo, 1999). Nutritional imbalance is also common in infected plants, mainly concerning the mineral potassium (Malavolta et al., 1993).

*Xylella fastidiosa* is a fastidious xylem-limited bacterium and has been identified as the causal agent of CVC (Leite Junior and Leite, 1991; Chang et al., 1993; Lee et al., 1993; Hartung et al., 1994). This bacterium is transmitted by sharpshooter leafhoppers (Roberto et al., 1996). Other strains of *X. fastidiosa* cause a range of economically important plant diseases including Pierce's disease of grapevine (PD), alfafa dwarf, phony peach disease, among others (Purcell and Hopkins, 1996).

CVC-affected plants have leaves with water deficiency symptoms, associated with significant decreases in the net photosynthesis and transpiration rates (Machado et al., 1994). Goodwin et al. (1988b) found decreased net photosynthesis and transpiration rates, as well as decreases in stomatal conductance for grapevines affected by PD. Goodwin et al. (1988b) related PD with low leaf water potential and increased xylem flow resistance. These same findings have also been reported for peach trees with phony peach disease (Andersen and French, 1987). Machado et al. (1994) and Oliveira et al. (2000) suggested that these processes may be due to stomatal dysfunction, increased water resistance through xylem vessels, and/or decreased water uptake making these plants more sensitive to water deficiency.

There are three possible hypotheses for the pathogenicity of *X. fastidiosa*: xylem blockage, plant biorregulators imbalance, and bacterial phytotoxins (Hopkins, 1989).

Lee et al. (1982) suggested that if the bacteria produced any phytotoxins, these could cause direct vascular plugging by high molecular weight compounds and/or as chemical compounds disturbing leaf metabolism, causing the leaf marginal necrosis typical of PD. There are many reports about the involvement of plant biorregulators affecting responses of plants infected with *X. fastidiosa* (French and Stassi, 1978; Hopkins and Thompson, 1984; Hopkins, 1985). However, the theory of water stress due to xylem occlusions has received better acceptance because of the recently published genome sequence of *X. fastidiosa* (Simpson et al., 2000).

Little is known about the causes of decreased leaf gas exchange rates in plants infected with *X. fastidiosa* and their probable interactions with stomatal physiology. Thus, this work tried to investigate variables related to stomatal responses in order to establish the role of stomatal aperture and carboxylation efficiency on photosynthesis of sweet orange plants infected with *X. fastidiosa*.

## MATERIAL AND METHODS

The experiment was conducted with 2.5 year-old sweet orange plants (*Citrus sinensis* L. Osbeck cv. Pêra) on 'Rangpur' lime (*Citrus limonia*) rootstock, cultivated in 100 L pots in a screenhouse at the Campinas Experimental Center (Campinas-SP, Brazil) in early March, 1999. Three of these plants were previously inoculated with *X. fastidiosa* by using the spliced approach grafting method (Medina et al., 1998a) and presented high incidence of foliar symptoms (16.4–39.8 % of leaves showing typical CVC chlorosis), measured by a CVC disease index, according to Amorin et al. (1993). Three additional plants were healthy. The bacterial presence was confirmed before and after the measurements through the polymerase chain reaction (PCR), specific for *X. fastidiosa* (Beretta et al., 1997).

Plants were randomly selected and taken to the laboratory, where they remained for 2 days for leaf gas exchange and water potential ($\Psi_w$) measurements. During the measurements, the plant was kept under an artificial system that provided photosynthetic photon flux density (*PPFD*) varying from 800 (at a distance of 0.5 m from the lamps) to 1200 (just beneath the lamps) $\mu mol.m^{-2}.s^{-1}$, provided by 7 mercury high pressure lamps of 400 W each. In order to minimize the heat irradiated by these lamps, a transparent acrylic box with a continuous water flux was placed beneath them. Air temperature and relative humidity were kept at 27 ± 1°C and 59 ± 2 %, respectively. During the 2 days used for measurements, the whole system was turned on at 06:00 a.m. and turned off at 06:00 p.m., giving the plant a photoperiod of 12 h. Therefore, this two-day period was important for plant aclimatization and also provided an extra day for checking or rejecting data.

At the time of measurements, the plants used were not in flower but had several developing small fruits. Gas exchange measurements were carried out using an open-gas portable photosynthesis system (LI-6400, LI-COR, Lincoln, Nebrasca – USA), an infra red gas analyser (IRGA). The air temperature inside the photosynthesis system cuvette was always set at 25°C, with a vapor pressure deficit (*VPD*) of ≅ 1.2 kPa and relative humidity at 65-70 %. The *VPD* in the cuvette was controlled by a high-precision dew point generator (Li-610 portable dew point generator, LI-COR, Lincoln, Nebrasca – USA). The rates of net photosynthesis (*A*) and transpiration (E), as well as the stomatal conductance to water vapor (*g*) were calculated by the LI-6400 data analysis program, which uses the Von Caemmerer and Farquhar (1981) general gas exchange formula.

The response of net photosynthesis to *PPFD* was carried out using an artificial quartz red light emitting diodes (*LED*) source, controlled with a quantum sensor located inside the leaf cuvette; the curve was obtained by varying the *PPFD* from 1,100 μmol.m$^{-2}$.s$^{-1}$ to zero. The response curve of A versus *PPFD* was then adjusted according to the following equation (Prado and de Moraes, 1997):

$$A = A_{max} [1 - e^{-k(Q - Qc)}] ,$$

where $A_{max}$ is the maximum net photosynthesis rate; k is the constant of adjustment; Q is the PPFD and Qc is the light compensation point. The apparent quantum yield (Φ) was estimated applying the next equation:

$$\Phi = k . A_{max} . e^{(k.Qc)}.$$

The response of net photosynthesis to internal leaf $CO_2$ concentration (*A/Ci* curve) was determined at 1000 μmol. m$^{-2}$.s$^{-1}$ of *PPFD*. The measurements started at 350 μmol.mol$^{-1}$ of $CO_2$. Once the steady state was reached, the $CO_2$ concentration was gradually lowered to 30 μmol.mol$^{-1}$ and then increased stepwise up to 1,200 μmol.mol$^{-1}$. The $CO_2$ was injected into the open circulating gas-stream of the photosynthesis system using its proper auto controlled $CO_2$ injector. Twelve to fifteen sequential measurements of net photosynthesis were taken for each *A/Ci* curve. Net photosynthesis values were plotted against the respective internal leaf $CO_2$ concentrations (*Ci*) to produce an *A/Ci* response curve. As net photosynthesis approached zero, $CO_2$ became the limiting factor for photosynthesis and the initial slope of *A/Ci* line represents the carboxylation efficiency (Farquhar and Sharkey, 1982).

The relative effect of stomatal resistance on photosynthesis (S%) was estimated by the following equation (Farquhar and Sharkey, 1982):

$$S\% = [(ACi - ACe)/ACi] \times 100,$$

where *ACe* represents the net photosynthesis at an ambient external $CO_2$ concentration of 350 μmol.mol$^{-1}$ and *ACi* is the net photosynthesis when the internal leaf $CO_2$ concentration is set at 350 μmol.mol$^{-1}$. *ACi* represents the photosynthesis rate as if there were no stomatal limitation to A (Farquhar and Sharkey, 1982).

Net photosynthesis and corresponding internal $CO_2$ values for the linear portion of the response curve were subjected to linear regression analysis in order to determine the slope. Correlations between *Ci* and *Ce* ($CO_2$ concentrations inside the chamber system – external $CO_2$ concentration) permitted the calculation of the $CO_2$ compensation point.

Leaf water potential ($\Psi_w$) was measured by the isopiestic thermocouple psychrometric technique (Boyer and Knipling, 1965). For this variable, an average of 10 samples (10 leaves) per plant were taken.

This study was conducted in a completely randomized experimental design, with two treatments (healthy and CVC-affected plants) and six replications. Mean values were subjected to one-way analysis of variance ($p \leq 0.01$ and $p \leq 0.05$) and compared using the Tukey's test, at 5 % of probability.

## RESULTS AND DISCUSSION

As expected, the inoculated plants presented positive results for PCR tests, while the non-inoculated ones were negative. CVC-affected plants showed typical CVC-chlorosis on leaves, well distributed in the canopy, although this chlorosis rarely surpassed 25 % of the leaf surface according to the disease index used (Amorin et al., 1993). The non-inoculated plants remained healthy.

$\Psi_w$ was significantly ($p \leq 0.05$) lower in CVC-affected plants (-1.47 ± 0.27 MPa) when compared to healthy ones (-1.03 ± 0.27 MPa) (table 1). When $\Psi_w$ of healthy plants was compared to $\Psi_w$ of diseased ones, we confirmed that xylem blockage was in fact occuring. Despite the fact that the data were obtained from different techniques, the $\Psi_w$ values were similar to the results presented by Machado et al. (1994). These authors and Medina et al. (1999) suggested that there is strong evidence supporting the hypothesis of *X. fastidiosa* causing xylem occlusion that restricts water supply to the mesophyll, thus explaining the low values of transpiration rates and stomatal conductance, which in turn lead to the low net photosynthesis rates observed in CVC-affected plants.

**Table 1.** Angular coefficient of the carboxylation efficiency, $CO_2$ conpensation point, and leaf water potential in healthy and CVC-affected 'Pera' sweet orange plants on 'Rangpur' lime rootstock

| Treatments | Carboxylation efficiency[a] ($\mu mol.m^{-2}.s^{-1}$) | $CO_2$ compensation point (Ce, µmol.mol) | Leaf water potential (MPa) |
|---|---|---|---|
| Healthy | 0.0476 ± 0.01 a | 43.15 ± 13.52 a | - 1.03 ± 0.27 a |
| CVC-affected | 0.0261 ± 0.01 b | 84.53 ± 12.56 b | -1.47 ± 0.27 b |

[a] Means ± standard deviation of 13 measurements on six plants per treatment. The same letters in columns are not significantly different from each other ($p \leq 0.05$).

Transpiration rates of healthy and CVC-affected plants measured using a heat balance sap flow gauge showed that infected plants, on average, had only half of the transpiration rates observed in healthy plants (Oliveira et al., 2000). Also, Bosco (2001) found that sweet orange plants (cv. 'Pêra' and''Natal') infected with *X. fastidiosa* presented a decrease of about 60 % in the sap flow. This is a clear indication of xylem blockage by the bacteria, ultimately supporting our data of lower $\Psi_w$ in infected plants.

Figure 1 shows lower photosynthesis rates for diseased plants even when ambient external $CO_2$ concentration was set at 350 $\mu mol.mol^{-1}$. Figure 2 shows the responses of *A*, *E* and *g* to the *PPFD* variation. Figure 3 shows the Ci response. *A*, *E* and *g* were significantly lower in CVC-affected plants (figure 3).

The lower values of net photosynthesis and transpiration rates are probably related to the lower stomatal conductance. But the decrease in net photosynthesis may also be a response of the lower stomatal conductance and carboxylation efficiency found in CVC-affected plants (figure 1). This decrease in stomatal conductance may be associated with the lower $\Psi_w$. Machado et al. (1994) also reported a decrease in these variables under field conditions in CVC-affected 'Pêra' and 'Valência' sweet orange trees. Machado et al. (1999) and Medina et al. (1998b) have related the decrease in g values with the decrease in $\Psi_w$, when $\Psi_w$ was less than –1.0 MPa in 'Valência' sweet orange plants submitted to soil water deficit.

CVC caused a decrease of 57.5, 30.3, and 50.2 % in *g*, *E* and *A*, respectively. Machado et al. (1999) observed a linear correlation between *E* and *g* and a curvilinear correlation between *A* and *g*. In other words, for equivalent decreases in stomatal conductance, the transpiration rate decreased at a higher proportion than the net photosynthesis rate. Thus, the lowest values of stomatal conductance in affected plants did not fully explain the decrease in photosynthesis, since for the same stomatal restriction water loss will be relatively more affected than $CO_2$ assimilation (Nobel, 1999). The decrease in *A* of CVC-affected plants seems to be promoted by other additional disturbances in the photosynthetic processes. Ribeiro (2002) also observed similar results working with healthy and CVC-affected 'Pêra' sweet orange plants. However, these data of lower leaf gas exchange rates in diseased plants do not indicate the bacteria's mechanism of action.

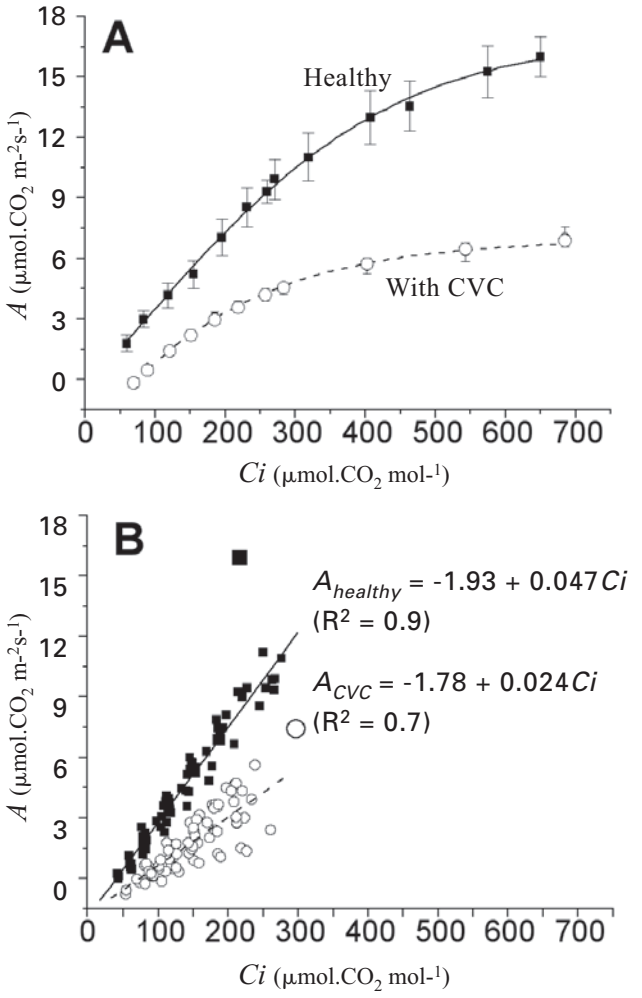


**Figure 1.** Net photosynthesis rates versus internal leaf $CO_2$ concentration (*Ci*) (*A/Ci* curves) (A), and carboxylation efficiency (B) of healthy and CVC-affected 'Pêra' sweet orange plants on 'Rangpur' lime rootstock.

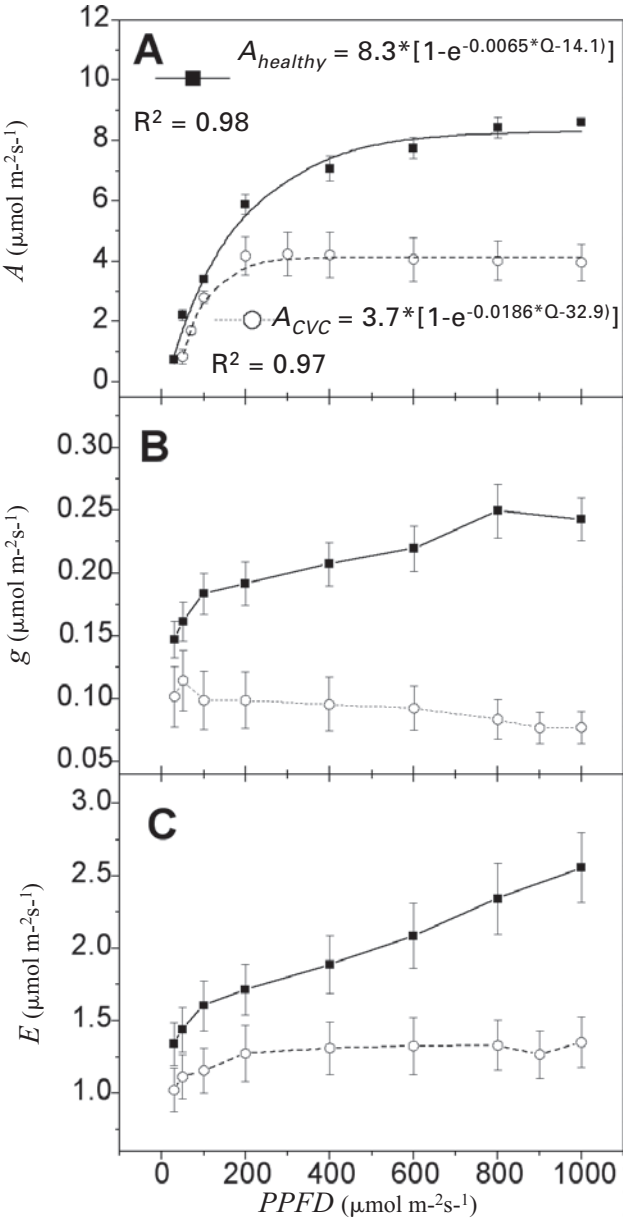


**Figure 2.** Mean values of net photosynthesis rates (A), stomatal conductance (B), and transpiration rates (*C*) in response to the photosynthetic photon flux density (*PPFD*) of healthy and CVC-affected'Pêra' sweet orange plants on 'Rangpur' lime rootstock

The light compensation point was lower in healthy plants (14.1 μmol. $m^{-2}.s^{-1}$) when compared to diseased ones (32.9 μmol. $m^{-2}.s^{-1}$) (figure 2). Increasing irradiance above the light compensation point results in a proportional increase in photosynthesis for both treatments, indicating that photosynthesis is limited by the amount of available light. This portion of the curve is limited by the rate of electron transport and by RuBP regeneration. Thus, the slope of the line gives the aparent quantum yield of photosynthesis for the leave (Jones, 1998). In healthy plants, this value was 0.059 μmol $CO_2$.(μmol photons)$^{-1}$, while it was 0.037 μmol $CO_2$.μmol photons$^{-1}$ in diseased ones, which correspond to the respective quantum yields of 16.9 and 26.8 μmol photons.μmol$^{-1}CO_2$, suggesting that the Calvin cycle efficiency, in terms of utilization of ATP and NADPH, was lower in CVC-affected leaves.

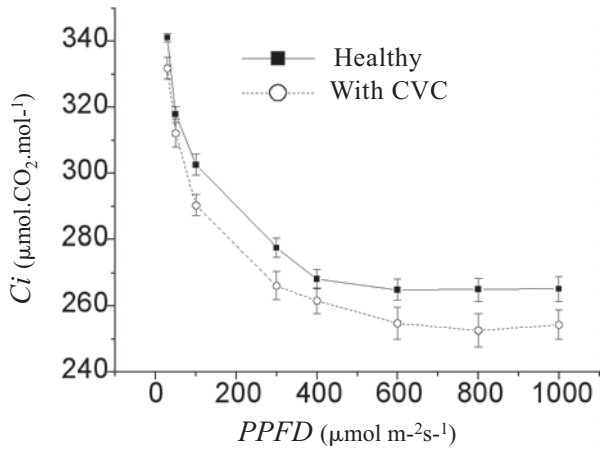


**Figure 3.** Mean values of internal leaf $CO_2$ concentration (Ci) versus the photosynthetic photon flux density (*PPFD*) of healthy and CVC-affected 'Pêra' sweet orange plants on 'Rangpur' lime rootstock.

At higher *PPFD*, the photosynthetic response to light starts to level off and reaches a saturation plateau. This point in CVC-affected plants was approximately 200, while it was 600 μmol.$m^{-2}.s^{-1}$ for healthy ones, indicating that factors such as electron transport reaction, Rubisco activity, or metabolism of triose phosphates have become more limiting in plants with CVC. Moreover, the maximum photosynthetic rate was more than twofold higher in healthy plants. Vu et al. (1986) and Machado et al. (1994) also found similar light saturation point and maximum photosynthesis rate for healthy plants at about 600 μmol photons.$m^{-2}.s^{-1}$.

The values of *Ci* in diseased plants was slightly lower when compared to healthy ones, although no significant differences were found (figure 3). Therefore, the substrate for photosynthetic activity apparently has not been restricted, but the calculation of *Ci* might not be reliable when $CO_2$ and water vapor fluxes are low, as occurs in infected plants. The problem could be the occurrence of patchy stomatal closure and the increasing importance of cuticular transpiration as stomata close, which may cause *Ci* to be overestimated, thus masking any stomatal effect on the photosynthetic reduction

(Cornic, 2000). However, the factor that may be decreasing $CO_2$ assimilation rates in diseased plants is not the substrate supply but other photochemical and biochemical factors.

The *A/Ci c*urves from healthy and CVC-affected plants (figure 1) were submitted to logarithmic regression. The relative effect of stomatal resistance on photosynthesis (S%) in diseased plants was about 30 % higher than in healthy plants (table 2). Nevertheless, although this stomatal response causes a depression in the photosynthesis rate, the linear portion of the *A/Ci* curves from both treatments were also compared (figure 1) and diseased plants presented a slope (carboxylation efficiency) that was approximately 45 % lower than the one found in healthy plants (table 1). Consequently, the $CO_2$ compensation point is higher in diseased plants than in healthy ones, indicating higher photorespiration in CVC-affected plants. The photorespiration could be a protective mechanism that avoids photoinhibition, mainly in $C_3$ plants (Hall and Rao, 1994), dissipating the excess ATP and NADPH, producing internal $CO_2$ (maintaining Rubisco activity), and consuming strong oxidants like $H_2O_2$ by action of catalysis (Lüttge et al., 1996).

Ribeiro (2002) observed that under affected mechanisms of stomatal closure and biochemical reactions, CVC probably promotes decrease in photosynthesis by increasing the alternative electron sink -such as photorespiration- through the increase in Rubisco oxygenase activity. This alternative excessive electron flux for photorespiration and Mehler reaction, due to the lower $CO_2$ assimilation rates in CVC-affected plants, probably blocks the photoinhibition and the $H_2O_2$ production as well.

All these results of lower leaf gas exchange rates, coupled to the affected photosynthetic metabolism in diseased plants, lead us to believe that one of the bacteria's mechanism of action is occurring. The adhesion of the bacterial colonies to the vessels, which blocks the xylem, results in a lack of water supply to the mesophyll, thus affecting the stomatal opening and the photosynthetic biochemical reactions.

The calculation of the relative effect of stomatal resistance on photosynthesis (S%) (table 2) shows that in diseased plants there is a more important effect of stomata depressing photosynthesis than in healthy plants. Stomatal conductance often decreases under increasing abiotic stresses, such as water defficiency (Farquhar and Sharkey, 1982). However, in the present case, the apparent water defficiency is one of the symptoms caused by CVC as a consequence of bacteria colonization of the xylem. Then, other factors may be acting over stomata and the photosynthetic apparatus.

Therefore, this idea would be in agreement with Machado et al. (1994) and Medina et al. (1999). Still, it is interesting to relate these results with the analysis of the linear portion of the *A/Ci* curves (figure 1). It can be observed that the estimation of carboxylation efficiency is lower in diseased plants, and this could indicate that even with a depression on photosynthesis caused by stomatal closure in diseased plants, the enzyme responsible for the carboxylation in the mesophyll is also altered. Similarly, the $CO_2$ compensation point is higher in CVC-affected plants when compared to healthy ones, indicating that diseased plants need twice the amount of $CO_2$ concentration in order to balance respiration and photorespiration when compared to heathy plants (table 1). The angular coefficient of the linear portion of the *A/Ci* curves also confirms these interpretations (table 1).

Would all these physiological disturbances encountered in CVC-affected plants be more related to the lack of water supply in the mesophyll, caused by the bacteria xylem blockage, or is it more linked with some phytotoxin produced by the bacteria? Although primarily defended by Hopkins (1989) as a hypothesis, the presence of any phytotoxin can not be ignored. It would be of scientific interest to determine the time when all these disturbances in leaf gas exchange start,

**Table 2.** Mean values of net photosynthesis at different internal leaf $CO_2$ concentrations (*ACi* and *ACe*); relative effect of stomatal resistance on photosynthesis (S%), and angular coefficient of the *A/Ci* curves in healthy and CVC-affected 'Pera' sweet orange plants on 'Rangpur' lime rootstock

| Treatments | *ACi* [a]µmol $m^{-2}$ $s^{-1}$ | *ACe* [b]µmol $m^{-2}$ $s^{-1}$ | S%( %) |
|---|---|---|---|
| Healthy | 12,08 ± 1,35 a | 9,83 ± 1,15 a | 18,7 ± 2,1 a |
| CVC-affected | 5,50 ± 1,77 b | 4,16 ± 1,37 b | 24,4 ± 3,3 b |

[a]*ACi* = net photosynthesis when internal leaf $CO_2$ concentration is 350 µmol $mol^{-1}$
[b]*ACe* = net photosynthesis when ambient external $CO_2$ concentration is 350 µmol $mol^{-1}$
Means ± standard deviation of 13 measurements on six plants per treatment. The same letters in columns are not significantly different from each other ($p \leq 0.05$).

and if possible, determine the exact time of the decrease in carboxylation efficiency and stomatal closure in relation to the period between inocculation and symptoms outbreak. Then, it could be investigated if the lower photosynthesis rates are not just related to the chlorotic areas on the leaf, or in other words, if the stomatal dysfuction is already present in a non-symptomatic leaf of an infected branch. Or would any phytotoxin be already causing these physiological disturbances before the symptoms outbreak? Queiroz-Voltan and Paradela Filho (1999) observed that chloroplasts from chlorotic areas of leaves with CVC symptoms were almost completely destroyed. The same observation was made by Queiroz-Voltan et al. (1998) in coffee plants infected with *X. fastidiosa*.

Simpson et al. (2000) found genes in the genome sequence of *X. fastidiosa* that may be involved in the production of phytotoxins. However, these same authors apparently gave more importance to the xylem blockage hypothesis.

In fact, the matrix composed of extracellular polysaccharides (EPSs - xanthan gum), in which *X. fastidiosa* is observed in the xylem, may contribute to bacteria-vessel adhesion, ultimately blocking the xylem. EPS synthesis can significantly enhance the colonization of phytopathogenic bacteria. EPS-defective strains of *Erwinia stewartii* presented a reduced virulence partly because they spread more slowly than the wild type in the vascular system of maize plants (Braun, 1990). In a similar study, Saile et al. (1997) used mutants to show that EPSs production increased the dissemination of *Ralstonia solanacearum* in tomato stem tissue. In a study with *Pseudomonas syringae* pv. *syringae*, Yu et al. (1999) demonstrated that alginate, a kind of EPS, has been shown to reduce pathogen desiccation stress and assist in the formation of microbial biofilms. Also, desiccation stress stimulated alginate synthesis in both *P. syringae* and *Pseudomonas aeruginosa* (DeVault et al., 1990; Singh et al., 1992), thereby increasing resistance to dehydration (Ophir and Gutnick, 1994).

Medina et al. (1999) observed that it took longer for CVC-affected plants to reach null photosynthesis rate values when they were submitted to 1-3 series of water deficiency. When plants were rewatered, they reported that diseased plants recovered more slowly than healthy ones. So, the disease coupled with water deficiency caused more physiological damages. Perhaps, in their case, some EPS probably played a role on the dissemination/colonization of *X. fastidiosa* during water deficit.

Abscisic acid (ABA) is also related to stomatal physiology. However, in the case of CVC-affected plants, it seems that ABA may be more related to the decrease in soil water content when the plants are submitted to soil water deficit than to the disease itself (Gomes et al., 1999). But Goodwin et al. (1988a) found higher concentration of ABA in the leaves of plants with PD when compared to healthy grapevines.

These results indicate that xylem blockage really occurs. From our results it could be concluded that the low net photosynthesis and transpiration rates and stomatal conductances (figure 2) would be an effect of low $\Psi_w$ because of the xylem obliteration. This could lead to a lower water supply for photosynthesis in the mesophyll, especially to produce the adequate turgor pressure and consequent stomatal opening. Furthermore, we suggest that in diseased plants the stomata have a more important effect on depressing photosynthesis than in healthy plants, thus characterizing an important stomatal dysfunctioning.

However, we could also imply that the carboxylation efficiency is also decreased in CVC-affected plants, as shown in figure 1. In other words, the photosynthetic metabolism is affected as well. Therefore, if the xylem occlusion by the bacteria causes water stress in the mesophyll, which in turn leads to stomatal closure and ultimately affects enzymatic processes related to photosynthesis, it would be reasonable to expect the same response in healthy plants under water deficiency. Accordingly, Vu and Yelenosky (1988) observed that healthy 'Valência' sweet orange plants subjected to water deficit showed a decrease in the activation and total activity of the Rubisco enzyme.

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## REFERENCES

Amorim L, Bergamin Filho A, Palazzo D, Bassanezi RB, Godoy CV, Torres GAM (1993) Clorose variegada dos citros: uma escala diagramática para avaliação da severidade da doença. Fitopatol. Bras. 18:174-180.

Andersen PC, French WJ (1987) Biophysical characteristics of peach trees infected with phony peach disease. Physiol. Mol. Plant Pathol. 31:25-40.

Beretta MJG, Barthe GA, Ceccardi TL, Lee RF, Derrick KS (1997) A survey for strains of *Xylella fastidiosa* in citrus affected by citrus variegated chlorosis and citrus blight in Brazil. Plant Dis. 81:1196-1198.

Bosco AD (2001) Transpiração em laranjeira (*Citrus sinensis* L. Osb.) infectada *com Xylella fastidiosa* Wells. Piracicaba, Universidade de São Paulo. Dissertação de Mestrado.

Boyer JS, Knipling EB (1965) Isopiestic technique for measuring leaf water potentials with a thermocouple psychrometer. Proc. Natl. Acad. Sci. USA 54:1044-1051.

Braun EJ (1990) Colonization of resistant and suceptible maize plants by *Erwinia stewartii* strains deffering in exopolysaccharide production. Physiol. Mol. Plant Pathol. 36:363-379.

Chang CJ, Garnier M, Zreik L, Rossetti V, Bové JM (1993) Culture and serological detection of the xylem-limited bacterial causing citrus variegated chlorosis and its identification as a strain of *Xylella fastidiosa*. Curr. Microbiol. 27:137-142.

Cornic G (2000) Drought stress inhibits photosynthesis by decreasing stomatal aperture - not by affecting ATP synthesis. Trends Plant Sci. 5:187-188.

DeVault JD, Kimbara K, Chakrabarty AM (1990) Pulmonary dehydration and infection of cystic fibrosis: evidence that ethanol activates alginate gene expression and induction of mucoidy in *Pseudomonas aeruginosa*. Mol. Microbiol. 4:737-745.

Farquhar GD, Sharkey TD (1982) Stomatal conductance and photosynthesis. Annu. Rev. Plant Physiol. 33:317-345.

French WJ, Stassi DL (1978) Response of phony-infected peach trees to gibberellic acid. HortScience 13:425-429.

Gomes MMA, Medina CL, Machado EC, Lagôa AMMA, Machado MA (1999) Quantificação do ácido abscísico em laranjeiras 'Pêra' infectadas pela'*Xylella fastidiosa* e submetidas à deficiência hídrica. Summa Phytopathol. 25:29. (Abstract)

Goodwin PH, De Vay JE, Meredith CP (1988a) Physiological responses of *Vitis vinifera* cv. "Chardonnay" to infection by the Pierce's disease bacterium. Physiol. Mol. Plant Pathol. 32:17-32.

Goodwin PH, De Vay JE, Meredith CP (1988b) Roles of water stress and phytotoxins in the development of Pierce's disease of the grapevine. Physiol. Mol. Plant Pathol. 32:1-15.

Hall DO, Rao KK (1994) Photosynthesis: Studies in biology. Cambridge University Press, Cambridge.

Hartung JS, Beretta J, Brlansky RH, Spisso J, Lee RF (1994) Citrus variegated chlorosis bacterium: axenic culture, pathogenicity, and serological relationships with other strains of *Xylella fastidiosa*. Phytopathology 84:591-597.

Hopkins DL (1985) Effects of plant growth regulators on development of pierce's disease symptoms in grapevine. Plant Dis. 69:944-946.

Hopkins DL (1989). *Xylella fastidiosa*: xylem-limited bacterial pathogen of plants. Annu. Rev. Phytopathol. 27:271-290.

Hopkins DL, Thompson CM (1984) Seasonal concentration of the pierce's disease bacterium in 'Carlos' and''Welder' muscadine grapes compared with 'Schuyler' bunch grape. HortScience 19:419-420.

Jones HG (1998) Stomatal control of photosynyhesis and transpiration. J. Exp. Bot. 49:387-398.

Laranjeira FF (1997) Dez anos de clorose variegada dos citros: o que sabemos?. Laranja 18:123-141.

Laranjeira FF, Palazzo DA (1999) Danos qualitativos à produção de laranja 'Natal' causados pela clorose variegada dos citros.'Laranja 20:77-91.

Lee RF, Beretta MJG, Hartung JH, Hooker ME, Derrick KS (1993) Citrus variegated chlorosis: confirmation of a *Xylella fastidiosa* as the causal agent. Summa Phytopathol. 19:123-125.

Lee RF, Raju BC, Nyland G, Goheen AC (1982) Phytotoxin(s) produced in culture by the Pierce's disease bacterium.'Phytopathology 72:886-888.

Leite Junior RP, Leite RMVBC (1991) Associação de *Xylella fastidiosa* com a clorose variegada dos citros. Summa Phytopathol. 17:7. (Abstract)

Lüttge U, Kluge M, Bauer G (1996) Les cytosomes. In: Botanique: Traité fondamental, pp.137-145. Lavoisier, Tec & Doc., Paris, France.

Machado EC, Quaggio JA, Lagôa AMMA, Ticelli M, Furlani PR (1994) Trocas gasosas e relações hídricas em laranjeiras com clorose variegada dos citros. Rev. Bras. Fisiol. Veg. 6:53-57.

Machado EC, Medina CL, Gomes MMA (1999) Teor de água no substrato de crescimento e fotossíntese em laranjeira 'Valência'. Rev. Bras. Fisiol. Veg. 58:217-226.

Malavolta E, Prates HS, Vitti GC, Pinto WBS (1993) Novas observações sobre o "amarelinho" ou clorose variegada dos citros. Laranja 14:177-200.

Medina CL, Nunes WMC, Machado MA, Machado EC (1998a) Método prático para transmissão de *Xylella fastidiosa* em mudas de citros. Fitopatol. Bras. 23:212. (Abstract)

Medina CL, Machado EC, Pinto JM (1998b) Fotossíntese de laranjeira 'Valência' enxertada sobre quatro porta-enxertos e submetida à deficiência hídrica. Bragantia 57:1-14.

Medina CL, Gomes MMA, Machado EC, Lagôa AMMA, Machado MA (1999) Fotossíntese, condutância estomática e relações hídricas em laranjeira 'Pêra' infectada com *Xylella fastidiosa*, submetida à sucessivos ciclos de deficiência hídrica. Rev. Bras. Fisiol. Veg. 11:37-38. (Abstract)

Nobel PS (1999) Leaves and fluxes. In**:** Physicochemical and environmental plant physiology. 2nd edn., pp. 293-349. Academic Press, San Diego, USA.

Oliveira RF, Machado EC, Marin FR, Medina CL (2000) Sap flow rates and stomatal conductance of sweet orange 'Pêra' (*Citrus sinensis* L. Osb.) infected by *Xylella fastidiosa*. In: Abstracts of the IX Congress of the International Society of Citriculture. Orlando, USA, pp. 161.

Ophir T, Gutnick DL (1994) A role for exopolysaccharides in the protection of microorganisms from desiccation. Appl. Environ. Microbiol. 60:740-745.

Prado CHBA, de Moraes JPAPV (1997) Photosynthetic capacity and specific leaf mass in twenty woody species of cerrado vegetation under field conditions. Photosynthetica 33:103-112.

Purcell AH, Hopkins DL (1996) Fastidious xylem-limited bacterial plant pathogens. Annu. Rev. Phytopathol. 34:131-151.

Queiroz-Voltan RB, Paradela Filho O (1999) Caracterização de estruturas anatômicas de citros infectados com *Xylella fastidiosa*. Laranja 20: 55-76.

Queiroz-Voltan RB, Paradela Filho O, Carelli MLC, Fahl, JI (1998) Aspectos estruturais de caffeeiro infectado com *Xylella fastidiosa*. Bragantia 57: 23-33.

Ribeiro RV (2002) Influência da temperatura na fotossíntese de laranjeira 'Pêra' com clorose variegada dos citros. Piracicaba, Universidade de São Paulo. MSc Thesis.

Roberto SR, Coutinho A, De Lima JEO, Miranda VS, Carlos EF (1996) Transmissão de'*Xylella fastidiosa* pelas cigarrinhas *Dilobopterus costalimai*, *Acrogonia terminalis* e *Oncometopia facialis* em citros. Fitopatol. Bras. 21:517-518.

Rossetti V, Negri JD (1990) Clorose variegada dos citros - revisão. Laranja 11:1-14.

Rossetti V, Garnier M, Bové JM, Beretta MJG, Teixeira AR, Quaggio JA, Negri JD (1990) Présence de bactéries dans le xylèm d'orangers atteints de chlorose variégéé, une nouvelle maladie des agrumes au Brésil. C. R. Acad. Sci. Ser III 310:345-349.

Saile E, McGarvey JA, Schell MA, Denny TP (1997) Role of extracellular polysaccharide and endoglucanase in root invasion and colonization of tomato plants by *Ralstonia solanacearum*. Phytopathology 87:1264-1271.

Simpson AJG, Reinach FC, Arruda P, Abreu FA, Acencio M, Alvarenga R, Alves LMC, Araya JE, Baia GS, Baptista CS, Barros MH, Bonaccorsi ED et al. (2000) The genome sequence of the plant pathogen *Xylella fastidiosa*. Nature 406:151-157.

Singh S, Koehler B, Fett WF (1992) Effect of osmolarity and dehydration on alginate production by fluorescent pseudomonads. Curr. Microbiol. 25:335-339.

Von Caemmerer S, Farquhar GD (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta 153:376-387.

Vu JCV, Yelenosky G, Bausher MG (1986) CO2 exchange rate, stomatal conductance, and transpiration in attached leaves of 'Valencia' orange. HortScience 21: 143-144.

Vu JCV, Yelenosky G (1988) Water deficit and associated changes in some photosynthetic parameters in leaves of 'Valencia' orange (*Citrus sinensis* [L.] Osbeck). Plant Physiol. 88:375-378.

Yu J, Peñaloza-Vázquez A, Chakrabarty AM, Bender CL (1999) Involvement of the exopolysaccharide alginate in the virulence and epiphytic fitness of *Pseudomonas syringae* pv. *syringae*. Mol. Microbiol. 33:712-720.