A Fogging System Improves Antioxidative Defense Responses and Productivity in Tomato

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ABSTRACT. Crops cultivated during summer in greenhouses and screen-covered structures (screenhouses) are negatively affected by stressful temperatures and vapor pressure deficit (VPD), which in turn influence yield and product quality. Fogging systems have been proposed as cooling methods to mitigate the adverse effects of high evaporation and excess temperatures in greenhouses. To evaluate the possible action of the fogging system on antioxidant response in cherry tomato (Solanum lycopersicum) fruit production, we studied the behavior of reactive oxygen species (ROS) scavenger enzymes such as superoxide dismutase, catalase, guaiacol peroxidase, enzymes involved in the ascorbate-glutathione cycle (Halliwell-Asada cycle), and compounds with antioxidant functions such as ascorbate, glutathione, proline, and polyamines. Fogging-screenhouse treatments, SF (a fogging system inside the screenhouse) and SFS (fogging system with a plastic sheeting, to maintain the microclimate created by the fogging system in the screenhouse) showed the best performance in terms of radiation with a percentage reduction of incident radiation on the crop of 18% and 37% and a mean reduction of maximum VPD values of 0.4 and 0.2 kPa, respectively, compared with the unfogging treatment (S). These improved environmental conditions, besides promoting the highest activities of ROS-scavenging enzymes and Halliwell-Asada cycle, the redox state of the ascorbate, and a low proline:free putrescine ratio, would explain the increase in commercial weight of fruit by 21% and 17% in SF and SFS, respectively, with respect to S.

ADDITIONAL INDEX WORDS. Solanum lycopersicum, environmental stress, climatic control strategies, oxidative stress

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Tomato, a horticultural annual with a worldwide distribution and enormous economic value, has beneficial effects on human health, offering high contents of natural antioxidants (Gómez-Romero et al., 2010). According to the Food and Agriculture Organization of the United Nations (2010), Spain harvested 4.3 million tonnes of tomato production, representing 25% of European production and placing it in the eighth position as the world producer with China, the United States, and India being the leading producers. The Mediterranean climate is characterized by long, hot summers (the ambient temperature exceeding 35°C at around midday in summer), high solar radiation flux (the daily solar radiation integral reaching 30 MJ·m⁻²), dusty and dry weather (relative humidity of the ambient air dropping below 10% at about noon), and limited water resources (Abdel-Ghany et al., 2012). These extreme environmental conditions induce numerous biochemical responses, including a proliferation of ROS, which disrupt the normal metabolism of plants, causing lipid peroxidation, protein denaturation, and DNA damage (Almeselmani et al., 2006). ROS content is controlled by an antioxidant system including low molecular antioxidant (ascorbate, glutation), and antioxidative enzymes such as superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX), and enzymes involved in the ascorbate–glutathione cycle (Halliwell-Asada cycle) ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), and glutathione reductase (GR) (Turhan et al., 2008). Increased activities of antioxidant enzymes develop plant tolerance and have also been reported in several higher plants under abiotic stresses (Yang et al., 2011). For a lettuce (Lactuca sativa) crop, exposure to strong light (1000 to 1200 μmol·m⁻²·s⁻¹) was associated with increased antioxidant capacity, manifesting as higher ascorbate (AsA) and glutathione (GSH) levels (Zhou et al., 2009). The activities of SOD, APX, GPX, and CAT increased after exposure to high temperature stress (30 to 38°C) in tomato plants (Chang et al., 2009). The combination of two environmental factors was studied by Havaux and Tard (1996) on tomato leaves subjected at 35°C for 2 h and short exposure to a radiation of 1000 μmol·m⁻²·s⁻¹ during 4 min. These researchers observed a rapid increase in thermostability of photosystem II to light. With respect to the effect that variations in climatic conditions trigger in oxidative metabolism in tomato fruit, Rosales et al. (2006) reported a decline in antioxidant enzymes such as APX and MDHAR, which augmented H₂O₂ and dehydroascorbate (DHA), respectively, in tomato fruit when light and heat stresses...
were superimposed, exceeding the optimum for tomato growth (temperatures often rise to 30 to 40 °C and light intensity can reach 1000 to 2000 μmol·m⁻²·s⁻¹).

Plant antioxidant defenses also depend on aminoated compounds such as proline (Pro) and polyamines (PAs), the accumulation of which is another of the most common stress responses in plants (Serraj and Sinclair, 2002). Environmental stress such as excess heat can induce the accumulation of Pro, which protects membranes and proteins against the adverse effects of temperature extremes by scavenging ROS at high concentrations in many plant species (Kaul et al., 2008). Rosales et al. (2007) demonstrated that the influence of high temperature and high solar radiation increased the Pro content as a result of diminished protein synthesis or increased hydrolysis to amino acids, coinciding with the highest values in malondialdehyde (MDA) content and lipo-oxygenase activity. PAs regulate the enzyme activities of plants (Asthir et al., 2012). These compounds may be good substrates for peroxidase activity by removing H₂O₂ in the apoplast and they could have a role in membrane stabilization because of an interaction with Ca²⁺ (Bouchereau et al., 1999). Cheng et al. (2009) reported a significant increase in activities of SOD, APX, GPX, and CAT in tomato plants that overexpressed the S-adenosylmethionine decarboxylase gene [which provides the aminopropyl groups to form spermidine (Spd) and spermine (Spm)] bolstering tolerance to high temperatures. Most research on the concentration of Pro and PAs in plants has been conducted on leaves, and only limited research is available on fruit. Questions remain about the role that PAs play in stress tolerance. The aim of the present work was to evaluate the microclimate in relation to tomato yield under different environmental conditions using fogging as well as combining fogging with additional plastic sheeting over the screenhouse and assessing the repercussions on oxidative metabolism, Pro metabolism, and free PA concentration in cherry tomato fruit from plants grown under a Mediterranean climate.

Materials and Methods

Plant material and growth conditions

Cherry tomato seedlings (cv. Alina) were grown for 30 d in a tray with wells 3 × 3 × 10 cm in a nursery (Semillero Saliplant S.L., Granada, Spain). Maxifort rootstock was grafted when the seedlings had developed three to four true leaves. Two weeks after grafting, the tomato plants were transferred to a screenhouse located in the Institute of Research and Training in Agriculture and Fisheries (IFAPA) Center Camino de Purchil, Granada, Spain (lat. 37°10'21" N, long. 3°38’10” W). The experiment was conducted from June to Nov. 2010. Plants were grown in soil at a density of 2.2 plants/m². Water and fertilizer were delivered by an automated drip irrigation system using a complete nutrient solution with an electrical conductivity (EC) of 1.9 dS·m⁻¹. The pH of the nutrient solution was measured daily and, when necessary, corrected with 1.0 M H₃PO₄ to maintain the pH at 6.2 to 7.0. The growth period was 152 d during the summer. Plants were grown in soil at a density of 2.2 plants/m². Water and fertilizer were delivered by an automated drip irrigation system using a complete nutrient solution with an electrical conductivity (EC) of 1.9 dS·m⁻¹. The pH of the nutrient solution was measured daily and, when necessary, corrected with 1.0 M H₃PO₄ to maintain the pH at 6.2 to 7.0. The growth period was 152 d during the summer. All lateral shoots were removed periodically. The plants were topped at the time that would allow complete last-truss maturation before the end of the production cycle because of the decline in temperature and radiation in October to November.

Measurements of environmental parameters

Over the entire fruit production cycle, air temperature (T°) and environmental humidity (RH) were measured using HMP45 probes (Vaisala, Helsinki, Finland) and VPD calculated from the VPD at 100% RH minus actual air vapor pressure measurement. Incident solar radiation was measured using pyranometer sensors (Model SKS1110; Skye Instruments, Llandrindod Wells, U.K.), situated in the screenhouse (Fig. 1). A data logger (CR-10; Campbell Scientific, Logan, UT) stored the average values for three measurements every 30 min.

The climatic parameters of incident radiation, air temperature, and VPD on a typical sunny day of the cycle were recorded. Data were expressed as the daily maximum T°, RH, and VPD from 1200 to 1800 hR. The daily integral radiation was calculated from the daily solar radiation data stored. The data shown were determined from anthesis to development and ripening of the fruit analyzed.

Experimental designs and fogging treatments

Treatments are described as a greenhouse structure covered with a black-and-white polyethylene monofilament screen of 9 × 6 strands/cm² from an enclosure called a screenhouse. Measurements were made in three distinct screenhouse compartments involving: 1) no environmental control; 2) active environmental control limited to a low-pressure fogging system; and 3) plastic sheeting coupled with a fogging system to prevent evaporative losses through the screenhouse (Fig. 1). The treatments began 22 d after transplanting (DAT) and were maintained as long as conditions allowed.

The cooling effect of the fogging system in the SF and SFS treatments was activated between 1200 and 1800 hR, when VPD was higher than 2.5 kPa inside the screenhouse. The density of low-pressure nozzles (7 L·h⁻¹) was 0.13 nozzles/m². The complimentary plastic sheeting had a high content of ethylene vinyl acetate and an average thickness of 200 μm. The plastic sheeting was deployed simultaneously with the fogging system (Fig. 1). In each sampling, the total yield, commercial yield, and the mean fruit weight were collected from the six center rows of plants. Uniformly ripe healthy fruit, at the red-ripe stage, were harvested at 87 DAT. Samples of fresh tissues from the tomato fruit were used to analyze the parameters described below.

Antioxidant metabolism

Concentrations of MDA and H₂O₂ in fruit extract. The MDA concentration was measured following the method of Heath and Packer (1968) and the result was expressed as absorbance at 532 to 600 nm per gram fresh weight. The H₂O₂ content of fruit samples was colorimetrically measured as described by Mukherje and Choudhuri (1983). The absorbance in the supernatant was measured at 415 nm. The result of H₂O₂ concentration was expressed as millimoles per gram fresh weight.

Antioxidant enzymatic activities in fruit extract. SOD (EC 1.15.1.1) activity was assayed by monitoring the inhibition of the photochemical reduction of nitroblue tetrazolium following the methods of Beyer and Fridovich (1987) with some modifications. SOD activity was expressed as the change in absorbance at 290 nm per minute per milligram of protein. CAT (EC 1.11.1.6) activity was determined by following the consumption of H₂O₂ at 240 nm for 5 min (Nakano and Asada, 1981). The GPX (EC 1.11.1.7) activity was determined by the increase in absorbance at 470 nm as a result of guaiacol oxidation (Nakano and Asada, 1981).
The enzymes APX (EC 1.11.1.11) and GR (EC 1.6.4.1) were assayed following Rao et al. (1996). APX activity was determined by registering the absorbance change at 290 nm. GR activity was measured after monitoring the oxidation of nicotinamide adenine dinucleotide phosphate at 340 nm for 3 min. APX activity was expressed as the change in absorbance at 290 nm per minute per milligram of protein, and GR activity was expressed as the change in absorbance at 340 nm per minute per milligram of protein. DHAR (EC 1.8.5.1) in fruit extracts was measured at 265 nm for 3 min after the change in absorbance resulting from the formation of AsA (Nakano and Asada, 1981). In addition, the enzyme MDHAR (EC 1.6.5.4) was assayed by registering the change in absorbance of the samples at a wavelength of 340 nm (Foyer et al., 1989).

**ASCORBATE AND GLUTATHIONE ASSAY.** The extraction and quantification of total AsA, reduced AsA, and DHA in fruit extracts followed the method of Law et al. (1992). The results were used to quantify the total AsA concentration, whereas the reduced AsA was quantified in the same way as the previous procedure, replacing 0.1 mL of dithiothreitol with 0.1 mL of distilled H₂O. Finally, the DHA concentration was deduced from the difference between total AsA and reduced AsA. The result of ascorbate forms was expressed as mmol per gram fresh weight. Reduced GSH, oxidized GSH (GSSG), and total GSH (reduced GSH + GSSG) were the recycling assay initially described by Noctor and Foyer (1998). GSH levels were estimated as the difference between total GSH and GSSG. The results were expressed as micromoles per gram fresh weight.
**Proline Metabolism.** For the determination of the free Pro concentration, leaves were homogenized in 5 mL of ethanol at 96%. The insoluble fraction of the extract was washed with 5 mL of ethanol at 70%. The extract was centrifuged at 3500 g for 10 min and the supernatant was preserved 4°C for the Pro determination (Irigoyen et al., 1992).

Glutamate dehydrogenase (GDH) activity was determined according to the method of Igarashi et al. (2006). Pyrroline-5-carboxylate synthetase (P5CS) extraction was carried out according to Sumithra et al. (2006). Leaves were homogenized with extraction buffer containing 100 mM Tris-HCl (pH 7.5), 10 mM β-mercaptoethanol, 10 mM MgCl2, and 1 mM phenyl methyl sulfonyl fluoride and then centrifuged at 10,000 g for 15 min. The supernatant was used for enzyme assays. For ornithine amine transferase [OAT (EC 2.6.1.13)] and proline dehydrogenase [PDH (EC 1.5.99.8)] extraction, leaves were homogenized in 100 mM K-phosphate buffer (pH 7.8). The homogenate was filtered and centrifuged at 12,000 g for 20 min (4°C) (Charest and Phan, 1990).

**Polyamine Analysis.** For the identification of PAs, 3 g of fresh leaves was homogenized in 4 mL of 6% (v/v) cold perchloric acid (PCA), kept on ice for 1 h, and then centrifuged at 21,000 g for 30 min. The pellet was extracted twice with 2 mL of 5% PCA and recentrifuged. The three supernatants were pooled and used to determine the levels of free PAs. The supernatant was benzoylated in accordance with the method of Aziz and Larher (1995). The high-performance liquid chromatography (HPLC) was carried out with ultraviolet detector analyses (HPLC/ultraviolet) were carried out using an Agilent 1100 series HPLC (Agilent, Waldbronn, Germany).

**Statistical analysis**

Data were analyzed using one-way analysis of variance and Fisher’s protected least significant difference test to separate means at the 0.05 P level.

**Results**

**Screenhouse Environmental Conditions and Their Effects on Yield.** Overall daily radiation was reduced by 18% with the use of a cooling system (SF), achieving reductions of 32% with the installation of plastic sheeting over the fogging system (SFS) (Fig. 2A). After starting the fogging (1200 hr), the maximum temperature in SF declined 2°C with respect to S and this level remained stable throughout the functioning period (Fig. 2B). However, SFS rose 2°C, surpassing 35°C (Fig. 2B). In terms of maximum daily VPD, SF diminished 0.8 kPa, whereas SFS was 0.5 kPa (Fig. 2C). Table 2 presents the time course of the integral of incident solar radiation, the maximum
The mean value of integral solar radiation into the screenhouse during the fog operation (1200 to 1800 HR) was \( \approx 16 \text{ MJ} \cdot \text{m}^{-2} \cdot \text{d}^{-1} \), this being 18% higher than in SF and 32% higher than in SFS (Table 1). The use of the fogging system (SF) lowered temperatures 1 °C with respect to the maximum \( T^a \) in S, whereas the SFS treatment registered the highest value [35 °C (Table 1)]. With respect to the maximum percent RH, in the fogging treatments, SF and SFS, the mean value of this parameter increased by 9% and 6%, respectively, over the study period as opposed to that of S (Table 1). The maximum VPD value was reached in S followed by SFS and SF with a reduction of 0.2 and 0.4 kPa, respectively (Table 1).

In SF, the total cherry tomato yield over the warm period was 20% and 15% higher than in S and SFS, respectively (Table 2). Commercial yield presented the same dynamics, registering the maximum value in SF, an intermediate value in SFS, and the lowest value in S (Table 2). Fresh weight of SF fruit increased by \( \approx 27\% \) and 16% more than S and SFS fruit, respectively (Table 2). Fruit subjected to the SF treatment presented the highest values in fruit dry weight, surpassing by 10% the weight of the fruits of other treatments (Table 2).

**Concentrations of \text{H}_2\text{O}_2 \text{ and MDA in fruit extract.}** Environmental stress increased oxygen-induced cell damage, causing lipid peroxidation in membranes measured by MDA content. In our work, the \text{H}_2\text{O}_2 \text{ concentration significantly increased in the S treatment by 22% and 33% in comparison with the SF and SFS treatments, respectively} (Fig. 3A). MDA content indicated a significant increase in the S treatment of 21% and 32% in relation to SF and SFS, respectively (Fig. 3B). Fruit subjected to the SF treatment presented the highest values in fruit dry weight, surpassing by 10% the weight of the fruits of other treatments (Table 2).

**HALLIWELL-ASADA CYCLE.** The SFS reached maximum values in the enzymes of the APX, DHAR, and GR cycles (Figs. 5A, 5C, and 5D). However, the treatments S and SF showed a similar trend in APX, DHAR, and GR activity, consistently presenting lower activity than in SFS (Figs. 5A, 5C, and 5D). On the contrary, no significant differences were detected in the MDHAR activity (Fig. 5B).

The total AsA content measured in tomato fruit represented an increase of 7% and 17% in S compared with that in SF and SFS, respectively (Table 3). The reduced AsA content was more pronounced in the SF treatment followed by S and least for SFS (Table 3). On the contrary, the DHA content showed a significant decline in SF as opposed to S, whereas SFS registered an intermediate value (Table 3). Thus, the redox state, measured as the ratio reduced ASA:total ASA in the tomato samples, proved higher in SF with 44% of the total reduced AsA (Table 3), whereas the reduced AsA content represented only 34% of the total AsA in the S treatment and 30% in SFS (Table 3).

GSH acts as a disulphide reductant to protect thiol groups on enzymes, regenerate ascorbate, and to react with singlet oxygen and hydroxyl radicals. In the present work, none of the different GSH forms presented significant differences in their concentration in any of the treatments studied (Table 4). Therefore, the redox state in relation to the GSH content showed no significant differences, either, in any treatment (Table 4).

**Proline metabolism and free \text{PAs} concentration.** The content of Pro showed a decrease of \( \approx 40\% \) at this amino acid level in tomato samples subjected to different environmental conditions (Table 5). Pro metabolism and accumulation depended on its synthesis by GDH, P5CS, and OAT activities and also the degradation activity by PDH. These activities are shown in Figure 5E–H. S treatment showed the highest levels of synthesis enzymes, GDH and P5CS, with an increase of 66% and 51% compared with SFS (Fig. 5E–F). However, OAT activity reached its highest level in the treatment SFS with a rise of 28% compared with S and SF that registered the lowest activity (Fig. 5G). Degradative activity caused by PDH increased significantly in SFS with respect to SF and S, the latter falling by more than 50% (Fig. 4C).

### Table 1. Average climate data during the month preceding the harvest of tomato fruit grown under a screenhouse (S), screenhouse and fogging system (SF), and screenhouse with a fogging system and plastic sheeting (SFS).

| Treatment | Integral radiation [mean ± se (MJ m\(^{-2}\) d\(^{-1}\)] | Avg maximum air temp [mean ± se (°C)] | Avg maximum RH [mean ± se (%)] | Avg maximum VPD [mean ± se (kPa)] |
|-----------|-------------------------------------------------|-------------------------------------|-------------------------------|----------------------------------|
| S         | 16.0 ± 0.3                                      | 33.4 ± 0.5                          | 62.7 ± 2.9                    | 3.5 ± 0.2                        |
| SF        | 13.1 ± 0.3                                      | 32.4 ± 0.3                          | 72.1 ± 2.6                    | 3.1 ± 0.1                        |
| SFS       | 16.0 ± 0.3                                      | 33.4 ± 0.5                          | 62.7 ± 2.9                    | 3.5 ± 0.2                        |

\( ^a \text{Differences between means were compared by Fisher’s least significant test} (P = 0.05). \text{Means followed by the same letter in the same column do not differ significantly.} \)

### Table 2. Mean values for total yield, commercial yield, average, and dry weight of tomato fruit grown under a screenhouse (S), screenhouse and fogging system (SF), and screenhouse with a fogging system and plastic sheeting (SFS).

| Treatment | Total yield [mean ± se (Mg ha\(^{-1}\))]| Commercial yield [mean ± se (g/fruit)]| Fruit dry wt [mean ± se (%)] |
|-----------|----------------------------------------|---------------------------------------|-----------------------------|
| S         | 11.62 ± 0.56 b\(^*\)                   | 11.29 ± 0.54 b                        | 8.93 ± 0.02 b               |
| SF        | 14.55 ± 1.15 a                         | 14.46 ± 1.14 a                        | 9.94 ± 0.32 a               |
| SFS       | 12.38 ± 0.98 ab                        | 12.10 ± 0.95 ab                       | 8.90 ± 0.11 b               |

\( ^a \text{Differences between means were compared by Fisher’s least significant test} (P = 0.05). \text{Means followed by the same letter in the same column do not differ significantly.} \)
reached maximum activity in the treatment SFS and SF (Fig. 5H), whereas S decreased by ≈60% compared with other treatments (Fig. 5H).

Free PAs were measured in tomato fruit samples under different environmental conditions (Table 5). Putrescine (Put) concentration did not show significant differences among any of the treatments. Nevertheless, Put was the most free PA in our work, being the only amine form detected in S treatment with a concentration of 77% and 52% on total free PAs in SF and SFS, respectively. Spd significantly increased in the SFS treatment with a rise of 60% over the second treatment with the highest values (SF), whereas S was not detected in any of the PA concentrations. Spm was not detected in tomato samples. The ratio Pro:free Put significantly increased in S to 30% higher than fogging treatments, SF and SFS, which registered the lowest ratio of these amino compounds (Table 5).

**Discussion**

Vegetative development of crops grown in greenhouses under Mediterranean conditions is subject to stressful environmental conditions as a result of high incident radiation levels (Lorenzo et al., 2003). These environmental variables can severely limit crop productivity as well as quality (Rosales et al., 2006). Most reports focus on explaining how shading, ventilation, and evaporative cooling affect the greenhouse microclimate (Kittas et al., 2001), demonstrating that shading (Baille et al., 2001) and evaporative cooling (Katsoulas et al., 2009) are effective in relieving greenhouse heat load under dry and sunny summer conditions in the Mediterranean area. In the present study, SF and SFS showed 18% and 32% reductions in radiation, respectively, in comparison with S (Table 1). The dispersion of water in the air by fogging reduced the maximum mean T°a from 33.4 to 32.4 °C in the case of SF. However, an increase of 2.2 °C was recorded for SFS (Table 1) as a result of the strengthening of the greenhouse effect and/or the lack of ventilation after the installation of the plastic sheeting over the fogging system. The maximum mean VPD of SF and SFS was 3.1 and 3.3, respectively, compared with 3.5 kPa in the S treatment (Table 1).

Katsoulas et al. (2009) concluded that fogging affected the weight of marketable fruit more significantly (by nearly 14%) when greenhouse air was cooled compared with non-cooled conditions. That is, the fresh average weight of SF fruit was ≈27% and 16% higher than that of S and SFS fruit, respectively, and the S treatment also manifested the higher value dry weight of tomato fruit (Table 2). This finding coincided with the lowest temperature and VPD values (Tables 1 and 2).

It is well known that environmental conditions affect the oxidative metabolism in tomato fruit. Under low radiation conditions, with VPD values inside the screenhouse below 3.3 kPa, showed lower H2O2 and MDA concentrations in SF and SFS treatments than appeared in S (Fig. 3A–B), where fruit were subjected to high radiation conditions (Table 1). Plants subjected to abiotic stress tended to overproduce ROS in different plant tissues (Pinheiro et al., 2004). SOD, CAT, and GPX, among other enzymes, constitute the first line of defense, detoxifying O_2^- radicals and removing the H_2O_2 production contributing to the antioxidant defense system (Candan and Tarhan, 2012). Lipid peroxidation was not induced by H_2O_2 formation (SOD activity) in the S treatment, but rather possibly by slow detoxification, as confirmed by CAT and GPX activities (Figs. 3 and 4A). The GPX activity (Fig. 4C)
proved inversely proportional to the lipid peroxidation (Fig. 3), because the minimum GPX activity in treatment S (Fig. 4C) coincided with the maximum accumulation of H2O2 and MDA (Fig. 3A–B). In short, these results suggest that the unfavorable environmental conditions in treatment S, especially attributed to solar radiation, VPD, and RH, impeded GPX activation, which appeared in our work as one of the key H2O2-detoxification processes in cherry tomato fruit.

The Halliwell-Asada cycle constitutes an important detoxification pathway for dissipating H2O2 and other reactive oxygen radicals in chloroplasts (Sgherri et al., 2003). In our work, as occurred for GPX activity (Fig. 4C), the SFS treatment registered the maximum values for the enzymes of the Halliwell-Asada cycle: APX, DHAR, and GR (Figs. 5A, 5C, and 5D). This would explain the low H2O2 concentration and therefore the minimum lipid peroxidation found in this treatment (Fig. 3). The increase in these enzymatic activities in SFS may be the result of the temperature reached in this treatment, which exceeded 35°C (Table 1). Rosales et al. (2006) observed an increase in the Halliwell-Asada cycle enzymes such as APX, DHAR, ascorbate oxidase and, to a lesser extent, MDHAR in a cherry tomato crop when maximum Ta exceeded 35°C.

The ascorbate detoxification system removed greater amounts of H2O2 and thereby prevented this compound from reaching high toxicity levels by accumulating in plant tissues. Therefore, higher levels of AsA were maintained with lower levels of membrane damage than in control plants after exposure to strong light and high temperatures in Arabidopsis thaliana plants (Wang et al., 2010). Thus, the content in reduced AsA in SF was greater than in the rest of the screenhouse treatments (Table 3). Also, Jimenez et al. (2002) have postulated that the redox state (reduced AsA:total AsA) increased as tomato fruit matured, implying less demand for reduced AsA (SFS) and/or a more efficient reduction of oxidized DHA in red-ripe fruit as occurred in the SF treatment (Table 3). In conclusion, a high ratio of reduced AsA to total AsA is essential to eliminate ROS in cells, and the SF treatment presented the best redox state in all the treatments, given that 44% total AsA content was found in the reduced form (Table 3).

A key plant strategy used for adaptation to damaging abiotic factors is stress-induced accumulation of other low-molecular metabolites as would be the case of Pro and PAs (Radyukina et al., 2010).

Biochemical studies indicate that Pro biosynthesis through the glutamate pathway dominates under stress conditions (Kavi Kishor et al., 2005). Pro accumulation was highest in the S treatment as a result of GDH, whereas P5CS increased and PDH activity decreased (Table 5; Fig. 5E–H). On the other hand, SF and SFS treatments underwent a reduction in P5CS activity and stimulation of OAT activity, this being described by Ruiz et al. (2002) and Sánchez et al. (2001) as a resistance mechanism for tolerating several types of environmental stress (Fig. 5F–G). This fact represented a reduction of 40% in Pro concentration against S treatment (Table 5). Pro has been demonstrated to scavenge hydroxyl radicals and singlet oxygen, thus providing protection against ROS-induced cell damage (Matysik et al., 2002). Fruit samples subjected to S treatment showed the highest Pro values, coinciding with a rise in H2O2 and MDA levels (Table 5; Fig. 3). Hence, several authors have observed that Pro accumulation is not an adaptive feature but rather only a stress symptom (Sánchez-Rodríguez et al., 2010). Pro degradation could improve the energy status by being directly

Fig. 5. (A) Ascorbate peroxidase (APX), (B) monodehydroascorbate reductase (MDHAR), (C) dehydroascorbate reductase (DHAR), (D) glutathione reductase (GR), (E) glutamate dehydrogenase (GDH), (F) pyrroline-5-carboxylate synthetase (P5CS), (G) ornithine aminotransferase (OAT), and (H) proline dehydrogenase (PDH) activities on tomato fruit extract in a screenhouse (S), screenhouse and fogging system (SF), and screenhouse with a fogging system and plastic sheeting (SFS). Vertical bars indicate mean values ± SE (n = 12) and differences between means were compared by Fisher’s least significant difference test (P = 0.05). Means followed by the same letter do not significantly differ.
coupled with the respiratory electron transport system and adenosine-5'-triphosphate (ATP) production (Lawlor, 1995). In addition, PDH activity could act as an effective response mechanism against oxidative stress, directly participating in detoxifying ROS and reducing their formation (Rosales et al., 2007). The climate control achieved by the cooling system provided in the treatment SF an increase of 47%, which increased further with the effect of the plastic sheeting in SFS with 13% in the activity of PDH in tomato samples subjected to such conditions, showing a positive correlation between PDH activity and reduced ROS concentration in treatments SF and SFS (Figs. 3 and 5H).

Enhanced tolerance is always correlated with elevated levels of PAs such as Put and/or Spd and Spm (Alcázar et al., 2010). Flores and Galston (1982) proposed accumulated PA concentration as a mechanism to alleviate osmotically stressed tissues, regulating water losses by increasing intracellular osmolality. Nevertheless, Bais and Ravishankar (2002) defined Put accumulation as the cause of stress-induced injury and could have drastic consequences for the regulation of nitrogen metabolism, protein synthesis, and the maintenance of cell pH as well as ion homeostasis. In the present study, the Put pool is turned to Spd synthesis in SF and SFS treatments (Table 5). In the screenhouse under the Mediterranean climate (S), it appears to prompt characteristic metabolic changes during which Put accumulates to high concentrations in tomato samples, whereas in SF and SFS treatments, PA levels remain essentially balanced, increasing the Spd concentration (Tables 1 and 5). According to Cheng et al. (2009), heat-stressed tomato plants with higher free Spd and Spm levels were accompanied by a pronounced increase in antioxidant enzyme activity and lower MDA levels (Tables 1 and 5; Figs. 3 and 5). Aziz and Larher (1995) postulated that an accumulation of Put and the maintenance of a high level of free Spd in rape (Brassica napus) leaf improved response to low water potential under osmotic stress. The free Put and Spd concentration and total free PAs in SF and SFS fruit alleviated osmotic stress caused by these stressful environmental conditions, providing high tomato weight per fruit with a gain of 27% and 14%, respectively (Tables 1, 2, and 5).

A competitive correlation occurs in the synthesis pathways of PAs and Pro for a common precursor, glutamate, especially while plants are under severe stress (Tonon et al., 2004). Zhao et al. (2001) showed an increase in Pro and a decrease in PAs when barley (Hordeum vulgare) plants were subjected to salt stress. This hypothesis is supported by Santa-Cruz et al. (1999), who reported that tomato plants under salinity had lower Put contents and Pro accumulated instead. Similarly, Delauney and Verma (1993) demonstrated that a flux through these competing pathways is extensively altered in favor of the Pro route, especially through γ-glutamyl-P, glutamate semialdehyde, and pyrroline-5-carboxylate (GDH and P5CS activities) in moth bean (Vigna aconitifolia) submitted to salt stress. Under summer stress conditions in the Mediterranean area, S treatment resulted in a high Pro:free Put ratio, precisely with high GDH and P5CS activities, whereas fog system treatments (SF and SFS) showed the lowest relation (Tables 1 and 5; Fig. 5E–F). Su and Bai (2008) also observed that a negative correlation between Pro and PA contents might be related to the conversion of PA degradation product to Pro, contributing to α-ketoglutarate, ATP, and nicotinamide dinucleotide production needed for the synthesis of Pro (Mutlu and Bozcuk, 2007).

**Conclusion**

Crops cultivated during summer in the Mediterranean region are negatively affected by stressful environmental conditions, which in turn influence yield and product quality. In this work, we demonstrate that, in a cherry tomato crop grown in a screenhouse during summer in a Mediterranean basin, a fogging system and plastic sheeting coupled with a fogging system improved the climatic conditions by reducing incident solar radiation and VPD. SFS registered a higher maximum air temperature causing the highest levels of total free PAs and Spd to be accompanied by a pronounced surge in antioxidant enzyme activity such as GPX and Halliwell-Asada cycle (APX, DHAR, and GR) causing lower MDA levels. On the contrary, tomato fruit in an unfogged treatment (S) presented a higher level of Pro and a low induced oxidative metabolism, which resulted in ROS accumulation. Therefore, we propose to

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**Table 3.** Total ascorbate (AsA), reduced AsA, dehydroascorbate (DHA), and redox state [AsA + (AsA + DHA)] in tomato fruit grown under a screenhouse (S), screenhouse and fogging system (SF), and screenhouse with a fogging system and plastic sheeting (SFS).

| Treatment | Total AsA [mean ± SE (mmol g⁻¹ fresh weight)] | Reduced AsA [mean ± SE (mmol g⁻¹ fresh weight)] | DHA [mean ± SE (mmol g⁻¹ fresh weight)] | [AsA + (AsA + DHA)] [mean ± SE (redox ratio)] |
|-----------|-----------------------------------------------|-----------------------------------------------|----------------------------------------|-----------------------------------------------|
| S         | 4.34 ± 0.21 a*                                | 1.48 ± 0.09 b                                | 2.85 ± 0.14 a                          | 0.34 ± 0.01 b                                |
| SF        | 4.10 ± 0.11 a                                 | 1.79 ± 0.10 a                                | 2.25 ± 0.14 b                          | 0.45 ± 0.01 a                                |
| SFS       | 3.60 ± 0.12 b                                | 1.11 ± 0.04 c                                | 2.49 ± 0.12 ab                         | 0.38 ± 0.02 b                                |

* Differences between means were compared by Fisher’s least significant test (P = 0.05). Means followed by the same letter in the same column do not differ significantly.

**Table 4.** Total glutathione (GSH), reduced GSH, glutathione disulfide (GSSG), and redox state [GSH + (GSH + GSSG)] in tomato fruit grown under a screenhouse (S), screenhouse and fogging system (SF), and screenhouse with a fogging system and plastic sheeting (SFS).

| Treatment | Total GSH [mean ± SE (µmol g⁻¹ fresh weight)] | Reduced GSH [mean ± SE (µmol g⁻¹ fresh weight)] | GSSG [mean ± SE (µmol g⁻¹ fresh weight)] | [GSH + (GSH + GSSG)] [mean ± SE (redox ratio)] |
|-----------|-----------------------------------------------|-----------------------------------------------|------------------------------------------|-----------------------------------------------|
| S         | 4.50 ± 0.35 a*                                | 2.91 ± 0.32 a                                | 1.59 ± 0.10 a                           | 0.64 ± 0.02 a                                |
| SF        | 4.10 ± 0.11 a                                 | 2.43 ± 0.12 a                                | 1.68 ± 0.06 a                           | 0.59 ± 0.02 a                                |
| SFS       | 4.01 ± 0.37 a                                 | 2.36 ± 0.31 a                                | 1.65 ± 0.11 a                           | 0.52 ± 0.05 a                                |

* Differences between means were compared by Fisher’s least significant test (P = 0.05). Means followed by the same letter in the same column do not differ significantly.
define the low ratio Pro:free Put as an effective as a biomarker of environmental stress in cherry tomato fruit; it implies a better balance in the water and antioxidant response on tomato fruit.

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