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Photosynthetic and antioxidant responses of Mexican lime (*Citrus aurantifolia*) plants to *Citrus tristeza virus* infection

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The effect of *Citrus tristeza virus* (CTV) infection on photosynthetic activity and antioxidant metabolism was analysed in plants of the highly susceptible citrus genotype Mexican lime (*Citrus aurantifolia*). Two virus isolates differing in their virulence (the severe T318 and the mild T385) were used in the experiments. CTV infection caused a reduction in photosynthetic capacity in infected plants. This limitation was mainly due to a reduction in the carboxylative efficiency whereas the limitation of CO_2 diffusion through the stoma had lower impact. The virus did not damage the antennae and did not reduce the efficiency of light harvesting complexes. Oxidative damage occurred in infected plants, as evidenced by the increase in malondialdehyde levels. Indeed, CTV infection caused an increase in ascorbate peroxidase activity in new shoots developed in infected plants during the 2 years of the experiment. Data suggest that the H_2O_2 removal machinery was not damaged as a result of stress but the defence mechanism was overwhelmed with time due to the continuing pressure of biotic stress.

Keywords: antioxidant activity, F_V/F_M, NPQ, photosynthesis, quantum yield

Introduction

Citrus is the most economically important fruit tree worldwide, with more than 131 million tonnes of fruit produced in 2012 on more than 8·7 million ha (FAO, 2012). Among the viral diseases affecting citrus, 'tristeza' has the greatest impact worldwide (Moreno *et al.*, 2008). *Citrus tristeza virus* (CTV) has caused the death of millions of citrus trees in Argentina, Brazil, South Africa, USA and Spain; moreover, the disease keeps spreading into new areas, either by propagation of infected buds or transmitted by different aphid species (Saponari *et al.*, 2013).

Natural CTV hosts are restricted to two genera within the family Rutaceae, namely *Citrus* and *Fortunella*. Among citrus genotypes, Mexican lime (*Citrus aurantifolia*) is known to be the most susceptible to CTV (Moreno *et al.*, 2008).

As described in Moreno *et al.* (2008), CTV may cause three different syndromes depending on virus strains and on the plant species, namely tristeza, stem pitting and seedling yellows. Tristeza, the most dramatic syndrome, causes, in some cases, a quick decline that could lead to the death of the infected trees. Stem pitting seems to be initiated by interruption of meristematic activity at limited areas of the cambium that results in irregular radial growth with local depression at the activated points. Seedling yellows is characterized by stunting, production of small pale or yellow leaves, a reduced root system and, sometimes, a complete arrest of growth of susceptible genotypes.

CTV is a filamentous plant virus with flexible virions composed of one molecule of single-stranded RNA of positive polarity, which has been completely sequenced, and one species of coat protein with molecular weight 25 kD (Karasev *et al.*, 1995). The genome structure of CTV has been well characterized and abundant data on the transcriptional changes induced in plants infected by CTV is available (Gandía *et al.*, 2007; Liu *et al.*, 2012). Although it is well known that infection results in the alteration of plant physiology, there is a lack of information on how CTV infection affects photosynthetic machinery, antioxidant activity and metabolic processes in citrus plants.

Photosynthesis is tightly regulated and its efficiency is strongly dependent on external abiotic and biotic factors influencing the status of the photosynthetic machinery. Chlorophyll fluorescence can be used as a diagnostic tool for photosystem II (PSII) and, therefore, as a marker of the impact of a specific stress situation on plant performance. In abiotic stress studies, chlorophyll fluorescence has been extensively used to describe changes in photosynthesis (Calatayud *et al.*, 2006; López-Climent *et al.*, 2008; Arbona *et al.*, 2009). Soil flooding and salt stress induce a progressive impairment of the photosynthetic machinery in citrus plants (López-Climent *et al.*, 2008; Arbona *et al.*, 2009). For virus-infected plants, there is a considerable disagreement regarding the alterations in chlorophyll fluorescence parameters. Whereas maximum fluorescence yield in dark-adapted leaves (F_V/F_M) did not change in *Eupatorium makinoi* infected by a geminivirus

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(Funayama *et al.*, 1997), it was significantly reduced in *Nicotiana tabacum* (Ryšlavà *et al.*, 2003) and in *Oncidium* (Chia & He, 1999) after virus infection.

The oxidation of water by the PSII complex results in the production of molecular oxygen that can also act as a potential electron acceptor, resulting in the formation of reactive oxygen species (ROS). These ROS, such as the superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\cdot}OH$), and singlet oxygen ($^1O_2$) are important stress signalling molecules. Activated oxygen or oxygen-free radicals have been associated with numerous physiological disorders of plants (Bartwal *et al.*, 2013). ROS cause direct damage to plant cells through oxidation of biological components such as nucleic acids, proteins and lipids. Plants have developed an intricate defence response network of lipophilic and hydrophilic antioxidant compounds and enzymes that provide protection against conditions of excessive oxidative damage (Bartwal *et al.*, 2013). Among the enzymatic systems, ascorbate peroxidase (APX), catalase (CAT) and others greatly contribute to coping with the environmentally induced oxidative stress and their activities have been used to evaluate stress responses in plants. It has been reported that tolerance to different environmental stresses correlates with an increased production of enzymes involved in the detoxification of ROS (Arbona *et al.*, 2003, 2008; Kukavica *et al.*, 2005).

Although there are reports on the photosynthetic performance in some crops after virus infection, the effect of CTV infection on citrus photosynthetic performance has not been investigated. In this work, the effects of CTV infection on Mexican lime (a highly susceptible genotype) plants were analysed to better understand how biotic stress affects physiological and biochemical processes in citrus plants. To gain knowledge on the changes in photosynthetic machinery, gas exchange and chlorophyll fluorescence parameters were compared between infected and healthy plants. To test the relationship between the antioxidant mechanisms and the severity of the stress imposed by two CTV isolates (the virulent T318 and the mild T385) in Mexican lime plants, oxidative damage, in terms of leaf malondialdehyde (MDA) concentration together with antioxidant enzyme activities (APX and CAT), was measured.

Materials and methods

Plant material, virus isolates and inoculation of Mexican lime plants

Experiments were performed using Mexican lime (*C. aurantifolia*) plants. The isolates T318 and T385 used in this study are part of a collection kept at the Instituto Valenciano de Investigaciones Agrarias (Moncada, Spain) and were kindly provided by Dr Pedro Moreno. T385 is a mild isolate that only induces inconspicuous vein clearing in Mexican lime, whereas T318 is a severe isolate inducing strong vein clearing and stem pitting in Mexican lime and other citrus species (Moreno *et al.*, 1993). These isolates were maintained in container-grown sweet orange plants propagated on *Carrizo citrange* (*C. sinensis* × *P. trifoliata*) rootstock in an insect-proof greenhouse.

For this study, Mexican lime seedlings were graft-inoculated with two bark pieces from either healthy plants or those infected with one of the two CTV isolates (six plants per treatment). Limes were grown in a temperature-controlled greenhouse (18/26°C night per day, photoperiod 6 h dark per 18 h dark) and 60–85% relative humidity using an artificial potting mix (50% sand and 50% peat moss). During this period, plants were watered three times a week with a half-strength Hoagland solution (Gómez-Cadenas *et al.*, 2002; Arbona *et al.*, 2006). CTV infection was confirmed by ELISA with monoclonal antibodies 3DF1 and 3CA5 (Cambra *et al.*, 2000) and by appearance of symptoms. Plants were kept under the described culture conditions for 2 years.

For oxidative damage and enzyme activity analyses, young sprouts with leaves were collected at different developmental stages during the active growing period and immediately frozen in liquid nitrogen.

Chlorophyll fluorescence parameters

Measurements were performed with an OS 1-FL portable fluorometer (Opti-Sciences). Five replicate plants per treatment were randomly chosen and maximum dark-adapted chlorophyll fluorescence, [$F_V/F_M = (F_M - Fo)/F_M$], was measured after 30 min of dark adaptation in four different leaves. Quantum yield [$\Phi_{PSII} = (F_M' - Fs)/F_M'$] was measured in the same leaves after actinic light adaptation. Non-photochemical quenching [$NPQ = (F_M - F_M')/F_M'$] was calculated. F_V/F_M indicates the maximum chlorophyll yield after modulated light pulse emission; the variation in Fo is related to damage in the PSII reaction centres whereas changes in F_M refer to alterations in the ability to reduce Q_A. F_M' is the maximum fluorescence in leaves under regular PAR (actinic radiation) and Fs is the minimum; Φ_{PSII} gives information about the non-cyclic electron transport from PSII to PSI. All terminology and calculations were performed according to Calatayud *et al.* (2006) and López-Climent *et al.* (2008).

Gas exchange

Leaf-gas exchange parameters were measured with an LCpro+ portable infrared gas analyser (ADC Bioscientific Ltd) under ambient CO_2 and humidity. Supplemental light was provided by a PAR lamp at 1000 μmol m^{-2} s^{-1} photon flux density and air flow was set at 150 μmol s^{-1}. After instrument stabilization, measurements were taken on four mature leaves (from an intermediate position on the stem) in each of the four plants chosen per treatment. Net CO_2 assimilation rate (μmol m^{-2} s^{-1}; A), the ratio of intercellular to ambient CO_2 concentration (Ci/Ca) and stomatal conductance (mol m^{-2} s^{-1}; g_s) were measured.

Malondialdehyde concentration and antioxidant enzyme activity

Malondialdehyde concentration was measured following the procedure described in Hodges *et al.* (1999). Plant material was homogenized in 5 mL 80% cold ethanol (Panreac) using a tissue homogenizer (Ultra-Turrax; IKA-Werke). Homogenates were centrifuged at 4°C to pellet debris and different aliquots of the supernatant were mixed either with 20% trichloroacetic acid (TCA; Panreac) or a mixture of 20% TCA and 0·5% thiobarbituric acid (Sigma-Aldrich). Both mixtures were allowed to react in a waterbath at 90°C for 1 h. After this time, samples were

cooled in an ice bath and centrifuged. Absorbance at 440, 534 and 600 nm was read in the supernatant against a blank. The MDA concentration in the extracts was calculated as described in Arbona *et al.* (2008).

Protein extraction was performed using a prechilled mortar and pestle in an ice bath. Briefly, 0·5 g frozen plant material was extracted in 2·5 mL phosphate-buffered saline (PBS) using sea sand as an abrasive. After extraction, the mortar was rinsed with another 2·5 mL buffer that was also collected. The homogenate was filtered through two layers of muslin cloth. The different buffers used for enzyme extraction were the following: for APX, 50 mM PBS pH 7·1 supplemented with 1 mM sodium ascorbate, 0·1 mM EDTA and two drops of Triton X-100 (Panreac); and for CAT, 50 mM PBS pH 6·8. Homogenates were centrifuged at 2360 ***g*** for 45 min at 4°C and the supernatants were collected for determination. The APX activity (EC 1·11·1·11) was determined following the depletion in absorbance at 290 nm because of ascorbic acid (Asa) consumption, and CAT (EC 1·11·1·6) was assayed using the hydrogen peroxide-dependent reduction of titanium chloride. Protein content in extracts was assessed by means of the protein-dye binding method using Coomassie blue G-250 (Sigma-Aldrich). Enzyme activity was expressed as arbitrary units per mg protein. Further details on enzyme assays are given in Arbona *et al.* (2003).

Statistical analysis

Data mean comparisons were performed with STATGRAPHICS PLUS v. 5.1 software (Statistical Graphics Corporation). One-way analysis of variance (ANOVA) was used to compare mean values among the different treatments. The least significant difference (LSD) test at $P \leq 0{\cdot}05$ was followed to assess significant differences.

Results

Symptoms of CTV infection in Mexican lime plants

The first symptoms of viral infection in Mexican lime plants were observed 4 weeks after inoculation. Eight weeks after the onset of the experiments, the symptoms of CTV infection were evident in all inoculated plants. The primary symptoms observed in plants infected with both isolates were vein clearing in young and mature leaves, and leaf cupping. In the case of plants infected with the severe isolate CTV (T318), vein clearing developed into corking of the main vein. Twenty-four weeks after the inoculation, corking was extended to the secondary leaf veins and the death of 15% of the new sprouts was recorded (data not shown).

During the second year after inoculation, a reduction in the leaf size was observed in leaves regardless of the severity of the isolate used for the inoculation. Vein corking was the most evident symptom in plants infected with T318, and a general leaf abscission together with the death of new branches (including those with 15 to 22 leaves) occurred in these plants.

Chlorophyll fluorescence parameters

Measurements of chlorophyll fluorescence and gas exchange parameters during the second year of the experiment are only included for plants infected with the mild CTV isolate because infection with the severe isolate T318 resulted in low and highly variable values.

Leaves of healthy plants maintained F_V/F_M levels around 0·8 throughout the 2-year experimental period. CTV infection affected this parameter during the first year after inoculation; from 17 to 39 weeks after inoculation, infected plants showed a significant decrease in F_V/F_M levels with respect to healthy plants, regardless of the virulence of the virus isolate (Fig. 1a.I). However, from week 41 to the end of the first year after inoculation, infected plant values of F_V/F_M were similar to healthy plants. During the second year after infection, leaves infected with CTV T385 presented F_V/F_M values significantly lower than those measured in non-infected plants at all data points (Fig. 1a.II).

Biotic stress reduced Φ_{PSII} in Mexican lime plants (Fig. 1b). At the first data point, infection caused a 4·4% decrease in Φ_{PSII} in plants inoculated with the mild isolate and a 5·5% reduction in those inoculated with the severe CTV isolate, in relation to control values. During the second year, PSII quantum efficiency could only be measured in plants infected with the mild isolate. Throughout this period, virus infection caused a drastic decrease in Φ_{PSII} as shown in Figure 1b.II. For example, 88 weeks after inoculation, Φ_{PSII} in infected plants was 18·6% lower than in healthy plants.

CTV infection did not have a consistent effect on Fo values (Fig. 1c). In most of the data points Fo values were similar between infected and healthy plants. However, at weeks 19 and 92, infected plants showed Fo values lower than healthy ones. In contrast, at weeks 31 and 41 an increase in Fo occurred as a consequence of virus infection.

At the beginning of the experimental period, NPQ drastically increased (1·8-fold at week 17) in leaves of plants infected with both CTV isolates (Fig. 1d). From this week and for the rest of the first year of study, NPQ values showed no clear trend. During the second year of the experiment, CTV infection induced a significant decrease in NPQ values in leaves of plants inoculated with T385 isolate (23·0% reduction at week 86; Fig. 1d.II).

Gas exchange parameters

In general, lower A and g_s and higher Ci/Ca were found in leaves of infected plants in comparison with non-infected ones. As shown in Figure 2a, CTV infection induced a decrease in A throughout the experimental period, this reduction being stronger in plants infected with the severe isolate T318. At week 21, net CO_2 assimilation rate in leaves of plants infected with T385 declined until reaching values 42·2% lower than those in healthy plants. By 86 weeks after infection, A in T385 infected plants decreased further to reach values 59·3% lower than the control (Figure 2a.II).

In general, stomatal conductance decreased after CTV infection, regardless of the virulence of the isolate (Fig. 2b). During the first year after virus inoculation,

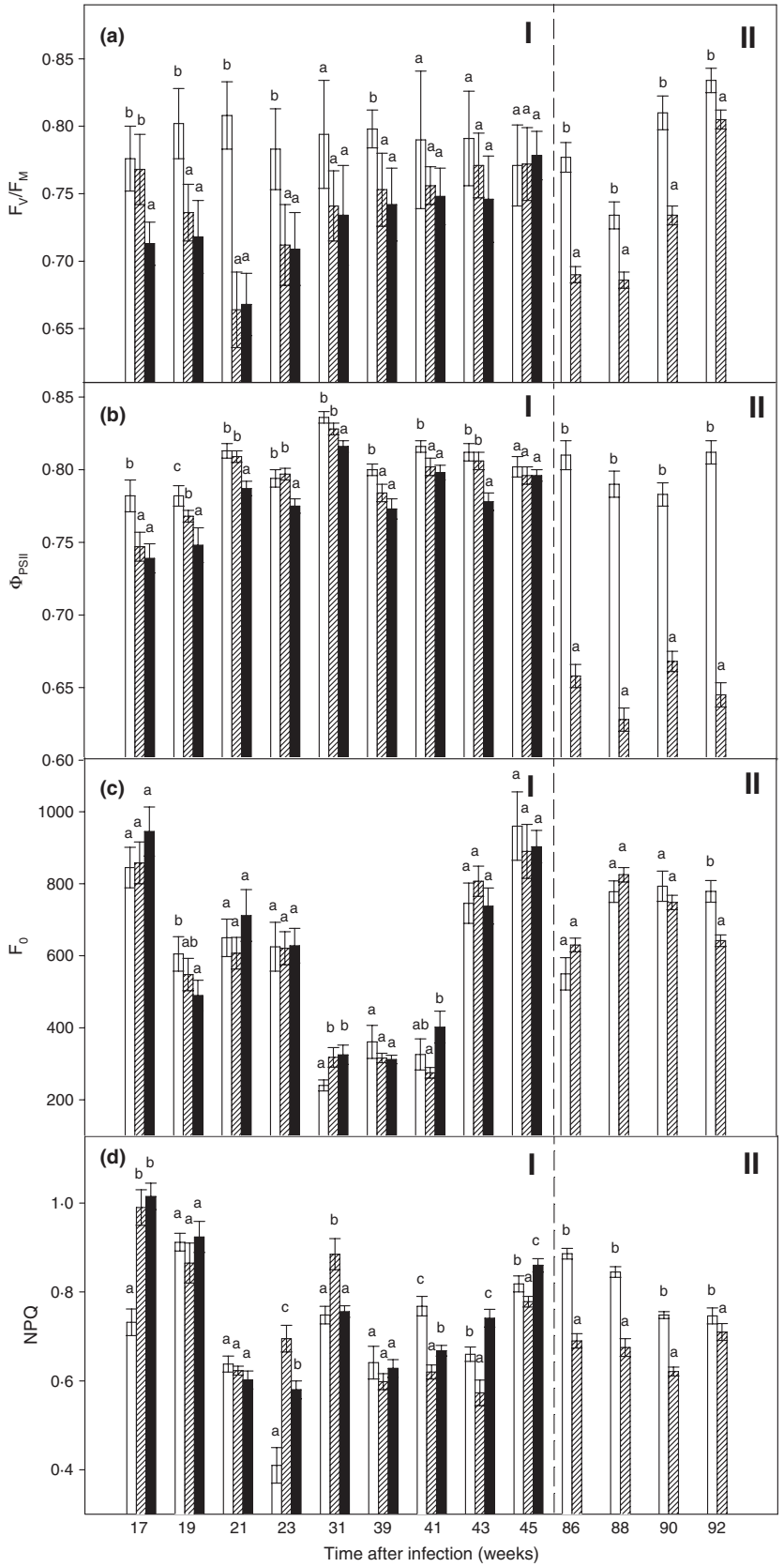

Figure 1 Chlorophyll fluorescence parameters in Mexican lime leaves after CTV infection. (a) Maximum fluorescence yield in dark-adapted leaves (F_V/F_M). (b) Quantum yield in light-adapted leaves (Φ_{PSII}). (c) Basal fluorescence in dark-adapted leaves (Fo). (d) Non-photochemical quenching (NPQ). I: first year after infection, II: second year after infection. Symbols denote (□) control plants, (■) plants infected with the severe T318 isolate, and (▨) plants infected with the mild T385 isolate. Data are mean values of 20 independent measurements ± standard error. Different letters denote significant differences at $P \leq 0{\cdot}05$ on each date.

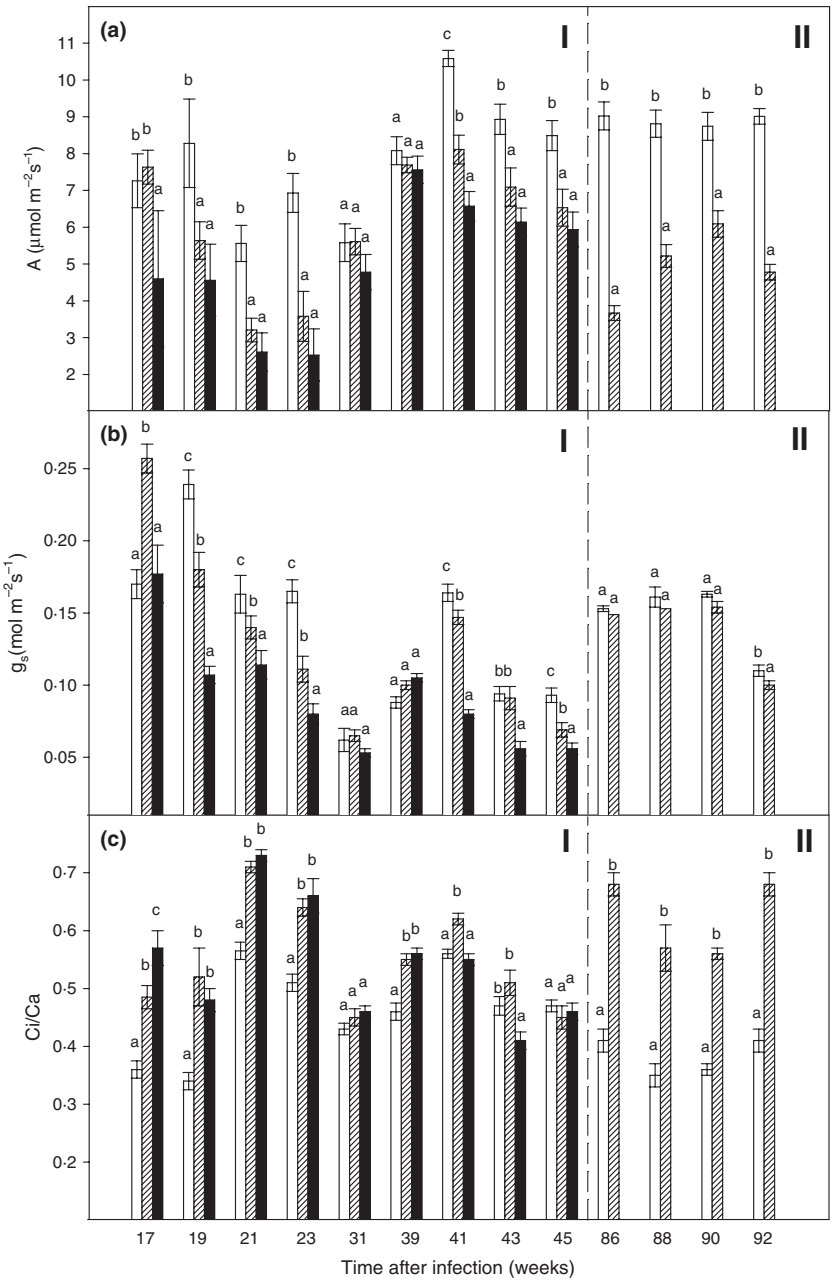

Figure 2 Gas exchange parameters in Mexican lime plants after CTV infection. (a) Net CO_2 assimilation rate (A). (b) Stomatal conductance (g_s). (c) Intercellular to ambient CO_2 ratio (C_i/C_a). I: first year after infection, II: second year after infection. Symbols denote (□) control plants, (■) plants infected with the severe T318 isolate, and (▨) plants infected with the mild T385 isolate. Data are mean values of at least 20 independent measurements ± standard error. Different letters denote significant differences at $P \leq 0{\cdot}05$ on each date.

leaves of plants infected with the severe isolate T318 exhibited the lowest g_s values, being, for example, 51·2% lower than in non-infected plants at week 41. During the second year of experiment, similar values of g_s were recorded in leaves of healthy and infected plants although at the end of the experimental period, a slight decrease in infected plants was observed (Fig. 2b.II).

Despite some variability, the ratio of intercellular to ambient CO_2 concentration generally increased in leaves of infected plants during the first year after infection (Fig. 2c.I). From week 19 to 39 there were no significant differences between plants infected with isolates T385 or T318 although at week 41 and 43, leaves of plants infected with the mild virus isolate T385 showed higher values of this parameter. During the second year, there was a drastic increase in Ci/Ca in leaves of infected plants (1·6-fold; Fig.2c.II).

MDA concentration

Oxidative damage in response to CTV infection was studied in leaves of Mexican lime in terms of MDA concentration (Fig. 3). Biotic stress increased MDA content in sprouts at all stages of development, both in the first and second years after inoculation. Throughout the experimental period the differences between healthy and

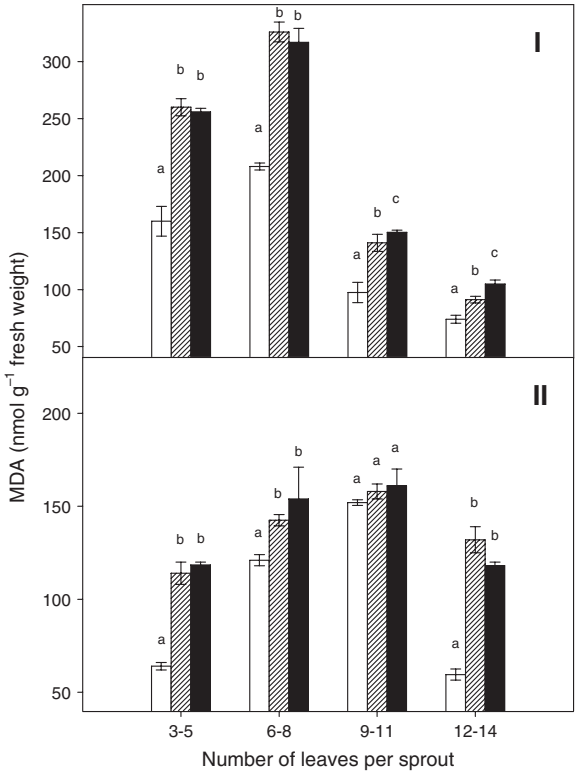

Figure 3 Malondialdehyde (MDA) concentration in shoots of Mexican lime at different stages of development after CTV infection. I: first year after infection, II: second year after infection. Symbols denote (□) control plants, (■) plants infected with the severe T318 isolate, and (▨) plants infected with the mild T385 isolate. Data are mean values of six independent replicates ± standard error. Different letters denote significant differences at $P \leq 0{\cdot}05$ at each stage of sprout development.

infected plants were statistically significant. During the first year, sprouts of 3–5 and 6–8 leaves exhibited maximum differences, MDA concentration being 1·6-fold higher in infected plants than in healthy plants.

Antioxidant enzymatic activity

APX activity was higher in infected plants at all stages of development. However, there were no differences in APX activity between shoots of plants infected with the two virus isolates at any stage of shoot development (Fig. 4a). During the second year of the experiment differences in APX activity between inoculated and non-infected plants drastically increased. APX activity in infected sprouts at early developmental stages (3–5 leaves) was 2·0-fold higher than in non-infected ones. This increase was more pronounced in infected sprouts at later developmental stages (9–11 and 12–14 leaves), reaching values 2·5- and 2·7-fold higher than those determined in healthy leaves, respectively.

Contrary to what was observed in APX, virus infection induced a reduction in CAT activity (Fig. 4b). During the first year after inoculation, differences in CAT activity were statistically significant between infected and healthy plants at all stages of sprout development. During the second year, CTV infection only induced reductions in CAT activity in the youngest and the oldest sprouts.

Discussion

In general, citrus genotypes are hosts for CTV, but there is a wide diversity in their response to viral infection, which is isolate-dependent. Mexican lime was chosen as a citrus model in this study because of its high susceptibility to CTV (Moreno *et al.*, 2008).

Whereas many studies have been directed towards understanding the structure, genetics, pathogenicity determinants and transport of viruses in plants, much less is known about the impact of a virus infection on plant physiology. Therefore, the present work studied the effect of CTV infection on chlorophyll fluorescence and gas exchange parameters, malondialdehyde concentration (to evaluate the oxidative damage) and antioxidant enzyme activity, in Mexican lime plants infected either with a mild or severe CTV isolate.

Although there are some reports on photosynthetic performance after virus infection (Chia & He, 1999; Ryšlavà *et al.*, 2003; Funayama-Noguchi & Terashima, 2006; Song *et al.*, 2009), until now there have been no investigations of photosynthetic responses of citrus plants to infection by CTV. The results of this study indicate that photosynthetic ability was reduced by CTV infection in Mexican lime plants, regardless of the virulence of the isolate used for infection. Biotic stress caused by CTV reduced Φ_{PSII} values in infected plants by about 5%. It has been reported than under abiotic stress conditions, reductions in Φ_{PSII} are associated with increases in NPQ (Osmond *et al.*, 1999; López-Climent *et al.*, 2008), suggesting an attempt to dissipate excess energy. These findings are in concordance with those observed during the first stages of virus infection; 17 weeks after inoculation, NPQ values strongly increased, regardless of the virus isolate. However, no study has examined, to date, the long-term effect of CTV infection on photosynthesis. Results of the present investigation show that, with the progress of infection, NPQ values in leaves of infected plants tended to decrease, being 23·0% lower than those determined in healthy plants at 86 weeks after infection. This could indicate an over-excitation of the photochemical system leading to an accumulation of reduced electron acceptors. This may in turn increase the accumulation of reactive radicals, which may further injure PSII components. A similar trend in NPQ values associated with virus infection has been reported in *Nicotiana benthamiana* plants infected with *Pepper mild mottle virus* (Pérez-Bueno *et al.*, 2006). Those authors proposed that virus-induced disturbances of the Benson–Calvin cycle could lead to an increase of the intra-thylakoidal pH gradient contributing to the NPQ increase during virus infection.

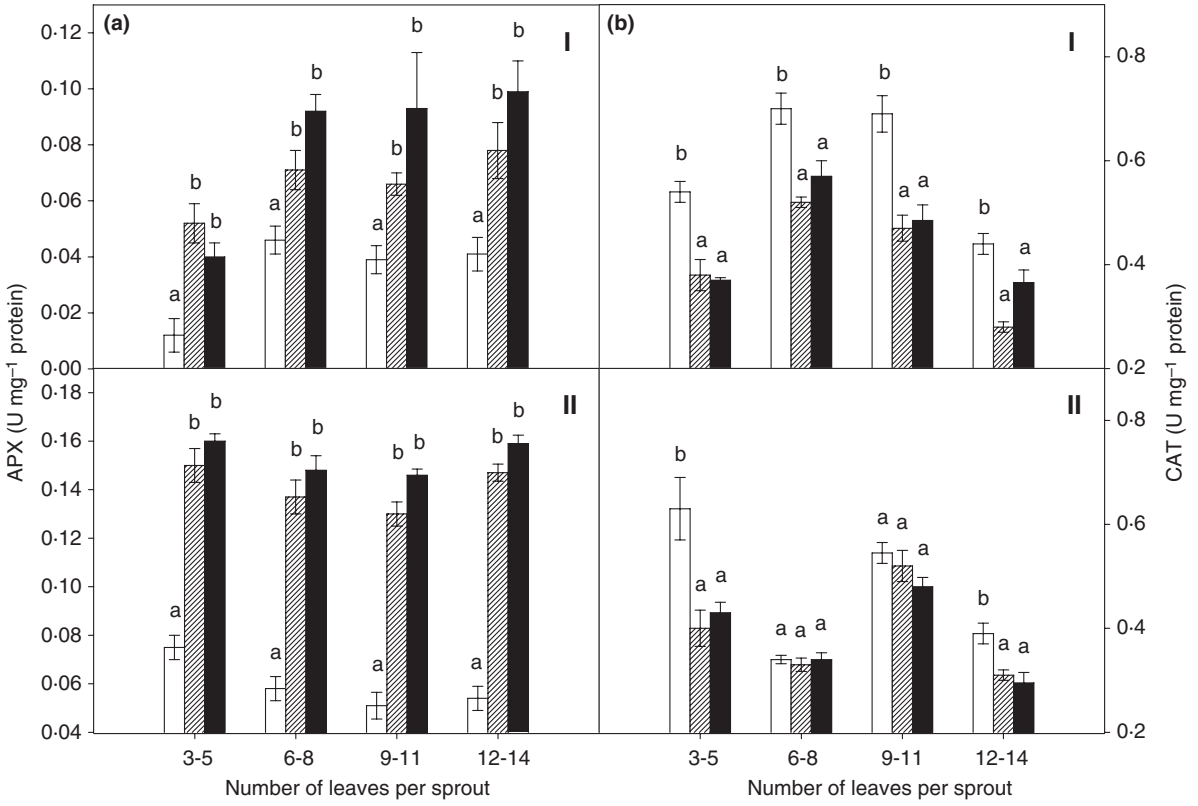

Figure 4 Antioxidant enzymatic activity in shoots of Mexican lime at different stages of development after CTV infection. (a) Ascorbate peroxidase activity (APX). (b) Catalase (CAT) activity. I: first year after infection, II: second year after infection. Symbols denote (□) control plants, (■) plants infected with the severe T318 isolate, and (▨) plants infected with the mild T385 isolate. Data are mean values of six independent replicates ± standard error. Different letters denote significant differences at $P \leq 0{\cdot}05$ at each stage of sprout development.

Contrary to that observed in citrus plants under abiotic stress conditions (Arbona *et al.*, 2009), CTV infection did not cause an increase in Fo, which suggests that virus infection did not damage the antennae and, therefore, did not reduce the efficiency of the light-harvesting complexes. The decrease in *A* observed in the present work together with the concomitant increase in Ci/Ca and reductions in F_V/F_M and Φ_{PSII}, suggest that limitations in photosynthetic activity induced by CTV in citrus are caused mainly by the reduction in carboxylative efficiency, whereas the limitation of CO_2 diffusion through the stoma seems to have a lower impact. In contrast to these findings, no evident changes in F_V/F_M were detected in peach plants infected for a long term with *Plum pox virus*, although the decrease in NPQ values in those plants reflected a reduced capacity for dissipation excess of light energy and an increase of reactive species of oxygen was detected (Hernandez *et al.*, 2004).

In plants growing under stress conditions, the lack of effective mechanisms for energy dissipation in a defective photosynthetic system, together with the increase of alternative electron sinks, may cause more electrons to divert to photorespiration and/or the Mehler reaction instead of being used in photosynthetic processes. This would cause an increase in active oxygen species and therefore result in higher oxidative damage. Results described in this work suggest that the lack of increase in NPQ in response to infection may be responsible, at least in part, for the MDA accumulation.

Several enzymatic activities greatly contribute to coping with oxidative stress and their activities have been used to evaluate stress responses in plants (Arbona *et al.*, 2008). However, under different biotic stress conditions, antioxidant enzymatic activities vary considerably. Usually, there is an increase in APX activity in wounded tissues (Samsone *et al.*, 2011) or in those damaged by chewing herbivores (Hu *et al.*, 2009). In contrast, phloem-sucking aphids cause a decrease in APX activity together with an increase in peroxidase activity in the affected tissues (Khattab, 2007). A decrease in antioxidant capacity in stressed tissues results in higher levels of ROS that may contribute to further injury (Bartwal *et al.*, 2013). The results of the present study show an increase in APX activity in new shoots developed in infected plants during the two years of the experiment. These data suggest that the machinery responsible for the removal of H_2O_2 was not damaged as a result of stress. However, CAT activity decreased significantly, especially

during the first year of infection. This may have occurred because the damage was localized in chloroplasts (where APX is active) whereas the CAT enzyme is localized in peroxisomes and is absent from chloroplasts (Singh *et al.*, 2010). Therefore, the results indicate a certain capacity of infected Mexican lime plants for H_2O_2 detoxification. However, the accumulation of MDA in infected plants would indicate that part of the ROS escape from the detoxification system, due to the continuous pressure exerted by the stress, and thereby cause an increase in lipid peroxidation.

It has been proposed that alterations in the activities of reactive oxygen species-scavenging enzymes could be a key step in the activation of the phytopathogenic response (De Gara *et al.*, 2003). In the present investigation, MDA levels and antioxidant enzymatic activities did not correlate with the differences in virulence between the two isolates. The APX activity, which plays an essential role in ROS scavenging, increased to the same extent in plants infected with either the severe or mild virus isolate. In addition, CAT activity decreased to the same extent in plants infected with either isolate, in agreement with results found in other host–pathogen systems (Hernandez *et al.*, 2004). In contrast, Hakmaoui *et al.* (2012) reported that the antioxidant response and the extent of oxidative stress in *N. benthamiana* plants correlated with the different virulence of isolates. This disagreement between investigations could be attributed to the different systems used in the trials. The present work was carried out with a woody species and the first effects of viral infection were estimated weeks after infection. However, Hakmaoui and collaborators performed the experiments with an herbaceous species and results were measured between 7 and 28 days after the infection.

It can be concluded from the current investigation that CTV infection caused impairment of the photosynthetic machinery, although the antennae complex was not affected and the availability of CO_2 in the substomatal cavity was not a limiting factor. Restrictions in photosynthetic activity induced by CTV seem related to a reduction in the carboxylative efficiency. As a consequence of the defective photosynthetic system, infected leaf cells suffered oxidative damage. Although infected plants exhibited some ability for H_2O_2 detoxification by activating APX activity, this defence mechanism was overwhelmed with time due to the continuing pressure of biotic stress.

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