Impacts of Pre- and Postbloom Sprays of Tryptophan on Calcium Distribution within ‘Red Jonaprince’ Apple Trees and on Fruit Quality

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Abstract. The aim of this study was to examine the effects of tryptophan (L-TRP) sprays on calcium (Ca) distribution within mature ‘Red Jonaprince’ apple (*Malus domestica* Borkh.) trees and on fruit quality. Trees were sprayed with L-TRP before flowering (at the green and pink bud stages and when 5% to 10% of flowers were open), after flowering (at petal fall and 14 and 28 days later), and both before and after flowering. In each spray treatment, 50 g of L-TRP per ha was applied. Other trees were sprayed with calcium chloride (CaCl₂) six times during the growing season at rates ranging from 6 to 9 kg·ha⁻¹. Plants that were not sprayed with L-TRP or CaCl₂ served as controls. The results found that the studied spray treatments did not affect yield, mean fruit weight, apple skin russeting and blush, seed set, mean seed weight, or acidity of fruit at harvest. Prebloom L-TRP sprays enhanced the concentrations of both free indole-3-acetic acid (IAA) in fruitlets (by ≈230% compared with those of the control plants) and Ca in fruitlets and fruit (on average by 18% compared with the control combination) but did not affect the leaf Ca concentrations. Apples from trees sprayed with L-TRP before flowering had lower starch index (SI) values at harvest than those of the control plants. Postbloom L-TRP sprays increased leaf Ca concentration, but had no effect on apple Ca concentration or fruit quality at harvest. Combined pre- and postbloom L-TRP sprays did not improve the effectiveness of this amino acid. Overall, preharvest CaCl₂ sprays increased leaf and fruit Ca concentrations and decreased fruit SI. Apples sprayed with CaCl₂ had lower soluble solids concentrations (SSCs) and were firmer than fruit from control trees. Prebloom L-TRP sprays are effective in improving apple Ca concentration, at least for triploid varieties that have small seed numbers.

Calcium deficiency in apple (*M. domestica* Borkh.) flesh is a serious problem for many varieties (Wójcik, 2004). Apples with a low Ca status are sensitive to cracking, sunburn, and some physiological disorders (bitter pit, cork spot, superficial scald, water core, senescent and internal breakdown, and Jonathan spot). The storability of such fruits is lowered (Faust and Shear, 1972; Raese,

1996; Shear, 1975). Moreover, Ca-deficient apples are also susceptible to bitter rot, and gray and blue mould caused by *Gleosporium* spp., *Botrytis cinerea* Pers., and *Penicillium expansum* Link. pathogens, respectively (Conway et al., 2002; Fallahi et al., 1997; Sams and Conway, 1987).

It is estimated that only 5% to 10% of Ca absorbed by the roots in a given year is sequestered into apples (Wójcik, 2004). Calcium deficiency in apple flesh is related to limited transport rate of this nutrient into the fast-growing fruit when their surface to volume ratio decreases (Shear and Faust, 1970). In most apple-growing regions, preharvest Ca sprays are recommended to produce Ca-sufficient fruit (Wójcik, 2009).

The efficiency of exogenous Ca absorption by apples is dependent on many factors

such as the variety (Wójcik et al., 1998), the developmental stage of the fruit (Harker and Ferguson, 1988; Raese and Drake, 2000), the number of sprays applied in a growing season (Le Grange et al., 1998; Wójcik, 2001a), the Ca salt used (Raese and Drake, 2002), the presence of the surfactants in the fertilizer (Harker and Ferguson, 1991), the spraying technique (Wójcik, 2001b), and the air temperature and humidity during and immediately after spraying (Michalczuk and Kubik, 1984). Therefore, it is not surprising that in many studies, preharvest Ca sprays have not increased apple Ca concentration, and/or reduced Ca-related disorders (Askew et al., 1960; Baxter, 1960; Jackson, 1962; Martin et al., 1960, 1965; Sadowski et al., 1965; Smock et al., 1962; Stevenson, 1962; Stiles, 1964; Wójcik, 1999). Thus, given the unreliable impact of preharvest Ca sprays on apple Ca concentration and the considerable costs associated with spraying in a season (particularly with the use of CaCl₂ fertilizers, which cannot be mixed with fungicides), it is necessary to find treatment procedures that would be both effective and inexpensive to guarantee the production of Ca-sufficient apples.

As an alternative to preharvest Ca sprays in apple orchards, applications of auxins have been studied by Bangerth (1976), Martin et al. (1976), and Looney (1977). In those studies, it was assumed that the application of exogenous auxins (IAA; 1-naphthaleneacetic acid; 1-naphthylacetamid; or 2,4,5-trichlorophenoxyacetic) can stimulate Ca movement into the fruit. This assumption appears to be justified because it has been demonstrated that Ca uptake into fruit is affected by auxins produced by the developing seeds and/or by basipetal auxin transport from the fruit (Benson and Stahly, 1972; Stahly and Benson, 1970). Unfortunately, application of auxins did not always lead to an increase in apple Ca levels. According to Basak (1999), the primary reasons for the lack of a positive impact of auxin sprays on apple Ca levels include inappropriate term of the treatment, increased fruit size, causing dilution of Ca concentration in the flesh, and rapid auxin degradation and conjugation of this hormone in plant tissues. High costs and a long legislative process for auxin-containing preparations (classified by the European Union as growth regulators) are also critical factors limiting their use in plant production.

To overcome the problems related to use of auxins, application of the amino acid L-TRP, a precursor of IAA (Pattison et al., 2014; Zhao, 2011), appears to be justified. To date, the effects of foliar sprays of TRP have been examined for some fruit crops such as navel oranges (*Citrus sinensis*), Clementine mandarins (*Citrus reticulata*), Valencia oranges, and pomegranates (*Punica granatum* L.) (El-Sayed et al., 2014; Hanafy et al., 2012; Khuong et al., 2010; Pillitteri et al., 2010). However, in none of those studies, the relation between L-TRP sprays and the Ca status of plants was examined. Therefore, the aim of our experiment was to examine the effects of L-TRP sprays on increasing Ca

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partitioning to the fruit and leaves. Additional purpose was to assess fruit quality as a result of L-TRP treatments.

Materials and Methods

The study was conducted in 2014–15 in a commercial apple orchard in Central Poland (lat. 50°2'N and long. 21°51'E). The mean annual temperature and the total precipitation in this region are 8.3 °C and 490 mm, respectively. The average temperature and rainfall during the vegetative period (May–October) are 14.9 °C and 302 mm, respectively.

The experimental apple field was planted in Spring 2008 on sandy loam soil (Albic Luvisol). Before the beginning of the study (Fall 2013), soil samples were taken for analysis from the surface layer (0–30 cm) of herbicide strips along the tree rows, at a distance of 25–30 cm from an emitter (the edge of the wetting zone), delivering 2.7 dm³ of water/h. The composite soil sample consisted of 10 subsamples. Sample was dried at room temperature, thoroughly mixed by a mechanical mixer until homogeneous, and sieved through a 1-mm plastic mesh screen. Chemical and physical properties of the soil were as follows—pH: 6.5; the bulk density: 1.5 g cm⁻³ (mean from the depth of 0–15 cm and 16–30 cm); the particle contributions of sand (1–0.05 mm): 63%, silt (0.05–0.002 mm): 29%, and clay (<0.002 mm): 8%; surface area: 89 m²·g⁻¹. The content of organic matter was 16 C kg⁻¹, total nitrogen (N): 761 mg·kg⁻¹, exchangeable Ca: 26 cmol·kg⁻¹, and available phosphorus (P): 54 mg·kg⁻¹, potassium (K): 187 mg·kg⁻¹, magnesium (Mg): 40 mg·kg⁻¹, boron (B): 3.7 mg·kg⁻¹, iron (Fe): 1654 mg·kg⁻¹, manganese (Mn): 89 mg·kg⁻¹, zinc (Zn): 11.1 mg·kg⁻¹, and copper (Cu): 5.1 mg·kg⁻¹. pH was determined potentiometrically at a ratio of one part soil to 2.5 parts 1 M KCl after shaking for 24 h (Mercik, 2004), the bulk density of undisturbed soil using the core method (Tisdall, 1951), the particles of sand, silt, and clay by the aerometric method of Casagrande and Prószyński as described by Ostrowska et al. (1991), the surface area by the ethylene glycol monoethyl ether method (Carter et al., 1965), total C and N by the dry combustion method at a temperature of 950 °C in the presence of pure oxygen (Allison, 1965) and by the Dumas combustion method (Buckee, 1994), respectively, P and K using a solution of Ca lactate (at a pH of 3.6), Mg using a solution of 0.0125 M Ca chloride (Ostrowska et al., 1991), Ca using a 1 M NH₄ acetate solution (Ostrowska et al., 1991), and Fe, Mn, Zn, Cu, and B by means of 1 M HCl extraction reagent (Mercik, 2004). Nitrogen and C were determined using a Leco TruSpec CNS analyser (Leco Corp., St. Joseph, MI) equipped with a thermal conductivity detector. Extractable K, Mg, Ca, Fe, Mn, Cu, Zn, and B were determined by inductively-coupled plasma spectroscopy (Thermo Jarrell Ash, Franklin, MA), and extractable P by a spectrophotometer (Cintra 916; GBC, Dandenong, Australia), using the

molybdenum blue reaction (Ostrowska et al., 1991).

On the basis of the above soil data, we can claim that before the start of the experiment pH was within the optimal range of 6.0 to 6.8 proposed by Wójcik (2009) for apple trees. Soil concentrations of available/exchangeable P, Mg, Ca, Fe, Mn, Fe, B, Zn, and Cu were also within the optimal ranges recommended by Sadowski et al. (1990) for macronutrients and by Mercik (2004) for micronutrients. Only soil K availability was high.

'Red Jonaprince' apple trees grafted on M.9 rootstock were selected for this study because the fruits of this variety are frequently Ca deficient (Wójcik, 2009). Trees were planted at a spacing of 3.5 × 2 m (1428 plants/ha) and trained as a spindle to a height of 2.5 m.

To ensure sufficient pollination of the experimental trees, 'Golden Hornet' (*Malus × zumi*) pollinizers were planted both at the beginning and end of each row, consisting of 80 trees. Two to three days before the onset of 'Red Jonaprince' tree flowering, two beehives were introduced into the field (0.3 ha) to optimize fruit set. Flowers/fruitlets of the experimental trees were not thinned chemically. Only deformed, damaged, and/or diseased fruitlets were removed by hand. This treatment was performed immediately after "June drop" and again 1 month later. In both years, the number of hand-thinned fruitlets was insignificant compared with harvested fruit (data not provided).

Soil moisture was maintained near field capacity from May to September by drip irrigation with emitters placed every 60 cm. Soil moisture was monitored by a tensiometer placed in the soil at a depth of 30 cm, at a distance of 20 cm from an emitter. The trees grew in 1.5 m-wide herbicide strips maintained by applications of Roundup 360 SL (glyphosate; Monsanto Europe, Antwerp, Belgium) and Basta 150 SL (ammonium glufosinate; Bayer CropScience, Monheim, Germany). Sod in the interrows was mowed six times in 2014 and four times in 2015.

During the experimental period, only N was applied annually. Ammonium nitrate (34N–0P–0K) was broadcast over the surface of the herbicide strips at a rate of 80 kg·ha⁻¹ N divided into two equal parts: half of the annual rate was applied at the swollen bud stage and the remaining amount was applied immediately after flowering. The above N rate and timing and mode of application were in accordance with the recommendations for apple trees grown on soil with low organic matter status (Wójcik, 2009).

Before initiation of the study and during the experimental period, trees were not treated with plant growth regulators. Control of pathogens and pests was performed according to recommendations for integrated production of apples (Sobczewski et al., 2013).

The apple trees were sprayed with L-TRP (pure grade; Sigma-Aldrich, Poznań, Poland) in the following variants: 1) three prebloom sprays, at the stages of green and pink buds

and when 5% to 10% flowers were open, 2) three postbloom sprays, at the stage of petal fall and again 14 and 28 d later, and 3) six pre- and postbloom sprays at the same plant development stages as in 1 and 2. In each spray treatment, L-TRP was applied at a rate of 50 g·ha⁻¹. To each TRP-containing spray solution, the nonionic surfactant Tween® 20 (polyoxyethylene sorbitan monolaurate; Sigma-Aldrich, St. Louis, MO) was added at a rate of 0.5 dm³·ha⁻¹.

Other apple trees were sprayed with CaCl₂ (commercial flake, 78% CaCl₂) six times in a growing season. The first spray treatment was made at 42 d after petal fall and the subsequent treatments were made at 14 d intervals. In both years, the last CaCl₂ spray was performed at 12 d before commercial harvest. The rates of CaCl₂ in the sprays were 6 kg·ha⁻¹ in the first three treatments, 7 kg·ha⁻¹ in the fourth spray, 8 kg·ha⁻¹ in the fifth spray, and 9 kg·ha⁻¹ in the final sprays. CaCl₂ rates and the timing of spraying were in accordance with the recommendations given by Wójcik (2009) for varieties sensitive to Ca-related disorders.

All sprays of L-TRP and CaCl₂ were performed in the morning when the surfaces of the aboveground parts of the plants were dry. Spray treatments were made using a motorized backpack sprayer with ≈500 dm³ of water per ha. Over the entire period of the experiment, the same trees were used for the treatments under evaluation. Trees not sprayed with L-TRP or CaCl₂ served as the control.

Over the 2-year duration of the experiment, the same trees were treated. The study was conducted using a complete randomized block design with four replications for each treatment (including the control). Each experimental plot consisted of 10 trees. Each plot within a row was separated by two trees and there was buffer row between experimental rows.

Leaf Ca concentrations were determined at 42 and 70 d after petal fall (but before the first and third spray of CaCl₂, respectively) and immediately after harvest (at 123 and 119 d after flowering in 2014 and 2015, respectively). The uppermost, fully expanded leaf (with stem) was taken from the current season's shoots located at the periphery of the crown. From each plot for all treatments, 200 leaves were collected. To remove any external, bound Ca with the cuticle structure, the leaves were gently rinsed with Alconox® detergent (Sigma-Aldrich), 0.01 M HCl, and double-deionized water. Samples were dried at 60 °C in a forced-draft oven and ground in a Wiley stainless steel mill. Leaf samples were then microwave digested in closed vessels in nitric acid (model MLS 1200; Milistone, Inc., Monroe, CT). The above methods for removal of Ca residues from leaf surface and preparation of leaf samples for analysis were in accordance with the procedures of the Agrochemical Laboratory of the Research Institute of Horticulture, Skierniewice, Poland (Kingston and Haswell, 1997). Calcium was determined by means of an inductively coupled plasma spectrometer and expressed on a dry weight (DW) basis.

Free IAA concentration in fruitlets was determined for 30 “King” fruitlets (without pedicels) per plot taken at 42 d after petal fall from the peripheral zone of the canopy at a height of 1.5–2.0 m above the soil surface. Immediately after harvest, fruitlets were frozen on dry ice, freeze-dried, and ground to a fine powder. Further sample preparation (extraction, purification, and derivatization) and determination of free IAA were performed according to the procedures described by Nakurte et al. (2012). Briefly, samples were supplemented with indole-3-propionic acid (IPA) as an internal standard (10 nmol·g⁻¹ fresh weight) and extracted with 100% methanol (5 mL·g⁻¹ fresh weight) for 24 h at 4 °C in the dark. Crude extracts were cleared by filtrating through 8-µm filters (Whatman no. 2 filter paper; Whatman International Ltd., Maidstone, Kent, England) at room temperature. The resulting mixtures were pooled and evaporated to the aqueous phase, which was subsequently adjusted to pH 10 with 1 M KOH and partitioned against 100% ethyl acetate. After centrifuging the mixture (4000 g_n × 10 min), the aqueous phase was separated and adjusted to pH 2.8 with 1 M acetic acid and partitioned against 100% ethyl acetate. After being spun down at 16,000 g_n for 2 min, the upper organic phase was recovered and completely dried and then dissolved in 300 µL of 100% methanol.

Detection and quantification of free auxins were performed with a high-performance liquid chromatography (HPLC) system equipped with ultraviolet and fluorescence detectors (Agilent 1200 series HPLC; Agilent Technologies, Santa Clara, CA). The optimum separation of auxins was achieved under reversed phase conditions using a Waters Nova-Pak C18 column (3.9 × 150 mm, 5-µm particles; Waters Co. Ltd., Milford, MA) at a flow rate of 1 mL·min⁻¹. The mobile phase was a linear gradient from 10% (v/v) methanol/acetic acid to 90% (v/v) methanol in 30 min. IAA and IPA were monitored at a wavelength of 273 nm and at 282 nm (Ex) and 360 nm (Em). Free IAA concentrations in fruitlets were expressed on a fresh weight basis.

The number of well-formed seeds in an apple was evaluated on the samples used for the determination of Ca at harvest. Each fruit was cut equatorially and the number of seeds that appeared to be viable was counted. After removing seeds from the fruit, they were weighed.

Fruit Ca concentration was determined in the same manner as leaf samples, using a 30-fruit sample per plot. “King” fruitlets/fruit were collected from the peripheral zone of the canopy at a height of 1.5–2.0 m above the soil surface. Fruit were rinsed with detergent, HCl, and deionized water. Seeds and the pedicel were removed. From each fruitlet/fruit, two radial slices were cut from opposite sides. Fruit samples were dried in a forced-draft oven at 75 °C for 72 h and ground to pass through a 40-mesh screen. Further preparation of the samples and Ca determination procedures were the same as for leaf samples. Fruit Ca concentrations were expressed on a DW basis.

Total fruit yield was measured separately for each plot. Commercial harvest was determined based on the SI and flesh firmness (FF) of apples from the control trees. Optimal values of the SI and FF for ‘Red Jonaprince’ apples are 5–7 and 75–80 N, respectively (recommendations of the Department of Storage and Processing of Fruits and Vegetables at the Research Institute of Horticulture, Skierniewice, Poland).

Quality features of fruit included the single fruit weight, russetting, blushing, FF, SSC, SI, and titratable acidity (TA). Mean fruit weight and fruit skin russetting and blushing were calculated/determined on an ≈20 kg bulk fruit sample per plot. Russetting and skin blushing were rated according to a 5-degree scale where 1 = the lack of russetting/blush, 2 = russetting/blush on <25% of surface, 3 = on 25% to 50% of surface, 4 = on 51% to 75% of surface, and 5 = >75% of surface. FF, SSC, SI, and TA of apples were measured/evaluated on 30 fruits of similar size taken randomly from each of the 20 kg fruit samples per plot. Firmness was measured on two opposite peeled sides of each fruit using a penetrometer EPT-1R (Lake City Technical Products, Kelowna, British Columbia, Canada) with an 11-mm diameter tip. SSC of the juice from each fruit was measured with Atago PR-101 (Atago Co., Ltd, Tokyo, Japan) electronic refractometer at 20 °C. SI was estimated on a 3-mm equatorial slice by an iodine test using a scale from 1 (100% hue of the cross-sectional area of the fruit) to 10 (0% hue). TA was determined by titrating the fruit homogenate with 0.1 N NaOH to pH 8.1 using an automatic titrator Mettler Toledo DL 50 Graphix (Mettler-Toledo AG., Schwerzenbach, Switzerland). TA was expressed as malic acid content.

All data were subjected to a one-way analysis of variance. Differences among means of five treatments were evaluated separately for each growing season using Duncan’s multiple range test at $P \leq 0.05$. The data on the number of seeds per fruit were square root-transformed as outlined by Szczepański and Rejman (1987). The analyses were performed using the software Statistica 10 (StatSoft Polska, Krakow, Poland).

Results and Discussion

The pattern of leaf Ca concentration was similar in both years (Table 1). At 42 d after

petal fall, only postbloom L-TRP sprays or pre- and postbloom L-TRP sprays were able to increase leaf Ca concentrations. At 70 d after petal fall, leaves of trees treated with CaCl₂, postbloom sprayed with L-TRP or pre- and postbloom sprayed with L-TRP contained more Ca than leaves sprayed with L-TRP only before flowering; the efficiencies of those spray treatments were comparable. At harvest, the highest leaf Ca concentrations were recorded on CaCl₂-sprayed trees. At harvest, postbloom sprays of L-TRP and pre- and postbloom sprays of L-TRP resulted in increase of leaf Ca status (on average for both combinations for 2 years by 14% compared with the control) but not as much as CaCl₂ sprays (on average for 2 years by 41% compared with the control). Given that increases in leaf Ca concentrations from L-TRP-sprayed plants after petal fall and combined before and after flowering were comparable at all stages, we can state that the above effect resulted only from the postbloom application of L-TRP. Enhanced Ca concentrations in the uppermost leaves might be caused by increased production of auxins in those tissues. This appears likely because it has been demonstrated that the synthesis pathway of IAA (the most physiological active auxin) is dependent on the quantity of free L-TRP in developing leaves (Normanly, 1987; Zhao, 2011).

Free IAA concentration in fruitlets differed among the different treatments (Table 2). Fruit from trees prebloom sprayed with L-TRP or pre- and postbloom sprayed with L-TRP contained significantly more free IAA than fruit from the control plots; concentrations of free IAA in fruitlets were increased by 254% and 213% on trees prebloom L-TRP sprayed and 209% and 173% on plants pre- and postbloom L-TRP sprayed in 2014 and 2015, respectively. As the effects of the above L-TRP sprays were comparable, it can be concluded that the increase in free IAA in the fruitlets was only the result of prebloom L-TRP sprays. Thus, postbloom L-TRP sprays had no effect on the concentration of free IAA in fruitlets. This indicates that L-TRP deposited on surface of the aerial plant parts after flowering was not involved in the synthesis of IAA in the seeds, which were the source of auxins in the fruit (Pattison et al., 2014).

The number of seeds in an apple ranged from 3.7 to 4.2 and was not influenced by the

Table 1. Changes in leaf calcium concentrations of ‘Red Jonaprince’ apple trees as a result of sprays of tryptophan (L-TRP) and calcium chloride.

Treatments	Leaf Ca concn (%)					
	2014			2015		
	Days after flowering					
	42	70	123	42	70	119
Prebloom L-TRP sprays	0.43 a ^z	0.56 a	1.24 a	0.45 a	0.58 a	1.21 a
Postbloom L-TRP sprays	0.52 b	0.67 b	1.43 b	0.58 b	0.68 b	1.32 b
Pre- and postbloom L-TRP sprays	0.53 b	0.69 b	1.42 b	0.57 b	0.69 b	1.33 b
Ca-chloride sprays	0.42 a	0.66 b	1.84 c	0.41 a	0.70 b	1.56 c
Control: no L-TRP and Ca-chloride	0.41 a	0.54 a	1.22 a	0.43 a	0.59 a	1.18 a

²Means within column with the same letter are not significantly different by Duncan’s multiple range test at $P \leq 0.05$.

spray treatments (Table 2). A weak set of seeds in 'Red Jonaprince' apples is not surprising because it is a triploid variety that produces both seeded and seedless fruit (Jackson, 2003). In our study, the seed number in apples seldom exceeded seven (data not shown). Also, mean seed weight did not differ among the treatments (Table 2). Thus, the increased concentrations of free IAA in fruitlets on trees prebloom sprayed with L-TRP did not result from improved seed set or seed weight. This indicates either enhanced IAA synthesis by individual seeds or changes in the transformation between free and conjugated IAA in fruit tissues.

Fruit Ca concentration was affected by the tested spray treatments (Table 3). At 42 d after petal fall, fruitlets from trees prebloom sprayed with L-TRP or pre- and postbloom sprayed with L-TRP contained more Ca than fruitlets of the control plants. At 70 d after petal fall, CaCl₂ sprays, prebloom L-TRP sprays or pre- and postbloom L-TRP sprays increased fruit Ca concentrations; the effects of those spray treatments were comparable. At harvest, CaCl₂-treated fruit contained the most Ca; compared with the control fruits, fruit Ca concentration was increased by 42% in 2014 and 48% in 2015. Apples from trees prebloom sprayed with L-TRP or pre- and postbloom sprayed with L-TRP also contained more Ca than the control plots. Fruit Ca concentrations were increased by 21% and 28% for prebloom L-TRP sprays and by 20% and 25% for pre- and postbloom L-TRP sprays in 2014 and 2015, respectively. Given that prebloom sprays and pre- and postbloom sprays of L-TRP had similar impacts on fruit Ca levels, it appears that only prebloom L-TRP sprays were able to enhance Ca status. We suggest that enhanced fruit Ca concentrations caused by prebloom L-TRP sprays were related to the increase of free IAA in their tissues. This hypothesis appears to be justified because Bangerth (1976) has demonstrated that Ca accumulation in apple fruit is regulated primarily by polar basipetal IAA transport from fruit. A similar relation between fruit auxin levels and Ca transported into fruit tissues has been reported by Cutting and Bower (1989) for avocado (*Persea americana* Mill.), by Banuelos et al. (1987) for tomato (*Lycopersicon esculentum* L. Mill.), and by Source et al. (2011) for kiwifruit (*Actinidia deliciosa*).

Fruit yields of 'Red Jonaprince' apple trees were not influenced by the studied treatments, averaging 35.5 and 36.3 tons per ha in 2014 in 2015, respectively (data not shown). Taking into account yields attained in intensive apple orchards in Poland, obtained yields in our study were moderate.

The spraying of CaCl₂/L-TRP did not affect mean fruit weight (Table 4). This suggests that increased concentrations of free IAA in fruitlets on the trees prebloom sprayed with L-TRP did not influence fruit size, although it is known that auxins participate in cell division and elongation.

Apple skin coloring and russeting were unaffected by spray treatments, averaging 4.5

Table 2. Effects of sprays of tryptophan (L-TRP) and calcium chloride on concentration of free IAA in 'Red Jonaprince' apple fruitlets at 42 d after petal fall and seed set.

Treatments	IAA concn (ng·g ⁻¹ fresh wt)		No. of seeds per fruit		Mean seed wt (mg)	
	2014	2015	2014	2015	2014	2015
Prebloom L-TRP sprays	3.9 b ²	4.7 b	3.7 a	3.9 a	48 a	51 a
Postbloom L-TRP sprays	1.3 a	1.9 a	4.1 a	4.1 a	49 a	50 a
Pre- and postbloom L-TRP sprays	3.4 b	4.1 b	3.8 a	3.9 a	50 a	48 a
Ca-chloride sprays	1.5 a	1.8 a	4.0 a	4.2 a	48 a	50 a
Control: no L-TRP and Ca-chloride	1.1 a	1.5 a	4.1 a	4.0 a	49 a	49 a

²Means within column with the same letter are not significantly different by Duncan's multiple range test at $P \leq 0.05$.

Table 3. Effect of sprays of tryptophan (L-TRP) and calcium chloride on 'Red Jonaprince' apple calcium concentration.

Treatments	Fruit Ca concn (mg·kg ⁻¹ dry wt)					
	42 d after petal fall		70 d after petal fall		119 (123) d after petal fall ²	
	2014	2015	2014	2015	2014	2015
Prebloom L-TRP sprays	1,654 b ³	1,703 b	1,152 b	1,341 b	245 b	301 b
Postbloom L-TRP sprays	1,487 a	1,487 a	958 a	1,087 a	189 a	256 a
Pre- and postbloom L-TRP sprays	1,692 b	1,691 b	1,123 b	1,382 b	231 b	311 b
Ca-chloride sprays	1,520 ab	1,490 a	1,198 b	1,352 b	285 c	352 c
Control: no L-TRP and Ca-chloride	1,466 a	1,510 a	980 a	1,121 a	192 a	248 a

²Fruit were harvested at 119 and 123 d after petal fall in 2014 and 2015, respectively.

³Means within column with the same letter are not significantly different by Duncan's multiple range test at $P \leq 0.05$.

Table 4. Effect of sprays of tryptophan (L-TRP) and calcium chloride on 'Red Jonaprince' apple quality at harvest.

Treatments	Mean fruit wt (g)		Flesh firmness (N)		Starch index (1–10)		Solids soluble concn (%)	
	2014	2015	2014	2015	2014	2015	2014	2015
Prebloom L-TRP sprays	190.1 a ²	192.7 a	83 a	81 a	4.8 b ³	4.5 b	14.4 b	13.9 b
Postbloom L-TRP sprays	188.2 a	190.5 a	81 a	83 a	6.5 c	6.4 c	14.1 b	14.1 b
Pre- and postbloom L-TRP sprays	185.8 a	189.2 a	81 a	80 a	4.7 b	4.8 b	14.3 b	13.8 b
Ca-chloride sprays	188.7 a	194.9 a	92 b	91 b	3.2 a	3.9 a	13.4 a	13.0 a
Control: no L-TRP and Ca-chloride	186.0 a	191.5 a	80 a	80 a	6.2 c	6.6 c	14.5 b	14.2 b

²Means within column with the same letter are not significantly different by Duncan's multiple range test at $P \leq 0.05$.

³The higher value, the less starch.

and 1.2 in 2014 and 4.3 and 1.3 in 2015, respectively (data not shown).

Only CaCl₂-sprayed apples were significantly firmer than apples of the control trees (Table 4). Improved firmness of CaCl₂-treated fruit can be attributed to increased Ca concentrations in the tissues. In many studies, a positive relationship has been reported between apple firmness at harvest and the Ca status in apple flesh (Ferguson, 1984; Poovaiah et al., 1988; Stow, 1993; Wójcik, 2004). According to Conway et al. (2002), the impact of Ca on apple firmness is related to the maintenance of cell wall integrity via the binding of carboxyl groups within the polygalacturonate polymers. It should be noted that despite increased Ca concentration in apples from trees prebloom sprayed with L-TRP, firmness was comparable to those of the control plants.

The SI values of fruit differed among the treatments (Table 4). The lowest SI values were recorded in the CaCl₂-sprayed apples.

Fruit from trees prebloom sprayed with L-TRP or pre- and postbloom sprayed with L-TRP had also lower SI values than fruit of the control plants. However, the efficiencies of the above spray treatments were similar, indicating that only prebloom L-TRP sprays changed the starch content in apple flesh. Given that apples from trees sprayed with CaCl₂ or prebloom sprayed with L-TRP had lower SI values, we suggest that the ripening process of those fruits was retarded. The retardation of the apple ripening process via CaCl₂ sprays or prebloom L-TRP sprays can be attributed to the increased Ca concentrations in their tissues. Negative correlations between apple Ca status and ripening rate have been reported in the studies of Faust and Shear (1972) and Marcelle (1990).

Only CaCl₂ sprays changed the SSC in fruit (Table 4). Apples treated with CaCl₂ had lower SSC than the control plants.

Fruit acidity was unaffected by the treatments, averaging 0.687% and 0.671%

in 2014 and 2015, respectively (data not shown).

Conclusions

The results of this study showed that prebloom sprays of L-TRP at a rate of 50 g·ha⁻¹ at the green and pink bud stages and at the onset of flowering enhanced 'Red Jonaprince' apple Ca concentrations at harvest despite the absence of an effect on leaf Ca concentrations. Increased fruit Ca status as a result of prebloom L-TRP sprays corresponded to enhanced concentrations of free IAA in fruitlets, suggesting the regulation of Ca transport into apples by auxins. Moreover, prebloom TRP sprays also retarded fruit ripening. However, the effects of prebloom TRP sprays on fruit Ca concentrations and the retardation of ripening were less pronounced than with the six sprays with CaCl₂ applied after 42 d after petal fall. We conclude that prebloom TRP sprays in apple orchards are effective in improving fruit Ca status, at least for triploid varieties. This treatment should be applied as a supplement to preharvest Ca sprays. However, a factor limiting application of the prebloom sprays of TRP is high price of this amino acid (≈150 USD spray/ha). Thus, it is necessary to refine and optimize the number of prebloom sprays and/or rates of L-TRP.

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