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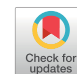
Preharvest application of methyl salicylate, acetyl salicylic acid and salicylic acid alleviated disease caused by *Botrytis cinerea* through stimulation of antioxidant system in table grapes



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ABSTRACT

The main goal of this study was to describe impact of preharvest application of methyl salicylate (MeSA), acetyl salicylic acid (ASA) and salicylic acid (SA) on the reduction of disease caused by *Botrytis cinerea* in two table grape cultivars ('Crimson' and 'Magenta'). Based on previous studies, MeSA and SA were applied at 0.1 and 0.01 mM for both cultivars, while ASA was applied at 1 mM in 'Crimson' and 0.1 mM in 'Magenta'. At time of harvest, berry maturity-quality attributes, bioactive compounds and antioxidant enzymes were determined. In addition, grapes were artificially inoculated with *B. cinerea* spores, and the berries were ranked for visual decay incidence after 5 days of inoculation. Salicylates preharvest treatments led to higher total acidity, content of bioactive compounds and activity of antioxidant enzymes in treated than in control berries. The application of salicylate derivatives induced resistance to *B. cinerea* spoilage, since higher percentage of berries with no symptoms was observed and on the contrary, the highest percentages of berries were obtained in control grapes. All preharvest treatments with SA, ASA and MeSA alleviated postharvest disease caused by *B. cinerea* probably due to increasing levels of phenolic compounds and activity of antioxidant enzymes, although the best results were obtained with MeSA at 0.1 mM. Also, for this treatment and dose, higher quality properties, such as higher concentrations of ascorbic, succinic and fumaric acids, were observed compared with no treated-grapes.

1. Introduction

The grape (*Vitis vinifera* L.) commercialisation chain is very demanding in terms of preserving fruit quality. Table grape is subjected to long storage periods before reaching its final destination, and there are risks of various postharvest losses (Champa, 2015), representing up to 25% and 50% of total production in industrialized and developing countries. Grey mould caused by *Botrytis cinerea* has been reported as a major postharvest disease of grapes (Martínez-Romero et al., 2007; Saito et al., 2019), which has a negative impact on the quality of fresh grapes such as weight loss, colour fading, accelerated softening, and reduction of shelf life, all of these causing severe economic losses. The control of this disease is very difficult since postharvest treatments with synthetic fungicides or SO_2 are not allowed in several countries due to their adverse effects on food safety and the environment (Youssef et al., 2015).

Salicylic acid (SA) and its derivatives, acetyl salicylic acid (ASA) and methyl salicylate (MeSA), are naturally occurring compounds ubiquitously distributed in the whole plant kingdom and classified under the group of plant hormones having diverse regulatory roles in the metabolism of plants (Hayat and Ahmad, 2007). SA has emerged as a key plant defence hormone with critical roles in different aspects of plant immunity, and is involved in systemic acquired resistance (SAR) demonstrated in several plant tissues, including fruits (Zhang and Li, 2019). Accumulation of SA plays a significant role in stimulation of local defence at initial infection site as well as in the distant tissues that are infection free for induction of SAR, while MeSA serves as a long-distance SAR signal which occurs via phloem or throughout other tissues and even in the outer parts of the plant due to its volatile nature (Nazar et al., 2017). Recently, enhanced disease resistance upon exogenous SA application has been reported in different fruits species including, tomato, pepper, orange and banana, among others (Koo et al., 2020).

As postharvest treatments several approaches have been reported to

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reduce the incidence and severity of *B. cinerea* in table grapes. Thus, essential oils, such as eugenol, thymol, or menthol added to modified atmosphere packages (MAP) improved the storability of table grapes and delayed rates of rachis deterioration and berry decay (Serrano et al., 2008; Valverde et al., 2005a). These essential oils maintained higher total phenolics and total antioxidant activity in both skin and flesh of the berries (Valero et al., 2006). Carvacrol vapour treatment of grape inoculated with *B. cinerea* inhibited fungal growth in a dose-dependent manner, suggesting that carvacrol could be used as alternative to the use of synthetic fungicides or SO_2 (Martínez-Romero et al., 2007). Other essential oils, such as sage oil, lavender oil, mint oil, and tea tree oil can effectively inhibit the growth of *B. cinerea* and the antifungal effects are dose dependent, the best antifungal effect being found from mint oil (Xueuan et al., 2018). These authors also reported that volatile vapour worked better than direct contact, suggesting that essential oils cause changes in membrane permeability and cell wall damage. On the other hand, edible coatings have shown efficacy in controlling fruit decay. For instance, *Aloe vera* gel was able to reduce microbial counts for both mesophilic aerobic and yeast and moulds on table grapes over storage with benefits in reducing berry decay (Valverde et al., 2005b).

As preharvest treatments, synthetic fungicide sprays may provide an alternative to the control of postharvest grey mould, although fungicide resistance in *B. cinerea* can result in the failure of disease control (Saito et al., 2019), apart from safety regulations. Preharvest application with several compounds has shown benefits in terms of improving quality on table grapes. For instance, methyl jasmonate (MeJA) has recently reported to affect the ripening process on table grape depending on concentration, since 5 and 10 mM delayed ripening while 1, 0.1 and 0.01 mM accelerated the maturation (García-Pastor et al., 2019). With respect to salicylates, their preharvest application improved the quality and enhanced the nutritive and bioactive compounds parameters at harvest and during storage in several fruit commodities, such plum (Martínez-Esplá et al., 2017, 2018) and sweet cherry (Giménez et al., 2015, 2017). In sweet cherry, an effect of preharvest SA treatments on reducing decay during storage was also reported (Yao and Tian, 2005) as well as in pears (Cao et al., 2006). In apricot, it has been recently reported that SA treatment, 7 and 2 days before harvesting decreased decay rate attributed to *Alternaria alternata* during cold storage, due to increased phenolic content, antioxidant capacity and activity of peroxidase (POD) and phenylalanine ammonia lyase (PAL) activities (Cui et al., 2020). Specifically in table grape, just in one previous paper the effects of preharvest SA treatments on reducing berry decay incidence during storage has been reported in 'Flame Seedless' cultivar (Champa et al., 2015).

However, as far as we know, there are no reports on the effect of salicylates applied as preharvest treatments in reducing the incidence and severity of the disease caused by *B. cinerea*. The aim of this study was to apply SA, ASA and MeSA as preharvest treatments and find out their effects on the incidence and severity of decay on table grapes inoculated with *B. cinerea*, as well the possible mechanism of action involved in the alleviation of this fungal disease.

2. Materials and methods

2.1. Plant material and field experimental design

The experiments were carried out in 2018 in a commercial plot of vineyards in Calasparra (Murcia, Spain) using two seedless table grape (*Vitis vinifera* L.) cultivars 'Crimson' (11-years old vine) and 'Magenta' (8-years old vine). Before the onset of veraison, the vineyards were preharvest treated with distilled water (control), salicylic acid (SA), acetyl salicylic acid (ASA) or methyl salicylate (MeSA) (purchased from Sigma-Aldrich, Madrid, Spain). For 'Crimson', three treatments were performed on June 26th (T1), on July 13th at veraison (T2) and on August 6th (T3). The applied doses were 0.01 mM SA, 1 mM ASA and 0.1 mM MeSA. For 'Magenta', three treatments were also performed on June 22nd (T1), on July 10th at veraison (T2) and on July 6th (T3). For this cultivar, the applied doses were 0.01 mM SA, 0.1 mM ASA and 0.1 mM MeSA. These concentrations were chosen based on previous experiments in two growing seasons (2016 and 2017), in which the best results for these treatments in terms of yield, berry maturity-quality and bioactive compounds were obtained. All treatments were performed by foliar spray application of 1 L per vine, containing 0.5% Tween 20 as surfactant. Treatments were made at sunrise and during favourable weather conditions, where rainfall or winds were not forecasted for the following 24 h. Pruning, thinning, fertilization and irrigation were carried out during the experiments according to local cultural practices for table grape without any use of fungicides. A completely randomized block design with five replicates of three vines for each cultivar and treatment was established. Clusters were harvested when berries reached the characteristic size, colour and soluble solid content (°Brix) of each cultivar in order to pick up full mature grapes. Fig. 1S shows a scheme of the experimental design.

2.2. Soluble solids content, titratable acidity and ripening index

A first set of bunches was used to determine the berry maturity-quality characteristics. Ten berries were sampled from each replicate (5 bunches, total 50 berries) and cultivar, and then rachis and peduncles were separated, cut and ground to obtain a homogeneous juice sample, in which total soluble solids (TSS) content were determined in duplicate with a digital refractometer Atago PR-101 (Atago Co. Ltd., Tokyo, Japan) at 20 °C, and expressed as g 100 g^{-1} (mean ± SE). Total acidity (TA) was determined also in duplicated in the same juice by automatic titration (785 DMP Titrino, Metrohm) with 0.1 N NaOH up to pH 8.1 and results (mean ± SE) expressed as g tartaric acid equivalent 100 g^{-1} fresh weight (FW). Ripening index (RI) was calculated as the ratio between TSS and TA. Data were the mean ± SE of five replicates.

2.3. Individual sugars and organic acids

The juice used for TSS and TA determination was centrifuged at 10,000 × *g* for 10 min and the supernatant was filtered through a 0.45 μm Millipore filter and then injected into a high-performance liquid chromatography (HPLC) system (Hewlett-Packard HPLC series 1100) to quantify individual sugars and organic acids. The elution system consisted of 0.1% phosphoric acid running isocratically with a flow rate of 0.5 mL min^{-1} through a Supelco column (Supelcogel C−610H, 30 cm 7.8 mm, Supelco Park, Bellefonte, PA, USA). Organic acids were detected by absorbance at 210 nm and sugars by refractive index detector. Results were expressed as g 100 g^{-1} at harvest. A standard curve of pure sugars and organic acids purchased from Sigma (Poole, UK) was used for quantification. Results were the mean ± SE of five replicates.

2.4. Skin bioactive compounds, antioxidant activity and antioxidant enzymes

Fourth set of 30 berries from each replicate were used for the determination of phenolics, anthocyanins (total and individual), antioxidant capacity and antioxidant enzymes activity. The berries were separated from the rachis, as well as the peduncle, and were peeled to separate the skin from the flesh. Both tissues were immediately frozen in liquid N_2, milled with mortar and pestle and kept at −80 °C until analysis. To extract total phenolics 1 g of skin tissue was manually ground in a mortar and pestle with 5 mL of water: methanol (2:8) containing 2 mM NaF (to inactivate polyphenol oxidase activity and prevent phenolic degradation) and then, sonicated in an ultrasonic bath for 60 min. The homogenate was centrifuged at 10,000*g* for 15 min, and phenolics were quantified in the supernatant using the Folin-Ciocalteu reagent as previously reported (Martínez-Esplá et al., 2017). Results

(mean ± SE) were expressed as mg gallic acid equivalent 100 g^{-1} FW. To extract total anthocyanins, 1 g of frozen skin tissue and 5 mL of methanol: formic acid: water (25:1:24, v/v/v) were manually ground, as same as previously, and then sonicated in an ultrasonic bath for 60 min and after that centrifuged at 10,000*g* for 15 min. Total anthocyanin concentration was measured by reading absorbance at 520 nm in an UNICAM Helios-α spectrophotometer (Cambridge, UK), and expressed as mg of malvidin 3-glucoside equivalent (molar absorption coefficient of 27,000 $M^{-1} cm^{-1}$ and molecular weight of 493.4 g mol^{-1}) per 100 g FW (mean ± SE). The supernatant was filtered through a 0.45 µm PVDF filter (Millex HV13, Millipore, Bedford, MA, USA) and used for individual anthocyanin quantification by HPLC analysis as previously reported (Martínez-Esplá et al., 2017). Chromatograms were recorded at 520 nm. Anthocyanin standards were: malvidin 3-glucoside for 'Magenta' and peonidin 3-glucoside for 'Crimson' cultivar (purchased from Sigma-Aldrich, Germany). Results are the mean ± SE of five replicates.

To measure total antioxidant activity (TAA), 1 g of skin tissue were manually homogenized in a mortar with 5 mL of 50 mM phosphate buffer pH = 7.8 and 5 mL of ethyl acetate. The homogenate was centrifuged at 10,000*g* for 15 min at 4 °C and the upper and lower fractions were used to quantify lipophilic (L-TAA) and hydrophilic total antioxidant activity (H-TAA), respectively. As previously described (Sayyari et al., 2011a), H-TAA and L-TAA were determined in duplicate in each extract using a reaction mixture containing 2,20-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), horseradish peroxidase enzyme and its oxidant substrate (hydrogen peroxide), in which $ABTS^{+}$ radicals are generated and monitored at 730 nm. The decrease in absorbance after adding the grapes extract was proportional to TAA of the sample which was calculated by using a calibration curve made with Trolox ((*R*)-(+)-6-hydroxy-2,5,7,8-tetramethyl-croman-2-carboxylic acid) (0–20 nmol) from Sigma Aldrich (Madrid, Spain), and results were expressed as mg of Trolox Equivalent (TE) 100 g^{-1} and were the mean ± SE of five replicates.

Crude extracts to measure peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX) enzymes in berry skin were performed by homogenizing 1 g of frozen tissue with 5 mL of phosphate buffer 50 mmol L^{-1}, pH 6.8, containing 1% (w/v) polyvinylpyrrolidone and 1.0 mmol L^{-1} ethylenediamine-tetraacetic acid. Then, the extracts were centrifuged at 10,000*g* for 30 min at 4 °C and the supernatant was used for the quantification as reported previously (Zapata et al., 2017). Briefly, for POD activity, the reaction mixture contained 200 µL of extract in a final volume of 3 mL of 50 mmol L^{-1} phosphate buffer (pH 7.0), 12 mmol L^{-1} H_2O_2 and 7 mmol L^{-1} guaiacol. The increase of absorbance at 470 nm during 1 min was measured and POD activity was expressed as U min^{-1} g^{-1} (where U was defined as an increase of 0.01 absorbance min^{-1}). For CAT, 100 µL of extract were added to 3 mL of reaction mixture containing 15 mmol L^{-1} H_2O_2 and 50 mmol L^{-1} phosphate buffer (pH 7.0). The decrease of absorbance at 240 nm during 1 min was measured and CAT activity was expressed as U min^{-1} g^{-1} (where U was defined as a decrease of 0.01 absorbance min^{-1}). Finally, for APX quantification, the reaction mixture contained 200 µL of extract in 3 mL of 50 mmol L^{-1} potassium phosphate (pH 7.0), 0.5 mmol L^{-1} ascorbic acid and 1.0 mmol L^{-1} H_2O_2. The decrease of absorbance at 290 nm from time 0 to 60 s was measured and APX activity was expressed in terms of units of enzymatic activity (U min^{-1} g^{-1}), with one enzymatic unit (U) being defined as a decrease of 0.01 ascorbate min^{-1}. Results are the mean ± SE of five replicates.

2.5. Experimental design of berry inoculation

One set of 120 berries from each of the five replicates were used for the inoculation experiment with *Botrytis cinerea*. Berries of the clusters were separated from the rachis by individually cutting them with scissors, without damaging them and maintaining the peduncle. Once selected the most homogeneous samples, these grapes were disinfected in a water bath with 100 ppm of chlorine during 1 min and allowed to dry spread on filter papers. Once dry, the 120 berries from each replicate, treatment and cultivar were placed into a plastic box with lid (30 × 15 × 5 cm) to inoculate them. Previously to inoculation process, these grapes were injured with a sterile lancet inside a laminar flow hood. Berry wound was always made on the right side attached to the peduncle and was 6 mm in deep (Fig. 1S). The fungus used in this study was *B. cinerea* CECT21000 (Spanish collection of type cultures) and routinely cultured on potato dextrose agar (PDA). The spores of *B. cinerea* were collected and diluted with sterile water until reaching the concentration of 7500 CFU mL^{-1} and used as stock. Five replicates of 120 single berries per treatment were inoculated by spraying them with this spore suspension of *B. cinerea* until runoff. Each berry received 900 spores, then air dried for 30 min, and boxes were closed slightly, allowing the evaporation of water excess and the oxygen and CO_2 exchange, and incubated for 5 days at 25 ± 1 °C with 80–85% relative humidity.

2.6. Visual decay incidence

Grapes were inspected at fifth day after inoculation and considered spoiled based on a visual scale of six hedonic points named as stages: S0, S1, S2, S3, S4 and S5. The evolution and visual appearance of the fungus growth was different for each table grape cultivars, and in turn the meaning of the S0 to S5 decay incidence scale was established as follow (photographs at the bottom of Figs. 1 and 2). In 'Magenta' cultivar the decay incidence scale was: S0, without damage; S1, wound browning; S2, microbial growth covering 1–2 mm of wound; S3, microbial growth covering 3–4 mm of wound; S4, microbial growth covering 4–5 mm of wound and even showing mycelial growth; S5, all the wound covered (6 mm) with the fungus and mycelium was observed. For 'Crimson' cultivar, this scale was: S0, without damage; S1, wound browning; S2, microbial growth covering the wound (6 mm); S3, microbial growth, covering a quarter of the berry and showing mycelial growth; S4, microbial growth, covering the half of the berry and

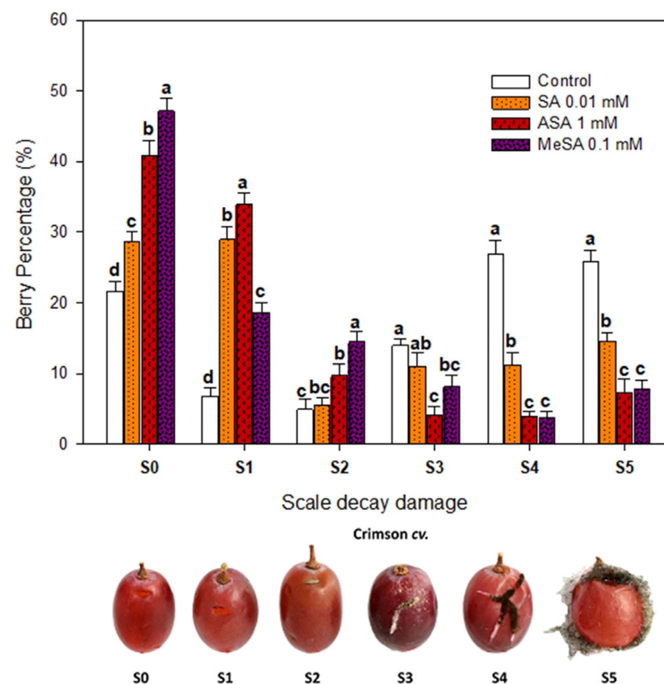

Fig. 1. Percentage of decayed berries according to the scale of visual aspect scale (S0–S5) of decay incidence in 'Crimson' table grape as affected preharvest treatments with control, salicylic acid (SA), acetyl salicylic acid (ASA) and methyl salicylate (MeSA). Data are the mean ± SE. Data are the mean ± SE. Different letters show significantly differences ($P < 0.05$) among treatments.

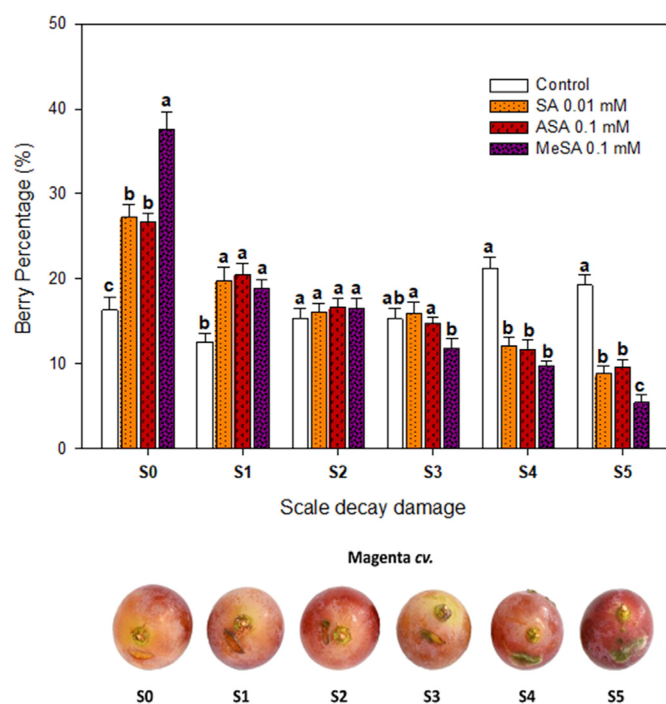

Fig. 2. Percentage of decayed berries according to the scale of visual aspect scale (S0–S5) of decay incidence in 'Magenta' table grape as affected preharvest treatments with control, salicylic acid (SA), acetyl salicylic acid (ASA) and methyl salicylate (MeSA). Data are the mean ± SE. Data are the mean ± SE. Different letters show significantly differences ($P < 0.05$) among treatments.

showing mycelial growth and softening; S5, microbial growth, covering the whole berry and showing mycelial growth and softening. Results were expressed as percentage of spoiled grapes in each stage based on the total number of fruits per box (mean ± SE of five replicates).

2.7. Statistical analysis

All statistical analyses were performed using the SPSS software package v. 12.0 for Windows. A one-way analysis of variance (ANOVA) was also performed to determine the significance of mean differences among treatments and cultivars, using HSD Duncan's test to examine if differences were significant at $P < 0.05$. Differences were indicated using different letter designations.

3. Results and discussion

3.1. Effect of SA, ASA and MeSA on *Botrytis cinerea* disease

Recently harvested grape berries, from both control and preharvest salicylate treated vines, were artificially injured and inoculated by spraying them with 7500 UFC mL^{-1} of *B. cinerea* suspension. After 5 days of inoculation, the disease incidence and severity were visually ranked in a six hedonic scale, and results are shown in Fig. 1 ('Crimson') and Fig. 2 ('Magenta'). For both cultivars similar results were obtained. The lowest decay incidence ($P < 0.05$) was obtained in those berries treated with MeSA, since the percentages of berries with absence of damage (S0) were 47 and 38%, for 'Crimson' and 'Magenta', respectively. On the other hand, this treatment showed the lowest percentage of berries with severe disease (S4 and S5) with values of 3–7% and 5–9%, respectively. The ASA treatment also showed good control of *B. cinerea* disease, but with lower performance than in MeSA-treated grapes. The most severe symptoms were shown in control grapes, with percentages of S4-S5 stages of 26–27 and 20–21% for 'Crimson' and 'Magenta', respectively. Moreover, in berries from control vines the lowest percentage at S0 stage (absence of symptoms), 26 and 16% for 'Crimson' and 'Magenta', respectively was obtained.

These results clearly demonstrated that preharvest treatment with salicylates, and especially MeSA, were able to induce resistance of table grape to be colonized by *B. cinerea*. There is no literature about the effect of preharvest application of ASA or MeSA on inducing fruit resistance to pathogen attack for comparative purposes, although a few reports are available about preharvest treatments with SA. Thus, preharvest treatments with SA delayed decay during storage in sweet cherry (Yao and Tian, 2005), pears (Cao et al., 2006) and apricot (Cui et al., 2020), in the last commodity the effect being attributed to increases in POD and PAL activities. In grapes, just in one paper has been reported the effect of preharvest SA treatments on reducing decay during storage in 'Flame Seedless' cultivar (Champa et al., 2015). On the other hand, many studies have shown that postharvest treatment with SA or MeSA at appropriate concentrations could enhance resistance to pathogens in postharvest of fruits and vegetables such as mango, sweet cherry and pomegranate (Sayyari et al., 2011a; Valverde et al., 2015; Zheng et al., 2006). Accordingly, disease development in tomato fruit caused by *B. cinerea* was effectively suppressed by MeSA treatment in terms of percentage of disease incidence and the lesion area (Min et al., 2018). In apples, SA was totally effective in controlling blue mould caused *Penicillium expansum* as well as on maintaining the fruit quality characteristics related to weight loss, TSSS and TA (da Rocha Neto et al., 2015, 2016). These authors proposed as mechanism of action to the fact that SA caused leakage of the pathogen's proteins to the medium, measured by lipid damage, and intracellular disorganization.

3.2. Bioactive compounds and antioxidant enzymes

Grape polyphenols are characterised by a large range of chemical structures and can be found in the skin, flesh and seed of the berry. Grape skin contains flavanols, flavonols, anthocyanins, and stilbenes, which concentration of these phenolic compounds is affected by cultivar and environmental factors during the growth and development. The interest in these compounds is based on their beneficial effects for human health mainly due to their well-known antioxidant activity and capacity to scavenge free radicals (Doshi et al., 2015; Flamini et al., 2013).

In our study, all preharvest treatment enhanced the concentration of total phenolics, total anthocyanins and total antioxidant activity (TAA) due to hydrophilic (H-TAA) and lipophilic compounds in the grape skin for both cultivars (Fig. 3). Total phenolics in control grapes were 177 ± 10 and 167 ± 9 mg 100 g^{-1}, for 'Crimson' and 'Magenta', respectively, this concentration being significantly ($P < 0.05$) increased in SA, ASA and MeSA-treated berries, up to ~270 and 310 mg 100 g^{-1} for 'Crimson' and 'Magenta', respectively. It is worth noting that no significant differences ($P < 0.05$) were observed among phenolic content in SA, ASA and MeSA treated berries. Total antioxidant activity (TAA) was determined separately in hydrophilic (H-TAA) and lipophilic (L-TAA) extracts, although values were higher for H-TAA than L-TAA. However, both parameters were enhanced in all treated ($P < 0.05$) grapes (1.5-fold) with respect to controls for both cultivars.

Both cultivars are red seedless table grapes, although 'Crimson' has a purple while 'Magenta' has a light-red colour due to the occurrence of anthocyanins, which are responsible for the pigmentation of these cultivars. As shown in Fig. 3, the concentration of total anthocyanins was significantly higher in 'Crimson' (~70 mg 100 g^{-1}) than in 'Magenta' (~30 mg 100 g^{-1}) and thus it reflects the differences in colour. Similar to the other bioactive compounds, the concentration of anthocyanins was significantly enhanced ($P < 0.05$) in treated grapes with respect to controls. Moreover, the individual profile of anthocyanins by HPLC was analysed (Table 1) and 5 anthocyanins were identified in both cultivars: Delphinidin-3 glucoside (Dl-3 gluc), Cyanidin-3 glucoside (Cy-3 gluc), Petunidin-3 glucoside (Pt-3 gluc), Peonidin-3

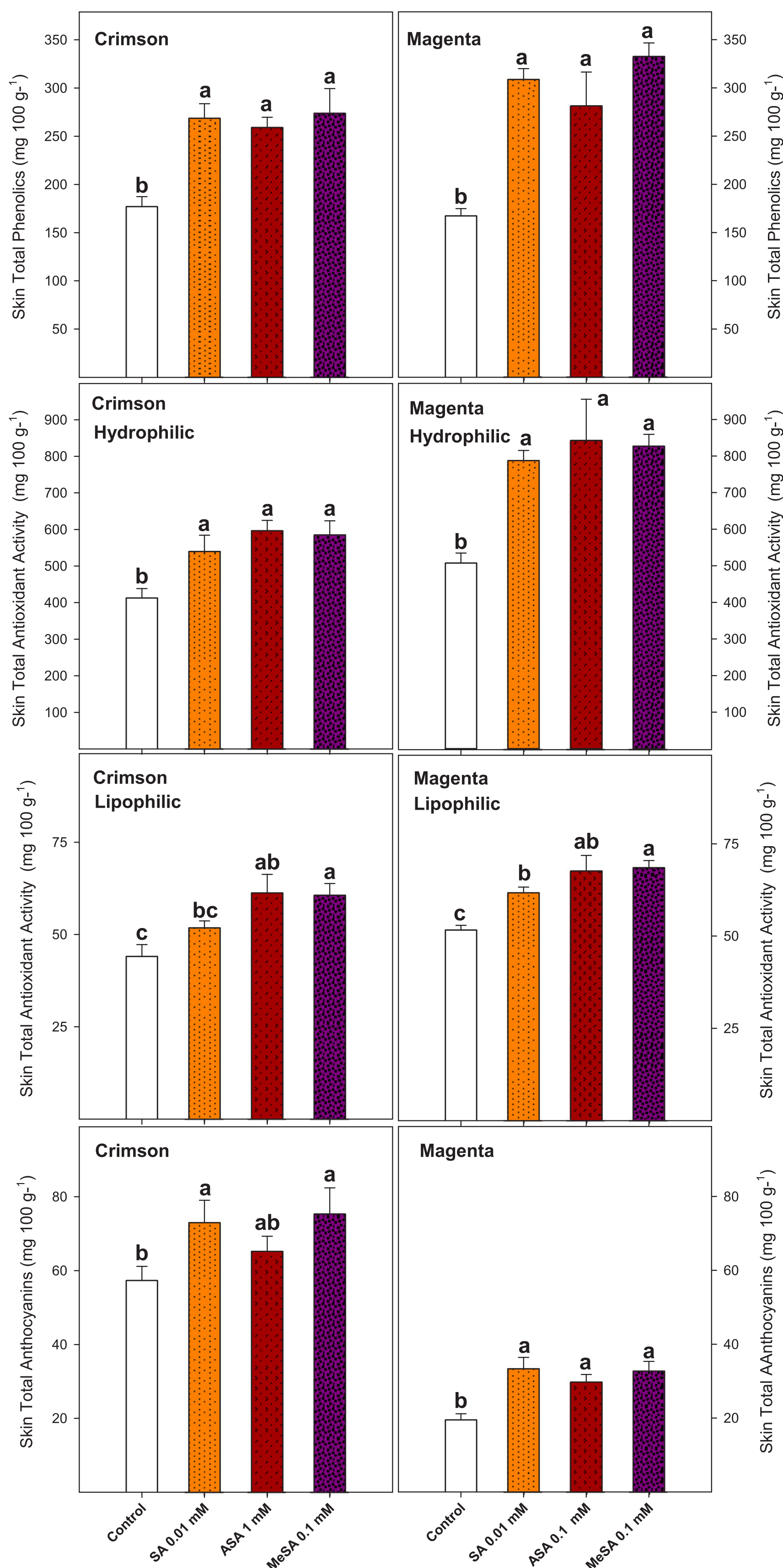

Fig. 3. Bioactive compounds the skin of two grape cultivars 'Crimson' and 'Magenta' as affected preharvest treatments with control, salicylic acid (SA), acetyl salicylic acid (ASA) and methyl salicylate (MeSA). Data are the mean ± SE. Different letters show significantly differences ($P < 0.05$) among treatments.

Table 1
Levels of individual anthocyanins (mg 100 g^{-1}) in the skin of two table grape cultivars at harvest as affected by treatment. Delphinidin-3 glucoside (Dl-3 gluc), Cyanidin-3 glucoside (Cy-3 gluc), Petunidin-3 glucoside (Pt-3 gluc), Peonidin-3 glucoside (Pn-3 gluc) and malvdin-3 glucoside (Mv-3 gluc).[a]

	Dl-3 gluc	Cy-3 gluc	Pt-3 gluc	Pn-3 gluc	Mv-3 gluc
'Crimson'					
Control	1.16 ± 0.28 a	0.61 ± 0.17 a	0.96 ± 0.23 a	18.21 ± 1.25 b	6.08 ± 0.61 b
SA 0.01 mM	0.78 ± 0.14 ab	0.46 ± 0.13 a	0.69 ± 0.14 a	26.94 ± 2.12 a	9.35 ± 0.78 a
ASA 1 mM	0.37 ± 0.14 b	0.16 ± 0.03 b	0.31 ± 0.08 b	22.34 ± 1.95 ab	7.77 ± 0.61 ab
MeSA 0.1 mM	0.96 ± 0.16 a	0.72 ± 0.17 a	0.91 ± 0.17 a	26.35 ± 2.51 a	8.88 ± 1.07 a
'Magenta'					
Control	1.35 ± 0.49 a	0.22 ± 0.07 a	0.76 ± 0.19 a	4.02 ± 0.75 a	5.18 ± 0.53 b
SA 0.01 mM	1.78 ± 0.51 a	0.23 ± 0.09 a	0.90 ± 0.26 a	5.53 ± 1.31 a	7.75 ± 0.91 a
ASA 0.1 mM	2.20 ± 0.48 a	0.34 ± 0.11 a	1.01 ± 0.18 a	5.15 ± 0.35 a	7.26 ± 0.43 a
MeSA 0.1 mM	2.29 ± 0.41 a	0.36 ± 0.09 a	1.15 ± 0.19 a	5.35 ± 0.86 a	7.35 ± 1.08 ab

[a] For each cultivar and parameter different letter following the mean are significantly different ($P < 0.05$) among treatments.

Table 2
Enzyme activity (U min^{-1} g^{-1}) of ascorbate peroxidase (APX), catalase (CAT) and peroxidase (POD) in the skin of two grape cultivars at harvest as affected by treatments.[a]

	APX	CAT	POD
'Crimson'			
Control	247 ± 9 b	471 ± 44 c	119 ± 4 c
SA 0.01 mM	268 ± 8 b	666 ± 42 b	134 ± 7 bc
ASA 1 mM	267 ± 9 b	580 ± 33 bc	139 ± 4 b
MeSA 0.1 mM	296 ± 6 a	829 ± 58 a	159 ± 7 a
'Magenta'			
Control	192 ± 9 c	380 ± 23 c	78 ± 2 c
SA 0.01 mM	254 ± 8 b	543 ± 21 b	87 ± 4 bc
ASA 0.1 mM	244 ± 9 b	680 ± 44 a	94 ± 2 b
MeSA 0.1 mM	278 ± 7 a	719 ± 35 a	113 ± 5 a

[a] For each cultivar and parameter different letter following the mean are significantly different ($P < 0.05$) among treatments.

glucoside (Pn-3 gluc) and malvdin-3 glucoside (Mv-3 gluc). In 'Crimson', the major anthocyanin was Pn-3 gluc followed by Mv-3 gluc while in 'Magenta' Mv-3 gluc and Pn-3 glu were the major anthocyanins and found at similar concentrations. As expected, all treated grapes showed significant ($P < 0.05$) higher concentration in these major individual anthocyanins than controls.

On the other hand, the activity of the antioxidant enzymes ascorbate peroxidase (APX), catalase (CAT) and peroxidase (POD) in the skin of the two grape cultivars at harvest was significantly ($P < 0.05$) enhanced due to all salicylate treatments. This effect was especially important for MeSA-treated berries which had the highest levels of APX, CAT and POD while control grapes showed the lowest activities (Table 2).

There are a number of mechanisms involved in improving the resistance of fresh produce to diseases by postharvest treatments with salicylates, e.g. changes in phenolic biochemistry, increased accumulation of H_2O_2, increased activity of antioxidant enzymes, induction of pathogenesis-related (PR) proteins and defence-related enzymes (Nazar et al., 2017; Zhang and Li, 2019). In the case of table grapes two of the above mechanisms of action are supported by data, that is the increase of phenolic compounds, including anthocyanins, and the greater activity of the antioxidant enzymes.

The increase in total phenolic compounds has been regarded as an indicator for plant defence response since they are able to scavenge free radicals (Beckman, 2000). Reactive oxygen species (ROS) are accumulated in plants under pathological and senescence conditions, leading to oxidative injury. It is well-known that APX, CAT, and POD are the key enzymes involved in ROS elimination, and the decrease in them may lead to high levels of ROS, as has been shown in control grapes. Therefore, the higher levels of antioxidant enzymes in treated berries could be involved in fruit resistance against *B. cinerea*. Accordingly, postharvest treatment with MeSA at 0.05 mM was effective in reducing disease incidence and severity of *B. cinerea* inoculated on tomato (Zhang et al., 2017). In addition, this treatment also enhanced the antioxidant enzymes CAT and POD as well as led to higher content of total phenolics, which agrees with our results on table grape. Chilling injury is another physiological disorder due to abiotic stress by low temperature storage, and salicylates have proved their efficacy on alleviating the chilling injury associated symptoms, such as skin browning, spots and weight loss. Thus, blood oranges treated with MeSA induced an accumulation of APX and CAT and the chilling injury was reduced (Habibi et al., 2019).

3.3. Effect of SA, ASA and MeSA on berry maturity-quality parameters

Among the flavour metabolites, sugar and organic acid compositions, which are measured through total soluble solids (TSS) and titratable acidity (TA), are most commonly associated with the taste of

Table 3
Levels of total soluble solids (g 100 g^{-1}), total acidity (g 100 g^{-1}), TSS/TA ratio (ripening index), glucose (g 100 g^{-1}), and fructose (g 100 g^{-1}) in two table grape cultivars at harvest as affected by treatments.[a]

	TSS (g 100 g^{-1})	TA (g 100 g^{-1})	TSS/TA ratio	Glucose (g 100 g^{-1})	Fructose (g 100 g^{-1})
'Crimson'					
Control	19.44 ± 0.28 b	0.56 ± 0.02 b	34.78 ± 0.63 a	9.02 ± 0.08 b	7.33 ± 0.08 c
SA 0.01 mM	19.87 ± 0.13 b	0.58 ± 0.01 b	34.25 ± 0.47 ab	9.16 ± 0.06 b	7.71 ± 0.08 b
ASA 1 mM	18.32 ± 0.25 c	0.64 ± 0.02 a	28.63 ± 0.73 c	8.62 ± 0.15 c	6.66 ± 0.15 d
MeSA 0.1 mM	20.63 ± 0.17 a	0.63 ± 0.01 a	32.74 ± 0.63 b	9.94 ± 0.06 a	8.50 ± 0.04 a
'Magenta'					
Control	16.70 ± 0.18 b	0.68 ± 0.02 b	24.55 ± 0.76 a	8.31 ± 0.12 b	7.01 ± 0.11 b
SA 0.01 mM	16.76 ± 0.13 b	0.64 ± 0.01 b	26.19 ± 0.70 a	8.07 ± 0.10 b	7.07 ± 0.08 b
ASA 0.1 mM	15.27 ± 0.09 c	0.78 ± 0.03 a	19.57 ± 0.79 c	7.63 ± 0.09 c	5.29 ± 0.05 c
MeSA 0.1 mM	17.93 ± 0.18 a	0.83 ± 0.02 a	21.61 ± 0.41 b	9.18 ± 0.09 a	7.34 ± 0.07 a

[a] For each cultivar and parameter different letter following the mean are significantly different ($P < 0.05$) among treatments.

Table 4
Concentration of individual organic acids two table grape cultivars at harvest as affected by treatment. Major organic acids (tartaric, citric and malic acids were expressed in g 100 g^{-1}) while minor organic acids (ascorbic, succinic and fumaric acids were expressed in mg 100 g^{-1}).[a]

	Tartaric acid	Malic acid	Citric acid	Ascorbic acid	Succinic acid	Fumaric acid
'Crimson'						
Control	0.27 ± 0.03 b	0.15 ± 0.01 b	0.09 ± 0.02 a	15.4 ± 0.11 c	0.08 ± 0.02 b	0.53 ± 0.04 b
SA 0.01 mM	0.35 ± 0.02 a	0.15 ± 0.01 b	0.08 ± 0.01 a	15.5 ± 0.19 c	0.12 ± 0.01 b	0.45 ± 0.02 b
ASA 1 mM	0.34 ± 0.01 a	0.17 ± 0.01 ab	0.04 ± 0.01 b	16.4 ± 0.24 b	0.16 ± 0.11 b	0.45 ± 0.02 b
MeSA 0.1 mM	0.38 ± 0.02 a	0.19 ± 0.01 a	0.07 ± 0.01 ab	22.6 ± 0.12 a	3.33 ± 0.06 a	0.74 ± 0.04 a
'Magenta'						
Control	0.32 ± 0.02 c	0.22 ± 0.03 a	0.07 ± 0.02 a	15.7 ± 0.09 b	4.85 ± 0.05 c	0.54 ± 0.02 b
SA 0.01 mM	0.41 ± 0.02 b	0.24 ± 0.02 a	0.11 ± 0.02 a	15.9 ± 0.22 b	5.64 ± 0.11 b	0.63 ± 0.05 ab
ASA 0.1 mM	0.44 ± 0.03 ab	0.24 ± 0.01 a	0.10 ± 0.01 a	15.7 ± 0.16 b	5.63 ± 0.19 b	0.47 ± 0.07 b
MeSA 0.1 mM	0.49 ± 0.02 a	0.26 ± 0.01 a	0.08 ± 0.01 a	19.3 ± 0.21 a	8.16 ± 0.12 a	0.84 ± 0.09 a

[a] For each cultivar and parameter different letter following the mean are significantly different ($P < 0.05$) among treatments.

fruits, including table grapes. Clusters were harvested when berries reached the characteristic size, colour and TSS (°Brix) of each cultivar in order to pick up full mature grapes. However, preharvest treatment significantly affected ($P < 0.05$) the content of TSS and TA at harvest (Table 3). For both cultivars, MeSA at 0.1 mM significantly increased the content of both TSS and TA with respect to control grapes, while a reduction in TSS was observed for 'Crimson' and 'Magenta' treated with ASA 1 and 0.1 mM, respectively. However, preharvest treatment with SA did not show significant differences comparing with control berries. Similarly, the ripening index or ratio TSS/TA revealed that ASA treatment induced a delay of ripening process. Between cultivars, 'Crimson' showed higher TSS (18–20 g 100 g^{-1}) than 'Magenta' (16–17 g 100 g^{-1}), while total acidity was lower in 'Crimson' (0.5–0.6 g 100 g^{-1}) than on 'Magenta' (0.6–0.8 g 100 g^{-1}). It is well known that different cultivars had different levels of TSS and TA. In a survey of 129 grape cultivars from Europe, North America and Japan, the content of TSS differed with average values of 16.5, 16.7 and 17.2, respectively (Shiraishi et al., 2010). Table grape cultivars are classified into two groups on the basis of their sugar composition: hexose accumulators (Type 1), which accumulate fructose, glucose, and trace amounts of sucrose, and sucrose accumulators (Type 2), which accumulate fructose, glucose, and a large amount of sucrose. Accordingly, both 'Crimson' and 'Magenta' belong to Type 1 cultivars. In fact, as shown in Table 3, only glucose and fructose were detected by HPLC-RI, and sucrose was not detected. Similarly to TSS, glucose and fructose were enhanced in grapes treated with MeSA and reduced in those treated with ASA, showing a high correlation between TSS and the content of sugars ($R^2 = 0.896$).

With respect to organic acids (Table 4), tartaric acid was found at the highest concentration followed by citric and malic acids, while ascorbic, succinic and fumaric acids were considered as minor. The concentration of organic acids was different depending on treatment and cultivar. All treated grapes with salicylates showed the maximum concentration of tartaric acid. Tartaric acid is synthesized in many plants, but accumulates in high quantities in the fruit of only a few genera, most significantly members of the *Vitaceae* family (Valero and Serrano, 2010), and thus considering a characteristic organic acid of grapes. With respect to ascorbic, succinic and fumaric acids, only grapes treated with MeSA at 0.1 mM had significant ($P < 0.05$) higher concentrations compared with controls and other treated grapes. Interestingly, from these results it can be highlight the increase of ascorbic acid or vitamin C. Many studies have investigated the effect of salicylates treatments on soluble solids, sugar content and acidity in fresh produce, most of them being applied as postharvest application. Generally, higher content of soluble solids, sugars and organic acids were found during storage of salicylate-treated fruits (Giménez et al., 2017; Habibi et al., 2019) and may be associated with lower metabolism, as occurred in MeSA-treated grapes. On the contrary, postharvest treatment with SA or ASA in pomegranate did not change the content of TSS or TA (Sayyari et al., 2011b), while in sweet cherry was reduced (Valero et al., 2011).

4. Conclusions

In this report we demonstrated for the first time that preharvest application of SA, ASA and MeSA induced resistance of table grapes to be colonized with *B. cinerea*. The mechanism of action involved in this effect could be the increased levels of phenolic compounds and the activity of antioxidant enzymes APX, CAT and POD, although the best results were obtained with MeSA at 0.1 mM in both table grape cultivars. These preharvest treatments also showed benefits in term of table grape quality, such as higher TSS, TA, sugars and organic acids, especially tartaric and ascorbic acid. In addition, bioactive compounds and antioxidant activity are also enhanced by preharvest salicylate treatments.

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Declaration of competing interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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