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Chlorophyll fluorescence as a tool for modern turfgrass phenotyping

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Abstract

In an effort to introduce a technological tool for turfgrass phenotyping, a custom-built fluorometer was developed to measure the F_{690}/F_{730} chlorophyll fluorescence waveband index that compares distinct properties of the fluorometric plant signature. Assessments of *Poa pratensis* revealed differences in the physiological condition of plants related to stress induced by *Fusarium* varieties. Results show that canopy/light source proximity needs to be standardized for an effective use of the index since an increase in excitation radiation led to a significant decline (R = -0.87) of the value. Interestingly, no changes in the index were observed for different exposure times (up to 5 s) of plants to incident excitation light. Further investigations included the additional cool-season species *Agrostis stolonifera*, *Festuca rubra commutata*, *Festuca arundinacea* and *Lolium perenne*, resulting in a consistent index yield for both plants that were dark- and light-conditioned over 30 minutes before the measurement. The findings suggest, that the index is particularly useful to estimate plant health under field conditions, despite it being generally hard to specify the stress.

1. Introduction

Grasslands cover almost 40% of the world's land surface (Zillmann et al. 2014), giving *Poaceae* an important role in the global ecosystem. Those species classified as turfgrasses are of economic interest due to their use in the landscaping, sports, and the construction of recreational sites (Vines and Zhang 2022). Despite their importance, turfgrass breeding is technologically vastly underdeveloped and often relies mainly on methods with limited comparability, such as visual scorings (Morris and Shearman 1999; Eickmeier 2007). Therefore, the development of new tools and technologies is highly anticipated to increase standardization and reliability of the measured data in the breeding process.

The detection of chlorophyll fluorescence (ChlF) signals is one of the tools gaining more attention in the phenotyping sector. Screenings involve various bioenergetic stress parameters, providing valuable insights in the photosynthetic reactions (Lichtenthaler and Rinderle 1988; Schreiber et al. 1988). Besides pulse amplitude modulation (PAM) fluorescence which needs to be measured in specifically pre-conditioned plants (Schreiber 2004), waveband emission features do also indicate stress response (Lichtenthaler 1996) while coming with its own set of application challenges. Despite some investigations discussing PAM application in turfgrasses (e.g. Itam et al. 2023; Li et al. 2024), experimental conditions for the waveband index F_{690}/F_{730} have never been evaluated for these species. The index compares the two spectral peaks of ChlF emission spectrum with the 730 nm peak being the fluorescence from the reabsorbed 690 nm emission signals which are part of the typical absorption spectrum of the photosynthetic apparatus (PA) (Lichtenthaler and Rinderle 1988;

Terjung 1998). Generally, the index is known to correlate with both chlorophyll content (Lichtenthaler et al. 1990a) and plant stress response (Lichtenthaler 2021), thus potentially enabling detection of breeding targets such as the green color of grasses, or tolerance to biotic stresses (e.g. pests) (European Union 2025).

The objectives of this research are therefore the technical conception and validation of a ChlF approach that specifically matches turfgrass application requirements:

- Development of a ChlF-based canopy scan approach for the potential detection of phenotyping parameters in turfgrass genotypes: Usually leaf-clip chambers are employed for the majority of ChlF-assessments, but for the use in turfgrass they lack applicability since the focus lies on the canopy instead of the single leaf.
- Evaluation of F_{690}/F_{730} index's susceptibility to variations in factors such as excitation irradiance, exposure time and photosynthetic apparatus saturation level: These factors are commonly discussed in ChlF research as they target photoinduced damages, fluorometric decay (see Kautsky curve) and physiological dependencies corresponding to the electron transport chain.

2. Data, Methods and Approach

The custom-built instrument to investigate laser-induces fluorescence (LIF) (Figure 1) consisted of a 1000 mW, 450 nm diode laser for excitation at the top, and a signal collection and processing segment at the device bottom. The system featured an integrating sphere made from TiO_2-coated polystyrene with an entrance for the laser beam, sample area for the turfgrass canopy, and an opening port for a factory calibrated HDX spectrometer (Ocean Optics). Both OD2 600 nm longpass filters (Edmund Optics) and baffles were introduced to protect the sensor from saturation in the excitation region. During experiments, the relative intensities of the target wavebands at 690 and 730 nm were measured and used to calculate the corresponding index. The assembly was mounted via a frame made from threaded rods and hardboard plastics (e.g. acrylic). Each measurement was performed after the turfgrass canopy was pressed down with a metal mesh, to homogenize canopy structure, thus increasing reproducibility and standardization comparable to the leaf-clip procedure. Excitation at surface level was set to 2000 µmol $m^{-2}\,s^{-1}$ which is equal to maximum fluorescence (F_m) conditions of the species involved, estimated with a LI-6800 (LI-COR). Therefore, previous tests were performed according to LI-COR standard procedures (Hupp 2024). Prior to excitation, all plants were dark-adapted for 30 minutes.

To prove the laser fluorometer's basic functionality and visualize common fluorescence spectra effects, demonstrative measurements were performed in both healthy and stressed populations of *Poa pratensis*. Stressed individuals showed a chlorotic decay caused by infections with various *Fusarium* strains. For assessing the index at different intensities of excitation radiation, an LI-250A Light Meter (LICOR) was positioned at seven various distances from the sample area and the measured photon flux densities were converted into irradiances. Consequently, four index yields were collected at each of these different heights for *P. pratensis* to represent exposure to different radiation intensities, followed by linear regression analysis of the data. The time-stability of the index was studied through further measurements of *P. pratensis*, with data being collected five times at every 500 ms for up to 5 s after the start of laser excitation. Further, index yields of five different turfgrass species (*Poa pratensis, Agrostis stolonifera*, *Festuca rubra commutata*, *Festuca arundinacea* and *Lolium perenne*) were compared under dark- and light-adapted states of the PA ten times each. After initial dark acclimation, light adaptation was achieved by exposure of the plants to artificial LED light at 25 µmol m^{-2} s^{-1} for 30 minutes. The last two examinations were analyzed using ANOVA and Tukey-HSD post-hoc.

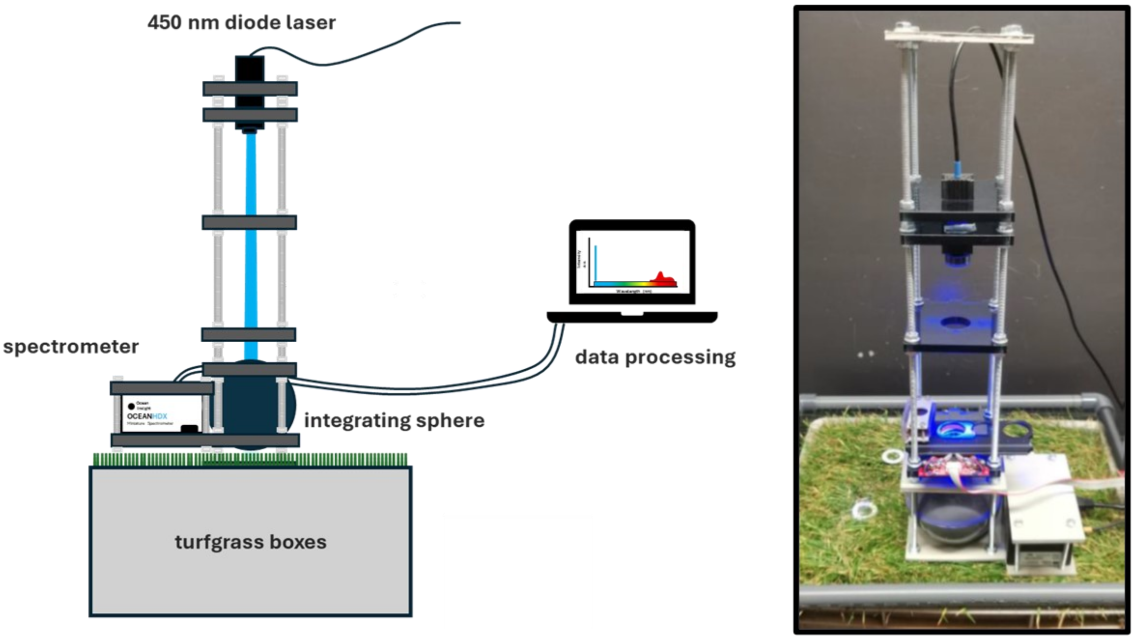

Figure 1: Sketch (left) and actual use case of the custom-built laser fluorometer.

3. Results and Discussion

Monitoring the F_{690}/F_{730} index under varying distances (irradiances) showed a strong negative correlation (Figure 2a), consistent with findings by Thoren (2007). While the author highlighted uncertainty of the effects origin, it emphasizes the importance of consistent excitation intensity during this kind of ChlF screenings. Increasing exposure times did not significantly alter the index within the observed timeframe (Figure 2b). Meanwhile, the overall fluorescence spectrum integral showed visual deterioration every 500 ms, known as Kautsky-Effect (Kautsky and Hirsch 1931), induced by increases in non-photochemical quenching, deteriorating signals after reaching F_m usually within under one second after initial exposure to excitation (Maxwell and Johnson 2000). Furthermore, no significant differences in index yields were observed among species between dark- and light-adapted PAs of the plant species involved (Figure 2c). This observation opposes the established PAM assessment, where saturation of the electron transport chain is a crucial characteristic for measurement output (Maxwell and Johnson 2000). Furthermore, these findings suggest, that exposure time and diurnal lighting can be disregarded as general factors, allowing assessments during regular working hours in the plant breeding industry. Additionally, it is possible to raise even more fluorescence traits under a continuous excitation e.g. by measuring blue (F_{450}) and green (F_{520}) fluorescence (Bürling et al. 2011; Lichtenthaler 2021). Both could be obtained before the F_{690}/F_{730} acquisition, as long as the entire process does not exceed the investigated 5 s timeframe. Furthermore, the LIF tool successfully visualized stress in *P. pratensis* by comparing healthy and stressed plants. The rising index values under increasing stress matches both, literature comparison of green (1.0) versus chlorotic plants (1.7) (Lichtenthaler et al. 1990b) and healthy (1.0) versus infected (1.3) plants (Bürling et al. 2011) with the latter study also showing a proportional F_{730}-decrease (Figure 3). Yet it is notable, that our plants experienced stress levels exceeding those of other authors, thus explaining higher values. Even under standardized application, major future concept limitations are due to the disentanglement of stress kinds (Kalaji et al. 2014).

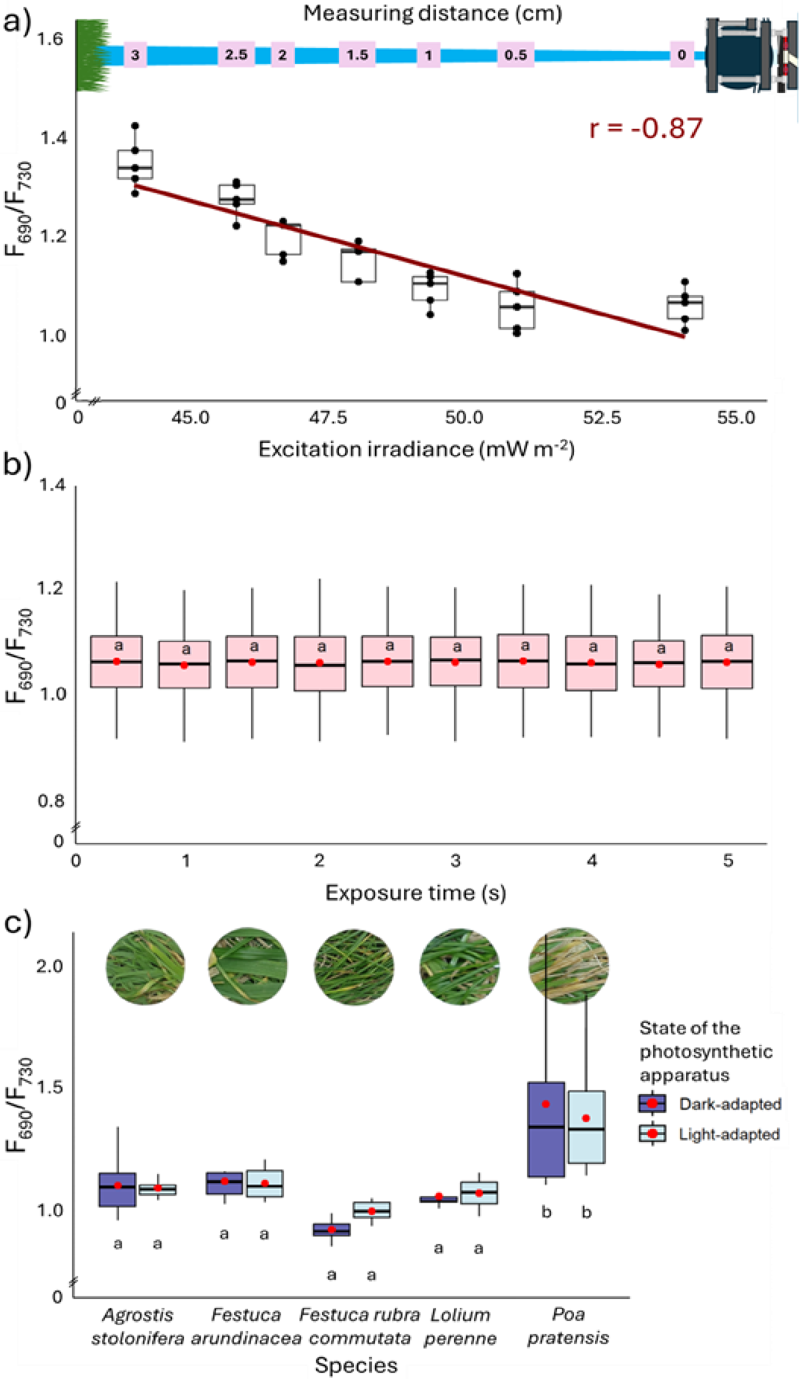

Figure 2: F_{690}/F_{730} index values: a) Varying excitation irradiance/proximity of *Poa pratensis* canopy and integrating sphere, b) Different times of exposure to 450 nm radiation before spectral data acquisition measured at surface level of mesh-covered grass canopy, c) Measurement for dark- ($0.05\ \mu mol\ m^{-2}\ s^{-1}$) and light- ($25\ \mu mol\ m^{-2}\ s^{-1}$) adapted state of the photosynthetic apparatus (ANOVA, $p \leq 0.001$; Tukey-HSD, boxes with different letters indicate significant difference, n = 28 for a), n = 50 for b), n = 100 for c). Red dots indicate the mean).

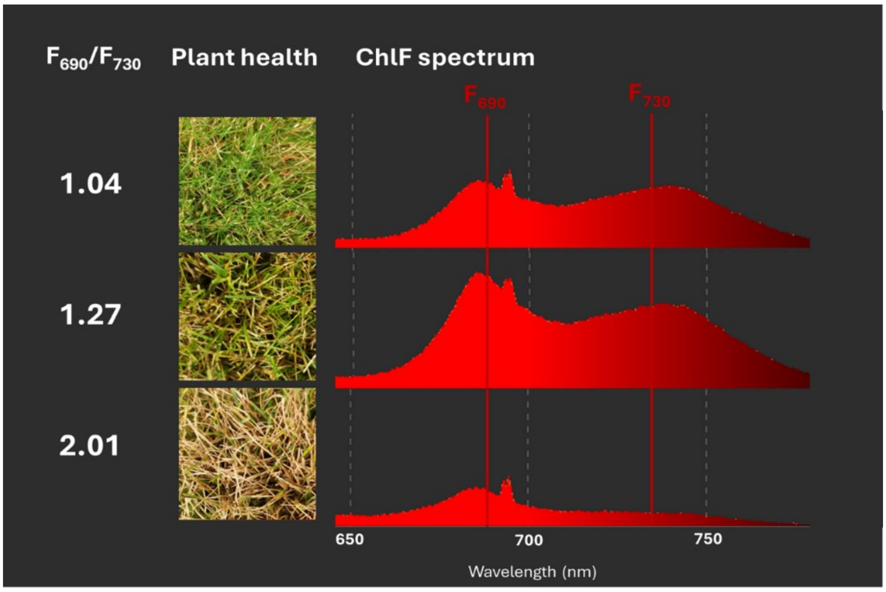

Figure 3: Raw signatures of chlorophyll fluorescence and F_{690}/F_{730} index yields in *Poa pratensis* subjected to different stress levels as consequence of a *Fusarium infection*.

4. Conclusions

This study underscores the need for a fundamental understanding of the technological operating conditions required for valid assessments of the F_{690}/F_{730} index in turfgrass phenotyping. Practical approaches of devices like this, need to be further evaluated and modified to facilitate reliable stress measurement. Methodological establishment into turfgrass breeding could provide valuable insights into plant physiology, exceeding the limitations of common visual or reflectance-based procedures. Eventually, an integration into state-of-the-art phenotyping platforms such as drone vehicles will become inevitable to enable an efficient and modern high-throughput phenotyping (HTP). Since mowing robots already spread within the industry, they provide unprecedented opportunities of monitoring.

Acknowledgements

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