

## Evaluation of *Xylella fastidiosa* Effects on Leaf Gas Exchange of Pera Sweet Orange Grafted on Rangpur Lime Rootstock

G. Habermann, E. C. Machado, J. D. Rodrigues, and C. L. Medina

**ABSTRACT.** In the last 10 yr, the Brazilian citriculture has been suffering from a devastating disease called citrus variegated chlorosis (CVC) which is caused by the bacterium *Xylella fastidiosa*. To evaluate *X. fastidiosa* effects on some physiological parameters of orange plants, we measured leaf photosynthesis, transpiration rates, and stomatal conductance of healthy and CVC-affected 2.5 yr-old Pera sweet orange grafted on Rangpur lime rootstock. The diseased plants did not have visible leaf symptoms but had the bacteria in their tissues. Eight months after grafting with *X. fastidiosa*-infected twigs, net photosynthesis vs. internal CO<sub>2</sub> concentration were analyzed and estimation of rubisco enzyme (ribulose-1,5-biphosphate carboxylase/oxygenase) efficiency was determined under standard conditions. Results showed there were no significant effects of *X. fastidiosa* on gas exchange rates of these leaves under our test conditions.

Citrus variegated chlorosis (CVC) has been observed since 1987 in São Paulo and Minas Gerais States, Brazil (16). During the last 5 yr, CVC has spread rapidly in nurseries and commercial groves. CVC is presently causing a yearly loss of about \$100 million, just in São Paulo State (10). The disease is characterized by zinc deficiency symptoms and chlorosis, which usually occurs in older leaves. In recently affected trees, the disease is very irregular and symptoms may affect only a tree sector. Trees which have been affected for a period of time, show variegated chlorosis throughout the canopy. As leaves mature, small light-brown, gummy lesions appear on the leaf underside corresponding to the chlorotic areas on the upperside. The lesions on the leaf underside may become dark brown or even necrotic and are somewhat raised. Fruit ripen early on CVC-affected trees, have a higher sugar content, but they are unmarketable because of their hard rind and small size (1).

*Xylella fastidiosa*, limited to the xylem of CVC-affected plants, has been associated with diseases in other economically important plants. CVC-affected plants have leaves with water deficiency symptoms and are associated with significant

decreases on the net photosynthesis and transpiration rates (13). 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 (13). The timing of infection and development of visible drought stress-like symptoms or even chlorotic lesions and decreases in gas exchange rates may be important. Thus, these symptoms could be the result of alterations in leaf gas exchange rates if the bacteria were producing phytotoxins which act to produce vascular plugs by high molecular weight compounds or as a chemical disturbing leaf metabolism (8). Symptoms could also result from increasing water stress in a leaf which leads to an increased concentration of solutes and enzyme crystallization in dehydrated cells (3, 9).

In this paper, we tried to evaluate photosynthesis, transpiration rates, and stomatal conductance in healthy and asymptomatic CVC-affected Pera sweet orange plants. We also analyzed the CO<sub>2</sub> assimilation curve when the leaf internal CO<sub>2</sub> concentration varied to determine the relative effect of stomatal resistance on photosynthesis as well as the *in vivo* rubisco enzyme efficiency.

## MATERIALS AND METHODS

This study was conducted in Campinas, São Paulo State, Instituto Agrônomo de Campinas (Núcleo Experimental de Campinas). The plants were grown in a screenhouse to completely avoid the presence of sharpshooters which are CVC vectors (12).

Six 2.5 yr-old Pera sweet orange plants grafted on Rangpur lime rootstock were cultivated in 25 L pots. Six other Rangpur lime plants were cut into a double wedge form at 5 to 10 cm from the ground for top-grafting with *X. fastidiosa* infected branches of CVC-infected Pera sweet orange plants (11).

Approximately 8 mo after inoculation with *X. fastidiosa* by top-grafting, leaves of healthy and CVC-inoculated plants were assayed by PCR, in order to detect the bacteria in its tissues (2).

Gas exchange measurements were done in a laboratory at 26°C, under 1,000  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  of photosynthetic active radiation (PAR) that was filtered through 8 cm of running tap water to remove infrared radiation produced by the lamps. A closed infrared gas analyzer system (IRGA) (LI-6200 Portable Photosynthesis System, LI-COR, Lincoln, NE) with a 0.25 L leaf chamber was used for all measurements. All measurements were conducted using two fully expanded mature leaves randomly chosen in each plant.

Intact leaves were inserted into the leaf chamber and allowed to acclimate for approximately 5 min to 350 to 360  $\mu\text{mol}\cdot\text{mol}^{-1}$   $\text{CO}_2$  at the light intensity previously mentioned. After acclimation, net photosynthesis approached zero before injecting  $\text{CO}_2$  into the system.

$\text{CO}_2$  was injected into the closed circulating gas-stream loop of the IRGA with a syringe, until the atmospheric  $\text{CO}_2$  concentration in the sample chamber reached approximately 1,200  $\mu\text{mol}\cdot\text{mol}^{-1}$ .

Twenty-five sequential measurements of net photosynthesis were taken over an interval of 20 to 25 min, as photosynthesis depleted the  $\text{CO}_2$  concentration in the sample-chamber to near the  $\text{CO}_2$  compensation point.

Net photosynthesis (*A*) values were plotted against the respective internal  $\text{CO}_2$  concentrations (*C<sub>i</sub>*) to produce an *A/C<sub>i</sub>* response curve. As net photosynthesis approached zero,  $\text{CO}_2$  became the limiting factor for photosynthesis and the initial slope of *A/C<sub>i</sub>* line represents the activity of rubisco (5) (Fig. 1, line BC).

Net photosynthesis and corresponding internal  $\text{CO}_2$  values for the linear portion of the response curve were subjected to linear regression analysis to determine the slope.

In addition to rubisco, analysis of the *A/C<sub>i</sub>* response curve permitted calculation of the relative effect of stomatal resistance on photosynthesis (*S%*). Its effect can be determined when net photosynthesis, at ambient external  $\text{CO}_2$  concentration of 350  $\mu\text{mol}\cdot\text{mol}^{-1}$ , (*AC<sub>e</sub>*), is subtracted from net photosynthesis when the internal  $\text{CO}_2$  concentration of the leaf is at 350  $\mu\text{mol}\cdot\text{mol}^{-1}$ , (*AC<sub>i</sub>*) (Fig. 1). *AC<sub>i</sub>* represents a rate as if there was no stomatal limitation to *A* (5). The resulting value then must be divided by the rate of net photosynthesis when internal  $\text{CO}_2$  is at 350  $\mu\text{mol}\cdot\text{mol}^{-1}$  (*AC<sub>i</sub>*). Thus, *S%* can be estimated by the equation below (5):

$$S\% = [(AC_i - AC_e)/AC_i] \times 100 \quad (\text{Equation 1})$$

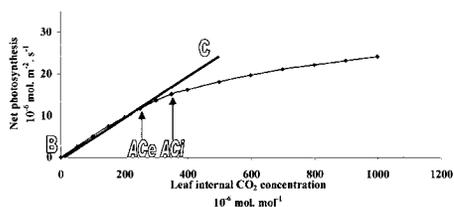


Fig. 1. Schematic representation of an *A/C<sub>i</sub>* curve.

TABLE 1  
MEAN VALUES OF NET PHOTOSYNTHESIS, TRANSPIRATION RATE, AND STOMATAL CONDUCTANCE IN HEALTHY AND CVC-AFFECTED SWEET ORANGE PLANTS

Plants	Net photosynthesis $\mu\text{mol. m}^{-2} \cdot \text{s}^{-1}$	Transpiration mmol $\text{m}^{-2} \cdot \text{s}^{-1}$	Stomatal conductance mol. $\text{m}^{-2} \cdot \text{s}^{-1}$
Diseased	$8.0 \pm 3.0$	0.114	0.0021
Healthy	$7.7 \pm 1.6$	0.103	0.0021
	ns <sup>z</sup>	ns	ns

<sup>z</sup>ns = not significantly different by *t*-student's test ( $P \leq 0.01$ ).

This study was conducted in a completely randomized experimental design, with two treatments (healthy and diseased plants) and six replications. Mean values were subjected to one-way analysis of variance and *t*-student's test at 1% level of probability.

**RESULTS AND DISCUSSION**

All inoculated plants had *X. fastidiosa*, but did not show visible symptoms on leaves, and non-inoculated plants were negative for the PCR test. Results from the gas exchange measurements showed that there were no significant differences on net photosynthesis and transpiration rates, and stomatal conductances between healthy and CVC-affected plants (Table 1).

The analysis of the *A/Ci* response curve data revealed that plants with *X. fastidiosa* in their tissues had the same net photosynthesis as the plants without the bacteria in their tissues (Figs. 2 and 3). The limiting effect of the stomates on net photosynthesis was not altered by this bacteria, at least 8 mo after inoculation. The values for both inoculated and non-inoculated plants were around 46% (Table 2). In addition to the non-significant main effects, the analysis of the initial slope of the *A/Ci* curves demonstrated that the rubisco efficiency was not modified or mediated because the *A/Ci* slopes of inoculated and non-inoculated plants were the same (Figs. 4 and 5). These results suggest that 8 mo after inoc-

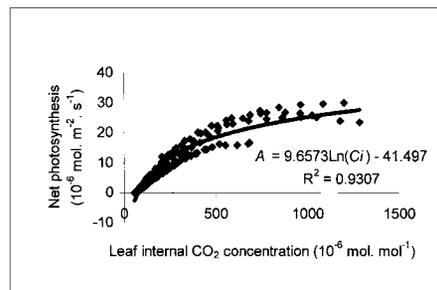


Fig. 2. *A/Ci* curves of CVC-affected sweet orange plants.

ulation was not long enough for the bacteria to cause any alterations on leaf gas exchange characteristics.

Visible symptoms did not appear later. The pots were too small for the adequate growth of roots and they were pot-bound. Flowers were cut in the previous flowering season which produced plants without carbohydrate sinks which may have lead to starch accumulation in leaves (both in healthy and diseased plants as observed by microscopy) which developed a thicker mesophyll. This might have influenced the bacteria and its

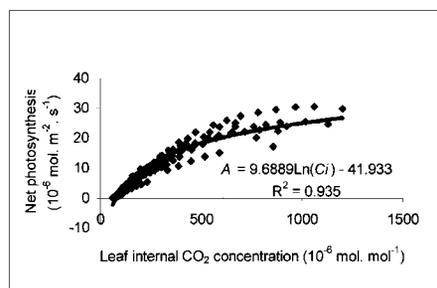


Fig. 3. *A/Ci* curves of healthy sweet orange plants.

TABLE 2  
MEAN VALUES OF THE RELATIVE LIMITING EFFECT ON THE PHOTOSYNTHESIS OF  
HEALTHY AND CVC-AFFECTED SWEET ORANGE PLANTS

Plants	$ACe^z$	$ACi^y$	$S\%^x$	$VC^w$ of $S\%$
Diseased	$8.0 \pm 3.0$	$14.6 \pm 2.2$	$46.6 \pm 13.8$	29.6
Healthy	$7.7 \pm 1.6$	$14.3 \pm 2.1$	$45.8 \pm 10.4$	22.7
	ns <sup>v</sup>	ns	ns	

<sup>z</sup> $ACe$  = Net photosynthesis when ambient external  $CO_2$  concentration is  $350 \mu\text{mol}\cdot\text{mol}^{-1}$ .

<sup>y</sup> $ACi$  = Net photosynthesis when internal leaf  $CO_2$  concentration is  $350 \mu\text{mol}\cdot\text{mol}^{-1}$ .

<sup>x</sup> $S\%$  = Stomatal resistance to photosynthesis and is calculated as  $[(ACi - ACe) / ACi] \times 100$

<sup>w</sup> $VC$  = Coefficient of variation

<sup>v</sup>ns = not significantly different by *t*-student's test ( $P \leq 0.01$ ).

interaction with the plant by not allowing the visible leaf symptoms to develop. Furthermore, these leaves started falling from the branches before completing 6 mo of age.

There are three possible mechanism(s) of pathogenesis that might be causing water deficiency symptoms in CVC-affected plants: i) phytotoxin; ii) growth regulator imbalance; and iii) xylem occlusion. Lower leaf water potential occur in plants with CVC when compared to healthy plants (13, 14). Similar findings have been reported for peach trees infected with *X. fastidiosa* (4).

Leaves of healthy and water-stressed CVC-affected plants had higher levels of abscisic acid (ABA), but this was not the case when healthy or CVC-affected plants were well-watered (7). These authors worked with symptomatic plants, which were inoculated by spliced-approach grafting, a much more effective method to transmit CVC that starts showing leaf symptoms within 3 to 4 mo (15). We used the top-grafting method which did not appear to be as effective because 8 mo after inoculation there were no leaf symptoms. Timing and density of bacteria accumulation inside the plant influences the level of infection (6). This interaction may help explain why no differences between treatments were found and may help explain why spliced approach grafting is more efficient to transmit the disease. Unfortunately, there are no other methods that provide effective information

about the density of *X. fastidiosa* populations in plant tissues (2) relative to the parameters tested here.

We conducted a similar study in sweet orange plants inoculated by spliced-approach grafting. CVC-affected plants showed lower values of net photosynthesis, transpiration rates and stomatal conductances and higher  $S\%$ . CVC-affected plants also had high incidence of leaf chlorosis and leaf water deficiency symptoms on affected branches (data not yet published).

Apparently, decreases in leaf gas exchange characteristics do not occur until disease symptoms are visible. Thus, if the xylem occlusion theory explains *X. fastidiosa*'s pathogenesis, symptoms could develop similarly to those reported for water stress in many species (3, 9). However, there have been no studies that monitor the progression of the physiological changes in CVC-affected plants in relation to time after inoculation.

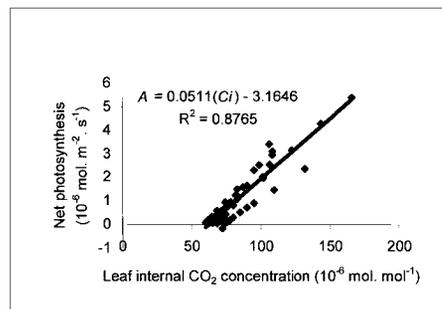


Fig. 5. Rubisco enzyme activity in healthy sweet orange plants.

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