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Methyl salicylate treatments of sweet cherry trees increase antioxidant systems in fruit at harvest and during storage

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ABSTRACT

Sweet cherry trees (*Prunus avium* L.) were treated with methyl salicylate (MeSa) at 1 mM at 3 key events on fruit development on-tree to analyze the effect of MeSa on bioactive compounds, total antioxidant activity (TAA) and antioxidant enzymes at harvest and during 28 days storage. The experiments were performed during two consecutive years, by using two sweet cherry cultivars, 'Sweet Heart' and 'Sweet Late' in 2013 and another more cultivar, 'Lapins', in 2014. Both total phenolics and anthocyanins content were significantly higher in MeSa-treated than in control fruit at harvest and during storage, leading to fruit with higher hydrophilic TAA (H-TAA). The activity of the antioxidant enzymes catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX) and superoxide dismutase (SOD) decreased along storage for all cultivars and experimental years, although those fruit treated with MeSa exhibited higher activities of these antioxidant enzymes than controls. Thus, MeSa treatment of cherry trees could increase health-promoting properties of cherry fruit consumption, due to its effect on increasing antioxidant and bioactive compounds, with additional effect on delaying the fruit postharvest senescence process by increasing the activity of the enzymes involved in reactive oxygen species (ROS) scavenging.

1. Introduction

Methyl salicylate (MeSa) is a volatile plant compound synthesized from salicylic acid having a role in plant growth and development, plant defense-mechanism, plant responses against several abiotic stresses as well as in the fruit ripening process (Hayat and Ahmad, 2007; Kumar, 2014). Thus, postharvest treatment with exogenous MeSa decreased chilling injury (CI) of tomato (Fung et al., 2006), sweet pepper (Fung et al., 2004) and mango (Han et al., 2006) fruits by protecting cell wall structure and cell membranes from dysfunction caused by lipid peroxidative injury. Accordingly, in pomegranate fruit, postharvest treatment with MeSa reduced significantly the CI symptoms, by maintaining membrane structure and its selective permeability leading to lower values of electrolyte leakage. In addition, other parameters related to fruit quality, such as fruit firmness, total soluble solids and total acidity were also maintained in MeSa treated fruit while significant losses occurred in control pomegranates (Sayyari et al., 2011a). Moreover, the content of total phenolics and total anthocyanins as well as the antioxidant activity in the arils increased along storage, although these increases were significantly higher in MeSa-treated than in control pomegranates, showing that MeSa has potential postharvest applications for reducing CI, maintaining quality, and improving the health benefits of pomegranate fruit consumption by increasing its antioxidant capacity and bioactive compounds (Sayyari et al., 2011a; Valero et al., 2015).

Reactive oxygen species (ROS), such as superoxide radical ($O_2^{\bullet -}$), peroxide radical ($O_2^{\bullet 2-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet -}$), are inevitably generated in plant cells as a consequence of normal metabolism, mainly in reactions catalyzed by oxidase and lipoxygenase and in β-oxidation of fatty acids. The ROS content in plant cell is dependent on their producing systems and scavenging mechanism, both enzymatic and non-enzymatic ones (Apel and Hirt, 2004). Non-enzymatic antioxidant compounds are reduced forms of ascorbate and glutathione, tocopherols, phenolics, alkaloids and carotenoids, while enzymatic scavenging mechanisms include mainly superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX). SOD detoxifies $O_2^{\bullet -}$ free radicals by converting them to O_2 and H_2O_2, which is further converted to H_2O and O_2 by CAT, APX and POD. CAT catalyzes the decomposition of hydrogen peroxide to

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water and oxygen, while APX uses ascorbate and H_2O_2 as substrates producing water and dehydroascorbate as products, the last one being converted to ascorbate by glutathione reductase enzyme. In addition, H_2O_2 can be also reduced to water by POD, by using organic molecules such as phenols as electron donor (Tareen et al., 2012). However, in spite of the presence of these efficient antioxidant systems, oxidative damages still occur in plant cells either due to uncontrolled production or inefficient scavenging of ROS. Thus, since the overall process of fruit ripening is considered as a functionally modified protracted form of senescence, associated with ROS accumulation (Hodges et al., 2004), the presence of high content of antioxidant compounds and high activity of antioxidant enzymes could lead to a delay of the fruit postharvest ripening process and to maintain fruit quality attributes for longer periods (Mondal et al., 2009; Kumar et al., 2014).

In this sense, in fruits such as mango and sugar apple, it has been shown that postharvest salicylic acid (SA) treatments led to lower superoxide free radical production and lipoxygenase activity and increases in the activity of SOD, CAT, and APX antioxidant enzymes during storage as compared with control fruit (Ding et al., 2007; Mo et al., 2008). Similar increases in these antioxidant enzymes were observed in peach fruit after postharvest SA treatment (Tareen et al., 2012). Even in sweet cherry, treatments with SA three days before harvesting or after harvesting increased POD activity and its transcript levels, which was related to the reduced fungal infection found in SA-treated fruit, especially when SA was applied to fruit at earlier maturity stages (Yao and Tian, 2005; Chan et al., 2008).

In previous reports, we have found that postharvest treatments of sweet cherry with SA or acetylsalicylic acid (ASA), which are close analogues of MeSa, delayed the postharvest ripening process, manifested by lower color changes and acidity and firmness losses, and maintained higher content of bioactive compounds and antioxidant activity during storage as compared with control fruit (Valero et al., 2011). Moreover, treatments of sweet cherry trees with SA or ASA, by foliar spray, during on-tree cherry growth and ripening, increased fruit weight and quality attributes (such as color and firmness) at commercial harvest, as well as the concentration of total phenolics and anthocyanins, which led to fruits with higher antioxidant activity, in both hydrophilic and lipophilic fractions (Giménez et al., 2014). By other hand, similar MeSa treatments of sweet cherry trees also increased fruit size and quality properties, such as firmness and TSS, at time of harvesting, showing also a significant effect on maintaining sweet cherry organoleptic properties along storage (Giménez et al., unpublished data). However, as far as we know, there is no scientific literature about the possible effect of MeSa treatment, either at pre- or postharvest application, on sweet cherry antioxidant systems. Then, the aim of this research was to evaluate for the first time the effect of preharvest MeSa treatments of sweet cherry trees on antioxidant compounds and the activity of the antioxidant enzymes SOD, CAT, POD and APX, at harvest and during prolonged cold storage. To achieve these objectives, the experiments were performed in two consecutive years, 2013 and 2014, by using two and three cherry cultivars, respectively.

2. Materials and methods

2.1. Plant material and experimental design

The experiments were performed in two consecutive years, 2013 and 2014 spring–summer periods. In 2013, two sweet cherry cultivars, 'Sweet Heart' and 'Sweet Late', cultivated in a commercial plot from "Fincas Toli S.L." located at Jumilla (38.473800 N, −1.323861 W, Murcia, Spain) were used and in 2014 another cultivar, 'Lapins', cultivated in a commercial plot for "Cerezas Aitana" located at Alcoy (38.780634 N, −0.443124 W, Alicante, Spain) was also used. Both locations are close each other with similar environmental and climatic conditions, and cherry tress grown under similar cultural practices. All cultivars are grafted on the 'Santa Lucía 64' rootstock and planted at 3 × 4 m. Three trees were selected completely at random for each cultivar and treatment: control (distilled water), and MeSa at 1.0 mM. This MeSa concentration was shown as appropriate in terms of increased fruit quality attributes (Giménez et al., unpublished data). Freshly prepared solutions (containing 0.5% of Tween 20) were foliar sprayed with a mechanical mist sprayer (7.5 L/tree) and repeated at three dates of the growth cycle, which corresponded to key events in fruit developmental process, according to previous experiments (Díaz-Mula et al., 2009; Giménez et al., 2014): T1 (at pit hardening), T2 (initial color changes) and T3 (onset of ripening). Sweet cherry fruit were harvested at commercial ripening stage which corresponded to S2 stage according to Serrano et al. (2009), and immediately transferred to the laboratory. Then, 90 fruits, homogeneous in color and size and without visual defects were selected from each tree or replicate, cultivar, year and treatment and randomly grouped in 3 lots of 30 fruits, which were stored in a cold room at normal atmosphere at 2 °C and RH of 85%. One lot was taken at random from each replicate after 0, 14 and 28 days of cold storage and transferred for 1 day at 20 °C and RH of 70%, afterward the analytical determinations were performed. The edible portion of the fruit from each lot was cut in small pieces to obtain a homogeneous sample, frozen in liquid N_2, mixed and stored at −20 °C until total anthocyanins, total phenolics, antioxidant activity, in both hydrophilic and lipophilic fractions and SOD, CAT, POD and APX activities were determined in duplicate for each sample.

2.2. Total phenolics, total anthocyanins, total carotenoids and total antioxidant activity determination

Phenolic extraction was performed by using 2 g of frozen tissue and 10 mL of water:methanol (2:8) containing 2 mM NaF (to inactivate polyphenol oxidase activity and prevent phenolic degradation) as previously described (Serrano et al., 2009) and quantified using the Folin–Ciocalteu reagent and results (mean ± SE) were expressed as mg gallic acid equivalent 100 g^{-1} fresh weight (FW). Total anthocyanins were extracted and determined according to previously reported (Serrano et al., 2005) and calculated as cyanidin 3-glucoside equivalent (molar absorption coefficient of 23,900 L cm^{-1} mol^{-1} and molecular weight of 449.2 g mol^{-1}) and results (mean ± SE) expressed as mg 100 g^{-1} FW. Total carotenoids were extracted according to Valero et al. (2011). Briefly, 2 g of sweet cherry fruit were extracted with acetone and shaken with diethyl ether and 10% NaCl for separation of the two phases. The lipophilic phase was washed with Na_2SO_4 (2%), saponified with 10% KOH in methanol, and the pigments were subsequently extracted with diethyl ether, evaporated and then made up to 25 ml with acetone. Total carotenoids were estimated by reading the absorbance at 450 nm and expressed as mg of β-carotene equivalent 100 $^{-1}$ FW, taking into account the $\varepsilon^{1\%}$cm = 2,560 and results were the ±SE.

TAA was quantified as previously described (Serrano et al., 2009) by homogenizing 2 g of tissue in 10 mL of 50 mM Na-phosphate buffer at pH 7.8 and 3 mL of ethyl acetate. After centrifugation at 10,000 g for 15 min at 4 °C, rhe upper fraction was used to quantify total antioxidant activity due to lipophilic compounds (L-TAA) and the lower for total antioxidant activity due to hydrophilic compounds (H-TAA), in a reaction medium containing 50 μL of the extract, 2 mM of the chromophore 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium

salt, 15 μM H_2O_2, and 25 μM horse radish peroxidase enzyme in 50 mM Na-phosphate buffer (pH 7.8) or methanol, for H-TAA and L-TAA, respectively, in a total volume of 1 mL. A calibration curve was performed with Trolox ((R)-(+)-6-hydroxy-2,5,7,8-tetramethyl-croman-2-carboxylic acid) (0–20 nmol) from Sigma (Madrid, Spain), and the results (mean ± SE) are expressed as mg of Trolox equivalent 100 g^{-1} FW.

2.3. Antioxidant enzymes

Crude extract for SOD, CAT, APX and POD enzymes was performed by homogenizing 5 g of frozen samples with 10 mL of phosphate buffer 50 mM, pH = 7.0 containing 1% (w/v) polyvinylpyrrolidone and 1 mM ethylenediaminetetraacetic acid (EDTA). The homogenate was centrifuged at 15,000 × *g* for 30 min at 4 °C and the supernatant used for enzyme assay. POD was determined as previously reported (Zapata et al., 2014), in a reaction mixture containing 50 mM phosphate buffer pH = 7.0, 12 mM H_2O_2, 14 mM guaiacol and 100 μL of enzymatic extract in a total volume of 3 mL. The increase of absorbance at 470 nm, due to guaiacol oxidation, was measured for 1 min. One enzymatic unit (*U*) was defined as 0.01 absorbance increase per minute, and peroxidase activity expressed as U min^{-1} mg $protein^{-1}$. For CAT and APX activities, the protocol described by Zhang et al. (2013) was used. In brief, for CAT, 100 μL of the above extract were added to 2.9 mL of reaction mixture containing 15 mM H_2O_2 and 50 mM phosphate buffer pH = 7.0. The degradation of H_2O_2 was measured by the decrease of absorbance at 240 nm during 1 min. One enzymatic unit (*U*) was defined as 0.01 absorbance decrease per minute, and CAT activity expressed as U min^{-1} mg $protein^{-1}$. For APX quantification the assay mixture contained 50 mM potassium phosphate pH = 7.0, 0.5 mM ascorbic acid and 1 mM H_2O_2. The decrease of absorbance at 290 nm during 1 min was measured. One enzymatic unit (*U*) was defined as the amount of enzyme that oxidizes 1 μmol of ascorbate per minute, and APX activity expressed as U min^{-1} mg $protein^{-1}$. SOD activity was determined photochemically as described in Zhang et al. (2013) with slight modifications. The reaction solution contained 50 mM phosphate buffer, pH 7.8, 5 mM methionine, 100 μM EDTA and 65 μM nitro-blue-tetrazolium (NBT). To 2.9 mL of this solution were added 25 μL of enzyme extract and 40 μL of 0.15 mM riboflavin. The tubes were then placed in a fluorescent light incubator (40 W, 10 min) and the formation of blue formazan was monitored by recording the absorbance at 560 nm. One unit (U) of SOD activity is defined as the amount of enzyme that causes a 50% inhibition of NBT reduction under assay conditions. The results are reported as U mg $protein^{-1}$. For all antioxidant enzyme activities results are the mean ± SE. Total protein content in the enzyme extract was quantified according to Bradford (1976).

2.4. Statistical analysis

The experiments were carried out by using a completely randomized design. Experimental data from each cultivar and year were independently subjected to ANOVA. Sources of variation for each cultivar and year were treatment and storage time. Mean comparisons were performed using HSD the Duncan's multiple test to examine if differences were significant at $p < 0.05$. All analyses were performed with SPSS software package v. 22.0 for Windows (SPSS, 2011). Correlations were performed between H-TAA and total anthocyanins or total phenolics and between L-TAA and carotenoids concentration.

3. Results and discussion

3.1. Phenolics, anthocyanins and antioxidant activity

It has been reported that total phenolic concentration in sweet cherry fruit increases during the last weeks of fruit development on tree, reaching final values ranging from 80 to 200 mg 100 g^{-1}, depending on cultivar, ripening stage, agronomic practices and environmental conditions (Serrano et al., 2005; Usenik et al., 2008; Díaz-Mula et al., 2009; Ballistreri et al., 2013). As it is shown in Fig. 1,

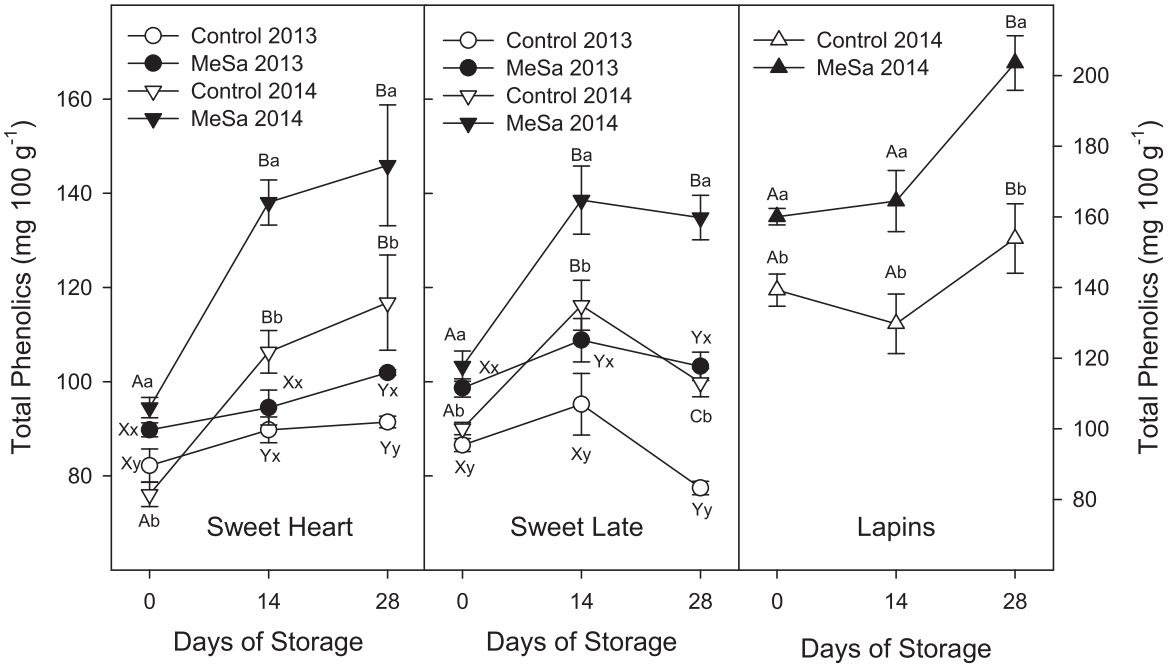

Fig. 1. Total phenolic concentration in control and methyl salicylate (MeSa) treated 'Sweet Heart' and 'Sweet Late' cultivars, for 2013 and 2014 year experiments (left scale), and in 'Lapins' cultivar for 2014 experiment (right scale), during storage at 2 °C + 1 day at 20 °C. Data are the mean ± SE. Different lower case letters show significant differences between control and treated fruit for each sampling date in 2013 (x, y) and in 2014 (a, b), while capital letters show significant differences along storage time in 2013 (X, Y) and in 2014 (A, B, C).

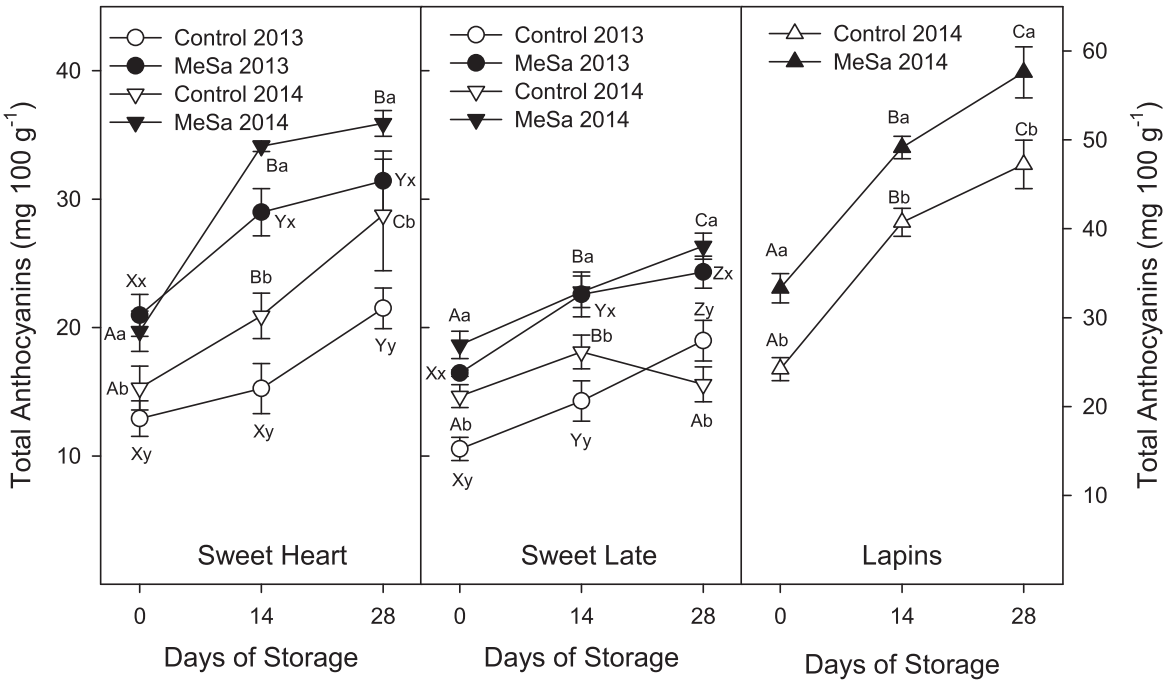

Fig. 2. Total anthocyanin concentration in control and methyl salicylate (MeSa) treated 'Sweet Heart' and 'Sweet Late' cultivars, for 2013 and 2014 year experiments (left scale), and in 'Lapins' cultivar for 2014 experiment (right scale), during storage at 2 °C + 1 day at 20 °C. Data are the mean ± SE. Different lower case letters show significant differences between control and treated fruit for each sampling date in 2013 (x, y) and in 2014 (a, b), while capital letters show significant differences along storage time in 2013 (X, Y, Z) and in 2014 (A, B, C).

phenolic concentration at harvest in control fruit was similar in 'Sweet Heart' and 'Sweet Late' cultivars for both, 2013 and 2014 years (≈75–80 mg 100 g^{-1}), while 2-fold higher concentration was found in 'Lapins' cultivar (≈140 mg 100 g^{-1}), showing that this cultivar has elevated content of phenolics as compared with others (Díaz-Mula et al., 2009; Serradilla et al., 2012). However, in MeSa-treated cherries phenolic concentration was significantly ($p < 0.05$) higher than in controls, in the three cultivars and for both years. Phenolic concentration increased in control fruit along storage, although in 'Sweet Late' cultivar a decrease occurred after prolonged storage, which could be attributed to an advancement of the ripening and senescence processes in this cultivar, as previously reported for other sweet cherry cultivars (Gonçalves et al., 2004; Serrano et al., 2009; Valero et al., 2011). However, in treated cherries total phenolic

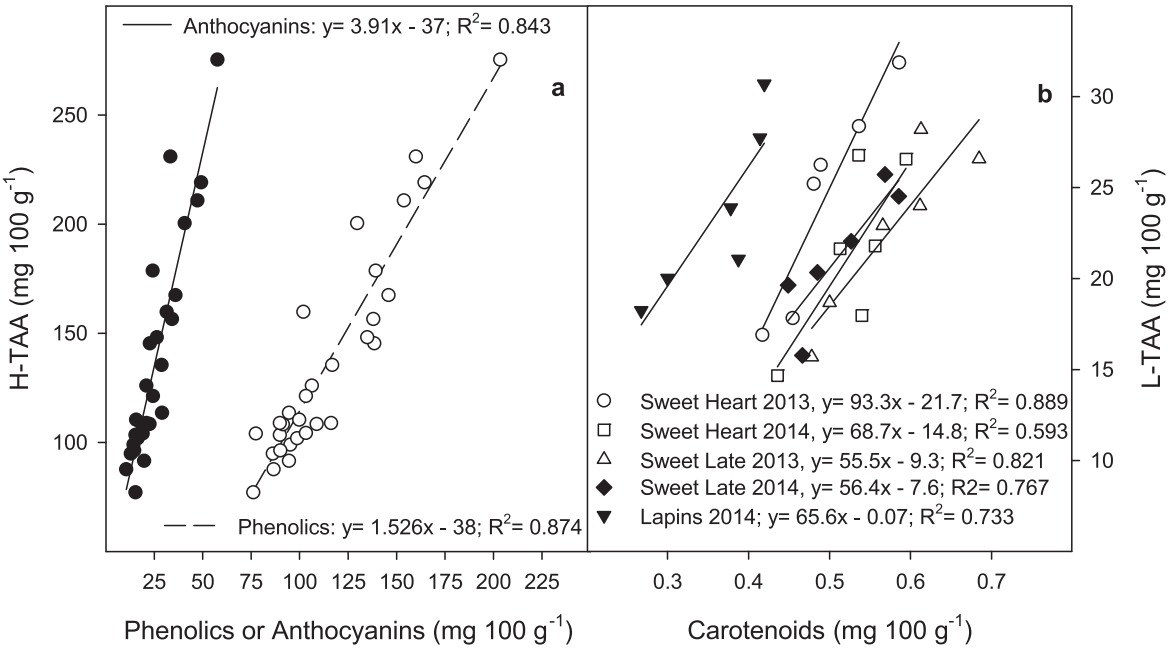

Fig. 3. Correlation between hydrophilic antioxidant activity (H-TAA) and total anthocyanin or total phenolic concentration (a), taking into account all data from all years and cultivars, and correlations between lipophilic antioxidant activity (L-TAA) and carotenoids concentration (b) taking into account data from control and treated cherries for each cultivar and growing season.

concentration was significantly ($p < 0.05$) higher than in controls along the storage period. A similar pattern to that of total phenolic compounds was found for total anthocyanin content (Fig. 2), that is, it increased along storage in control and treated fruit for all cultivars and years, except for control 'Sweet Late' in the year 2014, in which anthocyanin content increased during the first 14 days and then decreased during the rest of storage. Nevertheless, as commented above for phenolic content, preharvest MeSa treatments led to fruit with significantly ($p < 0.05$) higher anthocyanin concentration, at harvest time and along storage in the three cherry cultivars and for both experimental years.

No previous papers are available about the effects of preharvest MeSa treatment on fruit bioactive compounds for comparative purposes, although some evidences of postharvest treatments exist. Thus, postharvest MeSa treatments (at 0.1 or 0.01 mM) of pomegranate significantly increased total phenolics and anthocyanins with respect to controls during long term storage (Sayyari et al., 2011a). Similar results were obtained in pomegranates treated with ASA, a close analogue of MeSa (Sayyari et al., 2011b). By other hand, postharvest treatment of sweet cherry with SA or ASA, led to fruit with higher phenolic and anthocyanin concentrations along storage, as compared with those found in control cherries (Valero et al., 2011). Accordingly, the postharvest treatment of cornelian cherry fruit with 1 or 2 mM SA increased total phenolics, flavonoids and anthocyanins, due to the effect of SA on inducing phenylalanine ammonia lyase (PAL) activity, which catalyzes the first and committed step in the phenyl propanoid pathway and is therefore involved in the biosynthesis of phenolic

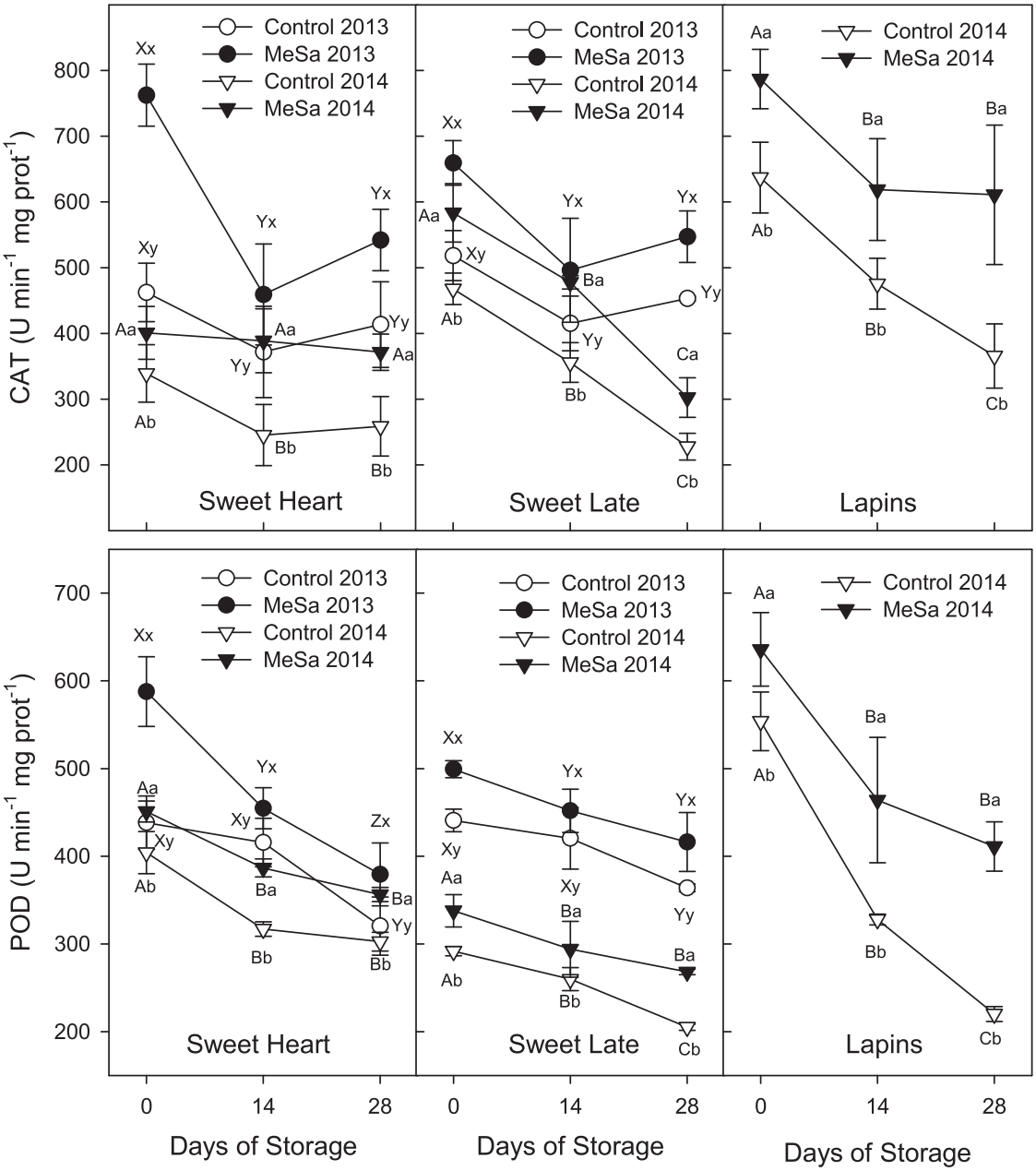

Fig. 4. Catalase (CAT) and peroxidase (POD) activities in control and methyl salicylate (MeSa) treated 'Sweet Heart' and 'Sweet Late' cultivars, for 2013 and 2014 year experiments and in 'Lapins' cultivar for 2014 experiment, during storage at 2 °C + 1 day at 20 °C. Data are the mean ± SE. Different lower case letters show significant differences between control and treated fruit for each sampling date in 2013 (x, y) and in 2014 (a, b), while capital letters show significant differences along storage time in 2013 (X, Y, Z) and in 2014 (A, B, C).

compounds such as flavonoids, anthocyanidins and phenylpropanoids (Dokhanieh et al., 2013). Similar increases of phenolic content, via stimulating the activity of PAL have been reported in apricot fruit after post-harvest SA treatments (Wang et al., 2015). In addition, treatment of grape berry with SA induced the accumulation of PAL mRNA and the synthesis of new PAL protein (Chen et al., 2006).

Total antioxidant activity in the hydrophilic extracts (H-TAA) was also higher at harvest time in MeSa-treated cherries than in controls, for the three cherry cultivars and for both years. This H-TAA increased along storage and was 2-fold higher in 'Lapins' than in 'Sweet Heart' and 'Sweet Late' (data not shown). In fact, H-TAA was highly correlated with both, total anthocyanin ($R^2 = 0.843$) and total phenolic ($R^2 = 0.874$) concentrations (Fig. 3), taking into account all data from 2013 and 2014 years of control and treated fruit of the three cultivars along the storage. These results show that anthocyanins and other phenolics could be the main hydrophilic compounds responsible for antioxidant activity of cherry fruit, as previously reported (Díaz-Mula et al., 2009; Ballistreri et al., 2013; Giménez et al., 2014). Thus, MeSa, as a safe signaling molecule, could enhance nutritional quality and improve health promoting properties of cherry fruit consumption, given the reported effect of anthocyanins and other phenolics on reducing the risk of several diseases, such as cancer, diabetes, Alzheimer's and cardiovascular diseases (McCune et al., 2011; Norberto et al., 2013). By other hand, L-TAA was very low as compared with H-TAA and no significant effect of MeSa treatment was observed on L-TAA, either at harvest time or along storage (data not shown). Accordingly, carotenoids concentration at harvest was ≈0.45–0.50 mg 100 g^{-1} in 'Sweet Heart' and 'Sweet

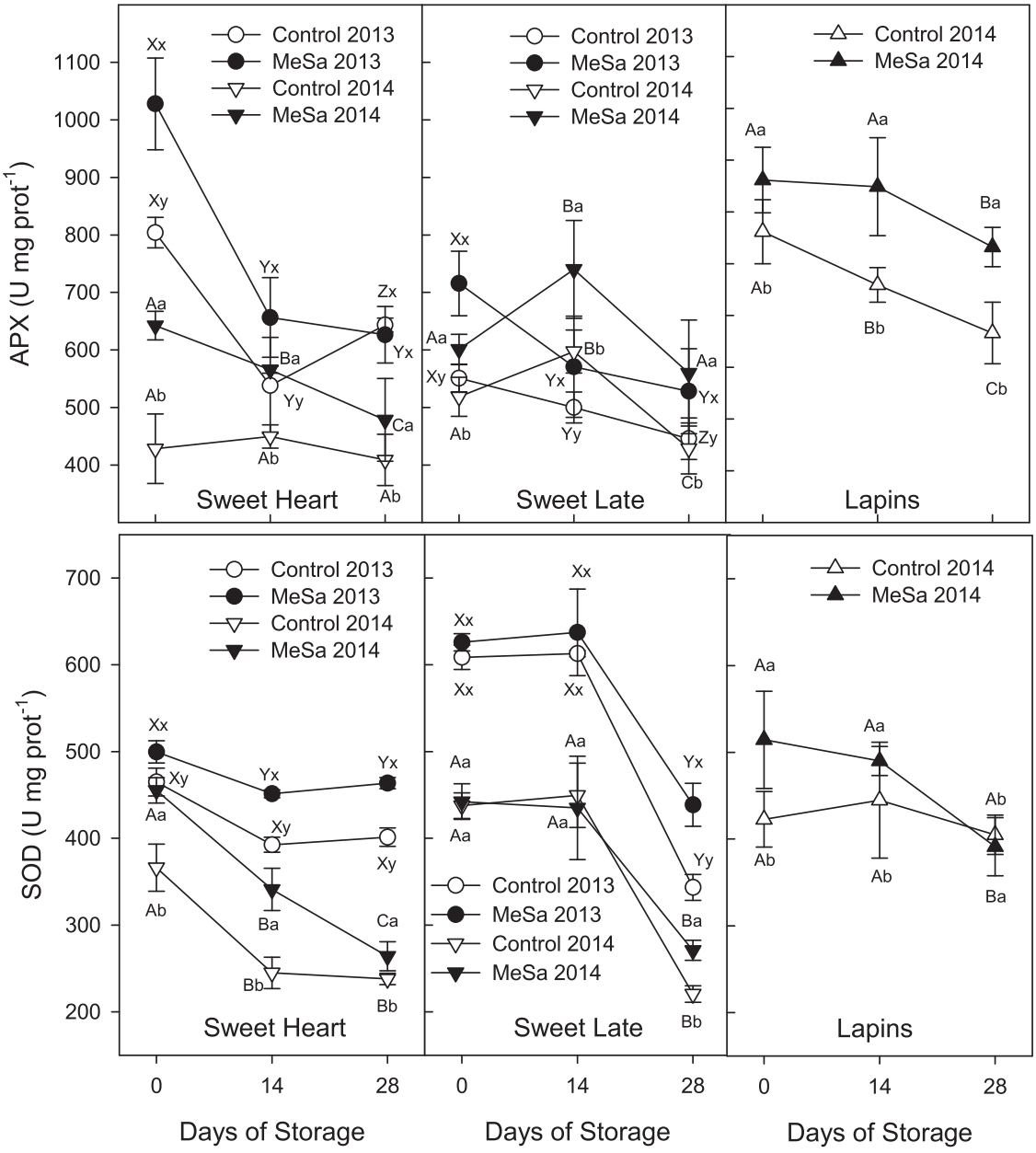

Fig. 5. Ascorbate peroxidase (APX) and superoxide dismutase (SOD) activities in control and methyl salicylate (MeSa) treated 'Sweet Heart' and 'Sweet Late' cultivars, for 2013 and 2014 year experiments and in 'Lapins' cultivar for 2014 experiment, during storage at 2 °C + 1 day at 20 °C. Data are the mean ± SE. Different lower case letters show significant differences between control and treated fruit for each sampling date in 2013 (*x*,*y*) and in 2014 (a, b), while capital letters show significant differences along storage time in 2013 (X, Y, Z) and in 2014 (A, B, C).

Late' for 2013 and 2014 and slightly lower for 'Lapins' (≈0.40 mg $100\,g^{-1}$), without significant effect of MeSa treatments, except for 'Sweet Heart' in 2014, in which carotenoids concentration at time of harvest was significantly higher than in controls (0.54 ± 0.02 and 0.43 ± 0.01 mg $100\,g^{-1}$, respectively). Carotenoids concentration increased along storage in control and treated fruit of all cultivars, although without significant differences ($p < 0.05$) attributed to treatment (data not shown). However, a high correlation was found between L-TAA and carotenoids (Fig. 3) in the three sweet cherry cultivar and in both growing season, taking into account data for control and treated fruit ($R^2 = 0.593$–0.889), which would indicate that carotenoids are the main lipophilic bioactive compounds contributing to L-TAA. However, since L-TAA was ten-fold lower than H-TAA, the main compounds contributing to the antioxidant capacity or sweet cherry are hydrophilic ones, as previously reported (Valero et al., 2011; Díaz-Mula et al., 2012), which have been significantly ($p < 0.05$) increased by preharvest MeSa treatments.

3.2. Antioxidant enzymes

SOD, CAT, POD and APX have been implicated in senescence and stress responses in vegetable tissues, since efficient activity of these antioxidant enzymes is essential for avoiding ROS caused damages (Hodges et al., 2004; Kumar et al., 2014). The present results show a general trend in those antioxidant enzymes, that is, decreases along storage, although important differences existed between control and treated cherries, among cultivars and growing seasons. CAT activity at harvest was significantly higher in MeSa treated cherries than in control in the three studied cultivars, and data for 2013 year were higher than those from 2014 in 'Sweet Heart' and 'Sweet Late' cultivars (Fig. 4). CAT activity decreased along storage, especially from day 0 to 14, although CAT activity was significantly higher in MeSa-treated than in control fruit, for all cultivars and growing seasons. A similar behavior was observed for POD activity, that is, POD deceased along storage and the activity was significantly higher in MeSa treated cherries than in controls (Fig. 4). For this antioxidant enzyme the highest activity at harvest was found in 'Lapins' and comparing both years, higher values were found in 2013 than in 2014 for both 'Sweet Heart' and 'Sweet Late' cultivars. APX activity at harvest was also higher in treated than in control fruit, although its evolution along storage was dependent on year and cultivar. Thus, in 'Sweet Heart' cultivar decreases in APX activity along storage were observed in samples of 2013 and in treated fruit of 2014, while no significant changes occurred in control fruit of 2014. APX activity also decreased during storage in control and treated fruit of 'Sweet Late' in 2013 experiment, as well as in 'Lapins' cultivar, while in control and treated 'Sweet Late' cherries in 2014 experiments this antioxidant enzyme increased from harvest until day 14 and then decreased (Fig. 5). Nevertheless, APX activity was always higher in treated than in control fruit. Finally, a general decrease along storage was also found in SOD activity, with values being significantly higher in MeSa-treated fruit than in controls, except for 'Sweet Late' cultivars, for which significant differences were just found at the last sampling date (Fig. 5).

In previous reports, it has been found that several postharvest treatments with significant effect on delaying the fruit postharvest ripening and senescence processes maintained higher activity of these antioxidant enzymes. Thus, in sweet cherry CAT and POD activities were higher in chitosan-coated fruit than in controls (Dang et al., 2010). Accordingly to the present results, He et al. (2013) reported decreases in CAT and POD activities during storage of sweet cherry, although the vacuum cooling treatment before storage led to higher activities in treated than in control cherries and delayed the postharvest ripening process. Moreover, postharvest treatments of sweet cherry with hexanal or 1-methylcyclopropene led to increases in SOD activity and lower decreases in APX activity during storage as compared with control cherries (Sharma et al., 2010). In addition, SOD, CAT and POD activities were also increased in peach fruit by postharvest treatments with SA, although in this fruit these antioxidant enzymes increased along storage at 0 °C (Tareen et al., 2012). Moreover, postharvest treatment of mango and sugar apple fruit with SA maintained higher levels of SOD, CAT and APX activities during storage as compared to those found in control fruit, which were accompanied by lower superoxide anion content and lower lipoxigenase activity (Ding et al., 2007; Mo et al., 2008). Superoxide radical accumulation during storage was also reduced in apricot by 1.0 and 2.0 mM SA treatment, due to an increase on SOD activity (Wang et al., 2015). This is of great importance, since the accumulation of $O_2^{\bullet}-$ may activate the formation of mere reactive ROS like $OH^{\bullet}$ or $^1O_2$, leading to peroxidation of membrane lipids and proteins and to acceleration of senescence procecess (Hodges et al., 2004; Mondal et al., 2009). Thus, the increased activity of these antioxidant enzymes, together with increased concentrations of antioxidants compounds, found in sweet cherry fruit (at harvest and during storage), as a consequence of MeSa treatments, could contribute to cleaning the ROS generated during the postharvest ripening process and in turn, to delay the postharvest ripening and senescence processes. These effects could explain the maintenance of fruit quality attributes during prolonged storage found in sweet cherries from MeSa treated trees (Giménez et al., unpublished data) as well as in pomegranate (Sayyari et al., 2011a), orange (Ahmad et al., 2013), mango (Ding et al., 2007), sugar apple (Mo et al., 2009), table grape (Khalil, 2014), or peach (Wang et al., 2006) after postharvest treatment with SA or ASA.

In conclusion, overall results show that preharvest treatment of sweet cherry trees with MeSa led to fruit with increased content on antioxidant compounds, such as total phenolics and anthocyanins, at harvest time and during storage, leading to increase in nutritional quality and to improve health promoting properties of cherry fruit. In addition, the increase in antioxidant enzymes by MeSa treatments may result in a high ROS scavenging potential, and in turn in delaying senescence process leading to the preservation of fruit quality attributes.

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