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Influence of post-harvest ozone application on the Epicarp of 'Pedro Sato' guava fruits under storage conditions

Influência da aplicação de ozônio pós-colheita no Epicarp de frutos de guava 'Pedro Sato' sob condições de armazenamento

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ABSTRACT

The expanding concern of consumers and public health authorities about the presence of pesticide residues on food has encouraged research on alternative methods to control post-harvest diseases. The objective of this study was to evaluate the influence of post-harvest ozone application on the sensibility of 'Pedro Sato' guava fruits under storage conditions. The experiments were performed in split-plot scheme: the plots were ozone concentrations (0, 65, 95, 185, 275, 370 and 460 µg L^{-1}) injected at 2 L min^{-1} into a fumigation chamber, and subplots were days of evaluation or harvest (0, 1, 3, 5, 7 and 9 days) in a completely randomized design with 3 replicates. 'Pedro Sato' guava fruits responded to ozone-induced oxidative stress at concentrations higher than 185 µg L^{-1}, causing visible anomalies, with green intervenal spots and red-brownish pustule on the epicarp. Although the mechanisms of ozone application on guava fruits are not entirely elucidated, it is known that the mechanisms that justify the leaf symptoms on this crop, exposed to different ozone concentrations, may help clarifying the observed anomalies on the epicarp when fruits are subjected to post-harvest ozone application.

Keywords: Ozone; Post-harvest periodization; Guava; Oxidative stress.

RESUMO

A crescente preocupação dos consumidores e autoridades de saúde pública sobre a presença de resíduos de pesticidas nos alimentos incentivou a pesquisa de métodos alternativos para controlar as doenças pós-colheita. O objetivo deste estudo foi avaliar a influência da aplicação de ozônio pós-colheita na sensibilidade de goiabas 'Pedro Sato' em condições de armazenamento. Os experimentos foram realizados em esquema de parcelas subdivididas: as parcelas foram concentrações de ozônio (0, 65, 95, 185, 275, 370 e 460 µg L-1) injetadas a 2 L min-1 em uma câmara de fumigação, e as subparcelas foram dias de avaliação ou colheita (0, 1, 3, 5, 7 e 9 dias), em delineamento inteiramente casualizado, com 3 repetições. A goiabeira 'Pedro Sato' respondeu ao estresse oxidativo induzido pelo ozônio em concentrações superiores a 185 µg L-1, causando anomalias visíveis, com pontos de intervenção verdes e pústula marrom-avermelhada no epicarpo. Embora os mecanismos de aplicação de ozônio em goiabeiras não sejam totalmente elucidados, sabe-se que os mecanismos que justificam os sintomas foliares desta cultura, expostos a diferentes concentrações de ozônio, podem ajudar a esclarecer as anomalias observadas no epicarpo quando os frutos são submetidos a pós aplicação de ozônio de colheita.

Palavras-chave: Ozônio; Periodização pós-colheita; Goiaba; Estresse oxidativo.

1 INTRODUCTION

Over the last years, Brazilian agriculture has integrated to its activities new crop species, as well as expanding production areas. One of these activities it fruit farming, due to the increasing fruit demand from the population who seek a more balanced and healthy diet. Among the many fruit tree species, guava stands out by its excellent quality and acceptance.

'Pedro Sato' cultivar has an outstanding position both in the national and international market due to its economic importance for fresh consumption, thereby resulting in great productivity, and specially for fresh juice production, due to its high nutritive value associated with a pleasant taste, attractive color, and high vitamin C content (SOUZA & NETO, 2009).

Ripening is the most studied phase in fruit post-harvest field because in this phase composition changes occur with great intensity. It must be noted that the product which will receive any type of post-harvest technology should have great quality, otherwise several fungal and bacterial diseases and physiological disorders will lead to immeasurable post-harvest losses (SILVA, 1982; EVANGELISTA, 1999).

Anthracnose, a disease caused by the fungus *Colletotrichum gloeosporioides*, is a challenge even in fruit allocated to exportation which were subjected to a phytosanitary treatment (LIMA FILHO et al., 2003), since the fungus survives at least one year in soil, plant and old fruit and leaf lesions (BAILEY et al., 1992). Hence, pathological deteriorations during post-harvest are considered the main reason for economic and fruit quality losses along the marketing chain.

Alternative control methods for post-harvest diseases can decrease or substitute the use of chemical products, following the world concern about environment and food security. Among such alternative methods, ozone under gaseous form or dissolved in water has stood out for its important performance on the reduction of apple rot incidence (PUIA et al., 2004), on the increase of kaki shelf life (SALVADOR et al., 2006) and on the decrease of bacterial load on strawberries and raspberries (BIALKA et al., 2007).

Guava leaves (*Psidium guajava* L.) 'Paluma' stand out by showing great susceptibility to atmospheric ozone (FURLAN et al., 2007), with visible symptoms characterized by dark dotted color (reddish) on its upper surface, which allows the use of such species as ozone pollution bioindicator. Although leaves from 'Paluma' guava demonstrated such behavior and leaves from 'Pedro Sato' guava responded with less intensity to ozone-induced oxidative stress (MORAES et al., 2011), little is known about fruit sensibility after gaseous ozone exposure.

Therefore, the objective of this study was to evaluate gaseous ozone effect on the sensibility of the epicarp of 'Pedro Sato' guava fruits to different concentrations of ozone, under storage conditions.

2 MATERIAL AND METHODS

We have used 'Pedro Sato' guava fruits that were harvested in the maturation stage 1 (dark green skin) (AZZOLINI et al., 2004) and physiologically developed, with mean hue angle (°h) of 117.76 °h at harvest. Guava fruits were selected and standardized according to their maturation stage and absence of anomalies, and then subjected to disinfection with 2% sodium hypochlorite (NaClO) solution, and placed on counters to dry under laboratory conditions.

Gaseous ozone was obtained from an ozone generator (Figure 1) based on the dieletric discharge method, developed by the Department of Physics from the Technological Institute of Aeronautics (ITA), São José dos Campos, SP, Brasil. We used, as an input source, humidity-free oxygen with purity of 90±3%, derived from a Mark 5 Oxygen Concentrator.

Figure 1 - Oxygen concentrator and ozone generator.

Ozone concentration was determined through the iodometric method (CLESCERL et al., 2000).

In order to evaluate the effect of ozone application on fruit sensibility, the gas was injected at concentrations of 0, 65, 95, 185, 275, 370 and 460 μg L-1 at 2 L min-1 for 60 min, into the fumigation chambers containing 48 fruits (Figure 2). Thereafter, the fruits were packaged in expanded polystyrene trays, inside plastic boxes, where they remained for nine days, under ambient conditions (23±2 °C e 70±2% UR).

Figure 2 - Fumigation chambers (maximum capacity: 60 L) developed and manufactured entirely with acrylic material with sealing system composed of silicone contours and fast clamps. Each chamber accommodated 48 guava fruits**.**

Subsequently to the storage period, fruits were relocated to the Laboratory of Structural Biology for their exterior aspect imaging analysis. Images were generated with a Nikon D700 camera attached to a binocular stereoscopic lens (1.0 and 5.0X magnification) Olympus Model SZH10. The following variables were used in the exterior aspect of 'Pedro Sato' guava fruits: color and epicarp firmness.

2.1 EXPERIMENTAL DESIGN

The experiment was performed in split-plot scheme, with plots being ozone concentrations (0, 65, 95, 185, 275, 370 and 460 μg L^{-1}) and subplots being the evaluation or harvest days (0, 1, 3, 5, 7 and 9 days) in a completely randomized design with 3 replicates of

5 fruits per experimental unit. Data were subjected to descriptive analysis using the Sistema para Análises Estatísticas, SAEG 9.0 software (SAEG, 2007).

3 RESULTS AND DISCUSSION

Ozone-induced anomalies were characterized by green spots and presence of red-brownish pustules on fruit epicarp (Figure 3). Such anomalies were observed four days after ozone exposure at concentrations higher than 185 μg L^{-1}. As the storage period and the maturation process progressed, the anomalies started to become more noticeable, but restricted to the fruit epicarp. Similar symptoms were described by several researchers (FURLAN et al., 2007 PINA & MORAES, 2007, 2011; TRESMONDI & ALVES, 2011) when reporting the effect of ozone in 'Paluma' and 'Pedro Sato' guava fruits (MORAES et al., 2011). Therefore, the anomalies observed in the present study are in accordance with the standard found in the literature (SÁNCHES et al., 2002; NOVAK et al., 2003).

Figure 3 - 'Pedro Sato' guava fruits with epicarp anomalies, caused by ozone application at concentrations of 0, 65, 95, 185, 275, 370 e 460 μg L-1 after nine days of storage. Images: José Lino Neto.

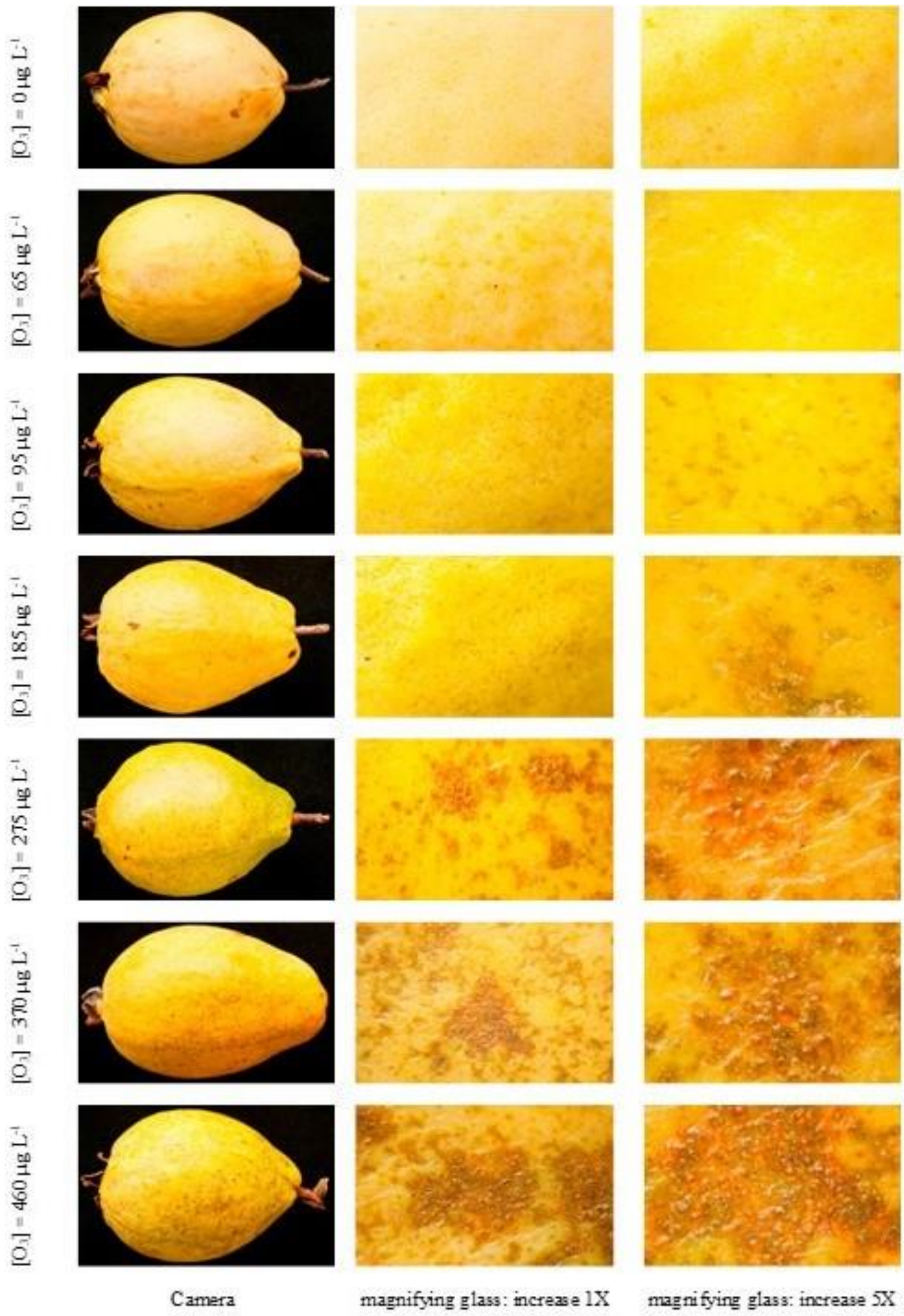
There is no information about the physiological disturbances that may lead to skin (epicarp) anomalies of guava fruits, regardless the variety, subjected to ozone application.

The expression of symptoms such as red-brownish spots are commonly named pigmentation or tanning, being a chronical injury resulting from the development and collection of phenolic pigments inside the cells, like anthocyanins, polymerized quinones, or proteins (KRUPA & MANNING, 1988; HEATH et al., 2009).

When plants absorb ozone, it can lead to an intensive chain production of reactive oxygen species (ROS) (MUDD, 1996; PELL et al., 1997). ROS are characterized in a chemical group that acts as a oxidative agent, including oxygen radicals (superoxide radical - O_2•, hydroxyl radical - OH•, and hydroperoxyl radical - HO_2•) and oxygen derived non-radicals (hydrogen peroxide H_2O_2 and singlet oxygen $^1O_2$) (MITTLER, 2002; HALLIWELL, 2006).

Although the mechanisms of ozone application on guava fruits are not entirely elucidated, it is known that the mechanisms that justify the leaf symptoms on this crop, exposed to different ozone concentrations, may help clarifying the observed anomalies, such as green spots red-brownish pustule development (Figure 4), on fruit epicarp under post-harvest ozone application.

Figure 4 - ‘Pedro Sato’ guava fruits presenting typical anomalies on te epicarp, caused by ozone application at concentrations of 0, 65, 95, 185, 275, 370 e 460 µg L-1 after nine days of storage . Images: José Lino Neto.



Due to the visual aspect of the epicarp, ‘Pedro Sato’ guava fruits became unfit for fresh consumption, but they can be used for industrial purposes. The anomalies observed in the present studies were restrained to the fruit epicarp, and may have not affected its pulp; similar conditions as girdling (WATANABE et al., 2011).

4 CONCLUSIONS

'Pedro Sato' guava fruits responded ozone-induced oxidative stress at concentrations higher than 185 μg L^{-1}, which lead to visible injuries with development of green spots and red-brownish pustules on the fruit epicarp.

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