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Artigo/Article

Efficacy of pre-harvest sprays to reduce rind pitting on Benny Valencia orange

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SUMMARY

Post-harvest pitting is a non-chilling related physiological rind disorder that the reduces external quality of various cultivars. The primary cause of post-harvest rind pitting of Valencia orange fruit is unknown, however, incidence thereof is aggravated by a change in post-harvest relative humidity (RH) and rind water status. The incidence of this disorder is erratic due to a wide range of factors influencing fruit susceptibility. Various plant growth regulators were evaluated to reduce the incidence of pitting in Benny Valencia fruit. Pre-harvest foliar sprays were performed in orchards during the 2014-2015 seasons in a complete randomised block design (n=10). The application of 2,4-D (2,4-dichlorophenoxyacetic acid, 10 mg L^{-1}) at 50% petal drop or after physiological fruit drop reduced the incidence of post-harvest rind pitting by 38% and 56%, respectively. Application of s-abscisic acid (s-ABA, 400 mg L_{-}^{1}) (Protone®) and thiabendazole (TBZ, 4000 mg L^{-1}) (ICA-TBZ®) 14 days before harvest reduced the incidence of this disorder by 43% and 51%, respectively. No negative effects of the foliar sprays in the form of chemical burn or loss of fruit quality were recorded. These results offer a new direction in the possible control of the incidence of this post-harvest physiological disorder by reducing susceptibility prior to harvest.

Index terms: physiological rind disorder, flavedo, auxins, abscisic acid, gibberellic acid, thiabendazole.

Eficácia de pulverizações pré-colheita para reduzir *pitting* na laranja Benny Valencia

RESUMO

O *pitting* é um distúbio fisiológico, que ocorre na casca nos frutos de citros, relacionado à falta de refrigeração que reduz a qualidade externa de várias cultivares. A causa primária em frutos de laranja de Valência é desconhecida, no entanto, a incidência é agravada por uma mudança na condição de umidade relativa (UR) pós-colheita e estado da água da casca. A incidência deste transtorno é devido a uma ampla gama de fatores que influenciam a susceptibilidade à fruta. Vários reguladores de crescimento de plantas foram avaliados para reduzir a incidência *pitting* em frutos de Benny Valência. Realizaram-se pulverizações foliares, em pré-colheita, em pomares durante as safras 2014-2015 em um delineamento de blocos ao acaso completo (n=10). A aplicação de ácido 2,4-D (ácido 2,4-diclorofenoxiacético, 10 mg L^{-1}) a 50% de gota de pétala ou após queda de fruta fisiológica reduziu a incidência de picada de casca pós-colheita em 38% e 56%, respectivamente.

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A aplicação do ácido s-abscísico (s-ABA, 400 mg L^{-1}) (Protone®) e tiabendazol (TBZ, 4000 mg L^{-1}) (ICA-TBZ®) 14 dias antes da colheita reduziu a incidência deste transtorno em 43% e 51%, respectivamente. Não foram registrados efeitos negativos das pulverizações foliares sob a forma de fitotoxidez ou perda de qualidade da fruta. Esses resultados oferecem uma nova direção no controle possível da incidência deste transtorno fisiológico em pós-colheita, reduzindo a suscetibilidade antes da colheita.

Termos de indexação: transtorno de causa fisiológica, flavedo, auxinas, ácido abscísico, ácido giberélico, tiabendazol.

INTRODUCTION

Non-chilling post-harvest pitting of citrus fruit is a physiological rind disorder that diminishes external fruit quality resulting in economic losses. The disorder originates in the transitional zone of the flavedo-albedo with epidermal cells of the flavedo, oil glands and deeper layers of the albedo initially unaffected (Agusti et al., 2001). As the disorder progresses, affected areas turn bronze in colour, most likely due to the oil gland content being released into intercellular spaces resulting in enzymatic oxidation (Agusti et al., 2001). The primary cause or mechanism of the disorder is unknown, however variation in relative humidity (RH) during post-harvest handling and resultant changes in the rind water status seem to be involved in the susceptibility to develop this disorder in Navel oranges (Alférez et al., 2003) and Marsh grapefruit (C. *paradisi* Macf.) (Alférez & Burns, 2004). Rind pitting or staining differ from oleocellosis, as the causal mechanism responsible is not related to any mechanical damage to the oil glands as in oleocellosis (Knight et al., 2002).

In citriculture, plant growth regulators (PGRs) or synthetic phytohormones are used pre- and post-harvest to improve fruit quality. The synthetic auxins, 3,5,6 trichloro-2-pyridiloxyacetic acid (3,5,6 TPA) and 2,4-dichlorophenoxy acetic acid (2,4-D) reduced fruit splitting in Nova mandarin (Greenberg et al., 2006; Stander et al., 2014). Gibberellic acid (GA_3) reduces the incidence of multiple rind disorders, such as creasing in Valencia and Navel orange fruit (Fidelibus et al., 2002). In citriculture, foliar application of s-abscisic acid (s-ABA) is not currently used, but in viticulture 0.2 g L^{-1} is applied at veraison to enhance berry colour development (Cantín et al., 2007; Peppi et al., 2006). However, it has been reported that the ABA level in the Navel orange fruit rind influences the incidence of certain physiological rind disorders such as post-harvest pitting and chilling injury (CI) (Alférez et al., 2005a). Post-harvest application of thiabendazole (TBZ), a well know fungicide is used in citrus pack-houses to control green and blue mould (*Penicillium spp*.). However it also reportedly reduces the incidence of chilling injury in Tarocco orange fruit (Schirra & Mulas, 1995; Schirra et al., 1998), Star ruby grapefruit (*C. paradisi* Macf.) (Schirra et al., 2000) and Navel orange fruit (Hordijk, 2013).

Most physiological disorders are thought to develop a sensitively to a disorder during fruit development in the orchard. For example, the negative impact of inadequate light levels has been shown in resulting in increased sensitivity of Nules Clementine mandarin in terms of rind breakdown (Cronje et al., 2013). Subsequent to preharvest fruit developmentally a postharvest stress such as a change in vapour pressure deficit (temperature and RH) could trigger the disorder (Alférez et al., 2003). If a reduction in susceptibility of citrus fruit could be affected by a management action, such as improved light management for Nules Clementine mandarin (Cronje et al., 2013) could lead to a commercial solution for a rind disorder. The mechanism of action of TBZ reducing chilling injury is unknown; however, due to the known efficacy in the same fruit part i.e. collapse of the flavedo the question was asked if this chemical could have an impact on a non-chilling physiological disorder as well. Benny Valencia orange fruit are exported from South Africa at arrange of temperatures from -0.6°C to 4°C depending on the import market regulations. This cultivar is therefore not a chilling injury sensitive cultivar but in contrast, it is susceptible to non-chilling related disorders such as rind pitting and staining.

The objective of this study was therefore to reduce the sensitivity of Benny Valencia orange fruit by evaluating the efficacy of various PGRs and the fungicide TBZ to reducing pitting of Valencia oranges when applied as pre-harvest foliar sprays. Elucidation of the mode of action of TBZ or the various PGRs tested did not form part of this study, as the initial aim was to determine efficacy.

MATERIALS AND METHODS

The trails were conducted on Benny Valencia orange orchards, at Riverside (23°48'02.51S 30°27'27.20E), and The Plains (23°50'23.94S 30°25'55.82E), in the Letsitele valley in Limpopo, the main Valencia orange production region of South Africa.

To determine the impact of postharvest dehydration followed by rehydration as a stress to induce pitting (Alférez et al., 2003) the following experiment was conducted prior to large-scale testing. Two samples of twenty fruit, uniform in size and colour, were harvested per tree from ten healthy and uniform Benny Valencia trees in both the Riverside and The Plains orchards during 2015. One sample received a stress treatment before being placed into cold storage (4.5°C) and the other was placed into cold storage directly after harvest. The stress treatment involved dehydrating fruit at 25°C and 50% RH (VPD of 1.55 kPa) for three days followed by 1 day at 20°C with 99% RH (VPD of 0.02) before being placed in cold storage at a temperature (4.5°C) not know to result in chilling injury of this cultivar. This stress treatment was used to induce pitting in order to evaluate the efficacy of the various foliar treatments. All fruit, therefore, followed a similar handling and storage protocols: treatments application in the orchard, harvest, postharvest pitting induction stress, cold storage (4.5°C for 21 days), 7 days shelf life storage (ambient conditions) and lastly evaluation of pitting incidence. Fruit were evaluated for postharvest pitting incidence index (PPI) as follows: Fruit were inspected and rated from 0 (no post-harvest pitting) to 4 (severe post-harvest pitting) (Figure 1), and a post-harvest pitting index (PPI) was calculated as follows:

$$\mathrm{PPI}(0-4) = \frac{\sum \left[\begin{array}{l} Rating\ (0-4) \times \\ number\ of\ number\ fruit \\ within\ each\ class \end{array} \right]}{Total\ number\ of\ fruit} \qquad (1)$$

The efficacy of foliar applied PGR's and the fungicide TBZ, on pitting of Benny Valencia was tested over two seasons. In all experiments applications were made on healthy and uniform trees with buffer trees between selected trees and buffer rows between rows. In addition, the control trees were always sprayed with water and 0.05 ml·L^{-1} Break-Thru® in order to ensure that any efficacy could be related to the product alone All foliar treatments were applied using a Stihl mist blower (SR 450, Andreas Stihl AG & Co., KG, Germany) at approximately 3 L of the mixture per tree.

During 2015 treatments were applied at Riverside in a randomised complete block design with ten replicate trees (n=10). The following products where applied: 2,4-D [2,4-D, Dow AgroSciences (Pty) Ltd.] at 10 mg L^{-1} at 50% petal drop (PD) or before physiological fruit drop, 3,5,6 TPA [Maxim®, ARYSTA Lifescience SA (Pty) Ltd.] at 10 mg L^{-1} before physiological fruit drop, GA_3 [Progibb®, Philargo SA (Pty) Ltd.] at 20 mg L^{-1} during January and TBZ [ICA-TBZ, ICA international chemicals (Pty) Ltd.] at 4000 mg L^{-1} two weeks before harvest. A non-ionic wetting agent [Break-Thru®, Villa Crop Protection (Pty) Ltd.] was also added at 0.05 mg L^{-1} Break-Thru® to spray solutions. Thirty fruit were sampled per tree during commercial harvest (22 June 2015) before receiving the pitting inducing stress treatment and placed into cold storage (21 days at 4.5°C) before being evaluated for pitting.

Published reports on the use of foliar applied s-ABA in citriculture could not be found and therefore s-ABA (Protone®, Valent BioSciences) was applied at three different concentrations (200 mg L^{-1}), (400 mg L^{-1}) and (800 mg L^{-1}) mixed with 0.05 mg L^{-1}Break-Thru® to trees (n=8) each in a randomised complete block design on 21 June 2015 at Riverside. Ten fruit were harvested per tree 1 day after application (DAA) as well as 8 and 15 DAA. After harvest

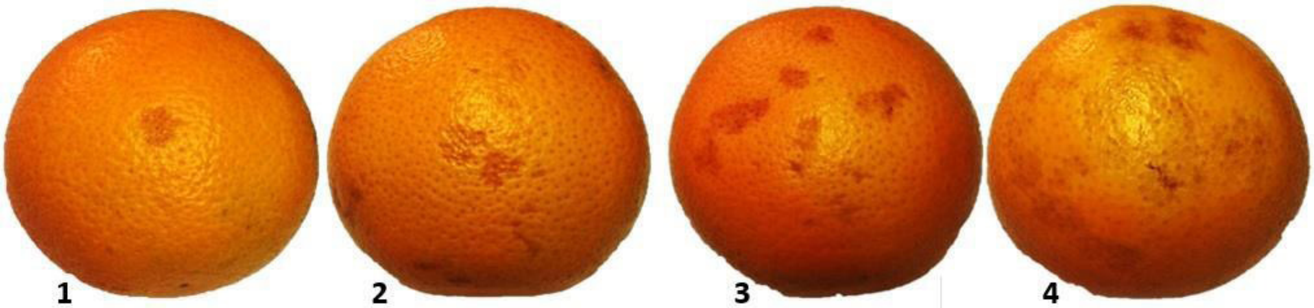

Figure 1. Chart of pitting incidence as used in all the trials: 1 low to 4 very high incidences.

fruit received the stress treatment and placed into cold storage before being evaluated for pitting.

During the 2016 season, only the efficacy of later applications (2 weeks before harvest) was evaluated to further gain information on the possible efficacy of these treatments. The following treatments were applied at Riverside one and two weeks before harvest to trees (n=6): 2,4-D at 10 mg L^{-1}, TBZ at 4000 mg L^{-1} and s-ABA at 400 mg L^{-1} and 800 mg L^{-1}, plus 0.05 mg L^{-1} Break-Thru® to each spray solution. Thirty fruit per tree were sampled during commercial harvest (30 June 2016). After harvest fruit received the stress treatment and placed into cold storage before being evaluated for pitting.

To test the commercial feasibility of s-ABA to reduce this disorder 4 blocks of 40 uniform and healthy trees each was selected in a historically susceptible orchard at Riverside during 2016. A commercial foliar sprayer was used to apply 400 mg L^{-1} s-ABA plus 0.05 ml·l^{-1} Break-Thru® at a rate of 3000 l·ha^{-1} to 20 trees per block. Three bins of fruit (each ± 400 kg) were harvested for each treatment per block and commercially packed where after three cartons (± 100 fruit per carton) of each treatment per block was stored at 4.5°C for 21 days.

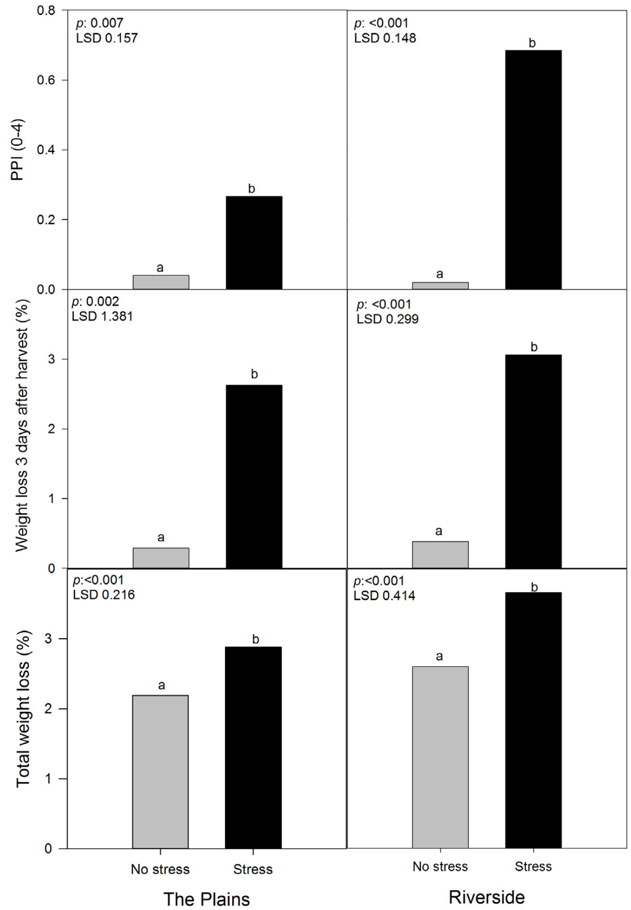

Figure 2. Post-harvest pitting index weight loss (%) 3 days after harvest and total weight loss (%) of Benny Valencia orange fruit from The Plains and Riverside after 21 days of storage at 4.5°C. Stressed fruit were dehydrated for 3 days at 25°C and 50% RH and rehydrated for 1 day at 18°C and 99% RH. Control fruit were placed into cold storage after harvest. Different letters indicate significant difference at 95% level ($p \leq 0.05$).

Data collection, evaluation and statistical analysis

Fruit weight was measured directly after harvest, after dehydration and after storage with a balance (Kern KB 3600-2N, Kern & Sohn GmbH | Ziegelei 1 | 72336 Balingen - Germany).

PPI (ranked) data (as discussed above) and weight loss (%) were analysed using the one-way ANOVA, two-way ANOVA (test interaction) and non-parametric one-way ANOVA (Kruskal-Wallis test, when data was not normally distributed) with SAS Enterprise guide v.5.1 (SAS Institute, Cary, NC, USA). Each treatment was compared with the other using Fischer least significance differences (LSD).

RESULTS

The post-harvest water stress of Benny Valencia orange fruit from both orchards resulted in significantly higher PPI and total weight loss after the 3-day stress treatment as well as after 21 days storage at 4.5°C (Figure 2). This stress was therefore used in all instances to induce pitting and evaluate the efficacy of chemical treatments.

During 2015, the foliar-applied treatments influenced PPI. All the treatments significantly (at a 90% level) reduced the incidence of post-harvest pitting except GA_3. TBZ significantly reduce weight loss during dehydration and total weight loss (Figure 3). In general, a reduction in post-harvest pitting was found with increasing concentration of s-ABA over all three harvesting dates. No interaction was found between s-ABA concentration and delay in sampling date (days after application-DAA). The two higher concentrations of 400 mg L^{-1} and 800 mg L^{-11} s-ABA reduced pitting incidence, however only significantly so if applied at 800 mg L^{-1} and harvested 15 DAA (Figure 4).

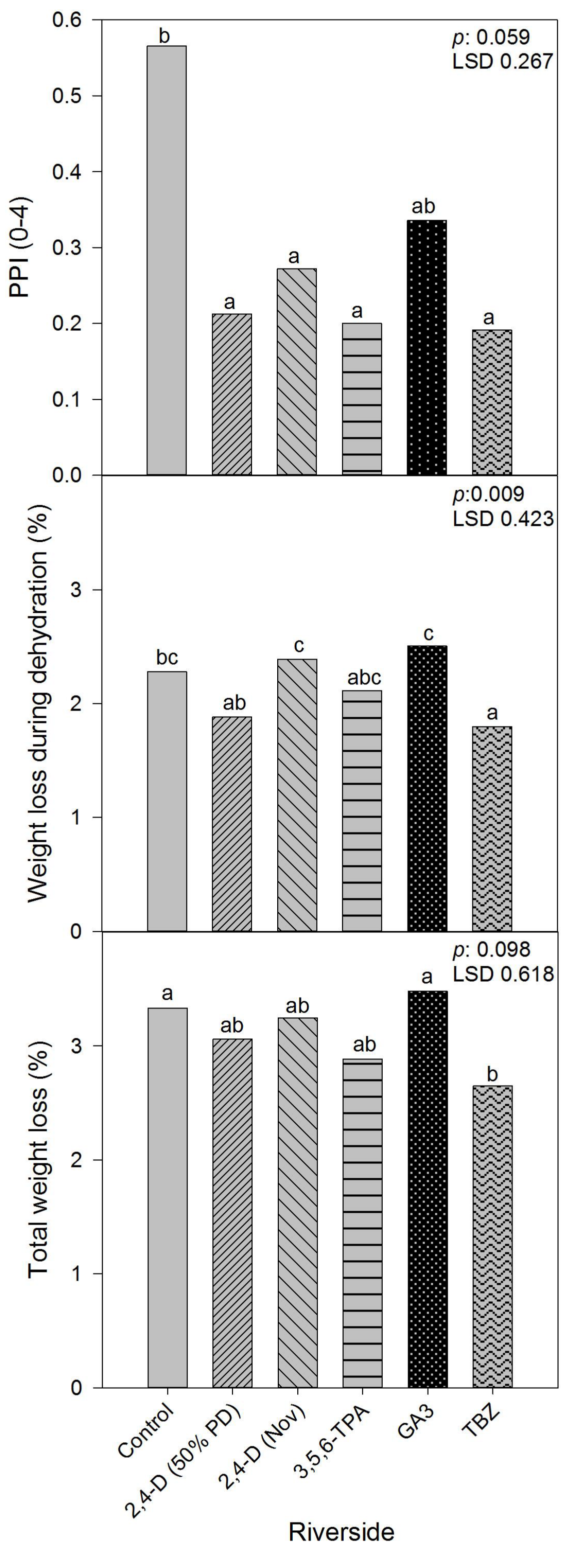

Figure 3. Post-harvest pitting index (PPI) (0-4), weight loss (%) during dehydration and total weight loss (%) of Benny Valencia orange fruit treated with foliar applied PGR's during the 2015 season at Riverside. Different letters indicate significant difference at 90% level ($p \leq 0.1$).

The 400 mg L^{-1} treatment concentration reduced the disorder at this harvesting date, at a 90% significance level. The time of harvest after application followed a similar trend with the two later harvest dates, 8 and 15 DAA, resulting in a reduction of pitting incidence (Figure 5). Fruit harvest 8 DAA and 15 DAA had a significantly lower incidence of this disorder than fruit harvested 1 DAA at a concentration of 400 mg L^{-1}. At 800 mg L^{-1} pitting was significantly reduced in fruit harvested 15 DAA however the reduction at 8 DAA was not significant. During 2016 no significant reduction was found on both the late season PGR trials in single tree applications and the s-ABA commercial trial (Figures 6 and 7)

DISCUSSION

These trials were the first to document the negative effect of a change in RH and therefore Vapour Pressure Deficit (VPD) during post-harvest storage resulting in post-harvest pitting of Benny Valencia oranges. The stress applied to the fruit by dehydration for three days at low RH then transferred to high RH for one day before storage resulted in 90% higher pitting incidence compared to fruit kept at a constant high RH. These results concur with results on Navel orange (Alférez et al., 2003) and grapefruit (Alférez & Burns, 2004; Alférez et al., 2005b) indicating the possibility of a similar mode of action. Alférez et al. (2003) hypothesized that when fruit was stored at low RH, a high VPD between the rind and the atmosphere results in water loss and a reduction in water potential (Ψ_w) of the albedo and flavedo cells. At a high VPD and RH a rehydration occurs which was thought to cause the rind collapse due to the possible involvement of phospholipase membrane degradation in the rind (Alférez et al., 2008). Thus this transfer of dehydrated Benny Valencia fruit from low to high RH which caused rind pitting could possibly be due to a similar mechanism that initiate this post-harvest disorder.

The efficiency of foliar sprays to reduce the incidence of pitting depended on the active ingredient and concentration of the chemical used. In addition, research into treatments to reduce pitting and to determine the efficacy of a treatment is complicated by the large variation in pitting susceptibility between seasons as well as orchards in the same area. The most promising treatments, were the application of 10 mg L^{-1} 2,4-D at 50% PD or 2,4-D and 3,5,6-TPA after physiological fruit drop. The reduction in pitting by these auxins could be attributed to their ability to

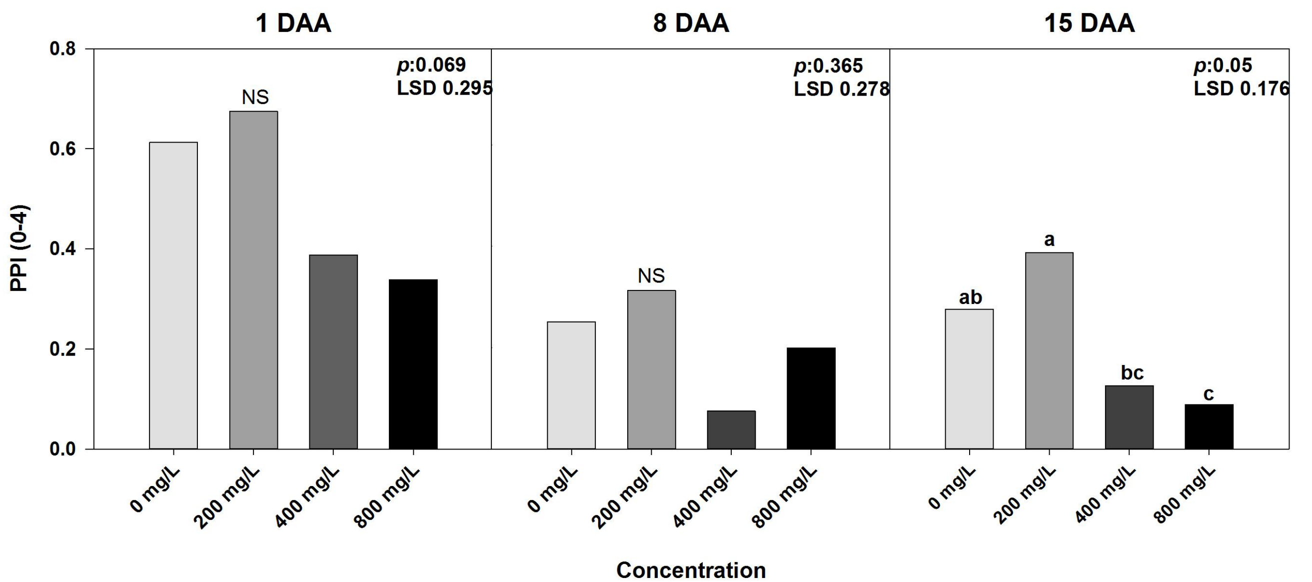

Figure 4. Concentration effect on post-harvest pitting index (PPI) (0-4) of Benny Valencia orange fruit harvested 1 Day after application (DAA), 8 DAA and 15 DAA, treated with four different concentrations of s-ABA 0.0 g L^{-1}, 0.2 g L^{-1}, 0.4 g L^{-1} and 0.8 g L^{-1}. Different letters indicate significant difference at 95% level (p≤0.05).

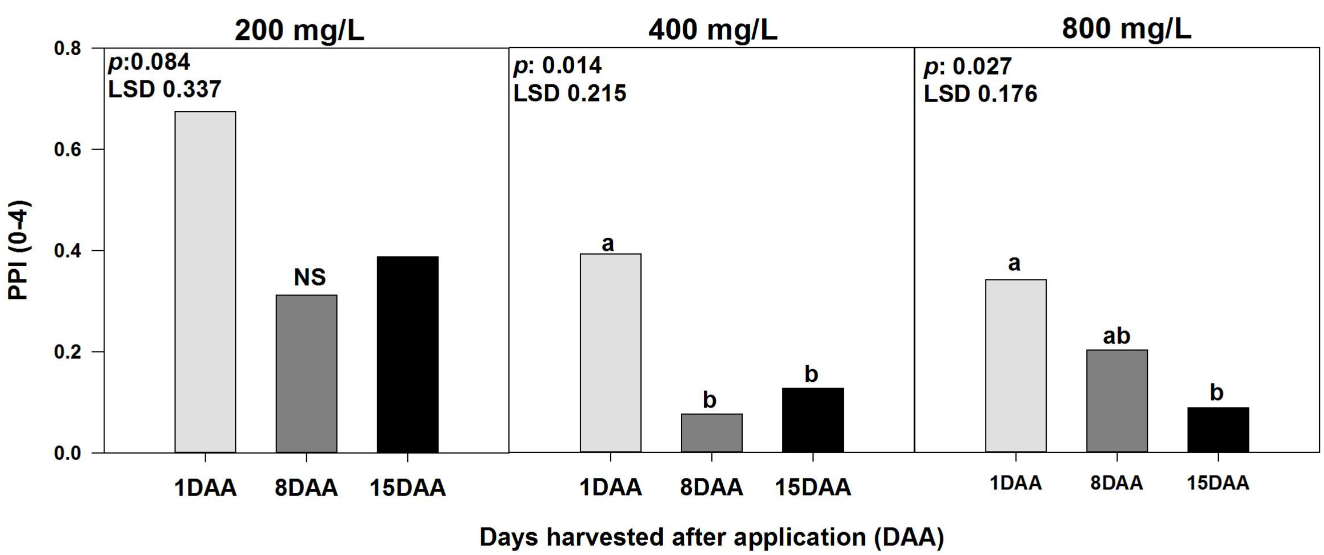

Figure 5. Timing effect on post-harvest pitting index (PPI) (0-4) of Benny Valencia orange fruit harvested 1 Day after application (DAA), 8 DAA and 15 DAA, treated with four different concentrations of s-ABA 0.0 g L^{-1}, 0.2 g L^{-1}, 0.4 g L^{-1} and 0.8 g L^{-1}. Different letters indicate significant difference at 95% level (p≤0.05).

influence fruit growth and development. Auxins are known to increase fruit sink strength and dry matter accumulation (Agustí et al., 2002) as well as increase the fruit peduncle diameter (Mesejo et al., 2003) which enhances transport capacity of the vascular system, to transport water and nutrients (Bustan et al., 1995). The growth of rind cells during stage II of fruit development is also promoted by 2,4-D and this can lead to increased rind thickness (Duarte et al., 2006; Stander et al., 2014). Thus any one or a combination of these effects of auxin on rind development and fruit growth could contribute to a less susceptible rind due to a possible improved nutrient accumulation of minerals and carbohydrates in the flavedo. The efficacy of 2,4-D to reduce pitting was also observed during the

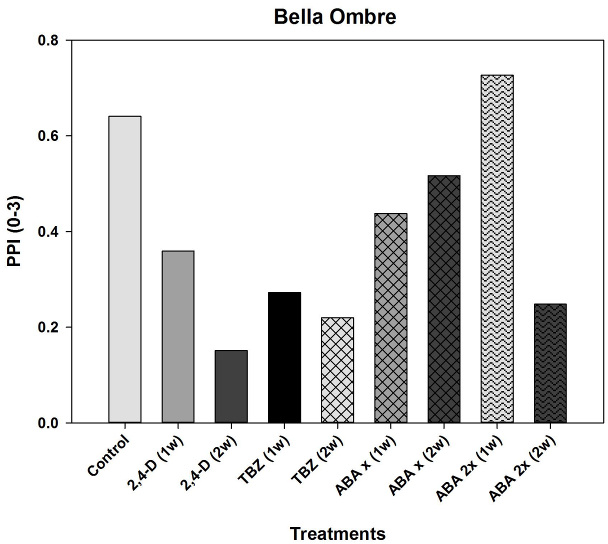

Figure 6. Post-harvest pitting index(PPI)(0-3) of Benny Valencia orange fruit treated with foliar applied PGR's one or two weeks before harvest.

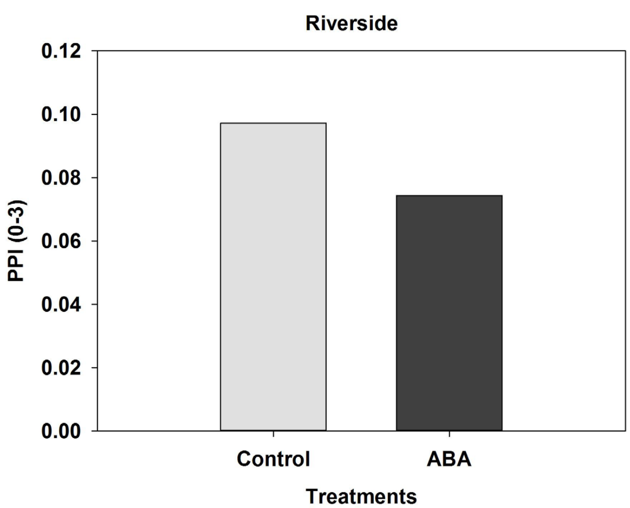

Figure 7. Post-harvest pitting index(PPI)(0-3) of Benny Valencia orange fruit commercially treated with 0.4 g L^{-1} s-ABA two weeks before harvest. Post-harvest fruit were commercially packed and received a stress treatment of three days at low humidity and 25°C and one day at high humidity and 18°C.

second season when 10 mg L^{-1} 2,4-D was applied one or two weeks before harvest and reduced the disorder by 44% and 76%, respectively, however, the results were not statistically significant due to the low incidence of the disorder.

Application of TBZ as a pre-harvest spray to reduce the incidence of pitting has not been previously documented. In this study foliar application of TBZ, one week before harvest at 4000 mg L^{-1} significantly reduced the incidence of post-harvest pitting of Benny Valencia orange (Figure 3) without exceeding the maximum residue level (MRL) for TBZ of 5 ppm. Even though the effect of pre-harvest applied TBZ on post-harvest pitting has not been previously studied the effectiveness of TBZ to reduce chilling injury during cold storage has been reported (Schiffmann-Nadel et al., 1972). Subsequently, it was shown that application of TBZ as 1% active ingredient 14 days before harvest reduced chilling injury of Tarocco blood oranges (Schirra et al., 2002). The mode of action through which TBZ reduces post-harvest pitting in unknown, however a reduction in post-harvest pitting incidence and weight loss during dehydration was found. Thus TBZ could possibly reduce the incidence of pitting by reducing postharvest water stress during the dehydration proses (Figure 3).

Pre-harvest foliar application of s-ABA on citrus, reduced pitting incidence in the first season when pitting incidence was high. The concentration of 200 mg L^{-1} s-ABA, -recommended for use in viticulture to improve colour development, - was too low, however the higher concentrations of 400 mg L^{-1} and 800 mg L^{-1} reduced PPI. The mode of action of the s-ABA is unknown but a definite lag phase between application and sampling in efficacy suggest a gradual change being affected. The delay in action was also recorded in weight loss with a significant reduction during dehydration of fruit harvested 15 DAA (data not shown). These results indicate that the effect of s-ABA on weight loss was possibly only temporary during the stress period as total weight loss did not differ. The phospholipids membrane degradation enzymes (PLA2 and PLD) are modulated by the water balance in the rind and ABA signalling appears to be involved in their gene expression (Romero et al., 2013, 2014). Alférez et al. (2003) found similar results on Pinalate orange, where the incidence of post-harvest pitting was reduced with post-harvest ABA application. Pre-harvest application of this PGR has not been documented for this purpose in citriculture and by further in-depth studies under various conditions and cultivars it may be possible to develop this active ingredient into a novel technology to control post-harvest pitting of citrus fruit.

In conclusion, post-harvest pitting of Benny Valencia orange is a physiological rind disorder aggravated by changes in post-harvest RH and temperature. The synthetic auxins 2,4-D applied at 50% PD of after physiological fruit drop and 3,5,6-TPA applied after physiological fruit drop significantly reduced the disorder when applied at 10 mg L^{-1}. In a novel approach, the pre-harvest application of s-ABA and the fungicide TBZ also reduced the disorder. An application of 10 mg L^{-1} 2,4-D or 3,5,6-TPA after physiological fruit drop over a opportunity to elucidate the development of fruit susceptibility to postharvest pitting as the known impact of this auxin on sink strength and subsequent changes in rind physiological condition (Mupambi et al., 2015: Stander et al., 2014). In addition, fruit should be packed and placed within the cold chain as soon possible to minimise post-harvest environmental variation and water stress resulting in dehydration followed by rehydration of the rind which results in pitting development in susceptible fruit.

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