

The Impact of Foliar Application of Calcium Fertilizers on the Quality of Highbush Blueberry Fruits Belonging to the 'Duke' Cultivar

Ireneusz OCHMIAN

West Pomeranian University of Technology in Szczecin, Laboratory of Orchardring at the Department of Horticulture, Słowackiego 17, 71-434 Szczecin, Poland; ochir@o2.pl; ireneusz.ochmian@zut.edu.pl

Abstract

The studies were conducted in the period of 2010-2011, in the Laboratory of Orchardring at the West Pomeranian University of Technology in Szczecin, and related to the bushes of the highbush blueberry of the Duke cultivar. The authors examined the impact of foliar calcium fertilizers (Calcinit, Calcium chloride, Fruton Calcium Calcium, Folanx Ca29 Lebosol Calcium Forte Calcium Forte) on the fruit size and firmness, their chemical composition, content of macronutrients in the foliage and fruits, and their colour. It was found that the applied fertilizers have varied impact on the examined attributes. The bushes sprayed with the Lebosol Calcium Forte and Calcinit preparations had big fruits with a high content of K and Mg, and dark foliage. The application of the Lebosol Calcium Forte fertilizer increased the content of polyphenols and vitamin C in the fruits. The smallest amounts of such compounds were determined in the fruits sprayed with the Calcinit fertilizer. The highest firmness and resistance to mechanical damage were achieved in the case of the fruits collected from the bushes sprayed with the foliar fertilizers containing calcium chloride, such as Fruton Calcium and Folanx Ca29. It was determined that the application of the calcium preparations resulted in the increase of the calcium content in the fruits and foliage. The highest amounts of this element were determined in the case of the plants sprayed with the Folanx Ca29 and Lebosol Calcium Forte preparations.

Keywords: calcium, chemical composition, color, firmness, *Vaccinium corymbosum*

Introduction

The cultivars of the highbush blueberry derive from the *Vaccinium* sp. plants, which grows in the wild in soils that are poor in nutrients. In effect, the fertilizer requirements of the blueberry, as compared to other fruit plants, are relatively low (Gough, 1994; Pliszka, 2002; Smolarz, 2003). However, high yielding, intensive irrigation and use of mulches make it necessary to complement and maintain the constant levels of macro- and micronutrients in plants and soil profiles (Pormale *et al.*, 2009). One of the aims of the producer is to preserve the high post-harvest quality of fruit. An important indicator of fruit freshness and attractiveness is their firmness, especially after the storage period. The mechanical strength of the fruit peel and pulp depends, to a large extent, on the condition of their cell membranes, and in particular, on the chemical composition of pectins connecting plant cells to form tissues (Starck, 2007). The major role in this process is carried out by calcium, which, by forming a part of phospholipids, contributes to the stabilisation of cell membranes, thus affecting their permeability (Szweykowska, 2004; White and Broadley, 2003). The deficiency or transport disorder of calcium results in a number of physiological diseases, thus decreasing, among others, the storage and commercial value of the fruit (Shear, 1975). An increasing importance is placed on the role of calcium as a secondary infor-

mation transmitter. In combination with calmodulin, this element participates in several basic metabolic processes (Snedden and Fromm, 2001; Zhang and Lu, 2003).

Plant roots absorb calcium from the soil solution in the form of Ca^{2+} ions (Clarkson, 1993; White, 2001); however, the high content of humus leads to the limitation on the quantity of free calcium ions, while organic acids form chelate complexes with Ca^{2+} ions (Starck, 2007). A low pH of the substrate affects the high concentrations of Al^{3+} , Fe^{2+} , and Mn^{2+} ions, inhibiting the up taking of calcium ions (Clarkson and Sanderson, 1971; Haynes, 1986). An alternative solution is, therefore, to supply calcium fertilizers by spraying the plants. The calcium supply to the foliage does not influence the concentration of this element in the fruits (Saure, 2005). For this reason, the best results are achieved when the solution of calcium preparation is applied directly to the fruit surface. The best assimilation of calcium is achieved by young fruit (Schlegel and Schönherr, 2002), since the permeability of cuticle is then the highest (Petit-Jimenez *et al.*, 2009), and the properly functioning stomatal apparatus provides an easy passage for Ca^{2+} .

The study describes the foliar application of calcium fertilizers with varied chemical composition to the bushes and the quality of highbush blueberry fruits, their size, colour, firmness and content of minerals and organic components.

Material and methods

The studies were carried out in the years 2010-2011 in the Laboratory of Orchardring at the Department of Horticulture West Pomeranian University of Technology in Szczecin, on a production farm. 'Duke' blueberry bushes were planted in the spacing of 1.2 × 2.0 in the podzolic soil of the VI valuation class. The experiment followed a randomised sub-block design (3 blocks, 5 plants in each block). The content analysis of the soil minerals showed a very high level of magnesium, medium levels of phosphorus and calcium, and a low level of potassium. In spring, the nitrogen fertilization was applied at a dose of 45 kg N/ha.

Within the growing period, the bushes were sprayed 4 times with calcium solutions (every 10 days ranging from 1 June of the decade), starting from the fruit setting until the full wetting of the foliage. Liquid calcium fertilizers, designed for foliar fertilization of plants and fruit, were used, together with the Silwet[®]Gold preparation enhancing the adhesion properties.

Calcinit - 0.5% solution:

Ca - 19.0%, N 15.5%, (NO₃⁻-14.5%, NH₄⁺-1.0%)
(Yara Poland)

Calcium chloride - 0.5% solution:

CaCl₂ -78-80%, NaCl -3-3.5%
(Inowrocławskie Zakłady Chemiczne Soda Mątwy S.A.)

Frucon Calcium Calcium - 1% solution:

Ca 17.5% calculated as CaO
(Spiess - Urania Chemicals GmbH)

Folanx Ca29[®] Ca29 - 1% solution:

Calcium formate
(LANXESS Distribution GmbH)

Lebosol Calcium Forte Calcium Forte - 1% solution:

Ca-13.5%, Mn-1.5%, Zn-0.5%
(Lebosol Calcium Forte Dünger GmbH).

Immediately after the harvests - 3 times from the third decade of July - the unit weight, dimensions and firmness of the fruits were determined (plunger 25 mm), as well as their resistance to mechanical damage (a 3-mm plunger) by using a non-destructive device connected to the FirmTech2 computer. At any time, a set of measurements was performed on 100 fruits. The chemical composition of fruit was also determined: total acidity expressed as citric acid (PN-90/A-75101/04) was determined using a titration method, as well as the content of vitamin C and nitrates by means of test strips read by the electronic refractometer, RQfleks10 (Merck), and the content of total extract using the refractometric method.

The blueberries collected at each harvest were frozen and stored at the temperature of -31°C. For research purposes, the required amount of berries was defrosted. The content of polyphenols and macronutrients was determined in these fruits. The total content of phenols, expressed as gallic acid, was estimated in methanol extractions, using the Folin-Ciocalteu reagent (Singleton and

Rossi, 1965). For macroelemental analyses in plant material, one hundred leaves of plants were sampled each year at the beginning of August. The estimation of the content of minerals was carried out in accordance with the Polish Standard (PN). After mineralisation, the total nitrogen content was determined with the Kjeldahl method (Ostrowska *et al.*, 1991). The content of K and Ca was measured with the atomic emission spectrometry, whereas the Mg content was determined by means of flame atomic absorption spectroscopy using the SAA Solaar instrument. The phosphorus content was determined with the Barton method at a wavelength of 470 nm, whereas the sulphur content with the turbidimetric method at a wavelength of 490 nm, employing the spectrophotometer Marcel s 330 PRO.

Prior to the first harvest of fruits, the index of leaf greenness was measured (after 5 leaves in 25 replicates for each fertilizer), using the Chlorophyll Meter SPAD-502 (Minolta) apparatus, and the foliage was taken for chemical analyses. In autumn, the mean foliage area was measured using the DIAS scanner connected to the computer.

Leaf (in which the green index is measured - 5 leaves in 25 replicates for each fertilizer) and fruit colour (per 100 fruits each time a set of fertilizer) were measured in a transmittance mode by means of a Konica Minolta CM-700d spectrophotometer in 1-cm-thick glass trays. Measurements were conducted in a CIE L*a*b* system [L* white (100) black (0), a* green (-100) red (+100), b* blue (-100) yellow (+100)], through a 10° observer type and D65 illuminant.

In order to determine the significance of differences, a one-factor analysis of variance was carried out, followed by the assessment of the significance of differences using the Tukey's test. To determine the relation between the applied fertilizers and fruit firmness, the results obtained were subjected to an agglomerative cluster analysis and classified into groups in a hierarchical order by means of the Ward's method. The statistical analyses were performed using the Statistica software.

Results and discussion

Size of fruit

Fruit size and firmness are of decisive importance for the harvest quality. The results showed a high variability of these parameters, resulting from the application of foliar calcium fertilizers (Tab. 1). The biggest fruit was collected from the bushes sprayed with the Lebosol Calcium Forte (343 g) and Calcinit (331 g) fertilizers, their diameter and height exceeding 20 mm. The smallest fruits were obtained from the bushes sprayed with calcium chloride and the Frucon Calcium fertilizer containing calcium chloride. The weight of 100 fruits was, respectively, 247 g and 278 g, which was less than the mass of the fruit control group (291 g). The results indicate that the fruits taken from all bushes are of big size, the average mass of the 'Duke'

blueberry being 1.5-1.9 g (Burkhard and Lynch1, 2009; Ehlenfeldt and Prior, 2001). Stückerath *et al.* (2008) having applied the preparation based on calcium oxide, also obtained smaller-size fruits (1.94 g), as compared to the control group (2.54 g).

Fruit firmness and diameter

Fruit firmness is of decisive importance in assessing, among other things, the fruit resistance to mechanical damage. It is commonly assumed that such a phenomenon is less intensive in fruits well supplied with calcium (Fallahi *et al.*, 1997; Sams *et al.*, 1993). The studies have confirmed the above assumption, which shows that the majority of fertilizers (calcium chloride, Folanx Ca29 and Fruton Calcium) enhances the firmness of the fruit, as compared to the control group (Tab. 1), both along the vertical axis (fruit height) and horizontal axis (fruit diameter). In contrast, all applied fertilizers have increased the fruit resistance to puncture. In comparison with other fruits, Duke cultivars are not very firm. Regardless of the fertilizers used, they show less firmness (control 325; sprayed 339-411 G·mm⁻¹), compared to the fruits of Sierra (440) and Patriot cultivars (512 G·mm⁻¹) (Ochmian *et al.*, 2007, 2009). It was also (Duan *et al.*, 2011) determined that the fruits of this cultivar were less firm than those of the Eliot cultivar. The findings on the influence of calcium on fruit firmness are ambiguous. The fruits soaked in the calcium solution after the harvest were more resistant to crushing (Hanson *et al.*, 1993). Likewise, the fruits of the O'Neal cultivar were firmer than the fruits collected from the control group of

bushes, but the results for the Bluecrop (Angeletti *et al.*, 2010) cultivar showed a different trend.

The cluster analysis of the relationships between the applied fertilizers and fruit firmness is assessed along the vertical axis (fruit height) and horizontal axis (fruit diameters), and the puncture force of the fruits (Fig. 1). The fertilizers were divided into four groups. The fertilizers, the impact of which was the most similar on the studied attributes, were classified into one group. The Calcinit and Lebosol Calcium Forte, Fruton Calcium and Folanx Ca29 fertilizers showed the most similar results with regard to the means. The fruits from the control groups and those sprayed with calcium chloride constituted two separate groups of means.

The chemical composition of fruits

The research results in that field failed to show any influence of the applied calcium preparations on the extract content of the highbush blueberry fruit, which was at the level of 13.2% (control), but no more than 13.7% (Folanx Ca29). This is the range, within which the extract from the fruits of the Duke cultivar (Duan *et al.*, 2011) lies, and of other cultivars, it ranges from 10% to 14.6% (Prior *et al.*, 1998; Skupień, 2006).

Typically, highbush blueberry fruits contain a small amount of organic acids. The application of calcium fertilizers resulted in the additional reduction of the organic acid content in the fruits while increasing the juice pH, as compared to the control group (Tab 1.). The fruits taken from the control group of bushes showed the highest con-

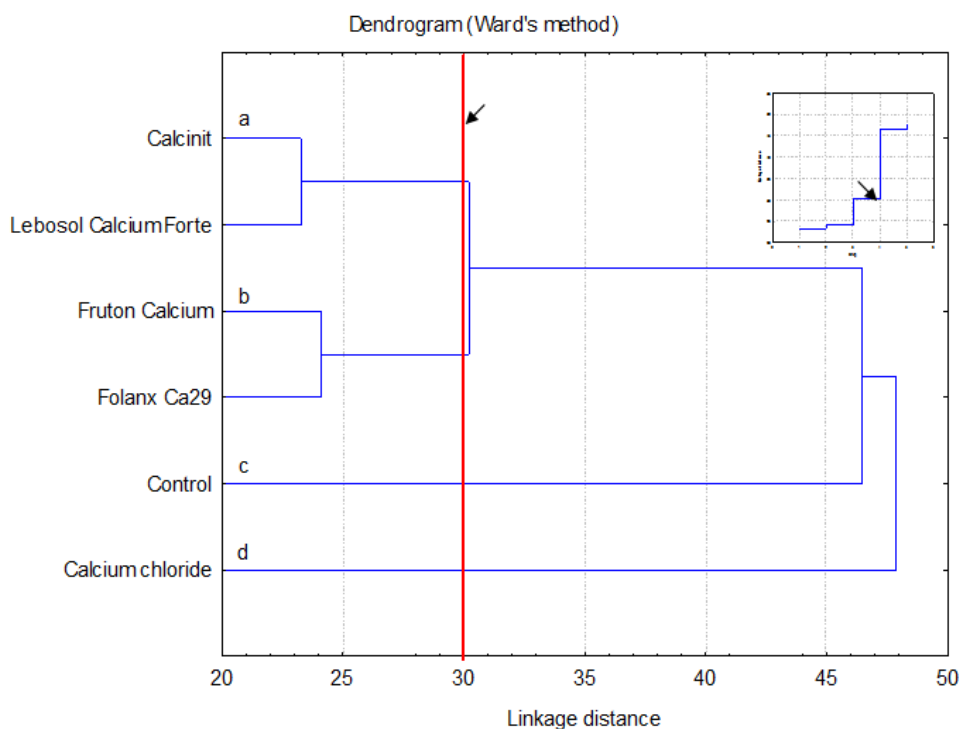


Fig. 1. Dendrogram of cluster analysis for fertilizers based on average for firmness.

The vertical line indicate the cut-off used to form the groups (a-d)

tent of organic acid of 0.75 g and the lowest juice pH (pH 3.62). The acidity of the fruits collected from the bushes sprayed with calcium fertilizers ranged from 0.52 to 0.75 g, while the pH was 3.85. These parameters are similar to those obtained by Duan *et al.* (2011).

The application of the majority of calcium fertilizers had an adverse effect on the content of polyphenols and vitamin C in the fruits. The content of these substances was higher in the control group of fruits (polyphenols 175 mg, vit. C 139 mg). Only the use of the Lebosol Calcium Forte fertilizer, the chemical composition of which significantly differs from those of other fertilizers, resulted in the fruits of similar composition to that of the fruit control group. The level of polyphenols in the Duke cultivar (138 - 182 mg·100 g⁻¹) was much lower than the values determined by Moyer *et al.* (2002): 274 mg·100 g⁻¹, Prior *et al.* (1998): 305 mg·100 g⁻¹ or Bunea *et al.* (2011): 424 mg·100 g⁻¹. The discoloured zone of the blueberry fruits is located around the fruit perimeter, and the research by other scientists shows that the content of polyphenolic compounds is generally higher in the fruit epidermis than in the fruit pulp (Chang *et al.*, 2000). The fruits of the cultivars under study were much bigger than indicated in the references. The research by Ochmian (work in press) indicated that the total area of the small fruits was 20-25% bigger than that of the big fruits, for 100 g of fruits. It may be the answer to the question why the fruits of the Duke cultivar having big fruits contain less polyphenolic compounds.

The assessment of the nitrogen content in the highbush blueberry fruits revealed a strong influence of calcium preparations, especially of the Calcinit and Fruton Calcium fertilizers. It was discovered that the fruits sprayed with these preparations had the highest nitrogen levels of, respectively, 31.3 and 36.7 mg·kg⁻¹. Nevertheless, these values are sufficient to allow the fruits to be consumed even by children. The control groups of fruits and those sprayed with the Folanx Ca29 fertilizer contained the smallest

amount of nitrogens, i.e. a little above 20 mg. These values are similar to those obtained by other scientists, who determined the content of nitrogen in fruits at levels ranging from 15.5 to 34.7 mg NO₃ (Ochmian *et al.*, 2009, 2010; Ostrowska and Ściążko, 1996).

Colour

The aim of the experiment was also to examine the leaf greenness index, the colour and size of the foliage. The devices called chlorophyll meters facilitate the assessment of the total content of the chlorophyll, based on the highly correlated leaf greenness index (Gregorczyk and Raczyńska, 1997; Pacewicz and Gregorczyk, 2009). It was found that the impact of the applied fertilizers on the foliage colour, expressed both as SPAD index and L*a*b*, is varied (Tab. 2). Definitely, the darkest foliage (parameter L* 32.39), containing the highest amount of substances, that give a blue (b* 30.74) and green colour (a* -14.78 and SPAD 44.5), was determined in the case of the bushes fertilised by calcium nitrate. Such a result was most probably attributable to the chemical composition of the fertilizer, namely the high content of nitrogen, as evidenced by the analyses of the foliage collected from such bushes, which contained the highest amount of nitrogen (Tab. 3). A very similar effect was achieved by using the Lebosol Calcium Forte fertilizer. The sprayed plants also showed the biggest foliage. The application of the remaining fertilizers resulted in the foliage with parameters similar to those of the leaves collected from the control group of bushes. The only way to decrease the value of b* parameter was to apply calcium in the chloride form and the Folanx Ca29 fertilizer.

According to Krzewińska *et al.* (2010), the medium content of the chlorophyll in the foliage of the Bluecrop cultivar, (SPAD index), was determined at the level of 39-48 units. The higher values were determined in the autumn season, compared to the measurements taken in spring.

Tab. 1. Some characteristics of the highbush blueberry 'Duke' fruits in dependence on fertilizing (mean for 2010–2011)

Variable	Applied fertilizers					
	Calcinit	Calcium chloride	Fruton Calcium	Folanx Ca29	Lebosol Calcium Forte	Control
100 fruits mass (g)	331 cd*	247 a	278 ab	305 bc	343 d	291 b
Fruit height (mm)	20.2 c	14.8 a	17.1 b	18.0 b	20.1 c	17.6 b
Fruit diameter (mm)	23.0 bc	16.1 a	20.6 b	21.3 bc	23.6 c	20.4 b
Fruit firmness measured on height axis (G mm)	357 ab	411 b	369 b	373 b	339 a	325 a
Fruit firmness measured on diameter axis (G mm)	173 a	223 b	204 b	182 ab	166 a	147 a
Puncture the fruit diameter axis (G mm)	125 ab	153 b	140 b	149 b	138 b	98 a
Soluble solids (%)	13.5 a	13.3 a	13.4 a	13.7 a	13.5 a	13.2 a
Titrate acidity (g·100 mL ⁻¹)	0.52 a	0.61 ab	0.65 ab	0.55 a	0.59 a	0.75 b
Juice pH	3.81 b	3.76 ab	3.71 ab	3.83 b	3.85 b	3.62 a
Total polyphenol (mg·1000 mL ⁻¹)	138 a	154 b	165 bc	168 bc	182 d	175 cd
Vitamin C (mg·1000 mL ⁻¹)	98 a	117 ab	121 bc	104 a	139 c	133 c
NO ₃ (mg·1000 mL ⁻¹)	31.3 bc	26.7 ab	36.7 c	22.4 a	27.3 ab	20.1 a

*Explanation: Different letters within columns indicate significant differences by Tukey's HSD (Honestly Significant Difference) test at $p < 0.05$

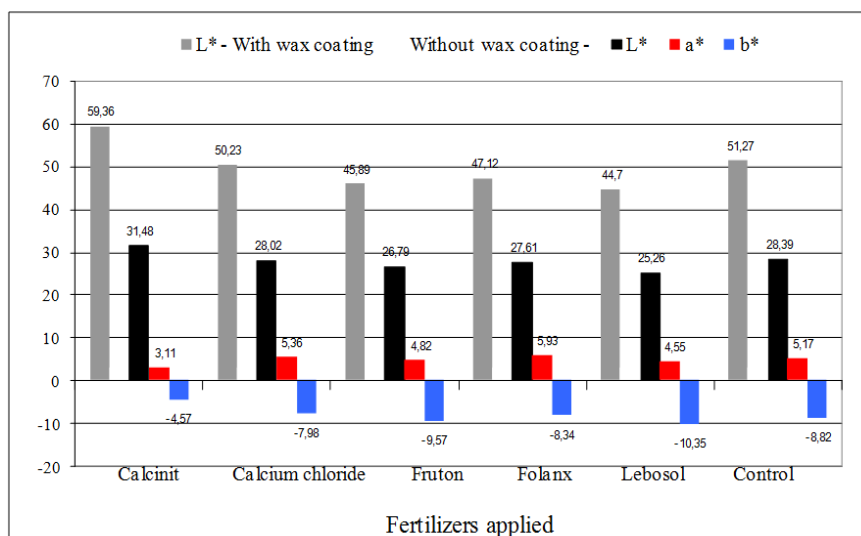


Fig. 2. The color of highbush blueberry 'Duke' depending on the applied calcium fertilizers

The estimation of the fruit colour showed that the fruits sprayed with the Calcinit fertilizer were the brightest, which is true for fruits both with and without a wax coating (L^* parameter - Fig. 2). The application of the remaining fertilizers resulted in darker fruits than those from the control group. The darkest were fruits collected from bushes sprayed with the Lebosol Calcium Forte fertilizer. Such fruits also had the highest content of fruit pigments, giving a blue colour (b^* parameter). The fruits sprayed with the Calcinit showed the lowest values of the b^* and a^* parameters, which determine the content of the pigments giving a red colour. The fruits of the highbush blueberry showed the values of the a^* and b^* parameters, which were similar to those of chokeberry fruits; however, they were brighter: blueberry fruits without a coating have L^* from 25.26 to 31.48), and chokeberry fruits have L^* from 16.14 to 21.59 (Ochmian *et al.* 2012).

Minerals in the foliage

The foliage was collected for analysis in August, since according to Pliszka (2002), the earlier date of leaf collection is not recommended due to the relatively high dynamics of changes in the content of minerals. The higher content of calcium, both in the fruits and foliage of the

'Duke' blueberry, was determined as a result of the application of foliar calcium fertilizers (Tab. 3), though these differences were not always significant. The biggest increase of the calcium content, compared to the control group, was estimated as a result of the application of the Lebosol Calcium Forte and Folanx Ca29 preparations. Angeletti *et al.* (2010) claims that the application of foliar calcium fertilization resulted in a 10% increase of calcium in the fruits, compared to the control group. Stückrath *et al.* (2008) found that the concentration increase of this preparation contributed to the increase of the calcium content in fruits. By applying the preparation based on calcium oxide to the plants, they achieved a level of calcium content in the fruits of 0.0102%. The results of their own study revealed that the calcium content in the fruits was at least three times higher, ranging from 0.034% (control) to 0.061% (Lebosol Calcium Forte).

The application of the Calcinit fertilizers resulted in the biggest increase of N (2.47%), K and Mg content, both in the foliage and fruits. The application of the Lebosol Calcium Forte fertilizer also resulted in the increase of K and Mg content in the fruits. The high content of nitrogen in the foliage and fruits is undoubtedly caused by the chemical composition of the fertilizer, which contains 15.5% N.

Tab. 2. Characteristics of leaves of the highbush blueberry 'Duke' in dependence on the applied calcium fertilizers

Variable	Applied fertilizers						
	Calcinit	Calcium chloride	Fruton Calcium	Folanx Ca29	Lebosol Calcium Forte	Control	
Green index – SPAD	44.5 b*	35.7 a	36.9 a	39.4 a	42.8 b	38.4 a	
Leaf color	L^*	32.39	45.08	39.77	40.13	36.42	42.42
	a^*	-14.78	-7.18	-9.16	-10.38	-14.06	-11.95
	b^*	30.74	15.73	14.08	16.56	28.31	26.31
Leaf area (cm ²)	17.8 b	15.9 a	16.1 a	16.5 ab	17.9 b	17.1 ab	

*Explanation: Different letters within columns indicate significant differences by Tukey's HSD (Honestly Significant Difference) test at $p < 0.05$

Tab. 3. The content of macrolelements in fruits and leaves of highbush blueberry 'Duke' depending on the applied calcium fertilizers

Nutrient	Applied fertilizers						
	Calcinit	Calcium chloride	Fruton Calcium	Folanx Ca29	Lebosol Calcium Forte	Control	
Fruits	Ca	0.045 ab*	0.052 ab	0.047 ab	0.056 b	0.061 b	0.034 a
	N	1.14 c	0.63 ab	0.54 a	0.81 b	0.69 a	0.57 a
	P	0.075 ab	0.092 bc	0.055 a	0.121 d	0.084 b	0.110 cd
	K	0.61 c	0.27 a	0.39 ab	0.46 ab	0.63 c	0.55 bc
	Mg	0.052 b	0.027 a	0.033 a	0.039 ab	0.048 b	0.044 ab
Leaves [% dry matter]	Ca	0.48 ab	0.77 c	0.86 c	0.63 bc	0.75 c	0.37 a
	N	2.47 c	1.54 ab	1.34 a	1.85 b	1.93 b	1.71ab
	P	0.29 a	0.22 a	0.25 a	0.17 a	0.31 a	0.22 a
	K	0.41 c	0.35 abc	0.26 a	0.29 ab	0.37 bc	0.33 abc
	Mg	0.28 c	0.17 ab	0.12 a	0.19 ab	0.22 bc	0.16 a

* Explanation: Different letters within columns indicate significant differences by Tukey's HSD (Honestly Significant Difference) test at $p < 0.05$

The chemical analysis of the foliage indicated that the content of macronutrients was at the optimum level, and only the N content in the foliage sprayed with the Calcinit was too high, whereas the K content in the foliage collected from the plants sprayed with the Fruton Calcium and Folanx Ca29 fertilizers, and from the control group of plants, was too low. A similar level of the macronutrient content in the foliage was shown by Krzewińska *et al.* (2010), with the exception of Ca content, which was found to be much lower (0.21-0.32%), as shown in the authors' own experiment (control 0.37; fertilised plants 0.48-0.86%). It shows that all plants sprayed with calcium accumulated the highest amounts of this element.

Conclusions

The biggest fruits in terms of mass and size were collected from the bushes sprayed with Lebosol Calcium Forte and Calcinit fertilizers; however, these fertilizers did not improve the firmness of the fruits. The highest firmness and resistance to mechanical damage was achieved in case of the fruits collected from the bushes sprayed with the foliar fertilizers containing calcium chloride, such as Fruton Calcium and Folanx Ca29. The fruits collected from the bushes sprayed with the Lebosol Calcium Forte preparation had the highest content of polyphenols and vitamin C. The highest content of calcium in the fruits was found in the case of the plants sprayed with the Lebosol Calcium Forte and Folanx Ca29 preparations, who also picked up the P contents. Likewise, the high levels of K and Mg were found in the fruits that were sprayed with the Calcinit and Lebosol Calcium Forte fertilizers, and N - both in the foliage and fruits - when the Calcinit fertilizer was used. It was showed that the fertilizers had a similar impact on the colour and SPAD. The 'Duke' foliage, sprayed Calcinit and Lebosol Calcium Forte preparations, showed the highest Green Index, the darkest colour (L*) and the highest content of compounds that give a green (a*) and blue (b*) colour. The 'Duke' fruits, sprayed with calcium fertil-

izers, had a brighter fruit peel (L*), both with and without a wax coating, and contained less compounds that give a blue colour (b*), compared to the control group.

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