

Quality of 'Isabel' grape treated pre-harvest with CaCl_2 and citrus biomass-based elicitor

Qualidade de uva 'Isabel' tratada na pré-colheita com CaCl_2 e elicitor à base de biomassa cítrica

Leonardo da Silva Santos¹; Silvanda de Melo Silva^{2*}; Ana Lima Dantas³; Antônio Fernando da Silva¹; Antonio Augusto Marques Rodrigues¹; Gerciane Cabral da Silva⁴; Luciana Cordeiro do Nascimento²; Rejane Maria Nunes Mendonça²

Abstract

The high rate of fruit decay and berry drop reduces quality and increases postharvest losses in 'Isabel' grapes, requiring pre-harvest and postharvest management practices available to the small-scale farmer, which maintain quality and extend postharvest life of the clusters. The objective of this study was to evaluate the effect of the pre-harvest application of CaCl_2 and Citrus Biomass-based elicitor in 'Isabel' grapes, in the maintenance of quality during storage at room conditions under ambient and modified atmospheres. The experiment was conducted in a randomized block design with 8 replicates, in the field located at the municipality of São Vicente Férrer-PE, Brazil. 'Isabel' vines were treated 28 days before harvest with: Citrus Biomass-based elicitor (CB), Citrus Biomass-based elicitor + CaCl_2 (CB + C), CaCl_2 (C) and Control (T) – without application. Grapes harvested in the commercial maturity were stored in a $4 \times 2 \times 7$ factorial arrangement, with 4 treatments (applied in the field), 2 storage conditions, ambient (AA) and modified (AM) atmospheres, under room conditions (25 ± 2 °C and $75 \pm 2\%$ RH) and 7 evaluation periods in four replicates. The application of CaCl_2 and CB-based elicitor reduced the berry drop index (55 and 75%, respectively), the decay and weight loss of 'Isabel' grape clusters. These treatments, associated or not, increased MA efficiency in maintaining cluster quality. The 'Isabel' grape berry drop index was directly influenced by the SS/TA ratio, pH and fruit decay index.

Key words: Modified atmosphere. Berry drop. Resistance inducers. Decay. *Vitis labrusca*.

Resumo

O alto índice de degrana e podridão das bagas reduzem a qualidade e elevam as perdas pós-colheita em uvas 'Isabel', demandando técnicas de manejo na pré e pós-colheita acessíveis ao pequeno produtor, que mantenham a qualidade e ampliem a vida útil pós-colheita dos cachos. Assim, este trabalho teve por objetivo avaliar o efeito da aplicação na pré-colheita de CaCl_2 e elicitor à base de biomassa cítrica em uvas 'Isabel' na manutenção da qualidade durante o armazenamento na condição ambiente sob atmosferas ambiente e modificada. O experimento foi conduzido em blocos casualizados a campo, no município de São Vicente Férrer-PE com 8 repetições. Videiras 'Isabel' foram tratadas, 28 dias antes da

¹ Mestres em Agronomia, Estudante de Doutorado, Programa de Pós-Graduação em Agronomia, PPGA, Centro de Ciências Agrárias, CCA, Universidade Federal da Paraíba, UFPB, Areia, PB, Brasil. E-mail: silvasantosleonardo@hotmail.com; digfernando@hotmail.com; antonioaugustomr@yahoo.com.br

² Profs. Drs., PPGA/CCA/UFPB, Areia, PB, Brasil. E-mail: silvasil@cca.ufpb.br; luciana.cordeiro@cca.ufpb.br; rejaneufpb@yahoo.com.br

³ Dr^a, Pesquisadora, PDJ/CNPq/PPGA/UFPB – PPGA, Areia, PB, Brasil. E-mail: dantas.ana.lima@gmail.com

⁴ M.e, Ciência e Tecnologia de Alimentos, Pesquisador, Empresa Estadual de Pesquisa Agropecuária da Paraíba, EMEPA, João Pessoa, PB, Brasil. E-mail: gercycs@hotmail.com

* Author for correspondence

colheita, com: elicitor de Biomassa Cítrica (BC), elicitor de Biomassa Cítrica + CaCl₂ (BC+C), CaCl₂ (C) e Testemunha (T) – sem aplicação. Cachos colhidos na maturação comercial foram armazenados em arranjo fatorial 4×2×7, sendo 4 tratamentos (aplicados no campo), 2 condições de armazenamento, atmosferas ambiente (AA) e modificada (AM), sob condição ambiente (25±2°C e 75±2% de UR) e 7 períodos de avaliação em quatro repetições. A aplicação de CaCl₂ e do elicitor BC reduziu o índice de degrana (55 e 75%, respectivamente), a podridão e a perda de massa dos cachos de uva ‘Isabel’. Estes tratamentos, associados ou não, aumentaram a eficiência da AM em manter a qualidade dos cachos. O índice de degrana de uva ‘Isabel’ foi influenciado diretamente pela relação SS/AT, pH e índice de podridão das bagas.

Palavras-chave: Atmosfera modificada. Degrana. Indutores de resistência. Podridão. *Vitis labrusca*.

Introduction

Grape production has great socioeconomic importance in Brazil, with a harvested area of 1,499,353 t in approximately 79,094 ha in 2015, with production concentrated in the South, Northeast and Southeast regions. Pernambuco state is known as the largest producer in the Northeast, and second overall in Brazil (IBGE, 2016), mainly in the mid-São Francisco Valley. However, other regions of the state are promising, such as the municipality of São Vicente Férrer-PE, with more than 600 ha of ‘Isabel’ grape (*Vitis labrusca* L.), mainly destined for the fresh fruit market (EMBRAPA, 2012; SILVA et al., 2012), whose production is predominantly comes from small-scale family farms, a promising employment and income alternative. Thus the ‘Isabel’ grape variety has adapted very well to the Northeast region of Brazil (EMBRAPA, 2012), and is a variety known for its high productivity and high sugar accumulation potential (SATO et al., 2009).

However, high levels of post-harvest losses of the ‘Isabel’ grape are mainly due to the high rate of berry drop and decay after harvesting. In this context, several techniques have been applied in postharvest grape storage, such as the use of refrigeration and modified atmosphere associated with the application of calcium chloride (DANNER et al., 2009; SILVA et al., 2012), also applied pre-harvest (RUIZ-GARCÍA et al., 2012; PORTU et al., 2015) as well as the association of pre-and postharvest treatments in other fruits, such as apples (SHARMA et al., 2013) and plums (MANGANARIS et al., 2008), aiming to promote an increase in fruit postharvest

life, thus resulting in increased prospects of greater profitability.

The application of calcium chloride, either during the development of cluster of grapes or in postharvest, in order to increase the postharvest life, has conferred greater resistance to handling, reducing the loss of mass, decay and berries abscission (TECCHIO et al., 2009; SILVA et al., 2012), the incidence of disease (DANNER et al., 2009; DEVI; KUMARI, 2015) and promoting a decrease in the activity of polyphenoloxidase and the consequent browning of the raquis during storage (DANNER et al., 2009).

Additionally, the application of elicitors or resistance inducers has been used to induce plant resistance against pathogens and emerges as an ecological alternative (DEVI; KUMARI, 2015; SAAVEDRA et al., 2016; ZHU et al., 2016), being safer to people and to the environment, and generally of lower cost and phytosanitary efficiency already proven in some pathosystems, including grapevines (GOMES et al., 2011; PINTO et al., 2012). In this context, the association of these inducers with calcium chloride in pre-harvest management may provide greater postharvest protection against pathogens that cause decay, and can be used safely in family farming.

In apples, metabolomic studies indicate a relationship between resistance to disease and phenolic profiles (SUN et al., 2017). Thus, among the elicitors, the composition based on citrus biomass, ascorbic acid and citrus phytoalexins stands out, which induce systemic resistance acquired in plants

(RSA) (GOMES et al., 2011). These agents induce plant tissues to self-synthesize phytoalexins in order to reduce damage caused by pathogens and have been tested in alternative control of plant diseases, *in vitro* and *in vivo* assays being used to prove the controlling action (ZHU et al., 2016). However, there are few studies that show the implications of their use on postharvest quality of fruits, especially associated with CaCl₂ and non-climacteric fruits such as grapes.

Thus, the objective of this study was to evaluate the effect of the pre-harvest application of calcium chloride and elicitor based on citrus biomass, associated or not, in 'Isabel' grapes, aiming to maintain postharvest quality during storage under ambient and modified atmospheres.

Material and Methods

The 'Isabel' (*Vitis labrusca* L.) grapes were produced on a commercial farm, conducted in a family farming system, in an irrigated vineyard, 3 years old, in the municipality of São Vicente Férrer-PE, Vale do Sirijí. The spacing was 3 x 2 and the plants were tied in a flat wire system, the production pruning was done twice a year, since two harvests were produced per year, in addition to green pruning. The experimental design was performed in randomized blocks, with eight replicates, composed of three 'Isabel' grape plants. 28 days before harvesting, when the clusters were at the beginning of pigmentation, four treatments were applied: Citrus Biomass-based elicitor (3 mL L⁻¹) – CB, Citrus Biomass-based elicitor (3 mL L⁻¹) + CaCl₂ (3%) – CB + C, CaCl₂ (3%) – C and Control (T) – no application. The treatments were applied with a pressure sprayer on 24 plants per treatment in all directions from the center of the plant to the edges until the solution began to drip from the clusters, considering the border of three rows of plants, between treatments.

After 28 days of application of the treatments, 'Isabel' grapes clusters were harvested at commercial maturity, considering the minimum

SS content of 14%, with uniform clusters being selected and damaged fruit discarded and taken to the laboratory. In a completely randomized design, the clusters were stored (four per replicate) in a factorial scheme 4 × 2 × 7, with 4 treatments (from the field); 2 storage conditions, modified (15 ± 25 cm styrofoam trays coated with polyvinyl chloride film – PVC, 14 µm thick) and ambient atmospheres, maintained under ambient conditions (24 ± 2 ° C and 75 ± 2% RH); and 7 evaluation periods every 2 days (0, 2, 4, 6, 8, 10 and 12 days).

The fruits were evaluated for the incidence of decay (%), determined by the difference of weight between the healthy and diseased fruits of each cluster; berry drop index (%), determined by the difference between the weight of the clusters and berry dropped fruits, after manually shaken five times, according to the methodology described by Silva et al. (2012); and fresh weight loss (%), obtained through daily weighing, considering the difference relative to the initial weight.

The color of the grape raquis was determined by the Minolta CM-508d Colorimeter, measuring the following variables: *L* *, which corresponds to lightness and varies from 0 (black) to 100 (white); the chromatic components, *a* * representing the transition from green to red, and *b* * representing the transition from blue to yellow (SILVA et al., 2012). The following were also evaluated: soluble solids – SS (%), determined by direct reading with ABBE bench type refractometer, with temperature control at 20 ° C (MASCARENHAS et al., 2010); titratable acidity – TA (g of 100 g⁻¹ tartaric acid pulp) by titration with 0.1 M NaOH and SS/TA ratio, determined by dividing SS and TA (AOAC, 2005).

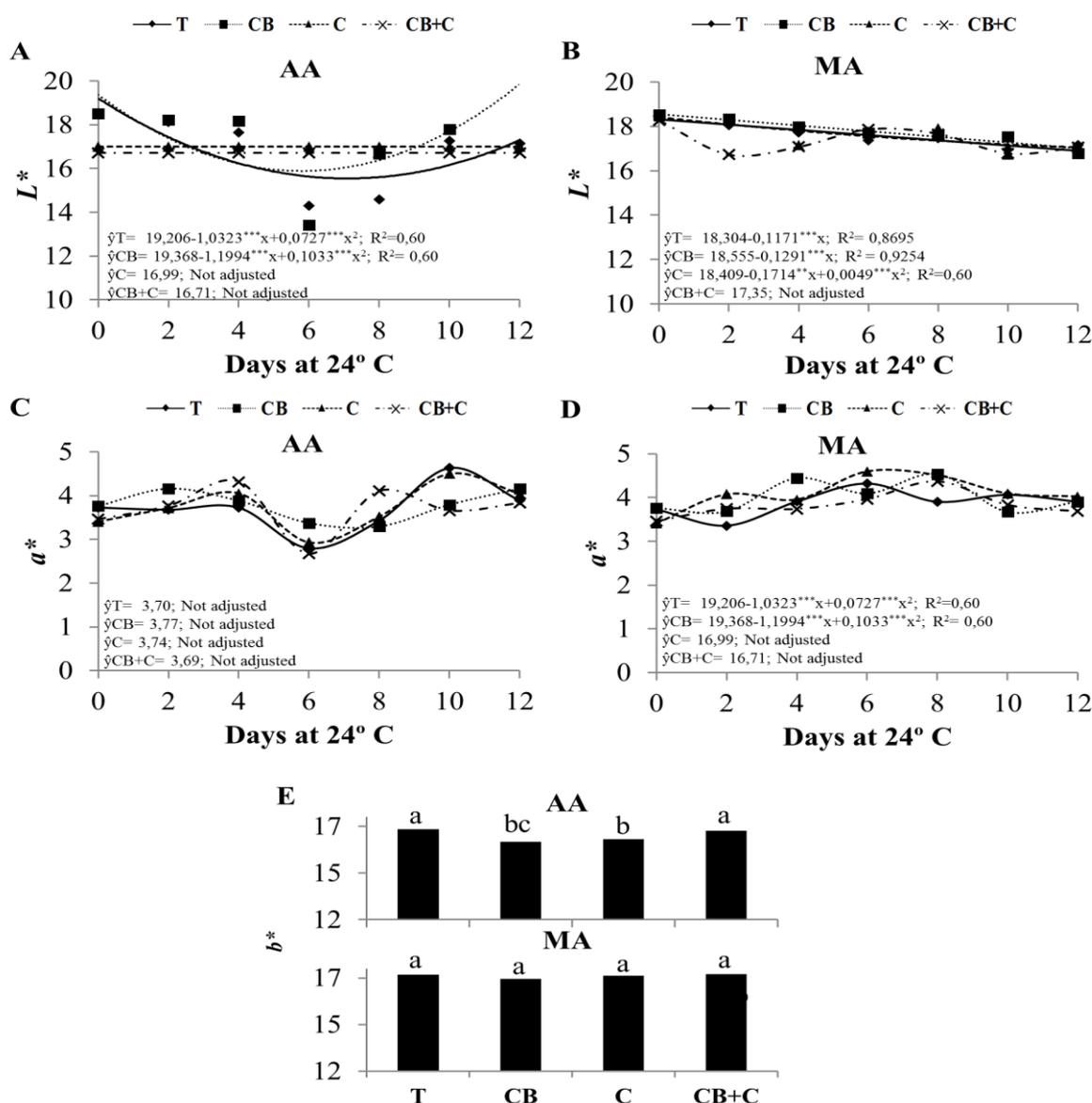
The data were submitted to analysis of variance and regression by SAS® 9.3 software (2011) and the means compared by the Tukey test (p≤0.05). In order to identify the main contributors in firmness and berry drop, a simple correlation analysis was performed between these and the physicochemical characteristics, using the statistical software JMP® 10.0.0.

Results and Discussion

The color of 'Isabel' grapes did not show marked changes during storage, indicating a reduced influence of the treatments applied in the pre-harvest in the color of the fruit (Figure 1). However, the fruit slightly lost lightness (L^*) during storage (Figure 1A and 1B). In turn, the variable a^* was not

influenced by the treatments, with slight evolution during storage, in both storage atmospheres (Figure 1C and 1D). The variable b^* , similarly, showed little change during storage, with lower values for the clusters maintained in ambient atmosphere (Figure 1E), and when treated with CaCl_2 alone or associated with the elicitor (Figure 1F), indicating a slightly more intense bluish color.

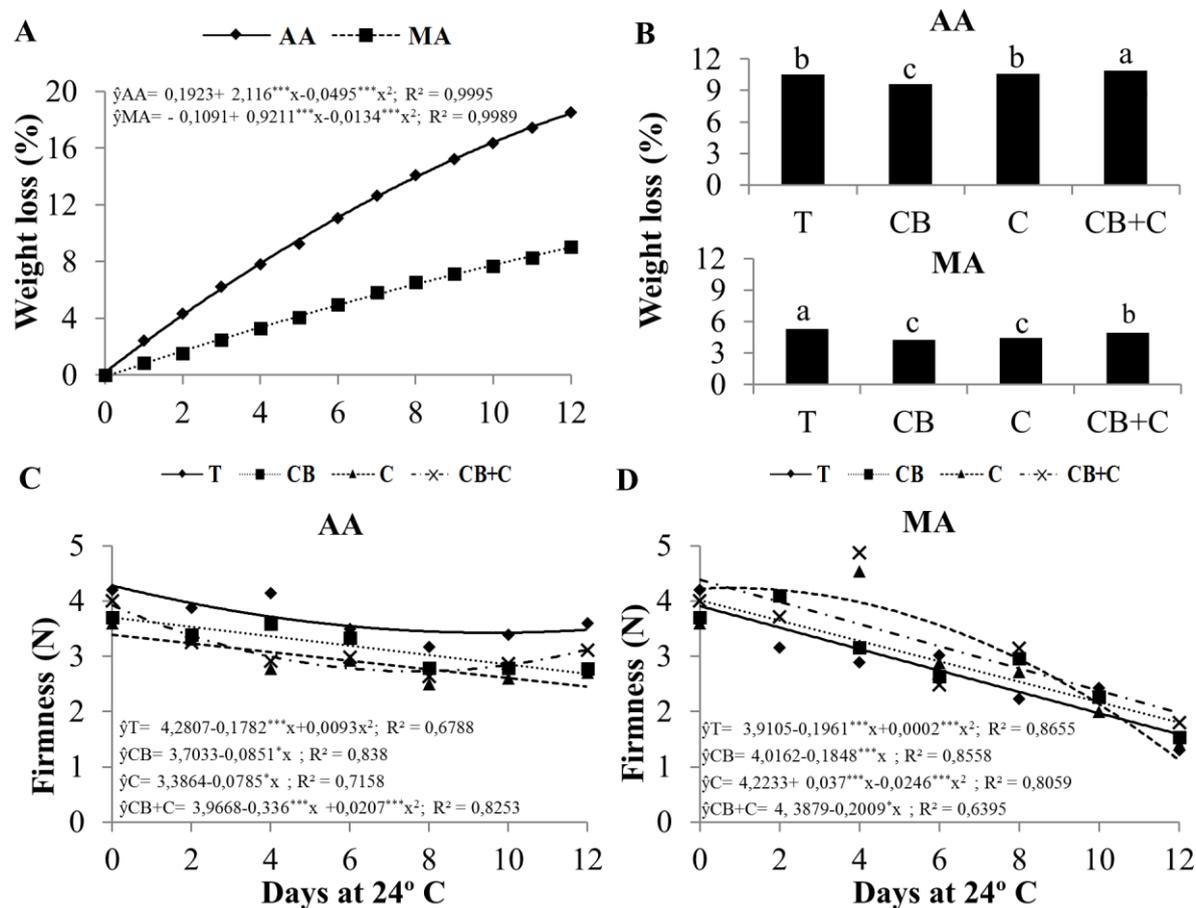
Figure 1. Color parameters L^* (A and B), a^* (C and D) and b^* (E), during storage ($24 \pm 2^\circ \text{C}$ and $75 \pm 4\% \text{ R.H.}$) of treated 'Isabel' grapes during pre-harvest with Citrus Biomass-based elicitor (CB), CaCl_2 (C), Citrus Biomass-based elicitor + CaCl_2 (CB + C) and Control – without application (T). AA = Ambient Atmosphere and MA = Modified Atmosphere.



These small changes in the skin color of 'Isabel' grapes during storage follow a typical behavior for non-climacteric fruits, which should be harvested at the maturity of consumption, corresponding to the physiological maturity (CHERIAN et al., 2014), in which the characteristic color of the cultivar is fully developed, but also indicates the beneficial effect of the treatments in maintaining the characteristic coloration of the berries. The pre-harvest application of CaCl₂ and the citrus biomass-based elicitor (CB) minimized the loss of fresh mass of 'Isabel' grapes

during storage, a clearer effect on clusters kept under a modified atmosphere (Figure 2B). In turn, the association of CaCl₂ and CB promoted greater weight loss (Figure 2B), indicating that when applied together, they may have caused tissue stress. The use of the modified atmosphere reduced the loss of weight by approximately 50%, regardless of the treatment used (Figure 2A). This effect was clearer in clusters from vines treated with citrus biomass (CB) and CaCl₂ (C).

Figure 2. Weight (A and B) and firmness (C and D) loss of 'Isabel' grapes during storage (24 ± 2 ° C and 75 ± 4% R.H) treated during pre-harvest with Citrus Biomass-based elicitor (CB), CaCl₂ (C), Citrus Biomass-based elicitor + CaCl₂ (CB + C) and Control – without application (T) elicitor. AA = Non-modified Atmosphere and MA = Modified Atmosphere.



The use of CaCl_2 and citrus biomass-based elicitor, associated or not, resulted in softer fruit during storage in ambient atmosphere (Figure 2C). The firmer fruits of the control, however, may be due to the greater weight loss of this treatment, which, due to the wilting, present greater resistance to penetration (SILVA et al., 2012). In contrast, clusters of all treatments maintained the fruit firmer than the control under modified atmosphere, especially when the CaCl_2 was applied pre-harvest, remaining more firm until the eighth day (Figure 2D). Probably, the influence of the pre-harvest treatments was not evidenced when the grapes were kept in ambient atmosphere due to their high weight loss, causing intense dehydration in the fruit, making the skin more resistant to pressure.

The pre-harvest application of calcium chloride promotes the strengthening of the cell wall of the fruit, providing greater resistance to the tissues, reducing, in addition to the fresh weight loss, the incidence of diseases in postharvest, being these responses closely related to increased calcium concentration in tissues (MADANI et al., 2014). 'Isabel' grapes treated postharvest with CaCl_2 under ambient and modified atmospheres showed reduced fresh weight loss (SILVA et al., 2012). However, the postharvest efficacy in maintaining fruit firmness seems to be higher when the use of CaCl_2 is combined with other bioregulators, such as 1-MCP, associated with hydrothermal treatment, as reported for guavas (CRUZ et al., 2015).

The pre-harvesting use of CaCl_2 also led to the reduction of mass loss during storage of 'Venus' grapes (DANNER et al., 2009) and provided apples with greater firmness, especially when combined with postharvest hydrothermal treatment (SHARMA et al., 2013). In plums, treated with CaCl_2 associated with other bioactivators, Manganaris et al. (2008) reported both the reduction in weight loss and retention in firmness during storage in plums, providing a higher resistance to fruits in postharvest.

The application of citrus biomass-based elicitor and CaCl_2 , associated or not, did not influence pH,

soluble solids content (SS) and titratable acidity (TA) of 'Isabel' grapes during storage (Figure 3). The content of SS was higher in the fruit maintained under ambient atmosphere (Figure 3A), probably due to the higher weight loss, whereas the TA showed a linear decrease, independently of the atmosphere (Figure 3B). For the SS/TA ratio, lower values were recorded for the clusters treated with citrus biomass-based elicitor when kept under modified atmosphere, differing only from the fruit treated with the elicitor associated with CaCl_2 , which promoted a higher ratio (Figure 3C). However, the SS content was lower, higher TA and SS/TA lower than that of 'Isabel' grapes from the São Francisco Valley (MASCARENHAS et al., 2010), indicating a higher aptitude of the Paraíba grapes for winemaking. In turn, the 'Isabel' grape produced in Londrina, PR, presented SS, TA and SS / TA ratio close to those reported herein (KOYAMA et al., 2014).

The SS content was maintained during storage, corroborating with the behavior of non-climacteric fruits and having been harvested with complete maturation, since the increase of sugars occurs during the skin degreening due to the advance of fruit ripening on the plant (CHERIAN et al., 2014). The SS were kept higher in clusters kept under ambient atmosphere, probably due to greater weight loss. Thus, postharvest SS increase was more related to the increase in the concentration of soluble compounds due to the loss of moisture during storage (DANNER et al., 2009). For banana, the treatment with an natural analogue of salicylic acid increased the disease resistance and delayed ripening, resulting in lower soluble solids (ZHU et al., 2016).

The decay index during storage under ambient atmosphere was influenced by the pre-harvest treatments, mainly by the use of the elicitor based on citrus biomass, associated or not with CaCl_2 , presenting a reduction at the end of the storage in more than 50% of the decay in relation to the control (Figure 4A). However, the use of the

modified atmosphere reduced further the decay for all treatments, especially when CaCl₂ or the elicitor (Figure 4B) was applied in isolation in the clusters pre-harvest. Thus, that minimized the incidence of decay, indicating its effectiveness in reducing

the incidence of rot in 'Isabel' grapes postharvest, allowing the reduction of the application of synthetic fungicides and, therefore, lower risks to consumer health.

Figure 3. Soluble Solids (A), titratable acidity (B) and SS / TA ratio (C) of 'Isabel' grapes during storage (24 ± 2 °C and $75 \pm 4\%$ R.H) treated during pre-harvest with Citrus Biomass-based elicitor (CB), CaCl₂ (C), Citrus Biomass-based elicitor + CaCl₂ (BC + C) and Control – no application (T) base. AA = Non-modified Atmosphere and MA = Modified Atmosphere (A and B, n = 4; C, n = 28).

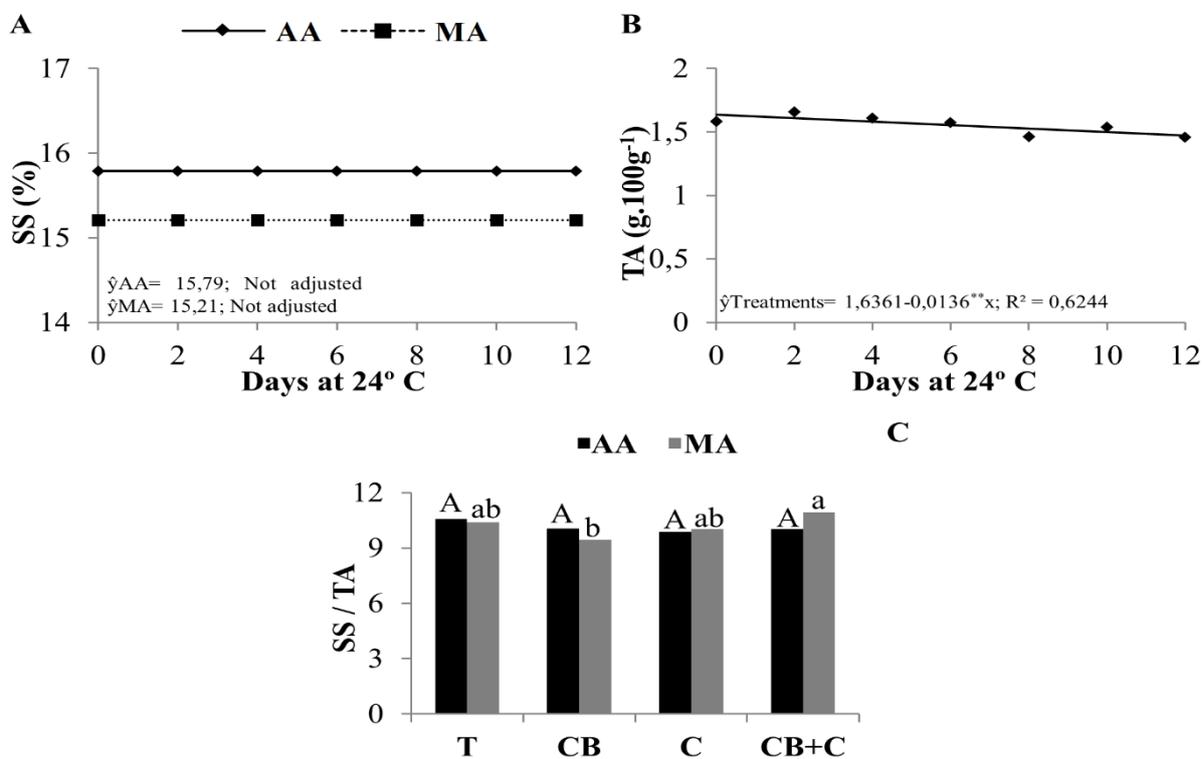
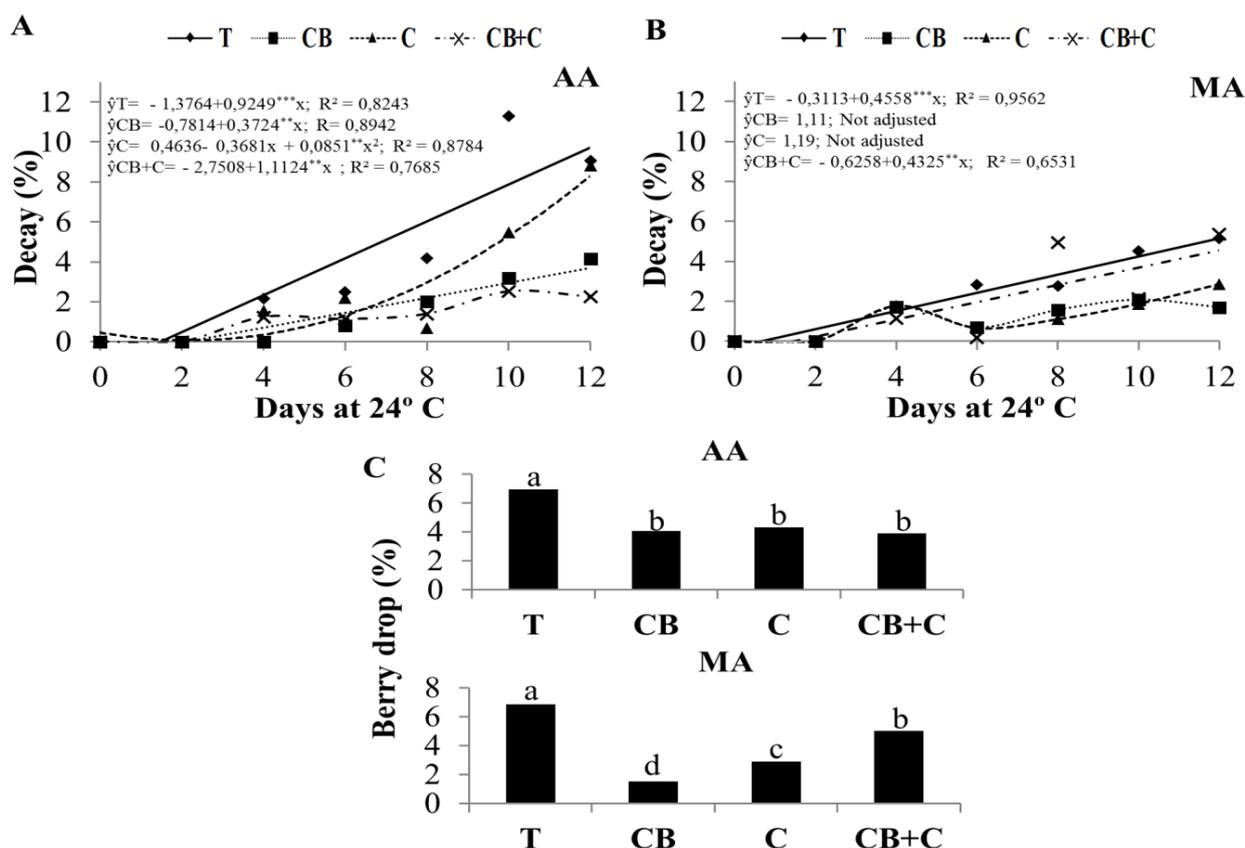


Figure 4. Percentages of decay under ambient atmosphere (A) and modified atmosphere (B) and berry drop (C) of ‘Isabel’ grapes during room storage ($24 \pm 2^\circ\text{C}$ and $75 \pm 4\%$ R.H) treated during pre-harvest with Citrus Biomass-based elicitor (BC), CaCl_2 (C), Citrus Biomass + CaCl_2 (BC + C) and Control – no application (T). AA = Ambient Atmosphere and MA = Modified Atmosphere. (A and B, n = 4, C and D, n = 28).



Silva et al. (2012), using postharvest CaCl_2 , also reported a decrease in ‘Isabel’ grape decay with 1,0 and 2,0% doses, especially when stored under modified atmosphere, however, this reduction was well below the one reported in this work, when CaCl_2 was applied pre-harvest. Tecchio et al. (2009) also attributed the reduction of the rate of decay to the use of CaCl_2 . The use of Agro-Mos[®] associated with K^+ phosphite and/or fungicides decreased the incidence of anthracnose and mildew during the development of the clusters (PINTO et al., 2012). Gomes et al. (2011) reported that the use of citrus biomass alone resulted in control above 70%, indicating the action of citrus bioflavonoids and phytoalexins (base of product composition) on the activation of systemic defense responses (SUN et

al., 2017), as herein for ‘Isabel’ grapes. The use of CaCl_2 in guava postharvest reduced the incidence of anthracnose, but showed little influence on fruit firmness and pH. However, SS and TA values were higher up to the 0,5% CaCl_2 dose (CRUZ et al., 2015).

The application of CaCl_2 and elicitor based on citrus biomass significantly reduced the rate of berry drop of the clusters of ‘Isabel’ grapes during storage (Figure 4C), especially when under modified atmosphere (MA). It was observed that the berry drop index was reduced by about 75% and 60% by the pre-harvest application in the clusters of the citrus biomass (BC) or CaCl_2 (C) isolated, respectively, under MA. However, the combination of citrus biomass plus CaCl_2 (BC + C) was less effective that

each treatment applied isolated in reducing berry drop under MA. In turn, under ambient atmosphere (AA) the berry drop was reduced by about 55% and, irrespective of the treatment, the use of C, elicitor BC or the combination of both (BC + C) were efficient in reducing berry drop as related to the control. However, in clusters kept under AA the combination BC + C seemed to be more effective in reducing berry drop.

Other studies have also shown the efficiency of the application of CaCl₂, either in pre-harvest (DANNER et al., 2009) or postharvest (SILVA et al., 2012) in 'Isabel' grapes, as well as for 'Dona Zilá' and 'Tardia de Caxias' grapes, which treated with 3% CaCl₂, after two months of cold storage, showed resistance to berry drop, as well as the reduction of the browning of the raquis and weight loss (BRACKMANN et al. 2002). However, the results of this study clearly indicate that the application of CaCl₂ and a pre-harvest resistance inducer had an impact on reducing the rate of berry drop and the development of decay, maintaining quality and prolonging postharvest life of 'Isabel' grape.

The berry drop is due to the increase of the enzymatic activity of the cell wall that acts in the

degradation of the pectin and cellulose, in the zones of abscission between the pedicel and the berry of the grapes (DENG et al., 2007). Thus, the association of the resistance elicitor to the active sites of the catalytic enzymes of these zones of abscission can be a mechanism that minimizes the metabolic rate, inhibiting the activity of the enzymes involved in the berry drop during the storage of 'Isabel' grapes.

Under the conditions of this experiment, the firmness presented a low negative correlation with the pH and the decay rate of the berries, indicating an inverse relationship between sanitation and berry integrity aspects, as observed at the end of storage (Table 1; Figures 3D, 4A and 4B). On the other hand, the berry drop index correlated positively and moderately with pH and decay, so that the increase in decay and pH values of the berries were related to the increase in the berry drop during storage in ambient conditions, with the increase (low, positive correlation), also, in the SS content and the SS/TA ratio (Table 1). Together, these results indicate the effectiveness of the pre-harvest application of CaCl₂ and the resistance inducer based on citrus biomass in the maintenance of the quality and food safety of Isabel' grapes.

Table 1. Correlation between firmness and berry drop and quality aspects (L^* , a^* , b^* , SS, TA, SS / TA, pH and Decay), during storage ($24 \pm 2^\circ \text{C}$ and $75 \pm 4\% \text{R.H}$) of 'Isabel' grapes treated during pre-harvest with Citrus Biomass-based elicitor (CB), CaCl₂ (C), Citrus Biomass-based elicitor + CaCl₂ (CB + C) and Control – without application (T).

Variable	By Variable	Correlation	Significance	-0,8	-0,6	-0,4	-0,2	0	0,2	0,4	0,6	0,8
Firmness	L^*	0,0047	0,9464									
	a^*	-0,1281	0,0651									
	b^*	0,0445	0,5232									
	SS	-0,0567	0,4158									
	TA	0,0558	0,4233									
	SS / TA	-0,0876	0,2083									
	pH	-0,2558	0,0002*									
	% Decay	-0,2075	0,0026*									
% Berry drop	L^*	-0,0840	0,2483									
	a^*	-0,0456	0,5128									
	b^*	-0,0928	0,1826									
	SS	0,1391	0,0451*									
	TA	-0,1347	0,0525									
	SS / TA	0,1723	0,0128*									
	pH	0,3717	<,0001*									
	% Decay	0,3788	<,0001*									

Conclusions

The CaCl_2 and the citrus biomass-based elicitor used isolated in pre-harvest on 'Isabel' grapes minimize weight loss, berry drop index and decay independently of the storage atmosphere.

The association of CaCl_2 and the elicitor based on citrus biomass potentiates the effect of the modified atmosphere, reducing the berry drop in 55% and 75%, respectively, and decay in 50% during room storage.

The berry drop index and the firmness of the 'Isabel' grape fruits, treated in the pre-harvest with CaCl_2 and elicitor based on citrus biomass, are mainly influenced by the rate of decay and pH.

Together, the pre-harvest application isolated of CaCl_2 or the elicitor based on citrus biomass alone is effective in minimizing decay and berry drop of 'Isabel' grape, consisting in a practical, safe, efficient, and environmentally sustainable alternative as well as for producer and consumer health.

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