

## Article

# The Effect of Methyl Anthranilate-Based Repellent on Chemical Composition and Selected Physiological Parameters of Sweet Cherry (*Prunus avium* L.)

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**Abstract:** Maturing sweet cherry fruits are exposed to damage caused by birds. In order to repel birds from orchards, it is increasingly common to use repellents based on plant extracts and compounds that undergo complete biodegradation. One such compound is methyl anthranilate (2-aminobenzoic acid methyl ester), which has an irritating effect on birds' senses of taste and smell. The objective of this study was to assess the effect of methyl anthranilate-based repellent (commercial name: Goose Chase/Fruit Shield) on the chemical composition and selected physiological parameters of sweet cherry cv. 'Burlat'. The repellent containing methyl anthranilate (26.4%) was applied in the form of a foliar spray seven days prior to fruit harvest at a 1% concentration (500 dm<sup>3</sup>·ha<sup>-1</sup> of working liquid). In the study, methyl anthranilate-based repellent was found to have no influence on the chemical composition of fruits and leaves or the selected physiological parameters of sweet cherry cv. 'Burlat'. Moreover, the use of methyl anthranilate did not affect the cracking susceptibility of sweet cherry fruits. This compound may constitute an interesting alternative to support sweet cherry orchard protection against birds and in organic cultures.

**Keywords:** repellent; cv. Burlat; physiological parameters; chemical composition; cracking of fruits



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## 1. Introduction

The ripening fruits of numerous tree and shrub species are exposed to damage from birds. It is an increasingly common practice to use repellents based on plant extracts and fully biodegradable compounds to repel birds from agricultural crops, from areas where they are undesirable (e.g., golf courses), and from areas where they may pose a direct threat to the health and safety of people (airports) [1–4]. One such compound is methyl anthranilate (2-aminobenzoic acid methyl ester). This compound has an irritating effect on the senses of taste and smell in birds. It constitutes a natural component of fruits of numerous plant species, including grapevines, oranges, and strawberries. This compound undergoes rapid biodegradation (within 7–8 days), which, in the case of protecting numerous orchards, constitutes an undisputed advantage. Moreover, it has not been demonstrated to have any negative impact on human health. It is a commonly used substance in the production of foods and drugs [1,5,6]. Many authors [1,2,5,7–10] have stated that the efficiency of repellents containing methyl anthranilate depends on several factors, including the protected plant species and bird species causing damage in its culture, the applied concentration, the frequency of spraying, and the weather (the spray must be repeated

after rainfall). Presently, several methyl anthranilate-based repellents are available on the market, including Bird Stop and RejeX-it. Currently, no reports are available regarding the influence of methyl anthranilate on plants; however, Avery [1], Curtis et al. [2], and Avery et al. [5] stated that at higher concentrations, it may demonstrate phytotoxic effects for blueberry and grapevines, resulting in leaf damage. Considering all these factors, this study was conducted to assess the influence of a methyl anthranilate-based repellent (commercial name: Goose Chase/Fruit Shield) on the chemical composition and selected physiological parameters of sweet cherry cv. 'Burlat'.

## 2. Materials and Methods

### 2.1. Experimental Design

A single-factor experiment was established in the form of random blocks in five repetitions (one repetition consisted of two trees). The two-year experiment was conducted in a production orchard in the township of Karwowo (53°22' N and 14°26' E), near Szczecin in northwest Poland. The study was conducted on fourteen-year-old trees of sweet cherry cv. 'Burlat' that were grafted on a "PHL-A" rootstock and grown at a spacing of 4 × 3 m. The repellent containing methyl anthranilate (26.4%), with the commercial name Goose Chase/Fruit Shield (manufactured by Bird-X Inc., Elmhurst, IL, USA), was applied in the form of a foliar spray at a 1% concentration (500 dm<sup>3</sup> · ha<sup>-1</sup> of working liquid) seven days prior to fruit harvest. A blank solution containing water was applied in the form of a foliar spray at the same time and dose. Each treated tree was at a distance of 15 m from a corresponding control tree, which prevented their direct contact.

### 2.2. Chemical Determinations in Fruit and Leaves

For the study, 100 fully ripe fruits were collected from each tree. Fifty fruits were homogenized to perform chemical determinations in the fresh fruits. The remaining 50 fruits were dried and mineralized in order to determine the content of mineral nutrients. Sweet cherry leaves were collected directly after the fruit harvest from the middle portion of annual increments and from the crown perimeter at the height of 1.5–2.0 m. A total of 25 leaves were obtained from each tree.

The dry weight content of the plant material was determined using the dry oven test at 105 °C until a constant weight was obtained. The total soluble solids (TSS) content was determined by a refractometer Atago Pol 1. The total acidity (TA) of the fruits was determined by the titration of a water extract of sweet cherry homogenate with 0.1 N NaOH to the end point of pH 8.1. On the basis of the content of the extract and the total acidity of the fruits, the maturity index (MI) was calculated from the formula  $MI = TSS/TA$  [11]. The content of L-ascorbic acid in the fruits was measured by a reflectometer Merck RQflex 10 [12]. A fruit sample (5 g) and 20 cm<sup>3</sup> of oxalic acid (1%) were mixed, homogenized for 1 min, and then filtered. Polyvinylpyrrolidone (PVPP) (500 mg) was added to 10 cm<sup>3</sup> of the filtered sample to remove phenols and 5–7 drops of H<sub>2</sub>SO<sub>4</sub> (25%) were added to reduce the pH to below 1. The results were expressed as mg L-ascorbic acid 100 g<sup>-1</sup> FW. The content of nitrates and nitrites was quantified with the reflectometer RQflex 10 (Merck) according to the protocol for the juice of red fruit (Merck, Nitrate in Red Colored Fruit Juices).

For the determination of polyphenols and flavonoids, and antioxidant activity and capacity, methanol extracts were prepared. For the extraction of the antioxidants, 5 g of fruits was treated with 50 cm<sup>3</sup> methanol at room temperature by stirring. This procedure was repeated at least five times until the extraction solvent became colorless. The obtained extracts were filtered over Whatman No.1 filter paper, the filtrate was collected, and then methanol was removed by a rotary evaporator at 40 °C. The residues were dissolved in methanol in a 50 cm<sup>3</sup> volumetric flask. The total polyphenol content of the fruit extracts was determined using the Folin–Ciocalteu reagent [13]. The fruit extracts (100 cm<sup>3</sup>) were mixed with 500 cm<sup>3</sup> of the Folin–Ciocalteu reagent and 1.5 cm<sup>3</sup> of 20% sodium carbonate. The mixture was shaken thoroughly and topped up to 10 cm<sup>3</sup> 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 concentrations of gallic acid. The total flavonoid contents were determined by the Kumaran and Karunakaran [14] method using quercetin as a reference compound. The fruit extract ( $1\text{ cm}^3$ ) was mixed with  $1\text{ cm}^3$  of a 2% aluminium trichloride solution in methanol and a drop of acetic acid, and then diluted with methanol to  $25\text{ cm}^3$ . The absorption at 415 nm was read after 40 min. Blank samples were prepared from  $1\text{ cm}^3$  of the plant extract and a drop of acetic acid, and then diluted to  $25\text{ mL}$  with methanol. Data were used to estimate the total flavonoid contents using a standard curve obtained from various concentrations of quercetin. The total antioxidant capacity of the extracts was assessed by the phosphomolybdenum method according to the procedure of Prieto et al. [15]. The assay is based on the reduction of Mo(VI)–Mo(V) by the extract and the subsequent formation of a green phosphate/Mo(V) complex at acid pH. Then,  $0.3\text{ cm}^3$  of the extract was combined with  $3\text{ cm}^3$  of the reagent solution (0.6 M of sulfuric acid, 28 mM of sodium phosphate, and 4 mM of ammonium molybdate). The tubes containing the reaction solution were incubated at  $95\text{ }^\circ\text{C}$  for 90 min. Then, the absorbance of the solution was measured against the blank at 695 nm using a spectrophotometer (Shimadzu, UV-1800, Kyoto, Japan) after cooling to room temperature. Methanol ( $0.3\text{ cm}^3$ ) was used in the place of the extract as the blank. The antioxidant activity was 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. [16]. The plant extract ( $0.1\text{ cm}^3$ ) was added to  $3\text{ cm}^3$  of a 0.004% methanol solution of DPPH. The absorbance at 517 nm 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 nitrogen content in the fruits and leaves was determined using the Kjeldahl distillation method: after ‘wet’ mineralization in a concentrated sulfuric acid, the phosphorus content was determined using the colorimetric method described by Barton, and the sulfur content was determined with nephelometry. The potassium and calcium contents in the leaves and fruits were determined using flame photometry, while magnesium, copper, manganese, zinc, nickel, cadmium, and lead contents were determined with atomic absorption spectrometry (Perkin Elmer AAS 300, PerkinElmer, Waltham, MA, USA). The stock solution used to determine the total content of the analyzed elements (P, K, Ca, Mg, S, Cu, Mn, Zn, Ni, Cd, and Pb) was obtained through the mineralization of the plant material in nitric (V) acid and perchloric (VII) acid at a ratio of 3:1.

### 2.3. Determination of Physiological Parameters

The determination of physiological parameters of sweet cherry leaves was performed on two dates: during the harvesting maturity phase (1st date of measurement) and 14 days after the fruit harvest (2nd date of measurement). The fully developed leaves from the mid-portion of long shoots, distributed on the perimeter of the crown at the central portion of its height, were used for the analyses. Measurements of leaf gas exchange parameters consisted of determining the intensity of  $\text{CO}_2$  assimilation (A) and transpiration (E), the stomatal conductance for water ( $g_s$ ), and the concentration of  $\text{CO}_2$  in intercellular spaces of chlorenchyma ( $c_i$ ). These were performed using a portable gas analyzer TPS-2, PP Systems, and working in an open system equipped with a PLC4 measurement chamber. The analyzer cuvette conditions were set to a constant supply of carbon dioxide at a concentration of 370 ppm ( $\mu\text{mol CO}_2\text{ mol}^{-1}\text{ air}$ ), humidity equal to ambient humidity, and lighting equal to 2053 PAR ( $\mu\text{mol m}^{-2}\text{ s}^{-1}$ ) that was provided by a light unit that came with the cuvette. The air temperature was measured with a silicon bandgap temperature sensor. The measurements were performed at an air temperature of  $22\text{--}25\text{ }^\circ\text{C}$ . The measurements were performed in 50 repetitions. On the basis of the obtained assimilation and transpiration intensity results, the photosynthetic water use efficiency was calculated ( $\omega_F$ ) using the ratio of the intensity of assimilation to transpiration. The content of assimilation pigments was

determined for the same leaves for which gas exchange parameters had been determined. The chlorophyll content was determined using the method described by Arnon et al. [17] with the modification provided by Lichtenthaler and Wellburn [18]. The carotenoid content was determined using the Hager and Meyer–Bethenrath method [19].

#### 2.4. Determination of Fruit Cracking Index

In order to determine the cracking index (CI), 50 additional fruits were collected from each tree and soaked in distilled water for 6 h. The CI was calculated using the formula  $CI = (5a + 3b + c) \times 100/5d$ , where *a* is the number of fruits with cracks after 2 h, *b* is the number of fruits with cracks after 4 h, *c* is the number of fruits with cracks after 6 h, and *d* is the total number of fruits in the sample [20].

#### 2.5. Statistical Methods

The resulting data were subjected to a one-way analysis of variance in a random block arrangement. To determine the significance of differences between the means, Duncan's confidence half-intervals were calculated at a significance level  $p = 0.05$ . Statistical calculations were carried out using Statistica 12.5 software (StatSoft Poland, <http://www.statsoft.pl/>). The data shown in the tables consist of mean values from two years of research.

### 3. Results and Discussion

According to Aronov and Clark [21] and Ahmad et al. [10], methyl anthranilate is environment-friendly, undergoes rapid biodegradation, and is easily obtainable (easy to produce). Askham [22] believes that the use of methyl anthranilate decreases sweet cherry damage by birds and does not influence the appearance, color, or taste of fruits. This opinion is shared by Mikiciuk et al. [23], who demonstrated that the use of a repellent based on methyl anthranilate significantly reduces the extent of damage caused by birds in sweet cherry orchards. Moreover, as stated by the aforementioned authors, it does not influence the weight of sweet cherry fruits.

Sweet cherry fruits are valued due to their delicious taste and nutritional value, which are both largely dependent on the sugar, organic acid, anthocyanin, and polyphenol contents of the fruit [24,25]. The obtained results concerning the content of extracts in fruits, their total acidity, and the value of MI have been confirmed in the literature. According to numerous authors, the extract content in sweet cherry cv. 'Burlat' ranges from 11.2% to 18.4%, the total acidity ranges from 0.25 to 1.2 g of malic acid·100 g<sup>−1</sup> FW, and the MI ranges from 16.4 to 29.0 [24–34]. In this study, it was found that the applied methyl anthranilate-based repellent had no influence on the content of extract in fruits and their total acidity. Moreover, the agent did not have any impact on the fruit MI (Table 1).

The antioxidative properties of sweet cherry fruits depend on a range of factors, including the cultivar, the cultivation system, weather conditions, and agrotechnical procedures [24,34,35]. The content of ascorbic acid in sweet cherry cv. 'Burlat' fruits was similar to the data provided in the literature [36] and it ranged from 22.6 to 23.3 mg (100 g)<sup>−1</sup> FW. No influence of the applied repellent on its content in the fruits was found (Table 1). According to many authors [24,26,31,36–38], the total content of polyphenols in 'Burlat' cultivar fruits ranges from 48 to 141 mg gallic acid·100 g<sup>−1</sup> FW. In the present study, its level ranged from 81.8 to 82.6 mg gallic acid (100 g<sup>−1</sup>) FW, and, similar to ascorbic acid, no influence of methyl anthranilate on this trait was found. According to Nizioł-Lukaszewska et al. [39], the content of flavonoids in 'Burlat' cv. fruits is 16.4 mg (100 g<sup>−1</sup>) FW, while Telesiński et al. [40] stated a value of 15.5 mg quercetin (100 g<sup>−1</sup>) DM. A fruit characterized by a similar flavonoid content was used in the present study (Table 1). The repellent did not influence the content of these compounds in the fruits. No impact of the methyl anthranilate-based repellent on the capacity and antioxidative activity of the tested fruits was found (Table 1). The values of capacity and antioxidative activity determined in the study are in line with data provided in the literature [34,35,40,41]. The

obtained results indicate that methyl anthranilate does not influence the content of sugars, organic acids, and antioxidants and their proportions in the fruits. Furthermore, it does not affect their taste and health properties, which are the most significant characteristics for consumers. When methyl anthranilate undergoes biodegradation, it loses its specific odor and does not affect the sensory parameters of fruits [22].

**Table 1.** The influence of methyl anthranilate on biochemical parameters and content of nitrates and nitrites in fruits of sweet cherry cv. ‘Burlat’.

Feature	Control	Methyl Anthranilate	Feature	Control	Methyl Anthranilate
Total soluble solids (TSS) (%)	13.5 ± 0.49 a *	13.5 ± 1.28 a	L-ascorbic acid (mg (100 g) <sup>-1</sup> FW)	22.6 ± 2.93 a	23.3 ± 2.52 a
Total acidity (TA) (g malic acid (100 g) <sup>-1</sup> FW)	0.77 ± 0.10 a	0.74 ± 0.10 a	Total polyphenols (mg gallic acid (100 g) <sup>-1</sup> FW)	81.8 ± 4.67 a	82.6 ± 3.26 a
Maturity index (MI)	17.5 ± 1.90 a	18.2 ± 2.27 a	Total flavonoids (mg quercetin (100 g) <sup>-1</sup> FW)	15.6 ± 0.74 a	15.3 ± 1.31 a
N-NO <sub>3</sub> (mg (100 cm) <sup>-3</sup> juice)	2.49 ± 0.51 a	2.33 ± 0.60 a	Antioxidant capacity (mg equivalent of ascorbic acid (100 g) <sup>-1</sup> FW)	158.1 ± 9.16 a	160.6 ± 8.72 a
N-NO <sub>2</sub> (mg (100 cm) <sup>-3</sup> juice)	0.43 ± 0.12 a	0.40 ± 0.11 a	Antioxidant activity (%DPPH)	39.7 ± 2.24 a	39.0 ± 3.54 a

\* Data are presented as mean ± SD. Means assigned identical letters do not differ significantly at the level of significance  $p = 0.05$ .

The content of dry weight and mineral components in the leaves and fruits of sweet cherry depends on a variety of factors, including cultivar, rootstock used, fertilization, foliar feeding, and irrigation [42–45]. Use of the repellent did not influence the content of dry weight, nitrates, nitrites, macro and micronutrients, or heavy metals in the leaves and fruits of the ‘Burlat’ cultivar (Tables 1–3). The amount of mineral components determined in the leaves and fruits remained within the values provided in the literature [42–46].

The repellent did not influence the content of the determined assimilation pigments (chlorophyll a, b, total chlorophyll, and carotenoids) for sweet cherry leaves (Table 4). Moreover, no impact of this agent on the ratio of chlorophyll a to chlorophyll b could be found. The ratio remained in the range from 2.19 (1st date of measurement, methyl anthranilate) to 2.37 (2nd date of measurement, control) and it was lower than the values provided for the ‘Burlat’ cultivar by Gonçalves et al. [47] and Viljevac et al. [48]. In the study of Pilarski et al. [49], the value of chlorophyll a/b ratio in leaves of sweet cherry cv. Hedelfinger was approximately 2.60–2.70.

**Table 2.** The influence of methyl anthranilate on content of dry matter and macronutrients in leaves and fruit of sweet cherry cv. ‘Burlat’.

Feature	Leaves		Fruit	
	Control	Methyl Anthranilate	Control	Methyl Anthranilate
Dry matter (%)				
	37.4 ± 1.99 a *	37.9 ± 2.48 a	13.1 ± 0.63 a	13.8 ± 0.41 a
Macronutrient (g·kg <sup>-1</sup> DM **)				
N	31.8 ± 3.86 a	33.5 ± 3.12 a	9.18 ± 1.69 a	9.05 ± 1.35 a
P	3.71 ± 0.21 a	3.86 ± 0.20 a	3.14 ± 0.31 a	3.12 ± 0.17 a
K	16.3 ± 0.84 a	16.5 ± 0.58 a	11.2 ± 1.10 a	11.7 ± 0.60 a
Ca	19.4 ± 0.75 a	21.8 ± 3.16 a	2.03 ± 0.28 a	1.86 ± 0.13 a
Mg	4.91 ± 0.37 a	4.96 ± 0.30 a	1.45 ± 0.23 a	1.43 ± 0.32 a
S	0.88 ± 0.015 a	0.89 ± 0.014 a	0.02 ± 0.001 a	0.02 ± 0.002 a

\* Data are presented as mean ± SD. Means assigned identical letters do not differ significantly at the level of significance  $p = 0.05$ . \*\* DM—dry matter.



**Table 3.** The influence of methyl anthranilate on content of micronutrients, lead, and cadmium in leaves and fruit of sweet cherry cv. ‘Burlat’.

Element	Leaves		Fruit	
	Control	Methyl Anthranilate	Control	Methyl Anthranilate
	(mg kg <sup>-1</sup> DM **)			
Mn	14.3 ± 0.49 a*	14.4 ± 0.37 a	2.42 ± 0.29 a	2.56 ± 0.27 a
Zn	14.1 ± 1.13 a	14.7 ± 0.51 a	2.71 ± 0.34 a	2.68 ± 0.22 a
Cu	7.41 ± 0.44 a	7.75 ± 0.32 a	4.36 ± 0.69 a	4.32 ± 0.45 a
Ni	1.89 ± 0.09 a	1.85 ± 0.05 a	2.15 ± 0.33 a	2.14 ± 0.31 a
Pb	2.50 ± 0.28 a	2.57 ± 0.24 a	1.28 ± 0.04 a	1.28 ± 0.10 a
Cd	0.02 ± 0.002 a	0.03 ± 0.005 a	0.02 ± 0.002 a	0.02 ± 0.002 a

\* Data are presented as mean ± SD. Means assigned identical letters do not differ significantly at the level of significance  $p = 0.05$ . \*\* DM—dry matter.

**Table 4.** The influence of methyl anthranilate on the content of assimilation pigments in leaves of sweet cherry cv. ‘Burlat’.

Assimilation Pigment	I Term of Measurement		II Term of Measurement	
	Control	Methyl Anthranilate	Control	Methyl Anthranilate
Chlorophyll a (mg g <sup>-1</sup> FW)	1.87 ± 0.196 a *	1.82 ± 0.182 a	1.90 ± 0.091 a	1.97 ± 0.108 a
Chlorophyll b (mg g <sup>-1</sup> FW)	0.79 ± 0.058 a	0.83 ± 0.053 a	0.82 ± 0.068 a	0.89 ± 0.196 a
Chlorophyll a/b	2.37 ± 0.075 a	2.19 ± 0.079 a	2.32 ± 0.244 a	2.21 ± 0.358 a
Chlorophyll a + b (mg g <sup>-1</sup> FW)	2.66 ± 0.255 a	2.65 ± 0.235 a	2.72 ± 0.064 a	2.86 ± 0.290 a
Carotenoids (mg g <sup>-1</sup> FW)	1.16 ± 0.083 a	1.10 ± 0.111 a	1.17 ± 0.161 a	1.20 ± 0.147 a

\* Data are presented as mean ± SD. Means assigned identical letters do not differ significantly at the level of significance  $p = 0.05$ .

The conducted study did not demonstrate that the repellent affected the intensity of CO<sub>2</sub> assimilation and transpiration or the photosynthetic water use efficiency in photosynthesis for the leaves of sweet cherry cv. ‘Burlat’ (Table 5). The CO<sub>2</sub> assimilation and transpiration intensity ranged from 10.4 (2nd date of measurement, control) to 11.9 μmol m<sup>-2</sup> s<sup>-1</sup> (1st date of measurement, methyl anthranilate) and from 1.84 (2nd date of measurement, methyl anthranilate) to 2.01 mmol m<sup>-2</sup> s<sup>-1</sup> (1st date of measurement, methyl anthranilate), respectively. Lenahan and Whiting [50] and Gonçalves et al. [47] reported that the assimilation rate in sweet cherry leaves was from 4.5 to 10.0 mmol m<sup>-2</sup> s<sup>-1</sup> and from 7.0 to 17.0 μmol m<sup>-2</sup> s<sup>-1</sup>. According to Lichev and Berov [51], the intensity of transpiration in sweet cherry leaves on various rootstocks ranged from 0.8 to 2.1 mmol m<sup>-2</sup> s<sup>-1</sup>.

**Table 5.** The influence of methyl anthranilate on the parameters of gas exchange in the leaves of sweet cherry cv. ‘Burlat’.

Parameter of Gas Exchange	I Term of Measurement		II Term of Measurement	
	Control	Methyl Anthranilate	Control	Methyl Anthranilate
A * (μmol m <sup>-2</sup> s <sup>-1</sup> )	11.2 ± 0.74 a **	11.9 ± 0.86 a	10.4 ± 1.09 a	11.1 ± 0.82 a
E (mmol m <sup>-2</sup> s <sup>-1</sup> )	1.86 ± 0.12 a	2.01 ± 0.18 a	1.97 ± 0.21 a	1.84 ± 0.11 a
g <sub>s</sub> (mol m <sup>-2</sup> s <sup>-1</sup> )	0.040 ± 0.003 a	0.051 ± 0.015 a	0.062 ± 0.014 a	0.059 ± 0.011 a
c <sub>i</sub> (μmol mol <sup>-1</sup> )	145.2 ± 10.55 a	152.4 ± 8.51 a	164.2 ± 7.94 a	158.4 ± 9.21 a
ω <sub>F</sub> (mmol mol <sup>-1</sup> )	6.02 ± 0.41 a	5.92 ± 0.55 a	5.28 ± 0.65 a	6.03 ± 0.37 a

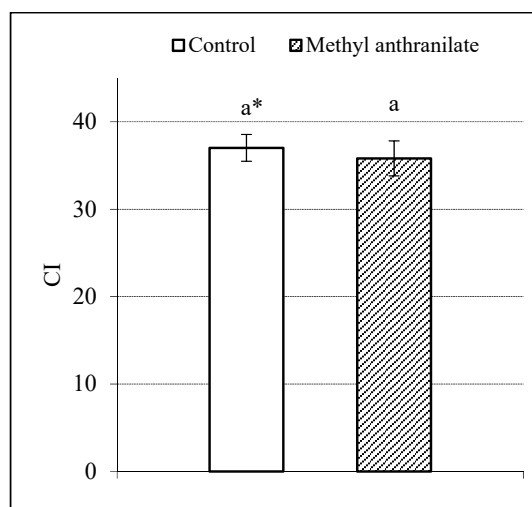
\* A: assimilation CO<sub>2</sub>. E: transpiration. g<sub>s</sub>: stomatal conductance for water. c<sub>i</sub>: concentration of carbon dioxide in the intercellular spaces. ω<sub>F</sub>: index of water use in the photosynthesis. \*\* Data are presented as mean ± SD. Means assigned identical letters do not differ significantly at the level of significance  $p = 0.05$ .

Gonçalves et al. [47] reported that stomatal conductance to water in the leaves of a few studied sweet cherry cultivars could amount to 0.09 mol m<sup>-2</sup> s<sup>-1</sup>. In our study, the value of stomatal conductance for water in ‘Burlat’ cultivar leaves did not exceed 0.062 mol m<sup>-2</sup> s<sup>-1</sup>. The tested repellent did not affect the value of this physiological trait of sweet cherry (Table 4). Its impact was further absent with regards to CO<sub>2</sub> concentration in intercellular spaces of chlorenchyma, which ranged from 145.2 (1st date, control) to

164.2  $\mu\text{mol mol}^{-1}$  (2nd date, control). Higher values of  $\text{CO}_2$  concentration in the leaves of several tested sweet cherry cultivars were found by Gonçalves et al. [47], ranging from 230 to 280  $\mu\text{mol mol}^{-1}$ .

Some authors point out that the use of methyl anthranilate, especially at higher concentrations, may damage the leaves of certain plant species [1,2,5]. According to Curtis et al. [2], the phytotoxicity of the methyl anthranilate depends of the formulation. The most phytotoxic formulations are aqueous suspensions because of the difficulties of maintaining this formulation in a uniform suspension on the leaf's surface. In our study, the methyl anthranilate was used in a starch-encapsulated formulation, which improved the mixture's stability and minimized phytotoxicity. In the presented study, no chlorotic or necrotic lesions of leaves caused by using methyl anthranilate were determined.

A considerable issue in sweet cherry culture is the phenomenon of fruit cracking during the maturation stage. Cracking is caused by excessive rainfall during maturation, and the mechanism of the phenomenon has not been fully understood. The yield loss caused by sweet cherry fruit cracking may reach up to 90% [52]. According to Yamamoto et al. [53], this phenomenon can be reduced in several ways: through the cultivation of cultivars that are resistant to cracking; the application of a protective coat against rain; and the spraying of trees with various chemical substances, particularly those containing calcium. Considering the above factors, it is important to determine the effect of agents used in the period preceding maturation and during the phase of maturation on cracking. The conducted study excluded the negative impact of methyl anthranilate-based repellent on fruit cracking in the field, as it was found that it does not affect the cracking index of fruits of sweet cherry cv. 'Burlat' (Figure 1).



**Figure 1.** Influence of methyl anthranilate on cracking indices (CI) of fruit of sweet cherry cv. 'Burlat'; \* Data are presented as mean  $\pm$  SD. Means assigned identical letters do not differ significantly at the level of significance  $p = 0.05$ .

#### 4. Conclusions

Methyl anthranilate is a substance that undergoes rapid biodegradation; moreover, it does not affect the taste of the fruits, the environment, or human health. Used as a repellent, it reduces sweet cherry yield losses caused by birds, yet it must be noted that its efficiency depends on a range of factors, including the weather. The study included a number of tests to determine the influence of methyl anthranilate on both chemical composition and physiological parameters. All of the results were negative and confirmed that methyl anthranilate has no significant effects on fruit quality. Moreover, the use of methyl anthranilate-based repellent did not impact the susceptibility of sweet cherry fruits to crack. Considering the above, it appears that this compound may constitute an

interesting alternative to support sweet cherry orchard protection against birds and in organic cultures.

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## References

1. Avery, M.L. Evaluation of methyl anthranilate as a bird repellent in fruit drops. *Proc. Vertebr. Pest Conf.* **1992**, *15*, 130–133.
2. Curtis, P.D.; Merwin, I.A.; Pritts, M.P.; Peterson, D.V. Chemical repellents and plastic netting for reducing bird damage to sweet cherries, blueberries, and grapes. *Hort. Sci.* **1994**, *29*, 1151–1155. [\[CrossRef\]](#)
3. Harris, R.E.; Davis, R.A. Evaluation of the efficacy of products and techniques for airport bird control. *LGL Rep. Aerodr. Saf. Branch Transp. Can.* **1998**, *3*, 107.
4. Simon, G. A short overview of bird control in sweet and sour cherry orchards—Possibilities of protection of bird damage and its effectiveness. *Int. J. Hort. Sci.* **2008**, *14*, 107–111. [\[CrossRef\]](#)
5. Avery, M.L.; Primus, T.M.; Defrancesco, J.; Cummings, J.L.; Decker, D.G.; Humphrey, J.S.; Davis, J.E.; Deacon, R. Field evaluation of methyl anthranilate for deterring birds eating blueberries. *J. Wildl. Manag.* **1996**, *60*, 929–934. [\[CrossRef\]](#)
6. Olbricht, K.; Grafe, C.; Weiss, K.; Ulrich, D. Inheritance of aroma compounds in a model population of *Fragaria* × *ananassa* Duch. *Plant Breed.* **2008**, *127*, 87–93.
7. Clark, L.; Mason, J.R. Interaction between sensory and postingestional repellents in starlings: Methyl anthranilate and sucrose. *Ecol. Appl.* **1993**, *3*, 262–270. [\[CrossRef\]](#)
8. Eisemann, J.D.; Werner, S.J.; O'Hare, J.R. Registration considerations for chemical bird repellents in fruit crops. *Outlooks Pest Manag.* **2011**, *22*, 87–91. [\[CrossRef\]](#)
9. Mason, J.R.; Clark, L. Evaluation of methyl anthranilate and activated charcoal as snow goose grazing deterrents. *Crop Prot.* **1995**, *14*, 467–469. [\[CrossRef\]](#)
10. Ahmad, S.; Saleem, Z.; Jabeen, F.; Hussain, B.; Sultana, T.; Sultana, S.; Al-Ghanim, K.A.; Al-Mulhim, N.M.A.; Mahboob, S. Potential of natural repellents methylanthranilate and anthraquinone applied on maize seeds and seedlings against house sparrow (*Passer domesticus*) in captivity. *Braz. J. Biol.* **2018**, *78*, 667–672. [\[CrossRef\]](#)
11. Falcão, L.D.; Chaves, E.S.; Burin, V.M.; Falcão, A.P.; Gris, E.F.; Bonin, V.; Bordignon-Luiz, M.T. Maturity of Cabernet Sauvignon berries from grapevines grown with two different training systems in a new grape growing region in Brazil. *Cienc. Inv. Agr.* **2008**, *35*, 321–332. [\[CrossRef\]](#)
12. Pantelidis, G.E.; Vasilakakis, M.; Manganaris, G.A.; Diamantidis, G. Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries, blackberries, red currants, gooseberries and Cornelian cherries. *Food Chem.* **2007**, *102*, 777–783. [\[CrossRef\]](#)
13. Yu, L.; Haley, S.; Perret, J.; Harris, M.; Wilson, J.; Qian, M. Free radical scavenging properties of wheat extracts. *J. Agric. Food Chem.* **2002**, *50*, 1619–1624. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Kumaran, A.; Karunakaran, R.J. In vitro antioxidant activities of methanol extracts of five *Phyllanthus* species from India. *LWT* **2007**, *40*, 344–351. [\[CrossRef\]](#)
15. Prieto, P.; Pineda, M.; Aguilar, M. Spectrophotometric quantitation of antioxidant capacity through the formation of phosphomolybdenum complex: Specific application to the determination of vitamin E. *Anal. Biochem.* **1999**, *269*, 337–341. [\[CrossRef\]](#)
16. Braca, A.; Tommasi, N.D.; Bari, L.D.; Pizza, C.; Politi, M.; Morelli, I. Antioxidant principles from *Bauhinia terapotensis*. *J. Nat. Prod.* **2001**, *64*, 892–895. [\[CrossRef\]](#)
17. Arnon, D.J.; Allen, M.B.; Whatley, F. Photosynthesis by isolated chloroplast. *Biochim. Biophys. Acta* **1956**, *20*, 449–461. [\[CrossRef\]](#)
18. Lichtenthaler, H.K.; Wellburn, A.R. Determinations of total carotenoids and chlorophyll a and b of leaf extracts in different solvents. *Biochem. Soc. Trans.* **1983**, *11*, 591–592. [\[CrossRef\]](#)
19. Hager, A.; Mayer-Berthenrath, T. Die Isolierung und quantitative Bestimmung der Carotenoide und Chlorophyll von Blättern, Algen und isolierten Chloroplasten mit Hilfe Dunnschichtchromatographischer Methoden. *Planta* **1966**, *69*, 198–217. [\[CrossRef\]](#)



20. Weichert, H.; von Jagemann, C.; Peschel, S.; Knoche, M. Studies on water transport through the sweet cherry fruit surface: VIII. Effect of selected cations on water uptake and fruit cracking. *J. Am. Soc. Hort. Sci.* **2004**, *129*, 781–788. [\[CrossRef\]](#)
21. Aronov, E.V.; Clark, L. Degradation studies of the non-lethal bird repellent, methyl anthranilate. *Pest Sci.* **1996**, *41*, 355–362. [\[CrossRef\]](#)
22. Askham, L.R. Efficacy of methyl anthranilate as a bird repellent on cherries, blueberries and grapes. *Proc. Vertebr. Pest Conf.* **1992**, *15*, 137–141.
23. Mikiciuk, G.; Chelpiński, P.; Popiel, J. The comparison of the effectiveness of different methods of protection against birds, which are used in cherry-tree orchards. *Zesz. Probl. Post. Nauk Rol.* **2009**, *536*, 149–153. (In Polish)
24. Chockchaisawasdee, S.; Golding, J.B.; Vuong, Q.V.; Papoutsis, K.; Stathopoulos, C.E. Sweet cherry: Composition, postharvest preservation, processing and trends for its future use. *Trends Food Sci. Technol.* **2016**, *55*, 72–83. [\[CrossRef\]](#)
25. Vavoura, M.V.; Badeka, A.V.; Kontakos, M.; Kontominas, M.G. Characterization of four popular sweet cherry cultivars grown in Greece by volatile compound and physicochemical data analysis and sensory evaluation. *Molecules* **2015**, *20*, 1922–1940. [\[CrossRef\]](#)
26. Gonçalves, B.; Landbo, A.-K.; Knudsen, D.; Silva, A.P.; Moutinho-Pereira, J.; Rosa, E.; Meyer, A.S. Effect of ripeness and postharvest storage on the phenolic profiles of cherries (*Prunus avium* L.). *J. Agric. Food Chem.* **2004**, *52*, 523–530. [\[CrossRef\]](#)
27. Kurlus, R. Growth, yield and fruit quality in eight sweet cherry cultivars grafted on ‘Tabel Edabriz’ rootstock. *J. Fruit Ornam. Plant Res.* **2004**, *12*, 35–39.
28. Pérez-Sánchez, R.; Gómez-Sánchez, M.A.; Morales-Corts, M.R. Description and quality evaluation of sweet cherries cultured in Spain. *J. Food Qual.* **2010**, *33*, 490–506. [\[CrossRef\]](#)
29. Drkenda, P.; Spahić, A.; Spahić, A.; Begić-Akagić, A. Testing of ‘Gisela 5’ and ‘Santa Lucia 64’ cherry rootstocks in Bosnia and Herzegovina. *Acta Agr. Slov.* **2012**, *99*, 129–136. [\[CrossRef\]](#)
30. Stojanovic, M.; Milatovic, D.; Kulina, M.; Alic-Dzanovic, Z. Pomological properties of sweet cherry cultivars on Gisela 5 rootstock in the region of Sarajevo. In *Proceedings of the 3rd International Scientific Symposium “Agrosym 2012”, Jahorina, Bosnia and Herzegovina, 15–17 November 2012*; University of East Sarajevo: East Sarajevo, Republika Srpska, Bosnia and Herzegovina, 2012; pp. 183–187.
31. Sen, F.; Oksar, R.E.; Golkarian, M.; Yaldiz, S. Quality changes of different sweet cherry cultivars at various stages of the supply chain. *Not Bot. Horti Agrobot. Cluj Napoca* **2014**, *42*, 501–506. [\[CrossRef\]](#)
32. Maglakelidze, E.; Bobokasvili, Z.; Kakashvili, V.; Tsigriasvili, L. Biological and agricultural properties of sweet cherry (*Prunus avium* L.) cultivars in Georgia. *Int. J. Sci. Res.* **2017**, *6*, 796–803.
33. Girard, B.; Kopp, T.G. Physicochemical characteristics of selected sweet cherry cultivars. *J. Agric. Food Chem.* **1998**, *46*, 471–476. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Sirbu, S.; Niculaea, M.; Chiriță, O. Physico-chemical and antioxidant properties of new sweet cherry cultivars from Iași, Romania. *Agron. Res.* **2012**, *10*, 341–350.
35. Prvulović, D.; Malenčić, D.; Popović, M.; Ljubojević, M.; Ognjanov, V. Antioxidant properties of sweet cherries (*Prunus avium* L.)—role of phenolic compounds. *Int. J. Agric. Biosyst. Engin.* **2011**, *59*, 687–690.
36. Legua, P.; Domenech, A.; Martínez, J.J.; Sánchez-Rodríguez, L.; Hernández, F.; Carbonell-Barrachina, A.A.; Melgarejo, P. Bioactive and volatile compounds in sweet cherry cultivars. *J. Food Nutr. Res.* **2017**, *5*, 844–851. [\[CrossRef\]](#)
37. Kim, D.O.; Heo, H.J.; Kim, Y.J.; Yang, H.S.; Lee, C.Y. Sweet and sour cherry phenolics and their protective effects on neuronal cells. *J. Agric. Food Chem.* **2005**, *53*, 9921–9927. [\[CrossRef\]](#)
38. Usenik, V.; Fabčić, J.; Stampar, F. Sugars, organic acids, phenolic composition and antioxidant activity of sweet cherry (*Prunus avium* L.). *Food Chem.* **2008**, *107*, 185–192. [\[CrossRef\]](#)
39. Nizioł-Lukaszewska, Z.; Skrzyński, J.; Leja, M.; Zawiska, I. Comparison of several cherry cultivars grown in Małopolska in terms of phenolic compounds content. *Episteme* **2012**, *15*, 445–451. (In Polish)
40. Telesiński, A.; Mikiciuk, G.; Mikiciuk, M.; Stręk, M.; Płatkowski, M.; Statkiewicz, M. Effect of preharvest use of anti-cracking preparations on changes in selected parameters of sweet cherry fruits during frozen storage. *Folia Pomer. Univ. Technol. Stetin. Agric. Aliment. Pisc. Zootech.* **2016**, *330*, 179–186. [\[CrossRef\]](#)
41. Serrano, M.; Guilleán, F.; Martínez-Romero, D.; Castillo, S.; Valero, D. Chemical constituents and antioxidant activity of sweet cherry at different ripening stages. *J. Agric. Food Chem.* **2005**, *53*, 2741–2745. [\[CrossRef\]](#)
42. Koumanov, K.S.; Staneva, I.N.; Kornov, G.D.; Germanova, D.R. Sweet cherry fruit quality under fertigation. *Acta Hort.* **2016**, *1139*, 551–558. [\[CrossRef\]](#)
43. Stachowiak, A.; Bosiacki, M.; Świerczyński, S.; Kolasiński, M. Influence of rootstocks on different sweet cherry cultivars and accumulation of heavy metals in leaves and fruit. *Hort. Sci.* **2015**, *42*, 193–202. [\[CrossRef\]](#)
44. Mikiciuk, G.; Mikiciuk, M.; Moździerz, E.; Statkiewicz, M.; Chylewska, U. The effects of foliar nutrition with InCa fertilizer on the chemical composition of leaves and fruits of sweet cherry. *J. Ecol. Eng.* **2015**, *16*, 116–119. [\[CrossRef\]](#)
45. Malik, I.A.; Peer, F.A.; Ahmad, M. Influence of different nutrient sprays on fruit quality of cherry (*Prunus avium* L.). *Res. J. Chem. Environ. Sci.* **2018**, *6*, 68–76.
46. Mikiciuk, G.; Moździerz, E.; Mikiciuk, M.; Chelpiński, P. The effect of antitranspirant on the content of microelements and trace elements in sweet cherry leaves and fruits. *J. Ecol. Eng.* **2013**, *14*, 36–38. [\[CrossRef\]](#)
47. Gonçalves, B.; Moutinho-Pereira, J.; Santos, A.; Silva, A.P.; Bacelar, E.; Correia, C.; Rosa, E. Scion-rootstock interaction affects the physiology and fruit quality of sweet cherry. *Tree Physiol.* **2005**, *26*, 93–104. [\[CrossRef\]](#)

48. Viljevac, M.; Dugalić, K.; Mihaljewić, I.; Šimić, D.; Sudar, R.; Jurković, Z.; Lepeduš, H. Chlorophyll content, photosynthetic efficiency and genetic markers in two sour cherry (*Prunus cerasus* L.) genotypes under drought stress. *Acta Bot. Croat.* **2013**, *72*, 221–235. [[CrossRef](#)]
49. Pilarski, J.; Tokarz, K.; Kocurek, M. Comparison of photosynthetic pigment contents in stems and leaves of fruit trees: Cherry, sweet cherry. *Folia Hort.* **2007**, *19*, 53–65.
50. Lenahan, O.M.; Whiting, M.D. Physiological and horticultural effects of sweet cherry chemical blossom thinners. *Hort. Sci.* **2006**, *41*, 1547–1551. [[CrossRef](#)]
51. Lichev, V.; Berova, M. Effects of rootstock on photosynthetic activity and productivity in the sweet cherry cultivar ‘Stella’. *J. Fruit Ornam. Plant Res.* **2004**, *12*, 287–293.
52. Simon, G. Review on rain induced fruit cracking of sweet cherries (*Prunus avium* L.), its causes and the possibilities of prevention. *Int. J. Hort. Sci.* **2006**, *12*, 27–35. [[CrossRef](#)]
53. Yamamoto, T.; Satoh, H.; Wanatabe, S. The effects of calcium and naphthalene acetic acid sprays on cracking index and natural rain cracking in sweet cherry fruits. *J. Jpn. Soc. Hort. Sci.* **1992**, *61*, 507–511. [[CrossRef](#)]