

## EFFECTS OF FOLIAR SPRAYS OF IODINE AND SELENIUM ON VEGETATIVE, REPRODUCTIVE, AND FRUIT RESPONSES OF KORDIA SWEET CHERRY

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### ABSTRACT

Iodine (I) and selenium (Se) deficiency in the human body constitutes a significant health, social, and economic challenge. This study aimed to examine the effects of sprays of I and Se, applied alone or combined, on vegetative growth, yield, fruit quality, and fruit biofortification of Kordia sweet cherry (*Prunus avium* L.). The trees were sprayed with: (i) I and/or Se, four times in the early season at rates of 25 g ha<sup>-1</sup> and 15 g ha<sup>-1</sup> per spray, respectively; (ii) I and Se, once, 3 days before harvest, at rates of 50–100 g ha<sup>-1</sup> and 30–60 g ha<sup>-1</sup>, respectively; and (iii) I and Se in the early season as in combination (i), plus before harvest as in combination (ii), using the lowest rates of these nutrients. It was found that none of the spray combinations affected tree vigour, fruit yield, mean fruit weight, and fruit firmness and acidity. Early-season Se sprays delayed leaf fall and increased both chlorophyll level in leaves and soluble solid concentration in fruit. Pre-harvest spray treatments decreased leaf chlorophyll concentration, and caused leaf damage and defoliation. The highest amounts of I and Se in fruit were found in trees sprayed with these nutrients in the early season plus before harvest. Lower efficiency in biofortifying fruit with I and Se was observed for early-season sprays and for pre-harvest spray applied at the highest rates of I and Se. It is concluded that early-season sprays, alone or combined with a pre-harvest spray of I and Se, can be recommended for late-ripening sweet cherry cultivars.

**Keywords:** chlorophyll level, fruit biofortification, nutrients, *Prunus avium* L., soluble solid concentration

### INTRODUCTION

Sweet cherry (*Prunus avium* L.) is a deciduous stone fruit tree widely cultivated in temperate zones [Habib et al. 2017]. Its fruit is valued not only for exceptional organoleptic properties, such as flavour, aroma, and texture, but also for its nutritional and dietary value, as it is a rich source of vitamins (e.g., vitamin C and provitamin A), minerals, dietary fibre, and bioactive compounds, including flavonols and anthocyanins [Díaz-Mula et al. 2010, Serra et al. 2011]. For these reasons, sweet cherry is recognised as a functional food with health-promoting properties [Blando and Oomah 2019].

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Selenium (Se) and iodine (I) are essential trace elements for humans. Iodine deficiency results in endemic goitre and impairment of growth, reproductive capacity, and cognitive functions [Hatch-McChesney and Lieberman 2022]. Selenium plays a crucial role in preventing cardiovascular diseases and certain types of cancer, as well as in supporting brain development, reproduction, and immune function [Rayman 2000]. It is estimated that nearly 2 billion individuals are at risk of I deficiency disorders [Ittermann et al. 2020], while 0.5–1 billion people worldwide exhibit clinical signs of Se deficiency [Oumer et al. 2024].

It is believed that the primary cause of human malnutrition in I and Se is the limited phytoavailability of these microelements in soil, which is multifactorial and depends on numerous factors such as pH, salinity, redox potential, microbiological activity, and the contents of clay particles, iron-manganese oxides, organic matter, and antagonistic ions in the soil solution [Eich-Greatorex et al. 2007, Stroud et al. 2010, Medrano-Macias et al. 2016, Gonzali et al. 2017, Shahid et al. 2018, Song et al. 2018].

One of the strategies to mitigate human malnutrition in I and Se is the enrichment of edible crops with these nutrients through the application of I- and Se-containing fertilisers (agronomic biofortification). The use of such fertilisers is recommended in many I- and Se-deficient regions, as they are easy to apply and highly effective in biofortifying plants [Hartikainen 2005, White and Broadley 2009, Cakmak et al. 2017, Smoleń et al. 2019a, Oztekin and Buyuktuncer 2025]. Since foliar absorption of exogenously applied nutrients is generally more efficient than soil application [Fageria et al. 2009, Fernández and Eichert 2009, Kannan 2010], foliar fertilisation of I and Se should be preferred, at least for field crops with edible parts above the soil surface [White and Broadley 2009, Medrano-Macias et al. 2016, Cakmak et al. 2017, Gonzali et al. 2017, Smoleń et al. 2019b].

To date, no research has been conducted on the responses of sweet cherry to pre-harvest I sprays. Despite numerous studies on foliar sprays of Se in agricultural and horticultural crops, only two reports have addressed the effectiveness of sprays of this nutrient in sweet cherry cultivation [Wójcik 2024, Zhang et al. 2025]. These studies demonstrated that pre-harvest Se sprays were highly effective in biofortifying the fruit. However, the effects of combined Se and I sprays have not been investigated, despite the fact that, from an economic point of view, the simultaneous use of multiple nutrients in spray solutions should be preferable [Prom-u-thai et al. 2020]. Therefore, the goals of this study were to examine the effects of I sprays alone and combined I and Se sprays, applied either early in the season or shortly before harvest, on vegetative growth, yield, fruit quality, and fruit biofortification in sweet cherry.

## MATERIAL AND METHODS

### Study site and soil conditions

The study was conducted over two consecutive growing seasons (2024 and 2025) at the Dąbrowice Experimental Station (latitude 51°55'N, longitude 20°6'E, elevation 136 m above sea level), affiliated with the National Institute of Horticultural Research in Skierniewice, Poland. In the first year of the study, the mean annual air temperature and total precipitation were 10.5 °C and 367 mm, whereas in the following year they were 9.7 °C and 496 mm, respectively. During the vegetation period (April–September), the average air temperature and rainfall were 17 °C and 202 mm in 2024, and 15.5 °C and 318 mm in 2025, respectively (Table 1).

The experimental sweet cherry field was planted in 2015 in Albic Luvisols with the following properties in the 0–30 cm layer: 65% sand (0.05–2 mm), 19% silt (0.002–0.05 mm), 16% clay (<0.002 mm), bulk density 1.3 g cm<sup>-3</sup>, organic carbon 14 g kg<sup>-1</sup>, pH 6.7, redox potential 473 mV, 3.1 g kg<sup>-1</sup> active iron (Fe) oxides, 1.8 g kg<sup>-1</sup> active aluminium (Al) oxides, 13 mg kg<sup>-1</sup> active manganese (Mn) oxides, 67 mg kg<sup>-1</sup> available phosphorus (P), 103 mg kg<sup>-1</sup> available potassium (K), 51 mg kg<sup>-1</sup> available magnesium (Mg), 2.6 mg kg<sup>-1</sup> total I, 0.17 mg kg<sup>-1</sup> water-soluble I, 137 µg kg<sup>-1</sup> total Se, and 4.1 µg kg<sup>-1</sup> KCl-soluble Se. The soil parameters were determined on a composite sample consisting of 10 subsamples taken in autumn of 2023 from the surface of the herbicide strips along tree rows. Except for the determination of total and soluble Se in the soil, the analytical procedures and apparatus used to assess soil parameters were described previously by Wójcik and Wójcik [2021]. Protocols for determining total and soluble Se in the soil were provided by Wójcik [2024].

### Plant material and orchard management

Kordia sweet cherry trees grafted onto the Mazzard F12/1 rootstock (*Prunus avium*) were used as the experimental plant material. Kordia is commonly grown in many sweet cherry-producing regions because the fruits are large, firm, sweet, and resistant to splitting and brown rot caused by *Monilinia* spp. [Quero-García et al. 2017].

**Table 1.** Monthly air temperatures and precipitation in Kordia sweet cherry orchard

Parameter	I*	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
2024												
Average air temperature (°C)	-1.0	5.2	5.8	10.5	16.3	18.7	20.6	19.4	16.2	9.3	3.0	2.2
Precipitation (mm)	32.6	32.8	31.8	23.8	39.8	48.6	23.2	30.6	36.2	20.8	27.6	19.4
2025												
Average air temperature (°C)	2.5	-0.5	6.2	11.0	11.3	18.0	19.2	18.3	15.3	8.5	4.1	2.2
Precipitation (mm)	19.6	6.8	40.8	34.4	71.8	53.4	90.2	6.8	61.6	61.4	35.8	13.6

\* Month: I – January, II – February, III – March, IV – April, V – May, VI – June, VII – July, VIII – August, IX – September, X – October, XI – November, XII – December

The trees were planted in rows oriented north-south (each 150 m long) at a spacing of 4.5 m between rows and 2.5 m between trees within rows (888 trees ha<sup>-1</sup>). The tree canopies were trained as a spindle to a height of 3.5 m.

In the experimental field, Regina sweet cherry trees, planted in every second row, served as pollinators for the Kordia trees. To ensure successful pollination, two beehives were placed in the field before the onset of flowering.

Protection treatments against pathogens and pests were carried out according to the recommendations for integrated fruit production provided by Czarnocka et al. [2023].

Soil management involved maintaining herbicide-treated strips along the tree rows and mowing the sod in the alleyways. The 1.5-m-wide herbicide strips were maintained from early spring until current-season shoot growth was completed. The sod was mowed until autumn, when it reached a height of 15–20 cm.

Soil moisture was maintained at nearly field capacity through surface drip irrigation using a tensiometer (Soil-moisture Equipment Corp., Santa Barbara, CA, USA) inserted at a depth of 30 cm, 15 cm from an emitter.

Annually, ammonium nitrate and K-sulphate were broadcast together at the swollen-breaking bud stage over the soil surface of the herbicide strips at rates of 60 kg nitrogen (N) ha<sup>-1</sup> and 80 kg K ha<sup>-1</sup>. The amounts of N and K fertilisers used, as well as the mode and timing of application, were in accordance with the recommendations of Wójcik [2009] for integrated fruit production carried out under conditions of low organic matter content and optimal K availability in the soil.

### The combinations and experimental layout

Trees were sprayed with I as K-iodate (KIO<sub>3</sub>, 59.3% I, Sigma-Aldrich, Poznań, Poland) and/or Se as sodium selenate (Na<sub>2</sub>SeO<sub>4</sub>, 41.8% Se; Sigma-Aldrich, Poznań, Poland) in the following combinations: (i) four early-season sprays of I or Se, applied from 14 days after petal fall (BBCH 72) to 6 days before harvest (BBCH 81), at 12–15 day intervals, at a rate of 25 g I ha<sup>-1</sup> or 15 g Se ha<sup>-1</sup> per spray (100 g ha<sup>-1</sup> and 60 g ha<sup>-1</sup> per season, respectively), (ii) early-season combined I and Se sprays applied according to the schedule and rates described above, (iii) pre-harvest I and Se spray, applied 3 days before picking, at the rates per ha: 50 g I + 30 g Se, 75 g I + 45 g Se, or 100 g I + 60 g Se, referred to as low, medium, and high rates, respectively, and (iv) early-season I and Se sprays as in combination (ii) plus pre-harvest I and Se sprays at the rates of 50 g ha<sup>-1</sup> and 30 g ha<sup>-1</sup>, respectively. Trees that were not sprayed with either I or Se served as controls. The seasonal rates of I and Se fertilisers and the solution concentrations of the given spray treatments are presented in Table 2.

The spray treatments were applied by means of a motorised backpack sprayer (Solo 433H, Kleinmotoren GmbH, Sindelfingen, Germany), equipped with a flat-fan nozzle, at a pressure of 10 bar, using approximately 750 L of water per ha. To improve the wetting of the fruit surface, the non-ionic surfactant Tween® 20 (polyoxyethylene sorbitan monolaurate; Sigma-Aldrich, St. Louis, Missouri, USA) was added to the spray solutions at a rate of 0.3 L ha<sup>-1</sup>.

**Table 2.** Iodine and selenium rates and spray solution concentrations used in foliar sprays in Kordia sweet cherry orchard

Combination	I rate (g ha <sup>-1</sup> per season)	Se rate (g ha <sup>-1</sup> per season)	Spray solution concentration (%)
Early-season sprays of I	100	–	0.0055
Early-season sprays of Se	–	60	0.0047
Early-season sprays of I and Se	100	60	0.0102
	50	30	0.0204
Pre-harvest spray of I and Se	75	45	0.0306
	100	60	0.0408
Early-season sprays of I and Se plus pre-harvest spray of I and Se at low rates	150	90	0.0102* 0.0204**
Control	–	–	–

\* for early-season sprays, \*\* for preharvest spray

In both years of the study, the same trees were used for the tested combinations. The experiment was conducted using a completely randomised block design with four replications, with one replicate per combination in each row. Each plot consisted of three trees. To minimise cross-contamination of the applied sprays, plots within a row were separated by one tree, and a buffer row was placed between every two adjacent treated rows.

### Measurements and observations

Leaf blade injury, leaf chlorophyll concentration, leaf drop, and tree vigour were used as vegetative indices. Leaf spray damage was evaluated 20 days after harvest on a sample of 60 leaves from each plot, collected from the middle portion of current-season shoots. Damage was rated on a scale of 1 to 5, where 1 indicated no visible injury and 5 represented chlorosis/necrosis covering more than 75% of the leaf blade area.

Leaf chlorophyll level was assessed on the samples used for the determination of spray damage, using a handheld SPAD-502 Plus Chlorophyll Meter (Konica Minolta Optics Inc., Osaka, Japan). Two readings per leaf were taken on opposite sides of the blade, at the midpoint between the tip and the base of the lamina.

Leaf drop was assessed on 40 labelled current-season shoots per plot, located outside the tree canopy, at two time points per season: when 5–10% of the leaves had fallen from the control trees, and 30 days later.

Vigour was evaluated 4 weeks after harvest by measuring the total length of current-season shoots from the central tree within each plot.

Fruit yield was measured for each plot by harvesting fruits with stems attached at the stage of commercial maturity, evaluated based on fruit size and skin colour using the Royal Horticultural Society Colour Charts.

The fruit quality parameters included the mean fruit weight (with pedicel), firmness, soluble solid concentration (SSC), and titratable acidity (TA). These parameters were determined on 30 fruits for firmness measurement and 100 fruits for the remaining characteristics, randomly selected from 10-kg bulk samples per plot.

The average fruit weight was calculated using an electronic balance with an accuracy of 0.01 g (WPS2100/C/2; Radwag, Radom, Poland).

The firmness of the fruit, defined as the maximum force required to puncture the skin, was measured using a texture analyser (Zwick Roell Z010; Ulm, Germany) equipped with a 50 N load cell and a flat-ended cylindrical probe with a diameter of 3.18 mm. Firmness was measured once per fruit, placed horizontally on a stationary steel plate at mid-height, near the suture, at a crosshead speed of 50 mm min<sup>-1</sup>. Results were expressed in newtons (N).

The soluble solid concentration was measured in fruit juice using a temperature-compensated digital refractometer (Atago Co., Ltd., Tokyo, Japan), and 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). Results were expressed as malic acid content.

Iodine and Se in the fruit were determined in the edible parts (skin and flesh) based on 100 fruits, uniform in size and colour, free of defects, randomly taken from a 10-kg bulk sample from each plot. Fruit samples were rinsed with

0.01 M HCl, followed by double-deionised water. After drying with a paper towel, seeds and pedicels were removed, and the samples were then homogenised using a mill (Retsch GM 200, RETSCH GmbH, Haan, Germany).

Iodine was determined according to the procedure proposed by the Polish Committee for Standardisation [2008]. One gram of homogenised fruit, 15 mL of distilled water, and 1 mL of 25% TMAH (tetra-methylammonium hydroxide) were added to 30-mL Falcon tubes. After thorough mixing, the samples were heated at 90 °C for 3 h. The tubes were then filled with deionised water, mixed, centrifuged at 4500 rpm for 15 min and filtered through a 0.24 µm membrane.

Selenium was determined according to the protocol recommended by the Polish Committee for Standardisation [2012]. Briefly, 1 g of homogenised fruit was placed in a Teflon vessel with 5 mL of 65% HNO<sub>3</sub> and 2.5 mL of 30% H<sub>2</sub>O<sub>2</sub>, and microwave digested in a closed system using a three-step temperature programme: 130 °C for 5 min, 160 °C for 10 min, and 200 °C for 20 min. After cooling, the digest was transferred to 25-mL volumetric flasks, diluted with double-deionised water, and filtered through a 0.24 µm membrane filter.

The measurements of I and Se in the obtained filtrates were performed by inductively coupled plasma mass spectrometry (XSeries 2 ICP-MS; Thermo Fisher Scientific, Waltham, MA, USA), and their concentrations in the fruit were expressed on a fresh weight (FW) basis.

The accuracy of I and Se measurements was verified using tomato (*Solanum lycopersicum*) leaves (SRM 1573a) with a concentration of 0.085 mg I kg<sup>-1</sup> on a dry weight (DW) basis, and apple (*Malus domestica*) leaves (Standard Reference Material – SRM 1515) containing 0.05 mg Se kg<sup>-1</sup> DW.

All data were subjected to one-way analysis of variance (ANOVA). Leaf drop data were transformed using the formula  $y = \arcsin(x)$ . Analyses were performed separately for each growing season, and mean comparisons were analysed using Tukey's multiple range test at  $P < 0.05$  with Statistica 10 software (StatSoft Polska, Krakow, Poland).

## RESULTS

The early-season sprays of I and/or Se caused no leaf injury. However, combinations of pre-harvest sprays of I and Se, regardless of the doses used, resulted in leaf damage, with injury severity increasing as the rates of these nutrients increased. Leaf injury from trees sprayed with I and Se early in the season plus before harvest was comparable to that observed after pre-harvest sprays of I and Se at low rates (Table 3).

At the first assessment time point of leaf fall, only the pre-harvest sprays of Se and I at medium and high rates led to defoliation (Table 3). At the second time point, all pre-harvest spray combinations caused defoliation, with the greatest leaf loss observed in trees treated with I and Se at high rates. Compared to the control, early-season Se sprays, with or without I, inhibited the leaf fall process, whereas the combination of early-season I and Se sprays together with pre-harvest sprays of these nutrients did not affect leaf drop (Table 3).

Early-season I sprays did not influence leaf SPAD readings, while early-season Se sprays, with or without I, led to increased values of this parameter (Table 3). The pre-harvest spray combinations resulted in a decrease in leaf SPAD readings, with the greatest decline found in trees treated with the highest rates of Se and I (Table 3).

The total length of current-season shoots and fruit yield per tree did not differ among the tested combinations, averaging 44.9 m and 9.4 kg in 2024, and 50 m and 7.8 kg in 2025, respectively (Tables 3 and 4).

The mean fruit weight was not affected by the tested treatments (Table 4). The spray combinations also had no effect on fruit firmness and acidity (Table 4). Only early-season Se sprays, with or without I, resulted in increased SSC in fruit (Table 4).

All I spray treatments enhanced fruit I concentration (Table 5). Early-season I sprays, with or without Se, were more effective in improving fruit I levels than pre-harvest I and Se sprays at low and medium rates, and were as effective as a single pre-harvest spray of these nutrients at high rates. The highest fruit I concentrations were found when early-season Se and I sprays together with a pre-harvest spray of these nutrients were applied (Table 5).

All Se spray combinations increased fruit Se concentrations (Table 5). The highest fruit Se concentrations were found in the combination of early-season I and Se sprays combined with a pre-harvest spray of these nutrients. Compared to the above combination, pre-harvest spray treatments had a weaker impact on fruit Se concentration, although their effectiveness positively corresponded to the Se rate used. Early-season Se sprays, with or without I, had a greater influence on fruit Se concentration than pre-harvest I and Se sprays at low and medium rates, and simultaneously had a comparable impact to pre-harvest I and Se sprays at high rates (Table 5).

**Table 3.** Effects of iodine and selenium sprays on phytotoxicity, leaf drop, leaf greenness and vigour of Kordia sweet cherry trees

Spray treatment	Leaf injury (1-5)		Defoliation (%)				SPAD value**		Total length of one-year old shoots per tree (m)	
	2024	2025	2024		2025		2024	2025	2024	2025
			I term*	II term*	I term	II term				
Early-season sprays of I	1.0 ±0d	1.0 ±0d	7 ±1b	36 ±2c	7 ±2b	32 ±4b	41 ±1b	42 ±1b	44 ±4a	51 ±4a
Early-season sprays of Se	1.0 ±0d	1.0 ±0d	6 ±1b	27 ±3d	6 ±1b	20 ±3c	45 ±2a	45 ±1a	46 ±3a	54 ±6a
Early-season sprays of I and Se	1.0 ±0d	1.0 ±0d	7 ±1b	25 ±2d	7 ±2b	24 ±4c	45 ±2a	46 ±2a	47 ±3a	49 ±2a
Pre-harvest spray of I and Se at low rates	1.5 ±0.1c	1.6 ±0.3c	8 ±1b	44 ±4b	7 ±1b	33 ±3b	38 ±1c	39 ±1c	44 ±5a	49 ±2a
Pre-harvest spray of I and Se at medium rates	2.1 ±0.2b	2.2 ±0.2b	17 ±2a	45 ±2b	15 ±3a	41 ±4a	38 ±1c	37 ±1c	43 ±3a	51 ±6a
Pre-harvest spray of I and Se at high rates	2.9 ±0.1a	2.9 ±0.1a	17 ±2a	56 ±4a	20 ±3a	42 ±2a	35 ±1d	33 ±1d	44 ±4a	49 ±2a
Early-season sprays of I and Se plus pre-harvest spray of I and Se at low rates	1.6 ±0.1c	1.4 ±0.1c	7 ±2b	35 ±2c	9 ±1b	32 ±5b	38 ±1c	37 ±2c	46 ±2a	48 ±4a
Control	1.0 ±0d	1.0 ±0d	7 ±2b	37 ±2c	7 ±2b	32 ±4b	41 ±1b	42 ±1b	45 ±5a	49 ±3a

\* I and II terms of defoliation were assessed when 5-10% of leaves from the control trees fallen and 30 d later, respectively.

\*\* 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 Tukeys multiple range test at  $\alpha = 0.05$ .

**Table 4.** Effects of iodine and selenium sprays on yielding and fruit quality of the Kordia sweet cherry trees

Spray treatment	Fruit yield (kg tree <sup>-1</sup> )		Mean fruit weight (g)		Firmness (N)		Soluble solid concentration (%)		Titratable acidity (%)	
	2024	2025	2024	2025	2024	2025	2024	2025	2024	2025
Early-season sprays of I	9.4 ±0.6a	8.1 ±0.3a	9.7 ±0.4a	10.1 ±0.3a	4.7 ±0.2a	4.8 ±0.2a	17.0 ±0.2b	17.3 ±0.2b	0.88 ±0.02a	0.83 ±0.02a
Early-season sprays of Se	9.2 ±0.6a	7.9 ±0.7a	9.9 ±0.3a	10.3 ±0.4a	4.8 ±0.2a	4.7 ±0.2a	19.5 ±0.3a	19.1 ±0.2a	0.85 ±0.02a	0.81 ±0.02a
Early-season sprays of I and Se	9.2 ±0.6a	7.5 ±0.5a	9.8 ±0.4a	10.5 ±0.4a	4.7 ±0.3a	4.9 ±0.1a	19.1 ±0.2a	19.2 ±0.2a	0.87 ±0.02a	0.84 ±0.02a
Pre-harvest spray of I and Se at low rates	9.4 ±0.5a	7.7 ±0.4a	9.7 ±0.3a	10.3 ±0.4a	4.6 ±0.3a	4.8 ±0.1a	17.4 ±0.3b	17.1 ±0.2b	0.86 ±0.02a	0.84 ±0.02a
Pre-harvest spray of I and Se at medium rates	9.3 ±0.4a	7.6 ±0.4a	9.7 ±0.3a	10.3 ±0.2a	4.7 ±0.3a	4.8 ±0.1a	17.3 ±0.4b	17.5 ±0.2b	0.86 ±0.04a	0.83 ±0.02a
Pre-harvest spray of I and Se at high rates	9.3 ±0.6a	7.8 ±0.5a	9.4 ±0.3a	10.4 ±0.2a	4.6 ±0.2a	4.9 ±0.1a	17.3 ±0.4b	17.3 ±0.2b	0.87 ±0.04a	0.84 ±0.04a
Early-season sprays of I and Se plus pre-harvest spray of I and Se at low rates	9.6 ±0.5a	7.8 ±0.4a	9.7 ±0.3a	10.3 ±0.1a	4.6 ±0.3a	4.8 ±0.1a	17.3 ±0.2b	17.2 ±0.2b	0.86 ±0.04a	0.82 ±0.02a
Control	9.7 ±0.4a	7.7 ±0.5a	9.7 ±0.3a	10.3 ±0.2a	4.7 ±0.2a	4.8 ±0.2a	17.4 ±0.4b	17.3 ±0.2b	0.86 ±0.03a	0.84 ±0.02a

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

**Table 5.** Effects of iodine and selenium sprays in Kordia sweet cherry orchard on fruit concentration of these nutrients

Spray treatment	I concentration ( $\mu\text{g } 100 \text{ g FW}$ )		Se concentration ( $\mu\text{g } 100 \text{ g FW}$ )	
	2024	2025	2024	2025
Early-season sprays of I	17.3 $\pm$ 1.2b	17.6 $\pm$ 1.6b	0.1 $\pm$ 0e	0.1 $\pm$ 0e
Early-season sprays of Se	1.1 $\pm$ 0.1e	1.0 $\pm$ 0.1e	3.6 $\pm$ 0.2b	3.6 $\pm$ 0.1b
Early-season sprays of I and Se	17.8 $\pm$ 1.3b	17.2 $\pm$ 1.3b	3.4 $\pm$ 0.3b	3.5 $\pm$ 0.2b
Pre-harvest spray of I and Se at low rates	9.1 $\pm$ 0.8d	9.4 $\pm$ 0.6d	2.4 $\pm$ 0.1d	2.4 $\pm$ 0.2d
Pre-harvest spray of I and Se at medium rates	12.1 $\pm$ 1.5c	13.5 $\pm$ 1.6c	2.9 $\pm$ 0.1c	2.9 $\pm$ 0.1c
Pre-harvest spray of I and Se at high rates	15.9 $\pm$ 1.3b	16.6 $\pm$ 1.1b	3.5 $\pm$ 0.2b	3.5 $\pm$ 0.2b
Early-season sprays of I and Se plus pre-harvest spray of I and Se at low rates	23.7 $\pm$ 1.2a	23.5 $\pm$ 1.2a	5.6 $\pm$ 0.2a	5.5 $\pm$ 0.3a
Control	0.7 $\pm$ 0.4e	0.9 $\pm$ 0.2e	0.1 $\pm$ 0 e	0.09 $\pm$ 0e

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

## DISCUSSION

### Soil I and Se

The amounts of I and Se in edible plant tissues primarily depend on their bioavailability in the soil [Weng et al. 2009, Lopes et al. 2017]. Typical total I concentrations in soils range from 0.5 mg to 20 mg  $\text{kg}^{-1}$ , with an average of approximately 5 mg  $\text{kg}^{-1}$  [Whitehead 1984]. In Poland, agricultural soils contain 0.8–10 mg  $\text{kg}^{-1}$  of I [Kabata-Pendias and Pendias 1999]. In our study, the total soil I concentration (2.6 mg  $\text{kg}^{-1}$ ) was slightly above the lower limit of this range, and may be considered a low I status.

In most non-seleniferous soils, total Se concentrations range from 10  $\mu\text{g}$  to 2000  $\mu\text{g } \text{kg}^{-1}$  [White and Broadley 2009], with an average of 400  $\mu\text{g } \text{kg}^{-1}$  [Shahid et al. 2018]. Soil Se concentrations below 125  $\mu\text{g } \text{kg}^{-1}$  are considered deficient [Broadley et al. 2006], although Lyons et al. [2003] suggest that under certain soil conditions, plant Se deficiency may occur even at total soil Se concentrations as high as 600  $\mu\text{g } \text{kg}^{-1}$ . In the present study, the total soil Se concentration was 137  $\mu\text{g } \text{kg}^{-1}$ , indicating that this level can be regarded as deficient or low. Notably, the total soil Se concentration in our study was lower than the average Se concentration in coarse-textured soils (172  $\mu\text{g } \text{kg}^{-1}$ ) reported in Finland, where Se deficiency in edible plant parts was widespread until the late 1980s [Ylärinta 1983].

In the present study, the proportion of water-soluble I relative to its total concentration was 6.5%, which is consistent with the statement by Duborská et al. [2021] that, in most mineral soils, the soluble I pool accounts for up to 10% of total soil I content.

In the case of Se, the KCl-soluble fraction in the soil, considered plant-available, was low (4.1  $\mu\text{g } \text{kg}^{-1}$ ), representing approximately 3% of the total soil Se content. Similar proportions of the KCl-soluble Se fraction in mineral soils have been reported by Ylärinta [1983], Martens and Suarez [1997], Yanai et al. [2015], and Ali et al. [2017].

### Tree responses

Although I and Se are not considered essential micronutrients for plants, numerous studies have demonstrated that under certain soil and climatic conditions, they can exert beneficial effects on some physiological, biochemical, and/or metabolic processes in plant tissues [Gupta and Gupta 2017, Zhang et al. 2023].

Given the limited phloem mobility of both I and Se [Gupta and Gupta 2017, Shahid et al. 2018], which restricts their retranslocation from leaves and fruits to woody tissues, the tested sprays of these micronutrients in 2024 likely had a negligible effect on sweet cherry tree responses in the following year.

In our study, none of the early-season spray combinations caused leaf burn and defoliation (Table 3), indicating that the applied rates of I and Se (25 g and 15 g  $\text{ha}^{-1}$  spray $^{-1}$ , respectively) were safe for cherry trees. However, Zhang et al. [2025] reported that in sweet cherries grown under semi-arid conditions, Se spray treatments at a dose of 8 g  $\text{ha}^{-1}$  spray $^{-1}$  and a solution concentration of 0.005% resulted in leaf and fruit damage. In our study, the Se rate applied in early-season sprays was nearly twice that used by Zhang et al. [2025] while the spray solution

concentrations were comparable. Thus, we suggest that the absence of spray burn in our study may be attributed to the use of the surfactant Tween 20 in the spray solutions, as this adjuvant is commonly known to reduce the surface tension between above-ground plant parts and aqueous solutions, thereby improving, among other things, the uniformity of spray deposition on plant surfaces. As a result, the amount of spray solution deposited per unit area of leaf and fruit is reduced, thereby lowering the risk of phytotoxicity. This suggestion is particularly justified for sweet cherry, which produces relatively large leaves. Another factor that might differentiate the susceptibility of sweet cherry leaves and fruits to spray-induced damage is the sprayer used in our study (a motorised backpack sprayer operating at a high water volume). On one hand, this sprayer ensures thorough coverage of all leaves and fruits within the tree canopy, especially in the upper zone, but on the other hand it results in considerable solution loss due to spray drift beyond the canopy as demonstrated by Godyń et al. [2025].

In our study, only early-season Se sprays, with or without I, increased leaf SPAD readings (Table 3), indicating an enhanced chlorophyll level. Because early-season I sprays caused no change in leaf SPAD readings, the increased leaf chlorophyll levels were related to the application of Se in early-season sprays. Similarly, Zhang et al. [2025] demonstrated an increase in chlorophyll content in the leaves of sweet cherry grown under Se-deficient conditions following sprays of this nutrient at a rate of 4.8 g ha<sup>-1</sup> per spray. An increase in leaf chlorophyll induced by exogenously applied Se has also been reported for chicory (*Cichorium intybus* L.) by Malorgio et al. [2009], Chinese boxthorn (*Lycium chinense*) by Dong et al. [2013], spinach (*Spinacia oleracea* L.) by Saffaryazdi et al. [2012], and sweet basil (*Ocimum basilicum* L.) and lettuce (*Lactuca sativa* L.) by Hawrylak-Nowak [2008, 2013]. The positive relationship between Se content in leaves and chlorophyll level can be attributed to the maintenance of the structural integrity and functional stability of chlorophyll molecules by Se, which results from the enhanced capacity to scavenge reactive oxygen species, as reported by Khan et al. [2023] and Zhang et al. [2025].

Despite the elevated chlorophyll levels in the leaves of trees sprayed with Se early in the season, neither tree vigour nor cropping was affected by this treatment (Tables 3 and 4). In contrast, Zhang et al. [2025] reported that under Se-deficient conditions, pre-harvest sprays of this nutrient at a rate of 4.8 g ha<sup>-1</sup> per spray increased the fruit yield of sweet cherries, which was related to an enhanced photosynthesis rate. In the above-mentioned studies, the growth of sweet cherry trees was not evaluated.

In our study, only early-season Se sprays, regardless of the presence or absence of I, increased SSC in fruit (Table 4), suggesting that greater production of assimilates, resulting from enhanced leaf chlorophyll levels, was primarily transported to the fruit. Under similar soil conditions, Wójcik [2024] also demonstrated elevated SSC in Burlat sweet cherry fruit from trees sprayed with Se early in the season, despite the lack of vegetative response. Elevated SSC in Se-sprayed trees has also been reported for sweet cherry by Zhang et al. [2025], peach (*Prunus persica* Batsch.) by Pezzarossa et al. [2012], pear (*Pyrus communis* L.) by Deng et al. [2019], and citrus (*Citrus reticulata*) by Wen et al. [2021].

In contrast to early-season Se sprays, with or without I, all pre-harvest spray combinations of I and Se decreased leaf SPAD readings, likely due to spray burns that consequently reduced leaf chlorophyll content (Table 3) and photosynthetic potential. Simultaneously, pre-harvest sprays of I and Se had no effect on fruit size and internal quality characteristics (Table 4), indicating that leaf burns, induced during 3 days before harvest (even up to about 25% of the leaf blade) do not pose a risk of changes in the basic commercial attributes of sweet cherry fruit, at least for the Kordia variety. Under similar soil and climatic conditions, Wójcik et al. [2014] and Wójcik and Wójcik [2021] also found no negative effects of foliar sprays of I or Ca applied 7–10 days before harvest on the appearance and quality of apples and pears, despite the observed leaf injury.

It is worth noting that, despite leaf damage and partial defoliation of trees sprayed with I and Se before harvest, their growth and fruit yields over the subsequent two seasons were not affected (Tables 3 and 4). This suggests that the reduction in leaf assimilatory area immediately after the termination of shoot elongation in mature sweet cherry trees did not affect flower bud formation and, presumably, the reserve of assimilates in the woody organs of the tree. Sitarek [2004] also reported that, under Polish conditions, partial leaf damage immediately before or after the harvest of late-ripening sweet cherry varieties did not affect the yield potential of trees and fruit quality, including size, SSC, and acidity.

### Fruit biofortification

Sweet cherry fruit, as a fleshy fruit, is regarded as a poor dietary source of both I and Se [Kunachowicz et al. 2017], which was confirmed in our experiment, as the concentrations of these nutrients in fruit from the control trees averaged only 0.8 µg I 100 g<sup>-1</sup> FW and 0.1 µg Se 100 g<sup>-1</sup> FW (Table 4). Under similar soil conditions, Wójcik [2023] reported that the native concentrations of I and Se in apples were 2 µg 100 g<sup>-1</sup> FW and 0.2 µg 100 g<sup>-1</sup> FW,

respectively. In Germany, Budke et al. [2020, 2021] reported that I concentrations in apples varied from 0.14  $\mu\text{g}$  to 0.63  $\mu\text{g}$   $100\text{ g}^{-1}$  FW. Pappa et al. [2006] found that Se concentrations in fleshy fruits of different species were within the range of 0.14–0.63  $\mu\text{g}$   $100\text{ g}^{-1}$  FW.

According to the European Commission [2011], the recommended dietary intakes of I and Se for adults are 150  $\mu\text{g}$  and 55  $\mu\text{g}$  per day, respectively. Instytut Żywności i Żywienia (National Food and Nutrition Institute) [2017], along with many national and international organizations promoting healthy eating, recommend that, for adults, fruits and vegetables should constitute half of the daily diet, with fruit accounting for one-fourth of this portion. Accordingly, the daily intakes of I and Se from fruit are 18.7  $\mu\text{g}$  and 6.9  $\mu\text{g}$ , respectively. Thus, based on these calculations, the amounts of I and Se in one portion of sweet cherry fruit (100 g) from the control trees accounted for 3.7% and 1.4% of the target daily intakes of fruit-derived I and Se, respectively.

In our study, early-season I sprays increased fruit I level up to 17.5  $\mu\text{g}$   $100\text{ g}^{-1}$  FW on average (Table 5), covering 94% of the daily requirement for fruit-derived I. In the case of Se, fruit sprayed with this nutrient in the early season contained, on average, 3.5  $\mu\text{g}$  Se  $100\text{ g}^{-1}$  FW (Table 5), accounting for 51% of the recommended daily intake from fruit-derived Se. Under similar soil conditions, Wójcik [2023] found comparable Se levels in Burlat sweet cherry fruit following early-season sprays of this nutrient. However, Zhang et al. [2025] reported that despite applying a considerably lower total Se dose in foliar sprays over the season (25.6  $\text{g ha}^{-1}$  versus 60  $\text{g ha}^{-1}$ ), Se levels in Mei Zao sweet cherry fruit reached 21.5  $\mu\text{g}$  per 100 g FW. The reasons for such pronounced differences in the uptake efficiency of exogenously-applied Se by sweet cherry fruit between our study and that of Zhang et al. [2025] are difficult to explain.

In the present study, pre-harvest sprays of I and Se met from 49% to 89% and from 35% to 51% of the daily requirements for fruit-derived I and Se, respectively, with the highest levels recorded in the treatments applying the greatest rates of these nutrients. It is worth noting that pre-harvest I and Se sprays at the highest rates were comparably effective in biofortifying fruit as early-season sprays of I or Se (Table 5). The high efficiency of single pre-harvest spraying using high nutrient rates in increasing their fruit level has also been demonstrated for Ca in apples and pears [Wójcik et al. 2009, 2014], and I and Se in apples [Wójcik and Wójcik 2021, Wójcik et al. 2024].

Fruit levels of I and Se following early-season plus pre-harvest sprays of these nutrients met, on average, 126% and 80% of the daily requirements for fruit-derived I and Se, respectively. This indicates that the above sprays were highly effective in biofortifying fruit and can be considered for recommendation in sweet cherries. However, multiple sprays of I and Se during the season generate production costs. To reduce these costs, it is justified to combine the application of I and Se with pesticides and/or  $\text{CaCl}_2$  fertiliser, which is commonly used in sprays to decrease rain-induced fruit cracking. Combined I and Se sprays with  $\text{CaCl}_2$  should be particularly preferred because, as demonstrated by Wójcik [2023, 2024] the addition of this salt improved the uptake of exogenously applied I and Se by apples.

## CONCLUSIONS

The results of this study demonstrated that in mature Kordia sweet cherries grown in soil with low levels of I and Se, four sprays of these nutrients, applied alone or combined during fruit growth (up to 6 days before harvest) at rates of 25  $\text{g I ha}^{-1}$  and 15  $\text{g Se ha}^{-1}$  per spray, did not affect tree vigour and fruit yield, although Se-containing sprays increased leaf chlorophyll concentration and SSC in the fruit. A single combined I and Se spray at rates of 50–100  $\text{g ha}^{-1}$  and 30–60  $\text{g ha}^{-1}$ , respectively, applied 3 days before harvest, damaged leaves and caused partial defoliation, which, however, did not affect tree growth, yield, and fruit quality. The above sprays with the highest rates of I and Se were as effective in biofortifying fruit with these nutrients as early-season sprays. Thus, given that early-season sprays of Se improved both SSC and the level of this nutrient in fruit, these treatments can be implemented in sweet cherries. To biofortify fruit with I, early-season sprays of Se can be applied combined with I.

In the case of the impact of the tested I and Se spray combinations on fruit enrichment with these nutrients, the most successful strategy was the application of early-season sprays plus an additional spray of I and Se before harvest at rates of 50  $\text{g ha}^{-1}$  and 30  $\text{g ha}^{-1}$ , respectively. This approach largely covered the target dietary requirements for Se and fully met them for I from fruit-derived sources. Therefore, we also conclude that the proposed schedule of combined I and Se sprays during the early season and shortly before harvest should be recommended in sweet cherry orchards for biofortifying fruit with these nutrients, at least for the Kordia variety and other late-ripening cultivars. However, further research should focus on verifying the above-mentioned strategy for early-ripening sweet cherry varieties, as well as on reducing the costs of Se and I sprays by using Se- and I-containing spray solutions with pesticides, plant growth regulators, and/or stimulants.

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## AUTHORS' CONTRIBUTIONS

P.W. – conception and assumptions, analysing the results, writing a manuscript, W.P., A.S. – developing the methods, J.F., Z.B., A.S, W.P. – conducting research.

## DATA AVAILABILITY

We declare that this work has not been prepared using generative artificial intelligence (genAI) tools.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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