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Pre-harvest spray application of prohexadione-calcium and paclobutrazol improves rind colour and regulates fruit quality in M7 Navel oranges

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ABSTRACT

Sweet orange (*Citrus sinensis* L. Osbeck) cv. 'M7' Navel exhibits poor flavedo colour at harvest in Western Australia. Gibberellins are known to retard fruit colour development in citrus fruit, therefore the efficacy of different concentrations and timings of spray applications of two inhibitors of gibberellin biosynthesis such as prohexadione calcium (Pro-Ca) and paclobutrazol (PBZ) on flavedo colour development and fruit quality was investigated. The effects of an aqueous solution containing different concentration of Pro-Ca (200, 400, 600, 800, 1200, 1600 or 2000 mg L^{-1}), and PBZ (100, 250, 500, 1000, 1500 and 2000 mg L^{-1}) sprayed at 6 or 3 weeks before anticipated harvest (WBAH) on flavedo colour development particularly from yellow to deep orange and on the fruit quality were investigated during 2015 and 2016. Unsprayed trees were kept as control. The spray application of Pro-Ca (800 mg L^{-1}) resulted in decreased hue angle ($h°$) (57.5), and increased citrus colour index (CCI) (10.2) and, total carotenoid levels (38.3 mg kg^{-1}) in the flavedo during 2015. Furthermore, Pro-Ca (600 or 800 mg L^{-1}) showed enhanced levels of total carotenoids (39.2 and 38.3 mg kg^{-1}) respectively, during 2015. In 2016, a single spray application Pro-Ca (1200 mg L^{-1}) at 3 WBAH exhibited reduced $h°$ (56.6) and increased CCI (10.7), and levels of total carotenoids (36.8 mg kg^{-1}) as compared to the control. Additionally, a single spray application of Pro-Ca (800, 1200 or 1600 mg L^{-1}) was able to increase total carotenoids in the flavedo (32.7, 36.8 or 33.1 mg kg^{-1}) respectively. In 2016, Pro-Ca (800 mg L^{-1}) exhibited increased SSC (13.0%) and reduced TA (1.08%) in the fruit juice. A single spray application of PBZ (1000 mg L^{-1}) at 6 WBAH significantly reduced $h°$ (56.7) and increased CCI (10.6) and levels of total carotenoids in the flavedo (42.6 mg kg^{-1}). However, a single spray application of PBZ (1500 mg L^{-1}) at 3 WBAH showed lower $h°$ (55.4) and enhanced CCI (11.2) and level of total carotenoids (47.1 mg kg^{-1}) in the flavedo during 2016. In conclusion, pre-harvest spray application of Pro-Ca (800 and 1200 mg L^{-1}) applied at 6 and 3 WBAH respectively enhanced fruit colour. A single pre-harvest spray application of PBZ (1000 and 1500 mg L^{-1}) applied at 6 and 3 WBAH respectively improved fruit colour in early maturing 'M7' Navel.

1. Introduction

Early maturing cultivars have been introduced in Western Australia to capture the early market of Navel oranges. 'M7' Navel matures three weeks earlier than 'Navelina' Navel and can be harvested in May to cater for the early market. Fruit is usually sold at high prices due to its early presence in the market. 'M7' also holds its acid levels within the fruit a bit longer than 'Navelina' Navel (DAFWA, 2017). However, 'M7' does not colour properly due to the warm climate in Western Australian conditions. The poor and inconsistent flavedo colours reduce economic returns for 'M7' growers and partially green fruit are difficult to sell in domestic or overseas markets. The colour of the flavedo has a significant importance when consumers buy Navel oranges. Flavedo colour changes occur in citrus as a result of increased chlorophyll degradation and accumulation of carotenoids pigments (Eilati et al., 1969a; Goldschmidt, 1988). Colour break in citrus occurs when a decrease in chlorophyll concentration exposes the presence of carotenoids, resulting in the appearance of the first citrus colour of sweet orange (Goldschmidt, 1988; El-Zeftawi and Garrett, 1978). The flavedo colour of Navel oranges is due to the genetic makeup and is also influenced by growing location (Barry and Le Roux, 2010). Other factors, such as rootstocks (Rabe and Von Broembsen, 1995), nutrition (Reitz and Koo, 1960), light interception (Sites and Reitz, 1949), plant growth regulators (Garcia-Luis et al., 1986; Rehman et al., 2018), canopy size

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(Krajewski, 1997) and deficient irrigation (Peng and Rabe 1996) have been reported to affect flavedo colour. However, the cold temperatures in winter months have a pronounced effect on flavedo colour development (Young, 1961).

GA_3 has been known to delay chlorophyll degradation and inhibit carotenoids accumulation in the flavedo of citrus fruit (Coggins, 1992; Goldschmidt and Eilati 1970). The level of GA_3 was found to be lowest at the time of ripening in the flavedo of 'Satsuma' mandarin (Kuraoka et al., 1979). Furthermore, GA_3 applications before colour break reduced carotenoid accumulation and caused delayed colour development in Navel orange (Lewis and Coggins 1964). Young fruit and leaves are the major sites of gibberellin biosynthesis (Spiegel-Roy and Goldshmidt, 1996). Delay in the flavedo colour in citrus is due to higher levels of endogenous gibberellins (Garcia-Luis et al., 1985).

Prohexadione-Ca (Pro-Ca) (3-oxide-4-propionyl-5-oxo-3-cyclohexene-carboxylate) also known as Apogee® (27.5% Pro-Ca) and Regalis® (10% Pro-Ca) is a growth retardant used to inhibit vegetative growth in fruit trees (Evans et al., 1999 and Prive et al., 2006). Bizjak et al. (2013) reported that Pro-Ca application also improved anthocyanin accumulation in 'Braeburn' apple fruit resulting in the initiation of red colour for temporary and no effect was found during apple storage. Furthermore, Barry and Le Roux (2010) reported that foliar spray application of Pro-Ca (400 mg L^{-1}) applied as a double dosage 6 and 3 weeks before anticipated harvest has the potential to improve the flavedo colour of 'Nules' clementine, mandarin and 'Navelina' Navel oranges. Whilst, Barry and Le Roux (2010) tested only two concentrations (200 or 400 mg L^{-1}) of Pro-Ca in inducing colour in the flavedo of mandarin, sweet orange and lemon fruit, and suggested that higher concentrations are more consistent to accumulate carotenoids in the flavedo and proposed to optimise the application rate further. The effects of Pro-Ca application on sweet orange fruit quality are also yet to be investigated.

Paclobutrazol (PBZ) is another growth retardant which is known to inhibit gibberellin biosynthesis (Rademacher, 2000). Greenberg et al. (1992) reported that foliar or soil application of PBZ during the autumn increased a total number of spring flush shoots by 1.6–2.7 fold and reduced the percentage of vegetative shoots in 'Shamouti' orange trees. Smeirat and Qrunfleh, (1988) also reported that PBZ reduced shoot and internode length and increased shoot diameter in 'Lisbon' lemon. PBZ has been reported to reduce vegetative growth in 'Minneola' tangelo (Monselise et al., 1976), 'Valencia' sweet orange (Delgado et al., 1986). Gilfillan and Lowe (1985) reported that PBZ enhanced 'Satsuma' mandarin flavedo colour by 1–2 units. Monselise (1985) reported that PBZ caused a rapid change in the flavedo colour in 'Topaz' tangor. The effect of PBZ application in reducing vegetative growth in different fruit crops has been investigated in detail but its application on promoting the flavedo colour and fruit quality in 'M7' Navel need to be investigated.

As a prelude, gibberellins are known to retard citrus fruit colour development whilst the role of inhibitors of gibberellins biosynthesis such as Pro-Ca and PBZ in regulating fruit colour development and quality in 'M7' Navel grown under a Mediterranean climate of Western Australia warrants to be investigated. Therefore, the objective of the present investigation was to explicate the role of gibberellins biosynthesis inhibitors including Pro-Ca and PBZ in promoting fruit colour development particularly from yellow to deep orange, levels of total carotenoids in the flavedo and fruit quality with the pre-harvest spray application in 'M7' sweet orange

2. Materials and methods

2.1. Plant material

Various experiments were initiated on uniform size, 5-year-old early maturing 'M7' Navel grafted on Carrizo citrange (*Citrus sinensis* (L.) Osbeck *x Poncirus trifoliata* Raf.) rootstock in a commercial orchard located at Moora (30° 35′ S/115° 55′ E), Western Australia in 2015 and 2016. The trees were spaced 5.0 × 2.5 m between and within rows. The row direction was North-South orientation. All the experimental trees received similar cultural practices including nutrition, plant protection and irrigation except excremental treatments.

2.2. Experiment 1: Pre-harvest treatments of Pro-Ca at 6, 3 WBAH single sprays or double spray at 6 WBAH followed by 3 WBAH on fruit colour and quality in M7 Navel

An aqueous solution containing different concentrations (200, 400, 600 or 800 mg L^{-1}) using [Regalis® soluble granule containing active ingredients (100 g kg^{-1}) (Pro-Ca)] (Nufarm Australia Pty, Ltd, Laveston North, Victoria 3026) and 'Tween 20' (0.05%, v/v) as a surfactant. The whole trees were sprayed until run off at 6 WBAH on 8 April or 3 WBAH on 30 April as a single spray application or as double sprays applied at 6 WBAH followed by 3 WBAH in 2015. In 2016, higher concentrations of Pro-Ca (400, 800, 1200, 1600 or 2000 mg L^{-1}) were applied as a single spray at 3 WBAH on 30 April. Unsprayed trees were kept as a control. The layout of the experiment was randomised block design with two-factor factorial including Pro-Ca treatments and times of application in 2015. A single tree was treated as an experimental unit and included three replicates during 2015. In 2016, the layout of the experiment was randomised block design with one-factor factorial comprising only Pro-Ca treatments, replicated four times. Blemish-free twenty-five fruit were randomly harvested around the tree canopy. An air-conditioned vehicle was used to transport the fruit to Curtin Horticulture Laboratory. Fruit peel colour ($h°$ and CCI), levels of total carotenoids in the flavedo and fruit firmness were recorded. Various quality parameters of the fruit, including soluble solids concentration (SSC %), titratable acidity (TA %), SSC: TA ratio, ascorbic acid concentration and total antioxidants, in the juice were determined in both years.

2.3. Experiment 2: Pre-harvest treatments of PBZ single spray at 6 WBAH in 2015 and 3 WBAH on fruit colour and quality in M7 Navel during 2016

An aqueous emulsion containing different concentrations (100, 250, 500, or 1000 mg L^{-1}) of PBZ using [Payback® liquid containing active ingredients (250 g L^{-1}) of Paclobutrazol (PBZ)] (Crop Care Australasia Pty. Ltd, Murarrie, Qld 4172) and 0.05% 'Tween 20' as a surfactant were sprayed at 6 WBAH (8 April) onto whole trees until run off in 'M7' by using a sprayer (The Selecta Trolleypak Mk II, Acacia Ridge, Australia) during 2015. In 2016, higher concentrations (500, 1000, 1500 or 2000 mg L^{-1}) of PBZ were applied 3 WBAH (30 April). Control trees were kept unsprayed during both years. The randomised block design was used in the experiment. Single tree plot was treated as an experimental unit and replicated four times during both years. A single tree was assigned as an experimental unit in 2015 and 2016. The fruit (25 per tree) were randomly picked around the tree canopy to assess colour and other quality parameters. The fruit peel colour ($h°$ and CCI) and fruit firmness were assessed. The levels of total carotenoids in the flavedo SSC (%), TA (%), SSC/TA ratio, vitamin C and total antioxidants in the juice were determined in both years.

2.4. Fruit colour, carotenoids and fruit quality variables

2.4.1. Determination of the fruit colour

The colour coordinates (L*, a* and b*) were determined using a Colorflex EZ (45°/0° design) spectrocolorimeter (Hunter Lab, Hunter Associates Laboratory Inc., Reston, VA, 20190, USA) at three positions around the equatorial plane of the fruit by following the method described earlier (Rehman et al., 2018). The hue angle ($h°$) value was calculated as $h° = tan^{-1}\ b^*/a^*$ and CCI was calculated by using the following formula $\mathrm{CCI} = \frac{1000 . a}{L . b}$.