



Responses of ‘Burlat’ Sweet Cherry Trees to Selenium Fertilisation under Low Soil Selenium Conditions

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Abstract

Selenium (Se) malnutrition in humans primarily results from low soil Se availability. In this study, we examined the effects of soil application and preharvest Se sprays on the vegetative growth, yield response, Se nutrition, and fruit quality of ‘Burlat’ sweet cherry (*Prunus avium* L.) trees. Selenium was applied through (i) soil application at a rate of 250 g ha⁻¹ or 500 g ha⁻¹ in each season and in the first year of the study, respectively, and (ii) four preharvest sprays per season at a rate of 15 g ha⁻¹ spray⁻¹. Se sprays were applied with or without calcium chloride (CaCl₂). Leaves from Se-fertilised trees contained more Se and had enhanced SPAD readings. However, the Se treatments did not affect tree growth and fruit yield. Compared to the control fruit, Se concentrations in fruit from trees sprayed with Se and CaCl₂, trees sprayed with Se alone, and trees supplied with Se to the soil were increased 15-, 12-, and 8-fold, respectively. None of the Se treatments affected fruit size, and acidity and the soluble solids concentration (SSC) of the fruit. Only the combined sprays of Se and CaCl₂ decreased the incidence of rain-induced fruit cracking. None of the tested Se treatments resulted in leaf injuries. In sweet cherry orchards, at least for the ‘Burlat’ variety, grown in low Se soils, preharvest Se sprays with or without CaCl₂ can be recommended to biofortify the fruit with Se and increase SSC in the fruit.

Keywords Agronomic biofortification · Fruit quality · Orchard · Selenate

1 Introduction

Selenium (Se) is an essential trace nutrient for human health, playing critical roles in preventing cardiovascular diseases and certain cancers, as well as supporting brain development, reproduction, and the immune system (Rayman 2012). It has been documented that more than 40 human diseases are related to Se deficiency, causing serious economic and social problems in many regions of the world (Schiavon et al. 2020).

Human Se malnutrition is primarily caused by low Se phytoavailability in soils (Lopes et al. 2017), which depends on various soil factors, including Se status, pH, redox potential, clay particle and organic matter content, microbiological activity, and the presence of sulphate and phosphate ions

(Eich-Greatorex et al. 2007; Shahid et al. 2018; Stroud et al. 2010). The problem of Se malnutrition can be mitigated and even resolved by enriching plants with Se through breeding species/varieties with an increased capacity for Se uptake (breeding biofortification), introducing genes into crops responsible for efficient Se absorption mechanisms (transgenic biofortification), or applying Se-containing fertilisers (agronomic biofortification). The latter mode of Se biofortification is favoured due to its ease of use, and rapid results (Cakmak et al. 2017; Ros et al. 2016). However, the efficiency of Se fertilisation in improving Se status in plant tissues depends on the cultivated species/variety, plant organ, physicochemical and biological properties of soil, mode and technique of Se fertiliser application, and the form and rate of Se used (Gupta and Gupta 2017; Hartikainen 2005; Lyons and Cakmak 2012; Puccinelli et al. 2017; White 2016).

Thus far, studies on Se biofortification of the fruit of perennial crops have been conducted on peach (*Prunus persica* Batsch.), pear (*Pyrus communis* L.), apple (*Malus domestica* Borkh.), pomegranate (*Punica granatum*), citrus (*Citrus reticulata*), and olive (*Olea europaea* L.) (Babalar et al. 2019; D’Amato et al. 2018; Deng et al. 2019; Groth

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et al. 2020; Ning et al. 2013; Pezzarossa et al. 2012; Wen et al. 2021; Wójcik 2023; Zahedi et al. 2019). In these experiments, only the impacts of preharvest Se sprays on fruit response were evaluated. However, it seems justified to examine the effect of soil Se fertilisation on fruit enrichment with this nutrient, especially for trees grown in coarse-textured soils low in organic matter, where Se mobility in the soil profile is considerable (Shahid et al. 2018; Tian et al. 2016). Research on the efficiency of soil Se fertilisation in biofortifying fruit with this nutrient is even more justified because: (i) Se can be used at higher rates than in foliar sprays, and (ii) Se taken up by the roots is transported largely to the aboveground parts of plants, including reproductive organs (Sors et al. 2005; Sun et al. 2021).

Sweet cherry (*Prunus avium* L.) is primarily cultivated in temperate climate across more than 40 countries (Bujdosó and Hrodsko 2017). According to the Food and Agriculture Organization (FAO 2022), global sweet cherry production in 2022 reached 2.7 million tons. Sweet cherries are highly valued for exceptional taste, as well as nutritional and dietary values, as they are rich in vitamins, minerals, fiber, and bioactive compounds, including flavonols and anthocyanins (Ballistreri et al. 2013; Blando and Oomah 2019). Thus, sweet cherries are a valuable addition to the human diet because their regular consumption supports the potential preventive health benefits in relation to cancer, cardiovascular disease, diabetes, and inflammatory diseases (McCune et al. 2010; Zhang et al. 2005).

In this study, we hypothesised that Se fertilisation improve the Se nutrition of sweet cherry trees, and fruit quality. To test this hypothesis, we conducted experiment under low soil Se conditions, either incorporating Se into the soil or applying it in preharvest sprays. We also hypothesised that the addition of calcium chloride (CaCl_2), commonly used in preharvest sprays to reduce rain-induced fruit cracking in cherries, to the Se-containing spray solution enhances the uptake of exogenously applied Se by fruit.

2 Materials and Methods

2.1 Study Site and Soil Conditions

This experiment was conducted over two growing seasons (2021–2022) at the Experimental Station Dąbrowice (latitude $51^\circ 55' \text{N}$, longitude $20^\circ 6' \text{E}$, elevation 136 m above sea level) of The National Institute of Horticultural Research in Skierniewice, Poland. The mean annual air temperature and total precipitation at this site were 8.1°C and 560 mm in 2021, and 10.2°C and 461 mm in 2022, respectively. During the vegetation period (May–October), the average

temperature and rainfall amounted to 14.5°C and 467 mm in 2021, and 15.2°C and 394 mm in 2022, respectively.

The experimental orchard was established in 2014 on Albic Luvisols with the following parameters in the 0–30 cm layer: 66% sand; 18% silt; 16% clay; pH 5.9; bulk density, 1.3 g cm^{-3} ; redox potential, 456 mV; organic matter content, 14 g C kg^{-1} ; active iron (Fe) oxide content, 3.1 g kg^{-1} ; active aluminum (Al) oxide content, 1.8 g kg^{-1} ; active manganese (Mn) oxide content, 13 mg kg^{-1} ; available phosphorus (P) content, 56 mg kg^{-1} ; available potassium (K) content, 123 mg kg^{-1} ; available magnesium (Mg) content, 48 mg kg^{-1} ; total Se content, $137 \mu\text{g kg}^{-1}$; and soluble Se content, $4.1 \mu\text{g kg}^{-1}$. The above parameters were determined based on a composite soil sample consisting of 20 subsamples taken in the early spring of 2021 (before mineral fertilisers were applied) from the surface of herbicide strips along tree rows, 20 cm from a dripper. Except for total and soluble Se in the soil, the analysis procedures used in the present study were previously described by Wójcik and Wójcik (2021).

To determine the total soil Se concentration, microwave-assisted digestion with an aqua regia extraction was employed. Briefly, 0.5 g of soil was placed in a Teflon vessel with 5 mL of aqua regia [37% hydrochloric acid (HCl)/70% nitric acid (HNO_3) at a ratio of 3:1]. This suspension was kept at room temperature for 12 h and then microwave-digested in a sealed vessel at 180°C for 20 min under a controlled pressure of 0.76 MPa (MLS 1200, Milistone, Inc., Monroe, CT, USA). After cooling to room temperature, the solution was quantitatively transferred into a 25-mL volumetric flask, and brought to volume with deionized water. The solution was then filtered through a $0.45 \mu\text{m}$ membrane, and Se was determined using an inductively coupled plasma mass spectrometer (X-Series^{II} ICP-MS; Thermo Fisher Scientific, Waltham, MA, USA).

Soluble Se was determined following the modified procedure outlined by Shao et al. (2020). In brief, 1 g of soil was placed in a 15 mL polypropylene centrifuge tube, followed by 10 mL of 0.25 M KCl. The suspension was then shaken in a rotator at 200 rpm for 1 h at 25°C , subsequently centrifuged at 3500 g for 15 min, and filtered through a $0.45 \mu\text{m}$ membrane filter. Se in the supernatant was determined using ICP-MS.

The analytical protocols for the determination of soil Se involved the utilization of an internal standard of $500 \mu\text{g L}^{-1}$ of germanium and Standard Reference Materials (SRM) 2706, representing New Jersey soil with $300 \mu\text{g Se kg}^{-1}$.

2.2 Plant Material and Orchard Management

The study was performed on early-maturing ‘Burlat’ sweet cherries grafted onto Mazzard F12/1 rootstock. In central Poland, fruit of this variety ripen in the second week of June.

The trees were planted in north-south-oriented rows, spaced 4.5 m apart between rows and 2.5 m between trees within each row (888 trees ha⁻¹). Tree canopies were formed as a spindle up to a height of 3.5 m.

Since the third year of tree growth, a 1.5 m-wide herbicide fallow has been maintained along tree rows using post-emergence herbicides. Between tree rows, the sod was systematically mowed when its height reached 15–20 cm (6 and 8 times in 2021 and 2022, respectively).

In the experimental orchard, soil moisture was maintained at nearly field capacity from May to September through surface drip irrigation, consisting of a single line along the tree row (approximately 20 cm from the trunk) and pressure-compensated emitters, each delivering 1.75 L of water h⁻¹. The soil moisture was measured using a tensiometer (Soilmoisture Equipment Corp., Santa Barbara, CA, USA) inserted 15 cm from an emitter. The water used for irrigation had a pH of 7.2, and an electrical conductivity (EC) of 0.23 mS cm⁻¹ (the mean values for water samples taken annually in early spring). Water quantities of 228 L and 297 L per tree were applied *via* the irrigation system in 2021 and 2022, respectively.

Annually, nitrogen (N) and K fertilisers were broadcast at the swollen-breaking bud stage (BBCH 05–08) over the soil surface of the herbicide strips. Nitrogen was applied at a rate of 60 kg ha⁻¹ as ammonium nitrate (34-0-0), and K was used at a dose of 80 kg ha⁻¹ as K-sulphate (0-0-50). The rates of N and K, as well as the mode and timing of their application, were in accordance with the recommendations by Wójcik (2009) for integrated fruit production under conditions of a low soil organic matter content and optimal K availability.

Commercial protection treatments against pathogens and pests were applied following the recommendations of Sobiczewski et al. (2021).

2.3 The Tested Treatments and Experimental Layout

Trees were fertilised with Se as sodium selenate (Na₂SeO₄; 41.8% Se; Sigma-Aldrich, Poznań, Poland) through soil application or foliar spraying. Trees that were not treated with Se served as controls.

Soil Se fertilisation was applied either annually at a rate of 250 g ha⁻¹, or only in the first year of the experiment (2021) at a dose of 500 g ha⁻¹, which, regardless of the frequency of Se application, corresponded to 562 mg Se per tree and 128 µg Se per kg of soil in the 0–30 cm layer over the two-year study period. Soil Se fertilisation was applied at the swollen-breaking bud stage (BBCH 05–08), one day after broadcasting N and K fertilisers, by evenly spraying the surface of the herbicide strips along tree rows with

500 L of aqueous solution using a herbicide boom (BHh Królik; Landi-Tech, Stara Warka, Poland).

The Se sprays were performed 4 times within a season, commencing 10 days after petal fall (BBCH 72) and continuing up to 5 days before harvest (BBCH 81), at 7–9-day intervals at a rate of 15 g ha⁻¹ in each spray treatment, corresponding to 134.8 mg Se tree⁻¹ over 2 seasons. The Se sprays were applied with or without CaCl₂ (commercial flake, 78% CaCl₂, 28% Ca; CIECH, Warsaw, Poland) at a rate of 5 kg ha⁻¹ per spray. The spray treatments were applied to both sides of the tree row in the morning with 750 L of water ha⁻¹ using a motorised backpack sprayer (Solo 433 H, Kleinmotoren GmbH, Sindelfingen, Germany). Foliar Se sprays were applied when rainfall was not expected within the following 24 h. Air temperature and relative humidity (RH) during the Se sprays were within the ranges of 9–13 °C and 42–69% in 2021 and 9–14 °C and 45–66% in 2022, respectively.

Throughout the entire study period, the same trees were utilized for the tested combinations. The experiment was arranged in a completely randomised block design with four replications, and each combination was replicated within one row. Each plot consisted of 7 trees, with plots within a row separated by a single tree. To mitigate contamination between the rows fertilised with Se, a buffer row was maintained between every two adjacent treated rows.

2.4 Measurements and Observations

2.4.1 Soil Se

Total and soluble Se fractions were determined only in soil-Se-fertilised plots and control plots. Soil samples were collected after 2 cropping seasons (2022), 8 weeks after harvest, from the 0–30 cm layer, 20 cm from an emitter. Each composite sample consisted of 3 subsamples per plot. The soil samples were air-dried, crushed, and sieved through a 2-mm mesh screen. Analytical procedures to determine the total and soluble Se pools were identical to those used at the beginning of the study.

The loss of exogenously applied Se from the surface layer of the soil after two cropping seasons was calculated by subtracting the difference in the total Se concentration between the soil-Se-fertilised plot and the control plot from the Se rate used.

2.4.2 Tree Se

Selenium was determined in both the leaves (with petioles) and the edible parts of the fruit (skin and flesh). At harvest, a sample of 100 leaves from each plot were collected from the mid-portion of the current-season shoots. Fruit Se was determined on a 100-fruit sample from each plot taken

randomly from a 10-kg bulk sample. The fruits designated for analysis were uniform in size and colour and free from visual defects.

The samples of leaves and fruit were individually rinsed with 0.01 M HCl, followed by double-deionized water. Leaves were dried at 60 °C in a forced-draft oven until a constant weight was achieved and then ground in a variable speed rotor mill to pass through a 32-mesh screen. After removing the seeds and pedicels, the fruit were homogenized. In the plant material prepared in the above manner, Se was determined according to the procedure recommended by the Polish Committee for Standardization (PN-EN 16159, 2012). Briefly, 0.5 g of ground leaves or 1 g of homogenized fruit was transferred to a Teflon vessel, and 5 mL of 65% HNO₃ and 2.5 mL of 30% hydrogen peroxide (H₂O₂) were added. The mixture was then microwave-digested in a closed vessel using the following temperature procedure: 130 °C for 5 min, 160 °C for 10 min, and 200 °C for 20 min. After cooling to room temperature, the solution was transferred to 25-mL volumetric flasks, diluted with double-deionised water, and filtrated through a 0.24 µm membrane filter. In the filtrate, Se was determined using ICP-MS, and its amount was expressed on a dry weight (DW) basis for leaves or on a fresh weight (FW) basis for fruit.

2.4.3 Vegetative and Yield Responses

Vegetative responses were assessed based on leaf blade injury, leaf chlorophyll status, and tree vigour. Leaf damage was evaluated 12 weeks after harvest on a 50-leaf sample from each plot, taken from the middle portion of the current season shoots at a height of 2.0–2.5 m above the soil surface on a scale from 1 to 5, where 1 indicated no blade injury and 5 represented chlorosis/necrosis covering more than 75% of the leaf blade area.

The leaf chlorophyll content was assessed on the leaf samples used for determining spray injuries by means of a hand-held SPAD-502 Plus Chlorophyll Meter (Konica Minolta Optics Inc., Osaka, Japan). Two measurements were made on opposite sides of each leaf blade at the mid-point between the tip and the base of the lamina.

Tree vigour was evaluated annually, 8 weeks after harvest, on 3 randomly selected trees from each plot by measuring the total length of current season shoots per tree and calculating the average shoot length. These measurements were performed on the same trees in both years.

The fruit yield was weighed separately from each plot. Fruits with attached stems were hand-picked at the commercial maturity stage, determined based on fruit size and skin colour using the Royal Horticultural Society Colour Charts.

The accuracy of Se determination in plant material was verified using tomato (*Solanum lycopersicum* L.) leaves (SRM 1515) containing 0.054 mg Se kg⁻¹ DW and spinach (*Spinacia* L.) leaves (SRM 1570a) with a concentration of 0.117 mg Se kg⁻¹ DW.

2.4.4 Fruit Quality

Fruit quality assessment included mean weight, soluble solids concentration (SSC), titratable acidity (TA), and incidence of cracked fruit. These parameters were determined within 4 h after harvest.

The mean fruit weight (including pedicel attached) as well as the SSC and TA of the fruit were determined on 100 fruits of uniform size that were free from visual defects, randomly taken from the 10-kg fruit samples per plot. The average fruit weight was calculated using an electronic balance with an accuracy of 0.1 g, SSC was measured in fruit juice using a temperature-compensating digital refractometer (Atago Co., Ltd., Tokyo, Japan), while TA was determined by titrating the fruit homogenate with 0.1 N sodium hydroxide to pH 8.1, using a Mettler Toledo DL 50 Graphix automatic titrator (Mettler-Toledo AG., Schwerzenbach, Switzerland).

Fruit cracking was visually evaluated on 300 fruits randomly collected from a 10-kg bulk sample from each plot.

2.4.5 Statistical Analysis

All data were subjected to a one-way analysis of variance. The data for cracked fruit were transformed using the formula $y = \arcsin x$. Analyses were performed separately for each growing season using Tukey's multiple range test at $P \leq 0.05$ by means of Statistica 10 software (StatSoft Polska, Krakow, Poland).

3 Results

3.1 Soil Se

After two cropping seasons, the total soil Se concentrations in the soil-Se-fertilised plots were higher than those in the control plots (Table 1). Soil Se levels in plots fertilised annually with soil-applied Se were greater than in plots with a single soil Se fertiliser application (Table 1).

Regardless of the frequency of soil Se fertilisation, its application led to an increase in the soluble Se fraction (Table 1). Soil Se solubility did not differ between the soil-Se-supplied combinations (Table 1).

Selenium losses from the 0–30 cm soil layer depended on the frequency of soil Se fertilisation (Table 1). In plots

Table 1 Changes in soil selenium status at a depth of 0–30 cm in a ‘Burlat’ sweet cherry orchard after two cropping seasons following soil selenium fertilisation

Soil Se fertilisation	Soil Se concentration ($\mu\text{g kg}^{-1}$)		Loss of Se used ($\mu\text{g kg}^{-1}$)	
	Total Se	KCl-soluble Se	($\mu\text{g kg}^{-1}$)	%
Annual application	232 $\pm$ 4 a	6.5 $\pm$ 0.3 a	32 $\pm$ 5 b*	25 b**
Single application	182 $\pm$ 5 b	5.9 $\pm$ 0.3 a	82 $\pm$ 6 a*	64 a**
No Se - control	136 $\pm$ 4 c	4.0 $\pm$ 0.3 b	-	-

* Se loss from soil-Se-fertilised plot was calculated by subtracting the difference in the total Se concentration between the given Se-enriched combination and control plot from the used rate of Se

** Percentage Se loss calculated in relation to the used rate of Se

Means with the same letter within each column are not significantly different by Tukey’s multiple range test at $\alpha = 0.05$

receiving a single Se dose, Se losses from the surface soil layer were two and a half times greater than those with annual Se fertilisation (Table 1).

3.2 Tree Se

All Se treatments increased leaf Se concentrations, although their efficiency was affected by the mode of fertilisation (Table 2). The highest leaf Se concentrations were found in trees sprayed with Se and CaCl_2 . Leaf Se concentrations

from Se-sprayed trees and trees fertilised with soil-applied Se in a single dose were comparable. In 2021, leaves from trees supplied annually with soil-applied Se contained less Se than those from trees fertilised at a single dose of Se. However, in the subsequent season, leaf Se concentrations did not differ among the aforementioned combinations.

Compared to fruit from the control trees, fruit from Se-fertilised trees had higher concentrations of this nutrient (Table 2). Similar to the leaves, the highest fruit Se concentrations were found in trees sprayed with Se and CaCl_2 ; compared to the control fruit, its concentrations in those fruits were 15 times higher in 2021 and 16 times higher in 2022. Selenium sprays alone were more effective in increasing fruit Se than soil Se treatments. Fruit Se concentrations were comparable between trees fertilised annually with soil-applied Se and with a single dose.

3.3 Vegetative and Yield Responses

None of the tested Se treatments caused leaf injury (Table 3). Regardless of the mode of Se fertilisation, leaves from trees fertilised with this nutrient had increased SPAD readings (Table 3). SPAD readings for leaves from trees fertilised with Se were comparable.

The total length of one-year-old shoots and the average shoot length per tree were not affected by the Se treatment (Table 3).

Table 2 Effect of selenium fertilisation on concentrations of this nutrient in leaves and fruit of ‘Burlat’ sweet cherry trees

Means with the same letter within each column are not significantly different by Tukey’s multiple range test at $\alpha = 0.05$

Treatment	Leaf Se ($\mu\text{g kg}^{-1}$ DW)		Fruit Se ($\mu\text{g } 100 \text{ g}^{-1}$ FW)	
	2021	2022	2021	2022
Annual soil application of Se	851 $\pm$ 37 c	1023 $\pm$ 27 c	2.7 $\pm$ 0.2 c	2.3 $\pm$ 0.2 c
Single soil application of Se	1232 $\pm$ 28 b	1125 $\pm$ 38 bc	2.5 $\pm$ 0.3 c	2.4 $\pm$ 0.2 c
Se sprays	1243 $\pm$ 31 b	1273 $\pm$ 22 b	3.7 $\pm$ 0.2 b	3.6 $\pm$ 0.3 b
Se sprays with CaCl_2	1546 $\pm$ 34 a	1487 $\pm$ 23 a	4.8 $\pm$ 0.2 a	4.5 $\pm$ 0.3 a
No Se - control	41 $\pm$ 3 d	45 $\pm$ 2 d	0.3 $\pm$ 0.1 d	0.3 $\pm$ 0.1 d

Table 3 Effect of selenium fertilisation on vegetative and fruit responses of ‘Burlat’ sweet cherry trees

Treatment	Leaf injury (1–5)		SPAD value*		Total length of one-year old shoots per tree (m)		Average shoot length (cm)		Fruit yield (kg tree ⁻¹)	
	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
Annual soil Se application	1 $\pm$ 0 a	1 $\pm$ 0 a	47.5 $\pm$ 0.3 a	46.8 $\pm$ 0.2 a	55 $\pm$ 3 a	52 $\pm$ 4 a	53 $\pm$ 3 a	47 $\pm$ 4 a	9.5 $\pm$ 0.6 a	9.2 $\pm$ 0.5 a
Single soil Se application	1 $\pm$ 0 a	1 $\pm$ 0 a	47.3 $\pm$ 0.4 a	46.4 $\pm$ 0.2 a	57 $\pm$ 6 a	47 $\pm$ 4 a	51 $\pm$ 4 a	51 $\pm$ 3 a	9.4 $\pm$ 0.6 a	8.8 $\pm$ 0.8 a
Se sprays	1 $\pm$ 0 a	1 $\pm$ 0 a	47.3 $\pm$ 0.4 a	46.3 $\pm$ 0.4 a	56 $\pm$ 5 a	49 $\pm$ 5 a	55 $\pm$ 2 a	50 $\pm$ 4 a	9.8 $\pm$ 0.7 a	8.9 $\pm$ 0.3 a
Se sprays with CaCl_2	1 $\pm$ 0 a	1 $\pm$ 0 a	47.2 $\pm$ 0.3 a	46.8 $\pm$ 0.2 a	52 $\pm$ 3 a	51 $\pm$ 4 a	57 $\pm$ 3 a	48 $\pm$ 4 a	9.2 $\pm$ 0.5 a	9.3 $\pm$ 0.5 a
No Se - control	1 $\pm$ 0 a	1 $\pm$ 0 a	45.6 $\pm$ 0.3 b	44.5 $\pm$ 0.3 b	55 $\pm$ 4 a	52 $\pm$ 5 a	52 $\pm$ 3 a	53 $\pm$ 3 a	9.1 $\pm$ 0.4 a	9.2 $\pm$ 0.5 a

*The higher value the more intense the green colour of the leaves

Means with the same letter within each column are not significantly different by Tukey’s multiple range test at $\alpha = 0.05$

The Se treatments had no effect on fruit yield (Table 3). Across all combinations, fruit yields averaged 9.4 kg tree⁻¹ (8.3 tons ha⁻¹) in 2021 and 9.1 kg tree⁻¹ (8.1 tons ha⁻¹) in 2022.

3.4 Fruit Quality

The mean fruit weight was not affected by Se treatment, averaging 6.9 g and 6.5 g in 2021 and 2022, respectively (Table 4). Fruit from Se-fertilised trees had a higher SSC than those from control trees (Table 4). The efficiency of the tested Se treatments in increasing SSC in fruit was comparable. None of the Se treatments affected TA in the fruit (Table 4). Only the combined sprays of Se and CaCl₂ reduced fruit cracking (Table 4).

4 Discussion

In non-seleniferous soils, Se concentrations typically range from 10 µg to 2000 µg kg⁻¹ (Fordyce 2013), with a world average of 400 µg kg⁻¹ (Shahid et al. 2018). According to Kabata-Pendias (1998), most agriculturally utilized soils contain 100–600 µg Se kg⁻¹. In Poland, Dudka (1992) found that Se concentrations in topsoil ranged from 70 µg to 410 µg kg⁻¹. Soils with a Se concentration below 125 µg kg⁻¹ are considered Se deficient (Broadley et al. 2006). In the present study, the soil Se concentration from the control plots averaged 137 µg kg⁻¹.

In our study, despite increased total soil Se concentrations in soil-Se-fertilised plots (Table 1), its levels remained within the native range reported by Dudka (1992) for soils in Poland. Theoretically, after applying 500 g of Se per ha, the Se concentration in the topsoil layer (0–30 cm) at a bulk density of 1.3 g cm⁻³ should increase by 128 µg kg⁻¹, reaching 265 µg kg⁻¹. In plots fertilised annually with soil-applied Se or in a single dose, the total soil Se levels were 232 µg kg⁻¹ and 182 µg kg⁻¹, respectively (Table 1), indicating that Se losses from the topsoil layer were 33 µg kg⁻¹ (129 g Se ha⁻¹) for plots fertilised annually with Se and 83 µg kg⁻¹ (324 g Se ha⁻¹) for plots supplied with Se in the first year of the study. Since tree vigour, fruit yield,

fruit Se concentration, and leaf Se concentrations in the second year of the experiment did not differ between soil-Se-fertilisation combinations (Tables 2 and 3), we suggest that the increased loss of soil-applied Se in a single dose resulted from enhanced leaching of this nutrient down the soil profile over the two growing seasons. This is likely because the selenate ions used in our study are considered mobile in the soil due to their low affinity for adsorption on soil constituents, especially in coarse-textured soils low in organic matter (Dinh et al. 2019; Shahid et al. 2018).

Soil Se availability is most often measured using a KCl solution as an extractant (Kulp and Pratt 2004). In Finland, in Se-deficient soils, the KCl-soluble Se fraction accounted for 0.4–2.5% of the total soil Se (Keskinen et al. 2011). Similarly, in the United Kingdom, Stround et al. (2010) found that the available soil Se fraction accounted for 1.1–3.4% of the total Se amount. However, in China, in low Se soils, the contribution of the available Se pool to total Se status was as high as 10.9% (Qin et al. 2017). In our study, the KCl-soluble Se fraction accounted for 2.9% of the total soil Se amount in the control plots, 2.8% in plots fertilised annually with soil-applied Se, and 3.2% in plots supplied with a single Se dose, indicating that the KCl-soluble Se fraction did not correspond to the total soil Se concentration. This conclusion is not surprising, as it has been documented that soil Se solubility is influenced not only by the total Se amount but also by physicochemical and biological soil properties (Keskinen et al. 2009; Tolu et al. 2014; Wang et al. 2018).

Fleshy fruits are generally considered to be a poor Se source (Kieliszek 2019), which was confirmed in our study, as the fruit from the control trees contained only 0.3 µg Se 100 g⁻¹ FW (Table 2). This Se level is slightly higher than that found in apples (0.2 µg 100 g⁻¹ FW) by Wójcik (2023) under similar soil conditions but lower than those in kiwi (*Actinidia deliciosa*), mandarin (*Citrus reticulata*), and pear fruit (0.4–0.6 µg 100 g⁻¹ FW) reported by Pappa et al. (2006) in Greece.

For many plant species, soil Se application is less successful in biofortification than foliar sprays of this nutrient (Gupta and Gupta 2017; Ryant et al. 2020). In our study, under conditions of coarse-textured soil low in organic matter, soil Se fertilisation (regardless of application mode) was

Table 4 Effect of selenium fertilisation on quality parameters of ‘Burlat’ sweet cherry fruit at harvest

Treatment	Mean fruit weight (g)		SSC (%)		TA (%)		Percentage fruit cracked	
	2021	2022	2021	2022	2021	2022	2021	2022
Annual soil application of Se	6.8 ± 0.2 a	6.4 ± 0.1 a	14.3 ± 0.3 a	16.0 ± 0.1 a	0.62 ± 0.04 a	0.69 ± 0.03 a	7.2 ± 1.2 a	5.3 ± 0.8 a
Single soil application of Se	7.0 ± 0.2 a	6.6 ± 0.3 a	14.1 ± 0.2 a	16.1 ± 0.2 a	0.63 ± 0.04 a	0.65 ± 0.03 a	6.5 ± 0.9 a	4.8 ± 0.7 a
Se sprays	6.8 ± 0.3 a	6.5 ± 0.3 a	14.2 ± 0.2 a	15.9 ± 0.2 a	0.63 ± 0.04 a	0.68 ± 0.03 a	6.9 ± 0.6 a	4.2 ± 0.6 a
Se sprays with CaCl ₂	6.9 ± 0.2 a	6.6 ± 0.2 a	14.1 ± 0.2 a	16.1 ± 0.2 a	0.59 ± 0.03 a	0.65 ± 0.04 a	2.7 ± 0.7 b	0.5 ± 0.3 b
No Se - control	7.0 ± 0.2 a	6.6 ± 0.2 a	13.5 ± 0.3 b	15.2 ± 0.2 b	0.60 ± 0.04 a	0.64 ± 0.04 a	7.9 ± 1.0 a	5.2 ± 0.6 a

Means with the same letter within each column are not significantly different by Tukey's multiple range test at $\alpha = 0.05$

less effective in biofortifying fruit than preharvest sprays (Table 2), despite using a considerably higher rate of this nutrient, suggesting that a considerable portion of the soil-applied Se was leached beyond the main root system of the trees and fixed by soil constituents (mainly by organic matter), reducing its availability. Furthermore, compared to the Se concentrations in leaves and fruit from control trees, Se levels in these organs from soil-fertilised trees increased by a factor of 24 and 8.2, on average, respectively, indicating that Se was preferentially transported to leaves. This preferential Se movement to leaf tissues is particularly observed in trees that produce fruit low in protein (Ryant et al. 2020).

In the present experiment, the highest fruit Se concentrations was found from trees sprayed with Se and CaCl_2 (Table 2). Since Se sprays alone were less effective in increasing this nutrient in the fruit than sprays of Se and CaCl_2 (Table 2), we conclude that CaCl_2 enhanced the Se absorption rate by the fruit. Similarly, Lawson et al. (2016) demonstrated that the addition of a CaCl_2 -based fertilizer to an aqueous spray solution containing iodine increased the uptake of this micronutrient by the edible parts of butterhead lettuce (*Lactuca sativa*). The influence of CaCl_2 on the uptake of accompanying nutrients from the spray solution by plants can be attributed to the hygroscopic properties of the salt (Schönherr 2001). According to Fernandez et al. (2020), the point of deliquescence (POD) for CaCl_2 , defined as the RH at which the liquefaction of the salt into a solute occurs, is as low as 30% (at 20 °C). This means that if the RH is above this POD value, CaCl_2 remains in liquefaction form. Given that, during the period of the spray treatments tested, the RH was above 42%, we suggest that CaCl_2 in the Se-containing solutions maintained/prolonged their liquid state on the plant surface, thereby improving the Se uptake rate by the epidermal cells of the fruit.

The recommended dietary intake (RDI) of Se for adults is 55 µg per day (European Commission 2011). Consuming one serving of sweet cherry fruit (100 g) daily from control trees, trees supplied with soil-applied Se (for both tested application modes), and trees sprayed with Se alone or combined with CaCl_2 covered 0.5%, 4.5%, 6.7%, and 8.5% of the RDI, on average, respectively. However, according to the guidelines of the Institute of Food and Nutrition in Poland (2017), fruit and vegetables should constitute half of the daily diet for adults, with fruit comprising one-fourth of this amount. Based on this recommendation, the daily Se intake from fruit should be 6.9 µg. Thus, the Se contents in one serving of sweet cherry fruit from control trees, soil-Se-fertilised trees, and trees sprayed with Se or Se with CaCl_2 accounted for 4.3%, 36%, 52%, and 68%, on average, of the target fruit Se amount, respectively. It is worth noting that in many European countries, dietary guidelines recommend consuming more vegetables and fruit than half of

the diet. Following this guideline, consuming Se-enriched cherry fruit, particularly those sprayed with Se alone or with CaCl_2 , can fully meet the requirements for this nutrient from the fruit.

Under certain conditions, supplying plants with Se at low rates leads to improved physiological and biochemical processes related to plant growth (Ryant et al. 2020; Shahid et al. 2018). In our experiment, the leaves of Se-fertilised trees had enhanced SPAD readings (Table 3). Since SPAD readings of leaves are positively correlated with chlorophyll status, as documented for many plant species (Richardson et al. 2002; Uddling et al. 2007), we can state that Se fertilisation enhanced leaf chlorophyll content in our study. An increase in leaf chlorophyll caused by exogenously applied Se was also been found for sweet basil (*Ocimum basilicum* L.), lettuce (*Lactuca sativa* L.), spinach (*Spinacia oleracea* L.), and chicory (*Cichorium intybus* L.) by Hawrylak-Nowak (2008), Hawrylak-Nowak (2013), Saffaryazdi et al. (2012), and Malorgio et al. (2009), respectively.

The increased leaf chlorophyll concentrations in Se-fertilised trees can be attributed to the maintenance of the integrity and functionality of chlorophyll molecules through the scavenging of reactive oxygen species (ROS) by the antioxidative action of Se. The effect of Se on scavenging ROS in leaf tissues is particularly found under conditions of environmental stress, such as drought, salinity, high heavy metal content in soil, and extreme air temperatures (Lanza and dos Reis 2021; Khan et al. 2023). Considering the soil and climatic conditions in our experiment, we suggest that the increased chlorophyll status in the leaves of Se-fertilised trees probably occurred under conditions of moderate water stress. This is likely because the total amounts of precipitation in the given years of the study were lower (560 mm in 2021 and 467 mm in 2022) than the estimated water requirement of 700 mm for high-density sweet cherry orchards grown in coarse-textured soils under Polish conditions (Treder 2022).

It is worth emphasizing that the fruit from Se-fertilised trees had enhanced SSC (Table 4). The positive impact of Se fertilisation on SSC in fruits has also been demonstrated in peach by Pezzarossa et al. (2012), pear by Deng et al. (2019), in citrus by Wen et al. (2021), tomato by Zhu et al. (2016), grape (*Vitis vinifera* L.) by Zhu et al. (2019), and strawberry (*Fragaria ananassa*) by Mimmo et al. (2017).

Rain-induced fruit cracking is due to the direct absorption of water through the fruit skin (Sekse et al. 2005). ‘Burlat’ fruit are considered sensitive to cracking (Rozpara 2013). It has been demonstrated that sweet cherry fruit with high SSC are more susceptible to cracking (Simon 2006). However, in our studies, fruit from Se-fertilised trees were not more cracked despite enhanced SSC (Table 4), indicating that

SSC in sweet cherry fruit is not a crucial factor in increasing their cracking, at least for the ‘Burlat’ variety.

In the present study, only sprays of Se with CaCl_2 reduced fruit cracking (Table 4). Since Se sprays alone did not affect fruit cracking, the reduction in cracking of fruit sprayed with Se with CaCl_2 can be attributed to the presence of Ca. The effect of preharvest Ca sprays, including CaCl_2 , in reducing rain-induced fruit cracking has been well-documented in sweet cherries grown under various soil and climatic conditions (Correia et al. 2018; Wójcik et al. 2013).

5 Conclusions

This study demonstrated that both soil application and preharvest Se sprays improved the nutrition of this nutrient in ‘Burlat’ sweet cherry trees, although Se sprays were more effective. Furthermore, CaCl_2 addition to the spray solutions increased the Se absorption rates by leaves and fruit and reduced the incidence of rain-induced fruit cracking. Soil application and preharvest Se sprays also increased SSC in the fruit. Thus, we conclude that in sweet cherry orchards, at least for the ‘Burlat’ variety, grown in low Se soils, preharvest Se sprays can be recommended to biofortify fruit with Se and increase SSC in the fruit. For varieties prone to rain-induced fruit cracking or cultivated in dry regions with low RH during the season, combined sprays of Se and CaCl_2 should be preferred.

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Data Availability The data that support the findings of this study are available on request from the corresponding author.

Declarations

Conflict of Interest The author declares that there is no conflict of interest.

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