Midday depression of photosynthesis and effects of mist spray in citrus

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Abstract

Diurnal variations of gas exchange, chlorophyll a fluorescence and some related biochemical characteristics in sun-acclimated mature citrus leaves of mist-sprayed (treatment) and unsprayed (control) trees were compared on sunny days during summer to identify the environmental and physiological factors limiting carbon gain in citrus tree canopies. At midday, net photosynthesis and maximal photochemical efficiency of photosystem II (Fv/Fm) in citrus leaves decreased significantly under control conditions, but the decrease was mitigated by mist spraying. Although the content of malondialdehyde, hydrogen peroxide and activities of antioxidant enzymes increased at midday in both mist-sprayed and control leaves, they were much higher in control leaves than in mist-sprayed leaves. The level of D1 protein decreased significantly in control leaves at midday and then was partly recovered later, while that in treated leaves changed to a much lesser extent because of alleviation of photoinhibition by mist spraying. Both the fast and the slow phases of millisecond-delayed light emissions in treated citrus leaves were higher than those in control leaves, indicating that mist spraying protects the normal operation of the photosynthetic apparatus in leaves. Mist spraying also reduced leaf temperatures and the ratio of air to leaf vapour pressure deficit (ALVPD), leading to increases in stomatal conductance (gs) and alleviation of photoinhibition at midday. It is concluded that the decline of leaf gs under high-ALVPD conditions in summer is an important factor contributing to midday depression of photosynthesis in citrus, and mist spraying is effective in alleviating midday depression of photosynthesis in citrus leaves.

Introduction

Photosynthesis is the most important chemical reaction on the earth, by which plants convert CO2, water and other inorganic materials to organic compounds and oxygen. Nevertheless, leaves are usually exposed to high irradiances, high air temperature and high vapour pressure deficits (VPD). These environmental stresses cause strong midday depression of photosynthesis (Tenhunen et al., 1987).

Light is a prerequisite for photosynthesis, but too much light could cause photoinhibition of net photosynthesis (Pn); for example, maximum Pn of sun-acclimated leaves in outer surfaces of citrus canopies is light saturated (about 600–700 μmol m$^{-2}$ s$^{-1}$) at about one third of full sunlight density (Jifon & Syvertsen, 2003), so non-utilised excitation energy will accumulate to reduce photosynthetic efficiency, namely photoinhibition (Demmig-Adams, 1990). Photoinhibition is usually described as a sustained reduction in the quantum yield of photosynthesis. Photoinhibition severity is influenced by not only light intensity but also superimposition of other environmental stresses, such as high temperature, water availability or CO2 supply (Barber &
Andersson, 1992; Medina et al., 2002). Some reports showed that leaf temperature ($T_l$) or via the ratio of air to leaf vapour pressure deficit (ALVPD), rather than plant water status, is the determinant factor of midday depression (Xu & Shen, 2005). The increase of ALVPD with rise of $T_l$ might cause the decrease of stomatal conductance ($g_s$) in most plant species (Dai et al., 1992; Franks & Farquhar, 1999). To some extent, stomatal closure limits photosynthetic CO$_2$ uptake and decreases $P_n$ because it decreases CO$_2$ availability to limit the activity of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) and exacerbate photoinhibition (Crafts-Brandner & Salvucci, 2000; Salvucci & Crafts-Brandner, 2004). High $T_l$ also directly reduces photosynthesis because of inactivation of many chloroplast enzymes under oxidative stress (Dekov et al., 2000). Compared with other factors, VPD was regarded as a more important factor of causing midday depression of photosynthesis in many plants, such as Prosopis juliflora (Pathre et al., 1998), wheat (Xu & Shen, 2005), Vicia faba (Talbott et al., 2003) and citrus (Medina et al., 2002), but there is no agreements on their mechanisms because high VPD often only occurs for a short period at midday and its effect may interact with other stresses (Shirke & Pathre, 2004; Xu & Shen, 2005); therefore, it is difficult to confirm what is the dominant factor in the midday depression of photosynthesis because these environmental and physiological effects occur at the same time in natural conditions (Pathre et al., 1998; Murakota et al., 2000; Franco & Lütge, 2002; Pons & Welschen, 2003; Xu & Shen, 2005).

Citrus, an economically important plant worldwide, can be grown in various climatic conditions ranging from hot-humid equatorial climates to warm-subtropical or even cooler maritime climates (Spiegel-Roy & Goldschmidt, 1996). $P_n$ of citrus is lower than that of most C$_3$ crops (Chen & Zhang, 1994; Jifon & Syvertsen, 2003; Guo et al., 2006). Severe midday depression of photosynthesis in citrus has been found under high photosynthetic photon flux density (PPFD) and air temperature ($T_a$) in summer (Chen & Zhang, 1994), which leads to reduced growth, fruit yield and quality. Even under irrigation, $g_s$ of citrus leaf is particularly sensitive to change in VPD and decreases with the increases of $T_l$ and VPD (Syvertsen & Sal-yani, 1991). Some researchers reported that shading could alleviate midday depression of photosynthesis in citrus (Medina et al., 2002; Jifon & Syvertsen, 2003) and grapevine (Cartechini & Palliotti, 1995). However, shading might reduce CO$_2$ assimilation when PPFD is low in early morning and late afternoon hours and even could reduce productivity or yield (Stampar et al., 2001). Alternatively, spraying water droplet (mist spray) can directly decrease VPD and indirectly reduce $T_l$. Previous works showed that mist spraying could be used to alleviate midday depression of photosynthesis in some plants, such as wheat (Xu et al., 1987) and soybean (Zheng et al., 1994). Moreover, mist spray does not need extra apparatus because citrus plantation usually has irrigation equipment, with low cost for application in improving its productivity.

In the present study, an integrative analysis of diurnal changes in gas exchange, chlorophyll fluorescence and some biochemical changes related to midday depression of photosynthesis was performed in mist-sprayed and control leaves to identify factors that may contribute to midday depression of photosynthesis in citrus and determine the effectiveness of mist spraying in alleviating midday depression of photosynthesis.

Materials and methods

Plant material and experimental treatment

The experiment was conducted in the summer (from July to September) 2005, the hotter months in Hangzhou, China (30°15’N, 120°10’E). Six 2-year-old Satsuma mandarin (Citrus unshiu Marc.) plants, grown in 10-L plastic pots containing loam : peat : coarse sand = 7:3:2 in the greenhouse of the Huajiaji campus, Zhejiang University, were selected based on the same growth appearances. They were placed outdoors for acclimation for about 1 month (from July to August). All these potted trees were randomly divided into two groups. One group was mist sprayed once in every 10 min from 10:00 to 17:00 h on sunny days in summer with a plastic sprayer (HX50; Yuyao Dongxia Sprayer Plastic Industrial Co. Ltd, Ningbo, China); the other group was used as the control without any treatment. During mist spraying, the spray nozzle was adjusted to make water into mist droplets in order not to form water beads on leaf surface. The space between two groups was about 10 m for avoiding the effect of mist spraying on control. Irrigation and nutrient supplementa-

Environmental conditions and gas exchange measurements

PPFD and $T_a$ around the leaves were measured with a quantum sensor incorporating a thermocouple near the cuvette of a gas exchange system (Li-6400; Li-Cor Inc., Lincoln, NE, USA).

Mature and sun-acclimated leaves in exterior canopy positions were sampled for gas exchange measurements on a sunny and windless day, five times a day under $T_a$ (30–40°C) and air CO$_2$ concentration ($C_a$) 370–420 μmol mol$^{-1}$, with a given saturating incident PPFD of 1000 μmol m$^{-2}$ s$^{-1}$ from a 670-nm red light-emitting
diodes (LEDs) with 10% blue light (Habermann et al., 2003). Leaf net rate, $g_{l}$ to water, transpiration ($E$), sub-stomatal and air CO$_2$ concentration (C$_i$ and C$_a$), $T_e$ the ratio of ALVPD and air relative humidity (RH) were also measured using an open gas exchange system (Li-6400; Li-Cor Inc.) with an integrated red/blue LED chamber head (Li-6400-02B; Li-Cor Inc.) (Von Caemmerer & Farquhar, 1981). During the measurements, the flow rate was maintained at 500 μmol s$^{-1}$. Care was taken to avoid shading of quantum sensor during measurements. The air pumped into the gas exchange system was collected from a sheltered buffer volume to limit fast fluctuations and prevent external disturbances (Oso´rio et al., 2002) using the dark leaf clip-6 equipped with a day with a pulse-modulated fluorometer (PAM-2000; Walz). Light for measurements was provided by Halogen lamp, and the wavelengths above 710 nm are removed by a sheltered buffer volume to limit fast fluctuations (Osório et al., 2006). Air VPD was calculated according to RH and $T_a$ (Meng et al., 1999).

**Chlorophyll a fluorescence measurements**

The same leaf was used for chlorophyll a fluorescence measurements right after gas exchange measurements. Chlorophyll a fluorescence was determined four times a day with a pulse-modulated fluorometer (PAM-2000; Walz, Effeltrich, Germany). Before each measurement, the sample leaves were dark adapted for 20 min (Franco & Lützge, 2002) using the dark leaf clip-6 equipped with a miniature sliding shutter, which prevents light access to the leaf during the dark-adaptation period. The fluorometer was connected to a trifurcated fibre optic (2010-F) and the leaf during the dark-adaptation period. The fluorometer was subsequently filtered and centrifuged at 15 000 g for 30 min at 4°C, then the pellets were resuspended in 0.05% guaiacol, 10 mM H$_2$O$_2$ and the enzyme. Its activity was denoted by the increase in absorbance at 470 nm by guaiacol oxidation ($E = 26.6$ μmol cm$^{-1}$).

**Chemical quenching coefficient (NPQ) and the actual quantum yield of PSII photochemistry ($\Phi_{PSII}$) were calculated, respectively, as qP = $(F_m' - F_o')/(F_m' - F_o)$, NPQ = $(F_m' - F_o)/F_m'$ and $\Phi_{PSII} = (F_m' - F_o')/F_m'$ (Genty et al., 1989; Krall & Edwards, 1992).** All measurements were performed from 09:00 to 15:00 h with six replications. The temperature and VPD were not regulated in leaf chamber during the measurements.

**Assay of malondialdehyde and H$_2$O$_2$**

Lipid peroxidation in fresh sample leaves was determined by 2-thiobarbituric acid–malondialdehyde (MDA) adduct formation as described by Heath & Packer (1968). The H$_2$O$_2$ concentration was assayed by a method from that of Patterson et al. (1984).

**Assay of antioxidant enzymes**

The antioxidant enzymes in fresh samples leaves were extracted according to Cakmak & Marschner (1992). The catalase (CAT, EC 1.11.1.6), the superoxide dismutase (SOD, EC 1.15.1.1) and ascorbate peroxidase (APX, EC 1.11.1.11) activities were determined according to Guo et al. (2006).

The peroxidase (POD, EC 1.11.1.7) activity was assayed following the guaiacol method described by Cakmak & Marschner (1992) with some modifications. The reaction mixture contained 25 mM phosphate buffer (pH 7.0), 0.05% guaiacol, 10 mM H$_2$O$_2$ and the enzyme. Its activity was denoted by the increase in absorbance at 470 nm by guaiacol oxidation ($E = 26.6$ μmol cm$^{-1}$).

**Thylakoid membrane isolated and D1 protein determinations**

Leaf samples after chlorophyll a fluorescence measurement were immediately frozen in liquid nitrogen and stored at −86°C for the D1 protein assays. Thylakoid membranes were obtained by grinding the frozen leaf discs with 5 mM ice-cold extraction buffer (pH 7.8, 5 mM MgCl$_2$, 50 mM Trisicine, 100 mM sucrose, 10 mM NaF and 1 mM phenylmethylsulphonylfluoride). The mixture was subsequently filtered and centrifuged at 15 000 g for 30 min at 4°C, then the pellets were resuspended and prepared for chlorophyll analysis (30 μL sample + 1 mL acetone). The gel was loaded with 10 μg chlorophyll in each well.

Protein extracts were mixed with an equal volume of double concentration sample loading buffer and separated by 15% sodium dodecylsulphate–polyacrylamide gel electrophoresis with 4 M urea (SDS/urea–PAGE) (Laemmli, 1970). The separated proteins were electrophoretically
transferred to polyvinylidene fluoride (PVDF) membrane (Millipore, Bedford, USA) and then probed using a polyclonal antibody to the D1 protein. Horseradish peroxidase-conjugated antirabbit antibody was used as secondary antibody. Bound antibodies were visualised using enhanced chemiluminescence (ECL) detection kit (Amersham Pharmacia Biotech, Uppsala, Sweden). D1 protein levels from samples on immunoblots were quantified using ‘Quantity One’ software (Bio-Rad; Bio-Rad Laboratories, Hercules, CA, USA).

Measurements of millisecond-delayed light emission

Six attached leaves with good symmetry from exterior canopies of the control plants were selected for millisecond-delayed light emission (ms-DLE) measurements. One side of the leaf mid-rib was sprayed by a plastic sprayer (HX50; Yuyao Dongxia Sprayer Plastic Industrial Co. Ltd) as the treatment, and the other side was not sprayed as the control. A transparent plastic film was used to cover the control side of the leaf to avoid the disturbance of mist spraying. Mist spraying was performed every 10 min as described above. All the six whole leaves were illuminated by 1000 μmol m\(^{-2}\) s\(^{-1}\) for 2 h at 28°C and then cut along the leaf mid-rib into 12 half leaves (6 × 2), including six mist-sprayed half leaves and six control half leaves, then all half leaves were dark adapted for 30 min at 28°C. Ms-DLE was measured with a phosphoroscope built in our laboratory following the detailed instructions of Wraith & Crofts (1971) with modifications by Xu & Shen (1984). Our previous paper suggested that the proton released from oxidation of water could diffuse easily to the bulk aqueous solution in the lumen at higher temperature, while the dynamic structure of thylakoid membrane was more suitable for maintaining the localisation of the proton released from water oxidation around PSII at low temperature (Wei et al., 1998). Therefore, three mist-sprayed half leaves and three control half leaves were determined at 2°C (low temperature), the remaining six half leaves were determined at 28°C (higher temperature). Each half leaf was put in a polymethylmethacrylate cuvette with water and irradiated with white light (1500 μmol m\(^{-2}\) s\(^{-1}\)) from a projection lamp passing through a 2-cm-thick water layer. The holes on the rotating wheels were arranged by the measuring process with a series of 5.6 ms cycles for the excitation and measurement, that is 1 ms excitation followed by 4.6 ms darkness. The delayed light between 2.8 and 3.8 ms was measured after every flash with an EMI 9558B photomultiplier with a red glass filter. The signal passing through an amplifier was recorded continuously by a SC-16 light beam oscillograph, whose response time of the order of a millisecond was sensitive enough for measurement of the fast phase (<0.1 s) of ms-DLE (Xu & Shen, 1984; Wei et al., 1998). The ms-DLE signals were recorded by the ultraviolet-sensitised recording paper.

Statistical analysis

One-way analysis of variance and significant differences were performed to all experimental data with SPSS 13.0 software. Least significant difference test was used on a significance level of P < 0.05 or P < 0.01. Data are the means ± SE of at least three measurements.

Results

Environmental conditions

All measurements were performed in summer with typical high PPFD, \(T_a\) and VPD. The maximum of PPFD, \(T_a\) and VPD were, respectively, recorded around 2000 μmol m\(^{-2}\) s\(^{-1}\) at 11:00 h (Fig. 1A), 40.8°C at 13:00 h (Fig. 1B) and 5.29 kPa at 13:00 h (Fig. 1D). \(C_a\) decreased from 9:00 to 13:00 h with the minimal value of 370 μmol mol\(^{-1}\), but with a slight recovery after 15:00 h (Fig. 1C). Mist spraying could slightly decrease VPD, but it did not influence PPFD, \(T_a\) and \(C_a\) around the leaves.

Diurnal variations in daytime of leaf gas exchange in mist-sprayed and control leaves

During the experimental period, \(P_n\) sharply decreased from peak values at 9:00 h (9.34 μmol CO\(_2\) m\(^{-2}\) s\(^{-1}\) in

Figure 1 Diurnal variations in daytime of environmental parameters around control (○) and mist-sprayed (■) leaves selected on a sunny day for gas exchange measurements: (A) photosynthetic photon flux density (PPFD); (B) air temperature (\(T_a\)); (C) air CO\(_2\) concentration (\(C_a\)) and (D) vapour pressure deficits (VPD).
the control and 9.90 µmol CO$_2$ m$^{-2}$ s$^{-1}$ in the treatment), and its decline was slower in the treatment than that in the control (Fig. 1). From 9:00 to 13:00 h, the drop percentages of $P_n$ in the treatment and control were 33.8% and 67.8%, respectively, without recovery in the afternoon (Fig. 2A). The $g_s$ of leaves basically remained constant in the treatment despite a slight increase at 11:00 h, while it showed a continual decline in the control. Comparing with 9:00 h, $g_s$ at 13:00 h remained at 80 mmol H$_2$O m$^{-2}$ s$^{-1}$ in the treatment, while it decreased to 46.8 mmol H$_2$O m$^{-2}$ s$^{-1}$ in the control (Fig. 2B). The $T_l$ at 13:00 h reached 39.9°C in the treatment, while it was 41.0°C in the control; slight decreases of $T_l$ in the afternoon were observed in the treatment and control (Fig. 2C). Diurnal variation of ALVPD was similar to $T_l$ (Fig. 2F). ALVPD of leaves in the treatment and control reached the maximal values of 4.83 and 5.22 kPa, respectively, at 13:00 h, followed by slight decreases in the afternoon. At 15:00 h, both $T_l$ and ALVPD decreased significantly in the treatment. Following similar patterns of $g_s$, diurnal variations, leaves $E$ in the treatment and control reached the maximal value of 4.29 and 3.28 mmol H$_2$O m$^{-2}$ s$^{-1}$, respectively, at 11:00 h, after which a continual depression in control leaves was observed (Fig. 2D). In contrast to $P_n$, the CO$_2$ concentration ratio of sub-stomatal to air (C/C$_a$) increased from 0.5 to 0.7 in the treatment and control during daytime, and it was not significantly affected by the mist spray (Fig. 2E).

**Relationship of leaf temperature or air to leaf vapour pressure deficit to net photosynthesis or stomatal conductance**

Linear regression analysis showed that $P_n$ was highly responsive to changes of $g_s$ in control leaves ($R^2 = 0.689$, $P < 0.001$) than in mist-sprayed leaves ($R^2 = 0.280$, $P < 0.050$), which indicates that $P_n$ in control leaves is more influenced by $g_s$ than those in mist-sprayed leaves (Fig. 3A). In contrast to $g_s$, $T_l$ had a greatly negative effect on $P_n$ ($R^2 = 0.803$, $P < 0.0001$) in the control, suggesting that depression of $P_n$ in summer results from an increase of $T_l$ to a great extent (Fig. 3B). In the control, $g_s$ was significantly influenced by ALVPD ($R^2 = 0.3914$, $P < 0.05$) and by $T_l$ ($R^2 = 0.978$, $P < 0.0001$), especially under ALVPD higher than 3 kPa conditions. This indicates that midday depression of $g_s$ is always accompanied with the increases of ALVPD and $T_l$ (Fig. 3C and Fig. 3D). However, this negative relationship between $g_s$ and ALVPD is not tight in mist-sprayed leaves. These analyses suggest that ALVPD influence $T_l$ and $g_s$, which showed close relationship to photosynthesis in citrus leaves under summer conditions.

**Diurnal variations in daytime of chlorophyll a fluorescence**

As with midday depression of $P_n$, $F_v/F_m$, $F'/F'_m$ and $\Phi_P$ of leaves in the control continually decreased from 9:00 to 15:00 h with increasing PPFD and $T_a$ (Figs 1 and 4) but recovered to values similar to those of mist-sprayed leaves in the late afternoon (Fig. 4A~4D). During the measurement period, $F_o$ kept relative constant in both treatment and control leaves. At midday, $F_v/F_m$ of leaves in the control reached the minimal values of 0.49, while that in the treatment was much higher at 0.69 (Fig. 4A and 4B). Responses of $F'/F'_m$ and $\Phi_P$ showed similar patterns as $F_v/F_m$ but the range of differences was much smaller than that of $F_v/F_m$ (Fig. 4C and Fig. 4D). $qP$ in the treatment was slightly higher than that in the control, but the difference was not significant. NPQ kept relatively constant in the treatment, whereas it oscillated in the
control with increase at 11:00 and 15:00 h and decrease at
13:00 h. All these showed that photochemical efficiency
decreased at midday, which may affect photosynthesis.
The phenomenon could be ameliorated by mist spraying.

Diurnal variations in daytime of
malondialdehyde, H₂O₂ and antioxidant en-
yzymes

With increasing PPFD and Tᵢ, MDA and H₂O₂ content
increased significantly in both control and treated plants
after 09:00 h and reached a maximum at 13:00 h, then
with a little recovery in the late afternoon. The contents
of MDA and H₂O₂ of leaves in mist-sprayed leaves were
lower than that in the control during daytime. The varia-
tion of CAT activity in leaves was similar to the changes
of MDA and H₂O₂, but sharp decreases of CAT activity
were observed in both the treatment and the control in
the afternoon (Fig. 5A and Fig. 5B). In contrast to H₂O₂,
APX activity of leaves decreased from 9:00 to 13:00 h
but increased after 13:00 h in the treatment and control
(Fig. 5C). With increasing Tᵢ, POD activities of leaves
continually declined after 9:00 h in the treatment and
control during the experimental period, reaching the
minimum (about 60% of POD activities at 9:00 h) at
15:00 h in the control (Fig. 5D and Fig. 5E). While SOD
activities of leaves remained relatively constant from
9:00 to 11:00 h in the treatment and control, sharp de-
creases were observed from 11:00 to 13:00 h and a little
recovery appeared from 13:00 to 15:00 h (Fig. 5F).

Linear regression analyses showed that Pᵣ was nega-
tively related to H₂O₂ concentration in the treatment
(R² = 0.867, P < 0.05) and control (R² = 0.904,
P < 0.05). Similar correlations of F₄/F₅ to Pᵣ in the treat-
ment (R² = 0.914, P < 0.05) and control (R² = 0.935,
P < 0.05) were also observed (Fig. 6B). There were strong
negative, linear correlations between H₂O₂ concentration
and Φ₈₅ in the treatment (R² = 0.830, P < 0.05) and
control (R² = 0.918, P < 0.05) (Fig. 6C). Significant linear
correlation between contents of MDA and H₂O₂ were
observed, especially in the control (R² = 0.908, P < 0.05)
(Fig. 6D). These analyses suggest that H₂O₂ plays an
important role in photochemical efficiency of PSII that is
closely related to photosynthesis in summer.

Significant linear correlation between Pᵣ and Φ₈₅ were
observed, especially in the control leaves (R² = 0.81)
(Fig. 7), which revealed that the midday depressions of $P_n$ closely related to photochemical efficiency of PSII that was always affected by $H_2O_2$ and MDA of leaves.

Changes of D1 protein content in mist-sprayed and control leaves

D1 protein, one of the proteins of PSII reaction centre complex with rapid turnover, could be used to reflect the degree of photoinhibition. In the treatment and control, D1 was significantly degraded at 13:00 h relative to that at 09:00 h (Fig. 8). D1 protein in the treatment decreased to 54% at midday relative to that at 09:00 h and recovered to 83% at 15:00 h, while it decreased to about 20% at midday in the control relative to that at 09:00 h and recovered to 53% at 15:00 h. These data showed that mist-spraying leaves could somewhat protect D1 protein from degradation and improve photochemical efficiency of PSII under high PPFD, $T_a$ and VPD conditions.
Effects of mist spraying on millisecond-delayed light emissions in citrus leaves

The proton motive force (PMF) that could provide energy for ATP synthesis can be recorded on two phases of the ms-DLE: the fast phase of ms-DLE (uprising of ms-DLE within 0.1 s at the beginning of illumination with flashing light) was correlated with a rapid establishment of thylakoid membrane potential and the proton gradient that is related to the proton released from the oxidation of water, while the slow phase of ms-DLE (the part after the fast phase of ms-DLE, which turned into a steady level within a few seconds) was mainly related to the form of steady proton gradient across the thylakoid membrane (Wraight & Crofts, 1971; Xu & Shen, 1984). At 28°C, ms-DLE intensities in citrus leaves showed that both components of pmf were higher in the treatment, which indicates that mist spraying, to a certain extent, protects the photosynthetic apparatus from damage. At 2°C, both fast and slow phases increased compared with those at 28°C, which confirms the contribution of the localisation of the proton released from water oxidation around PSII. Moreover, the fast phase of mist-sprayed leaves showed higher signal intensity at 2°C than that at 28°C, suggesting that mist spraying alleviates the damage of PSII under strong light illumination (Fig. 9).

Figure 5 Diurnal variations in daytime concentrations of malondialdehyde (MDA) and H2O2 and activities of antioxidant enzymes in control (●) and mist-sprayed (○) leaves of citrus. (A) MDA; (B) H2O2; (C) catalase (CAT); (D) ascorbate peroxidase (APX); (E) peroxidase (POD); (F) superoxide dismutase (SOD). Asterisks (*) and (**) indicate significant difference at $P < 0.05$ and $P < 0.01$, respectively.
Discussion

Previous studies have shown that midday depression of photosynthesis is a common phenomenon in C3 plants. This phenomenon is thought to be mainly caused by stomatal closure, elevated respiration and photoinhibition resulting from strong light, high temperatures and VPD in summer (Medina et al., 2002; Pons & Welschen, 2003; Osório et al., 2006).

The present study showed that midday depression of \( P_n \) and \( g_s \) in citrus trees occurred in summer with high PPFD, \( T_a \) and VPD under atmospheric CO2 (Figs 1, 2A and 2B), which is consistent with previous observations on citrus (Chen & Zhang, 1994; Jifon & Syvertsen, 2003) and other C3 plant species (Pathre et al., 1998; Pons & Welschen, 2003). Our results also showed that \( P_n \) and \( g_s \) of leaves in the control declined from 9:00 to 15:00 h, but mist spraying significantly mitigated the midday depression of \( P_n \) and \( g_s \) in citrus trees (Fig. 2A and Fig. 2B) via reducing ALVPD (Fig. 2A and Fig. 2B). The high correlations of \( P_n \) to \( g_s \) (\( P < 0.05 \)) and \( g_s \) to ALVPD (\( P < 0.05 \)) in control leaves indicate that stomatal closure may play an important role in midday depression of photosynthesis in citrus leaves (Fig. 3). Similar results have been obtained in Arbutus unedo growing in a Mediterranean climate (Raschke & Resemann, 1986) and Populus deltoids, Prosopis juliflora and Acacia auriculiformis in India (Pathre et al., 1998; Shirk & Pathe, 2003, 2004). Although an increase in \( E \) was found in mist-sprayed leaves (Fig. 2D), leaf water use efficiency (WUE, WUE = \( P_n/E \)) was slightly increased by mist spraying (data not shown).

The negative relationship between \( T_l \) and photosynthesis in control leaves (Fig. 2A and Fig. 2C) suggests that high \( T_l \) may have contributed to mid-depression of photosynthesis. High temperature has been shown to decrease Rubisco activase activity, leading to deactivation of Rubisco (Salvucci et al., 2001; Salvucci & Crafts-Brandner, 2004). Because mist spray significantly increased \( g_s \) and photosynthesis with only slight decrease in \( T_l \) (Fig. 2A and Fig. 2C) and there was a tight relationship between \( g_s \) and photosynthesis, we think stomatal closure at midday is the main factor responsible for midday depression of photosynthesis. Mid-depression of photosynthesis, in turn, increases excessive excitation energy,
which may have led to the inactivation of PSII. Our results showed that the mid-depression of \( P_n \) control leaves were accompanied by the significant decrease in PSII activity (Fig. 4), which are similar to those obtained on nectarine trees under well-watered or water-stressed conditions in the Mediterranean region during summer (Oso´rio et al., 2006) and some species of citrus leaves, such as grapefruit (\( Citrus \) paradisi Macfady) (Jifon & Syvertsen, 2003) and ‘Pera’ orange (\( Citrus \) sinensis Osbeck) (Medina et al., 2002). Moreover, in control leaves, a slight increase in \( F_o \) and a acute decrease in thermal dissipation ability were found at midday (Fig. 4A and Fig. 4F), but almost no change in the proportion of reaction centres that remain open, suggesting that decline of PSII activity in citrus leaves at midday may be caused by PSII photodamage. In addition, the close relationship between \( \Phi_{\text{PSII}} \) and \( P_n \), particularly in control leaves (\( R^2 = 0.81 \)) (Fig. 7), indicated that the mid-depression of photosynthesis in citrus leaves was related to photoinhibition. In fact, a decline of PSII activity in plants exposed to photoinhibition conditions was noted because of the damage and significant reduction in turnover rates of D1 protein (the most sensitive protein in the PSII proteins to photoinhibition) (Vass et al., 1992; Aro et al., 1993, 1994; Melis, 1999). Generally, PSII photodamage is caused by reactive oxygen species (ROS) and D1 protein is an important target of ROS (Krause, 1988). In our study, the level of D1 protein was much lower at 13:00 h in the control than at 9:00 h (Fig. 8), which indicates that decline of \( P_n \) may be related to the damage of PSII reaction centre and D1 protein under strong light and high temperature via production of ROS such as \( \text{H}_2\text{O}_2 \) (Fig. 6A–6C). MDA, a product of lipid peroxidation, had been considered as an indicator of oxidative damage (Ji & Jiao, 2001). Contents of MDA and \( \text{H}_2\text{O}_2 \) showed slight increases at about 13:00 h, especially in the control (Fig. 5A); the MDA increment also could be associated with the increase of \( \text{H}_2\text{O}_2 \) content (Fig. 6D). As a result, the activities of APX, CAT and POD were...
triggered by the increase of H$_2$O$_2$ content. However, there was no difference detected in SOD activity between the control and the treatment (Fig. 5). The increases of CAT and POD activities in leaves under heavy metal or UV-B stress were also observed (Srivastava & Tel, 1992; Kondo & Kawashima, 2000). All these indicate that antioxidant enzymes could play a role in alleviating the photodamage of citrus leaves at midday. The fact that all the changes reflecting the damage of oxidative stress to PSII (such as H$_2$O$_2$) occurred in the present study (Fig. 6) when $g_s$ decreased below the suggested threshold (about 50 mmol H$_2$O m$^{-2}$ s$^{-1}$) is consistent with the notion that stomatal closure is an important factor for mid-depression of photosynthesis (Flexas et al., 2004). Recently, the relationship between stomatal closure at high light, generation of H$_2$O$_2$ and inhibition of photosynthesis through photoinhibition and enzyme oxidation were demonstrated (Zhou et al., 2007). Presumably, the reduction of thermal dissipation ability in citrus leaves at midday may be a consequence of a decreased ribulose 1,5-biphosphate (RuBP) regeneration (Lawlor & Corriol, 2002) and of ATP synthesis (Kanazawa & Kramer, 2002) that related to the $\Delta$PH trans-thylakoid membrane (Dilley, 2004; Krupenina & Bulychev, 2007).

Millisecond-delayed light emission of chloroplasts originating in PSII was related to a recombination process in the reaction centre where the transport of photoelectron formed to provide energy for ATP formation by pmf (Malkin, 1977; Avenson et al., 2005). Therefore, dynamics of chlorophyll ms-DLE reflect the proton motive force of thylakoid membrane for photophosphorylation. In the present study, the lower ms-DLE signal observed in control leaves after exposure to a moderate level of PPFD (1000 $\mu$mol m$^{-2}$ s$^{-1}$) indicates that excessive light energy probably has damaged thylakoid membranes of photosynthetic apparatus (Schrader et al., 2004; Murata et al., 2007). As the results showed, both fast and slow phases of ms-DLE in mist-sprayed leaves were higher than those in the control at two measuring temperatures (Fig. 9), which reveals that mist spraying not only reduced the dissipation of territorial proton around PSII but also alleviated the decrease of total proton grada-
tion. Apparently, mist spray has multiple benefits to the photosynthetic apparatus. The reduction of ALVPD maintained stomatal opening for CO$_2$ diffusion into leaves for photosynthesis. Moreover, higher pmf may trigger photoprotective processes of the photosynthetic reaction centres, such as xanthophyll cycle (Eskling et al., 1997; Takizawa et al., 2007).

In conclusion, the midday depression of photosynthesis in citrus is related to many environmental factors during midday, including high VPD, high temperature and high light. Although high temperature may directly decrease photosynthesis in citrus leaves, stomatal closure appears to be the main factors responsible for mid-depression of photosynthesis. Mid-depression of photosynthesis is associated with photoinhibition of PSI and degradation of D1 protein. Mist spraying is effective in alleviating mid-depression of photosynthesis in citrus leaves.

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