ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS

Volume 63 132 Number 4, 2015
http://dx.doi.org/10.11118/actaun201563041195

FOLIAR APPLICATION OF PHOSPHORUS IMPROVES APPLE FRUIT COLOR DURING RIPENING

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Abstract

ŠTAMPAR FRANCI, BIZJAK JAN, VEBERIČ ROBERT, JAKOPIČ JERNEJA. 2015. Foliar Application of Phosphorus Improves Apple Fruit Color During Ripening. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, 63(4): 1195–1200.

The red color of skin is a much desired property in apple production. For better red fruit coloration the applications of foliar fertilizers on the basis of calcium and phosphorus are used. In the present study 'Braeburn' apple trees were sprayed twice with Phostrade Ca (phosphorus), 5 and 3 weeks before harvest. In 7-days intervals fruit color, the content of flavonoids and enzyme activity involved in the synthesis of anthocyanins have been monitored. Foliar application of Phostrade Ca caused a more intense red skin color of apples and higher anthocyanin content. Their level increased during ripening, in treated apples the content of total anthocyanins was 20-fold higher at harvest while in control apples only 9-fold higher compared to the initial values. Cyanidin 3-galactoside was the most abundant anthocyanin (80 to 86% of total anthocyanins), followed by cyanidin 3-arabinoside, cyanidin 3-glucoside, cyanidin 3-xyloside and cyanidin 7-arabinoside. Prostrade Ca increased the levels of all quercetin glycosides, with the exception of quercetin 3-rhamnoside. No significant influence of Phostrade Ca on the content of hydroxycinnamic acids, dihydrochalcones, flavanols and total phenolics has been monitored. The activity of FHT and DFR increased during ripening but Phostrade Ca influenced only higher activity of DFR.

Keywords: phosphorus, anthocyanins, color development

INTRODUCTION

The red color of apple skin is derived mainly from a class of flavonoids called anthocyanins. The most abundant is cyanidin 3-galactoside (idaein), which presents more than 80% of all anthocyanins in apple skin. In minor amounts cyanidin 3-glucoside, cyanidin 3-arabinoside, cyanidin 3-xyloside, cyanidin 3-rutinoside, cyanidin 3-acetylglycoside and cyanidin 3,5-diglucoside have been detected in some cultivars (Gomez-Cordoves *et al.*, 1996; Lancaster, 1992).

In apples, anthocyanins are formed in the phenylpropanoid pathway. Phenylalanine (PAL) is the key enzyme linking primary and secondary metabolism. In the synthesis of flavonoids, chalcone synthase (CHS) and chalcone isomerase (CHI) lead to the formation of flavanones, which are then further converted by flavanone 3-hydroxylase (FHT) and dihydroflavonol 4-reductase (DFR) to leucoanthocyanidins (flavanols), which are the precursors of anthocyanins (Treutter, 2001).

The biosynthesis of anthocyanins in maturing fruit is a light-dependent process (Lancaster, 1992). Therefore, Ju *et al.* (1995) and Arakawa (1991) established that bagging increased synthesis of anthocyanins and Saure (1990) linked better light accessibility in the canopies and dwarfing rootstocks with higher anthocyanin levels. Other authors also report improved apple skin coloration in orchards, in which the floor has been covered with reflective film (Blanke, 2008; Jakopič *et al.*, 2010). Additionally, Arakawa (1991) confirmed that temperature effectively increases red color of apple. Cool temperatures achieved by micro sprinkler irrigation boost up the production of anthocyanins (Iglesias *et al.*, 2002). Genotype (Treutter, 2001), endogenous

ethylene (Whale *et al*., 2008) and abscisic acid (Kondo *et al*., 1991) are also among factors contributing to red apple skin color. Therefore, availability of carbohydrates in connection to crop load (Treutter, 2001), a regular level of nitrogen and suitable mineral nutrition (Awad and Jager, 2002; Angelis *et al*., 2011) are responsible for intense red coloration.

Researchers have reported that a superior red fruit coloration of apples could potentially be achieved with application of foliar fertilizers on the basis of calcium and phosphorus (Seniphos, Phostrade Ca, …). However, the mode of action of these products has not yet been explained. The most likely hypothesis links increased apple skin color and higher external fruit quality to changed mineral composition of apples, especially higher content of calcium ions (Larrigaudiere *et al*., 1996).

The effect is comparable to the application of Ethephon (2-chloroethylphosphonic acid) which is used for advanced ripening of many fruit species and also in experiments stimulating the synthesis of anthocyanins (Gomez-Cordoves *et al*., 1996; Larrigaudiere *et al*., 1996; Awad and Jager, 2002; Li *et al*., 2002). The advantage of Seniphos in comparison to Ethephon is that the former exhibits no negative influence on storage properties of fruits (Larrigaudiere *et al*., 1996).

Although Mata *et al*. (2006) reported that apples, which were sprayed twice with Prohexadione Ca developed a higher proportion of red color this was perhaps a result of higher lighting in the canopy because of diminished vegetative growth. However, Prohexadione Ca influences the synthesis of phenolic compounds (Halbwirth *et al*., 2006; Bizjak *et al*., 2012) as it decreases the activity of several enzymes: flavanon-3-hydroxilase (FHT), flavanol synthase (FLS) and anthocyanidin synthase (ANS). Consequently, it reduces the synthesis of anthocyanins and causes inferior red color of apple fruits. Bizjak *et al*. (2012) reported reduced red skin color of apples after spraying with Prohexadion Ca a few weeks before harvest.

In the present study the effect of phosphorus (Phostrade Ca) on apple skin color during the last few weeks prior to harvest has been investigated. To identify the mechanisms of the Phostrade Ca in apples, skin color, secondary metabolites as well as activity of selected enzymes in phenylpropanoid pathway have been analyzed.

MATERIAL AND METHODS:

Plant Materials

The experiment was carried in year 2010 out on 20 'Braeburn' apple trees (*Malus domestica* Borkh.) grafted on M9 rootstock, cultivated according to the integrated production. Phostrade Ca (23.6% P_2O_5 w/w, 4.3% CaO w/w and 3% N w/w) was applied on 10 trees in the concentration of 0.5% twice: 5 and 3 weeks before commercial harvest. Control trees were sprayed with water. For laboratorial analysis from fifteen random chosen fruits were harvested per treatment every 7 days till commercial harvest.

Fruit Color

Skin color was measured using a portable colorimeter (CR-200 Chroma;Minolta, Osaka, Japan) recording *a** color coordinates on the fruit surface (McGuire, 1992).

Analysis of Individual Phenolics

Apple skin was ground to a fine powder and 5 grams were extracted with 10 mL of methanol containing 3% (v/v) formic acid and 1% (w/v) 2,6-di-tert-butyl-4-methylphenol (BHT) in a cooled ultrasonic bath for 1 h. The samples were centrifuged, filtered and transferred to a vial for analyzes on HPLC-MS system (Thermo Scientific, San Jose, CA) and detected at 280 nm, 350 nm and 530 nm as described by Bizjak *et al*. (2013).

Analyses of Enzyme Activity

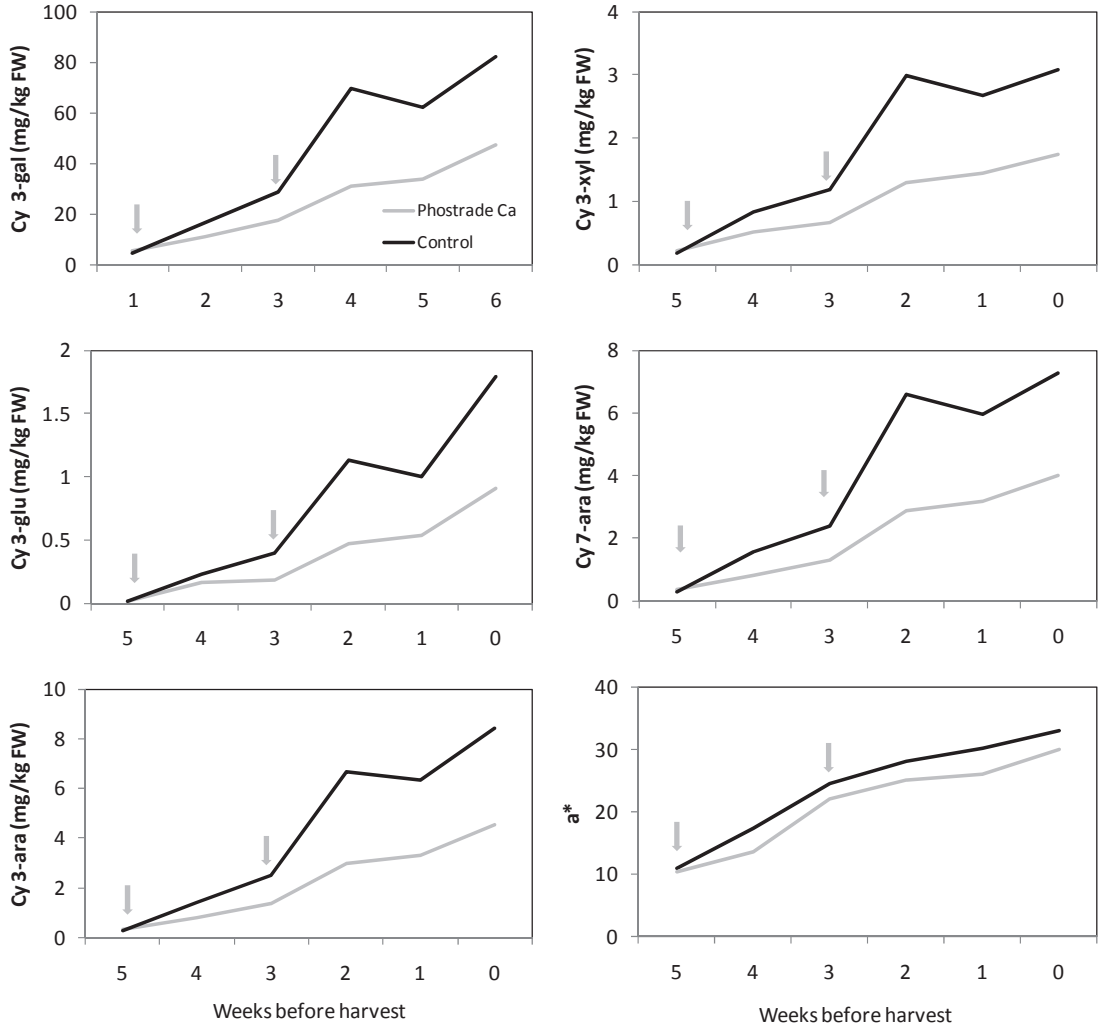
Shock-frozen apple skin was ground to powder with liquid nitrogen and 1 g was homogenized with 0.5 g quartz sand, 0.5 g Polyclar AT, and 6 mL 0.1 M Tris/HCl (containing 0.4% Na-ascorbate, pH 7.25) in a mortar. The homogenate was centrifuged and passed through a gel chromatography column (Sephadex G25 medium). Enzyme assays were performed as previously described (Slatnar *et al*., 2010). Activity of enzymes was calculated and expressed in $nkat \cdot g^{-1}$ FW.

Statistics

Statistical analysis was conducted using the Statgraphics Plus 4.0 program (Manugistics. Inc., Rockville, MD). All data were subjected to one-way analysis of variance including the treatment as factor. Significant differences among treatments were determined using the least significant difference test at the significance level 0.05.

RESULTS AND DISCUSSION

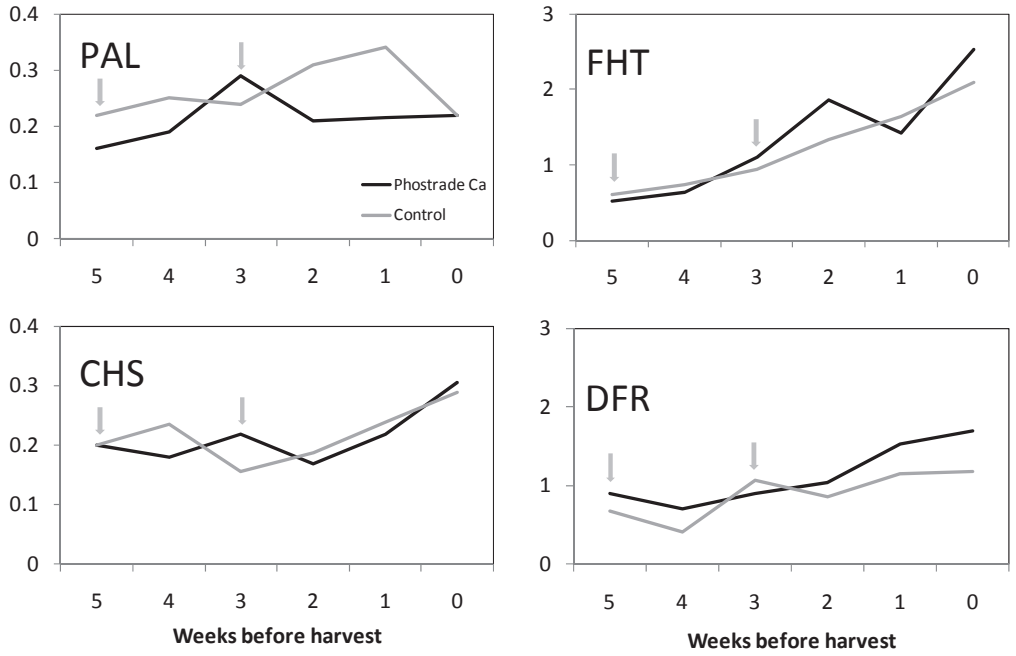
Foliar application of Phostrade Ca before ripening caused a more intense red skin color of apples confirmed by colorimetric measurements (Fig. 1). Higher values of parameter *a** in the Phostrade Ca treatment signify higher red intensity (McGuire, 1992). This is a result of increased anthocyanin synthesis in apple skin, which responded to Phostrade Ca treatment. The level of total and individual anthocyanins increased during ripening in both treatments. The comparison of anthocyanin level at harvest time and five weeks earlier revealed that the level was 20 fold higher in treated apples and only 9 fold higher in control apples (Fig. 1). Before the first application no significant differences in the total anthocyanin level between treated and untreated apples have been observed. Among anthocyanins, cyanidin 3-galactoside was



1: *The content of individual anthocyanins (Cy 3-gal = cyanidin 3-galactoside, Cy 3-xyl = cyanidin 3-xyloside, Cy 3-glu = cyanidin 3-glucoside, Cy 7-ara = cyanidin 7-arabinoside, Cy 3-ara = cyanidin 3-arabinoside) and colorimetrically determined color (a*) development during ripening in apple skin in the control and Phostrade Ca treatments. The arrows mark the time of Phostrade Ca application*

most abundant and represented from 80–86% total anthocyanin content. Other anthocyanins (cyanidin 3-arabinoside, cyanidin 3-glucoside, cyanidin 3-xyloside and cyanidin 7-arabinoside) were present in much lower amounts. Phostrade Ca induced an increase of all analyzed anthocyanins for 80–100% in comparison with the control treatment at harvest (Tab. I). A positive influence of phosphorus and calcium on apple color has been reported in different cultivars, such as 'Starking Delicious' (Gomez-Cordoves *et al*., 1996; Larrigaudiere *et al*., 1996), 'Fuji' (Li *et al*., 2002), 'Elstar' (Funke and Blanke, 2006) and 'Jonagold' (Wojcik and Wojcik, 2007). Our results of Phostrade Ca sprayings on anthocyanin synthesis are comparable to the results of Li *et al*. (2002), who reported improved red skin color and an increase in flavonoid content in apples, sprayed with Senifos (a mixture of P, K and N). Composition of Senifos is similar to Phostrade Ca. A positive influence of Senifos on apple skin color has been observed by Gomez-Cordoves *et al*. (1996) and Larrigaudiere *et al*. (1996) in 'Starking Delicious' apples. On the other hand, Awad and Jager (2002) did not confirm significant effects of Senifos on the content of anthocyanins and flavonols in 'Jonagold' apples.

In addition to anthocyanins, Phostrade Ca caused an increase of flavonols, among which quercetin 3-galactoside was the most abundant and accounted for 40% of total flavonols. Differences between treated and untreated apples were lower than in the case of anthocyanins but still significant (Tab. I). However, no significant differences of Phostrade Ca application on the content of hydroxycinnamic acids, dihydrochalcones, flavanols and total phenolics have been measured (Tab. I). Although the stimulating effect of phosphorus on color and flavonoid content in apples has been confirmed (Li *et al*., 2002; Gomes-Cordoves *et al*., 1996; Awad and Jager, 2002), the mechanism and function remain unknown. Li *et al*. (2002) reported that Seniphos increased PAL and CHI activity. A higher PAL activity



2: *Changes in enzyme activity (nkat/mg FW) of PAL, CHS, FHT and DFR in 'Braeburn' apples treated with Phostrade Ca and control during ripening. FW = fresh weight; PAL = phenylalanine ammonia lyase; CHS = chalcone synthase; FHT = flavanone 3-hydroxylase; DFR = dihydroflavonol 4-reductase*

I: *Content levels (mg/kg FW) of analyzed phenolic groups in 'Braeburn' apples treated with Phostrade Ca and the control at harvest time*

	Control	Phostrade Ca	
Anthocyanins	58.5	102.6	***
Flavonols	319.5	512.0	*
Dihydrochalcones	58.0	62.2	n.s.
Hyroxycynnamic acids	31.6	30.9	n.s.
Flavanols	266.3	297.4	n.s.

during synthesis of anthocyanins has also been reported by Larrigaudiere *et al.* (1996). However, some authors (Lister *et al.*, 1996; Saure, 1990) state that PAL is not the key enzyme in anthocyanin synthesis and CHS not a regulatory enzyme (Ju *et al.*, 1995) as the same patterns of change in activity of PAL and CHI have been observed in red and green-skin apple cultivars (Lister *et al.*, 1996). In our study, their activity did not change after Phostrade Ca application. During the ripening process, when increased synthesis of anthocyanins has been measured, the activity of FHT and DFR intensified. FHT activity increased 4-fold and DFR activity 2-fold during ripening. Furthermore, FHT and DFR activities were significantly correlated with anthocyanin accumulation ($P < 0.0001$). Also Slatnar *et al.* (2012) reported a higher FHT and DFR activity before harvest in red apple 'Florina' but not in yellow 'Golden Delicious'. However, significant influence of Phostrade Ca on higher enzyme activity has only been observed for DFR. On the basis of enzyme activity we can conclude that PAL, CHI/CHS, FHT and DFR enzymes are necessary for anthocyanin synthesis but higher activity of an individual enzyme not yet assures a higher synthesis of an individual group of phenolic compounds. A similar finding has been ascertained by Lister *et al.* (1996) who noted that PAL enzyme activity in 'Granny Smith' is quite high although this cultivar does not accumulate anthocyanins or only in very little amounts. Anthocyanin synthesis could be more dependent on availability of dihydroquercetin or leucocyanidin as precursor molecules and activity of ANS and GT, not analyzed in our study. Also Ju *et al.* (1995) reported the possibility that the transformation of dihydroquercetin to cyanidin could represent the checkpoint of their synthesis.

CONCLUSION

Foliar application of Phostrade Ca five and three weeks before technological harvest increased the level of anthocyanins and flavonols and caused intense red skin color of 'Braeburn' apples. Although PAL and CHS/CHI are involved in the synthesis of anthocyanins, their activity was not the limiting

factor for their synthesis. The color development of apple fruit is a complex process and the understanding of its steps, the role of enzymes, primary metabolites and individual nutrients would enable a progress of technical measures by which fruit coloration could be increased in problematic cultivars and in the conditions which are not optimal for red color development.

Acknowledgement

This research is part of the program Horticulture No. P4-0013-0481 funded by the Slovenian Research Agency.

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