Action of ethylene on postharvest of summer squash 'Menina Brasileira'

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ABSTRACT

The purpose of this study was to evaluate the sensitivity and the physiological responses of summer squash 'Menina Brasileira' to ethylene. Immature fruits were harvested and placed in 20 L sealed buckets, in which ethylene was applied at concentrations of 0.1, 1.0, 10, 100, and 1000 μL L^{-1} for 24 h. Fruits were placed in buckets with no ethylene as a control treatment. Thereafter, the fruits were taken out of the buckets and maintained on bench, wherein on days 0, 2, 4, 6, and 8, they were evaluated regarding the accumulated fresh weight loss, soluble sugars, reducing and non-reducing sugars, starch, total chlorophyll, content of malondialdehyde, and electrolyte leakage. Fruits of summer squash 'Menina Brasileira' showed sensitivity to exogenous ethylene with no weight loss stimulation. Additionally, the fruits exhibited small changes in nutritional quality attributes and changes in the external fruit appearance, including decreased chlorophyll content as well as damage to cell membrane characterized by increase in malondialdehyde content and electrolyte leakage. These changes were stimulated by increasing exogenous ethylene concentration.

Key words: *Cucurbita moschata*; chlorophyll; sugars; malondialdehyde; leakage of electrolytes.

RESUMO

Ação do etileno na pós-colheita de abobrinha 'Menina Brasileira'

O objetivo do trabalho foi avaliar a sensibilidade e as respostas fisiológicas da abobrinha 'Menina Brasileira' a diferentes doses de etileno. Os frutos foram colhidos, homogeneizados e colocados em baldes de 20 litros hermeticamente fechados, nos quais foram aplicadas doses de etileno nas concentrações de 0,1; 1,0; 10; 100 e 1000 μL L^{-1} durante 24 h, além do controle. Posteriormente, os frutos foram retirados dos baldes e mantidos sobre bancada, onde aos dias 0, 2, 4, 6 e 8 foram avaliados quanto à perda de peso fresco e teores de açúcar solúvel total, açúcar redutor e não redutor, amido, clorofila, conteúdo de malonaldeído e extravasamento de eletrólitos. Os frutos da abobrinha 'Menina Brasileira' apresentaram sensibilidade ao etileno exógeno sem estimular a perda de peso fresco. Ademais, os frutos exibiram pequenas alterações nos atributos de qualidade nutricional, além de mudanças no aspecto externo dos frutos, como diminuição do teor de clorofila e injúria por danos à membrana celular, caracterizada pelo aumento do conteúdo de malonaldeído e extravasamento de eletrólitos. Essas alterações foram estimuladas em resposta ao aumento da concentração de etileno.

Palavras-chave: *Cucurbita moschata*; clorofila; açúcares; malonaldeído; extravasamento de eletrólitos.

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INTRODUCTION

The summer squash (*Curcubita moschata*) is originated from the American continent, more precisely in the central area of Mexico (Carmo, 2009), being an important economical plant of the Cucurbitaceae family. In Brazil, fruits of *Cucurbita pepo* are consumed at immature stage of development and thus the action of ethylene on postharvest of this fruit must be known.

The summer squash is a fruit that has non-climacteric respiratory pattern (Kader, 2002). In this context, the degree of response and sensitivity to ethylene are dependent on the development stage, variety, and perception by the plant organ apart of your respiratory pattern (Giovannoni & Barry, 2007; Yoo *et al*., 2009; McAtee *et al*., 2013). For example, the use of ethylene at the pre-climacteric stage anticipates the ripening of climacteric fruits consequently. In non-climacteric fruits, quick increases occur in respiratory activity, which is immediately decreased, promoting changes in product quality with no reflect in physiological maturation (Paul *et al*., 2012). However, researches have shown that ethylene also regulates some aspects of ripening in non-climacteric fruits (Yamane *et al*., 2007; Paul *et al*., 2012; McAtee *et al*., 2013), which demonstrates that the literature has divergence in relation to action of ethylene on postharvest quality of non-climacteric fruits.

The responses of fruit to ethylene can be desirable, such as production of volatile substances and the reduction of astringency, and undesirable, as the increase of cell wall degrading enzymes, starch conversion into sugars, yellowing, among others (Yamane *et al*., 2007). Therefore, the aim of this study was to evaluate the level of sensitivity and the physiological responses of summer squash 'Menina Brasileira' to ethylene.

MATERIAL AND METHODS

Immature fruits at commercial stage of summer squash 'Menina Brasileira' were harvested and transported to the laboratory. After cleaning with a damp paper towel, fruits were placed in 20 L sealed buckets, in which 0 (control), 0.1, 1.0, 10, 100, and 1000 $\mu L\ L^{-1}$ of ethylene were applied for 24 h.

The experiment was conducted in a split plot, with the plots comprising the ethylene concentrations and the subplots the days of storage, in a completely randomized design with four repetitions. After exposure to ethylene, fruits were distributed on benches at 24 ± 2 °C, under photon flux of 10 $\mu mol\ m^{-2}\ s^{-1}$ from white fluorescent light and relative humidity of 60% for eight days. Evaluations were performed for loss of fresh weight, carbohydrates, chlorophyll, malonaldehyde (MDA), and electrolyte leakage every two days.

The quantification of total soluble sugars was performed according to the phenol-sulfuric method (Dubois *et al*., 1956). Reducing sugars were measured according to the methodology of Somogyi-Nelson (Nelson, 1944). For quantification of starch, the method described by McCready *et al*. (1950) was followed. For the evaluation of chlorophyll, four discs of the fruit peel were removed with a manual punch of 11 mm in diameter. The discs were weighed and placed in screw-top tubes containing 5 mL of N, N-dimethylformamide under cooling at 4 °C in the dark. After ten days, the chlorophyll content was determined by method of N,N-dimethylformamide (Inskeep & Bloom, 1985) in a spectrophotometer, with readings at wavelengths of 647 and 664.5 nm.

The lipid peroxidation was evaluated by quantifying the accumulation of MDA using the test thiobarbituric acid (TBA) (Cakmak & Horst, 1991). The absorbance of the supernatant fraction was measured at 532 nm and corrected for non-specific turbidity by subtracting the absorbance at 600 nm. The estimate of the amount of TBA-MDA complex was obtained using the absorption coefficient of 155 $mM^{-1}\ cm^{-1}$ and expressed as nmol g^{-1} fresh weight (Heath & Packer, 1968).

The electrolyte leakage was measured according to the method described by Lima *et al*. (2002). The initial electrical conductivity (L1) of the suspension liquid was read in a conductivity meter (Digimed, model MD-31) and expressed as a percentage of the total conductivity (L2). The permeability of the membrane was calculated as: El% = (L1 / L2) ×100.

The data were analyzed by regression analysis using the Statistical Analysis System SAEG (SAEG, 2007), version 9.1. The regression model was based on the significance of the regression coefficients using the t test at 5% probability, the coefficient of determination (R^2 = SQReg / SQtrat), and on the biological phenomenon under study.

RESULTS AND DISCUSSION

The fresh weight loss of the fruits increased linearly as a function of time regardless of the ethylene concentration applied (Figure 1). The rate of weight loss was not significant among the treatments. Thus, the mass loss detected is attributed to natural aging process of fruits (Cao *et al*., 2012) due to loss of dry matter consumed in the respiratory event and mainly by water loss through epidermis of the fruit (Finger & Vieira, 2007). Under experimental conditions, the losses ranged from 12.18 to 17.41% on the 5th day of storage, in which the fruits presented unacceptable quality for commercialization. Weight loss for vegetables in general is in order of 3 to 6%, which are sufficient to cause a marked decline in quality (Chitarra & Chitarra, 2005). However, in the current literature, there is no work related to the level of fresh weight loss tolerated for the summer squash.

A linear decrease of total soluble sugars (Figure 2) and reducing sugars (Figure 3) occurred along the evaluation period for all treatments. The reduction was greater for the dose of 10 µL L^{-1}, reaching a reduction of 22 and 35% for total soluble sugars and reducing sugars, respectively, at the end period of evaluation. The exogenous ethylene is a known inducer of respiration in non-climacteric fruits. Therefore, higher respiration rates and lower total soluble sugar content in response to increase in ethylene concentration are expected. However, this behavior was not observed in this study; the biggest reduction in total soluble sugar content was observed in the lower ethylene dose applied. Likewise, bananas exposed to treatment with propylene (Golding *et al*., 1998) and jiló (*Solanum gilo*) fruits treated with ethylene doses (Mendes, 2013), this kind of response was also not observed.

The reduction in the content of reducing sugars in all treated fruits indicated that these monosaccharides were used into metabolite processes. Respiratory activity that occurs in fruits is responsible for the decrease of these sugars, which are converted into trioses to enter in the glycolytic pathway and to follow the respiratory cycle (Taiz & Zeiger, 2013). This is a natural process in plant tissues, which consumes glucose and fructose as the main reducing sugars, resulting in lower concentration of these metabolites during the postharvest period. Furthermore, the utilization for the synthesis of sucrose, which is evidenced by the increases in the level of non-reducing

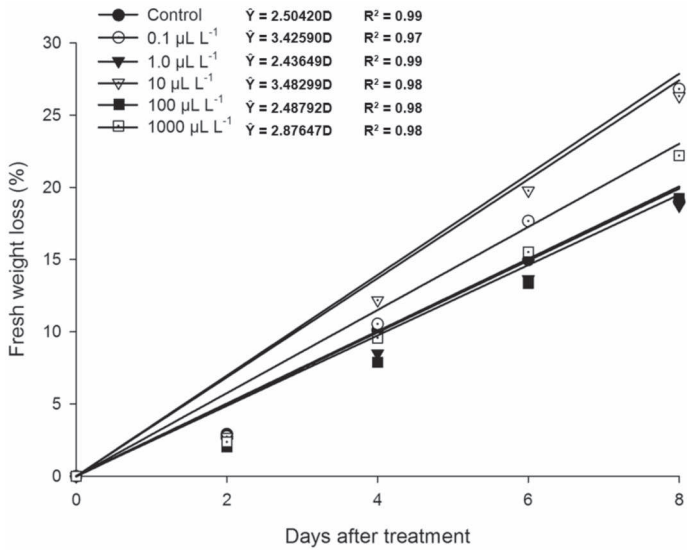

Figure 1: Estimate of fresh weight loss (%) in fruits of summer squash 'Menina Brasileira' treated with ethylene doses, depending on the number of days after treatments.

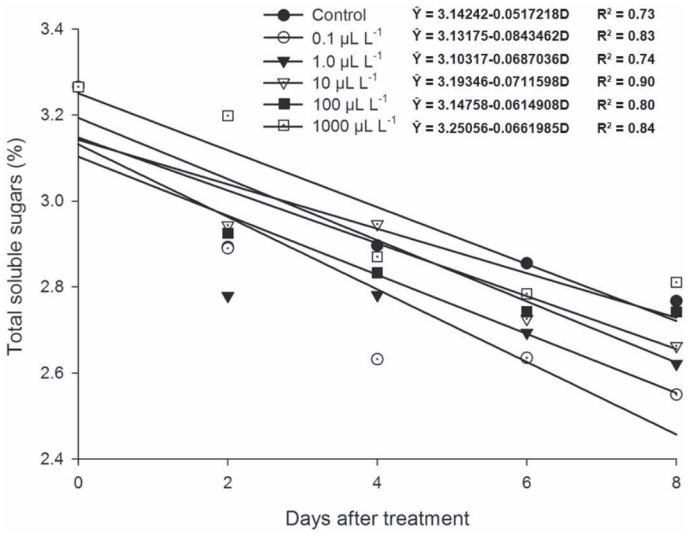

Figure 2: Estimate of total soluble sugars in fruits of summer squash 'Menina Brasileira' treated with ethylene doses, depending on the number of days after treatments.

sugars, is another factor that may be responsible for the decrease of reducing sugars (Figure 4).

Content of starch decreased over time, with greater reduction in higher doses of ethylene, 50.31% and 45.11%, respectively, in treatments with 100 and 1000 μL L^{-1} of ethylene over an eight-day period (Figure 5). Generally, the starch content in the immature squashes is low, less than 3%, although some varieties can reach higher levels (Stevenson *et al.*, 2007). In this experiment, it was determined that starch content in summer squash is about 1.03%. The reduction in starch content and the increase in non-reducing sugar content during the evaluation period suggest that a starch bioconversion into non-reducing sugar and/or starch into reducing sugar, and subsequently sucrose synthesis (non-reducing sugar) may have occurred. The increase in sucrose content is simultaneous with starch degradation and these transformations can precede the formation of glucose and fructose, being the main degradation pathway of starch (Taiz & Zeiger, 2013).

The chlorophyll content decreased by 67.97%, 69.63%, and 73.39% in response to the three highest ethylene doses (10, 100, and 1000 μL L^{-1}), respectively, after eight days of storage (Figure 6). The fruits treated with a dose of 1000 μL L^{-1} ethylene presented rapid degradation of chlorophyll in the early days of evaluation, with exponential behavior of falling (Figure 7). In the other treatments, this decrease was in a linear model over time with a lower rate in the control fruits. The effect of ethylene on chlorophyll was also observed in several other species. Ethylene

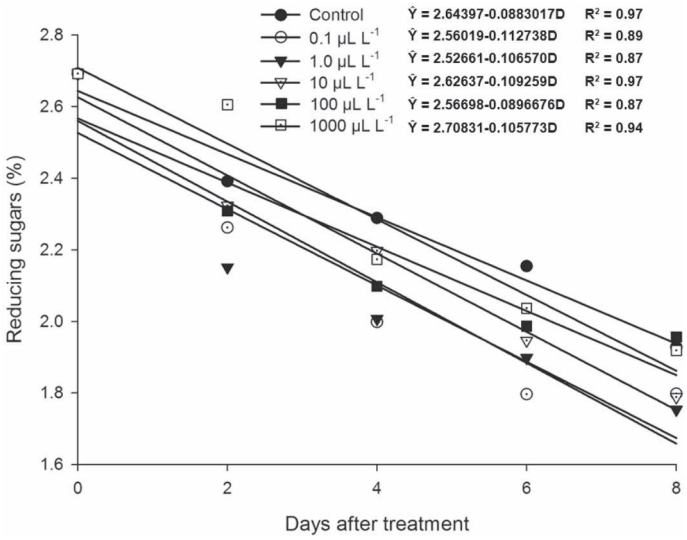

Figure 3: Estimate of the levels of reducing sugars in fruits of summer squash 'Menina Brasileira' treated with ethylene doses, depending on the number of days after treatments.

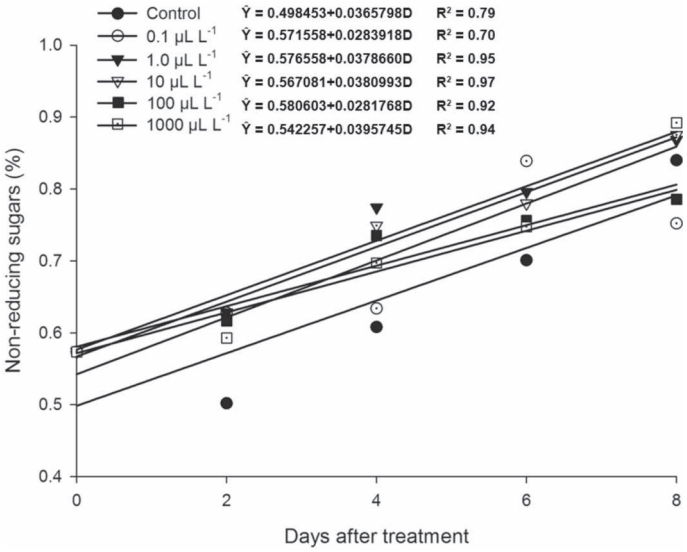

Figure 4: Estimate of non-reducing sugars in fruits of summer squash 'Menina Brasileira' treated with ethylene doses, depending on the number of days after treatments.

concentration of 0.1 µL L^{-1} was effective in reducing chlorophyll content (Mendes, 2013). Domingues *et al.* (2001) observed that the Ethephon (1000 mg L^{-1}) application in oranges accelerated the change in the color of the peel; however, no change in the internal quality of fruits was observed. The storage of peppers in an environment containing 100 µL L^{-1} ethylene resulted in color change due to decrease in chlorophyll content (Fox *et al*., 2005). The reduction in chlorophyll content of summer squash fruit may have occurred due to the increase in activity of the enzyme chlorophyllase. Paul *et al*. (2012) reported that ethylene, even in low concentrations in non-climacteric fruit, is associated with events involved in the maturation, among them, increases in chlorophyllase activity and consequent chlorophyll degradation.

Malondialdehyde is the marker of lipid peroxidation that has been used as an indicator of damage to the plant cell membrane. The findings indicated the occurrence of lipid peroxidation in all analyzed treatments. However, the fruits treated with 1000 µL L^{-1} of ethylene increased MDA and electrolyte leakage in response to higher ethylene doses (Figures 8 and 9) at the end of the evaluation period, indicating that these fruits suffered greater damage to the cell membrane. This fact shows that exposure of summer squash fruit to high doses of ethylene increases the permeability and decreases membrane integrity, which results in oxidation of lipids.

The direct consequence of damage to cell membranes by lipid peroxidation is the leakage of cellular contents to the outside. In this context, there was more electrolyte leakage to

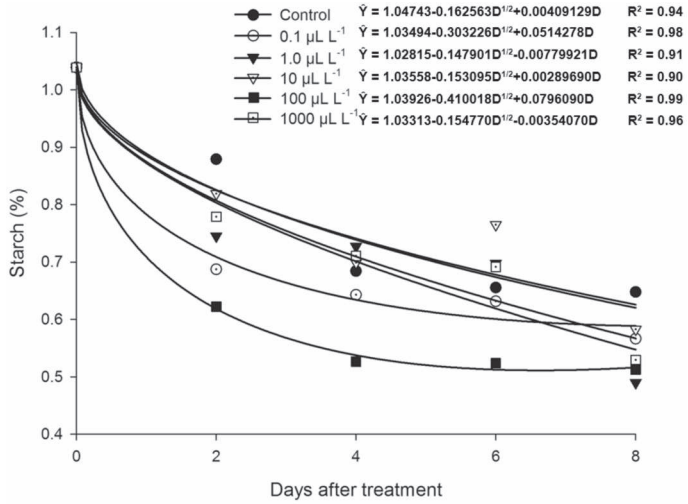

Figure 5: Estimate of the starch content in fruits of summer squash 'Menina Brasileira' treated with ethylene doses, depending on the number of days after treatments.

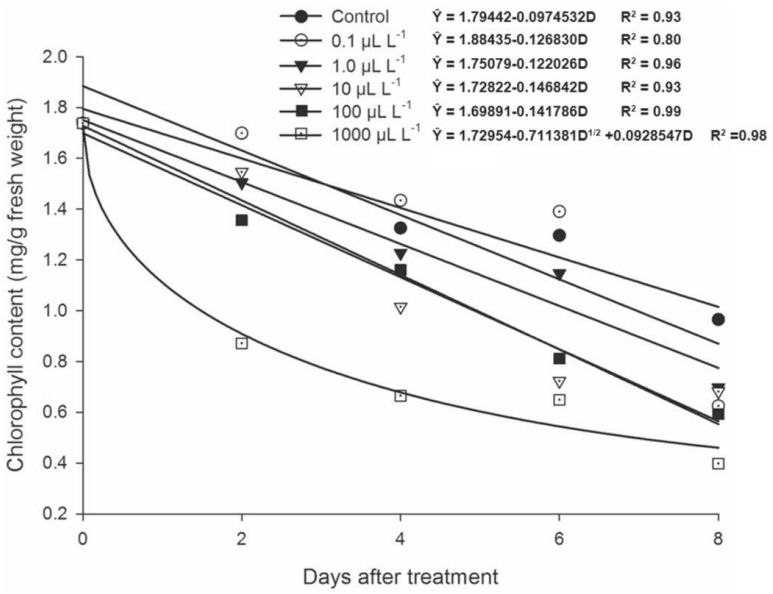

Figure 6: Estimate of chlorophyll content in fruits of summer squash 'Menina Brasileira' treated with ethylene doses, depending on the number of days after treatments.

fruits exposed at doses of 1000, 100, and 10 µL L^{-1} ethylene, demonstrating that higher doses of ethylene caused higher disruption of cell membranes (Figure 9). Interestingly, higher ion extravasation was observed in mango fruits in response to increasing ethylene doses applied (Silva, 2012). Likewise, tangerines subjected to treatments with 5 and 10 µL/L ethylene obtained higher percentages of electrolyte extravasation when compared with control treatment (Costa, 2009). This increase was probably caused by the rupture of cells, increasing the amount of ions in solution, contributing to increases in the electrical conductivity of the medium. The electrolyte leakage serves as a reference to membrane integrity or alteration of selective permeability (Maia *et al*., 2014). The changes are due to decrease in the fluidity of lipids and structural change and loss of membrane proteins. Thus, the electrolyte leakage, in association with the MDA, has been used as an indicator of damage to the plant cell membrane.

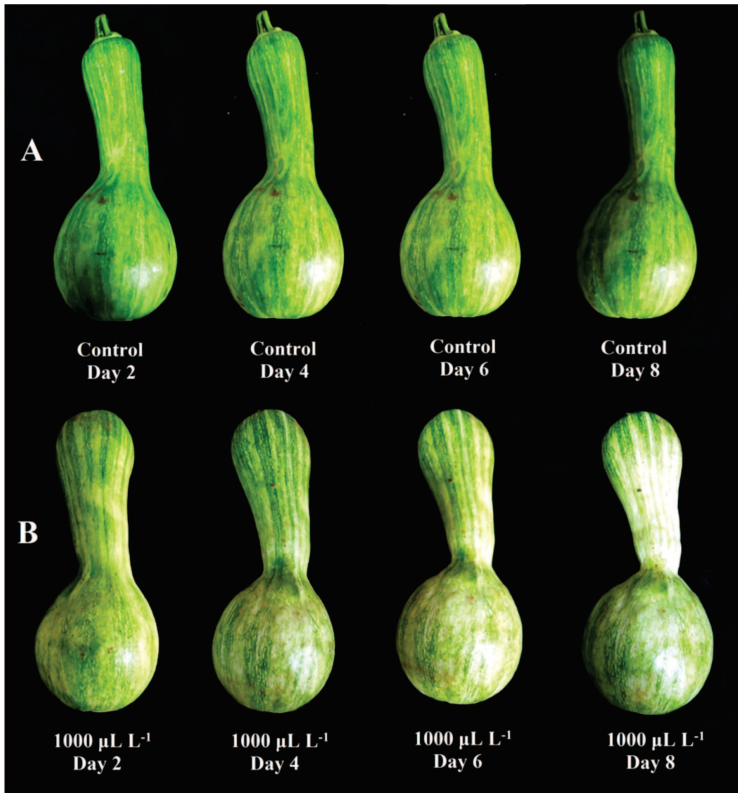

Figure 7: Appearance of summer squash 'Menina Brasileira' fruits in control treatment (A) and in response do ethylene dose of 1000 µL L^{-1} *(B).*

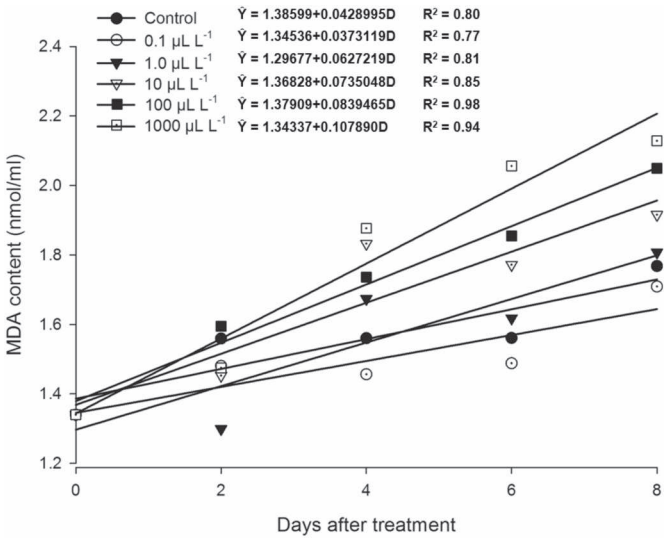

Figure 8: Estimate of MDA content in fruits of summer squash 'Menina Brasileira' treated with ethylene doses, depending on the number of days after treatments.

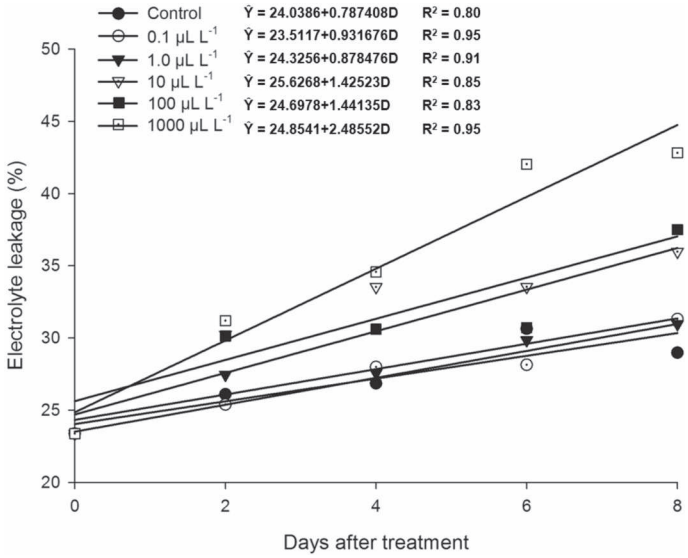

Figure 9: Estimate of electrolyte leakage (%) in fruits of summer squash 'Menina Brasileira' treated with ethylene doses, depending on the number of days after treatments.

The role of ethylene in postharvest deterioration of cucurbits is highly variable. Pumpkin and winter squash have low sensitivity to ethylene, while watermelon is detrimentally affected by ethylene, resulting in softening of the whole fruit (Kader, 2002). In cucumber, a study by Hurr *et al.* (2010) showed that the postharvest response of cucumber fruit to ethylene is highly dependent on developmental stage.

CONCLUSIONS

The fruits of summer squash 'Menina Brasileira' exhibit small changes in attributes of nutritional quality as well as in the external appearance, including decreased chlorophyll content and damage to cell membrane characterized by increase of malondialdehyde and electrolyte leakage as consequence of ethylene application.

Therefore, despite being non-climacteric fruit, these responses demonstrate that summer squash presents a sensibility to ethylene and, thus, strategies to control the action of this hormone on its postharvest quality must be established.

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