Insights into entangled variations in the red edge position and red to far-red ratios of soybean leaves

Gladimir V. Baranoski

Gladimir V. G. Baranoski, "Insights into entangled variations in the red edge position and red to far-red ratios of soybean leaves," Proc. SPIE 12727, Remote Sensing for Agriculture, Ecosystems, and Hydrology XXV, 127270J (17 October 2023); doi: 10.1117/12.2680204

Event: SPIE Remote Sensing, 2023, Amsterdam, Netherlands

Insights into Entangled Variations in the Red Edge Position and Red to Far-Red Ratios of Soybean Leaves

Gladimir V. G. Baranoski

Natural Phenomena Simulation Group, School of Computer Science, University of Waterloo, 200 University Avenue West, Waterloo, Ontario, N2L 3G1, Canada

ABSTRACT

Protein-rich soybean crops have a strategic importance for food production worldwide. Initiatives to increase their yield without compromising the environment include the use of remote sensing technologies to monitor their cultivation using spectral data. The red edge position (REP) is among the most used spectrally-derived information in this area. It is strongly correlated to the plants' chlorophyll contents and it can provide a reliable indication of changes in their nutrient status. Besides the availability of nutrients, the plants' photosynthetic capacity is also affected by other abiotic factors, notably light exposure. Variations in the red to far-red (R/FR) ratios of light impinging on soybean leaves are believed to trigger shade-avoidance responses that contribute to their photosynthetic efficiency. To date, the extent of possible connections between variations in the REP and R/FR ratios of soybean leaves remains unclear. In this paper, we address this open question using available measured spectral reflectance and transmittance data obtained for two groups of soybean specimens characterized by distinct chlorophyll contents. More specifically, we examine the impact that their distinct pigmentation levels have on their respective REP and R/FR ratios. The potential ramifications of our findings include not only the enhancement of the procedures employed in the monitoring and management of soybean crops through the combined use of these indices, but also the strengthening of the current knowledge about the intertwined physiological processes responsible for these plants' highly adaptive photosynthetic apparatus.

Keywords: soybean, reflectance, red edge position, red to far-red ratio, photosynthetic capacity, shade avoidance, precision agriculture, remote sensing.

1. INTRODUCTION

Soybean (*Glycine max* (L.) Merr., *Soja hispida*[1]) crops have substantially increased in importance for food production in the last decades.[2] Besides their high protein content, these C3 legumes can fix atmospheric nitrogen for their own growth, which minimizes the use of inorganic fertilizers in their cultivation.[3] Due to the high demand for soybean crops worldwide, a diverse array of monitoring (remotely and *in situ*) and management procedures have been proposed the increase their yield while mitigating detrimental effects (*e.g.*, the contamination of freshwater supplies due to the excessive use of fertilizers) to the surrounding ecosystems.[4]

These procedures are often associated with the use of spectral features relating the plants' foliar radiometric parameters (*e.g.*, reflectance and transmittance) to their nutrient (*e.g.*, nitrogen) status. Arguably, one the most employed of these spectral features is the *red edge*: the characteristic increase in foliar reflectance in the 680 to 800 nm region of the light spectrum. This feature was first described by Collins,[5] and it is directly associated with the combined effects of light absorption by pigments, notably the chlorophylls, and scattering by internal foliar structures (*e.g.*, cells and organelles).

The red edge is a relatively broad spectral feature. Thus, to facilitate comparisons of reflectance measurements obtained under different conditions and involving distinct species, it is often characterized in terms of the wavelength, termed the red edge position (REP), associated with its maximum slope.[6] This, in turn, corresponds to the peak value of the first derivative of the reflectance curve in the spectral region from 680 to 800 nm.

A noticeable decrease in chlorophyll contents is usually an indicator of nutritional stress, reduced photosynthetic capacity or senescence of plant leaves. It has been noted[7,8] that the REP and the leaves' chlorophyll

Remote Sensing for Agriculture, Ecosystems, and Hydrology XXV, edited by Christopher M. U. Neale, Antonino Maltese, Proc. of SPIE Vol. 12727, 127270J · © 2023 SPIE · 0277-786X · doi: 10.1117/12.2680204

contents are strongly correlated. Accordingly, the REP can be employed, for instance, in the evaluation of crop nutrient status, which can prompt in-season adjustments of fertilizer applications.[9]

Besides nutrient stress,[2] the photosynthetic capacity of soybean leaves may also be significantly affected by other abiotic factors such as distinct light exposure conditions.[10–12] Even though shade-tolerant soybean cultivars can be grown for commercial and scientific purposes,[12] soybean is a shade-avoiding species.[13] More specifically, variations in the red to far-red (R/FR) ratios of light impinging on shade-sensitive soybean leaves can trigger shade-avoidance responses (*e.g.,* petiole and internode elongation) with the purpose of increasing light capture.[10,14–16] Since leaves that develop under shade tend to present a reduced photosynthetic capacity,[12,16] this trait of soybean plants further contributes to the efficiency of their photosynthesis apparatus.

It has been stated that as light propagates through leaves forming soybean canopies, the R/FR ratios are decreased[13,17] and the resulting low R/FR ratios act as shade-avoidance signals.[3,13,18] We note that, although the REP and R/FR ratios of the light propagated (reflected and transmitted) by soybean leaves may be affected by changes in their chlorophyll contents, a possible connection between these features has not been quantitatively investigated to date. If such a connection exists, it can have relevant implications for the monitoring and management of soybean crops. In this paper, we assess this possibility through the analysis of the REP and R/FR ratios of soybean leaf specimens with different biochemical characteristics.

The values for these indices were obtained using measured spectral reflectance and transmittance data acquired for different soybean leaf specimens. Details about the employed spectral datasets along with the formulation used to obtain the specimens' REP and R/FR ratios are provided in the next section. In section 3, we present our preliminary findings and elaborate on their practical ramifications. Finally, in Section 4, we conclude the paper and outline directions for future investigations on the REP and R/FR ratios of plant leaves.

2. MATERIALS AND METHODS

The measured spectral reflectance and transmittance datasets employed in our investigation have been made available to the scientific community by the multidisciplinary LOPEX (Leaf Optical Properties Experiment) project.[19] This project involved spectral, morphological and biochemical measurements conducted on 120 leaf specimens representative of more than 50 woody and herbaceous species. These specimens were collected from trees and crops near the Joint Research Centre in Ispra, Italy, during periods of high phenological activity.

The LOPEX spectral reflectance and transmittance files used in this work are listed in Table 1. They consist in directional-hemispherical reflectance and transmittance curves from 400 to 2500 nm (with a resolution of 1 nm), which were measured considering an angle of incidence of 8° (with respect to the specimens' normal vector). These files were acquired for two groups of soybean leaf specimens, henceforth referred to as batch 1 and batch 2, characterized by markedly distinct chlorophyll contents. For the specimens in batch 1, the measured average chlorophyll a and b contents per fresh weight were 2.9 mg/g and 0.8 mg/g, respectively. For the specimens in batch 2, these contents were 0.09 mg/g and 0.05 mg/g, respectively. In Figs. 1 and 2, for illustrative purposes, we present the reflectance and transmittance data of two representative specimens, B1d and B2d, of batches 1 and 2, respectively.

Batch 1			Batch 2		
Leaf Specimen	Reflectance Spectral File	Transmittance Spectral File	Leaf Specimen	Reflectance Spectral File	Transmittance Spectral File
B1a	opex0219	opex0220	B2a	opex1549	opex1550
B1b	opex0221	opex0222	B2b	opex1551	opex1552
B1c	opex0223	opex0224	B2c	opex1553	opex1554
B1d	opex0225	opex0226	B2d	opex1555	opex1556
B1e	opex0227	opex0228	B2e	opex1557	opex1558

Table 1: LOPEX spectral reflectance and transmittance files employed in our investigation. The batch 1 files correspond to soybean leaf specimens with typical chlorophyll contents, while the batch 2 files correspond to soybean leaf specimens with low chlorophyll contents.

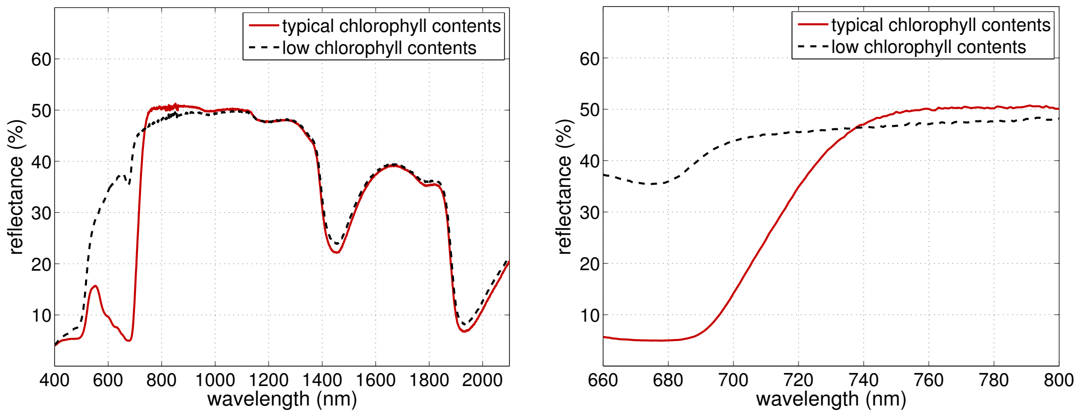

Figure 1: Reflectance data for two representative specimens, B1d and B2d, of batches 1 (characterized by typical chlorophyll contents) and 2 (characterized by low chlorophyll contents), respectively. Left: full spectra (400-2500 nm). Right: Zoom-in of the spectral region of interest.

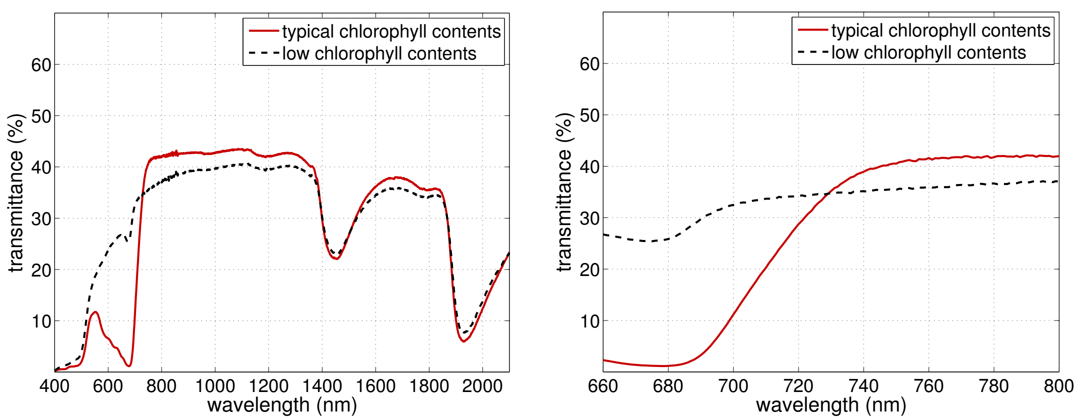

Figure 2: Transmittance data for two representative specimens, B1d and B2d, of batches 1 (characterized by typical chlorophyll contents) and 2 (characterized by low chlorophyll contents), respectively. Left: full spectra (400-2500 nm). Right: Zoom-in of the spectral region of interest.

To obtain the REP of the examined soybean leaf specimens, we computed the first derivative of their respective reflectance curves in the red region using a three point numerical differentiation formula.[20] More precisely, we employed the following expression to compute the first derivative at a given wavelength (λ):

$$\rho'(\lambda) = (\rho(\lambda + 10) - \rho(\lambda - 10)) \times 0.05, \quad (1)$$

where $\rho(\lambda)$ denotes the reflectance (at the wavelength λ) sampled from the specimens' reflectance curves. Also for illustrative purposes, we present in Fig. 3 the first derivatives computed for the reflectance curves (depicted in Fig. 1 right) of two representative specimens, B1d and B2d, of batches 1 and 2, respectively.

The light reaching the leaves of a soybean plant in a cultivated field can have a direct (sunlight) and an indirect component.[10] The latter may have been propagated, for instance, by the soil underneath the plants or by other leaves (belonging to the same plant or adjacent plants). To quantify the ratios of red to far-red propagated light, researchers often use as sampling references the wavelengths that correspond to the absorption peaks of chlorophyll (within the red and far-red bands of interest) obtained under *in vitro* conditions,[10] namely 660 nm and 730 nm respectively. Accordingly, we employed the following formula to quantify the red to far-red

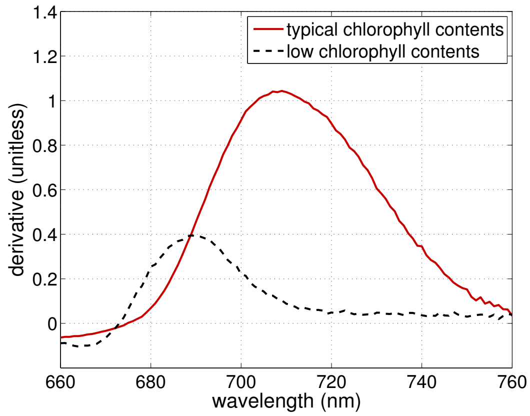

Figure 3: First derivatives of the reflectance curves (depicted in Fig. 1 right) of two representative specimens, B1d and B2d, of batches 1 and 2, respectively.

ratios of the light reflected by the selected specimens:

$$R/FR_\rho = \rho(660)/\rho(730). \tag{2}$$

It has also been observed that the chlorophyll absorption peaks are shifted under *in vivo* conditions to 645 nm and 735 nm, respectively.[14] Hence, for completeness, we also computed the red to far-red ratios of the light reflected by the selected specimens considering these wavelengths:

$$R/FR^*_\rho = \rho(645)/\rho(735). \tag{3}$$

Similarly, for the quantification of the red to far-red ratios of light transmitted by the selected specimens, we also used two formulas. In this case, the ratios, denoted R/FR_τ and R/FR^*_τ, are expressed by replacing the reflectance values by transmittance ($\tau(\lambda)$) values in Eqs. 2 and 3, respectively.

3. RESULTS AND DISCUSSION

In Table 2, we present the values computed for the the red edge position and red to far-red ratios of light reflected by the soybean leaf specimens belonging to batches 1 (characterized by typical chlorophyll contents) and 2 (characterized by low chlorophyll contents). Recall that the REP and the chlorophyll contents are strongly correlated.[7,8] Accordingly, as expected, the REP values computed for the batch 2 specimens were noticeable lower than the values computed for the batch 1 specimens.

On the other hand, the R/FR ratios of reflected light computed for the batch 2 specimens were noticeable higher than the values computed for batch 1 specimens. As, it can be observed in Table 3, the same trend was apparent with respect to the red to far-red ratios of transmitted light.

It is worth noting that among specimens belonging to the same batch, and thus characterized by similar chlorophyll contents, such a connection between the REP and the R/FR ratios could not be established as it can also be verified in Tables 2 and 3. For example, examining the values presented in Table 2, we notice that, while the REP of specimens B1c, B1d and B1a increase monotonically (707, 709 and 710, respectively), their R/FR_ρ ratios (0.1285, 0.1332 and 0.1122, respectively) and R/FR^*_ρ ratios (0.1430, 0.1499 and 0.1210, respectively) do not follow the same behaviour.

To the best of our knowledge, the observed inverse relationship between the REP and the R/FR ratios of light propagated by groups of soybean leaves with markedly distinct chlorophyll contents has not been reported in the literature to date. Although a larger number of measurements will be required to confirm this putative relationship, our preliminary findings suggest that a significant reduction in the chlorophyll contents of soybean

Batch 1				Batch 2			
Leaf Specimen	REP	R/FR_ρ	R/FR^*_ρ	Leaf Specimen	REP	R/FR_ρ	R/FR^*_ρ
B1a	710	0.1122	0.1210	B2a	690	0.7740	0.7759
B1b	707	0.1282	0.1399	B2b	686	0.7860	0.7301
B1c	707	0.1285	0.1430	B2c	685	0.7808	0.6987
B1d	709	0.1332	0.1499	B2d	689	0.8089	0.8062
B1e	709	0.1364	0.1479	B2e	688	0.6619	0.5604
Average	708.4	0.1277	0.1393	Average	687.6	0.7623	0.7142

Table 2: Values computed for the red edge position (REP) and red to far-red ratios (R/FR_ρ and R/FR^*_ρ) of light reflected by the soybean leaf specimens belonging to batches 1 (characterized by typical chlorophyll contents) and 2 (characterized by low chlorophyll contents).

Batch 1			Batch 2		
Leaf Specimen	R/FR_τ	R/FR^*_τ	Leaf Specimen	R/FR_τ	R/FR^*_τ
B1a	0.0836	0.1298	B2a	0.7814	0.7741
B1b	0.1044	0.1544	B2b	0.7882	0.7676
B1c	0.0782	0.1167	B2c	0.7861	0.7265
B1d	0.0651	0.0966	B2d	0.7690	0.7640
B1e	0.0749	0.1140	B2e	0.7450	0.6796
Average	0.0812	0.1223	Average	0.7739	0.7423

Table 3: Values computed for *in vitro* red to far-red ratios (R/FR_τ and R/FR^*_τ) of light transmitted by the soybean leaf specimens belonging to batches 1 (characterized by typical chlorophyll contents) and 2 (characterized by low chlorophyll contents).

leaves leads not only to an expected reduction in the REP, but also to an increase in R/FR ratios of light propagated through their canopies.

We remark that low R/FR ratios may act as shade-avoidance signals for soybean leaves.[13] Consequently, a substantial reduction of their chlorophyll contents followed by an increase in their R/FR ratios can diminish these plants' shade avoidance responses. This aspect, in turn, can be prejudicial to the plants' photosynthetic capacity and significantly reduce the yield of shade-avoiding soybean crops.[3,16]

Recall also that the REP is being routinely employed as an indicator of a number of factors affecting crop productivity. Its putative connection with the R/FR ratios, upon confirmation, may potentially extend its scope of applications to the monitoring of alterations in the plants' shade avoiding capabilities brought about by significant changes in their chlorophyll contents. Conversely, noticeable variations in the R/FR ratios of reflected light could potentially assist the detection of significant reductions in the plants' chlorophyll contents. We note that the calculation of the R/FR ratios requires fewer spectral samples (two) than those (three or four) usually used in REP estimations.[6]

Another aspect that can contribute to the reduction of soybean crops' yield is weed competition (interference).[21,22] For many weed species, the breaking of seed dormancy tends to be inhibited by low R/FR ratios and stimulated by relatively high R/FR ratios.[23,24] Again, if the observed connection between the REP and the R/FR ratios of light propagated by groups of soybean leaves with markedly distinct chlorophyll contents can be statistically confirmed, significant variations in the plants' REP can also be used to provide additional supporting data for weed management strategies.[22]

The ramifications of our preliminary findings may be also extended to intercropping cultivation systems. Soybeans are often intercropped with C4 grains such as corn (*Zea mays* L., maize).[13,25] These systems are implemented to increase land-use efficiency and crop yield.[3] However, intercropped/partner species tend to affect each other's light exposure conditions in ways that may be adverse to those purposes.[25] For instance, it has been pointed out through predictive simulations supported by measured data[24] that the R/FR ratios of light propagated by corn leaves increase as their chlorophyll contents decrease, notably as a result of nutrient stress.

Thus, light propagated by chlorophyll-depleted corn leaves may exacerbate the reduction of shade avoidance responses of neighbour soybean plants under nutrient stress.

Lastly, we also note that neighbour/partner crops, such as soybean and corn, can have distinct mechanisms of adaptation to stress factors. Accordingly, we believe that a deeper understanding about the circumstances in which their REP values can correlate with their R/FR ratios should be pursued. It would strengthen the foundation required for the design of more cost-effective procedures to evaluate (remotely and *in situ*) the crops' aggregated and individual health status.

4. CONCLUDING REMARKS

In this work, we have examined a possible connection between the REP and the R/FR ratios of soybean leaves. Our findings suggest that such a connection may be established when one considers the spectral responses of specimens with markedly distinct chlorophyll contents. Given the potential implications of such putative entanglement for the monitoring and management of mono- and intercropped crops, future laboratory and field experiments are warranted to obtain a comprehensive assessment of its photobiological basis. Furthermore, considering the ever-increasing demand for high-yield and environmentally-friendly crops, it would also be beneficial to extend such experiments to other cultivated plant species.

ACKNOWLEDGMENTS

This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC-Discovery Grant 238337).

REFERENCES

[1] Bird, G., "Haemagglutinins of Glycine soja," *Nature* **176**(4493), 1127–1128 (1955).

[2] Miransari, M., "Soybeans, stress, and nutrients," in [*Environmental Stresses in Soybean Production: Soybean Production*], Miransari, M., ed., Academic Press, Cambridge, MA, USA (2016). Volume 2.

[3] Yang, F., Feng., L., Liu, Q., Wu, X., Fan, Y., Raza, M., Cheng, Y., Chen, J., Wang, X., Yong, T., Liu, W., Liu, J., Du, J., Shu, K., and Yang, W., "Effect of interactions between light intensity and red-to- far-red ratio on the photosynthesis of soybean leaves under shade condition," *Environ. Exp. Bot.* **150**, 79–87 (2018).

[4] Hatfield, J., Gitelson, A., Schepers, J., and Walthall, C., "Application of spectral remote sensing for agronomic decisions," *Agronomy J.* **100**, S–117–S–131 (2008).

[5] Collins, W., "Remote sensing of crop type and maturity," *Photogrammetric Engineering and Remote Sensing* **44**, 43–55 (1978).

[6] Baranoski, G. and Rokne, J., "A practical approach for estimating the red edge position of plant leaf reflectance," *International Journal of Remote Sensing* **26**(3), 503–521 (2005).

[7] Gates, D., Keegan, H., Schleter, H., and Weidner, V., "Spectral properties of plants," *Applied Optics* **4**, 11–20 (1965).

[8] Zarco-Tejada, P., Pushnik, J., Dobrowski, S., and Ustin, S., "Steady-state chlorophyll *a* fluorescence detection from canopy derivative reflectance and *double-peak* edge effects," *Remote Sens. Environ.* **84**, 283–294 (2003).

[9] Fu, Y., Yang, G., Pu, R., Li, A., Li, H., Xu, X., Song, X., Yang, X., and Zhao, C., "An overview of crop nitrogen status assessment using hyperspectral remote sensing: Current status and perspectives," *European Journal of Agronomy* **124**, 126241:1–16 (2021).

[10] Smith, H., "Light quality, photoperception, and plant strategy," *Ann. Rev. Plant Physiol.* **33**, 481–518 (1982).

[11] Bunce, J., "Mutual shading and the photosynthetic capacity of exposed leaves of field grown soybeans," *Photosynth. Res.* **15**, 75–83 (1988).

[12] Yao, X., Li, C., Li, S., Zhu, Q., Zhang, H., Wang, H., Yu, C., St. Martin, S., and Xie, F., "Effect of shade on leaf phosynthesis capacity, light intercepting, electron transfer and energy distribution of soybeans," *Plant Growth Regul.* **83**, 409–416 (2017).

[13] Pausch, R., Britz, S., and Mulchi, C., "Growth and photosynthesis of soybean (*Glycine max* (L.) Merr.) in simulated vegetation shade: influence of the red to far-red radiation," *Plant Cell Environ.* **14**, 647–656 (1991).

[14] Kasperbauer, M., "Far-red light reflection from green leaves and effects on phytochrome-mediated assimilate partitioning under field conditions," *Plant Physiol.* **85**, 350–354 (1987).

[15] Franklin, K., "Shade avoidance," *New Phytologist* **179**, 930–944 (2008).

[16] Hitz, T., Hartung, J., Graeff-Hönninger, S., and Munz, S., "Morphological response of soybean *Glycine max* (L.) Merr.) cultivars to light intensity and red to far-red ratio," *Agronomy* **9**, 428:1–15 (2019).

[17] Sattin, M., Zuin, M., and Sartorato, I., "Light quality beneath field-grown maize, soybean and wheat canopies - red:far red variations," *Physiol. Plantarum* **91**, 322–328 (1994).

[18] Baranoski, G., "On the asymmetry of the red to far-red ratios of light propagated by the adaxial and abaxial surfaces of bifacial leaves," in [*International Geoscience and Remote Sensing Symposium - IGARSS 2020*], 4878–481, IEEE (2020).

[19] Hosgood, B., Jacquemoud, S., Andreoli, G., Verdebout, J., Pedrini, G., and Schmuck, G., "Leaf Optical Properties Experiment 93 (LOPEX93]," Tech. Rep. EUR 16095 EN, Institute for Remote Sensing Applications (Unit for Advanced Techniques), Ispra, Italy (1995).

[20] Burden, R. and Faires, J., [*Numerical Analysis*], PWS Publishing Company, Boston, fifth ed. (1993).

[21] Liu, J., Mahoney, K., Sikkema, P., and Swanton, C., "The importance of light quality in crop-weed competition," *Weed Research* **49**, 217–224 (2009).

[22] Green-Tracewicz, E., Page, E., and Swanton, C., "Light quality and the critical period of weed control in soybean," *Weed Science* **60**, 86–91 (2012).

[23] Pons, T., "Seed responses to light," in [*Seeds: The Ecology of Regeneration in Plant Communities*], Fenner, M., ed., 237–260, CABI Publishing, Wallingford, UK (2000).

[24] Baranoski, G., "Assessing the effects of nutrient stress on the red to far-red ratios of light transmitted by unifacial plant leaves," in [*International Geoscience and Remote Sensing Symposium - IGARSS 2020*], 5187–5190, IEEE (2020).

[25] Yang, F., Huanga, S., Gaoa, R., Liua, W., Yonga, T., Wanga, X., Wua, X., and Yanga, W., "Growth of soybean seedlings in relay strip intercropping systems in relation to light quantity and red:far-red ratio," *Field Crops Res.* **155**, 245–253 (2014).