Fruit quality of ‘Braeburn’ apple trees sprayed at post-bloom and preharvest with prohexadione-calcium and GA$_{4+7}$

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Abstract – The objective of this study was to assess the effects of post-bloom (PB) or preharvest (PH) treatments of ‘Braeburn’ apple trees with prohexadione-calcium (ProCa; 300 mg L$^{-1}$), an inhibitor of gibberellins biosynthesis, or GA$_{4+7}$ (300 mg L$^{-1}$) on fruit quality at harvest and after storage (four months at 0±0.5 °C, followed by five days of shelf life). PB treatments started 15 days after full bloom, with one application every week and six applications in total. PH treatments started five weeks before anticipated harvest, with one application every week and four applications in total. Control trees were sprayed with water. GA$_{4+7}$ PB increased length/diameter ratio, and GA$_{4+7}$ PH increased titratable acidity (TA) and flesh firmness and slightly reduced red color of the fruit at harvest. ProCa PB and PH reduced ethylene production rate, delayed starch degradation and reduced soluble solids content of the fruit at harvest, and ProCa PH maintained firmness at harvest and after storage and TA of the fruit after storage. ProCa PB or PH delayed the loss of skin green background color at harvest, while at both treatment timings reduced the development of skin red color, but with a much stronger effect when sprayed PH than PB.

Index terms: anti-gibberellin, ethylene, Malus domestica Borkh, maturity, skin color.

Qualidade de frutos em macieiras ‘Braeburn’ pulverizadas em pós-floração e pré-colheita com prohexadiona-cálcio e GA$_{4+7}$

Resumo - O objetivo deste trabalho foi avaliar os efeitos dos tratamentos em pós-floração (PF) ou pré-colheita (PC) de macieiras ‘Braeburn’ com prohexadiona-cálcio (ProCa; 300 mg L$^{-1}$), um inibidor da biossíntese de giberelinas, ou GA$_{4+7}$ (300 mg L$^{-1}$), na qualidade dos frutos na colheita e após o armazenamento (quatro meses a 0±0,5 °C, seguido por cinco dias de vida de prateleira). Os tratamentos PF começaram 15 dias após a plena floração, com uma aplicação a cada semana e seis aplicações no total. Os tratamentos PC começaram cinco semanas antes da previsão de colheita comercial, com uma aplicação por semana e quatro aplicações no total. Plantas controle foram pulverizadas com água. GA$_{4+7}$ pulverizado em PF aumentou a relação comprimento/diâmetro, e GA$_{4+7}$ pulverizado em PC aumentou acidez titulável (AT) e a firmeza de polpa, e causou leve redução na cor vermella dos frutos na colheita. ProCa, pulverizado em PF e PC, reduziu a taxa de produção de etileno, o índice de iodo-amido e o teor de sólidos solúveis dos frutos na colheita, e ProCa pulverizado em PC aumentou a firmeza de polpa na colheita e após o armazenamento, e a AT dos frutos após o armazenamento. ProCa, pulverizado em PF e PC, retardou a perda da cor de fundo (verde) e o aumento na cor vermella dos frutos na colheita, porém com maior efeito ao ser pulverizado em PC do que em PF.

Termos para indexação: anti-giberelina, etileno, Malus domestica Borkh, maturação, cor da epiderme.
Apple trees are often treated with gibberellins (GAs) during the first weeks after anthesis to reduce russetting and to promote fruit set or fruit elongation (GHOSH; HALDER, 2018), but this can advance fruit maturity and ripening and exacerbate storage breakdown (SHARPLES; JOHNSON, 1986). Rapid fruit growth promoted by GAs sprayed early in the season can impair the functionality of the xylem vessels and/or dilute Ca content in the fruit flesh, increasing the risk of Ca deficiency disorders in apples (DE FREITAS et al., 2016). Adversely, GAs sprayed early in the season can increase vegetative growth, leading to greater competition between leaves and fruit for Ca, and thus increasing fruit susceptibility to Ca deficiency disorders (AMARANTE et al., 2020). GAs are also sprayed at preharvest (PH) to delay fruit maturation and ripening in apples (TURNER, 1972).

Prohexadione-calcium (ProCa) spraying at post-bloom (PB) reduces the biosynthesis of active GAs in apple trees, inhibits vegetative growth (CLINE et al., 2008) and increases Ca content in the fruit (DE FREITAS et al., 2016; AMARANTE et al., 2020). However, orchard treatment with ProCa PB at high doses can impair red color development in apples (CLINE et al., 2008). In addition, treatment with ProCa PH might also impair red color development in some cultivars of apples (BIZJAK et al., 2012).

Gibberellins and anti-gibberellins are used commercially in apple orchards with different objectives, depending on phenological stage when trees are treated. However, for the best of our knowledge, this is the first research set to compare application timing (post-bloom or preharvest) of ProCa or GAs on apple fruit quality at harvest and after storage.

The study was carried out in 2018 in a commercial orchard, with 10 year old ‘Braeburn’ apple trees (grafted on M-7 rootstock) trained to a central leader and planted at medium density (2.0 m within-rows x 6.0 m between-rows). The orchard was located in Elk Grove, California, USA (38°21’23.27’’N, 121°32’13.62’’W, altitude of 123 m). The trees were sprinkler irrigated between May and August, and fruit thinned to two fruits per cluster to assure uniform tree crop load between treatments. Full bloom occurred on March 29th 2018.

Trees were sprayed with ProCa (300 mg a.i. L⁻¹; Apogee® with 27.5% ProCa) or GA₄+7 (300 mg a.i. L⁻¹; TypRus® with 2.0% GA₄+7), both products at PB or PH. PB treatments started 15 days after full bloom (DAFB), with one application every week and six applications in total. PH treatments started five weeks before anticipated harvest (125 DAFB), with one application every week and four applications in total. The trees were sprayed with a total volume of 1,000 L ha⁻¹ until “run off”. Control trees were sprayed with water.

The experiment followed a randomized block design, with five treatments and four blocks, each replicate consisting of four apple tree. Fifty fruit per replicate were harvested at commercial maturity (160 DAFB, on September 5th 2018). Twenty-five fruit per replicate were assessed at harvest in terms of fruit weight, length/diameter ratio, skin blush area (%), skin color (with a chroma meter Minolta CR-400, on the sun exposed and shaded sides of each fruit, at the L, C, and h° color space), starch index (1-5), titratable acidity (TA; % malic acid), soluble solids content (SSC; %), and flesh firmness (N), as described by Silveira et al. (2014). Fruit were also assessed for respiration (mL CO₂ kg⁻¹ hr⁻¹) and ethylene production (µL C₂H₄ kg⁻¹ hr⁻¹) rates at harvest, as described by Agar et al. (2000). Twenty-five fruit per replicate were cold stored for four months (at 0±0.5 °C/90-95% RH), and then assessed for TA, SSC, flesh firmness, and respiration and ethylene production rates following shelf life (5 days at 20±4 °C/60-70% RH).

Data on percentage of red skin color were transformed to arc sin [(x+5)/100]¹/² before statistical analysis. Treatment means were compared by Tukey’s test (p < 0.05) using the statistical program SAS (version 9.2).

Fruit weight at harvest was not different between treatments (data not shown). Only trees sprayed with GA₄+7 PB had fruit with a higher length/diameter ratio at harvest (Table 1). GAs treatment of apple trees during the first weeks after anthesis is known to increase the length/diameter ratio of the fruit (GHOSH; HALDER, 2018).
Fruit quality of ‘Braeburn’ apple trees sprayed at post-bloom and preharvest with prohexadione-calcium and GA$_{4+7}$

Table 1. Length/diameter (L/D) ratio, skin blush (%), skin color attributes ($L$, $C$ and $h^o$) and starch index of the fruit harvested from ‘Braeburn’ apple trees treated with GA$_{4+7}$ or ProCa post-bloom (PB) or preharvest (PH). Control trees were sprayed with water.

| Treatments   | L/D ratio | Skin blush (%) | Skin color attributes | Starch index (1-5) |
|--------------|-----------|----------------|-----------------------|--------------------|
|              |           |                | Sun exposed side      | Shaded side        |
|              |           |                | $L$ | $C$ | $h^o$ | $L$ | $C$ | $h^o$ |
| Control      | 0.867 b   | 50.6 a         | 49.8 c | 32.0 b | 49.0 c | 71.7 ab | 42.5 a | 110.2 b | 3.72 a |
| GA$_{4+7}$ PB| 0.931 a   | 54.0 a         | 51.0 bc | 35.4 a | 49.2 c | 72.7 a | 41.8 a | 106.3 c | 3.58 a |
| ProCa PB     | 0.880 b   | 53.1 a         | 53.1 bc | 29.8 c | 64.1 b | 69.1 c | 43.2 a | 112.1 ab | 3.12 b |
| GA$_{4+7}$ PH| 0.885 b   | 53.9 a         | 53.7 b | 32.1 b | 59.4 b | 70.9 b | 41.8 a | 111.1 ab | 3.42 ab |
| ProCa PH     | 0.893 b   | 24.7 b         | 58.6 a | 32.2 b | 77.1 a | 69.2 c | 43.2 a | 112.2 a | 3.22 b |
| CV (%)       | 3.99 b    | 25.8 b         | 6.35 | 6.24 | 18.7 | 2.23 | 2.02 | 2.12 | 15.2 |

Means (of four replicates) within columns followed by the same letter are not significantly different by Tukey’s test (p<0.05).

Both ProCa PB and PH treatments delayed the loss of green background color on the shaded side of the fruit (characterized by lower $L$ and higher $h^o$ values) in comparison to the control fruit (Table 1). ProCa PH significantly reduced fruit red skin color at harvest (Figure 1). These fruit had lower blush coverage on the skin and less intense red color on the sun exposed side of the fruit (characterized by higher $L$ and $h^o$ values) (Table 1). ProCa PB did not affect blush coverage of the skin, but reduced the intensity of skin red color on the sun exposed side of the fruit (characterized by higher $h^o$) in comparison to the control fruit (Figure 1; Table 1). ProCa reduces red color by reducing anthocyanin accumulation possibly due to the structural similarity between ProCa and 2-oxoglutaric acids (HALBWIRTH et al., 2006).

Figure 1. Skin color of fruit harvested from ‘Braeburn’ apple trees treated with gibberellins GA$_{4+7}$ or prohexadione-calcium (ProCa) at post-bloom (PB) or preharvest (PH). Control trees were sprayed with water.
GA$_{4+7}$, PB and PH did not affect skin background color (on the shaded side of the fruit) or skin blush (Figure 1). However, GA$_{4+7}$ PH reduced red color on sun exposed side of the fruit (characterized by higher $L$ and $h^°$ values) in comparison to the control, but its effect was less intense than ProCa PH (Figure 1; Table 1). GAs treatment close to commercial maturity delays fruit maturation on the tree (Turner, 1972), therefore delaying red skin color development.

ProCa PB and PH had no effect on respiration rate at harvest and after storage, and ethylene production rate after storage (Figure 2). For respiration rate at harvest, considering the same treatment time (PB or PH), there was a trend towards lower respiration in fruit from trees treated with ProCa than those treated with GA$_{4+7}$, but due to high variability the differences were not significant (Figure 2).

ProCa PB and PH reduced at harvest ethylene production rates (Figure 2), starch index (Table 1) and SSC (Figure 3), and ProCa PH increased at harvest TA of the fruit (Figure 3), in comparison to the control. ProCa reduces ethylene biosynthesis by the inhibition of 1-aminocyclopropane-1-carboxylic acid oxidase (RADEMACHER, 2000), therefore delaying fruit maturity at harvest. However, after storage, only fruit from trees treated with ProCa PH had higher TA and flesh firmness than the control (Figure 3).

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**Figure 2.** Respiration (top) and ethylene (bottom) production rates of the fruit at harvest and after storage (mean ± SE); fruit were harvested from ‘Braeburn’ apple trees treated with gibberellins GA$_{4+7}$, or prohexadione-calcium (ProCa) at post-bloom (PB) or preharvest (PH), and control trees. Within an individual graph, bars topped by the same letter were not significantly different by Tukey’s test (p<0.05).
Figure 3. Soluble solids content, titratable acidity and flesh firmness of apple fruit at harvest (left) and after storage (right) (mean ± SE); fruit were harvested from ‘Braeburn’ apple trees treated with gibberellins GA$_{1+,7}$ or prohexadione-calcium (ProCa) at post-bloom (PB) or preharvest (PH), and control trees. Within an individual graph, bars topped by the same letter were not significantly different by Tukey’s test (p<0.05).
GA\textsubscript{4+7} PH resulted in higher fruit TA and flesh firmness at harvest compared with control fruit (Figure 3). These results confirm the effect of GAs treatment close to commercial harvest on delaying fruit maturation (TURNER, 1972).

Treatment of apple trees with ProCa PB increases fruit calcium (Ca) content (DE FREITAS et al., 2016; AMARANTE et al., 2020). Calcium maintains higher fruit flesh firmness and reduces the incidence of many postharvest physiological disorders (DE FREITAS et al., 2016). Therefore, treatment with ProCa PB preserves fruit quality by reducing ethylene production and also increasing Ca content of the fruit.

The results show that treatment of apple trees with GA\textsubscript{4+7} PB increased the length/diameter ratio of the fruit at harvest, but had no effect on fruit quality attributes at harvest, and it might increase the risk of Ca-related postharvest physiological disorders (AMARANTE et al., 2020). However, GA\textsubscript{4+7} PH delayed the changes of TA and flesh firmness of ‘Braeburn’ apples at harvest. ProCa PB and PH reduces fruit ethylene production at harvest, but ProCa PH provides better preservation of fruit quality during cold storage than ProCa PB. However, ProCa PH impairs red color development of ‘Braeburn’ apples with negative impacts on fruit commercial value. Therefore, treatment with ProCa PB (instead of PH) is likely more suitable for apple genotypes that have red skin color to achieve the desirable effects of reduced ethylene production, delayed maturation, increased Ca content and reduced risk of Ca-related postharvest physiological disorders, with minor impacts on red color development. However, treatment with ProCa PH might be convenient for apple cultivars with green/yellow skin color to delay fruit maturation at harvest and improve postharvest quality of the fruit.

**References**

AGAR, I.T.; BIASI, W.V.; MITCHAM, E.J. Temperature and exposure time during ethylene conditioning affect ripening of ‘Bartlett’ pears. *Journal of Agriculture and Food Chemistry*, Washington, v.48, n.2, p.165-170, 2000.

AMARANTE, C.V.T.; SILVEIRA, J.P.G.; STEFFENS, C.A.; DE FREITAS, S.T.; MITCHAM, E.J. Post-bloom and preharvest treatment of ‘Braeburn’ apple trees with prohexadione-calcium and GA\textsubscript{4+7} affects vegetative growth and postharvest incidence of calcium-related physiological disorders and decay in the fruit. *Scientia Horticulturae*, Amsterdam, v.261, p.108919, 2020.

BIZJAK, J.; JAKOPIC, J.; SLATNAR, A.; STAMPAR, F.; STICH, K.; HALBWIRTH, H.; ZADRAVEC, P. VEBERIC, R. Late prohexadione-calcium application on maturing apple cv. ‘Braeburn’ fruit reduces anthocyanins and alters the phenolic content. *European Journal of Horticultural Science*, Stuttgart, v.77, n.4, p.154-162, 2012.

CLINE, J.A.; EMBREE, C.G.; HEBB, J.; NICHOLS, D.S. Performance of prohexadione-calcium on shoot growth and fruit quality of apple: Effect of spray surfactants. *Canadian Journal of Plant Science*, Ottawa, v.88, n.1, p.165-174, 2008.

DE FREITAS, S.T.; AMARANTE, C.V.T.; MITCHAM, E.J. Calcium deficiency disorders in plants. In: PEREKK, S. *Postharvest ripening physiology of crops*. Boca Raton: CRC Press, 2016. p.477-502.

GHOSH, S.; HALDER, S. Effect of different kinds of gibberellin on temperate fruit crops: A review. *The Pharma Innovation Journal*, New Delhi, v.7, n.3, p.315-319, 2018.

HALBWIRTH, H.; FISCHER, T.C.; SCHLANGEN, K.; RADEMACHER, W.; SCHLEIFER, K. J.; FORKMAN, G.; STICH, K. Screening for inhibitors of 2-oxoglutarate-dependent dioxygenases: Flavanone 3β-hydroxylase and flavonol synthase. *Plant Science*, Pullman, v.171, n.2, p.194-205, 2006.

RADEMACHER, W. Growth retardants: Effects on gibberellin biosynthesis and other metabolic pathways. *Annual Review of Plant Physiology and Plant Molecular Biology*, Palo Alto, v.51, n.1, p.501-531, 2000.

SHARPLES, R.O.; JOHNSON, D.S. Effects of some growth regulators on the ripening and storage quality of apples and pears. *Acta Horticulturae*, Leuven, n.179, p.721-730, 1986.

SILVEIRA, J.P.G., AMARANTE, C.V.T., STEFFENS, C.A., CORREA, T.R.C., PAES, F.N. Yield potential and fruit quality of apple trees treated with gibberellin and inhibitor of gibberellins biosynthesis. *Revista Brasileira de Fruticultura*, Jaboticabal, v.36, n.4, p.771-779, 2014.

TURNER, J.N. Practical uses of gibberellins in agriculture and horticulture. *Outlook on Agriculture*, London, v.7, n.1, p.14-20, 1972.