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SALICYLATE COMPOUNDS AND 1-METHYLCYCLOPROPENE ON POSTHARVEST OF 'VEGA' CUT ROSE

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Key words: Rosa hybrida L., salicylic acid, methyl salicylate, 1-methylcyclopropene, floral opening.

ABSTRACT

Cut roses are characterized as highly perishable products due to the intense metabolism during postharvest, mainly the rise in respiratory activity and fast senescence. This study evaluated the effects of applications of 0.05 mM methyl salicylate (MeSA), 1.0 mM of salicylic acid (SA) and 1.0 µL L^{-1} of 1-methylcyclopropene (1-MCP) in maintaining the quality postharvest of cut roses cv. Vega. After treatments for 12 h, roses were kept at 20±1°C and 65±5% RH in vases with deionized water. The flowers were evaluated every two days for ten days concerning respiratory activity, ethylene production, peroxidase (POD) activity, relative fresh weight (RFW), relative water content (RWC) of the and floral opening . The results show that SA and 1-MCP reduced the respiratory activity and ethylene production, increase POD activity, RFW and the RWC of the petals. However, the 1-MCP is the only that improves the floral opening.

COMPUESTOS SALICILATOS Y 1-METILCICLOPROPENO EN LA POST-COSECHA DE ROSA PARA CORTE 'VEGA'

Palabras claves: Rosa hybrida L., ácido salicílico, metil salicilato, 1-metilciclopropeno, aperture floral

RESUMEN

Las rosas de corte son caracterizadas por ser altamente perecedero debido al intenso metabolismo después de la cosecha, principalmente el aumento de la actividad respiratoria que lleva a un consumo rápido de sus reservas y a una rápida senectud. En este estudio fueron evaluados los efectos de la aplicación de 0,05 mM de metil salicilato (MeSA); 1,0 mM de ácido salicílico (AS) y 1,0 µL L^{-1} de 1-metilciclopropeno (1-MCP) para la manutención de la calidad postcosecha de rosas de corte cv. Vega. Después de los tratamientos las rosas fueron almacenadas a 20±1°C y 65±5% UR en vasos con agua desionizada. Las flores fueron evaluadas a cada dos días durante 10 días. Las variables analizadas fueron: taza respiratoria, producción de etileno, actividad de la peroxidasa (POD), peso fresco relativo (PFR) y contenido relativo de agua (CRA) de las pétalas y apertura floral. Fue observado que el AS y el 1-MCP redujeron la taza respiratoria y la producción de etileno y aumentaron la actividad de la POD, el PFR y el CRA. El 1-MCP fue el único tratamiento que mejoró la apertura floral.

INTRODUCTION

Cut flowers are characterized as highly perishable products due to the intense metabolism during postharvest, showing rise in respiratory activity and ethylene production (Mayak, 1987; Kumar et al., 2008). This high metabolic activity increases fresh weight loss and the use of carbohydrate reserves, causing a fast senescence of cut flowers. Therefore, the possibility to attenuate the respiratory activity related to a lower ethylene response has a remarkable importance in the postharvest of flowers, since this are fundamental traits to enable the metabolism maintenance and flower opening (Reid, 2002).

Some compounds that might be used to reduce these processes during postharvest are 1-methylcyclopropene (1-MCP), salicylic acid (SA) and methyl salicylate (MeSA). The 1-MCP binds itself irreversibly to the ethylene

receptor site, inhibiting the ethylene action and therefore also limiting its autocatalytic production. This results in a lower respiratory activity (Serek et al., 1995; Schotsmans et al., 2009). The SA, a natural phenolic compound, participates in the regulation of many plant physiological processes as scavenge of reactive oxygen species (ROS) under stress conditions, and also has a potential to decrease ethylene production by reducing the activity of 1-aminocyclopropane-1-carboxylic acid (ACO) (Leslie and Romani, 1988; Asghari and Aghdam, 2010). The MeSA has the same capacity of SA but in a volatile form, being produced by plants as a signal that induce local and systemic responses (Shulaev et al., 1997).

Although not classified as highly sensitive to ethylene, roses (*R. hybrida* L.) have a variable response between cultivars, and the stress that cut flowers suffer during harvest and postharvest may increase the ethylene sensitivity and/or production (Woltering and Van Doorn, 1988; Macnish et al., 2010). The 1-MCP has been shown to provide improvements on postharvest maintenance of ornamental plants, with positive results for some cultivars of roses (Macnish et al., 2010; Pietro et al., 2010), carnation (Karimi, 2014) and nasturtium (Silva and Finger, 2015). On the other hand, the salicylate compounds, which have been successfully studied for induction of systemic acquired resistance against pathogens (Vlot et al., 2009), also have potential to maintain the postharvest quality of flowers as observed in gladiolus (Ezhilmathi et al., 2007), roses (Alaey et al., 2011) and anthuriums (Promyou et al., 2012).

All the possibilities the salicylate compounds present could have a more incisive approach with a demonstrated potential to maintain postharvest quality. So was chosen another compound well known by keeping the postharvest quality (1-MCP) that also present the characteristics of being non-toxic and easy to apply. Therefore, the purpose of this study was to investigate the influence of MeSA and SA compared to 1-MCP on the metabolism and quality traits during the postharvest of cut rose.

MATERIALS AND METHODS

Plant material and experimental conditions

Stems of cut roses cv. Vega obtained from an open-field commercial grower were selected and transported for one hour in tap water to the laboratory at São Paulo University, Piracicaba, SP, Brazil, where were re-cut to a uniform length of 50 cm and placed in vases containing deionized water.

The vases with flowers were placed inside hermetic chambers of 186 L and exposed to the treatments for 12 hours in controlled environmental conditions of 20±1°C, 65±5% RH. The treatments were consisted of 0.05 mM of MeSA, 1 µL L^{-1} of 1-MCP, and 1 mM of SA. Because of the volatile nature of MeSA and 1-MCP the chambers lids were sealed and then these treatments were inserted throughout a sealable lateral opening. The MeSA was applied on a filter paper attached to the wall of the chamber, while the 1-MCP, obtained from EthylBloc™ (AgroFresh Inc., PA, USA) dissolved in water, was released from a sealed vial that was opened inside the chamber. The SA was dissolved in deionized water and applied via pulsing, and the control was deionized water.

Afterwards the water was replaced and the flowers were stored in the same environmental conditions under a 12 hour light cycle, with the water being replaced and the stems re-cut 1.5 cm every two days.

The following assessments were performed in six evaluations (day 0, 2, 4, 6, 8 and 10).

Respiratory activity and ethylene production

Three rose stems were placed into a 5 L glass container and hermetically sealed for 40 min at the same environmental conditions of the storage. For the respiratory activity and the ethylene production, a sample of 0.5 mL from the internal atmosphere was collected

through a silicone septum and measured by flame ionization (FID) gas chromatography (Trace GC Ultra, Thermo Electron Corporation, MA, USA). The respiratory activity was expressed as mg CO_2 kg^{-1} h^{-1} and ethylene production as nL C_2H_4 kg^{-1} h^{-1}.

Peroxidase (POD) activity (EC 1.11.1.7)

For the enzyme extraction 0.3 g of frozen petals were ground and homogenized with 5 ml of cold 0.2 M potassium buffer (pH 6.7) and 1 mg of polyvinylpyrrolidone-10 (PVP), centrifuged at 10,000 x g for 10 minutes at 4°C. For the quantification, 0.5 mL solution containing 20 mM hydrogen peroxide with 0.2 M phosphate buffer (pH 6.7), and 0.5 mL of solution containing 4 mM aminoantipyrine with 10 mM pyro-catechol were added to 1 mL of enzyme extract. The solution was incubated in a 30° C water bath for 5 min and the enzymatic reaction stopped with 2 ml of ethyl alcohol. The activity was determined spectrophotometrically (Libra S22, Biochrom Ltd, Cambridge, UK) by changes in absorbance at 505 nm and expressed in units per milligram of protein (U mg^{-1} protein). Protein content was determined according to Bradford (1976) using bovine serum albumin as standard.

Relative fresh weight (RFW)

The RFW was determined by obtaining the fresh weight in each evaluation (*FWe*), considering the 1.5 cm re-cut of every evaluation, and comparing with the initial fresh weight (*FWi*) using the equation: RFW (%) = (*FWe*-*FWi*)/*FWi**100, expressed as a percentage of the initial weight.

Relative water content (RWC)

The RWC was determined by obtaining the fresh weight (*FW*) of 10 mm disc from six petals, which were subsequently immersed in deionized water at room temperature for six hours to determine the turgid weight (*TW*) and then dried at 65°C to obtain the dry weight (*DW*). The relative water content was calculated using the equation: RWC (%) = (*FW*-*DW*)/(*TW*-*DW*)*100, expressed as a percentage of the initial fresh weight (Weatherley, 1950).

Floral opening

The floral opening was determined by grades using a pre-determined visual scale: 1 = sepals curved downwards, external petals starting to open; 2 = external petals opened, intermediate starting to open; 3 = intermediate petals opened, internal starting to open; 4 = whole flower opened.

Statistics

The experiment was designed completely randomized with four replications of three flowers. Data analyses were performed by analysis of variance (F test) using SISVAR statistical software 5.3. Multiple comparisons among the treatments with significant differences tested were conducted by using Tukey test ($P < 0.05$).

RESULTS AND DISCUSSION

Respiratory activity and ethylene production

In the first evaluation the roses presented a high respiratory activity of 514 mg CO_2 kg^{-1} h^{-1} that was maintained by the control in the second day (Figure 1a). All treatments had a decrease towards the fourth day, with 1-MCP and SA presenting the lowest activities with an average of 267 mg CO_2 kg^{-1} h^{-1}. These treatments showed a similar behavior during the evaluations, presenting a respiratory peak of 400 mg CO_2 kg^{-1} h^{-1} on the sixth day followed by a decrease until the tenth day, when they had an average of 288 mg CO_2 kg^{-1} h^{-1}. MeSA presented the respiratory peak on the eighth day, with 444 mg CO_2 kg^{-1} h^{-1} followed by a reduction to 355 mg CO_2 kg^{-1} h^{-1} on the tenth day. The control had an activity peak of 540 mg CO_2 kg^{-1} h^{-1} on the sixth day and presented a higher respiratory activity compared to the 1-MCP and SA in all evaluations.

The ethylene also presented a high production rate of 1.52 nL C_2H_4 kg^{-1} h^{-1} in the first evaluation that was maintained in the

second day by the control, but suffered a drop on the other treatments (Figure 1b). For all treatments was observed production peak on the eighth day sustained until the tenth day, except only for the control that wasn't detected in the last evaluation.

The results show that the roses expressed a similar pattern of respiratory activity and ethylene production. The high respiratory activity and ethylene production presented in the first evaluation are likely to be a response to the stress conditions caused by harvest and handling, as they were followed by markedly lower rates in the two subsequent evaluations (Coorts, 1973; Yakimova and Woltering, 1997). During the second day the 1-MCP and SA quickly lowered both rates, and that is a desirable trait mainly because since the ethylene is related to the increase on invertase activity and the respiratory activity consumes carbohydrates, it is likely to enable the flowers to save energetic resources to be used later during the vase life.

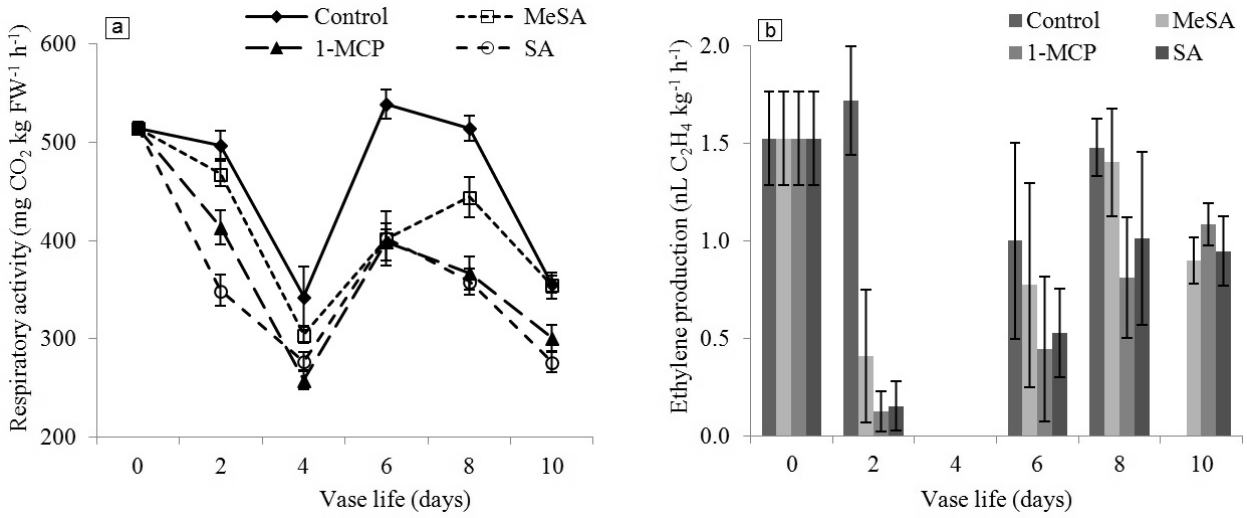

Figure 1. Respiratory activity (a) and ethylene production (b) of roses cv. Vega treated with methyl salicylate (MeSA), 1-methylcyclopropene (1-MCP) and salicylic acid (SA) stored at 20±1°C, 65±5% RH. Vertical bars represent the standard error of means (*n=4*).

The roses usually show a consistent and well defined respiratory climacteric and a small ethylene production during senescence, and these two events probably share a common trigger (Mayak, 1987; Kumar et al., 2008). Our results also show that the respiratory climacteric occurred prior to the ethylene peak, and that all the treatments had a lower peak compared to the control. Besides, the MeSA treatment also delayed the peak.

The 1-MCP is known for the direct inhibition of the ethylene action by binding to its receptor site. The SA, besides influencing the ACC conversion to ethylene, also enhances the ROS scavenging activity, possibly reducing the stress signaling which might ultimately lead to lower ethylene action and production (Leslie and Romani, 1988; Asghari and Aghdam, 2010). Similar results were found for 1-MCP application in roses cv. Vega and cv. Sparkle (Pietro et al., 2010; Nergi and Ahmadi, 2014) and in carnations (In et al., 2013), and were also found for SA application in gladiolus (Ezhilmathi et al., 2007).

Peroxidase activity

The initial evaluation had a POD activity of 1.59 U mg^{-1} protein followed on the second and fourth days by similar average activities of

1.69 and 1.76 U mg^{-1} protein, respectively (Figure 2). All the treatments had a peak in activity on the sixth day except the control. In this day, SA treatment showed value of 2.24 U mg^{-1} protein; MeSA and 1-MCP presented an average of 2.03 U mg^{-1} protein while control showed 1.73 U mg^{-1} protein. Starting on the sixth day there was decrease in the POD activity for MeSA, 1-MCP and SA while for the control there was increased. In the tenth day SA and 1-MCP were the lowest activities showing an average of 1.81 U mg^{-1} protein, while MeSA and control presented 1.99 and 2.25 U mg^{-1} protein, respectively.

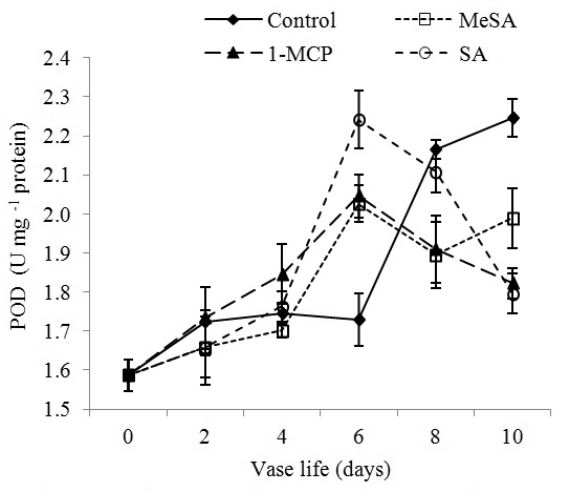

Figure 2. Peroxidase activity (POD) of roses cv. Vega treated with methyl salicylate (MeSA), 1-methylcyclopropene (1-MCP) and salicylic acid (SA) stored at 20±1°C, 65±5% RH. Vertical bars represent the standard error of means (*n=4*).

The POD is one of the scavenging enzymes responsible for catalyzing H_2O_2 decomposition. Our results show that in the control, the POD activity remained stable until the day of the climacteric peak and after that kept increasing until the last day. The 1-MCP and SA had a POD activity peak on the same day of the climacteric, so this reflection of the respiratory activity is likely an answer to the increase of the ROS production.

Nevertheless, the SA presented the highest POD activity peak and the MeSA had the POD peak prior to the climacteric. These are positive results since the salicylate compounds are used by the plant to generate stress tolerance, using their stress signaling proprieties to induce antioxidant enzymes, resulting in increased ROS scavenger activity. Plants under biotic and abiotic stress can also accumulate SA in order to cause a transient oxidative stress, which boosts the antioxidative capacity to prevent subsequent cellular damage, and it suggests that salicylate compounds help to delay senescence (Popova, 2013). Our results suggest that the treatments, especially the SA, might promote the ROS scavenging activity. Similar results of increased ROS scavenging activity by salicylate compounds were found in gladiolus (Ezhilmathi et al., 2007), roses cv. Yellow Island (Gerailoo and Ghasemnezhad, 2011) and anthurium (Promyou et al., 2012).

Floral opening

The roses treated with 1-MCP presented a better opening grades than the control in all evaluations, and better than MeSA an SA from the sixth day (Figure 3). The 1-MCP presented an opening grade of 3.5 on the sixth day followed by 3.7 on the eighth and tenth, while all the other treatments presented an average of 3.2 and 3.3, respectively. Only on the second day SA presented better floral opening than control.

Considering that the floral opening depends on the expansion of the petals cells, which is mainly dependent on the water uptake, the higher RWC indicates a higher potential for better floral opening. However, when the floral opening was analyzed, only the 1-MCP achieved improves results than control. According to Ma et al. (2008) the ethylene affects the gene expression of aquaporins (AQP), important structures that facilitate the water intake and consequently the cellular expansion, therefore also interfering in the flower opening. They also show that when roses were treated with 1-MCP the expression of the genes of AQPs was

induced, increasing petal water content during the flower opening and therefore improved it (Ma et al., 2008; Nergi and Ahmadi, 2014).

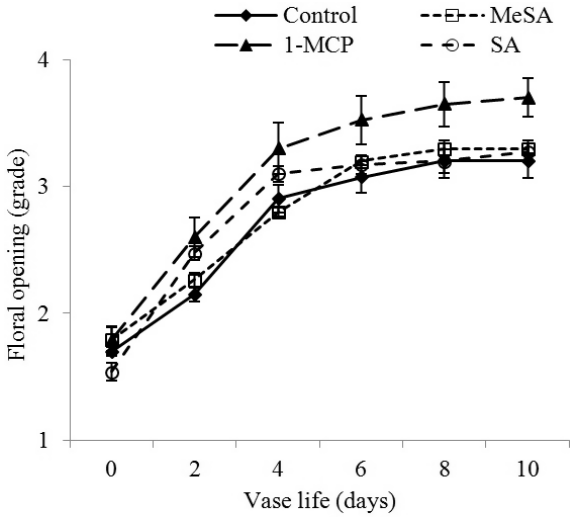

Figure 3. Floral opening of roses cv. Vega treated with methyl salicylate (MeSA), 1-methylcyclopropene (1-MCP) and salicylic acid (SA) stored at 20±1°C, 65±5% RH. Vertical bars represent the standard error of means (*n=4*).

Relative fresh weight and relative water content

All treatments increased the relative fresh weight on the second day. The 1-MCP and SA sustained the increase until the sixth day, presenting higher fresh weight than the control from the fourth day forward, having peaks of +4.5 and +3.5% while the control peak was +1.9% (Figure 4a). The MeSA had higher fresh weight than control only on the eighth and tenth days, but lower than other treatments. On the tenth day the average of 1-MCP and SA treatments presented a 6% higher fresh weight than the control.

The RWC of the petals treated with 1-MCP and SA was higher than control on all evaluations (Figure 4b). The MeSA presented higher RWC than control only on the fourth and sixth days. The greater difference was observed on the fourth day, with MeSA, 1-MCP and SA presenting an average RWC 3.6% higher than control.

The increase on the first evaluations of RFW is probably a result of the increased transpiration caused by the stress of harvest and transportation which lowered the initial weight, followed by a great water uptake by the stems in the following evaluations. The improved RFW, provided especially by 1-MCP and SA, shows a superior water balance likely related to the regulation of stress conditions that results in the lower respiratory activity, which therefore reduces the transpiration rates, and besides, maintains the carbohydrates concentration that provides an osmotic potential that favors the water uptake.

Reflecting the results of RFW, the RWC of the petals was improved by all treatments, mainly 1-MCP and SA. These results were also observed for 1-MCP treatments in roses cv. First Red and cv. Vega (Chamani et al., 2005; Pietro et al., 2010) and for SA treatments in roses cv. Black Magic, anthuriums and gladiolus (Alaey et al., 2011; Marandi et al., 2011; Promyou et al., 2012).

Although the SA and MeSA had similar results to 1-MCP in the other parameters, the 1-MCP controls exclusively the ethylene inhibitory stimulus, while the salicylate compounds interact with many hormones, including the auxins, notably important to cell expansion. The SA is reported to down-regulate the auxins production when the plant is under stress, and so it might explain the not so notable RWC and not improved floral opening (Iglesias et al., 2011). Also, the salicylate compounds might have caused a lack of metabolism stimulus that suppressed the use of energy reserves, and the results may also represent a delay of the complete opening by the salicylate compounds, which might enable a desirable extension of the total vase life, based on the better fresh weight and petal RWC presented compared to control.

In summary, the SA, MeSA and 1-MCP treatments result in lower postharvest metabolism of roses, mainly by reducing

respiratory activity and ethylene production, and favoring scavenger capacity and water balance of the flower stems. However, the 1-MCP was the only one to improve the floral opening.

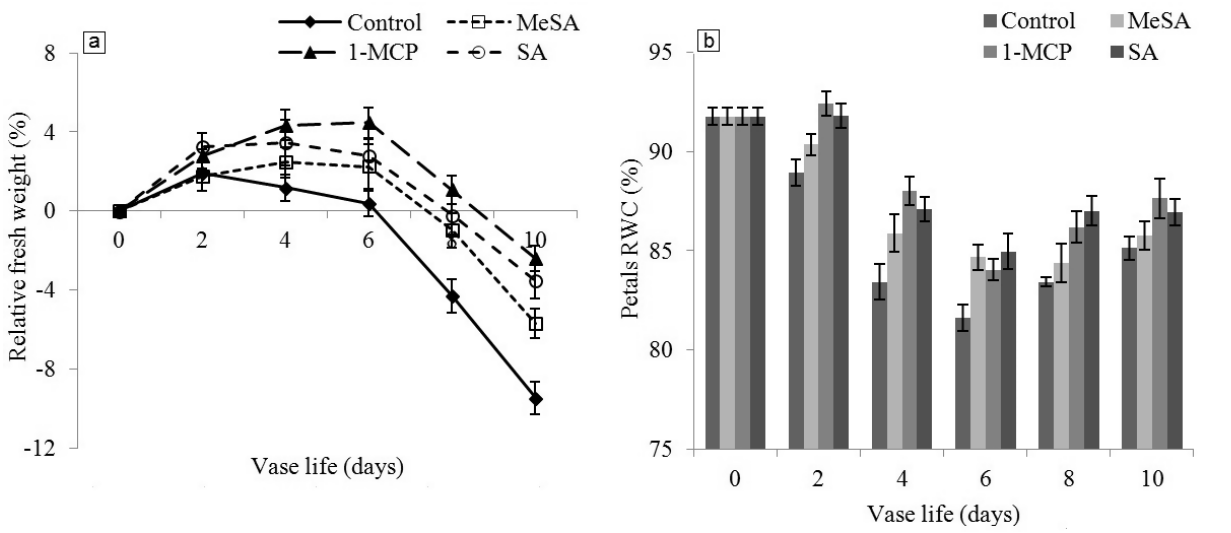

Figure 4. Relative fresh weight (RFW) (a) and relative water content (RWC) of petals (b), of roses cv. Vega treated with methyl salicylate (MeSA), 1-methylcyclopropene (1-MCP) and salicylic acid (SA) stored at 20±1°C, 65±5% RH. Vertical bars represent the standard error of means (*n=4*).

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