Modified atmosphere in the control of *Alternaria* rot on postharvest grapes

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Abstract

The aim of this study was to evaluate the control of *Alternaria* rot in 'Thompson Seedless' grapes by means of modified atmosphere. Polyamide bags were compared to high density polyethylene bags, combined or not with sodium metabisulphite (SO_2). Fruit from a commercial farm were selected and disinfested; then, they were injured with an entomological pin, inoculated with a suspension of *Alternaria alternate* at a concentration of 10^5 conidiam L^{-1} and kept in a wet chamber for 24 hours. Subsequently, grape boxes were placed inside specific bags according to the treatment and stored in a cold chamber at 2°C and 75% relative humidity during 40 days. From the 12^{th} day of storage, the disease incidence and the following physic chemical varieties were weekly evaluated until the 40^{th} day: weight loss, soluble solids content (SS), pH, titrable acidity (TA), SS/TA ratio, peroxidase activity, and CO_2 and O_2 concentrations. A completely randomized split-plot design was adopted with five replicates. Covering grape boxes with polyamide bags, even when sodium metabisulphite was not used, showed to be a feasible alternative to maintain the postharvest quality of 'Thompson Seedless' grapes, as well as to reduce rot caused by *A. alternata*.

Keywords: 'Thompson Seedless'; Postharvest; Disease; Blackspot; Peroxidase.

Introduction

The grape 'Thompson Seedless', 'Sultana' or 'Sultanina' is the most important fine table grape of worldwide consumption. It has a highly significant economic importance in the semiarid region of São Francisco Valley of Brazil, where it has expanded in the last years due to the increasing import demand. However, postharvest aspects, such as development of fungal diseases, can limit the commercialization of this variety, especially when long-term storage is required.

Etiological evaluations have demonstrated that the most frequent phytopathogens causing postharvest rots in the Sub-medium São Francisco Valley in Brazil are *Lasiodiplodia theobromae, Cladosporium herbarum, Aspergillus niger* and *Alternaria alternate* (Terao et al., 2009).

The symptoms of the infection caused by *A. alternate* is characterized by changes in the fruit epidermis coloration from brown to black, showing flat or depressed spots of well defined edges, which gradually evolve to large deteriorated areas that are shallow or highly necrosed

(Agrios, 2004). Swart et al. (1995) demonstrated that this fungus are able to colonize grapes even under refrigeration, penetrating the tissue by stomata, lenticels or micro cracks. Species of *Alternaria* identified causing rot on grapes were *A. alternata*, *A. arborescens* and *A. tenuissima* (Polizzotto et al., 2012).

To prevent the development of phytopathogens, grapes receive antifungal treatment, generally sulfur dioxide (SO_2) fumigated or generated from potassium metabisulphite ($K_2S_2O_5$) or sodium metabisulphite ($Na_2S_2O_5$) into packages containing the bunches (Camili et al., 2007; Retamales et al., 2003). According to Camili et al. (2007), alternative measures to the use of SO_2 are needed since this compound is an allergen for some individuals and has restrict use for some products of plant origin.

In the grape-exporting region at São Francisco Valley in Brazil, the use of packages made of high density polyethylene (HDPE), associated with SO_2, recommended for the control of *Botrytis cinerea*, is highly common. However, when the goal is to increase the shelf life in refrigeration, the use of this material is limited because some qualitative attributes are lost, more over the incidence of grape rot caused by *B. cinerea* in that region is very low to *Alternaria* rot (Terao et al., 2009), wherein the efficiency of control of that treatment is not still well established.

Alternatives to the use of chemical products have been studied, and the use of plastic packages made of low density materials (LDPE), polyamides (PA) and polypropylenes (PP) may be an effective alternative to preserve the product quality. Besides acting as a physical barrier, these materials modify the atmosphere, which is generally used to slow physiological processes such as the respiration intensity (Cia et al, 2007; Ferri et al., 2004). Furthermore, they can act indirectly on fungal control by activating the fruit defense mechanisms, preventing the development of phytopathogens (Neves et al., 2008). Among these mechanisms is the increase in the activity of peroxidase. This isoenzyme is considered a signalizer of several metabolic processes and is associated with resistance induction processes such as lignin synthesis and cell wall stiffening (Resende et al., 2003).

Thus, the aim of the present study was to investigate the effect of modified atmosphere, promoted by the use of plastic packages made of polyamides, as a method to control postharvest rot caused by *A. alternate* in 'Thompson Seedless' grapes produced in the semiarid region of São Francisco Valley of Brazil.

Material and methods

Preparation of fruits and inoculation

'Thompson Seedless' grape bunches were collected from commercial farm Special Fruit, located in Juazeiro - BA, Brazil, where the weather is hot and dry, semiarid, Bswh type, the annual rainfall is 571.5 mm and the annual average temperature is 26.4ºC, at latitude 9°24'133" South and longitude 40°20'513" West. The bunches were taken to the packaging shed of the farm for cleaning and classification according to the quality standards established by the European market.

At the Postharvest Pathology Lab of Embrapa Semiárido in Petrolina - PE, Brazil, the inoculation was made using a spore suspension of *A. alternara* isolatead justed to a concentration of the order of 10^5 conidiam L^{-1}.

From each grape bunch, ten berries were selected to receive injuries made with an entomological pin at a depth of approximately 2.0 mm. On the injury of each berry, 1µL inoculum suspension was applied by using a micropipette. After inoculation, the bunches were stored in plastic boxes with lids in a humid chamber for 24 hours and transferred to a cold chamber at 2ºC and relative humidity of 75%.

Treatment application

The following treatments were evaluated: T1–bag made of polyamide (PA) without sodium metabisulphite; T2–bag made of PA with sodium metabisulphite; T3–bag made of high density polyethylene (HDPE) without sodium metabisulphite; and T4–bag made of HDPE with sodium metabisulphite. T3 and T4 were used as controls since the adopted bags are commonly employed in the region, associated or not with sodium metabisulphite. For each treatment, there were five replicates constituted of two grape bunches stored in a transparent rigid plastic container. Storage was done in a cold chamber at 2ºC and 75% relative humidity during 40 days.

Following these 40 days of storage, physiological assessments were done for the disease incidence and physicochemical characteristics of the fruit such as: weight loss, pH, soluble solid content (SS), titrable acidity (TA), SS and titrable acidityratio (SS/TA), peroxidase (POD) activity, and gas (CO_2/O_2) concentration.

Weight loss was evaluated weekly by weighing the bunches individually, subtracting the weight of the previous week from current weight.

The pH was obtained by using a digital pHmeter, model NTpH2; SS was obtained with a digital refractometer (Instrutherm); TA was determined by means of titration with 0.1 N NaOH solution. POD activity was determined for method used by Clemente and Pastore (1998): after the mixture of the solution containing 2.75mL hydrogen peroxide at10% and the anhydrous sodium acetate buffer solution (50mM and pH5.5),1mL plant extract was added and, finally, 0.16 mL guaiacol at1% was also added. The result of this mixture was incubated at 30°C for 20 minutes and readings were carried out in a Shimadzu spectrophotometer, program UV Probeat 480 nm.

CO_2/O_2 concentrations were obtained with a gas analyzer (Check Point, PBI Dansensor) equipped with a needle of 20 Gand 0.9 mm diameter that was inserted in the package and allowed to suck the gases for one minute. Following the needle removal, the hole was sealed with adhesive tape to prevent gas loss.

Polyamide bags were constituted of liner-chain polymers, in micro perforated bags of 30 µm thickness, while high density polyethylene was constituted of ramified chain polymers in micro perforated bags of 20 µm thickness and ventilation rate between 0.5 and 2.0%.

Sodium metabisulphite ($Na_2S_2O_5$) the generator of SO_2 was applied using sachet/pad (98.5% of $Na_2S_2O_5$) containing 9 grams of active ingredient.

Experimental design was completely randomized in subdivided plots with five replicates. Results were subjected to analysis of variance (F test) by using the program ASSISTAT and the treatment means were compared according to Tukey's test at 1% probability and Scott-Knott test for analysis of POD activity.

Results and discussion

Grapes stored in bags made of PA combined with sodium metabisulphite (SO_2) (T2) had lower incidence of *Alternaria* rot, differing from the other treatments in all assessments (Figure 1). It is well established that fruits and vegetables subjected to modified atmosphere have low incidence of fungi in the postharvest, as well as longer shelf life, due to the balance between the gases inside the package, i.e., between CO_2 and O_2; in addition, decreased O_2 concentration favors the reduction in oxidative reactions, delaying the enzymatic darkening and other metabolic processes in the plant. Thus, low oxygen levels may limit the production of oxygen reactive species (Hodges et al., 2000), contributing to the maintenance of several metabolic reactions, preventing damage to molecules, such as proteins, lipids, carbohydrates and DNA.

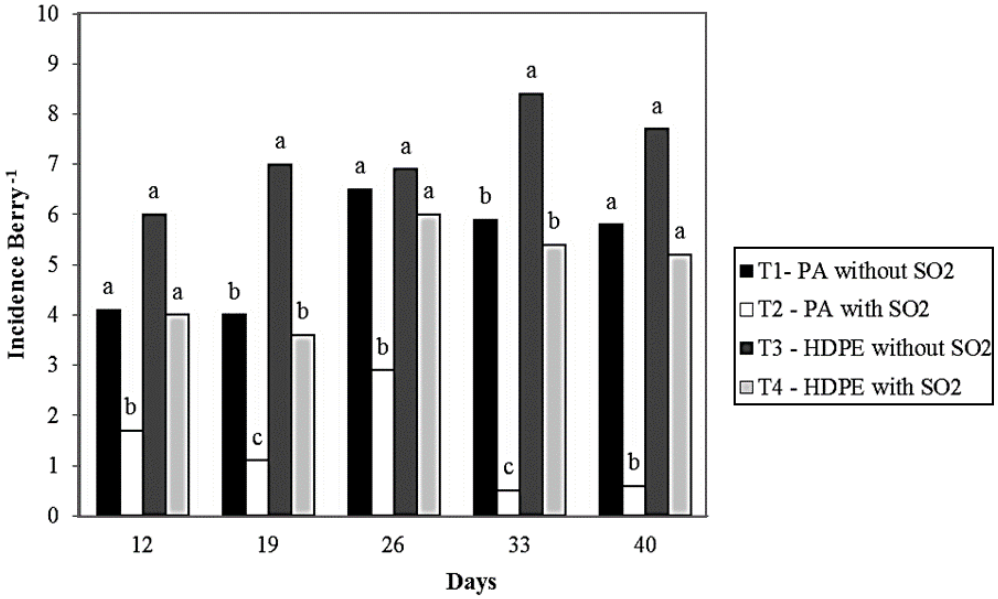

Fig.1. Incidence of *Alternaria alternata* in inoculated 'Thompson Seedless' grape bunches subjected to treatments with bags made of polyamide (PA) and high density polyethylene (HDPE), combined or not with sodium metabisulphite (SO_2), and stored in a cold chamber at 2ºC and 75% relative humidity, during 40days.

Means followed by the same letter in each assessment period do not significantly differ according to Tukey's test at 1% probability level.

Not only the packages act in antioxidant action mechanisms, but also the sodium metabisulphite, may have contributed to a lower incidence of *Alternaria* rot (Retamales et al., 2003).The action of sodium metabisulphite in inhibiting mycelia growth and spore germination of fungi species is already well established (Mills et al., 2004).

On the other hand, grapes packed with bags made of PA without sodium metabisulphite (T1) and with bag made of HDPE with sodium metabisulphite (T4) did not differ during the whole evaluation period, evidencing that PA, even not combined with sodium metabisulphite (T1),can be a feasible alternative to the currently common use of sodium metabisulphite. Results of several studies have shown that the use of CO_2 to obtain a modified atmosphere is

an alternative to the use of SO_2 in the grape quality maintenance (Artés-Hernàndez et al., 2004).

Gas concentration readings (Figures 2 and 3) evidenced that PA bags promoted a modified atmosphere for grapes kept in this bag, showing clear a decrease in the levels of O_2 and an increase in the levels of CO_2. The correlation between gas composition and *A. alternate* development inhibition was more evident when PA bag was associated with sodium metabisulphite (Figure 3).

Alternaria rot inhibition, due to the modified atmosphere, is possibly a result of the release of gases that are toxicto the fungi, such as ethanol and acetaldehyde, and also the increase of shelf life of the fruit, making it more resistant to fungi infection (Artés-Hernàndez and Tomás-Barberán, 2006).

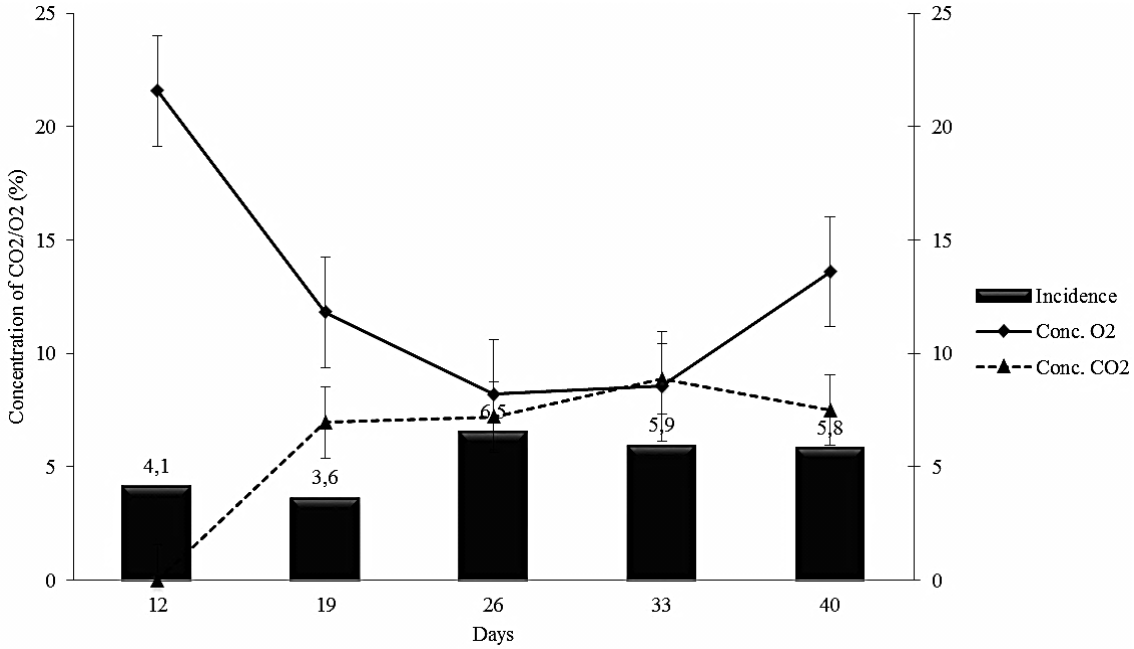

Fig.2. Incidence of *Alternaria alternata* and CO_2/O_2 concentrations under modified atmosphere (bag made of polyamide without sodium metabisulphite) in inoculated 'Thompson Seedless' grape bunches stored in a cold chamber at 2ºC and relative humidity of 75%, during 40 days.

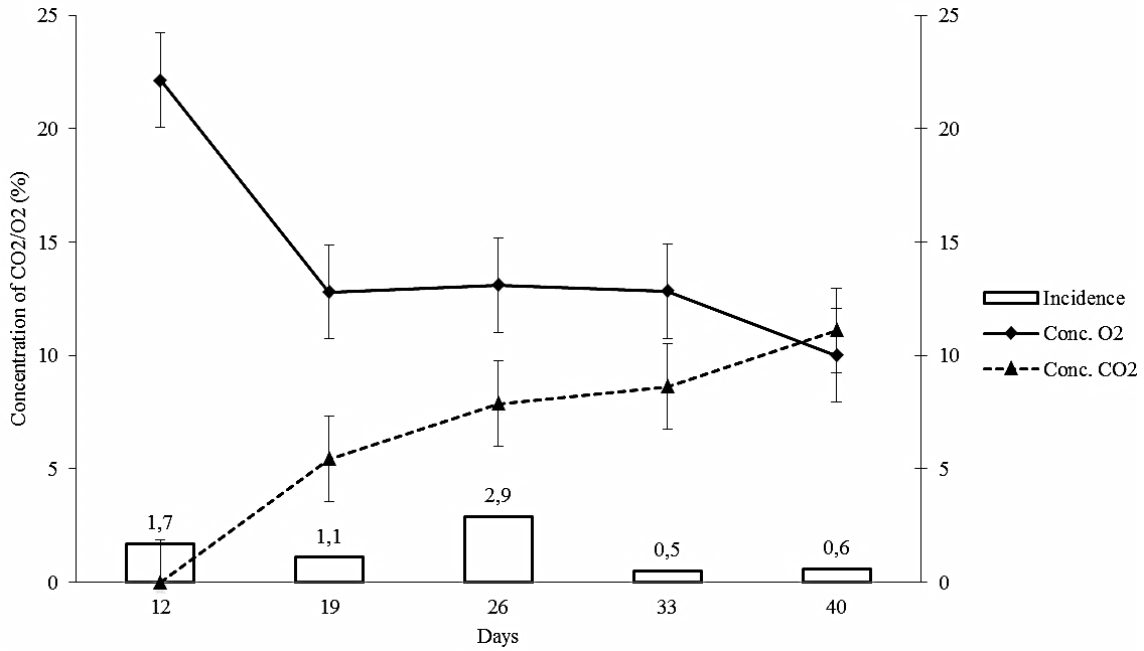

Fig.3. Incidence of *Alternaria alternate* and CO_2/O_2concentrations under modified atmosphere (bag made of polyamide with sodium metabisulphite) in inoculated 'Thompson Seedless' grape bunches stored in a cold chamber at 2ºC and relative humidity of 75%, during 40 days.

Considering the quality of grapes, weight loss did not significantly differ among treatments until the 33rd day (Table1). On the 40th day, however, there was a significant difference in weight loss between treatments using bag made of PA combined or not with metabisulphite (T1 and T2), and the treatment using bags made of HDPE with metabisulphite (T4), demonstrating that PA bags can prevent grape bunches from losing weight during long-term storage (Neves et al., 2008).

Table 1. Weight loss (%) of 'Thompson Seedless' grape bunches subjected to two types of package: polyamide(PA) and high density polyethylene (HDPE), combined or not with sodium metabisulphite (SO_2), after 12, 19, 26, 33 and 40 days of storage in a cold chamber at 2ºCand 75% relative humidity.

Treatments	Days (weight loss – g)				
	12	19	26	33	40
T1- PA without SO_2	5,56a	6,81 b	9,80ab	13,70 a	7,39b
T2- PA with SO_2	7,70a	10,75 ab	5,23 b	5,48 b	8,29b
T3- HDPE without SO_2	7,96a	8,04 ab	11,31 a	13,61 ab	10,34ab
T4- HDPE with SO_2	7,64a	14,42 a	10,24 a	12,10 ab	17,63a
CV (%)	29,84	28,99 *	26,26 *	31,04 *	29,64

Means followed by the same letter in the column do not significantly differ at 1%probability level according to Tukey's test.

There was no significant influence of the package type and the addition or not of metabisulphite on pH and SS. However, SS/TA ratio differed between the treatments using PA bags with and without SO_2 (T1 and T2) and the treatment using HDPE bags with SO_2 (T4) (Table 2).This datum is qualitatively interesting since it demonstrates that the package type is not a determining factor for these characteristics, but it influenced SS/TA ratio and weight loss after 40 days of storage in a cold chamber (Table 1 and 2), showing that PA bags seem to keep the fruit quality.

Table 2. Physicochemical variables: Hydrogen Potential[1], soluble solids content[2] (SS – ºbrix) and soluble solids and titrable acidity ratio[3] (RATIO) in 'Thompson Seedless' grape berries subjected to two types of package: polyamide (PA) and high density polyethylene (HDPE), combined or not with sodium metabisulphite (SO_2), at the beginning and at the end of storage (40 days) in a cold chamber at 2ºC and relative humidity of 75%.

Treatments	pH[1]		SS[2]		RATIO[3]	
	Initial	Final	Initial	Final	Initial	Final
T1- PA without SO_2	4.062	4.326 a	18.920	19.200 a	25.613	25.803ab
T2- PA with SO_2	4.062	4.258 a	18.920	18.840 a	25.613	25.001b
T3-HDPE without SO_2	4.062	4.184 a	18.920	18.940 a	25.613	24.762b
T4- HDPE with SO_2	4.062	4.184 a	18.920	19.640 a	25.613	28.307a
CV (%)		3,90		4,26		5.38

Means followed by the same letter in the column do not significantly differ at 1% probability level according to Tukey's test.

Concerning to enzymatic profile, already in the first week peroxidase activity tended to be increased by package promoting modified atmosphere (PA), combined or not with SO_2 and then reduced over the storage period. This may be a result of treatments controlling the disease incidence (Figure 4).

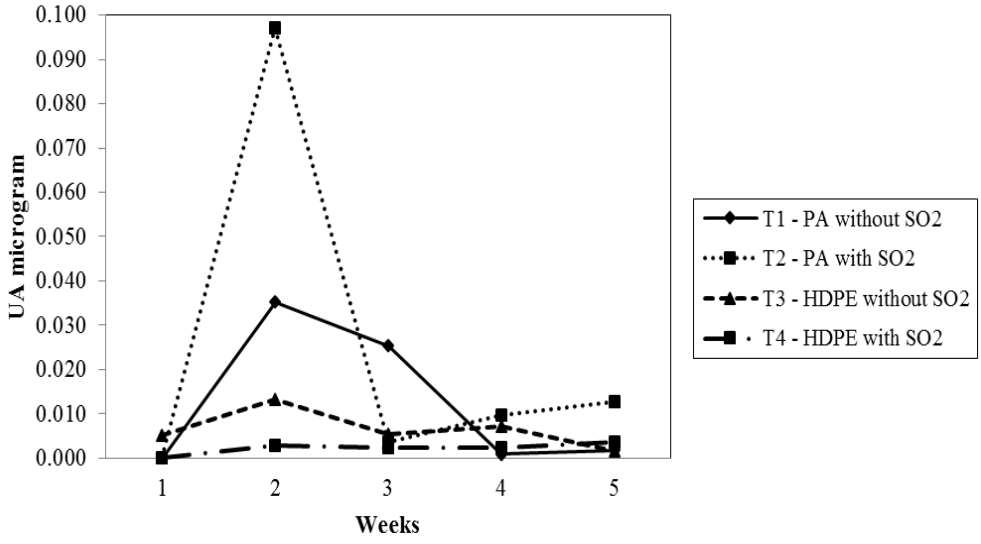

Fig.4. Activity of the enzyme peroxidase (POD) in inoculated 'Thompson Seedless' grape bunches subjected to two types of polyethylene packages: polyamide (PA) and high density polyethylene (HDPE), combined or not with sodium metabisulphite (SO_2), stored in a cold chamber at 2ºC and relative humidity of 75%, during 40days.

Increased peroxidase activity has been related to reduced fungal growth (Ipolito et al., 2000). Peroxidases are correlated with the beginning of resistance induction, suggesting that one of the roles of these enzymes is to control pathogenic fungi (Van loon et al., 1998). One of the first reactions of the cell against the presence of pathogens is the formation of reactive oxygen species (ROS), such as peroxides, superoxides and hydroxyls (Wang et al., 2007). Several authors have suggested a relationship between the production of ROS and the response sensitivity, including the activation of specific genes (Shirazu et al., 2000). Thus, increased POD activity could be related to the massive production of peroxides in an attempt to eliminate the excess free radicals, presenting therefore an antioxidant action.
This enzyme can also be toxic to phytopathogens by controlling the activity of hydrolase, which converts benzoic acid into salicylic acid (HSU et al., 2003; Lee et al., 2001; Mayer et al., 1987), which in turn acts on the plant resistance to pathogens.
Similar studies were carried out, in parallel to this research, and according to Camargo et al.(2012), packaging grapes in modified atmosphere, using PA bags, was efficient in controlling *Aspergillus niger*, that causes losses on grapes in field and during postharvest, in the same semiarid region.

Conclusion

The covering of grape boxes with polyamide film can be a feasible alternative to use of sodium metabisulphite to maintain the postharvest quality of 'Thompson Seedless' grape, as well as to reduce rot caused by *Alternaria alternata*.

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