A Passive Method for Detecting Vegetation Stress from Orbit: Chlorophyll Fluorescence Spectra from Fraunhofer Lines

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Abstract

Solar-stimulated chlorophyll fluorescence measured with the Fraunhofer line depth method has correlated well with vegetation stress in previous studies. However, the instruments used in those studies were limited to a single solar absorption line (e.g. 656.3 nm), obviating the red/far-red ratio (R/FR) method. Optics and detector technology have reached the level whereby multiple, very narrow Fraunhofer lines are resolvable. Thirteen such lines span the visible spectrum in the red to far-red region where chlorophyll fluorescence occurs. Fluorescence intensities at the 13 Fraunhofer line wavelengths were used to model emission spectra. The source data were collected for summer and fall bean crops (*Phaseolus vulgaris* L.) subjected to various levels of nitrogen fertilization. The intensities were adjusted to account for Fraunhofer line depth and atmospheric transmittance. Multiple R/FR fluorescence ratios, calculated from the modeled fluorescence spectra, correlated strongly with leaf chlorophyll concentration and well with applied nitrogen. The ratio yielding the best correlation with chlorophyll utilized red fluorescence at the 694.5 nm Fraunhofer line and far-red fluorescence at the 755.6 nm Fraunhofer line. Twenty R/FR ratios, each evaluated for the maximum differential between low and high (optimal) nitrogen treatments, ranked higher in some cases and lower in others, possibly related to the time of year the crops were grown and the stage of growth of the crops. Ratios with 728.9 nm and 738.9 nm in the denominator consistently ranked in the lowest and next lowest quartile, respectively. Ratios of the 656.3 nm Fraunhofer line and the 755.6 nm line consistently ranked highest for the summer crop. Ratios with 755.6 nm in the denominator ranked in the upper quartile for 10 out of 12 measurement dates. Differences in ratio ranking indicate that physiological conditions may be estimated using selected ratios of Fraunhofer lines within the context of R/FR analysis. A passive instrument designed to monitor R/FR chlorophyll fluorescence (i.e. vegetation stress) from orbit could be built today.

Keywords: agriculture, silviculture, plant stress, red/far-red ratio, line depth method.

Introduction

People involved in agriculture, silviculture, and the husbanding of our natural resources have been seeking methods to effectively monitor large areas of vegetation. Their concerns range from precision management of applied nutrients, mainly nitrogen, to pest control and disease containment. A measureable indicator of the health of vegetation is presented because a plant adjusts its levels of chlorophyll (Chl) in response to changes in its immediate environment. The relationship between leaf Chl concentration and the ratio of red (ca 685 nm) to far-red (ca 735 nm) Chl fluorescence has been established as one way in which plant response can be exploited (Lichtenthaler and Rinderle (1988); Hak et al., 1990).

For most annual crop species and deciduous trees and shrubs, during a leaf growth cycle the red/far-red (R/FR) fluorescence ratio decreases as the leaf Chl concentration increases, and increases with loss of Chl under conditions of stress, such as nutrient or water shortage or excess, high light or heat levels, and senescence. The increase in the ratio with loss of Chl is explained by a decrease in self absorption by Chl in the red spectral region (Krause and Weis, 1991; Gitelson et al., 1998). The R/FR fluorescence ratio has also been recognized to be an indicator of shorter term physiological activity (Lichtenthaler et al., 1986; Lichtenthaler, 1996). Incubating a leaf with the herbicide diuron, which acts to disrupt the electron transport pathway in the photosynthetic mechanism, greatly increases both red and far-red fluorescence. However, the red fluorescence is increased to a greater degree than is the far-red fluorescence, yielding an R/FR fluorescence ratio increase. The general terms of red and far-red fluorescence are used here because different authors state different specific wavelengths depending on the resolution of the instruments with which they are collecting their data, or on personal preference.

A variety of instruments have been used for the collection of Chl fluorescence data ranging from laboratory fluorescence spectrophotometers to laser induced fluorescence imagers. Each instrument has advantages and limitations. Active instruments, such as those for which the excitation source may be a laser, are not anticipated to be used at orbital altitudes, and may have limited use at aircraft altitudes. However, data collection from airborne or satellite platforms are essential for large area coverage. Passive instruments capable of detecting solar stimulated Chl fluorescence have been built for the observation of solar absorption features (Fraunhofer lines) and produce data based on the Fraunhofer line depth method (Plascyk, 1975; Carter et al., 1996; Kebabian et al., 1999). A prototype Fraunhofer line discriminator (FLD) has been used to collect imaging data from an airborne platform with encouraging results (Hemphill and Settle, 1981). The Fraunhofer line depth method determines luminescence, which is, in this case, a general term that includes fluorescence, by observing the intensity of the solar continuum (a) surrounding a Fraunhofer line, the intensity within the line (b), the corresponding line center measurement for a target (c), and the analogous target continuum (d). According to Plascyk (1975) the reflectance coefficient is calculated from the measurements as

$$R = \frac{(d-c)}{(a-b)} \tag{1}$$

and the luminescence coefficient is calculated as

$$L = \frac{d}{a} - R \text{, or } L = \frac{d}{a} - \frac{(d-c)}{(a-b)}. \tag{2}$$

A single set of measurements can therefore provide both the reflectance and fluorescence of a target at a specific Fraunhofer line wavelength. For the case of vegetation observations is it understood that the principle contributor to the luminescence coefficient is Chl fluorescence.

While the technique is effective and elegant, and has provided data that has correlated well with vegetation stress (McFarlane et al., 1980; Carter et al., 1990; Carter et al., 1996), these instruments had the disadvantage of being limited to a single Fraunhofer line for any given measurement, obviating the power of the R/FR method. Optics and detector technology have reached the level whereby multiple Fraunhofer lines, much narrower than lines used in the past (e.g. 656.3 nm), can be resolved. Several lines with absorption depths of at least 50 percent span the visible spectrum from 650 to 800 nm where Chl fluorescence occurs. This level of absorption has been determined to be well within the capability of a very high spectral resolution, filter based instrument to extract information from the incident radiance that could be collected at orbital altitudes (Cook, 1999), as well as from airborne or ground based platforms.

Objectives

When collected with a laboratory spectrophotometer, *in vivo* Chl fluorescence measurements yield a definitive spectrum. The signature shape is also seen in spectral data obtained with laser-induced remote sensing spectrometers operated at relatively short distances from the vegetation target. The object of this study was to model and predict the shape of the Chl fluorescence emission from orbital altitudes. The effects of wavelength dependent atmospheric transmittance and Fraunhofer line depth upon individual Fraunhofer lines were considered. Further, the correlation of multiple, adjusted Fraunhofer lines, and selected red/far-red ratios derived from them, with differing levels of nitrogen treatments and measured Chl concentrations were used to predict the best lines or ratios for the determination of vegetation health status from an orbital platform.

Materials and Methods

Excitation-emission-matrix (EEM) fluorescence data obtained from summer and fall bean crops subjected to various levels of nitrogen fertilization were used as the basis for modeling Chl emission spectra from individual measurements taken at thirteen Fraunhofer line wavelengths spanning the visible part of the solar spectrum from 650 to 780 nm. The EEM fluorescence data were collected with an Hitachi Instruments, Inc. F4500 fluorescence spectrophotometer calibrated and adjusted for corrected spectra generation according to the manufacturer's specifications. Excitation wavelengths, for the EEM, ranged from 300 to 600 nm in 10 nm steps, while emission intensities were measured at 5 nm intervals from 400 to 780 nm. Although these data were corrected for instrument characteristics during collection, the corrections were removed for this study to better simulate the spectra of field instruments. For the summer crop there were 10 replicates each for 5 levels of nitrogen treatment (20 to 100 mg kg^{-1} at 20 mg kg^{-1} steps). For the fall crop there were 6 replicates each for 5 levels of nitrogen treatment (40 to 120 mg kg^{-1} at 20 mg kg^{-1} steps). The data used for this study included three measurement dates from both crops; the earliest time of measurement when the leaves of interest had visually reached full flush, an intermediate time during the growth cycle, and a measurement taken when some of the leaves were visibly beginning senescence. The measurements were taken on the same leaves on each of the three collection dates throughout the crop growth cycle.

To model emission intensities at Fraunhofer line wavelengths, the integral of the excitation spectrum from 300 to 600 nm, interpolated between the two closest emission wavelengths at a 5 nm interval for each Fraunhofer line wavelength, was extracted from the EEM data sets. The derived intensities were adjusted for the percent absorbance, or line depth, of each Fraunhofer line, and for terrestrial atmospheric upwelling transmittance at 400 km altitude modeled with Modtran from PLEXUS. A validation of Modtran was conducted by Wang et al. (1996). Leaf Chl concentrations were measured using a calibrated Minolta SPAD-502 (Specialty Products Agriculture Division) hand-held Chl meter. Statistical analyses were conducted with the Prism® statistics package from GraphPad Software, Incorporated.

Results and Discussion

Figure 1 shows a typical spectrum for Chl fluorescence emission from 655 to 780 nm with an excitation wavelength of 470 nm. This spectrum of a healthy plant was extracted from an excitation-emission-matrix (EEM) data set for an optimal nitrogen treatment of 100 mg kg^{-1} collected with a laboratory fluorescence spectrophotometer. Also shown in Figure 1 is a typical spectrum for a plant under stress. The nitrogen treatment for this plant was 20 mg kg^{-1}.

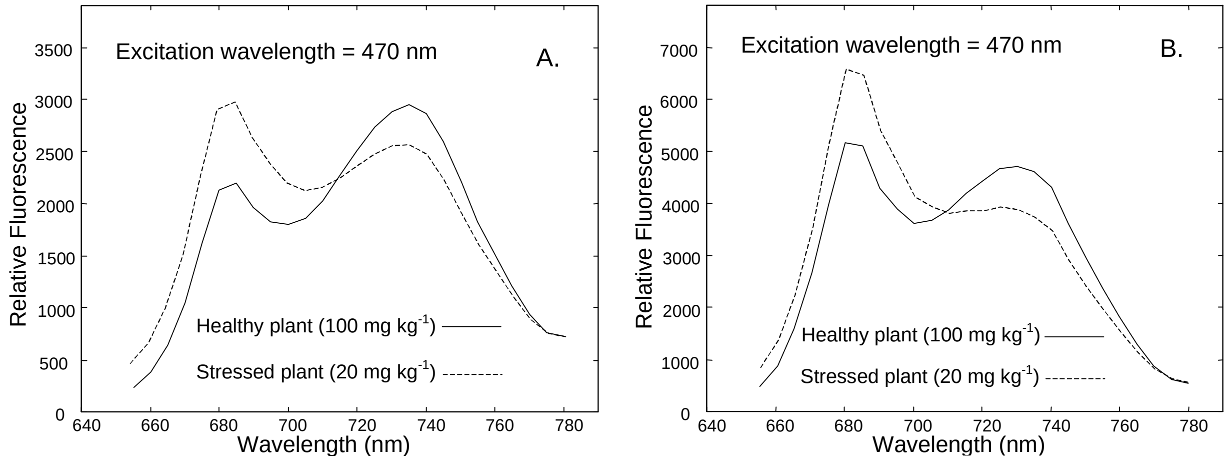

Figure 1. Typical corrected chlorophyll fluorescence spectra (A) for a healthy bean plant (*Phaseolus vulgaris* L.) with an optimal nitrogen treatment of 100 mg kg^{-1} and a stressed bean plant with a nitrogen treatment of 20 mg kg^{-1}. Also shown are typical uncorrected chlorophyll fluorescence spectra (B) for the same healthy and stressed bean plants.

The R/FR fluorescence ratios for the two spectra shown in Figure 1a are 0.791 and 1.218, respectively. These ratios are typical of those obtained from corrected spectra produced with laboratory spectrophotometers. The corrections remove the effects of the excitation source spectral characteristics, the instrument optics, and the detector spectral characteristics. For the two spectra shown above, the uncorrected R/FR ratios are 0.997 and 1.554, respectively, and those spectra are shown in Figure 1b. The distinction is important because most field instruments do not provide for corrected spectra. All data presented in the rest of this report are based on uncorrected spectra.

With the adjustment for line depth (A_{FL}) and atmospheric transmittance (T_{ATM}), at those same Fraunhofer line wavelengths, the modeled shape of the Chl fluorescence emission spectrum is radically different from that gathered with a laboratory spectrophotometer. Figures 2a and 2b below show the emission spectra for Chl fluorescence at an excitation of 470 nm, the modeled spectra derived from integrated excitation spectra at the thirteen Fraunhofer lines listed in Table 1, and the line depth and transmittance adjusted modeled spectra.

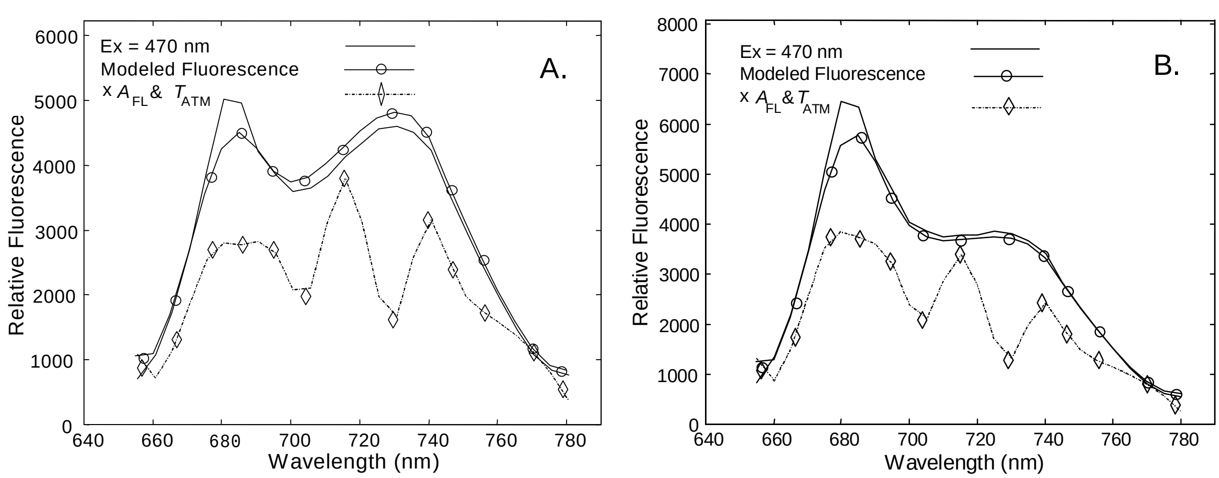

Figure 2. Chl fluorescence, modeled spectra, and adjusted spectra of the same healthy and stressed bean plants shown in Figures 1a and 1b, respectively.

The thirteen Fraunhofer lines chosen for examination and the modeling of the Chl fluorescence emission spectrum are listed in Table 1. Also included in the table are the percent absorbance, percent atmospheric transmittance, bandwidth in milliangstroms (mÅ), and the source of absorbance in the solar atmosphere. No significant correlation was achieved between the intensities modeled for these lines and either leaf Chl or applied nitrogen.

Table 1. Selected Fraunhofer lines and their associated properties.

Wavelength (nm)	Absorbance (%)	Bandwidth (mÅ)	Atm. Transmittance (%)	Element or ion Source
656.28	> 0.800	4020	0.6973	Hα
666.35	0.567	76	0.7295	Fe I
676.78	0.559	83	0.7411	Ni I
685.52	0.488	85	0.7429	Fe I
694.52	0.554	82	0.7308	Fe I
703.82	0.419	76	0.7511	Fe I
714.82	0.713	157	0.7308	Ca I
728.92	0.479	116	0.4230	Si I
738.94	0.537	144	0.7610	Fe I
746.23	0.501	119	0.7738	Cr I
755.56	0.519	98	0.7808	Ni I
769.90	> 0.800	154	0.7212	K I
778.06	~ 0.550	102	0.7885	Fe I

Twenty R/FR ratios were derived from five Fraunhofer lines in the red and four in the far-red part of the spectrum for all three measurement dates for both crops. The ratio utilizing red fluorescence at 694.5 nm and far-red fluorescence at 755.56 nm consistently yielded strong

Table 2. The coefficient of determination (r^2) values are presented for R/FR fluorescence ratios of modeled Fraunhofer line intensities versus leaf chlorophyll concentration as measured with a Minolta SPAD-502 chlorophyll meter.

Crop 1 Date 1	R/FR v Chl			10-Jul
R/FR	728.92	738.94	746.23	755.56
656.28	0.27	0.57	0.69	0.71
666.35	0.38	0.68	0.78	0.79
676.78	0.26	0.60	0.75	0.77
685.52	0.39	0.69	0.79	0.81
694.52	0.73	0.82	0.85	0.85
Crop 1 Date 2	R/FR v Chl			17-Jul
R/FR	728.92	738.94	746.23	755.56
656.28	0.62	0.83	0.87	0.88
666.35	0.77	0.86	0.89	0.90
676.78	0.64	0.78	0.85	0.86
685.52	0.69	0.80	0.86	0.87
694.52	0.80	0.85	0.89	0.90
Crop 1 Date 3	R/FR v Chl			21-Jul
R/FR	728.92	738.94	746.23	755.56
656.28	0.51	0.62	0.64	0.64
666.35	0.59	0.64	0.66	0.66
676.78	0.57	0.62	0.65	0.66
685.52	0.62	0.65	0.68	0.68
694.52	0.7	0.72	0.73	0.73

Crop 2 Date 1	R/FR v Chl			4-Sep
R/FR	728.92	738.94	746.23	755.56
656.28	0.01	0.28	0.56	0.60
666.35	0.00	0.31	0.56	0.59
676.78	0.00	0.22	0.48	0.52
685.52	0.02	0.33	0.55	0.58
694.52	0.33	0.57	0.66	0.66
Crop 2 Date 2	R/FR v Chl			11-Sep
R/FR	728.92	738.94	746.23	755.56
656.28	0.55	0.76	0.84	0.84
666.35	0.33	0.58	0.74	0.77
676.78	0.16	0.42	0.64	0.69
685.52	0.14	0.47	0.70	0.74
694.52	0.49	0.75	0.85	0.86
Crop 2 Date 3	R/FR v Chl			17-Sep
R/FR	728.92	738.94	746.23	755.56
656.28	0.48	0.61	0.67	0.68
666.35	0.50	0.60	0.66	0.67
676.78	0.42	0.56	0.64	0.66
685.52	0.43	0.59	0.66	0.68
694.52	0.59	0.68	0.71	0.72

correlation with leaf Chl concentration (Table 2). For the Date 2 measurement (when the beans had reached maximum growth) of Crop 1 (the summer crop), almost all ratio combinations, except those using 728.92 nm in the denominator, correlated strongly with measured leaf Chl concentration. For the Date 2 measurement of Crop 2 (the fall crop), ratios with both 656.28 and 694.5 nm in the numerator strongly correlated with measured Chl. Ratios with 728.92 nm in the denominator were, again, the exception.

The correlation between measured leaf Chl and applied nitrogen treatment levels were strongest for the second measurement date for each crop, with final collection date r^2 values falling below those calculated for the initial measurements (see Table 3). The stronger correlation between applied nitrogen and leaf Chl concentration occurs in the Date 2 measurements for both summer and fall crops when the measured leaves had reached full flush and not yet begun to senesce.

Table 3. Linear regression r^2 values for leaf Chl concentration versus applied nitrogen by crop and measurement date.

	Date 1	Date 2	Date 3
Crop 1 (summer)	0.72	0.75	0.72
Crop 2 (fall)	0.72	0.89	0.78

The strength of the correlation between the twenty R/FR fluorescence ratios and applied nitrogen (Table 4) varied in a fashion similar to those for the R/FR fluorescence ratio / leaf Chl concentration comparison. The ratio of 694.52 nm in the red and 755.56 nm in the far-red consistently provided the strongest correlation with applied nitrogen treatment.

Table 4. The coefficient of determination (r^2) values are presented for the R/FR fluorescence ratios of modeled Fraunhofer line intensities versus applied nitrogen treatments.

Crop 1 Date 1		RFR v N		10-Jul
R/FR	728.92	738.94	746.23	755.56
656.28	0.18	0.39	0.49	0.51
666.35	0.22	0.45	0.53	0.55
676.78	0.14	0.39	0.51	0.53
685.52	0.23	0.46	0.55	0.56
694.52	0.5	0.58	0.61	0.61
Crop 1 Date 2		RFR v N		17-Jul
R/FR	728.92	738.94	746.23	755.56
656.28	0.62	0.74	0.75	0.76
666.35	0.65	0.69	0.72	0.73
676.78	0.48	0.59	0.66	0.68
685.52	0.50	0.60	0.66	0.68
694.52	0.59	0.65	0.69	0.71
Crop 1 Date 3		RFR v N		21-Jul
R/FR	728.92	738.94	746.23	755.56
656.28	0.61	0.63	0.63	0.63
666.35	0.53	0.54	0.57	0.57
676.78	0.45	0.49	0.53	0.54
685.52	0.48	0.51	0.54	0.55
694.52	0.51	0.53	0.57	0.58

Crop 2 Date 1		RFR v N		4-Sep
R/FR	728.92	738.94	746.23	755.56
656.28	0.02	0.14	0.42	0.49
666.35	0.01	0.42	0.67	0.70
676.78	0.04	0.42	0.67	0.71
685.52	0.14	0.56	0.73	0.75
694.52	0.56	0.74	0.78	0.77
Crop 2 Date 2		RFR v N		11-Sep
R/FR	728.92	738.94	746.23	755.56
656.28	0.50	0.70	0.77	0.77
666.35	0.33	0.56	0.70	0.72
676.78	0.17	0.42	0.62	0.66
685.52	0.16	0.47	0.67	0.71
694.52	0.48	0.71	0.79	0.80
Crop 2 Date 3		RFR v N		17-Sep
R/FR	728.92	738.94	746.23	755.56
656.28	0.53	0.61	0.64	0.65
666.35	0.50	0.57	0.61	0.62
676.78	0.40	0.52	0.58	0.60
685.52	0.39	0.53	0.60	0.61
694.52	0.52	0.6	0.65	0.66

The twenty R/FR ratios were also evaluated for the maximum percent differential between low and high (optimal) nitrogen treatments (see Table 5). For the summer crop, the comparisons included 20 mg kg-1 v 100 mg kg-1 and 40 mg kg-1 v 100 mg kg-1. For the fall crop, the comparisons included 40 mg kg-1 v 120 mg kg-1 and 40 mg kg-1 v 100 mg kg-1.

Table 5. The ranking values for the percent difference between low and high nitrogen treatments (mg kg^{-1}) for Crop 1 (summer) and Crop 2 (fall). The wavelengths are rounded to the nearest nanometer.

Crop 1	Date 1		Date 2		Date 3		Overall	
Ratio	20 v 100	40 v 100	20 v 100	40 v 100	20 v 100	40 v 100	Sum	Average
656/755	17	20	20	20	20	20	117	20
666/755	18	18	18	18	19	18	109	19
656/746	12	19	19	19	17	19	105	18
677/755	19	16	17	17	18	16	103	17
686/755	20	14	15	15	16	17	97	16
666/746	13	17	16	16	15	15	92	15
695/755	16	15	13	13	12	14	83	14
677/746	14	13	14	14	14	12	81	13
686/746	15	11	12	12	13	13	76	12
656/739	7	10	11	11	11	11	61	11
695/746	11	12	10	10	8	10	61	10
666/739	8	9	9	9	10	9	54	9
677/739	9	8	8	8	9	7	49	8
686/739	10	7	6	6	7	8	44	7
695/739	6	6	5	5	6	5	33	6
656/729	2	5	7	7	5	6	32	5
666/729	3	4	4	4	4	4	23	4
677/729	4	3	3	3	3	2	18	3
686/729	5	1	2	2	2	3	15	2
695/729	1	2	1	1	1	1	7	1
Crop 2	Date 1		Date 2		Date 3		Overall	
Ratio	40 v 120	40 v 100	40 v 120	40 v 100	40 v 120	40 v 100	Sum	Average
677/755	18	19	19	19	20	20	115	20
666/755	17	15	20	20	19	19	110	19
686/755	19	20	17	17	17	17	107	18
656/755	12	10	18	18	18	18	94	17
677/746	14	16	16	15	16	16	93	16
695/755	20	18	13	13	12	12	88	15
666/746	13	13	15	16	15	15	87	14
686/746	15	17	12	12	13	13	82	13
656/746	11	7	14	14	14	14	74	12
695/746	16	14	11	11	11	11	74	11
677/739	8	11	10	9	10	10	58	10
666/739	7	8	9	10	9	9	52	9
686/739	9	12	7	7	7	7	49	8
695/739	10	9	6	6	6	6	43	7
656/739	6	2	8	8	8	8	40	6
677/729	3	5	5	3	5	5	26	5
666/729	2	3	4	5	4	4	22	4
686/729	4	6	2	2	2	2	18	3
656/729	1	1	3	4	3	3	15	2
695/729	5	4	1	1	1	1	13	1

Ratios of the 656.3 nm Fraunhofer line and the 755.6 nm line consistently ranked highest for the summer crop. Ratios with 728.92 nm and 738.94 nm in the denominator consistently ranked in the lowest and next lowest quartile, respectively. Ratios with 755.56 nm in the denominator ranked in the highest quartile for ten out of twelve comparisons. The two ratios completing the upper quartile were 656.28 v 746.23 for the summer and 676.78 v 746.23 for the fall.

Conclusions

The modeling of Chl fluorescence emission spectra based on Fraunhofer line data extracted from laboratory generated spectra and adjusted for individual Fraunhofer line depth and atmospheric transmittance has been accomplished. The validity of the model awaits the actual measurement, from orbit, of the thirteen Fraunhofer line intensities used in the model. An alternate method of validation would be an experiment wherein a location could be found that would allow the measurement of vegetation, from an oblique angle, through a column of atmosphere with an optical density equal to that encountered for an altitude of approximately 400 km (Rock, 1999).

While, no significant correlation was achieved between the individual intensities modeled for these lines and either leaf Chl or applied nitrogen, the R/FR fluorescence ratio, 694.52 nm / 755.56 nm, consistently yielded the best correlations with nitrogen treatment levels and strong correlations with measured leaf Chl concentration. That the best correlations with applied nitrogen occurred for the Date 2 measurements for both crops is explained by the fact that the best correlations between applied nitrogen and measured leaf Chl also occurred for the Date 2 measurements. Factors other than applied nitrogen (i.e. differences related to early development and senescence) have a stronger effect on the R/FR ratios for the Date 1 and Date 3 measurements for both crops. For the comparisons between low and high nitrogen treatment levels, which tend to accentuate the natural growth cycle differences, the 694.52/755.56 ratio consistently ranked in the second highest quartile and occasionally in the highest quartile. This strengthens the argument for the use of the 694.52/755.56 ratio as the choice for orbital measurements where instrument size and weight, time on target, and other constraints may limit an instrument to two wavelengths. The Fraunhofer line at 728.92 nm yielded low to high nitrogen treatment, differences of ratios consistently in the lowest quartile. This was most likely due to the low atmospheric transmittance at that wavelength (see Table 1); nearly half of the value measured for the remaining Fraunhofer line wavelengths. The 694.52/ 755.56 ratio would provide the best information on gradations of Chl fluorescence during any part of the growth cycle. However, for the purposes of determining gross differences, especially during early development or autumnal Chl breakdown, substituting of any of the other four red fluorescence Fraunhofer lines in the numerator would enhance the results. Because of its second highest ranking for both the summer and fall crops, the 666.35 nm Fraunhofer line would be the first choice for such a substitution. If an orbital instrument were to be operational only during summer or fall, but not both, better choices for the ratio would then be 656.28/755.56 for the summer and 676.78/755.56 for the fall, as these rank highest for the related crops. A suggestion that certain ratios may be more sensitive to plant physiological status related to differing environmental conditions can be found in the example of the five ratios in which 755.56 nm is in the denominator. While all these ratios are strongly correlated with Chl for the Date 2 measurements of the summer crop, the ratios with 666.35, 676.78, and 685.52 in the numerator are not strongly correlated with Chl for the Date 2 measurements of the fall crop. The two ratios at the extremes of the Chl emission spectrum remain strongly correlated with Chl, implying that something other than Chl altered the shape of the observed spectrum.

These results reveal good choices of Fraunhofer line ratios for preliminary instrument testing. Considering the current state of detector and optical technology, there is no reason not to build and launch an orbital Chl fluorescence detection instrument. An unexpected benefit of this study is that the specificity with respect to differing physiological conditions of vegetation, lacking in previous studies with passive fluorescence instruments due to the limitation of a single Fraunhofer line, may be overcome with the application of selected ratios of Fraunhofer lines within the context of R/FR analysis.

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