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Measuring Fluorescence as a Means to Evaluate the Physiological Reaction to Growth Retardant Applied to Manage Turf

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Abstract: This paper presents the effects of the physiological reaction of the main cool-season grass species used for turf in a temperate climate: perennial ryegrass, Kentucky bluegrass, and tall fescue, on a twice-a-year trinexapac-ethyl (TE) application in late spring and early autumn, seasons of rapid turf growth. The fully established turf plots in the split-plot system of three replicates, with three cultivars/species, were treated by TE (1.5 and 4.5 g/100 m^2). The 4.5 g/100 m^2 was harmful to Kentucky bluegrass. The perennial ryegrass responded by dose-dependent growth inhibition; 30–60% in spring and 25–40% in autumn for lower and higher doses, respectively. Tall fescue responded by 50% growth inhibition independently of concentration and season. Plant physiological responses, visualized as graphs of fluorescence data, revealed the stress of Kentucky bluegrass upon high TE dose. Based on principal component analysis (PCA) analysis, three groups were distinguished: perennial ryegrass varieties from high and low TE treatments and Kentucky bluegrass varieties from high TE. TE-dependent growth reduction with no significant quality decrease benefits the environment by reducing carbon footprint machine operations (mowing). Utilizing fluorescence measurement may help to manage turf physiology.

Keywords: *Lolium perenne* L.; *Poa pratensis* L.; *Schedonorus arundinaceus* Schreb.; plant growth regulator; lawn quality; Chl *a* fluorescence; JIP

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1. Introduction

Perennial ryegrass (*Lolium perenne* L.), Kentucky bluegrass (*Poa pratensis* L.), and tall fescue (*Festuca arundinacea* L. syn. *Schedonorus arundinaceus* L.) are the most used species among cool season turfgrasses on urban turfs. In a temperate climate zone, perennial ryegrass is used virtually in every turf grass seed mixture due to rapid seed emergence and high vigor of seedlings resulting in rapid turf establishment. As a component of well-managed turf, it is resistant to traffic, but unfortunately, it is also prone to diseases that develop in temperatures around zero centigrade [1]. Kentucky bluegrass, by contrast, germinates relatively slowly and takes longer to establish turf. Its significant advantage is forming rhizomes, which are more resistant to tearing, facilitating easier recovery (if tears happen) compared to the bunch-type perennial ryegrass or tall fescue [2]. Tall fescue creates a deep root system, making this species the most tolerant to drought among the three investigated species [3,4].

Late spring and early autumn are periods of frequent mowing in Europe and North America's temperate climate zones corresponding to timing of growth of grasses during optimal temperatures and light conditions. As a result, the biomass of clippings is about 5.3 times greater than in the middle of the summer [5,6]. The intensity of grass elongation growth in late spring/early autumn can be estimated as fourfold higher than in March and in November, at the beginning and the end of annual vegetation, and 2.5 times higher as compared to the midsummer (Figure 1).

Figure 1. Ideogram of changes in lawn growth intensity during the year in the temperate climate zone (52°12′58.536″ N, 20°38′43.008″ E) expressed as changes in grass height. Winter dormancy usually lasts from half of November until half of March.

Ignatieva et al. [7,8] estimated that, on a world scale, turfs occupy more than half of urban green areas as green carpets surrounding buildings, decorative elements in parks, and natural covers of stadiums and playgrounds. Although there is currently scientific evidence of the adverse effects of low-mown turfs on biodiversity, low-mown turfs are still a frequent priority for green architecture [9]. Low-mown properties also result from what is often pictured as a perfect yard [10,11]. In such places, the limits of turf care should be considered a pro-ecological action, reducing maintenance costs and the carbon footprint.

Mowing is still one of turf management's most energy-intensive cultural practices [12]. Continuous use of mowers powered by internal combustion engines results in release to the atmosphere: carbon mono and di-oxides, sulfur trioxide, and hydrocarbons (HC) and their derivatives. It was estimated that lawnmowers contribute 5.2 and 11.6% to the emission of carbon monoxide and non-methane hydrocarbons, respectively, compared to road transport emissions in Australia [13]. The meta-analysis of the mowing frequency impact on the environment shows positive ecological effects with reduced mowing frequency [5]. High turf maintenance costs consist of fossil-fuel-driven mowing, application of chemicals, and irrigation [14]. Fuel used for mowing is responsible for 0.85 kg of carbon equivalent (Ce) emitted to the atmosphere for 1 kg of gasoline and 0.94 kg for 1 kg of diesel fuel used [14]. According to Allaire et al. [15], frequently mowed sites emit up to 2.0 kg m^{-2} CO_2 annually.

Plant growth regulators (primarily acting as gibberellins' inhibitors) are commonly used to slow down plant elongation and, consequently, the number of mowings and accumulated biomass [16–20]. Growth inhibition results in a higher density of leaf mesophilic cells and thus higher chlorophyll concentration, seen as a darker green. It also affects turf density, tillering, longer greenness in fall and earlier greening up in spring, greater grass tolerance to shade, diseases, and unfavorable temperatures [17,21–24].

Our present research aimed to determine whether using a growth regulator contributes to the stress induction in plants and, if so, is this reaction the same for different species of grasses. Chl *a* fluorescence measurements are used as a screening method to detect plant physiological state.

In 1991 Syngenta introduced Primo maxx®, containing trinexapac-ethyl (TE), as an active substance [21], which suppresses the activity of the 3-β-hydroxylase enzyme required for the synthesis of gibberellin (GA1) active form. As the labeling of Primo maxx® gives divergent dosing recommendations for different years (Table 1), we decided to use a lowered dose to assess whether the TE dose reducing or overdosing could be possible. Three turf species, three varieties each, were studied under the two doses of TE treated in the field twice a year.

Table 1. Recommended doses [ml and g of active ingredient/100 m^2] of trinexapac-ethyl (TE) for application on turfgrass species: perennial ryegrass, Kentucky bluegrass, and tall fescue in different years, as specified on Primo maxx® (Syngenta) product label information. The trinexapac-ethyl concentration is 12 [%].

Recommended TE Doses Per 100 m^2 * (2018)	
Perennial ryegrass (1)	16–30 (1.8–3.4)
Kentucky bluegrass (2)	20 (2.3)
Tall fescue (3)	24 (2.7)
Mix (1) × (2)	24 (2.7)
Mix (1) × (2) × (3)	24 (2.7)

* 12% TE doses are given in ml/100 m^2 and in (g of active ingredient/100 m^2).

2. Materials and Methods

2.1. Plant Materials and Field Studies

Three grass species, each represented by three varieties, were used for the experiment: perennial ryegrass (*Lolium perenne* L.), varieties: Goalkeeper, Jackento, Top Gun; Kentucky bluegrass (*Poa pratensis* L.), varieties: Award, BlueChip, Liberator, and tall fescue (*Festuca arundinacea* L. syn. *Schedonorus arundinaceus* L.), varieties: Arid 3, Pixi and Stowell. The experiment was performed twice (in 2018 and 2019), in 3 replications in the split-plot system, in which the TE dose was the first factor and grass varieties were the second- factor. The experiment was run at the Plant Breeding and Acclimatization Institute-National Research Institute, Poland (GPS 52°12′49″ N, 020°38′33″ E), in an open area with full sun on sandy loam soil (58.4% of sand, 38.7% of silt, 2.9% of clay). The initial contents of macronutrients was (in mg/L of soil): N—16, P—88, K—130, Mg117 and Ca—1120. Soil pH_{KCl} was 7.24, and soil organic matter content 1.5%. The field size was 77 m × 25 m, and the size of a single plot of 2 m × 2 m. Seeds sowing rates were: 20 g/m^2 of perennial ryegrass, 10 g/m^2 Kentucky bluegrass, and 25 g/m^2 tall fescue [25].

The management practice consisted of once a week of mowing (at midday) to a height of 3 cm, beginning in April and continuing through to mid-November (in sum, 30 mowings per year). Ammonium nitrate, NH_4NO_3, was applied twice (2.5 kg/100 m^2) in the last weeks of both April and August. Additionally, plots were watered by a Rain Bird system equipped with SMRT-Y Soil Moisture Sensor (Rain Bird Ltd., Brentwood, UK), which turned on/off irrigation in response to soil moisture at 60–80% of soil water capacity. Spray applications of TE (Modus, Syngenta Ltd., Bracknell, UK; 250 g/L concentration) were made on fully established turf plots for two consecutive years (2018 and 2019) twice a year, at the beginning of May and at the transition between August and September, promptly after mowing and fertilization, during windless and sunny days at temperatures 20–23 °C and air humidity 55–70%, typical for the study's region. A Kombi Classic battery sprayer (Fleet Line Markers Ltd., Malvern, UK), generating 200 kPa (2 bar) pressure, was used to apply TE in doses: 0 (control), 1.5 (low), and 4.5 (high) [g/100 m^2].

2.2. Evaluation of TE Influence on Turfs

The turfgrass quality, grass height, clipping yield, chlorophyll content, and Chl a fluorescence were collected one and five weeks after TE application. Evaluations based on a 9-grade scale included: visual merit, turf density, and color. Visual merit is defined by the British Society of Plant Breeders Ltd. (2021) as "an overall measure of the suitability of the turf for its potential use. It is a combination of sward density, leaf width, disease resistance, color, and other factors which could influence appearance, such as "cleanness of cut" (Table 2) [26].

Table 2. Description of scale grades used for visual evaluation of lawn quality.

Scale Grades	Visual Merit	Turf Density	Turf Color
1	no plants	no plants	no plants
2	poorly looking	very weak	dried
3	weak	≤20%	yellow-brown turf
4	poor	≤45%	bleaching turf
5	sufficient	≤60%	gray-brown
6	average	>60%	green-blue
7	good	quite dense (ca. 80%)	light-green
8	very good	nearly 100% dense	green turf
9	ideal	fully dense turf	dark green

Grass height was measured on three randomly selected places per plot Using Prism Gauge (Bernhard and Company Ltd., Rugby, Warwickshire, UK). The dry mass of the clippings was determined after overnight drying at 105 °C. Clippings were collected separately from each plot using a Stiga Ltd. rotary mower 48 cm wide, with a basket (cutting height 3 cm).

Chlorophyll index (CI) was estimated using a contactless FieldScout Chlorophyll Meter CM100 (Spectrum Technologies, Inc., Plainfield, IL, USA) and expressed in relative units (0–999). Three measurements per plot were done at noon on a full sunny day.

Chl *a* fluorescence was measured using PocketPEA portable fluorimeter (Hansatech Instruments, King's Lynn, Norfolk, UK) in three replications per plot [27]. First, the fluorescence was induced in a leaf previously darkened for 30 min by red (627 nm), saturating light of 3500 μmol m^{-2} s^{-1}. Then, the first 3 s of fluorescence were registered, and the collected data were elaborated using PocketPEA dedicated software to extract the photosynthesis-related parameters [28,29]. Parameters list and their biophysical meaning: F_O (initial fluorescence value at 50 μs responding to radiation losses during migration of excitation energy induced by photons in chlorophyll antennas); F_M (maximum usually after 0.5–1 s responding to a total reduction of Photosystem II (PS II)); T_{FM} (time to reach FM); F_V (maximal variable fluorescence, a difference between maximal and minimal fluorescence); F_V/F_M (the ratio of variable and maximal fluorescence reflecting the force of the photosynthetic light reactions); F_V/F_O (the ratio of variable and initial fluorescence reflecting the efficiency of the water-splitting complex on the donor side of PS II); Area (the total area between the y-axis and increasing part of fluorescence curve on fluorescence plot reflecting the pool size of reduced acceptors); RC_{ABS} (the number of active reaction centers in chlorophyll antennas per absorption); $(1 - V_J)/V_J)$ (the measure of forwarding electron transport); PI_{ABS} (the performance index).

Additionally, the row fluorescence data were double normalized. The differences between the fluorescence values of TE-treated plants and the values measured on control plants, presented on a logarithmic time scale, formed the ΔW_{OJ} parabolic curves. On such graphs, the extremum with the negative value is characteristic of the plants in good physiological condition, contrary to the positive ones [28,30,31].

2.3. Climatic Data

The temperature and the precipitation were registered by automatic weather station MAWS101 (Vaisala, Finland) localized on the experimental fields.

The weather differed slightly from the multi-year average during the investigation (years 2018 and 2019) (Table 3). The annual average temperatures were higher by 2.0 °C in the first year and 2.6 °C in the next year than the long-term average (8.3 °C). Exceptions occurred only in February and March 2018, thus in months with no plant vegetation in a temperate climate. On the other hand, the annual sum of precipitations was lower (in 2018 by 4%, and in 2019 by 15%) than the long-term average, inducing the periodical droughts. The experimental fields were irrigated to eliminate the effects of drought.

Table 3. Mean month air temperatures and a sum of month precipitations during the experiment compared with typical values calculated as multiannual averages based on archival data (1995–2015).

	January	February	March	April	May	June	July	August	September	October	November	December
Years	Temperature [°C]											
1995–2015	−1.8	−1.0	2.8	8.1	14.2	16.9	18.5	18.2	13.3	8.3	2.8	−0.1
2018	0.9	−3.1	0.8	13.5	17.9	19.2	21.1	20.9	16.1	10.2	4.4	2.1
2019	−1.6	3.0	5.9	11.7	13.9	22.8	19.1	20.8	14.6	11.0	6.4	3.3
	Precipitation [mm]											
1995–2015	21.3	17.5	22.3	30.3	46.0	63.0	74.3	51.8	43.5	31.5	30.3	28.4
2018	26.0	8.8	16.4	26.0	73.2	25.2	80.0	34.4	34.0	28.0	28.8	63.8
2019	39.6	34.2	36.8	3.0	9.2	40.8	53.2	37.4	74.4	13.6	15.8	34.8

2.4. Statistical Analysis and Results Visualization

The two-way analysis of variance (ANOVA) for the split-plot experiment, at $p \leq 0.05$, and post-hoc Duncan test were applied for differences probability evaluation. Additionally, the Least Significant Difference (LSD) was calculated with the same accuracy. Detailed results of ANOVA are given in Supplementary Materials. In the main text results are presented in Figures. Principal component analysis (PCA), based on a correlation matrix algorithm, was performed for CI, all chlorophyll *a* fluorescence traits, and visually collected data. Two-year averages were used for the calculations. All calculations were made using STATISTICA® 13 for Windows (StatSoft (Europe) GmbH, Hamburg, Germany).

3. Results

3.1. Turf Visual and Biometric Ratings

The trinexapac-ethyl application resulted in variation of visual scores for different turf species but not among varieties within species (Figure 2, Supplementary Table S1). The Kentucky bluegrass was the most sensitive: a high TE dose irreversibly decreased the turf's visual merit, evaluated in spring was changing from 8 points in control to 3 after one week and 5 points after five weeks post-treatment. No regeneration post five weeks was detected in the autumn. On the contrary, the visual merit of perennial ryegrass was enhanced by 4 points, independently of the dose. The tall fescue turfs were ideal in all conditions. Turf density and color were less affected than visual merit. Turf density was, in general, high (7–9 points). Only the Kentucky bluegrass turfs were thinned via the high TE dose (5 points). Moreover, its color was light green, and after the TE treatment, it became even weaker. On the contrary, the perennial ryegrass color became darker via the TE treatment, independently of term and dose (increasing up to 8 points) (Figure 2, Supplementary Table S1).

The higher the TE dose, the slower regrowth rate, and thus lower biomass clipped, especially after the first week of TE application. The differences were visible between species (Figure 3) and slightly between varieties (Supplementary Table S2). In spring, a 1st-week post TE application, the growth of perennial ryegrass was inhibited by 30% and 60%, by lower and higher TE doses, respectively, whereas for other species, regardless of the growth retardant concentration (Kentucky bluegrass by 60% and tall fescue by 50%). In the 5th week, nearly no statistically proven differences between control and TE-treated plots were detected. The only exception was tall fescue treated by a higher TE dose, with regrowth ca. 10% higher than control. The regrowth patterns were repeated when the grass elongation growth was slower in the autumn. The ryegrass regrowth was inhibited by ca. 25 and 40% depending on TE dose, whereas the two other species by 50% regardless of TE concentration. After five weeks in fall, the TE influence on turfs regrowth was minor (Figure 3, Supplementary Table S2). The clipping yields aligned with the elongation growth (Supplementary Figure S1, Supplementary Table S2).

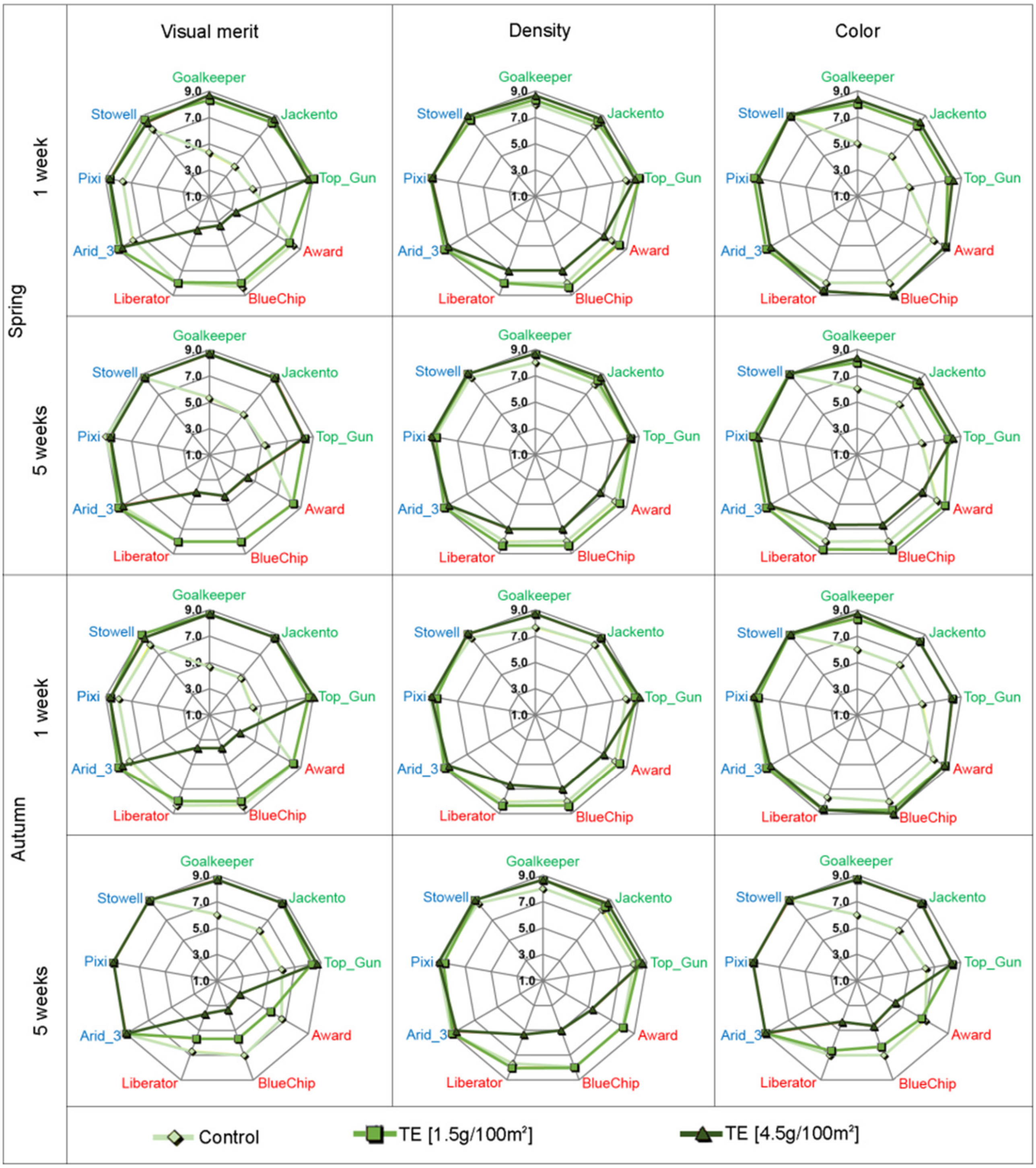

Figure 2. Radar plots for comparison of visual merit, turf density, and turf color. The statistical importance of differences is shown in Table S1. In addition, color code is used in inscriptions categorizing species: varieties names in green letters– varieties of perennial ryegrass: Goalkeeper, Jackento, Top Gun; varieties names in red letters– varieties of Kentucky bluegrass: Award, BlueChip, Liberator; varieties names in blue letters– varieties of tall fescue: Arid 3, Pixi and Stowell.

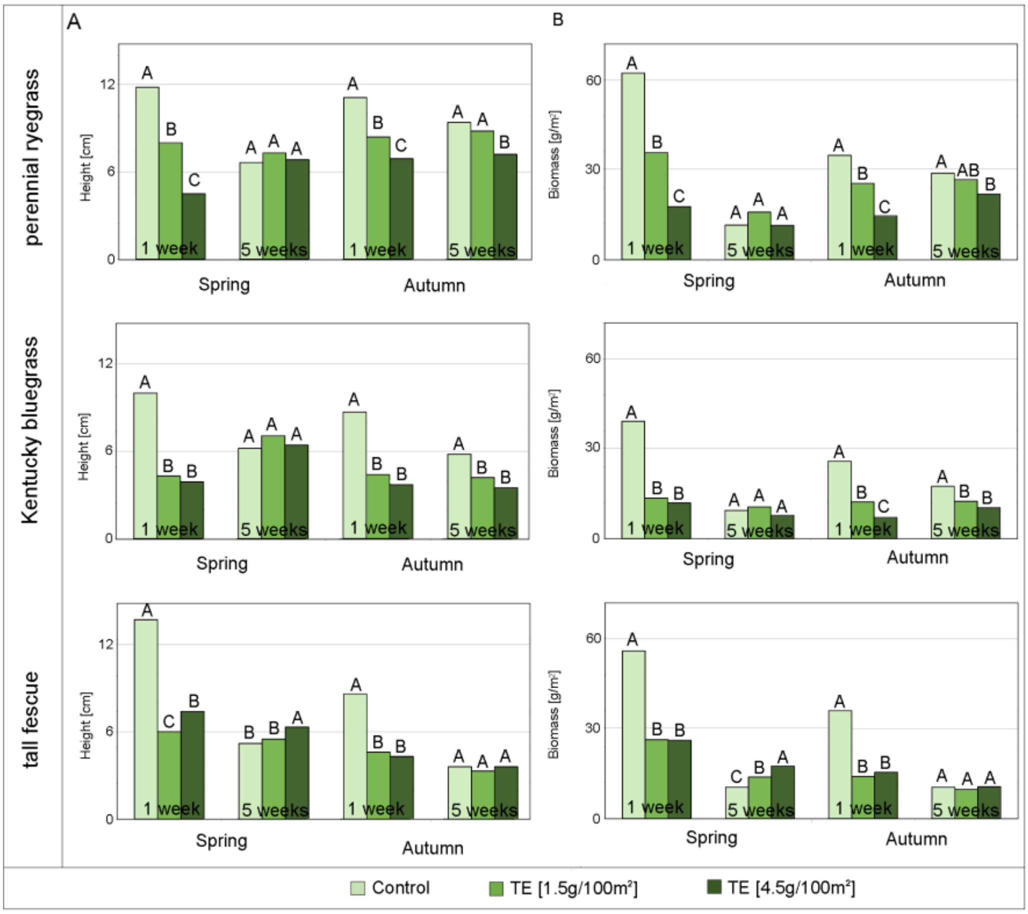

Figure 3. The effect of TE doses on grass regrowth in spring and autumn, after 1 and 5 weeks post-TE-application: (**A**) height [cm]; (**B**) Dried clippings biomass [g/100 m^2]. Letters designate homogeneous groups within the term, based on the post-hoc Duncan test, with probability ≥95%. ANOVA analysis is presented in Supplementary Materials Table S2.

3.2. TE Influence on the Chlorophyll Index (CI) and Chl a Fluorescence

The Chlorophyll Index (CI) reflects the turf greenness by giving information about the relative chlorophyll content in plants. The higher the CI, the greener the turf is. In the first-week post-TE treatment, the CI of control plots in spring was more elevated than treated ones except for the Goalkeeper variety of perennial ryegrass. In five weeks, in spring and autumn, CI was higher on TE-treated plots by 22% on average. Kentucky bluegrass and tall fescue (beside Pixi variety) responded to a single dose (on average by 60% CI increase, whereas perennial ryegrass to a triple dose by 80%) (Table 4).

Results of Chl *a* fluorescence measures were analyzed as double normalized fluorescence curves (Figure 4) and in the form of fluorescence parameters (Supplementary Table S3a,b) [28,31–33]. TE treatments at both terms and doses, for all species but Kentucky bluegrass, have a neutral or positive influence on photosynthesis. All ΔW_{OJ} curves have minor inflection points (Figure 4) beside the maximum of Kentucky bluegrass at the 5th-week post-high TE dose. On the other hand, a significant ΔW_{OJ} minimum was found for perennial ryegrass at the 5th week post-high TE dose, thus outperforming the other two species in terms of photosynthesis. Interaction between TE doses and cultivar was most evident in tall fescue (Supplementary Table S3a,b).

Table 4. Results of TE on Chlorophyll Index measured one and five weeks after TE treatments. Homogeneous groups were identified using Duncan's multiple comparisons.

Factor	Perennial Ryegrass			Kentucky Bluegrass			Tall Fescue		
	Goalkeeper	Jackento	Top Gun	Award	BlueChip	Liberator	Arid 3	Pixi	Stowell
				Spring (1 week)					
Control	289A	281A	287A	276A	264A	253A	236A	283A	266A
TE [1.5 g/100 m^2]	276A	267A	276A	235B	238B	234A	216B	212B	229B
TE [4.5 g/100 m^2]	249A	214B	200B	177C	171C	173B	221B	195C	193C
				Spring (5 weeks)					
Control	359B	348B	373B	386A	406A	401A	400B	393A	388B
TE [1.5 g/100 m^2]	383B	374B	336B	426A	435A	400A	458A	335B	432A
TE [4.5 g/100 m^2]	478A	462A	440A	326B	365B	345B	320C	338B	375B
				Autumn (1 week)					
Control	387A	386A	373A	452C	380A	393A	320B	380A	390A
TE [1.5 g/100 m^2]	305B	312B	335B	367B	310B	344B	291C	286C	333B
TE [4.5 g/100 m^2]	300B	285B	291C	271A	285B	305B	351A	312B	297C
				Autumn (5 weeks)					
Control	122C	139C	134C	170C	198B	221B	234B	222C	241C
TE [1.5 g/100 m^2]	294B	279B	283B	440A	414A	438A	438A	476A	483A
TE [4.5 g/100 m^2]	347A	327A	325A	218B	193B	286B	252B	343B	314B

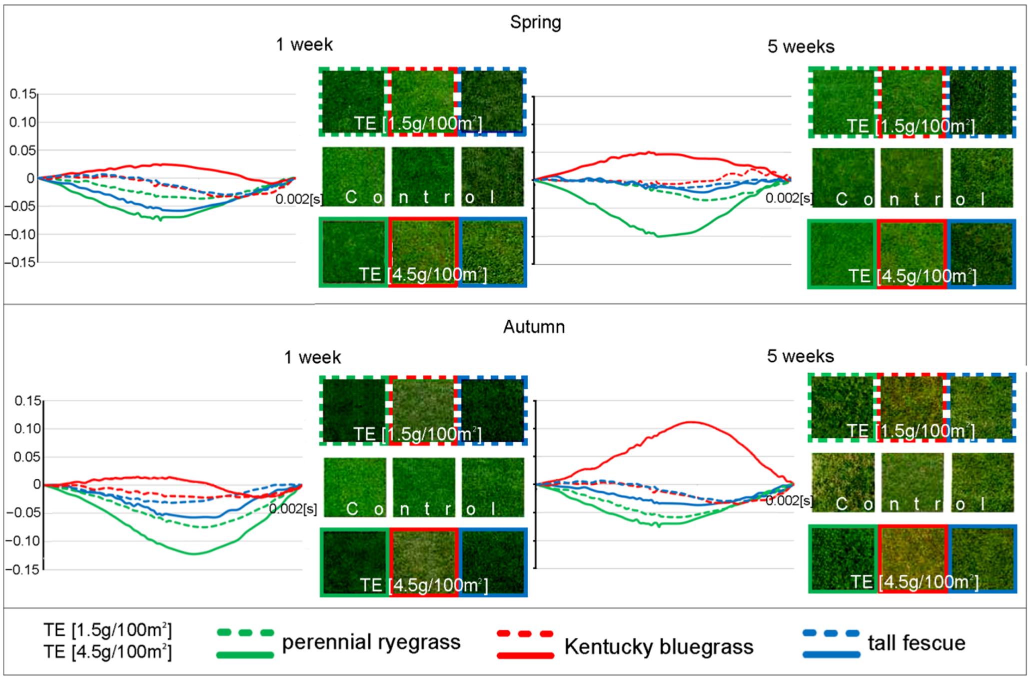

Figure 4. The influence of different TE doses, 1 and 5 weeks post-treatment, on chlorophyll a fluorescence was analyzed as ΔW_{OJ} curves. See methods for details of ΔW_{OJ}. The columns present turf photos of perennial ryegrass, Kentucky bluegrass, and tall fescue. Color code: green—perennial ryegrass; red—Kentucky bluegrass; blue—tall fescue.

The Chl *a* fluorescence parameters, the chlorophyll index, the turfgrass height, clipped biomass, and visual evaluation parameters were used for the principal component analysis (PCA) (Figure 5, Supplementary Table S3).

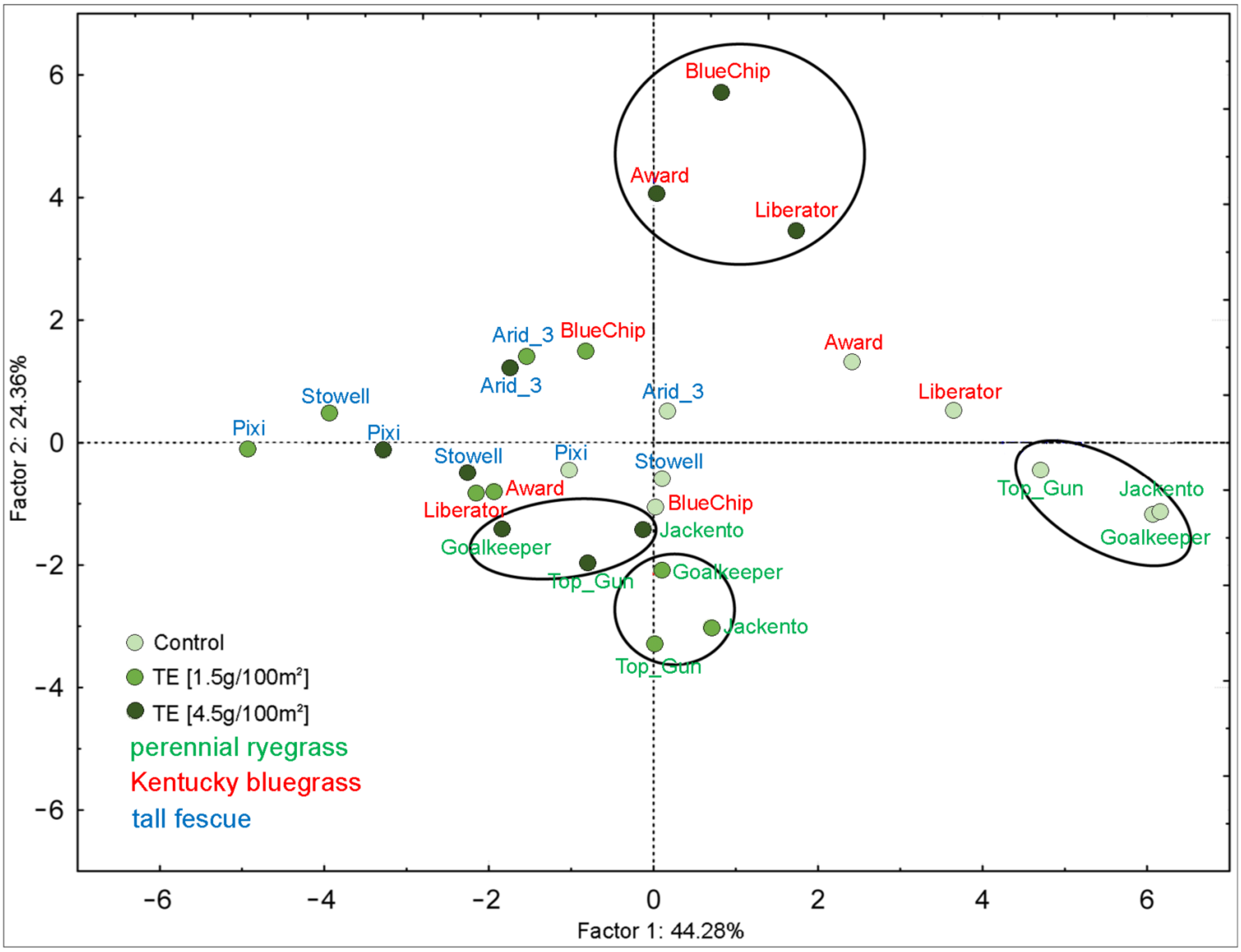

Figure 5. The distribution of tested varieties in the PCA plot is based on the data set: visual merit, turf density, turf color, chlorophyll index, and Chl *a* fluorescence parameters. In addition, color code is used for species: green—varieties of perennial ryegrass: Goalkeeper, Jackento, Top Gun; red—varieties of Kentucky bluegrass: Award, BlueChip, Liberator; blue—varieties of tall fescue: Arid 3, Pixi and Stowell.

Four factors explained over 90% of the cumulative variance (Supplementary Table S4). The first component (PC1) explains 45% of the variance and includes 11 out of 16 parameters used for PCA. Extreme negative weights (<−0.9) have: PI_{ABS} (performance index), F_V/F_O (efficiency of the water-splitting complex on the donor side of PSII), F_V/F_M (the ratio of variable and maximal fluorescence reflecting the force of the photosynthetic light reactions) and RC_{ABS} (the number of active reaction centers in chlorophyll antennas per absorption). The second component explains 26% of the variance and includes eight parameters, among which strong negative weights (<−0.7) belonged to F_O (initial fluorescence) and F_M (maximal fluorescence). Finally, factors 3 and 4 explain about 20% of the variability. PC3 components with positive weights (>+0.5) were: visual merit and turf density. PC4 components were: T_{FM} with a positive weight (>+0.8), and the Chlorophyll Index with a negative weight (<−0.6). PCA grouping in a two-dimensional coordinate system was as follows: perennial ryegrass, three groups of varieties: control, low, and high TE doses; Kentucky bluegrass, only one group separated upon high-TE treatment; tall fescue varieties not grouped (Figure 5).

4. Discussion

4.1. Effects of Trinexapac-Ethyl (TE) on Turf Physiology as Measured via Chlorophyll Index and Fluorescence Data

In general, the growth inhibition is accompanied by good visual merit of perennial ryegrass and tall fescue turfs; the deeper green of leaves resulting from the same number of chloroplasts in leaves of a lower leaf area in TE-treated plants [17,21–23,30–32]. However, our experiment shows growth inhibition of the Kentucky bluegrass without improvement of visual merit. That is in line with Meghyn et al. [32], who used 2.3 g/100 m^2 of TE to test the hybrid bluegrass's shade response (*Poa arachnifera* Torr. × *Poa pratensis* L.) in comparison with Kentucky bluegrass and tall fescue. Serensits et al. [20] used 1.7 g/100 m^2 of TE and Ervin and Koski [31,33] 2.7 g/100 m^2 with the same effect. According to our knowledge, there is no information about Kentucky bluegrass reaction to TE overdose. In our studies, Kentucky bluegrass' over-sensitive response to TE is manifested by the decreased turf quality and gray-green color. The worse physiological condition was confirmed by chlorophyll index (CI) and Chl a fluorescence data [34]. The fluorescence parameters and the ΔW_{OJ} curves demonstrated the stress of Kentucky bluegrass plants treated by a higher TE dose. Both gave information about the biophysical status of photosynthetic apparatus and showed a worse physiological state of Kentucky bluegrass [8,27,35–37]. The concentration of TE, which is harmful, depends on the species. Overdosing induces physiological stress, reflected by the reduction of the chlorophyll antenna and light-capturing complexes, slowing down the redox reactions at the PS II donor and acceptor sides and even along the entire electron transport chain, influencing the redox balance of the whole cell [28,29,38]. Aamlid et al. suggested a general rule when using Primo MAXX® on nordic golf courses: the dose should be reduced by half of that recommended by the manufacturer [16]. The perennial ryegrass response proportional to TE doses is typical, i.e., as seen in Sheikh Mohammadi et al. [22] or Głąb et al. [17]. The response of tall fescue in the way independent of TE dose suggests the lower TE requirements of that species. In our experiment, the CI and Chl *a* fluorescence parameters and the ΔW_{OJ} curves with visible minimum indicate a better physiological status of that species via TE treatment.

4.2. Benefits of the Study and Possibilities of the Use of TE in Turfgrass Management

Our experiment overviews the variation in turf response to TE, used twice per year, at months of the highest biomass formation during the season. Its usage reduced, on average, the clipped biomass by 30%. Besides subjective visual turf quality evaluation (Table 2), the non-invasive measuring devices were used to objectify an assessment of the lawn quality and to generate many parameters, which could be used for statistical inference.

Our research was carried out on separate species. Thus, the evaluation of species succession in the lawn calls for the further investigation of Kentucky bluegrass successes after perennial ryegrass during turf formation at a newly seeded area [38]. Since the Kentucky bluegrass is sensitive to TE. TE overdoses can seriously damage the freshly sown lawn. Such information is essential for green areas management. Further research should explore TE's influence on the species in turf mixtures and the degree of weed infestation.

4.3. Implications for Turf Management

Implications for turf management should be considered in two aspects, i.e., aesthetic, with local scope, and the carbon footprint aspect, at a global scale.

When using TE, the turf colonization by particular species should be considered due to differential sensitivity to TE treatment. Since perennial ryegrass (very tolerant to adverse impacts of TE) dominates in turfs in the first two years after sowing, and Kentucky bluegrass (sensitive) begins to dominate after that, TE overdoses should be avoided, especially on older turfs. Tall fescue has an intermediate turf establishment rate, slightly slower than perennial ryegrass but faster than Kentucky bluegrass. It is used in seed mixtures due to lower demand for water and fertilization, critical for lowering turf maintenance costs, and the pro-ecological importance of water saving in a warming climate [39].

We have shown that perennial ryegrass is very tolerant to TE doses, while Kentucky bluegrass is sensitive. High TE doses in the first years of the turf usage may result in the disappearance of a Kentucky bluegrass and the formation of a perennial ryegrass monoculture turf, which is sensitive to diseases due to the relatively low resistance of perennial ryegrass to diseases, for example, snow mold [1,40,41]. Such monoculture turf under low fertilization can become visually unattractive (gray-green color instead of dark green). So, balancing the proportion of fertilizers and growth retardants with an expectation of the turf's age, influencing the succession of species, is essential for turf visual appearance. If tall fescue dominates on the turf TE doses can be considered lower than 1.5 [g/100 m^2].

The present experiment may aid the choice of TE doses depending on the turf species composition. However, the TE doses should be as small as possible to obtain the highest turf quality and the lowest maintenance costs, jointly with the pro-ecological principle of sustainable development [42]. Additionally, our studies have shown that using the Fieldscout CM 1000 Meter, the detection of TE influence on grass species and its potential harmful effect is possible. A previously documented use of the CM 1000 Meter was for turf nitrogen fertilization effects or differences in savannah grass (*Axonopus compressus* (Sw.) P. Beauv.) responses to drought and soil compaction [42,43]. Measures of Chlorophyll a fluorescence make it possible to compare plants' physiological status upon the TE treatment.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy12081776/s1; Table S1: Results of ANOVA, two-way analysis of variance for the split-plot designed experiment, followed by homogeneous group identification using Duncan's multiple comparisons of turf ratings, Table S2: Results of ANOVA, two-way analysis of variance for the split-plot designed experiment, followed by homogeneous group identification using Duncan's multiple comparisons of phenotypic data (height, biomass, Chlorophyll index), Table S3. Results of ANOVA, two-way analysis of variance for the split-plot designed experiment, followed by homogeneous group identification using Duncan's multiple comparison procedures for (a) measured Chl *a* fluorescence parameters: F_O, F_M, F_V, T_{FM}, Area; (b) calculated Chl *a* fluorescence parameters: F_V/F_M, F_O/F_M, RC_{ABS}, $(1-V_J)/V_J$ and PI, Table S4: (a) Eigenvector values of principal components calculated for a complete dataset; (b) The loading weights of each dataset variable on each principal component, Figure S1. The correlation between grass height [cm] and clipped biomass [g/m^2]. Circles mark varieties of perennial ryegrass (pr), squares- Kentucky bluegrass (Kb), and triangles- tall fescue (tf).

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