DOI: 10.21005/AAPZ2018.48.4.09

FOLIA POMERANAE UNIVERSITATIS TECHNOLOGIAE STETINENSIS
Folia Pomer. Univ. Technol. Stetin., Agric., Aliment., Pisc., Zootech. 2018, 345(48)4, 91–104

*Grzegorz MIKICIUK, Małgorzata MIKICIUK[1], Arkadiusz TELESIŃSKI[1]*
*Małgorzata STATKIEWICZ, Urszula CHYLEWSKA*

# THE EFFECTS OF InCa FERTILIZER USED IN FOLIAR NUTRITION ON YIELD QUANTITY AND QUALITY AND SELECTED PHYSIOLOGICAL PARAMETERS OF SWEET CHERRY CV. 'BURLAT'

Department of Horticulture, West Pomeranian University of Technology, Szczecin, Poland
[1]Department of Plant Physiology and Biochemistry, West Pomeranian University of Technology, Szczecin, Poland

**Abstract.** Sweet cherry is an important crop, grown on all continents. A serious problem associated with sweet cherry cultivation is fruit cracking, occurring on rainy days during the ripening period. The yield loss caused by fruit cracking can be as high as 90%. The most common approach to reducing this adverse phenomenon is foliar fertilization with calcium-containing preparations. Only a few studies have focused on the impact of this macronutrient on fruit quality and content of bioactive substances in them and physiological properties of the trees. The aim of this study was to evaluate the effects of calcium foliar supplementation on fruit yield quality and quantity, fruit susceptibility to cracking and on selected physiological parameters of sweet cherry. The study involved 14-year old sweet cherry trees of 'Burlat' cultivar, grafted onto 'PHL-A' rootstock. The trees were sprayed with InCa fertilizer (8.0% N, 13.0% CaO, and 1.0% Zn). The fertilizer was applied three times, at a dose of 1.5 $dm^3 \cdot ha^{-1}$. The first foliar fertilization was performed at the beginning of the flowering period, and the next two took place at fourteen-day intervals. By reducing fruit cracking, InCa used in foliar nutrition significantly increased marketable yield of sweet cherry, without changing fruit weight, size or antioxidant properties. Application of the fertilizer caused a reduction in total acidity (TA) of the fruit and increased TSS/TA (total soluble solids/total acidity) ratio. Foliar application of InCa fertilizer increased the content of chlorophyll *a*, total chlorophyll and carotenoids in the leaves of 'Burlat' cultivar. The foliar nutrition with InCa did not change $CO_2$ assimilation rate, $CO_2$ concentration in leaf intercellular spaces and the value of relative water content (RWC).

**Key words:** *Prunus avium* L., calcium, cracking fruit, antioxidants, assimilation pigments, gas exchange.

## INTRODUCTION

Sweet cherry is an important crop, grown on all continents (Sîrbu et al. 2012; Stojanovic et al. 2012). A demand for sweet cherries increases every year, and over 85% of the harvested fruit are intended for direct consumption (Stojanovic et al. 2012). Therefore, profitability of these fruit production depends primarily on the yield quality. Many authors claim that popularity of sweet cherry fruit is due not only to their taste, but also to nutritious and health-promoting properties. Sweet cherries are rich in bioactive components, such as phenolic compounds that are known for their antioxidant properties and may provide some

Corresponding author: Grzegorz Mikiciuk, Department of Horticulture, West Pomeranian University of Technology, Szczecin, Juliusza Słowackiego 17, 71-434 Szczecin, Poland, e-mail: Grzegorz.Mikiciuk@zut.edu.pl

protection against neurodegenerative diseases (McCune et al. 2011; Prvulović et al. 2011; De Souza et al. 2014). According to Ferretti et al. (2010), the content of these compounds in the fruit depends on environmental factors and agrotechnical procedures. A serious problem associated with sweet cherry cultivation is fruit cracking, occurring on rainy days during the ripening period. Simon (2006) claims that yield loss caused by fruit cracking can be as high as 90%. The rain-triggered mechanism of fruit cracking is not sufficiently understood. This negative phenomenon can be reduced in many ways, including a selection of crack-resistant cultivars, using covers to protect the fruit against rain and spraying the trees with various chemicals (Yamamoto et al. 1992). The most common approach to reducing fruit cracking is foliar fertilization with calcium-containing preparations. Calcium increases a stability of cell walls by strengthening the bonds of pectin chains and reducing their solubility. It also decreases cell membrane permeability to water and is absorbed by cuticule, thus affecting the osmotic potential and limiting water penetration into fruit (Erogul 2014; Meland et al. 2014). Most studies on foliar fertilization of sweet cherry with calcium-containing fertilizers concern their effects on fruit cracking, and to a lesser extent the content of bioactive substances in the fruit or physiological properties of the trees. According to Ferreti et al. (2010) modern methods of calcium supplementation should not only provide protection against cracking, but also facilitate accumulation of bioactive compounds in plants and improve biological quality of the yield.

Therefore, the aim of this study was to evaluate the effects of InCa calcium containing fertilizer on fruit yield quality and quantity, fruit susceptibility to cracking and on selected physiological parameters of sweet cherry 'Burlat'.

## MATERIALS AND METHODS

A univariate experiment in a random block arrangement and five replications was carried out in the years 2011–2013, in an industrial orchard in Karwowo (53°22'N and 14°26'E) near Szczecin. The study involved 14-year old sweet cherry trees of 'Burlat' cultivar, grafted onto 'PHL-A' rootstock and growing at 4 x 3 m spacing. The trees were sprayed with InCa fertilizer based on CaT technology (manufacturer Plant Impact), containing 8.0% N, 13.0% CaO, and 1.0% Zn. CaT is a synthesized plant extract containing auxins, designed to activate a calcium-auxin pump and calcium channels to improve calcium supply levels (Arysta LifeScience 2013). The fertilizer was applied three times, at a dose of 1.5 $dm^3 \cdot ha^{-1}$ (300 $dm^3$ liquid per 1 ha). The first foliar fertilization was performed at the beginning of the flowering period, and the next two took place at fourteen-day intervals. Control trees were sprayed with distilled water. Marketable yield and mass of 1 fruit were determined by weight (to the nearest 10 g and 0.01 g). Fruit width (transverse diameter) and length (longitudinal diameter) were determined using an electronic caliper to the nearest 0.1 mm. Maximum transverse and longitudinal diameter of the analyzed fruit were measured. The weight and size were determined for 100 fruit in each repetition.

Total soluble solids content (TSS) was assessed by a digital refractometer Atago Pol 1 (Atago, Japan). Total fruit acidity (TA) was evaluated by titration of a water extract of sweet cherry homogenate with 0.1 N NaOH to the end point of pH 8.1 (measured with a multimeter

Elmetron CX-732, according to PN-90/A-75101/04). The content of L-ascorbic acid in fruit was measured by a reflectometer Merck RQflex 10 (according to Pantelidis et al. 2007). Fruit sample (5 g) and 20 $cm^3$ oxalic acid (1%) were mixed, homogenised for 1 min, and filtered. PVPP (polyvinylpolypyrrolidone) (500 mg) was added to 10 $cm^3$ of the filtered sample, to remove phenols, and 5–7 drops of $H_2SO_4$ (25%) were added, to reduce the pH to below 1. Results were expressed as mg L-ascorbic acid 100 $g^{-1}$ FW.

The content of nitrate and nitrite was quantified with the reflectometer RQflex 10 (Merck) according to protocol for the juice of red fruit (Merck, Nitrate in Red Coloured Fruit Juices). The sample was prepared by mixing 10 $cm^3$ of the juice and 500 mg PVPP for 1 min. The measurements of nitrate and nitrite were made after filtering the sample. To determine the content of polyphenols and flavonoids, as well as antioxidant activity and capacity, the harvested fruit were washed under running water and dried with a paper towel. For the extraction of the antioxidants, 5 g of fruits was treated with 50 $cm^3$ methanol at room temperature with stirring. This procedure was repeated at least five times until the extraction solvent became colourless. The obtained extracts were filtered over Whatman No.1 filter paper and the filtrate was collected, then methanol was removed by a rotary evaporator at 40°C. The residues were dissolved in methanol in 50 $cm^3$ volumetric flask.

The total antioxidant capacity of the extracts was assessed by the phosphomolybdenum method according to the procedure of Prieto et al. (1999). The assay is based on the reduction of Mo(VI)–Mo(V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acid pH. 0.3 $cm^3$ extract was combined with 3 $cm^3$ of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95°C for 90 min. Then the absorbance of the solution was measured at 695 nm using a spectrophotometer (Shimadzu, UV-1800) against blank after cooling to room temperature. Methanol (0.3 $cm^3$) in the place of extract is used as the blank. The antioxidant activity is expressed as the number of equivalents of ascorbic acid. The free radical scavenging activity of the extracts, based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined by the method described by Braca et al. (2001). Plant extract (0.1 $cm^3$) was added to 3 $cm^3$ of a 0.004% methanol solution of DPPH. Absorbance at 517nm was determined after 30 min, and the percentage inhibition activity was calculated from $[(A_0–A_1)/A_0]\cdot 100$, where $A_0$ is the absorbance of the control, and $A_1$ is the absorbance of the extract. The total polyphenol content of fruit extracts was determined using Folin–Ciocalteu reagent (Yu et al. 2002). Fruit extracts (100 $cm^3$) were mixed with 500 $cm^3$ of the Folin–Ciocalteu reagent and 1.5 $cm^3$ of 20% sodium carbonate. The mixture was shaken thoroughly and made up to 10 $cm^3$ using distilled water. Then the absorbance at 765 nm was determined.

These data were used to estimate the total polyphenol contents using a standard curve obtained from various concentration of gallic acid. The total flavonoid contents were determined by Kumaran and Karunakaran (2007) method using quercetine as a reference compound. 1 $cm^3$ of fruit extract was mixed with 1 $cm^3$ 2% aluminium trichloride solution in methanol and a drop of acetic acid, and then diluted with methanol to 25 $cm^3$. The absorption at 415 nm was read after 40 min. Blank samples were prepared from 1 $cm^3$ of plant extract

and a drop of acetic acid, and then diluted to 25 ml with methanol. Data were used to estimate the total flavonoid contents using a standard curve obtained from various concentration of quercetine.

Physiological parameters of sweet cherry leaves were evaluated twice, first during color change at the ripening stage (I measurement), and then at harvest maturity (II measurement). The analyses involved fully developed leaves from the central part of long shoots, located on the perimeter at mid-crown. Gas exchange parameters measured in leaf tissues included $CO_2$ assimilation intensity (A) and transpiration (E), stomatal conductance to water ($g_S$) and $CO_2$ concentration in chlorenchyma intercellular spaces ($c_i$). They were performed using a portable gas analyzer TPS-2, PP Systems, operating in an open system and equipped with a measuring chamber PLC4. The analyzer cuvette conditions were set to a constant supply of carbon dioxide at a concentration of 370 ppm (μmol $CO_2 \cdot mol^{-1}$ air), humidity equal to ambient humidity and lighting equal to 2053 PAR (μmol $\cdot m^{-2} \cdot s^{-1}$), provided by a light unit coming with the cuvette. Air temperature was measured with a silicon sensor. The measurements were performed at air temperature of 22–25°C. The measurements were repeated fifty times. The data describing assimilation intensity and transpiration were used to calculate photosynthetic water-use efficiency ($\omega_F$), determined as the ratio of assimilation to transpiration rate.

Assimilation pigments content was determined in the same leaves, in which the gas exchange parameters were assessed. Chlorophyll content was measured using a method described by Arnon et al. (1956) and modified by Lichtenthaler and Wellburn (1983), and carotenoids content was assessed according to Hager and Mayer-Berthenrath (1966). For the estimation of assimilation pigments content, a known mass of leaf (about 0.05 g) were homogenized in 10 $cm^3$ of 80% acetone. The homogenate was centrifuged at 2500 g for 10 minutes. The absorbance of the acetone extracts was measured at 440, 645 and 663 nm using a spectrophotometer (Marcel Mini). Relative water content (RWC) index in sweet cherry leaves was calculated as described by Yamasaki and Dillenburg (1999). Leaf material was weighed to determine the fresh weight and placed in distilled water for 24 h and then the turgid weight was recorded. Finally, the samples were dried in an oven at 80°C for 48 h and the dry weights were recorded. RWC was calculated as: RWC = [(Fresh weight – Dry weight)/ (Turgid weight-Dry weight)] x 100.

Rainfall, as a condition conducive to fruit cracking, was simulated by soaking the harvested fruit in a container filled with distilled water. The percentage of cracked cherry fruit was determined after 2, 4, 6, 12, 18, 24, 36 and 48 hours of soaking. Fruit cracking susceptibility was assessed for 100 fruit in each repetition. Cracking index (CI) was calculated according to Weichert et al. (2004).

The resulting data were subjected to one-way analysis of variance, in a random block arrangement. To determine the significance of differences between means, Duncan's confidence half intervals, at a significance level $\alpha = 0.05$ were calculated. Statistical calculations were carried out using Statistica 10 software. The data shown in the tables consists mean values from three years of research.

## RESULTS AND DISCUSSION

The use of InCa preparation caused a 16.2% increase in the marketable yield (Table 1). The observed yield increase, a direct result of using the investigated preparation, was manifested by a reduction in fruit cracking (Fig. 1). Fruit susceptibility to cracking is probably associated with their skin structure, and foliar fertilization with calcium increases its concentration in the epidermis, thus improving the integrity of the cell wall and reducing this adverse phenomenon (Lane et al. 2000; Demirsoy and Demirsoy 2004). Our results are consistent with the reports of many other authors who claimed that foliar supplementation with calcium reduced fruit cracking that may significantly deplete sweet cherry yield (Yamamoto et al. 1992; Rupert et al. 1997; Demirsoy and Bilgener 1998; Chełpiński et al. 2007; Balbontin et al. 2013; Erogul 2014). In our study, InCa preparation seemed to be the most effective in limiting fruit cracking over the first few hours of soaking in distilled water. After two hours of rainfall simulation, the fertilizer reduced fruit cracking by 67.7%, after four hours by 55.9%, and after six hours by 42.2%, in comparison to the control. After 48 hours, cracking in the fruit fertilized with InCa preparation was limited by 32.8%, as compared to the control (Fig. 1). The use of foliar fertilization with InCa formulation resulted also in a significant decrease of CI index (Fig. 2). Similar findings were published Weichert et al. (2004), who claimed that the use of calcium chloride markedly reduced CI index in 'Summit' cultivar.

Table 1. The influence of InCa fertilizer on yield, weight and size of fruit of sweet cherry cv. ‘Burlat’

| | Sum of marketable yield (2011–2013) [kg/tree] | Weight of 1 fruit [g] | Width [cm] | Length [cm] |
|---|---|---|---|---|
| Control | 41.9 a* | 7.93 a | 2.58 a | 2.38 a |
| InCa | 49.7 b | 7.82 a | 2.59 a | 2.37 a |
| Mean | 45.8 | 7.88 | 2.59 | 2.38 |

*Means assigned identical letters do not differ significantly at the level of significance α = 0.05.

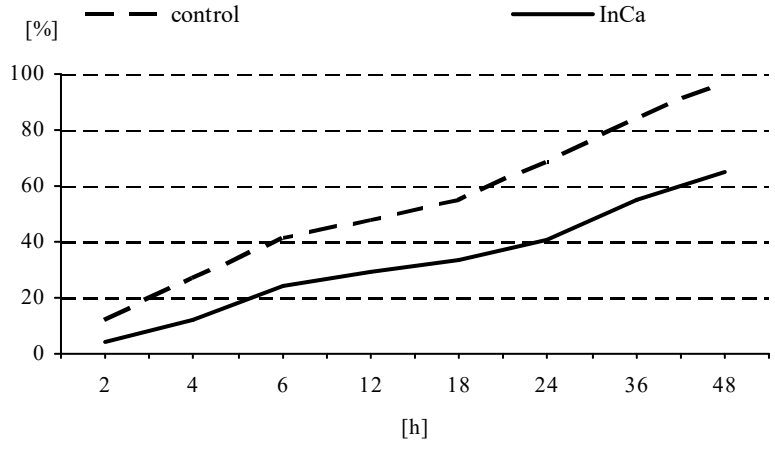


Fig. 1. Percentage share of cracked fruit in a sample, depending on the time of soaking in distilled water

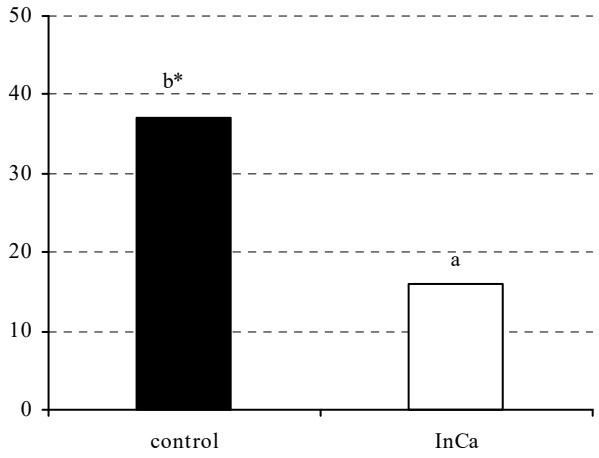


Fig. 2. The influence of InCa fertilizer on cracking indices (CI) of fruit of sweet cherry cv. 'Burlat'
*Means assigned identical letters do not differ significantly at the level of significance α = 0.05.

InCa treatment affected neither weight nor size (width, length) of the fruit (Table 1). Similar conclusion was published by Chełpiński et al. (2007) and Erogul (2014), who reported that calcium fertilization did not affect fruit weight. The fruit harvested in our experiment were characterized by slightly greater average weight, width and length than the fruit of the same cultivar studied by Kurlus (2004) and Bieniek et al. (2011). Weight, width and length of our fruit were similar to the values reported by Stojanovic et al. (2012), who found that average weight of 'Burlat' cv. fruit was 7.94 g, width 2.52 cm, and length 2.31 cm.

The preparation did not affect the content of soluble solids (TSS), and this result has been confirmed by other authors, who concluded that foliar fertilization with calcium did not change the amount of soluble solids (Table 2) (Yamamoto et al. 1992; Demirsoy and Bilgener 1998; Erogul 2014). The content of soluble solids (TSS) was similar to the values reported in other scientific papers. Gonçalves et al. (2004); Kurlus (2004), Pérez-Sánchez et al. (2010); Drkenda et al. (2012); and Stojanovic et al. (2012) claimed that the content of soluble solids in 'Burlat' cv. ranged from 11.2% to 15.3%. InCa preparation decreased a total acidity (TA) of the studied fruit and increased TSS/TA ratio (Table 2). However, different outcomes were achieved by Clayton and Biasi (2003) and Erogul (2014), who reported that fertilization with different forms of calcium did not affect the acidity of sweet cherry fruit. Total fruit acidity (TA) determined in our study was similar to the data reported in the literature. Girard and Kopp (1998), Sîrbu et al. (2012), Pérez-Sánchez et al. (2010), and Drkenda et al. (2012) reported that sweet cherry acidity may range from 0.3 to 1.2 g of malic acid · $100g^{-1}$ FW and largely depends on a cultivar and weather conditions in the growing season. According to Girard and Kopp (1998), TSS/TA ratio in sweet cherry ranges from 18.3 to 29.0. No effect of InCa fertilization on nitrate and nitrite accumulation in the fruit of the studied cultivar was found (Table 2). Considering the applicable standards, their content should be deemed as very low and acceptable for this type of foods (Commission Regulation No. 1881/2006).

Sweet cherries are an important source of numerous compounds exhibiting antioxidant properties (Prvulović et al. 2011). According to Sîrbu et al. (2012) and Prvulović et al. (2011) fruit antioxidant properties depend on many factors, including the species, cultivar, cultivation system, climatic conditions or agrotechnical procedures. L-ascorbic acid concentration in the study fruit seemed to be high, as according to Ferreti et al. (2010), Bieniek et al. (2011) and McCune et al. (2011) its content in sweet cherry is about 7 mg · 100g$^{-1}$ FW. However, Serrano et al. (2005) pointed out that it depended on fruit ripeness, and in ripe fruit its concentration may be as high as 28 mg · 100g$^{-1}$ FW (Table 2). No effect of InCa fertilization on the content of L-ascorbic acid was found in our study. Moreover, foliar calcium fertilization seemed not to affect polyphenol content in the fruit or fruit antioxidant capacity and activity (Table 3). Concentration range of sweet cherry fruit polyphenols reported in the literature is very wide and ranges from 410 to almost 3000 mg of gallic acid · kg$^{-1}$ FW (Kim et al. 2005; Serrano et al. 2005; Vangdal and Slimestad 2006; Ferretti et al. 2010; Usenik et al. 2010; McCune et al. 2011; Prvulović et al. 2011; Nizioł-Łukaszewska 2012), and in 'Burlat' cv. it is between 916 and 1410 mg gallic acid · kg$^{-1}$ FW (Gonçalves et al. 2004; Kim et al. 2005).

Polyphenol content in the study fruit was not high, but it fell within the range provided in the literature. Antioxidant capacity of 'Burlat' sweet cherries was quite high, as according to Serrano et al. (2005) and Sîrbu et al. (2012) its normal range in this species is from 500 to 1720 mg · kg$^{-1}$ FW (Table 3). Mean antioxidant activity of the analyzed fruit was 39.0% DPPH (Table 3), which was consistent with the results obtained by Prvulović et al. (2011). According to the above-cited authors, antioxidant activity of 'Burlat' sweet cherry fruit is approximately 36% DPPH. Flavonoid content, expressed as the amount of quercetin in sweet cherry fruit, ranges from 18 to 600 mg · kg$^{-1}$ FW (Harnly et al. 2006; Gonzáles-Gómez et al. 2010; De Souza et al. 2014). According to Nizioł-Łukaszewska et al. (2012), in 'Burlat' cultivar it amounts to 164 mg · kg$^{-1}$ FW. The present study demonstrated that the use of InCa preparation reduced flavonoid content in the fruit (Table 3).

Table 2. The influence of InCa fertilizer on content of soluble solids, total acidity, nitrates, nitrites and L-ascorbic acid in fruit of sweet cherry cv. 'Burlat'

| | Total soluble solids (TSS) [%] | Total acidity (TA) [g malic acid · 100 g$^{-1}$ FW] | TSS/TA | $N\text{-}NO_3$ | $N\text{-}NO_2$ [mg · 100 cm$^{-3}$ juice] | L-ascorbic acid [mg · 100 g$^{-1}$ FW] |
|---|---|---|---|---|---|---|
| Control | 13.3 a | 0.79 b | 16.8 a | 2.68 a | 0.69 a | 22.4 a* |
| InCa | 13.6 a | 0.53 a | 25.7 b | 3.20 a | 0.66 a | 19.6 a |
| Mean | 13.5 | 0.66 | 21.3 | 2.94 | 0.68 | 21.0 |

Explanations see Table 1.

Table 3. The influence of InCa fertilizer on content of total polyphenols, total flavonoids and on antioxidant capacity and activity of fruit of sweet cherry cv. 'Burlat'

| | Total polyphenols [mg gallic acid · kg$^{-1}$ FW] | Total flavonoids [mg quercetin · kg$^{-1}$ FW] | Antioxidant capacity [mg equivalent of ascorbic acid · kg$^{-1}$ FW] | Antioxidant activity [% DPPH] |
|---|---|---|---|---|
| Control | 838 a* | 155 b | 1565 a | 39.4 a |
| InCa | 827 a | 144 a | 1586 a | 38.6 a |
| Mean | 833 | 150 | 1576 | 39.0 |

Explanations see Table 1.

InCa preparation triggered an increase in chlorophyll *a*, total chlorophyll and carotenoid content in sweet cherry leaves at both measurements, i.e. at the ripening and at harvest maturity stage. For chlorophyll *b* such a correlation was observed only during the second measurement. Foliar fertilization with calcium did not affect *a* to *b* chlorophyll ratio. Its average values were 2.72 at the ripening stage and 2.47 at the harvest maturity stage (Table 4). Our results were similar to those presented in the literature. Gonçalves et al. (2005) reported that *a* to *b* chlorophyll ratio in the leaves of sweet cherry 'Burlat' cv. was 3.09. According to Viljevac et al. (2013), this ratio in cherry ranges from about 3.0 do 5.5.

Table 4. The influence of InCa fertilizer on content of assimilation pigments in leaves of sweet cherry cv. 'Burlat'

| | Chlorophyll a [mg · g$^{-1}$ FW] | Chlorophyll b | Chlorophyll a / b | Chlorophyll a + b [mg · g$^{-1}$ FW] | Carotenoids |
|---|---|---|---|---|---|
| I term of measurement | | | | | |
| Control | 1.51 a* | 0.57 a | 2.65 a | 2.08 a | 0.90 a |
| InCa | 2.18 b | 0.78 b | 2.79 a | 2.96 b | 1.25 b |
| Mean | 1.85 | 0.68 | 2.72 | 2.52 | 1.08 |
| II term of measurement | | | | | |
| Control | 1.87 a | 0.79 a | 2.37 a | 2.66 a | 1.16 a |
| InCa | 2.28 b | 0.89 a | 2.56 a | 3.17 b | 1.49 b |
| Mean | 2.08 | 0.84 | 2.47 | 2.92 | 1.33 |

Explanations see Table 1.

Photosynthesis and transpiration, and the photosynthetic water-use efficiency, determined as the ratio of $CO_2$ assimilation to transpiration, also called leaf gas exchange efficiency, are key yield-controlling physiological processes. Our study showed no effects of InCa preparation on $CO_2$ assimilation in sweet cherry leaves. It was on average 11.48 and 9.1 μmol · m$^{-2}$ · s$^{-1}$ in the leaves treated with the calcium preparation and control ones. The observed $CO_2$ assimilation rate was high. Lenahan and Whiting (2006) and Gonçalves et al. (2005) reported that the assimilation rate in sweet cherry leaves was from 4.5 to 10.0 and from 7.0 to 17.0 μmol · m$^{-2}$ · s$^{-1}$. Calcium fertilization affected the transpiration process in sweet cherry. At the ripening stage, greater intensity of transpiration was observed in the control leaves, while at the harvest maturity stage the transpiration was more intense in the InCa sprayed leaves. At the first measurement, greater photosynthetic water-use effectiveness, defined by the ratio of $CO_2$ diffusion rate through the stomata towards the leaf interior to the rate of water molecule diffusion in the opposite direction, was found in InCa treated plants. It resulted from the high intensity of $CO_2$ assimilation and low intensity of transpiration in these leaves. Foliar fertilization with calcium did not affect this physiological process during the second measurement (Table 5).

InCa application did not change the stomatal conductance to water, determined in the leaves of the studied species at fruit harvest maturity. A decrease in this parameter value was observed during the ripening phase in the calcium-supplemented plants. Gonçalves et al. (2005) pointed out that stomatal conductance to water in the leaves of a few studied sweet cherry cultivars could amount to 0.9 mol · m$^{-2}$ · s$^{-1}$. Stomatal conductance is controlled by the interaction of a number of environmental factors, e.g. air saturation deficit, soil water

availability (Sultana et al. 1999), and internal factors, such as $CO_2$ concentration in intercellular spaces or leaf water potential (Tuzet et al. 2003). InCa preparation did not affect $CO_2$ concentration in intercellular spaces of the studied sweet cherry cultivar, as its average value was 149.95 μmol · mol$^{-1}$ at the first and 147.06 μmol · mol$^{-1}$ at the second measurement (Table 5). In the study by Gonçalves et al. (2005), involving a few sweet cherry cultivars, $CO_2$ concentration was higher and ranged from 230 to 280 μmol · mol$^{-1}$.

Table 5. The influence of InCa fertilizer on the parameters of gas exchange in the leaves of sweet cherry cv. 'Burlat'

| | A [μmol · m$^{-2}$ · s$^{-1}$] | E [mmol · m$^{-2}$ · s$^{-1}$] | $\omega_F$ [mmol · mol$^{-1}$] | $g_s$ [mol · m$^{-2}$ · s$^{-1}$] | $C_i$ [μmol · mol$^{-1}$] |
|---|---|---|---|---|---|
| I term of measurement | | | | | |
| Control | 7.00 a* | 2.42 b | 2.89 a | 0.091 b | 157.71 a |
| InCa | 10.17 a | 1.96 a | 5.19 b | 0.052 a | 142.20 a |
| Mean | 8.59 | 2.19 | 4.04 | 0.071 | 149.95 |
| II term of measurement | | | | | |
| Control | 11.2 a | 1.86 a | 6.02 a | 0.040 a | 145.22 a |
| InCa | 12.8 a | 2.33 b | 5.49 a | 0.034 a | 148.91 a |
| Mean | 12.0 | 1.10 | 5.75 | 0.037 | 147.06 |

A – assimilation $CO_2$, E – transpiration, $\omega_F$ – index of water use in the photosynthesis, $g_s$ – stomatal conductance for water, $c_i$ –concentration of carbon dioxide in the intercellular spaces.
Other explanations see Table 1.

Modification of water metabolism is a common plant response to various environmental factors, and leaf relative water content (RWC) is one of the most important parameters in its assessment. The value of this index positively correlates with plant photosynthetic efficiency (David 2002; Tezara et al. 2002). Similarly as in the case of $CO_2$ assimilation rate, no effects of InCa preparation on the relative water content (RWC) in sweet cherry leaves were found (Fig. 3).

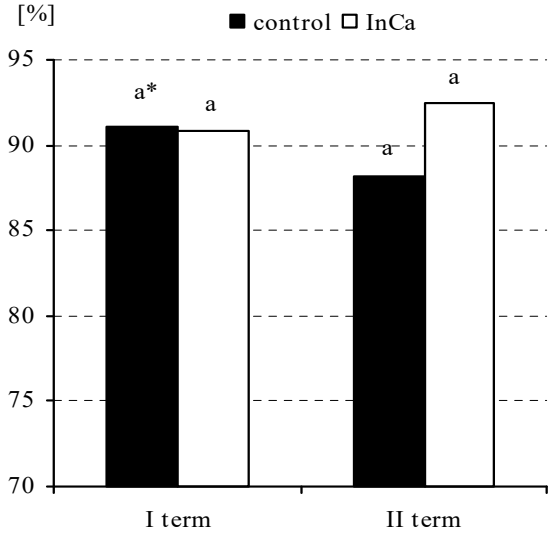


Fig. 3. The influence of InCa fertilizer on RWC in the leaves of sweet cherry cv. 'Burlat'
*Means assigned identical letters do not differ significantly at the level of significance α = 0.05.

## CONCLUSIONS

By reducing fruit cracking, InCa preparation significantly increased the marketable yield of sweet cherry, without changing fruit weight, size or antioxidant properties. Application of the fertilizer caused a reduction in total acidity of the fruit and improved palatability, expressed as TSS/TA ratio. Foliar application of InCa fertilizer increased the content of chlorophyll *a*, total chlorophyll and carotenoids in the leaves of 'Burlat' cultivar. The preparation did not change $CO_2$ assimilation rate, $CO_2$ concentration in leaf intercellular spaces and the value of relative water content (RWC). The effects of the preparation on transpiration intensity and efficiency of gas exchange were ambiguous and depended on the time of measurement.

## REFERENCES

**Arnon D.J., Allen M.B., Whatley F.** 1956. Photosynthesis by isolated chloroplast. Biochim. Biphys. Acta 20, 449–461.

**Arysta LifeScience.** 2013. Informations materials. [b.m.], Arysta LifeScience, 9.

**Balbontín C., Ayala H., Bastías R.M., Tapia G., Ellena M., Torres C., Yuri J.A., Quero-García J., Ríos J.C., Silva H.** 2013. Cracking in sweet cherries: A comprehensive review from a physiological, molecular, and genomic perspective. Chil. J. Agric. Res. 73(1), 66–72.

**Bieniek A., Kawecki Z., Kopytowski J., Zielenkiewicz J.** 2011. Yielding and fruit quality of Lithuanian sweet cherry cultivars grown under the climatic and soil conditions of Warmia. Folia Hort. 23(2), 101–106.

**Braca A., Tommasi N.D., Bari L.D., Pizza C., Politi M., Morelli I.** 2001. Antioxidant principles from *Bauhinia terapotensis*. J. Nat. Prod. 64, 892–895.

**Chełpiński P., Lewandowski J., Gembara J., Mikiciuk G.** 2007. Wpływ stosowania preparatów Wapnowit i Calcinit na pękanie owoców czereśni odmiany Burlat [Influence of Wapnowit and Calcinit on cracking of cherry fruits cv. 'Burlat']. Rocz. AR Pozn., Ogrodnictwo 383(41), 291–296. [in Polish]

**Clayton M., Biasi W.V.** 2003. Postharvest quality of 'Bing' cherries following preharvest treatment with hydrogen cyanamide, calcium ammonium nitrate, or gibberellic acid. Hort. Sci. 38(3), 407–411.

**Commission Regulation (EC) No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs,** https://eur-lex.europa.eu/legal-content/EN/ALL/?uri==CELEX:32006R1881, access: 20.04.2018.

**David W.** 2002. Limitation to photosynthesis in water stressed leaves: stomata vs. metabolism and the rol of ATP. Ann. Bot. 89, 871–885.

**De Souza V.R., Patrícia Pereira P.A.P., Da Silva T.L.T., De Oliveira Lima L.C., Pio R., Queiroz F.** 2014. Determination of the bioactive compounds, antioxidant activity and chemical composition of Brazilian blackberry, red raspberry, strawberry, blueberry and sweet cherry fruits. Food Chem.156, 362–368.

**Demirsoy L.K., Bilgener S.** 1998. The effects of preharvest chemical applications on cracking and fruit quality in 'Zirat', 'Lambert', and 'Van' sweet cherry varietes. Acta Hort. 468, 663–670.

**Demirsoy L., Demirsoy H.** 2004. The epidermal characteristics of fruit skin of some sweet cherry cultivars in relation to fruit cracking. Pak. J. Bot. 36(4), 725–731.

**Drkenda P., Spahić A., Spahić A., Begić-Akagić A.** 2012. Testing of 'Gisela 5' and 'Santa Lucia 64' cherry rootstocks in Bosnia and Herzegovina. Acta Agr. Slov. 99(2), 129–136.

**Erogul D.** 2014. Effect of preharvest calcium treatments on sweet cherry fruit quality. Not. Bot. Hort. Agr. Cluj-Napoca 42(1), 150–153.

**Ferretti G., Bacchetti T., Belleggia A., Neri D.** 2010. Cherry antioxidants: From farm to table. Molecules 15, 6993–7005.

**Girard B., Kopp T.G.** 1998. Physicochemical characteristics of selected sweet cherry cultivars. J. Agric. Food Chem. 46, 471–476.

**Gonçalves B., Landbo A.-K., Knudsen D., Silva A.P., Moutinho-Pereira J., Rosa E., Meyer A.S.** 2004. Effect of ripeness and postharvest storage on the phenolic profiles of cherries (*Prunus avium* L.). J. Agric. Food Chem. 52, 523–530.

**Gonçalves B., Moutinho-Pereira J., Santos A., Silva A.P., Bacelar E., Correia C., Rosa E.** 2005. Scion–rootstock interaction affects the physiology and fruit quality of sweet cherry. Tree Physiol. 26, 93–104.

**Gonzáles-Gómez D., Lozano M., Fernández-León M.F., Bernalte M.J., Ayuso M.C., Rodríguez A.B.** 2010. Sweet cherry phytochemicals: Identification and characterization by HPLC-DAD/ESI-MS in six sweet cherry cultivars grown in Valle del Jerte (Spain). J. Food Compos. Anal. 23, 533–539.

**Hager A., Mayer-Berthenrath T.** 1966. Die Isolierung und quanttaive bestimung der carotenoide und chlorophyll von blatern, Algen und isolierten Chloroplasten mit Hilfe Dunnschichtchromatographischer Methoden. Planta 69, 198–217.

**Harnly J.M., Doherty R., Beecher G.R., Holden J.M., Haytowitz D.B., Bhagwat S.** 2006. Flawonoid content of U.S. fruits, vegetables and nuts. J. Agric. Food. Chem. 54(26), 9966–9977.

**Kim D.O., Heo H.J, Kim Y.J., Yang H.S., Lee C.Y.** 2005. Sweet and sour cherry phenolics and their protecive effects on neuronal cells. J. Agric. Food Chem. 53(26), 9921–9927.

**Kumaran A., Karunakaran R.J.** 2007. In vitro antioxidant activities of methanol extracts of five Phyllanthus species from India. LWT 40, 344–351.

**Kurlus R.** 2004. Growth, yield and fruit quality in eight sweet cherry cultivars grafted on 'Tabel Edabriz' rootstock. J. Fruit Ornam. Plant Res. 12, 35-39.

**Lane W.D., Meheriuk M., McKenzie D.-L.** 2000. Fruit cracking of a susceptible, an intermediate, and a resistant sweet cherry cultivar. Hort. Sci. 35(2), 239–242.

**Lenahan O.M., Whiting M.D.** 2006. Physiological and horticulural effects of sweet cherry chemical blossom thinners. Hort. Sci. 41(7), 1547–1551.

**Lichtenthaler H.K., Wellburn A.R.** 1983. Determinations of total carotenoids and chlorophyll a and b of leaf extracts in different solvents. Biochem. Soc. Trans. 11, 591–592.

**McCune L.M., Kubota C., Stendell-Hollis N.R., Thomson C.A.** 2011. Cherries and health: A review. Crit. Rev. Food Sci. 51(1), 1–12.

**Meland M., Kaiser C., Christensen J.M.** 2014. Physical and chemical methods to avoid fruit cracking in cherry. AgroLife Sci. J. 3(1), 177–183.

**Nizioł-Łukaszewska Z., Skrzyński J., Leja M., Zawiska I.** 2012. Porównanie kilku odmian czereśni i wiśni uprawianych w Małopolsce pod względem zawartości związków fenolowych [Comparison of few varieties of cherry and sour cherry cultivated in Malopolska Region in respect of their phenolic content]. Episteme 15, 445–451. [in Polish]

**Pantelidis G.E., Vasilakakis M., Manganaris G.A., Diamantidis G.** 2007. Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries, blackberries, red currants, gooseberries and Cornelian cherries. Food Chem. 102, 777–783.

**Pérez-Sánchez R., Gómez-Sánchez M.A., Morales-Corts M.R.** 2010. Description and quality evaluation of sweet cherries cultured in Spain. J. Food Quality 33, 490–506.

**PN-90/A-75101/04.** Polish standard of preparation of fruit and vegetable samples and test methods. Determination of total acidity.

**Prieto P., Pineda M., Aguilar M.** 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of phosphomolybdenium complex: Specific application to the determination of vitamin E. Anal. Biochem. 269, 337–341

**Prvulović D., Malenčić D., Popović M., Ljubojević M., Ognjanov V.** 2011. Antioxidant properties of sweet cherries (*Prunus avium* L.) – role of phenolic compounds. World Acad. Sci., Eng. Technol. 59, 1149–1152.

**Rupert M., Southwick S., Weis K. Vikupitz J., Flore J., Zhou H.** 1997. Calcium chloride reduces rain cracking in sweet cherries. Calif. Agr. 51(5), 35–40.

**Serrano M., Guilleän F., Martinez-Romero D., Castillo S., Valero D.** 2005. Chemical constituents and antioxidant activity of sweet cherry at different ripening stages. J. Agric. Food Chem. 53, 2741–2745.

**Simon G.** 2006. Review on rain induced fruit cracking of sweet cherries (*Prunus avium* L.), its causes and the possibilities of prevention. Int. J. Hort. Sci. 12(3), 27–35.

**Sîrbu S., Niculaua M., Chiriţă O.** 2012. Physico-chemical and antioxidant properties of new sweet cherry cultivars from Iaşi, Romania. Agron. Res. 10(1–2), 341–350.

**Stojanovic M., Milatovic D., Kulina M., Alic–Dzanovic Z.** 2012. Pomological properties of sweet cherry cultivars on Gisela 5 rootstock in the region of Sarajevo, in: Book of Proceedings of Third International Scientific Symposium "Agrosym Jahorina 2012", Jahorina 15–17.11.2012. Bosnia and Herzegovina, University of East Sarajevo, 183–187.

**Sultana N., Ikeda T., Itoh R.** 1999. Effect of NaCl salinity on photosynthesis and dry matter accumulation in developing rice grains. Environ. Exp. Bot. 43, 211–220.

**Tezara W., Mitchall V., Driscoll S.P., Lawrol D.W.** 2002. Effects of water deficyt and its interaction with $CO_2$ supply on the biochemistry and phisiology of photosynthesis in sunflower. J. Exp. Bot. 375, 1781–1791.

**Tuzet A., Perrier A., Leuning R.** 2003. A coupled model of stmatal conductance, photosynthesis and transpiration. Plant Cell Environ. 26, 1097–1112.

**Usenik V., Fajt N., Mikulic-Petkovsek M., Slatnar A., Stampar F., Veberic R.** 2010. Sweet cherry pomological and biochemical characteristics influenced by rootstock. J. Agric. Food Chem. 58, 4928–4933.

**Vangdal E., Slimestad R.** 2006. Methods to determine antioxidative capacity in fruit. J. Fruit Ornam. Plant Res. 14(Suppl. 2), 123–131.

**Viljevac M., Dugalić K., Mihaljewić I., Šimić D., Sudar R., Jurković Z., Lepeduš H.** 2013. Chlorophyll content, photosynthetic efficiency and genetic marcers in two sour cherry (*Prunus cerasus* L.) genotypes under drought stress. Acta Bot. Croat. 72(2), 221–235.

**Weichert H., Jagemann C. von, Peschel S., Knoche M.** 2004. Studies on water transport through the sweet cherry fruit surface: VIII. Effect of selected cations on water uptake and fruit cracking. J. Amer. Soc. Hort. Sci. 129(6), 781–788.

**Yamasaki S., Dillenburg L.R.** 1999. Measurements of leaf relative water content in *Araucaria angustifolia*. Rev. Bras. Fisiol. Vegetal. 11(2), 69–75.

**Yamamoto T., Satoh H., Wanatabe S.** 1992. The effects of calcium and naphthalene acetic acid sprays on cracking index and natural rain cracking in sweet cherry fruits. J. Japan. Soc. Hort. Sci. 61(3), 507–511.

**Yu L., Haley S., Perret J., Harris M., Wilson J., Qian M.** 1995. Free radical scavenging properties of wheat extracts. J. Agric. Food Chem. 50, 1619–1624.

## WPŁYW DOKARMIANIA DOLISTNEGO NAWOZEM InCa NA WIELKOŚĆ I JAKOŚĆ PLONU ORAZ WYBRANE PARAMETRY FIZJOLOGICZNE CZEREŚNI ODMIANY ‘BURLAT’

**Streszczenie.** Czereśnia jest gatunkiem o dużym znaczeniu gospodarczym, uprawianym na wszystkich kontynentach. Dużym problemem w uprawie tego gatunku jest zjawisko pękania owoców, do którego dochodzi podczas deszczowych dni w okresie ich dojrzewania. Straty w plonie wywołane tym zjawiskiem mogą dochodzić nawet do 90%. Najpopularniejszym sposobem ograniczania pękania owoców czereśni jest dolistne dokarmianie roślin nawozami zawierającymi wapń. Stosunkowo niewiele badań koncentruje się na wpływie tego makroskładnika na jakość owoców i zawartość w nich substancji bioaktywnych oraz na cechy fizjologiczne drzew. W pracy przedstawiono wyniki badań nad wpływem preparatu

zawierającego wapń zarówno na wielkość i jakość plonu owoców oraz na ich podatność na pękanie, jak i na wybrane parametry fizjologiczne czereśni. Badania przeprowadzono na drzewach czereśni odmiany 'Burlat', uszlachetnionych na podkładce 'PHL-A'. Drzewa opryskiwano nawozem o nazwie handlowej InCa (8,0 % N, 13,0 % CaO i 1,0 % Zn). Nawóz zastosowano w dawce 1,5 $dm^3 \cdot ha^{-1}$ (300 $dm^3$ cieczy roboczej na 1 ha), w trzech terminach. Pierwszy zabieg dokarmiania dolistnego wykonywano na początku kwitnienia, następne dwa zabiegi – w odstępach czternastodniowych. Preparat InCa poprzez ograniczenie pękania owoców istotnie zwiększał plon handlowy, nie zmieniając ich masy, wielkości i właściwości antyutleniających. Zastosowanie nawozu, powodując zmniejszenie kwasowości ogólnej owoców (TA), wpłynęło na zwiększenie stosunku TSS/TA (zawartości ekstraktu do kwasowości ogólnej). Dolistna aplikacja nawozu InCa zwiększyła zawartość chlorofilu a, chlorofilu całkowitego i karotenoidów w liściach odmiany 'Burlat'. Nie stwierdzono wpływu stosowanego nawozu na natężenie asymilacji $CO_2$, stężenie $CO_2$ w przestworach międzykomórkowych liści oraz na wielkość wskaźnika RWC.

**Słowa kluczowe:** *Prunus avium* L., wapń, pękanie owoców, antyutleniacze, barwniki asymilacyjne, wymiana gazowa.