Environmental parameters and fertilisers as factors affecting the salicylic acid and total polyphenol contents in sport turfgrasses

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ABSTRACT

In 2019, we conducted a field experiment to test the effects of three different granular fertilizers on the salicylic acid and total polyphenolic contents in five different sport turfgrass mixtures under natural conditions. It was one of the first experiments assessing the natural resistance of sport turfgrass in Europe. In five terms, before and after fertilizers applications, we randomly collected turfgrass samples within each plot for chemical analyses. The turfgrass mixtures did not affect the average contents of salicylic acid and total polyphenols. The average contents of both compounds were influenced by the date of evaluation and fertilization scheme. The average content of salicylic acid was significantly the lowest under fertilization scheme C. The highest level of salicylic acid was detected on 18th June under scheme A and the lowest on 28th May, where the salicylic acid content ranged from 0.01 ± 0.00 mg.g⁻¹ under scheme A to 0.017 ± 0.00 mg.g⁻¹ under scheme B and 0.006±0.00 mg.g⁻¹ under scheme C. The content of total polyphenols was higher under fertilization schemes B and C. We conclude that the method of fertilization exerts a certain effect on the salicylic acid and total polyphenol contents in turfgrass mixtures, which also depends on environmental factors.

Introduction

Sport turfgrasses are faced with various biotic and abiotic stresses, which exert negative effects on their growth, development and productivity. Due to intensive management, sport turfgrasses are often susceptible to the outbreak of infectious diseases (Vincelli and Munshaw 2014). Disease resistance is the key factor to ensuring the quality of sport turfgrasses (Wiewióra et al. 2015).

The availability of mineral nutrients is essential for the growth and development of plants. They are important factors regulating various physiological and biochemical processes. A deficiency in any one of the elements affects plant metabolism, quality and disease resistance (Amtmann and Armengaud 2009). Some mineral elements, such as nitrogen and sulfur, are constituents of organic compounds that feed, attract or deter pathogens. Other materials, such as calcium and silicon, determine the mechanical properties of cell walls and influence physical barriers or palatability. Potassium fertilisation is generally advertised as improving plant health (Davis et al. 2018).

Salicylic acid (SA) (2-hydroxy benzoic acid) is a member of a diverse group of simple phenolic compounds involved in the regulation of plant growth and development (Kumar 2014; Khan et al. 2015). It is a defence-related plant hormone that plays a key role in resistance to different microbial pathogens (viruses, bacteria, fungi and oomycetes) (Koo et al. 2020). SA is identified as a crucial signalling molecule required for the expression of genes involved in the plant defence response (Halim et al. 2006) and a potent plant hormone because of its diverse regulatory roles in plant metabolism (Hayat et al. 2010). The level of SA in plants depends on the developmental stage of the plant and varies with environmental stresses (Arif et al. 2020; Zafar et al. 2021).

Plant cells produce two types of organic compounds that are classified as primary and secondary metabolites. Primary metabolites (phytosterols, acyl lipids, nucleotides, amino acids and organic acids) are compounds vital for plant photosynthesis, respiration, growth and development. Secondary metabolites are associated with the plant’s response to environmental factors...
Polyphenols are the most widely distributed class of plant secondary metabolites and naturally occur in nearly all plants. They are essential compounds that ensure plant growth, development and reproduction. In addition, they also contribute to the elimination of waste, pigmentation, defence against various pathogens and herbivores or serve as signalling molecules involved in recognising symbionts. Polyphenols are involved in signal transduction from the root to the shoot and assist with nutrient mobilisation (Sharma et al. 2019).

In response to abiotic stresses, the biosynthesis of secondary metabolites is usually increased in plants. Polyphenols are produced under optimal and suboptimal conditions in plants. Plants exhibiting increased synthesis of polyphenols under abiotic stresses usually show better adaptability to limiting environments (Sharma et al. 2019).

Plants have evolved a number of defence mechanisms to respond to pathogens. They react to pathogen attack by activating defence mechanisms that act locally and systemically (Durner et al. 1997). Systemic acquired resistance (SAR) is the increased ability of a plant to resist pathogen infection following an initial infection. SAR produced by pathogen attack is based on salicylic acid (SA) signalling (Segarra et al. 2006). SA occurs naturally in plants, and plants produce this molecule to defend themselves against pathogen infections (Malamy and Klessig 1992; Hayat et al. 2010). It has been known, that different fertilisation regimes can influence phenolic compounds in different cultivated crops, like wheat (Konopka et al. 2012), red raspberry (Stojanov et al. 2019) and also ornamental herbs (Onofrei et al. 2017).

The aim of this study was to determine how different environmental parameters, such as biotic and abiotic stresses, affect the contents of salicylic acid and total polyphenols in sport turfgrass mixtures and thus indirectly affect their resistance/tolerance to diseases and pests, which has been also presented in our previous studies.

**Materials and methods**

**Experimental design**

An experiment was performed in 2019 at the Laboratory Field (46° 03’ N, 14° 28’ E, 300 m a.s.l.) of Biotechnical Faculty (University of Ljubljana) in Ljubljana. The responses of five different sport grass mixtures (Table 1) to three different fertilisation schemes were studied. The experimental area (180 m²) was arranged into a split-plot design with three replications (blocks). Sport grass mixtures were sown randomly. Each sport grass mixture was fertilised with three different fertilisation schemes: scheme A (inorganic fertiliser with two soil improvers), scheme B (organic fertiliser with two soil improvers) and scheme C (inorganic fertiliser without soil improvers). A detailed list of applied fertilisers and soil improvers is presented in Table 2, together with dates of application/fertilisation.

Fertilisers and soil improvers were applied at a rate of 25 g.m⁻² and were all supplied by Vitalis Care Ltd. (Samobor, Croatia). The solid inorganic fertilisers NPK NovaTec® Classic 12-8-16(±3+TE), NovaTec® Triplo 15-9-15(±2+TE), and NovaTec® Suprem 21-5-10(±3+TE) were produced by Compo Expert (Münster/Westphalia, Germany). NPK, DCM Vital-Green 14-4-8 + 3MgO + Fe, DCM Grass-Care 6-3-20 + 3MgO + Fe, soil improvers DCM Vivilsol® Minigran®, and DCM Antagon were produced by DCM Ltd. (Grobendonkmesto, Belgium). DCM Vivilsol Minigran is a mixed organic soil improver rich in organic matter to which bacteria Bacillus sp. have been added. This soil improver of vegetable origin releases soil phosphorus and enhances its uptake by plant roots. Moreover, it produces an optimal plant and root health status. It is the ideal solution for soils with a poor structure and limited fertility. DCM Antagon is an organic improver containing a Trichoderma-based biostimulant. This soil improver is

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| Table 1. Sport turfgrass mixtures used in our study and the suppliers. |
|---------------------------------------------------------------|
| Sport turfgrass mixtures | Turfgrasses cultivars and their percentage in the mixture | Supplier |
|--------------------------|------------------------------------------------------------|----------|
| 1 | *Lolium perenne* L. ‘Silver Dollar’ (50%), *Lolium perenne* L. ‘Vantage’ (30%), *Lolium perenne* L. ‘Carlee’ (30%) | Tempoverde Ltd., Italy |
| 2 | *Lolium perenne* L. ‘Fabian’ (40%), *Lolium perenne* L. ‘Tetrasar’ (40%), *Lolium perenne* L. ‘Merchtwo’ (20%) | DLF Trifolium Ltd., Denmark |
| 3 | *Lolium perenne* L. ‘Greensway’ (25%), *Lolium perenne* L. ‘Tetragreen’ (20%), *Lolium perenne* L. ‘Greensky’ (40%), *Poa pratensis* L. ‘SR2100’ | DLF Trifolium Ltd., Denmark |
| 4 | *Lolium perenne* L. ‘RPR’ (100%) | Barenbrug Ltd., the Netherlands |
| 5 | *Lolium perenne* L. ‘Barminton’ (10%), *Poa pratensis* L. ‘Baron’ (10%), *Festuca arundinacea* L. ‘Barlexas’ II’ (25%), *Festuca rubra* L. ‘Bardiva’ (10%), *Festuca rubra* L. ‘Barustic’ (25%) | Barenbrug Ltd., the Netherlands |
enriched with an organic base fertiliser (NPK 4-3-2). It improves the soil structure and stimulates the development of a diverse and rich microbial soil life. It is indispensable after soil fumigation or steaming and suitable for various applications (DCM-info2021).

Weather parameters and their determination

Soil temperature (5 and 10 cm), air temperature (50 cm and 2 m), relative humidity (Rh) and soil water content (5 and 10 cm) were measured with an ‘IoT system for environmental parameter measurements (producer: Slovenian Forestry Institute: Laboratory for Electronic Devices, Ljubljana, Slovenia’). Data regarding the sum precipitation (mm) of rain and sum of sun radiation (h) were collected from a database at the Slovenian Environmental Agency (MeteoSi 2021).

Sampling of sport turfgrasses for chemical analysis

On 23rd May, 28th May, 18th June, 27th June, 12th July, 12th August, 26th August, 19th September, 1st October and 23rd October, turfgrass samples (above the ground plant part) were collected for chemical analyses. Three samples were collected from a specific fertilisation scheme within turfgrass species per block. Samples were collected, depending on the period (spring-autumn), between 5:00 pm and 9:00 pm and weather parameters were recorded on the day of sampling only until the beginning of sample collection. Samples were stored in a freezer (−80°C; producer: Sanyo) prior to lyophilisation. After lyophilisation with the lyophilizer LIO-10P (producer: Kambič laboratorijska oprema, Semič, Slovenia), we ground the material with a grinder (producer: IKA) to obtain powder for chemical analysis.

Determination of the salicylic acid content

Three millilitres of 90% aqueous methanol and 10 µL of the internal standard 3,4-DHBA (10 µg/µL) were added to 0.3 g of dry, milled plant tissue. The mixture was homogenised by vortexing and extracted for 10 min with ultrasound. Another 2.5 ml of 100% methanol were added to the remaining plant tissue and extracted again. After centrifugation, both supernatants were collected, and 40 µl of 10% aqueous NaOH were added and evaporated to dryness in a rotary evaporator. Dry matter was dissolved in 1 ml of 5% aqueous trichloroacetic acid and 3 ml of a mixture of ethyl acetate/cyclopentane (1/1). The extraction procedure was repeated two times. Both organic phases (top phase) were combined and evaporated again with a rotary evaporator at 40°C. Dry matter was dissolved in 600 µl of 100% methanol and filtered through 0.22 µm syringe filters (PET). In this sample, the free salicylic acid content was determined using HPLC.

The HPLC analysis was performed on an Agilent 1200 instrument equipped with DAD and FLD detectors (Agilent, CA, USA). The DAD wavelength was set to 290 nm, the excitation wavelength of the FLD detector was set to 305, and the emission wavelength was set to 407 nm. For elution, a gradient mobile phase was used with the following programme: 100% A for 1 min, followed by a gradient in 20 min to 0% A and 100% B, gradient to 100% An in 5 min and finally 5 min with an isocratic solution of 100% A and 0% B. Mobile phase A consisted of 20 mM sodium acetate diluted in 10% aqueous methanol, and phase B was 100% methanol.

### Table 2. Fertilisation schemes and the fertilisers used in our survey.

| Date of application | Applied fertilisers |
|---------------------|---------------------|
| 28th March, 2019    | A: NovaTec® Suprem 21-5-10(+3+TE) + DCM Vivisol® Minigran® + DCM Antagon  
B: DCM Vital-Green 14-4-8 + 3MgO + Fe + DCM Vivisol® Minigran® + DCM Antagon  
C: NovaTec® Suprem 21-5-10(+3+TE) |
| 17th May, 2019      | A: NovaTec® Suprem 21-5-10(+3+TE) + DCM Vivisol® Minigran® + DCM Antagon  
B: DCM Vital-Green 14-4-8 + 3MgO + Fe + DCM Vivisol® Minigran® + DCM Antagon  
C: NovaTec® Suprem 21-5-10(+3+TE) |
| 20th June, 2019     | A: NovaTec® Triplo 15-9-15(+2+TE) + DCM Vivisol® Minigran® + DCM Antagon  
B: DCM Vital-Green 14-4-8 + 3MgO + Fe + DCM Vivisol® Minigran® + DCM Antagon  
C: NovaTec® Triplo 15-9-15(+2+TE) |
| 23th July, 2019     | A: NovaTec® Classic 12-8-16(+3+TE) + DCM Vivisol® Minigran® + DCM Antagon  
B: DCM Grass-Care 6-3-20 + 3MgO + Fe + DCM Vivisol® Minigran® + DCM Antagon  
C: NovaTec® Classic 12-8-16(+3+TE) |
| 28th August, 2019   | A: NovaTec® Classic 12-8-16(+3+TE) + DCM Vivisol® Minigran® + DCM Antagon  
B: DCM Grass-Care 6-3-20 + 3MgO + Fe + DCM Vivisol® Minigran® + DCM Antagon  
C: NovaTec® Classic 12-8-16(+3+TE) |
| 3rd October, 2019   | A: NovaTec® Classic 12-8-16(+3+TE) + DCM Vivisol® Minigran® + DCM Antagon  
B: DCM Grass-Care 6-3-20 + 3MgO + Fe + DCM Vivisol® Minigran® + DCM Antagon  
C: NovaTec® Classic 12-8-16(+3+TE) |
The retention time of the internal standard was 2 min on the FLD detector and that of salicylic acid was 3.4 min on the DAD detector. All reagents were purchased from Sigma-Aldrich and were of analytical grade. The results are presented as mg of salicylic acid/g sample. At the same time, moisture content was determined, and all results are presented on a dry matter basis. The validation of the method showed a relative standard deviation of determinations ±3.16%.

**Determination of total polyphenol contents**

For the determination of total phenolic contents, procedures described by Ahmed et al. (2019). Extraction was performed on finely powdered plant tissue (0.1 g) with 50 ml of methanol (Sigma-Aldrich) at 20°C for 24 h on a shaker. Sample extracts were filtered through Sartorius 388 filter paper (12–25 μm) (Sartorius, Switzerland). Two milliliters of the extract were removed, and 4 ml of a 2% (w/w) aqueous solution of Na₂CO₃ (Sigma-Aldrich) and 5 ml of 10% Folin & Ciocalteu reagent (Sigma-Aldrich) were added. The sample was placed in the dark, and absorbance at 765 nm was measured using a UV-VIS spectrophotometer UV-1900 (Shimadzu, Japan) within 15–30 min. As a standard, a solution of gallic acid (Sigma-Aldrich) was used, and the results are presented as mg GAE/g sample. The moisture content was determined at the same time, and thus all results are presented on a dry matter basis. The validation of the method showed a relative standard deviation of determinations ± 2.04%.

**Data analysis**

Analysis of variance (ANOVA) was performed to establish the differences among sport grass mixtures within fertilisation schemes. Differences in average salicylic acid and average total polyphenol contents were analysed with ANOVA. Before analysis, each variable was tested for homogeneity of variance, and nonhomogeneous data were log (Y) transformed prior to ANOVA. Significant differences (P ≤ 0.05) between average values were identified using Duncan’s honestly significant difference multiple range test. All statistical analyses were performed using Statgraphics Centurion XVI software (Statgraphics Technologies Inc., USA), and the results are presented as the untransformed means ± standard errors (SE). We calculated correlations by performing a linear regression analysis (y = kx + n) between average weather parameters and salicylic acid/total polyphenol contents. Prior to the statistical analysis, the experimental results were statistically evaluated using the programme Statgraphics Centurion XVI (2009).

**Results**

**Average values and daily sum of weather parameters**

The highest average values of soil temperature (5 and 10 cm) and air temperature (50 cm and 2 m) on the sampling date were recorded on 27th June. The soil water content was the highest on the first two sampling dates. Detailed values are presented in Table 3. The highest average values for parameters in the period mentioned above were also detected one day before sampling. Detailed data are presented in Table 4. Sun radiation (h) and rainfall (mm) are in both tables presented as daily sum.

**Average content of salicylic acid**

According to the results of statistical analysis, the average content of salicylic acid was influenced by the

### Table 3. Average values/daily sum of weather parameters recorded on the date of sampling for the chemical analysis.

| Date of sampling | Soil temperature (5 cm) (°C) | Soil temperature (10 cm) (°C) | Sun radiation (h) | Air temperature (50 cm) (°C) | Air temperature (2 m) (°C) | Rh (%) | Rainfall (mm) | Soil water content (5 cm) (mV) | Soil water content (10 cm) (mV) |
|------------------|-----------------------------|-------------------------------|-------------------|-----------------------------|-----------------------------|-------|---------------|-------------------------------|-------------------------------|
| 23rd May         | 17.68 ± 0.51                | 16.69 ± 0.52                 | 1.9               | 15.79 ± 0.28                | 16.03 ± 0.45                | 86.59 | 2.55          | 7.30                          | 1007.81 ± 2.65                |
| 28th May         | 17.52 ± 0.64                | 17.25 ± 0.35                 | 1                 | 15.22 ± 0.65                | 15.28 ± 0.77                | 99.08 | 1.01          | 15.30                         | 1021.35 ± 0.77                |
| 18th June        | 24.06 ± 0.45                | 23.78 ± 0.42                 | 8.8               | 21.16 ± 0.45                | 21.30 ± 0.67                | 85.26 | 2.55          | 0.10                          | 800.52 ± 1.21                 |
| 27th June        | 27.10 ± 0.55                | 26.20 ± 0.21                 | 13.8              | 27.03 ± 0.56                | 27.60 ± 0.56                | 76.22 | 0.17          | 0                            | 897.81 ± 2.10                 |
| 12th July        | 23.24 ± 0.40                | 22.79 ± 0.22                 | 6.7               | 19.27 ± 0.72                | 19.44 ± 0.77                | 84.97 | 3.67          | 0.70                          | 943.65 ± 2.68                 |
| 12th Aug         | 25.79 ± 0.33                | 25.22 ± 0.30                 | 11.2              | 24.55 ± 0.25                | 25.03 ± 0.65                | 82.27 | 2.22          | 0                            | 810.94 ± 1.11                 |
| 26th Aug         | 23.33 ± 0.22                | 23.05 ± 0.27                 | 6.8               | 21.68 ± 0.45                | 21.89 ± 0.58                | 92.84 | 1.79          | 0                            | 827.50 ± 2.01                 |
| 19th Sept        | 17.51 ± 0.21                | 17.93 ± 0.25                 | 9.1               | 13.25 ± 0.45                | 13.59 ± 0.75                | 81.34 | 3.34          | 11.30                         | 911.35 ± 0.77                 |

Note: Sun radiation (h) and rainfall (mm) are presented as daily sum.
The content of salicylic acid was 0.03 ± 0.002 mg.g⁻¹ against 0.01 ± 0.002 mg.g⁻¹ of salicylic acid. The content of salicylic acid was 0.034 ± 0.003 mg.g⁻¹ of salicylic acid were detected under schemes A and B, respectively. Among the 10 sampling dates, the highest (0.045 ± 0.002 mg.g⁻¹) salicylic acid content was detected under scheme A. The lowest average salicylic acid content was detected on 28th May, when the salicylic acid content ranged from 0.01 ± 0.00 mg.g⁻¹ under scheme A to 0.017 ± 0.00 mg.g⁻¹ under scheme B and 0.006 ± 0.00 mg.g⁻¹ under scheme C. So, when turfgrass was fertilised with fertilisation scheme C, SA content was the lowest. A detailed description of all values is presented in Figure 1.

**Average total polyphenol contents**

Based on the results of the statistical analysis, the average total polyphenol content was influenced by the date of evaluation ($F_{9,147} = 4.64, P < 0.05$) and fertilisation scheme ($F_{2,149} = 3.27; P < 0.05$). We did not detect an effect of the sport turfgrass mixture ($F_{4,142} = 0.54; P = 0.7070$), interaction between the fertilisation scheme and sport turfgrass mixture ($F_{8,142} = 0.69; P = 0.6971$), interaction between the fertilisation scheme and date of evaluation ($F_{18,142} = 1.55; P = 0.1005$), or interaction between the sport turfgrass mixture and date of evaluation ($F_{36,142} = 0.91; P = 0.6141$) on the average content of salicylic acid. The content of salicylic acid was 0.03 ± 0.002 mg.g⁻¹ in all sport turfgrass mixtures. In general, the significantly lowest average salicylic acid content was detected under fertilisation scheme C, where 0.027 ± 0.002 mg.g⁻¹ was detected. On average, 0.034 ± 0.003 mg.g⁻¹ and 0.032 ± 0.002 mg.g⁻¹ salicylic acid were detected under schemes A and B, respectively. Among the 10 sampling dates, the highest (0.045 ± 0.01 mg.g⁻¹) salicylic acid content was detected on 18th June. On 18th June, 0.058 ± 0.02 mg/g salicylic acid was detected under scheme A. The lowest average salicylic acid content was detected on 28th May, when the salicylic acid content ranged from 0.01 ± 0.00 mg.g⁻¹ under scheme A to 0.017 ± 0.00 mg.g⁻¹ under scheme B and 0.006 ± 0.00 mg.g⁻¹ under scheme C. So, when turfgrass was fertilised with fertilisation scheme C, SA content was the lowest. A detailed description of all values is presented in Figure 1.

**Table 4.** Average values/daily sum of weather parameters recorded one day prior to sampling for the chemical analysis.

| Date of evaluation | Soil temperature (5 cm) (°C) | Soil temperature (10 cm) (°C) | Sun radiation (h) | Air temperature (50 cm) (°C) | Air temperature (2 m) (°C) | Rh (%) | Rainfall (mm) | Soil water content (5 cm) (mV) | Soil water content (10 cm) (mV) |
|--------------------|-----------------------------|-------------------------------|------------------|-----------------------------|-----------------------------|--------|---------------|-------------------------------|-------------------------------|
| 22nd May           | 15.73 ± 0.62                 | 15.24 ± 0.31                 | 3.1              | 14.55 ± 0.39                | 14.76 ± 0.55                | 90.81 ± 2.17 | 0.40            | 1010.94 ± 1.01                | 993.96 ± 2.88                 |
| 27th May           | 17.58 ± 0.27                 | 17.59 ± 0.35                 | 0.19             | 14.24 ± 0.42                | 14.27 ± 0.65                | 102.82 ± 0.61 | 2.70            | 1009.69 ± 2.65                | 995.63 ± 1.88                 |
| 17th June          | 24.28 ± 0.37                 | 23.97 ± 0.24                 | 7.6              | 21.61 ± 0.55                | 21.74 ± 0.73                | 80.96 ± 0.45 | 0.10            | 810.63 ± 2.21                | 761.15 ± 2.47                 |
| 26th June          | 25.89 ± 0.42                 | 25.15 ± 0.45                 | 14.3             | 24.99 ± 0.25                | 25.41 ± 0.87                | 80.54 ± 1.62 | 0.00            | 928.33 ± 2.38                | 880.52 ± 1.97                 |
| 11th July          | 21.76 ± 0.52                 | 21.53 ± 0.11                 | 9.3              | 17.86 ± 1.15                | 18.29 ± 0.87                | 76.39 ± 2.77 | 0.00            | 954.58 ± 3.25                | 902.19 ± 3.10                 |
| 11th Aug           | 25.09 ± 0.25                 | 24.89 ± 0.20                 | 7.2              | 23.16 ± 0.71                | 23.39 ± 0.56                | 86.15 ± 3.45 | 0.00            | 822.92 ± 1.20                | 765.10 ± 0.82                 |
| 25th Aug           | 22.88 ± 0.55                 | 22.66 ± 0.25                 | 8.3              | 20.46 ± 0.52                | 20.77 ± 0.47                | 93.33 ± 2.11 | 14.10           | 862.50 ± 2.22                | 747.08 ± 1.89                 |
| 18th Sept          | 18.57 ± 0.42                 | 19.09 ± 0.21                 | 0.19             | 14.26 ± 0.45                | 14.41 ± 0.56                | 93.95 ± 2.45 | 0.00            | 908.12 ± 0.99                | 839.54 ± 1.78                 |
| 30th Sept          | 17.91 ± 0.56                 | 17.86 ± 0.20                 | 7.3              | 17.63 ± 0.13                | 18.21 ± 0.45                | 89.10 ± 3.22 | 9.80            | 953.54 ± 0.83                | 892.81 ± 2.55                 |
| 22nd Oct           | 14.94 ± 0.81                 | 15.01 ± 0.35                 | 7.3              | 13.86 ± 0.29                | 14.68 ± 0.74                | 96.17 ± 3.74 | 0.00            | 910.62 ± 1.27                | 878.85 ± 3.01                 |

Note: Sun radiation (h) and rainfall (mm) are presented as daily sum.

**Figure 1.** Average content of salicylic acid (mg.g⁻¹) in turfgrass cultivated under each fertilisation scheme on different evaluation dates (for comparisons within the date of evaluation between fertilisation schemes, df = 2,142 (23rd May, F = 0.98, P < 0.05; 28th May, F = 2.07, P < 0.05; 18th June, F = 9.13, P < 0.05; 27th June, F = 7.77, P < 0.05; 12th July, F = 13.10, P < 0.05; 12th Aug, F = 14.22, P < 0.05; 26th Aug, F = 6.73, P < 0.05; 19th Sep, F = 7.22, P = 0.0732; 1st Oct, F = 12.12, P < 0.05; 23rd Oct, F = 10.20, P < 0.05)).
evaluation \( F_{36,149} = 0.91; \ P = 0.6141 \) on the average content of total polyphenols. The total polyphenol content was approximately 24 mg/g when fertilisation schemes B and C were applied. When fertilisation scheme A was applied, approximately 22 mg.g\(^{-1}\) total polyphenols were detected, which was significantly lower than the other values. In general, the average content of total polyphenols ranged from 24 mg.g\(^{-1}\) in sport turfgrass mixture 1–23 mg.g\(^{-1}\) in sport turfgrass mixture 3 and 22 mg.g\(^{-1}\) in sport turfgrass mixture 5. At the beginning of sampling (23rd May), the lowest content of total polyphenols was detected in turfgrass cultivated under fertilisation scheme A, as approximately 24 mg.g\(^{-1}\) of total polyphenols were detected. Total polyphenol content reached the highest values, when scheme B and C was applied. A detailed presentation of the results is provided in Figure 2.

**Correlations between average values of environmental parameters and contents of chemical substances on different sampling dates**

In all three schemes, negative correlation coefficients were also observed between the salicylic acid content in the samples and soil temperature at depths of 5 and 10 cm and air temperature at 0.5 and 2.0 m above the ground, but the highest correlation coefficient was still low \((-0.31)\). In scheme A, positive correlation coefficients were observed between the salicylic acid content in the samples and the values of the four parameters, as well as the relative humidity, with a maximum of 0.32 (Table 5).

In all three schemes, negative correlation coefficients were observed between the total polyphenol contents in the samples and the soil temperature at depths of 5 and 10 cm and air temperatures at 0.5 and 2.0 m above the ground on the day of sampling. All values were low and insignificant, and none exceeded \(-0.24\).

The correlation coefficients between the content of total polyphenols in the samples and precipitation and the soil water content at depths of 5 and 10 cm were positive. The highest, but still only moderate (0.64), correlation coefficient was observed in scheme C between the total polyphenol content and precipitation (Table 6).

In all three schemes, one day before sampling, negative correlation coefficients were observed between the salicylic acid content in the samples and the soil water content at depths of 5 and 10 cm, and relative air humidity. In each scheme, we confirmed the statistical significance for only one of the three correlation coefficients, and the strongest and most significant correlation, which was moderate in our case \((-0.66)\), was observed between the salicylic acid content in the samples and the soil water content at a depth of 10 cm.

In schemes B and C, negative correlation coefficients were also observed between salicylic acid content in the
Table 5. Correlation coefficients between average values/daily sum of some environmental parameters and the salicylic acid content on the sampling date.

| Environmental parameter | Statistical parameter | scheme A | scheme B | scheme C |
|-------------------------|-----------------------|----------|----------|----------|
| soil temperature (5 cm) | r 0.27                | −0.27    | −0.30    |
|                        | p 0.1442              | 0.1523   | 0.1040   |
| soil temperature (10 cm)| r 0.32                | −0.22    | −0.27    |
|                        | p 0.08                | 0.2298   | 0.1425   |
| sun radiation (h)      | r 0.27                | 0.07     | 0.12     |
|                        | p 0.08                | 0.125    | 0.125    |
| air temperature (50 cm) | r 0.19                | −0.31    | −0.30    |
|                        | p 0.30                | 0.0945   | 0.1030   |
| air temperature (2 m)  | r 0.19                | −0.30    | −0.28    |
|                        | p 0.3117              | 0.1052   | 0.1337   |
| Rh                     | r 0.09                | 0.07     | −0.05    |
|                        | p 0.6297              | 0.6447   | 0.7663   |
| Rainfall               | r −0.54               | −0.29    | −0.37    |
|                        | p 0.0017*             | 0.1127   | 0.04*    |
| soil water content (5 cm)| r −0.60              | −0.39    | −0.29    |
|                        | p 0.005*              | 0.0039*  | 0.1181   |
| soil water content (10 cm)| r −0.64             | −0.43    | −0.37    |
|                        | p 0.002*              | 0.0171*  | 0.0411*  |

Note: *p < 0.05.

Table 6. Correlation coefficients between average values of some environmental parameters and total polyphenol contents on sampling date.

| Environmental parameter | Statistical parameter | scheme A | scheme B | scheme C |
|-------------------------|-----------------------|----------|----------|----------|
| soil temperature (5 cm) | r −0.10               | −0.15    | −0.13    |
|                        | p 0.7752              | 0.6862   | 0.7102   |
| soil temperature (10 cm)| r −0.13               | −0.15    | −0.12    |
|                        | p 0.7366              | 0.6709   | 0.7425   |
| sun radiation (h)      | r −0.11               | −0.45    | −0.26    |
|                        | p 0.0810              | 0.0034   | 0.0981   |
| air temperature (50 cm) | r −0.15               | −0.15    | −0.23    |
|                        | p 0.6697              | 0.6829   | 0.5254   |
| air temperature (2 m)  | r −0.154              | −0.16    | −0.24    |
|                        | p 0.6706              | 0.6544   | 0.5011   |
| Rh                     | r −0.22               | 0.21     | −0.05    |
|                        | p 0.5356              | 0.5565   | 0.8778   |
| Rainfall               | r 0.29                | 0.53     | 0.64     |
|                        | p 0.4225              | 0.1127   | 0.04*    |
| soil water content (5 cm)| r 0.24               | 0.33     | 0.32     |
|                        | p 0.5106              | 0.3569   | 0.0462*  |
| soil water content (10 cm)| r 0.25              | 0.35     | 0.42     |
|                        | p 0.4865              | 0.3219   | 0.2332   |

Note: *p < 0.05.

Table 7. Correlation coefficients between average values of some environmental parameters on the day before sampling and content of salicylic acid.

| Environmental parameter | Statistical parameter | scheme A | scheme B | scheme C |
|-------------------------|-----------------------|----------|----------|----------|
| soil temperature (5 cm) | r 0.36                | −0.17    | −0.25    |
|                        | p 0.3026              | 0.6443   | 0.4798   |
| soil temperature (10 cm)| r 0.37                | −0.14    | −0.23    |
|                        | p 0.2843              | 0.6879   | 0.5162   |
| sun radiation (h)      | r 0.01                | −0.086   | −0.86    |
|                        | p 0.9274              | 0.0949   | 0.3941   |
| air temperature (50 cm) | r 0.35                | −0.13    | −0.14    |
|                        | p 0.3162              | 0.7360   | 0.7049   |
| air temperature (2 m)  | r 0.36                | −0.10    | −0.11    |
|                        | p 0.3007              | 0.7707   | 0.76     |
| Rh                     | r −0.39               | −0.08    | −0.08    |
|                        | p 0.2629              | 0.8220   | 0.8230   |
| Rainfall               | r −0.41               | −0.11    | −0.12    |
|                        | p 0.2388              | 0.7462   | 0.7348   |
| soil water content (5 cm)| r −0.58              | −0.45    | −0.35    |
|                        | p 0.0801              | 0.0501   | 0.0269*  |
| soil water content (10 cm)| r −0.66             | −0.37    | −0.30    |
|                        | p 0.0372*             | 0.0477*  | 0.0927   |

Note: *p < 0.05.

samples and soil temperature at depths of 5 and 10 cm and air temperature 0.5 and 2.0 m above the ground, but the highest correlation coefficient was still low (−0.25). In scheme A, positive correlation coefficients were observed between the salicylic acid content in the samples and the values of the four parameters listed above, although the highest correlation coefficient was still low, namely, 0.37 (Table 7).

In all three schemes, one day before sampling, negative correlation coefficients were observed between the total polyphenol content in the samples and the soil temperature at depths of 5 and 10 cm and air temperatures at 0.5 and 2.0 m above the ground. All values were low and insignificant, and none exceeded −0.25.

The values of the correlation coefficients were, with one exception, positive between the total polyphenol content in the samples and relative air humidity, precipitation and soil water content at depths of 5 and 10 cm, with the highest, but still only moderate (0.59), correlation coefficient observed in scheme A between the total polyphenol content and precipitation (Table 8).

Correlation between average total polyphenol content and average salicylic acid within fertilisation schemes

As presented in Table 9, negative moderate correlation was detected in schemes A and B, where average total polyphenol content provoked negative impact on salicylic acid, where in fertilisation scheme C, low correlation between total polyphenols and salicylic acid was detected.
Table 8. Correlation coefficients between average values of some environmental parameters one day before sampling and total polyphenol contents.

| Environmental parameter | Statistical parameter | scheme A | scheme B | scheme C |
|-------------------------|-----------------------|----------|----------|----------|
| soil temperature (5 cm) | r                     | -0.16    | -0.14    | -0.05    |
|                         | p                     | 0.6627   | 0.6966   | 0.8829   |
| soil temperature (10 cm)| r                     | -0.15    | -0.13    | -0.02    |
|                         | p                     | 0.6878   | 0.7274   | 0.9520   |
| sun radiation (h)      | r                     | 0.41     | -0.03    | -0.27    |
|                         | p                     | 0.0087   | 0.8640   | 0.0976   |
| air temperature (50 cm)| r                     | -0.21    | -0.25    | -0.20    |
|                         | p                     | 0.5505   | 0.0489   | 0.5867   |
| air temperature (2 m)  | r                     | -0.23    | -0.24    | -0.24    |
|                         | p                     | 0.5168   | 0.4922   | 0.5096   |
| rh                      | r                     | 0.04     | 0.48     | 0.18     |
|                         | p                     | 0.9171   | 0.1561   | 0.6126   |
| rainfall                | r                     | 0.59     | 0.48     | 0.37     |
|                         | p                     | 0.0856   | 0.0568   | 0.0742   |
| soil water content (5 cm)| r                      | 0.18     | 0.30     | 0.19     |
|                         | p                     | 0.6219   | 0.4041   | 0.5900   |
| soil water content (10 cm)| r                      | 0.19     | 0.17     | -0.02    |
|                         | p                     | 0.6284   | 0.6371   | 0.9520   |

Note: r represents the correlation coefficient, *p < 0.05.

Table 9. Impact of total polyphenols on salicylic acid content regarding different fertilisation schemes.

| Total polyphenols scheme A | Salicylic acid scheme B | Salicylic acid scheme C |
|----------------------------|-------------------------|-------------------------|
| r -0.58                    | /                       | /                       |
| p 0.0799                   | /                       | /                       |
| Total polyphenols scheme B | /                       | -0.60                   |
| r /                        | /                       | -0.60                   |
| p / 0.0674                 | /                       | -0.18                   |
| Total polyphenols scheme C | /                       | /                       |
| r /                        | /                       | 0.62                    |
| p /                        | /                       | 0.62                    |

Discussion

In one of the first European investigations of the natural resistance of sport turfgrasses, we examined how different fertilisation schemes altered the contents of endogenous salicylic acid and total polyphenols in different turfgrass mixtures. The effects of different grass mixtures on the contents of salicylic acid and total polyphenols were not statistically confirmed. Therefore, the results presented in this paper depend on the method of fertilisation and selected abiotic factors. Results, presented in this study are part of multiple year study, that was performed, and where we also performed monitoring of average yield (Zanelli et al. 2021).

Sport turfgrasses are susceptible to diseases that cause enormous economic losses. Frequent use of fungicides has contributed to environmental pollution and a loss of biodiversity (Aamlid et al. 2012; Coelho et al. 2021). The reduction in the use of fungicides has led to the search for other sport turfgrass preventive maintenance methods.

In recent years, sport turfgrass fertilisation has focused on the use of biostimulants (Yousfi et al. 2021), endophytes (Bradshaw and Pane 2020) and other environmentally friendly products. In our research, we wanted to determine how different fertilisation schemes affected the contents of endogenous salicylic acid and total polyphenols. For this purpose, we studied the effect of organic fertilisers with soil improvers and inorganic fertilisers with and without soil improvers (DCM Minigran Fertilizers 2021). In a field experiment, a product with antagonistic Trichoderma fungi and Bacillus bacteria was used as a soil improver. Sun radiation proved to have more constant impact on total polyphenol impact than on salicylic acid.

As ThariqJaveed et al. (2021) has reported, soil antagonistic fungus Trichoderma spp. improves nutrient utilisation in soil. As detected in our study, a higher salicylic acid content was recorded under schemes A and B, where products with the fungi Trichoderma spp. and Bacillus spp were used. During the period from 17th June to 26th July, when schemes A and B had the highest salicylic acid contents, we also recorded the highest temperatures in soil/air, which exceeded 21°C and reached 27°C, and precipitation was almost nonexistent. High temperatures were recorded between 25th and 26th August, when we also detected the highest salicylic acid levels in schemes A and B. In our opinion, we can conclude that the higher content of salicylic acid helps turfgrasses to better tolerate drought stress, which has already been established by Ervin et al. (2005), and in vegetables by Lin et al. (2020) and Zhi Zhong et al. (2020).

Cheng et al. (2020) reported that Bacillus megaterium affects the formation of salicylic acid in rice, which in turn alters the resistance of plants to diseases. What’s more, salicylic acid is an important factor contributing to the natural resistance of turfgrass to diseases, as already established by Liu et al. (2018). Regarding use of Bacillus subtilis and salicylic acid results in faster wheat growth and higher resistance to drought conditions (Lastochkina et al. 2020), but we can confirm this in our study because we did not monitor the yield in our field experiment, As reported in the study by Krishnan et al. (2016) of the responsiveness of grasses to high temperatures, the sampling period is very important when analysing the salicylic acid content in plants. Salicylic acid is also an important factor responsible for alleviating water stress (precipitation and soil water),
which has already been studied by Oliveira et al. (2019). This finding was also confirmed in our study, as we observed a moderate negative correlation between the amount of water in the soil (on the day of sampling and one day before sampling) and the content of salicylic acid. We determined a negative correlation between the amount of precipitation and the salicylic acid content. Results of our study have also confirmed, that when adding soil improvers in combination with fertilisers to the turfgrass pitches, content of total polyphenols effects negatively on content of salicylic acid. Stated finding haven’t been detected in previous studies.

We detected smaller differences in the polyphenol content than in salicylic acid content between different fertilisation schemes. Therefore, among the ten sampling dates, only one sample from scheme A (on 28th May) was confirmed to exhibit a significantly lower total polyphenol content, while in the other two cases (18th June and 1st October), a significantly lower total polyphenol content was detected in scheme A compared to scheme C. According to the fertilisation scheme, among other values, no significant difference was found. We concluded that the polyphenol content does not represent a typical turfgrass response to different fertilisation schemes.

For some plants, such as eggplants and tomatoes, higher temperatures result in higher levels of total polyphenols (Barbagallo et al. 2013; Helyes et al. 2015). Previous studies on turfgrasses have focused on the effect of drought stress on total polyphenol formation. Fuentesalba-Sandoval et al. (2020) did not observe a significant effect of water deficiency on the total polyphenol content, which was also confirmed in our study, where we confirmed a weak nonsignificant correlation one day before sampling, and we confirmed a moderately positive and significant effect of precipitation on the day of sampling.

Based on the results of the present study on sports turfgrasses, which represents the first of its kind in the world, we conclude that significant differences in the contents of salicylic acid and total polyphenols were not observed between different sport turfgrass mixtures, but sampling dates and fertilisation schemes exerted significant effects on the contents of both molecules. We detected a higher salicylic acid content in turfgrasses cultivated with organic and inorganic fertiliser schemes along with soil improvers, indicating that soil improvers exert a certain effect on the formation of salicylic acid in sport turfgrasses, regardless of whether they are used together with inorganic or organic fertilisers. Regarding total polyphenol contents, a similar effect of soil improvers was not confirmed, therefore we conclude that soil improvers in turfgrasses do not have a constant or predictable influence on polyphenol content.

We established that the method of fertilisation exerts a certain effect on the salicylic acid and total polyphenol contents, but the effect of fertilisation depends largely on environmental factors, especially temperature, precipitation, sun radiation and water content in the soil. Thus, we conclude that effective fertilisation is an extremely complex procedure that requires further basic and applied research for clarification.

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