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The Use of a New Ionic Derivative of Salicylic Acid in Sugar Beet Cultivation

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Abstract: The need for sustainable development in the context of pesticide use has been recognized by the European Union. The "Farm to Fork Strategy" indicates a goal of 50% reduction in pesticide use by 2030. To address this challenge, we used the concept of ionic liquids to modify known resistance inducers, i.e., a group of substances whose action is indicated as an alternative to fungicides. A new, patented substance developed by us, which is a choline 3,5-dichlorosalicylate, has been tested in the context of its use in sugar beet cultivation with the aim of controlling Cercospora leaf spot (CLS). The results suggest that the use of this substance in combination with one fungicide treatment reduces disease infection and produces yields very similar to the use of a standard protection program assuming the use of two fungicides. Such results provide the basis for further development of 3,5-dichlorosalicylate in terms of its use in agriculture. Thanks to its use, it was possible to resign from one fungicide treatment, while maintaining protection against CLS and yields at the same level as for the full fungicide protection program. Such an approach is in line with European Union policies.

Keywords: organic salts; salicylic acid; plant stimulant; plant resistance inducer; sugar beet cultivation; Cercospora leaf spot; technological sugar yield

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1. Introduction

More than half of the world's sugar beet production takes place in European Union countries. In 2022, Poland was the third largest producer of sugar beets in the EU, with a production of around 14 million tons of sugar beet from an area of 220,000 ha [1]. Sugar extracted from beet roots is used in food as well as fuel and pharmaceutical industries. Cercospora leaf spot (CLS) is the fungal disease that negatively affects sugar beet cultivation around the world. If *Cercospora beticola* Sacc., the causing agent of this disease, remains uncontrolled, a complete leaf dieback can occur on sugar beet plants. In such a situation, there is a risk of reduction in both sugar yield and beet root yield, as the plant has to restore its leaves [2]. Effective protection against this disease involves the use of chemical and non-chemical methods. As for non-chemical methods, it is recommended to use suitable crop rotation, deep tillage, and balanced nitrogen fertilization or to eliminate weeds infested by *C. beticola*. Another non-chemical method is sowing of sugar beet varieties resistant to CLS [3]. However, the basis for protection against this disease is still chemical treatments involving the use of fungicides. This type of protection, although the most effective, is not without difficulties in applying it in agricultural practice. Due to the short disease cycle,

high genetic variability, and production of numerous spores, *C. beticola* easily develops resistance towards active substances of fungicides [4,5]. This requires the appropriate rotation of the fungicide products used, which is significantly difficult due to the limited number of active substances approved for use in sugar beet cultivation [6]. Currently, in Poland, there are 52 different fungicide products, comprised of only four different groups of active substances: strobilurins, triazoles, succinate-dehydrogenase inhibitors, and morpholines. Taking into account the abovementioned aspects that make effective protection against this pathogen difficult, mainly due to the risk of acquiring resistance, other effective methods free from this risk are being sought. One such method is the use of systemic acquired resistance (SAR) inducers.

The phenomenon of SAR (systemic acquired resistance) constitutes one of the defence mechanisms of plants against pathogens [7]. The plant's response understood as the stimulation of its acquired resistance may be activated in response to the action of the pathogen, but also in response to the action of an elicitor that mimics the action of the pathogen. Such elicitors include substances of a natural or chemical origin [8]. Regardless of the elicitor of this mechanism characterized by the increased expression of pathogenic-related genes (PR genes) in the plant's tissues, the signal molecule in this process is salicylic acid [9].

Salicylic acid is a phytohormone that is also reported as a SAR inducer [10]. However, its efficiency in terms of resistance induction is moderate comparing to other known resistance inducers such as benzotiadiazoles [11]. Silverman et al. investigated the efficiency of inducing SAR induction by various derivatives of salicylic acid, substituting halogens such as fluorine, bromine, chlorine, or iodine at positions 3, 4, and 5 of the benzene ring manifested by the increased expression of PR1 genes in tobacco plants. Results of this work indicate that salicylic acid derivatives such as 3,5-dichlorosalicylic acid exhibit higher activity in terms of SAR induction, comparing to the activity of salicylic acid itself [12]. In response to either a pathogen attack or application of a SAR inducer, the synthesis of salicylic acid takes place, especially in distal tissues. Jasmonic acid and ethylene are also reported to participate in plant resistance, but their synthesis occurs to a greater extent when tissue damaged is caused by insects and during the elicitation of Induced Systemic Resistance. A vast body of evidence indicates that salicylic acid, jasmonic acid, and ethylene pathways constitute a signalling network, mutual positive and negative interactions.

Beyond its function in SAR induction, salicylic acid is also tightly associated with various aspects of plant growth and development, including thermogenesis, transpiration, photosynthesis, ion uptake, and cross talk with other hormones. This leads to the conclusion that the exogenous application of salicylic acid can be classified as an application of a biostimulant that positively influences plant growth and development and counteracts the adverse effects of abiotic stresses. Therefore, salicylic acid, and its derivatives, can be treated on an equal footing with other biostimulants such as humic and fulvic acids, chitosan, or plant-growth-promoting bacteria (PGPB). The positive impact of these biostimulants on the qualitative and quantitative parameters of sugar beet has been demonstrated in many studies; however, these results did not present an analysis related to the level of infestation with CLS disease [13–17].

Salicylic acid is also a signalling molecule associated with the phenomena called growth–immunity tradeoff [18,19]. Both those phenomena are regulated by salicylic acid [20]. The change in plant resource allocation associated with the induction of SAR can lead to a reduction in yield [21]. The improper application of the test substance understood as the use of its too high concentration, too many treatments during the growing season, or a too little interval between them, may stimulate this phenomenon. These aspects related to the application of a given SAR inducer constitute the main obstacle in introducing the use of such substances into agricultural practice.

To our best knowledge, most of the research is performed on salicylic acid itself and not its derivatives. Moreover, agricultural experiments with the use of SAR inducers are mainly conducted in controlled conditions, i.e., in a greenhouse, rather than in field

conditions. Our assumption regarding the selection of the substance was to focus on salicylic acid derivatives that have higher SAR activity than salicylic acid, and to subject it to chemical modifications in order to eliminate the drawback related to low solubility. Therefore, 3,5-dichlorosalicylic acid was the derivative and changed into an anionic form (3,5–dichlorosalicylate anion) and coupled with the choline cation responsible for increasing solubility of the compound and reducing phytotoxicity [22]. This approach was already successfully applied by our team for the derivatization of benzothiadiazoles and other structural analogues of salicylic acid [11,23,24].

The general aim of this study undertaken was to check the effect of using a new active substance, being an ionic derivative of salicylic acid, on the field cultivation of sugar beets. Such tests, apart from determining the effectiveness in ensuring protection against CLS, must be supplemented with the determination of parameters describing the yield. The reason for this is the occurrence of the growth–immunity tradeoff phenomenon, which is strongly related to the occurrence of the SAR phenomenon. Due to the fact that when the SAR phenomenon is triggered, the plant changes the allocation of its resources towards defence mechanisms, it may turn out that the cost of defence against a given disease will be a reduction in the plant's yield [19]. The signal molecule in this process, as in the case of the SAR induction process, is also salicylic acid [18].

Variants of treatment tested in this study were selected in such a way as to show the possibility of reducing the number of fungicide treatments by supplementing them with a new active compound responsible for plant resistance induction or even completely replacing the use of fungicides with such a compound. The definitive confirmation of this possibility required not only examining the degree of infection of sugar beet with *C. beticola*, but also demonstrating that there was no growth–immunity tradeoff causing a decrease in yields [21].

2. Materials and Methods

2.1. Tested Substance

The substance studied was choline 3,5-dichlorosalicylate obtained in our group, which is a derivative of salicylic acid whose resistance-inducing effect on plants has been described in the literature. The substance is the subject of patent application PCT/PL/2023/050110 [25]. The proposed substance was obtained with 99.9% purity. Due to the fact that the substance dissolves very well in water (>100 g/L), no formulation ingredients were added to the water solution. The adopted dosage of this substance per hectare of crops results from our previous tests and is 80 g per hectare.

2.2. Locations of the Experimental Fields and Their Characteristics

The experiment was carried out in a total of 4 experimental fields, meaning in 2 locations in the Kuyavian–Pomeranian Voivodeship in Poland in two consecutive years, i.e., 2021 and 2022. Characteristics of each experimental field are provided in Table 1. On each field, an appropriate crop rotation was maintained. The meteorological information for all experiments was obtained from the weather station of Falęcin and is shown in Figure 1.

Table 1. Description of experimental sites.

Year of Experiment	Name and Symbol of Location	Geographical Coordinates	Soil Characteristics	pH
2021	Skąpe (A)	53°19′20″ N 18°30′17″ E	sandy loam	7.3
2021	Falęcin (B)	53°13′06″ N 18°36′33″ E	sandy loam	6.9
2022	Skąpe (A)	53°19′20″ N 18°30′17″ E	sandy loam	6.9
2022	Falęcin (B)	53°13′06″ N 18°36′33″ E	sandy loam	6.7

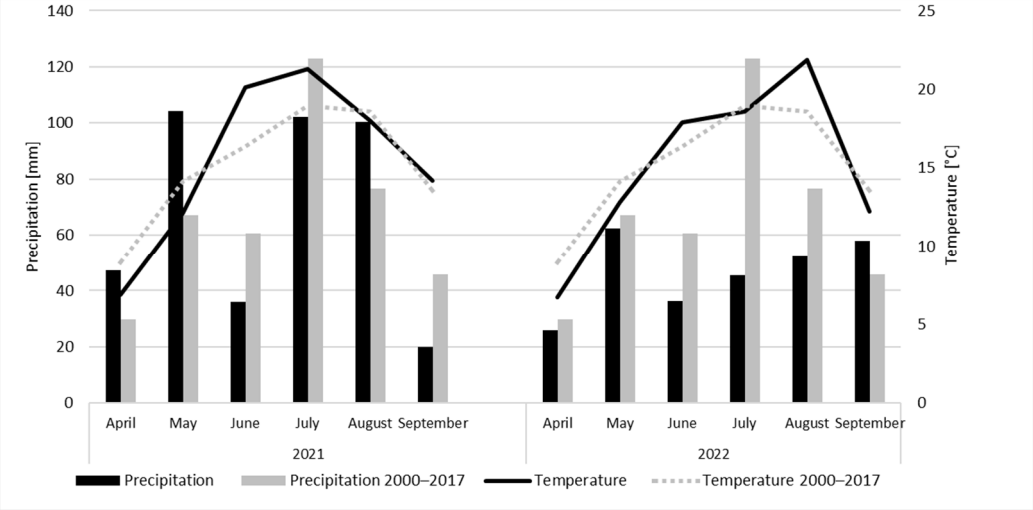

Figure 1. Weather conditions (average temperature and precipitation) at experimental location Falęcin in years 2021–2022.

2.3. Plant Material

Field studies were performed on sugar beet variety *Pacific* (Maribo, Denmark), which is a normal-type variety registered in 2018. As for its characteristics, plants of this variety are resistant to rhizomania (caused by BNYVV) and have increased resistance to Cercospora leaf spot (caused by *C. beticola*), powdery mildew (caused by *Erysiphe betae*), seedling rot, and beet root tip rot (caused by *Aphanomyces cochlioides* and *Fusarium* fungi).

2.4. Experimental Design

As for the experimental setup, per each variant of treatment, there were 4 replications organised according to randomised block design. Each replication constituted a plot consisting of 5 rows and had a total area of 20.25 m^2 (9 m long and 2.25 m wide). The seeds were sown with a 6-row drill at a row spacing of 0.45 m in early April. The spacing between plants was 0.18 m. Sugar beets were harvested manually from an area of 9.00 m^2 in early October.

All treatments with plant protection products and the tested ionic derivative of salicylic acid are presented in Tables 2 and 3. Spray application was made using a wheelbarrow sprayer with a spraying liquid output of 400 L per hectare.

Table 2. The detailed schedule of treatments performed in the experiments in 2021.

Variant of Treatment	The Treatment Number and Date				
	1 July 2021 I	**9 July 2021 II**	**15 July 2021 III**	**22 July 2021 IV**	**12 August 2021 V**
UTC					
SFP			Spyrale 475 EC		Eminent 125 ME
50% SFP			Spyrale 475 EC		
3,5diClSal	3,5diClSal	3,5diClSal		3,5diClSal	3,5diClSal
3,5diClSal (3 times) + 50% Fungicide	3,5diClSal	3,5diClSal	Spyrale 475 EC		3,5diClSal
3,5diClSal (4 times) + 50% Fungicide	3,5diClSal	3,5diClSal	Spyrale 475 EC	3,5diClSal	3,5diClSal

Spyrale 475 EC (ADAMA, Warsaw, Poland) active substances: fenpropidin at 375 g L^{-1}, difenoconazole at 100 g L^{-1}. Eminent 125 ME (UPL, Limited, Mumbai, India) active substance: tetraconazole at 125 g L^{-1}.

Table 3. The detailed schedule of treatments performed in the experiments in 2022.

Variant of Treatment	The Treatment Number and Date				
	5 July 2022 I	**12 July 2022 II**	**18 July 2022 III**	**27 July 2022 IV**	**22 August 2022 V**
UTC					
SFP			Spyrale 475 EC		Eminent 125 ME
50% SFP			Spyrale 475 EC		
3,5diClSal	3,5diClSal	3,5diClSal		3,5diClSal	3,5diClSal
3,5diClSal (3 times) + 50% Fungicide	3,5diClSal	3,5diClSal	Spyrale 475 EC		3,5diClSal
3,5diClSal (4 times) + 50% Fungicide	3,5diClSal	3,5diClSal	Spyrale 475 EC	3,5diClSal	3,5diClSal

Spyrale 475 EC (ADAMA, Warszawa, Poland) active substances: fenpropidin at 375 g L^{-1}, difenoconazole at 100 g L^{-1}. Eminent 125 ME (UPL Limited, Mumbai, India) active substance: tetraconazole at 125 g L^{-1}.

2.5. Assessment of C. beticola Infection

The assessment of the disease incidence and severity was made on all plants from three middle rows within each block separately, in consistence with the EPPO Standard scale [26]. It is a 10-point scale, where 0 means no symptoms of the disease, while 9 indicates completely destroyed foliage and the occurrence of new leaves produced by the plant (Table 4). The CLS incidence and severity were assessed on the day of harvesting sugar beets. For each repetition, assessment was made on 10 consecutive plants from each row.

Table 4. Description of EPPO scale for assessing level of infection with *C. beticola* and corresponding values of DSI.

Degree of Infection on the EPPO Scale	Description	Infected Leaf Area [%]	Corresponding Value of DSI [%] [1]
0	no symptoms	0	0.00
1	single spots on the leaf	0.1	11.11
2	single spots on the leaf	1	22.22
3	moderately numerous spots on the leaf that begin to merge	2	33.33
4	numerous spots on the leaf that are merged together	5	44.44
5	numerous spots on the leaves	10	55.56
6	numerous spots on the leaves	25	66.67
7	numerous spots on the leaves	50	77.78
8	numerous spots on the leaves	75	88.89
9	numerous spots on the leaves, new leaves begin to develop	95	100.00

[1] The description of the DSI calculation is presented in Section 2.6.

2.6. Assessment of Pathogen Infection

Apart from the CLS assessment made as described above, the disease severity index (DSI) was also used to express the data [27,28]. This index is a single index number for summarising the total effect of the disease on a single plant or a small sample of plants. Based on mean values for each assessed block, subsequent calculation was made according to the equation

$$\mathrm{DSI}[\%] \frac{\sum(\mathrm{classfrequency} \times \mathrm{scoreofratingclass})}{(\mathrm{totalnumberofobservations}) \times (\mathrm{maximaldiseaseindex})} \times 100 \quad (1)$$

2.7. Assessment of Qualitative Parameters of Yield

The evaluation of qualitative parameters of beet was performed using a Venema auto-analyser IIIG (Venema Consulting, Groningen, The Netherlands). As much as 30 kg of sugar beets harvested from each plot was a sample that was subjected to the analysis. After washing, sugar beets were ground to obtain a uniform pulp, which was then clarified with a 0.3% $Al_2(SO_4)_3$ solution. The fluorometric ortho-phthaldialdehyde (OPA) method was used to analyse the content of α-amino-N (AmN), while the flame photometry method was used to determine potassium and sodium content. Sucrose content (SC) in fresh taproots was determined by polarimetry. White sugar yield (WSY) was calculated according to the Brunswick formula [29,30]. Sugar processing losses were calculated according to the formula of Buchholz et al. [30]:

$$CT = 0.12 \times (K + Na) + 0.24 \times (N\text{-}\alpha\text{-amines}) + 0.48\ [\%], \quad (2)$$

where CT is the sugar processing losses [%]; K, Na, and N-α-amines are the potassium, sodium, and α-amino nitrogen content [mmol (100 g^{-1} fresh roots)].

Refined sugar contents were calculated according to the formula of Buchholz et al. [31]:

$$RSC = Pol - CT - 0.6\ [\%] \quad (3)$$

where RSC is the refined sugar content [%]; Pol is the biological sugar content in roots (sugar polarization) [% fresh weight]; CT is the sugar processing losses [%].

Technological sugar yield was calculated according to the formula of Trzebiński [31,32]:

$$YST = YR \times RSC \times 100^{-1}\ [t\ ha^{-1}] \quad (4)$$

where YST is the technological sugar yield [%]; YR is the root yield [t ha^{-1}]; RSC is the refined sugar content [%].

2.8. Statistical Analysis

A fixed-effects model of a three-way ANOVA with a general linear model according to the split–split-plot design (SPP) was applied to determine the combined effect of the year of this study, localization, and variants of treatment and their interactions on the root yield, sugar polarization, potassium content, sodium content, α-amino nitrogen content, and technological sugar yield. Homogenous groups were determined using the Tukey HSD test at $p = 0.05$. Calculations were made using Statistica 12.0 software (TIBCO, Palo Alto, CA, USA). Eta squared was used to gauge the effect size for the ANOVA model.

As for the DSI values for each experiment, the data were analysed from 4 blocks for each variant of treatment. All recorded and calculated data were evaluated by the analysis of variance (ANOVA), and the mean differences were compared by a post hoc test at a $p < 0.05$ level, according to Tukey's HSD. Statistical analyses were performed using OriginLab 2022 software for Windows (OriginLab Corp., Northampton, MA, USA).

3. Results

3.1. Weather Conditions

The meteorological data during all experimental periods are presented in Figure 1. The warmest month of 2021 was July. The average daily air temperature recorded in this month was 21.3 °C, which was 2.3 °C higher than the average for 2000–2017. In 2022, the highest average daily temperature was recorded in August (21.9 °C). It was higher than the August average daily temperature observed in the multi-year period by 3.3 °C.

The highest total precipitation during the sugar beet growing season was recorded in 2021. It amounted to 409.9 mm and was 7 mm higher than the average precipitation recorded during the same period of the year 2000–2017. In 2022, total precipitation was lower comparing to the average for 2000–2017. The difference in total precipitation between comparing periods was equal to 122.5 mm.

3.2. Assessment of Disease Severity

As for the disease severity, the DSI value was the highest for plants of UTC both in terms of the analysis of each of the locations separately and for the analysis based on mean values (Table 5). In general, DSI values in 2021 were lower comparing to DSI values in 2022. However, when analysing results for each location in a given year separately, it should be concluded that the general pattern of dependencies between the results remains very similar. As it was expected, the most efficient protection against *C. beticola* was provided by treatment of plants according to the SFP variant. In three of the four analysed experiments, the DSI values for the SFP variant and 3,5diClSal (4 times) + 50% Fungicide are at the same statistical level.

Table 5. Summary of the results for Disease Severity Index values for field experiment conducted in 2021 and 2022.

Variant of Treatment	Location A (2021)	Location B (2021)	Mean (2021)	Location A (2022)	Location B (2021)	Mean (2022)	Mean (2021 and 2022)
UTC	73.61 d	68.06 d	70.84 e	98.33 d	98.33 d	98.33 d	84.58 b
SFP	36.12 a	33.06 a	34.59 a	77.50 a	69.44 a	73.47 a	54.03 a
50% SFP	43.05 b	41.67 b	42.36 c	89.16 c	80.00 b	84.58 c	63.47 a
3,5diClSal	60.84 c	51.11 c	55.98 d	88.33 c	87.78 c	88.06 c	72.01 ab
3,5diClSal (3 times) + 50% Fungicide	42.23 b	38.62 ab	40.42 bc	84.72 bc	80.83 b	82.78 bc	61.60 a
3,5diClSal (4 times) + 50% Fungicide	36.39 a	33.34 a	34.86 a	81.94 ab	73.33 a	77.64 ab	56.25 a

Symbols for the table: within columns, mean values marked with the different letter differ significantly at $p = 0.05$, while mean values are not marked with a letter if differences are not significant. UTC—plants that were not treated either with fungicides or with choline 3,5-dichlorosalicylate, SFP—plants treated with fungicides 2 times, 50% SFP—plants treated with a fungicide 1 time, 3,5diClSal—plants treated with choline 3,5-dichlorosalicylate 4 times, 3,5diClSal (3 times) + 50% Fungicide—plants treated with choline 3,5-dichlorosalicylate 3 times and a fungicide 1 time, 3,5diClSal (4 times) + 50% Fungicide—plants treated with choline 3,5-dichlorosalicylate 4 times and a fungicide 1 time. The detailed schedule of treatments is provided in Tables 2 and 3.

3.3. Assessment of Qualitative and Quantitative Parameters of Yield

Mean values of qualitative and quantitative parameters of yield depending on the experimental factor are presented in Table 6. Significant changes in values of tested parameters are observed for the factor related to the year of conducting the experiment. Parameters such as root yield, sugar polarization, and technological sugar yield were higher in 2021, comparing to those observed in 2022. For the remaining parameters, i.e., the content of potassium, sodium, and α-amino nitrogen, the opposite situation is observed. As for the values of parameters analysed depending on the location, significant changes are observed in each of the parameters except from the technological sugar yield.

As for the analysis based on variants of treatment, both root yield and technological sugar yield are the lowest for plants of the UTC variant. The highest value of indicated parameters is observed for plants treated according to SFP. Comparing plants of variant 50% SFP and plants of variant 3,5diClSal, values for both root yield and technological sugar yield are at the same level. As for the variants 3,5diClSal (3 times) + 50% Fungicide and 3,5diClSal (4 times) + 50% Fungicide, although the values for the root yield parameter are not statistically at the same level, the values for the parameter of technological sugar yield are statistically at the same level.

Significant differences are not observed for sugar polarization. Although differences in parameters describing sodium and potassium content are observed, the differences are mostly statistically insignificant.

Table 6. Qualitative and quantitative parameters of yield depending on the experimental factors.

		Root Yield [t ha^{-1}]	Sugar Polarization [%]	Potassium Content [mmol 1000 g^{-1} of Pulp]	Sodium Content [mmol 1000 g^{-1} of Pulp]	α-Amino Nitrogen Content [mmol 1000 g^{-1} of Pulp]	Technological Sugar Yield [t ha^{-1}]
Year (A)	2021	65.85 •	19.34 •	35.12 ••	5.70 ••	5.76 ••	11.61 •
	2022	63.29 ••	17.21 ••	38.47 •	8.01 •	16.61 •	9.60 ••
Localization (B)	Location A	63.36 B	18.55 A	39.91 A	5.85 B	12.87 A	10.55 A
	Location B	65.78 A	18.00 B	33.67 B	7.86 A	9.50 B	10.67 A
Treatment (C)	UTC	58.57 d	18.14 a	37.09 ab	7.74 a	12.13 a	9.52 c
	SFP	69.04 a	18.29 a	38.46 a	6.48 a	11.85 ab	11.33 a
	50% SFP	63.08 c	18.33 a	35.92 b	6.59 a	9.77 b	10.41 b
	3,5diClSal	62.90 c	18.19 a	36.44 ab	7.23 a	11.58 ab	10.28 b
	3,5diClSal (3 times) + 50% Fungicide	66.25 b	18.36 a	37.05 ab	6.42 a	10.66 ab	10.95 a
	3,5diClSal (4 times) + 50% Fungicide	67.58 ab	18.33 a	35.80 b	6.68 a	11.12 ab	11.15 a

Symbols for the table: within the columns, mean values marked with different symbols (• or ••) differ significantly depending on the year of the experiment; means marked with different capital letters vary significantly depending on the localization of the experiment; means marked with different lowercase letters differ significantly depending on the variant of treatments at $p = 0.05$ according to Tukey's HS. UTC—plants that were not treated either with fungicides or with choline 3,5-dichlorosalicylate, SFP—plants treated with fungicides 2 times, 50% SFP—plants treated with a fungicide 1 time, 3,5diClSal—plants treated with choline 3,5-dichlorosalicylate 4 times, 3,5diClSal (3 times) + 50% Fungicide—plants treated with choline 3,5-dichlorosalicylate 3 times and a fungicide 1 time, 3,5diClSal (4 times) + 50% Fungicide—plants treated with choline 3,5-dichlorosalicylate 4 times and a fungicide 1 time. The detailed schedule of treatments is provided in Tables 2 and 3.

3.3.1. Root Yield

The analysis of main effects revealed that there was a significant influence of variants of treatment [$F(5,60)$—46.98, $p < 0.001$, η^2—0.593], localization [$F(1,6)$—71.77, $p < 0.001$, η^2—0.593], and the year of conducting the experiment [$F(1,6)$—218.63, $p < 0.001$, η^2—0.080] on the root yield. Of the three experimental factors tested, the root yield was mainly influenced by the treatment and the value of the η^2 coefficient was equal to 59.3%. The influence of the two remaining factors tested was lower, and the value of the η^2 coefficient related to the year was equal to 8.0%, while the value of the η^2 coefficient related to localization was equal to 7.1% (Table 7).

Table 7. Summary of the results for root yield for field experiment conducted in 2021 and 2022.

Source of Variance	Df_1	Df_2	Root Yield [t ha^{-1}]	
			F	η^2
Year (A)	1	6	218.63 ***	8.0
Localization (B)	1	6	71.77 ***	7.1
Treatment (C)	5	60	46.98 ***	59.3
A × B	1	6	4.30 ns	<1
B × C	5	60	4.31 **	5.4
A × C	5	60	2.31 ns	2.9

Df_1—degrees of freedom for the effect, Df_2—degrees of freedom for the corresponding error, η^2—eta squared (%), *** significant at $p < 0.001$, ** significant at $p < 0.01$, ns—not significant.

A three-way ANOVA revealed that there was a statistically significant interaction effect between the year of this study and variant of treatment on the root yield [$F(5,60)$—4.31,

p—0.002, η^2—0.054]. The effect explained 5.4% of the variability of the results, whereas the effect of the remaining interactions between the experimental factors was insignificant.

3.3.2. Sugar Polarization

The analysis of main effects revealed that there was a significant influence of localization [F(1,6)—20.98, p—0.004, η^2—0.054] and the year of conducting the experiment [F(1,6)—506.73, $p < 0.001$, η^2—0.810] on the sugar polarization. Tested variants of treatment did not influence the sugar polarization. Of the two significant factors, the year of the experiment explained 81% of the variability, while the localization factor explained 5.4% of the variability (Table 8).

Table 8. Summary of the results for sugar polarization for field experiment conducted in 2021 and 2022.

Source of Variance	Df_1	Df_2	Sugar Polarization [%]	
			F	η^2
Year (A)	1	6	506.73 ***	81.0
Localization (B)	1	6	20.98 **	5.4
Treatment (C)	5	60	<1 ns	<1
A × B	1	6	<1 ns	<1
B × C	5	60	<1 ns	<1
A × C	5	60	<1 ns	<1

Df_1—degrees of freedom for the effect, Df_2—degrees of freedom for the corresponding error, η^2—eta squared (%), *** significant at $p < 0.001$, ** significant at $p < 0.01$, ns—not significant.

A three-way ANOVA revealed the lack of significance for interactions between tested factors, in terms of their influence on sugar polarization.

3.3.3. Potassium Content

The analysis of main effects revealed that there was a significant influence of the year of conducting the experiment [F(1,6)—14.28, p—0.009, η^2—0.150], localization [F(1,6)—889.25, $p < 0.001$, η^2—0.521], and variants of treatment [F(5,60)—3.57, p—0.007, η^2—0.043] on the potassium content in pulp. This parameter was mainly influenced by the localization factor with the value of the η^2 coefficient equal to 52.1% (Table 9).

Table 9. Summary of the results for potassium content for field experiment conducted in 2021 and 2022.

Source of Variance	Df_1	Df_2	Potassium Content [mmol 1000 g^{-1} of Pulp]	
			F	η^2
Year (A)	1	6	14.28 **	15.0
Localization (B)	1	6	889.25 ***	52.1
Treatment (C)	5	60	3.57 **	4.3
A × B	1	6	2.39 ns	<1
B × C	5	60	2.67 *	3.2
A × C	5	60	3.30 **	4.0

Df_1—degrees of freedom for the effect, Df_2—degrees of freedom for the corresponding error, η^2—eta squared (%), *** significant at $p < 0.001$, ** significant at $p < 0.01$, * significant at $p < 0.05$, ns—not significant.

A three-way ANOVA revealed that there were two statistically significant interactions related to potassium content. The first of them was between localization and variants of treatment [F(5,60)—2.67, p—0.030, η^2—0.032], while the second was between the year of conducting the experiment and variants of treatment [F(5,60)—3.30, p—0.010, η^2—0.040].

3.3.4. Sodium Content

The analysis of main effects revealed that there was a significant influence of the year of conducting the experiment [$F(1,6)$—479.24, $p < 0.001$, η^2—0.754] and localization [$F(1,6)$—26.15, p—0.001, η^2—0.072] on the sodium content in the pulp (Table 10).

Table 10. Summary of the results for sodium content for field experiment conducted in 2021 and 2022.

Source of Variance	Df_1	Df_2	Sodium Content [mmol 1000 g^{-1} of Pulp]	
			F	η^2
Year (A)	1	6	50.90 ***	28.3
Localization (B)	1	6	30.71 ***	21.3
Treatment (C)	5	60	2.16 ns	4.8
A × B	1	6	<1 ns	<1
B × C	5	60	4.08 **	9.0
A × C	5	60	1.09 ns	2.4

Df_1—degrees of freedom for the effect, Df_2—degrees of freedom for the corresponding error, η^2—eta squared (%), *** significant at $p < 0.001$, ** significant at $p < 0.01$, ns—not significant.

A three-way ANOVA revealed a significant interaction between localization and variants of treatment [$F(5,60)$—4.08, p—0.002, η^2—0.090]. The effect explained 9.0% of the variability of the results. The influence of other interactions was not significant.

3.3.5. α-Amino Nitrogen Content

The analysis of main effects revealed that there was a significant influence of the year of conducting the experiment [$F(1,6)$—479.24, $p < 0.001$, η^2—0.754] and variants of treatment [$F(5,60)$—2.37, p—0.049, η^2—0.016] on the α-amino nitrogen content in pulp. This parameter was mainly influenced by the year of conducting the experiment with the value of the η^2 coefficient equal to 75.4% (Table 11).

Table 11. Summary of the results for α-Amino Nitrogen content for field experiment conducted in 2021 and 2022.

Source of Variance	Df_1	Df_2	α-Amino Nitrogen Content [mmol 1000 g^{-1} of Pulp]	
			F	η^2
Year (A)	1	6	479.24 ***	75.4
Localization (B)	1	6	26.15 **	7.2
Treatment (C)	5	60	2.37 *	1.6
A × B	1	6	11.23 *	3.1
B × C	5	60	1.28 ns	<1
A × C	5	60	<1 ns	<1

Df_1—degrees of freedom for the effect, Df_2—degrees of freedom for the corresponding error, η^2—eta squared (%), *** significant at $p < 0.001$, ** significant at $p < 0.01$, * significant at $p < 0.05$, ns—not significant.

A three-way ANOVA revealed the significant influence of interaction between the year of conducting the experiment and localization [$F(1,6)$—11.23, p—0.015, η^2—0.031]. This interaction explained 3.1% of the variability in the results (based on the η^2 coefficient value). The impact of the remaining interactions between factors was insignificant.

3.3.6. Technological Sugar Yield

The analysis of main effects revealed that there was a significant influence of the year of conducting the experiment [$F(1,6)$—557.60, $p < 0.001$, η^2—0.611] and variants of treatment [$F(5,60)$—29.98, $p < 0.001$, η^2—0.229] on the technological sugar yield. This parameter was mainly influenced by the factor related to the year of conducting the experiment with the value of the η^2 coefficient equal to 61.1%. The influence of variants of treatment was

lower comparing to that of the year of conducting this study, and was equal to 22.9%. The localization of the experiment did not influence the technological sugar yield (Table 12).

Table 12. Summary of the results for technological sugar yield for field experiment conducted in 2021 and 2022.

Source of Variance	Df_1	Df_2	Technological Sugar Yield [t ha^{-1}]	
			F	η^2
Year (A)	1	6	557.60 ***	61.1
Localization (B)	1	6	2.16 ns	<1
Treatment (C)	5	60	29.98 ***	22.9
A × B	1	6	3.77 ns	<1
B × C	5	60	3.42 **	2.6
A × C	5	60	1.84 ns	1.4

Df_1—degrees of freedom for the effect, Df_2—degrees of freedom for the corresponding error, η^2—eta squared (%), *** significant at $p < 0.001$, ** significant at $p < 0.01$, ns—not significant.

A three-way ANOVA revealed the significant influence of interaction between localization and variants of treatment [$F(5,60)$—3.42, p—0.008, η^2—0.026]. This interaction explained 2.6% of variance.

3.4. Summary of Results

For presented results, in addition to their statistical analysis, it is equally valuable to express them as a percentage decrease (for DSI values) and increase (for parameters describing quantitative and qualitative yield parameters) in relation to the untreated control. Such a comparison is presented in Table 13.

Table 13. Summary of the results for qualitative and quantitative parameters of yield and disease severity index values for field experiment conducted in 2021 and 2022. Mean values represent percentage decrease (for DSI values) and increase (for parameters describing quantitative and qualitative yield parameters) in relation to the untreated control.

Variant of Treatment	Root Yield	Sugar Polarization	Potassium Content	Sodium Content	α-Amino Nitrogen Content	Technological Sugar Yield	DSI
UTC	-	-	-	-	-	-	-
SFP	117.88%	100.83%	103.69%	83.72%	97.69%	119.01%	36.12%
50% SFP	107.70%	101.05%	96.85%	85.14%	80.54%	109.35%	24.96%
3,5diClSal	107.39%	100.28%	98.25%	93.41%	95.47%	107.98%	14.86%
3,5diClSal (3 times) + 50% SFP	113.11%	101.21%	99.89%	82.95%	87.88%	115.02%	27.17%
3,5diClSal (4 times) + 50% SFP	115.38%	101.05%	96.52%	86.30%	91.67%	117.12%	33.49%

UTC—plants that were not treated either with fungicides or with choline 3,5-dichlorosalicylate, SFP—plants treated with fungicides 2 times, 50% SFP—plants treated with a fungicide 1 time, 3,5diClSal—plants treated with choline 3,5-dichlorosalicylate 4 times, 3,5diClSal (3 times) + 50% Fungicide—plants treated with choline 3,5-dichlorosalicylate 3 times and a fungicide 1 time, 3,5diClSal (4 times) + 50% Fungicide—plants treated with choline 3,5-dichlorosalicylate 4 times and a fungicide 1 time. The detailed schedule of treatments is provided in Tables 2 and 3.

In none of the conducted experiments was there a decrease in root yield in relation to variant UTC. As it was expected, the highest increase in root yield was observed for standard fungicide treatment. As for the treatment according to variant 3,5diClSal, its increase in terms of root yield was comparable to the increase based on variant 50% SFP.

4. Discussion

European Union legislation indicates the need to undertake actions aimed at reducing the use of and dependence on pesticides. It is also related to the negative impact on the environment caused by the extensive use of pesticides. Therefore, it is of great importance to search for new methods providing efficient protection against pathogens. Of particular interest is the investigation of methods providing efficient protection against various pathogens, as the problem of limiting the amount of active substances available for use in plant protection applies not only to substances registered for use on sugar beet. In our previous studies, we have demonstrated that our new SAR inducers provide effective protection against various diseases on many different plant species [33–37]. Results of this study indicate the possibility of reducing fungicide treatments with the use of choline 3,5-dichlorosalicylate for providing protection against CLS. However, based on the results for variants of treatments tested in this work, the possibility of complete replacement of fungicide treatments with the tested SAR inducer was not proven.

As for the analysis of the results presented in this study, it should be noted that pathogen pressure was different in both experimental years. This is manifested by higher DSI values in 2022, comparing to those of 2021 (Table 5). Of note, this difference cannot be explained by the precipitation differences observed in Figure 1. However, precipitation is not the only factor influencing severity of *C. beticola.* The fungus is polycyclic within a sugar beet growing season. Conidia of *C. beticola* are produced most readily at temperatures from 15 to 23 °C and relative humidity greater than 60%. The dispersion of conidia occurs by wind, water splashes, running water, and insects. As for the environmental conditions that are favourable to the disease to develop, the following characteristics are indicated: day temperatures from 25 to 35 °C, night temperature of 16 °C, and prolonged periods of 90% to 95% of relative humidity or free moisture on leaves [38].

The difference in disease severity in both years is also manifested by differences observed for parameters related to quantitative and qualitative parameters of yield (Table 6). Parameters such as root yield, sugar polarization, and technological sugar yield were significantly higher in 2021, compared to the values observed in 2022. In turn, melassigenic elements were higher in 2022, compared to values of 2021. It is particularly visible in α-amino nitrogen content in pulp. Plants heavily infected by *C. beticola* rebuild their leaf apparatus using substances accumulated in the root, mainly sugar. Additionally, the content of molasses, especially sodium and α-amino nitrogen, increases in the roots of plants heavily infected by the talus moth, which further deteriorates their technological properties [39]. Of particular significance is the increase in the content of α-amino nitrogen, which is the main molasses-forming component [40]. An increase in the content of molasses in sugar beet roots is unfavourable because these ingredients hinder the crystallization of sugar. The percentage of sugar polarization is also susceptible to the degree of CLS disease infestation [41]. A higher degree of infection reduces the polarization of sugar. However, it should be noted that the technological sugar yield is mainly influenced by the root yield, and to a lesser extent by the sugar polarization and the content of melassigenic elements [41].

The statistical analysis of the influence of location on the experimental results was also performed and showed significant differences in the examined parameters (Table 6). They are most likely due to growing conditions, which may have differed in both locations. The main aim of this work was to demonstrate the effectiveness of choline 3,5-dichlorosalicylate in providing protection against *C. beticola*; thus, detailed research to determine the reasons for the differences in both experimental locations was not performed. Of note, although the differences between the studied parameters are statistically significant, their values do not differ significantly from each other, and for the most important of the studied parameters, i.e., technological sugar yield, the values of this parameter for both locations are not statistically different.

As for the analysis based on the variants of treatment factor, results can be discussed on several levels (Table 6). Firstly, the results of 3,5diClSal can be compared to the results of

variants SFP and 50% SFP. As expected, the best results were obtained for plants treated with SFP, while the results for the 50% SFP and 3.5diClSal variants are practically at the same level. Importantly, the technological sugar yield of these two results is at the same statistical level. However, when it comes to ensuring the effectiveness of protection against CLS disease, treatment of plants according to the 50% SFP variant resulted in providing more efficient protection (manifested by lower DSI value) compared to plants treated according to the 3.5diClSal variant. The second level of the analysis concerns the results obtained for combined variants' treatment, i.e., 3.5diClSal (4 times) + 50% Fungicide and 3.5diClSal (3 times) + 50% Fungicide. Technological yield of these two variants was significantly higher comparing to that of 50% SFP. Moreover, when comparing the value of this parameter for these two indicated variants of treatment and SFP, the yield values are at the same statistical level. As for the DSI values, only values obtained for variant 3.5diClSal (4 times) + 50% Fungicide and SFP do not differ significantly. However, it should be borne in mind that in the case of the two combined variants of treatment, it was necessary to perform a total of five or four treatments, compared to only two treatments performed in the SFP variant.

Based on presented results, better effects on sugar beet plants were achieved when the variant of treatment 3,5diClSal (4 times) + 50% Fungicide, and not 3,5diClSal (3 times) + 50% Fungicide, was applied. However, it remains to be determined whether the treatment in the fourth term was crucial and whether it is possible to reduce the number of treatments before the first fungicide treatment. The application of the SAR inducer before the standard fungicide application date is to induce plant resistance, before pathogen pressure occurred. Unfortunately, indications from a pathogen early-warning system may not appear early enough to make SAR inducer treatment dependent on them. Therefore, the date of the first application will most likely take place without appropriate grounds.

On the subject of the mechanisms of action of choline 3,5-dichlorosalicylate, it was already mentioned that salicylic acid has a role in both SAR induction and processes related to plant growth and development. This dual role is particularly visible in the context of the use of this substance in the cultivation of sugar beet. When the infestation with CLS disease is too extensive, plants need to rebuild leaves using the accumulated resources [41]. On the one hand, the activity related to providing protection against CLS disease allows the plant to reduce the level of infestation; on the other hand, the stimulating effect on the plant's growth processes has a positive influence on the amount of accumulated resources. In the specific case presented in this work, understood as the use of choline 3,5-dichlorosalicylate in a certain number of treatments and at a given concentration, the effectiveness of both these actions was not as significant as for the application of a standard fungicide program consisting of two fungicide treatments. However, comparing the results obtained for plants treated according to variant 3,5diClSal and plants of UTC, the activity attributed to the new ionic derivative of salicylic acid has been confirmed. This indicates the direction of further research on developing the optimal technology for using this SAR inducer, either in treatments consisting only of this active substance or in technology assuming the combined use of fungicides and SAR inducers. Such optimal technology must assume the selection of an appropriate concentration of a given SAR inducer, the selection of an appropriate number of treatments, and the development of a final application schedule. Once the program is developed, it will be justified to conduct more detailed research on the mechanism of action of this active substance and to describe in more detail the relationship between the activity related to SAR induction and biostimulation.

Another argument in the context of the future implementation of this active substance into agricultural practice is also the demonstration of an appropriate economic benefit for farmers, which will allow them to obtain an increase in yields at a level greater than the costs associated with the need to carry out additional treatments using the new active substance. Another solution that allows reducing the total number of treatments performed by the farmer may be the combined use of a fungicide and a SAR inducer in a single

application. In such a case, during one spraying, it will be possible to provide the plant with active substances that have complementary effects in the context of plant protection.

The process of developing technology for using SAR inducers, specially transferring the results of tests under controlled conditions to field conditions, still remains difficult. The efficiency of SAR inducers tested during field studies is prone to be influenced by different factors such as temperature or rainfall. Understanding of the impact of such factors is still insufficient [42]. However, despite these difficulties, SAR inducers constitute a promising alternative for active substances of plant protection products. The implementation of Regulation 2015/408/EC resulted in the introduction of a list of active substances of plant protection products that are to be removed from the market [43]. This will lead to a significant decrease in active substances authorized to trade, which negatively affects the possibility of ensuring the appropriate rotation of active substances used.

In parallel with this regulation, the "Farm to Fork Strategy" also applies and sets out a requirement for reducing the use of pesticides by 50% by 2030 [44]. Taking these indications into account, even ensuring the possibility of reducing the number of treatments by half, is of key significance and provides grounds for conducting further research on optimizing the use of choline 3,5-dichlorosalicylate in combination with fungicides.

5. Conclusions

In this study, we have shown the results of the application of a novel ionic derivative of salicylic acid, having a plant resistance inducer, in sugar beet cultivation. A combined variant of treatment consisted of a resistance inducer and fungicide (in a reduced number of treatments) and resulted in similar levels in terms of protection against *C. beticola* and obtaining technological sugar yield comparing to standard fungicide treatment (SFP). The variant of SFP included two applications, while that of combined treatment with a resistance inducer and fungicide consisted of either four or three applications of choline 3,5-dichlorosalicylate and one application of a fungicide. Based on presented results, better effects on sugar beet plants were achieved when the variant of treatment 3,5diClSal (4 times) + 50% Fungicide, and not 3,5diClSal (3 times) + 50% Fungicide, was applied.

Results of this study indicate the possibility of reducing fungicide treatments with the use of choline 3,5-dichlorosalicylate for providing protection against CLS. This creates the basis for further research on the development of protection programs assuming the combined use of resistance inducers and fungicides. However, based on the results for variants of treatments tested in this work, the possibility of complete replacement of fungicide treatments with the tested SAR inducer was not proven.

Although the protection with fungicides is cheaper than that with resistance inducers, it is expected to be phased out because of increasing resistance of pathogens to the active ingredients of fungicides and gradual withdrawal of particular chemical substances from plant protection. New active ingredients to be used for plant protection are few and far between and their introduction is preceded by a long and expensive registration process. The use of resistance inducers is not a cheap method of plant protection, but their use might become favourable in view of the EU strategy "From field to table", demanding the limitation of the use of pesticides by 50% by 2030.

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