ASSESSING THE EFFECTS OF NUTRIENT STRESS ON THE RED TO FAR-RED RATIOS OF LIGHT TRANSMITTED BY UNIFACIAL PLANT LEAVES

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ABSTRACT

Crops formed by C_4 plant species (*e.g.*, maize and sugarcane) continue to be in high demand for both food and biofuel production worldwide. In order to meet this demand while conserving agricultural resources, comprehensive scientific efforts need to be directed toward the reduction of crop yield losses, which are often associated with crop-weed competition. The germination of seeds from different weed species depends on the red to far-red ratio of light reaching them. In the case of seeds buried under C_4 plants, this light may have been transmitted through these plants' unifacial leaves. These, in turn, may have been subjected to varying levels of nutrient stress, notably those resulting from a lack of nitrogen. In this work, we investigate the red to far-red ratios of light transmitted through nitrogen-deficient unifacial leaves. Our findings suggest that this type of stress can lead to a further surge in yield losses by increasing the red to far-red ratios of light transmitted through the leaves and, thus, contributing to an increase in weed seed germination. Accordingly, our findings are expected to further the current understanding about stress factors limiting C_4 crop yields. Such an understanding is essential for the development of more effective technologies for the monitoring (remote and *in situ*) and management of these factors.

Index Terms— foliar transmittance, red to far-red ratio, nitrogen deficiency, seed germination, weed interference.

1. INTRODUCTION

Monocotyledonous C_4 crop species characterized by the presence of unifacial leaves, such as maize (*Zea mays L.*; corn) and sugarcane (*Saccharum officinarum* L.), are among the main sources of food for human and animal populations in many regions across the planet. They are also major suppliers of raw materials for biofuel production. Due to the increasing demand for C_4 crops, integrated scientific initiatives are required to increase their yield and reduce their use of freshwater and fertilizers. These initiatives include the combined use of satellite, ground-based and *in silico* (computational) data

in the detection and mitigation of stress factors that can negatively affect crop productivity and ecological sustainability.

The lack of nutrients is one of the most serious forms of abiotic stress limiting crop yield. It leads to a reduction in the plants' photosynthetic capacity since it causes a decrease in their photosynthetic pigment contents [1, 2]. In particular, nitrogen deficiency has been the focal point of investigations in this area due to the fact that this compound is arguably the most important nutrient for growing crops [3]. Moreover, while insufficient nitrogen supply reduces crop yield, excessive application of nitrogen-based fertilizers can result in the degradation of water sources and soil [2, 4], with detrimental effects to the environment and human health [4, 5].

Besides nutrient stress, a number of environmental processes, such as weed-interference, also contribute to substantial reductions of crop yield on a global scale [6, 7]. This process is driven by a direct crop-weed competition for water, nutrients and light. The seeds of many weed species are known to be photoblastic, *i.e.*, their germination is significantly affected by the amount and spectral quality of the impinging light. The latter aspect can be expressed in terms of the red to far-red ratio (R/FR) of light reaching these seeds [8].

For many species, the breaking of seed dormancy tends to be inhibited by low R/FR values, and stimulated by relatively higher R/FR values [9]. Considering that photoblastic weed seeds are capable of responding to small amounts of light and vast populations of them are found in arable soils [10], it is essential to acquire a deeper knowledge on the light exposure conditions leading to their germination. This will enable the application of more robust strategies for weed reduction.

To date, efforts in this area have been focused on the study of crop responses (*e.g.*, shade avoidance) to light propagated by weed species (*e.g.*, [6, 7, 11, 12]), and on the analysis of the spectral (*e.g.*, [8, 13, 14]) and spatial (*e.g.*, [15, 16, 17]) distributions of light within crop canopies. The compound effect of nutrient stress and weed-interference on C_4 crops remains to be unravelled. This work aims to make inroads toward the elucidation of these fundamental interconnected processes. To achieve this objective, we systematically investigate the effects of nitrogen dificiency on the red to far-red ratios of light transmitted by unifacial plants leaves. We then discuss their putative impact on weed seed germination.

2. INVESTIGATION FRAMEWORK

In this work, we employ an *in silico* investigation approach. It consists in conducting controlled experiments using a first-principles hyperspectral model of light interactions with unifacial plant leaves, known as ABM-U [18], and actual measured data [19]. These experiments involved the computation of directional-hemispherical transmittance curves for unifacial plant leaves subjected to varying levels of nitrogen deficiency. These curves were obtained considering an angle of incidence equal to $8°$ (for consistency with actual measured data [19]). To enable the full reproduction of our investigation outcomes, we made ABM-U available for online use [20] along with the supporting biophysical data (*e.g.*, refractive indices and extinction coefficients).

In our investigation, we considered two unifacial leaf specimens, henceforth referred to as M1 and M2, obtained from distinct maize plants. The actual transmittance curves measured for these specimens were made available in the LOPEX database [19], and are identified as spectral files 148 and 538, respectively. We employed these curves as references for our *in silico* experiments. In the characterization of specimens M1 and M2, we employed their respective LOPEX biochemical and structural datasets presented in Table 1.

Thickness and weight values presented in Table 1 correspond to measurements (considering a foliar area of 4.1 cm^2) performed on the actual specimens associated with the LOPEX spectral files 148 and 538. These values are used to calculate the concentration (mass divided by volume) of the absorbers in terms of g/cm^3 (Table 2) since their contents are given either in terms of mg per fresh weight (in the case of chlorophylls and carotenoids) and as percentage of dry weight (in the case of cellulose, lignin and protein) in the LOPEX database (as depicted Table 1).

The volume considered in these calculations corresponds to the sampled foliar area multiplied by the thickness of the mesophyll tissue, which we estimated to be 80% of the leaves' total thickness [21]. The values selected for the cuticle undulations, epidermis cell caps and mesophyll (spongy) cell caps were 10, 5 and 5, respectively. These values were derived from data available in the literature and also borne out by observations of cross sections of maize leaves [21].

As stated by Carter and Knapp [22], nitrogen stress can be closely simulated by varying the chlorophyll concentration of modelled leaves. Accordingly, we changed the chlorophyll concentrations of our modelled control specimens to obtain the radiometric quantities for the modelled nitrogen-stressed specimens. Moreover, experiments by Al-Abbas *et al.* [23], showed that an 80% reduction in nitrogen content was accompanied by negligible variations in the thickness and water content. We used this value as the upper bound for chlorophyll content reduction in our *in silico* experiments.

To quantify the ratios of red to far-red transmitted light, researchers often use as sampling references the wavelengths

Parameter	M1	M2
Chlorophyll a content ($mg\ g^{-1}$)	2.90	3.16
Chlorophyll b content ($mg\ g^{-1}$)	0.80	1.11
Carotenoids content ($mg\ g^{-1}$)	0.66	0.84
Protein content ($\%$)	26.55	24.09
Cellulose content ($\%$)	26.60	25.89
Lignin content ($\%$)	3.03	2.75
Thickness (cm)	0.0186	0.0224
Fresh Weight (g)	0.0688	0.0796
Dry Weight (g)	0.0170	0.0203

Table 1. Characterization datasets for specimens M1 and M2. The concentrations of chlorophylls and carotenoids are given as content per fresh weight. Protein, cellulose and lignin contents are given in terms of percentage of dry weight. Thickness and weight values were measured for the actual specimens used to obtain LOPEX spectral files 148 and 538.

Parameter	M1	M2
Chlorophyll a conc. (g/cm^3)	0.00328	0.00342
Chlorophyll b conc. (g/cm^3)	0.00090	0.00120
Carotenoids conc. (g/cm^3)	0.00075	0.00091
Protein conc. (g/cm^3)	0.07407	0.06656
Cellulose conc. (g/cm^3)	0.07421	0.07152
Lignin conc. (g/cm^3)	0.00845	0.00760

Table 2. Absorbers' concentration values used as input to the ABM-U model to simulate the spectral responses of specimens M1 and M2.

that correspond to the absorption peaks of chlorophyll (in the red and far-red regions of interest) obtained under *in vitro* conditions [8, 14], namely 660 and 730 nm respectively. Accordingly, we employ the following formula, henceforth referred to as *in vitro* red to far-red ratio, to quantify the spectral quality of the light transmitted by the selected specimens:

$$R/FR = \tau(660)/\tau(730), \qquad (1)$$

where $\tau(\lambda)$ denotes the transmittance value at the wavelength λ (in nm).

It has also been observed that the chlorophyll peaks are shifted under *in vivo* conditions to 645 and 735 nm, respectively [24]. Thus, for completeness, we also employ the following formula, henceforth referred to as *in vivo* red to far-red ratio, in the quantification of the spectral quality of the light transmitted by the selected specimens:

$$R^*/FR^* = \tau(645)/\tau(735). \qquad (2)$$

3. RESULTS AND DISCUSSION

Initially, we assessed the fidelity of the boundary instances of our *in silico* experiments. We started by computing transmittance curves (termed modeled-F) for the selected specimens

in their fresh states, which are characterized by the parameter values depicted in Tables 1 and 2. As it can be observed in Fig. 1, these curves closely agree with their measured counterparts. Accordingly, they were employed as the baselines (lower bounds) for our *in silico* experiments.

We then proceeded to compute transmittance curves (termed modeled-S) for the selected specimens in nitrogen stress states, which were associated with an 80% reduction in their nitrogen content [23]. These curves, which are also depicted in Fig. 1, were employed as the upper bounds for our *in silico* experiments. As it can be observed in their plots, such a reduction in the nitrogen content resulted in a noticeable increase in the specimens' transmittance in the 400 to 750 nm range, a behaviour also observed in actual transmittance measurements performed on nitrogen-stressed maize leaves [23]. Furthermore, since this increase occurs due to a reduction (by the same magnitude [23]) in the specimens' chlorophyll contents, its varying impact on the transmittance curves is associated with the bands of absorption maxima and minima of this photosynthetic pigment. This behaviour was also observed in the actual transmittance measurements [23]. These aspects indicate that the modeled-S (upper bound) curves are qualitative consistent with actual experimental observations reported in the literature.
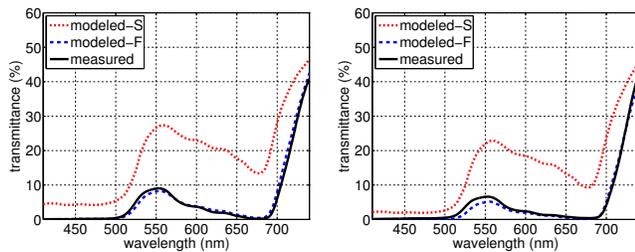

Fig. 1. Comparison of measured and modeled transmittance curves obtained for the M1 (left) and M2 (right) specimens. The modeled-B curves were computed considering the specimens in their fresh (baseline) states, which are characterized by the parameter values provided in Tables 1 and 2. The modeled-S curves were computed considering the specimens subject to a nitrogen stress associated with an 80% reduction in their chlorophyll contents [23].

Besides computing the transmittances curves for the boundary instances mentioned above, we also computed transmittance curves for intermediate variations in nitrogen (chlorophyll) content. These curves, plotted within the 640 to 740 nm range associated with the red to far-red ratios, are presented in Fig. 2. As it can be observed in their plots, a linear reduction of nitrogen (chlorophyll) content resulted in a non-linear increase in the transmittance of the selected specimens within the spectral region of interest.

As it can be observed in the graphs presented in Fig. 3, the variations in the specimens' transmittances (Fig. 2) were
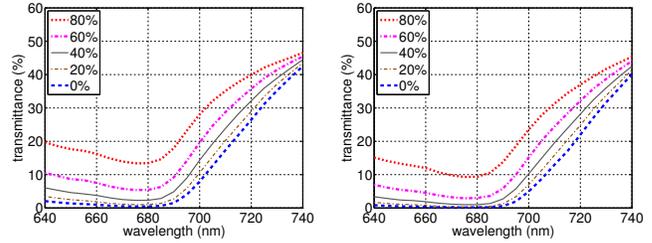

Fig. 2. Comparison of modeled transmittance curves computed for specimens M1 (left) and M2 (right) considering nitrogen stress states associated with reductions in their chlorophyll contents from 0 to 80% [23].

Fig. 3. Comparison of *in vitro* and *in vivo* red to far-red ratios of light transmitted by specimens M1 (left) and M2 (right) considering nitrogen stress states associated with reductions in their chlorophyll contents from 0 to 80% [23].
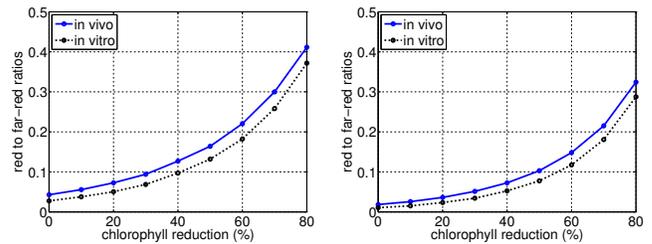
translated to non-linear variations in the corresponding red to far-red ratios. More precisely, the linear reduction of nitrogen (chlorophyll) content responsible for the non-linear increase in transmittance resulted in a nearly quadratic increase in both sets of red to far-red ratios, *in vivo* and *in vitro*, with the latter set depicting consistently lower values than the former.

We remark that many weed species are characterized by having photoblastic seeds whose germination is inhibited by low red to far-red ratios of impinging light. However, the higher these ratios, the higher the chance of breaking their dormancy [9]. For the selected specimens in their fresh states, we have obtained low simulated ratio values, which is consistent with measured ratio values provided in the literature [9, 14]. In addition, the results of our *in silico* experiments indicate that the red to far-red ratios of light transmitted by unifacial leaves tend to increase as they become subject to more severe levels of nitrogen dificiency. Hence, our findings suggest that nutrient stress, particularly in the case of nitrogen deficiency, is likely to contribute to an increase in weed germination, which would be detrimental to a crop yield already affected by the disturbance of the plants' photosynthetic apparatus due to the lack of this compound.

4. CONCLUSION AND FUTURE WORK

In this investigation, we have postulated that nitrogen deficiency, one of the most detrimental forms of abiotic stress af-

fecting the plants' photosynthetic capacity [23], can have an additional negative impact on agricultural yield. More specifically, our findings indicate that a limited supply of nitrogen, the pivotal plant nutrient from agricultural and ecological perspectives [2, 3, 4, 5], can elicit a significant increase in the red to far-red ratios of light transmitted by the unifacial leaves of C_4 crop species. These elevated ratios, in turn, can potentially increase the probability of weed seed germination and, consequently, the weed-crop competition for limited natural resources. Although further *in situ* experiments would be required to confirm these trends, our findings highlight the importance of closely monitoring and, if necessary, correctly adjusting the nitrogen levels of C_4 crops.

The cost-effective mitigation of crop losses, particularly through remote and *in situ* technologies for the detection of plants responses to stress, is among the grand challenges faced by precision agriculture. In order to tackle this challenge while maintaining ecological sustainability, more cooperative scientific efforts (*e.g.*, involving the pairing of high-fidelity modeling initiatives with *in situ* experimental procedures) need to be directed toward the gathering of comprehensive data and fundamental knowledge about the environmental and physiological processes leading to these losses. To contribute to the achievement of this common global goal, our future work in this area will be aimed at the predictive simulation and analysis of time-dependent photobiological phenomena [25] associated with these processes.

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