# A Narrow-Waveband Spectral Index That Tracks Diurnal Changes in Photosynthetic Efficiency*

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We present a new "physiological reflectance index" (PRI) isolated from narrow waveband spectral measurements of sunflower canopies. This index correlates with the epoxidation state of the xanthophyll cycle pigments and with the efficiency of photosynthesis in control and nitrogen stress canopies, but not in water stress canopies undergoing midday wilting. It is analogous in formulation to the broadband normalized difference vegetation index (NDVI) and uses reflectance at 531 nm and at a reference wavelength to minimize complications associated with diurnal sun angle changes. In conjunction with other methods, this index may lead to improved remote and ground-based estimates of canopy photosynthetic function.

## INTRODUCTION

Most remote sensing of vegetation uses broadband sensors to derive indices of vegetation cover. Perhaps the most widely used index is the normal-ized difference vegetation index (NDVI) (Tucker, 1979). Although NDVI correlates well with canopy features such as biomass, leaf area index (LAI), absorbed photosynthetically active radiation (APAR), and canopy photosynthetic capacity (Sellers, 1987), it often fails to capture dynamic physiological processes, which may occur on fine temporal and spectral scales. For example, drought-tolerant evergreens can undergo seasonal changes in light use efficiency and photosynthetic activity without equivalent changes in NDVI or canopy structure (Running and Nemani, 1988). Furthermore, many species under seasonal stress undergo dramatic midday "photosynthetic depressions" (Tenhunen et al., 1987), presumably without significant changes in canopy architecture.

At the leaf level, the xanthophyll cycle may be associated with diurnal reductions in photosynthetic efficiency, defined here as the net $CO_2$ uptake rate divided by the incident photosynthetic photon flux density (PFD). Under conditions of excess light, when the absorbed PAR exceeds the capacity of the photosynthetic reactions, the xanthophyll cycle pigment violaxanthin is deepoxidized to zeaxanthin via antheraxanthin, and this reaction is readily reversed under limiting light (Fig. 1; Demmig-Adams, 1990). Thus, the epoxidation state (EPS), which is a convenient way of expressing the relative concentrations of the three xanthophyll cycle pigments [see Eq. (1), below], may be a useful indicator of short-term

## The Xanthophyll Cycle
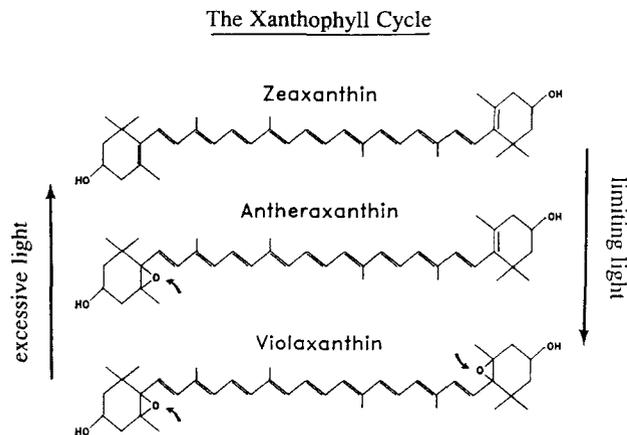


*Figure 1.* The xanthophyll cycle. Excessive absorbed PAR causes the deepoxidation of violaxanthin to zeaxanthin via antheraxanthin, and this process is reversed under limiting PAR. Small arrows indicate the epoxide groups involved in these biochemical transitions. The difference spectrum associated with these pigment conversions results in a detectable "signal" near 531 nm in the reflectance spectra of intact leaves and canopies. After Demmig-Adams (1990).

changes in photosynthetic activity. Remote detection of changes in EPS might be useful in monitoring photosynthetic activity of vegetation canopies.

The advent of portable radiometers with high spectral resolution offers new possibilities for examining dynamic physiological processes occurring on fine temporal and spectral scales. Laboratory studies of individual leaves have identified an absorbance feature at 505 nm (Bilger et al., 1989) and a reflectance feature at 531 nm (Gamon et al., 1990) associated with the conversion of violaxanthin to zeaxanthin. At the canopy level, Gamon et al. (1990) demonstrated a short-term correlation between EPS and reflectance at 531 nm ($R_{531}$) of a sunflower crop subjected to an artificial "dawn-to-midday" transition imposed by the sudden removal of shadecloth at noon. However, the isolation and interpretation of the "xanthophyll signal" from diurnal canopy reflectance spectra presented a bigger challenge due to the presence of overlapping spectral features unrelated to the xanthophyll signal.

The purpose of this article is to illustrate a simple method for extracting a useful "physiological reflectance index" (PRI) from diurnally changing reflectance signatures of sunflower canopies. We present results both from shade removal experiments that provided an abrupt transition from low to high PAR and from diurnal measurements

that followed gradual PAR changes under ambient sunlight. The PRI is contrasted with NDVI to illustrate the potential utility of narrow-bandwidth reflectance and to illustrate the complementary nature of these two vegetation indices. Although the methods presented here were aimed at isolating a "xanthophyll signal" indicative of photosynthetic activity, they may be applicable to other subtle and dynamic features in spectral reflectance.

## MATERIALS AND METHODS

Sunflower (*Helianthus annuus* L. cv. CGL 208) was used in all experiments. Seeds were planted in rows in late May 1989, and thinned to an average spacing of 55 cm (between rows) and 27 cm (within rows). Cultural conditions designed to obtain nitrogen-stress, water-stress, and control canopies (with ample irrigation and nitrogen) are described in Fredeen et al. (1991).

All measurements of intact canopies were conducted on 15 (water stress), 16 (control), and 17 (nitrogen stress) August 1989. The crop was in a late flowering stage, but flower heads were removed 1 day prior to canopy measurements to avoid complications due to their large visible reflectance (unpublished data). LAI was 4.2, 1.2, and 1.0 for the control, nitrogen-stress, and water-stress canopies, respectively. Dawn / noon leaf water potentials were $-0.21$ / $-0.82$, $-0.53$ / $-0.84$, and $-1.46$ / $-2.36$ MPa for the control, nitrogen-stress, and water-stress canopies, respectively. Clear skies prevailed on all three dates, except that morning measurements on the nitrogen-stress plot were made under persistent fog that lifted at approximately 10:35 PST.

An artificial "dawn-to-midday" light transition was applied to intact canopies in the field through sudden removal of a shadecloth (Gamon et al., 1990). This method produced a rapid transition from low to midday light levels without the large changes in sun angle, canopy structure, leaf movement, and atmospheric effects that typically occur over several hours. Diurnal canopy measurements under naturally changing light conditions were made on canopy regions adjacent to those used in the shade removal experiments.

Canopy radiance was detected with a narrow-bandwidth visible / near-infrared spectroradiometer

(10 nm bandwidth at half-maximum response) fitted with 15° FOV optics (Model SE590 with detector model CE390WB-R, Spectron Engineering, Inc., Denver, Colorado), and expressed as spectral reflectance after correction by the radiance of a level halon standard. Further details of the reflectance calculations are presented in Gamon et al. (1990). All spectral measurements were made in a nadir orientation from a vertical distance of approximately 4 m, allowing radiance measurements of a 1-m diameter area consisting of 8–10 plants.

Diurnal spectral reflectance measurements of the adaxial surfaces of individual leaves near the canopy top were made on 5 July 1990, with a second spectroradiometer (6 nm bandwidth at half-maximum response) equipped with an integrating sphere (LI-1800, LI-COR, Inc., Lincoln, Nebraska). Plants were in an early vegetative stage (LAI = 0.45) on this date, and cultural conditions were similar to the control canopy of 1989. Sample leaves were randomly chosen from horizontal, top-canopy leaves similar in orientation, sun exposure, and developmental stage to adjacent leaves used for xanthophyll-pigment and gas-exchange determinations (described below). Reflectance measurements were made by placing a leaf in the integrating sphere immediately prior to measurement, which took approximately 10 s. This short high-light exposure did not result in noticeable xanthophyll deepoxidation in dark-adapted leaves (data not shown).

Leaf disks (0.9 cm$^2$) punched from upper-canopy leaves were analyzed for xanthophyll cycle pigments by the HPLC procedure of Thayer and Björkman (1990) as reported in Gamon et al. (1990). The epoxidation state (EPS) was calculated from the area-based, molar concentrations of the three xanthophyll cycle pigments, violaxanthin ($V$), antheraxanthin ($A$), and zeaxanthin ($Z$), as follows:

$$EPS = (V + 0.5*A) / (V + A + Z). \qquad (1)$$

The relationships between PRI, EPS, NDVI, $R_{531}$, and photosynthetic efficiency were examined with Pearson correlation matrices (Systat v. 5.1 for Macintosh computers, Systat, Inc., Evanston, Illinois). In Tables 1 and 3, EPS is expressed as ln(1-EPS) because the Pearson correlation coefficients involved a linear model, and this loga-

rithmic transformation improved the linearity of relationships involving the PRI (data not shown).

The reported EPS values are means of three to five samples typically extracted and assayed together, and thus represent the means of three to five upper canopy leaves. Near-horizontal leaves near the canopy top were chosen because these leaves matched those used in leaf-level reflectance determinations and gas exchange measurements, and because they probably contributed most of the canopy radiance detected from above.

Using a portable gas-exchange system equipped with a 1-L leaf chamber (LI-6200, LI-COR, Inc., Lincoln, Nebraska), net $CO_2$ uptake was determined on three to six leaves similar in size, orientation, and canopy position to those samples for xanthophyll pigments and leaf reflectance. "Photosynthetic efficiency" was calculated as the net $CO_2$ assimilation rate divided by the PFD incident on the leaf during the measurement. Incident PFD was estimated by correcting the PFD measured by the sensor on the chamber housing by the mean transmittance of the chamber lid (approximately 0.835 in the vicinity of the leaf). Photosynthetic activity was expressed as photosynthetic efficiency to normalize for variation in incident PFD. Photosynthetic efficiency is also a physiological analog to the "dry matter yield" or "light-use-efficiency" coefficient commonly used in studies of net primary productivity (e.g., Monteith, 1977; Goward et al., 1985; Russell et al., 1989).

To minimize the effects on the "xanthophyll signal" of overlapping spectral features due to sun angle variation, we derived a physiological reflectance index (PRI) from $R_{531}$ and reflectance at a reference wavelength, $R_{REF}$, as follows:

$$PRI = (R_{REF} - R_{531}) / (R_{REF} + R_{531}), \qquad (2)$$

where $R_{531}$ is reflectance at 531 nm. The formulation of this PRI is analogous to that of NDVI (Tucker, 1979). $R_{531}$ was selected to represent EPS based on our previous findings that reflectance in this spectral region was strongly correlated with EPS under certain conditions (Gamon et al., 1990; see also Figs. 2 and 3). In leaf-level measurements employing the LI-1800 spectroradiometer, we used $R_{530}$ as the "xanthophyll" wavelength. Pearson correlation matrices using a range of reference wavebands in combination with 531 nm (or

530 nm at the leaf-level) suggested that there is sometimes a wide latitude in the choice of acceptable reference waveband, and that the ideal reference waveband for correlation with EPS varied slightly between experiments and treatments (see Results and Discussion).

To calculate NDVI, we first calibrated the spectroradiometer response against the spectral response of NOAA AVHRR Bands 1 and 2 (as reported in Sellers, 1987) and integrated the corrected narrow waveband radiance over these two broad bands. NDVI was then calculated from the integrated reflectance in these two bands as follows:

$$NDVI = (R_{NIR} - R_{RED}) / (R_{NIR} + R_{RED}), \quad (3)$$

where $R_{NIR}$ and $R_{RED}$ are reflectances in the near infrared and visible, respectively.

Our canopy spectroradiometer readings included a second-order instrument signal that led to errors in the calculated reflectance, appearing as gradual declines in reflectance at wavelengths above 800 nm. Reflectance in the visible and

shorter NIR wavelengths, and thus the PRI, was unaffected by this artifact. Empirical tests with and without blocking filters on the instrument fore-optics indicated that the error in our NDVI values resulting from this second-order instrument signal could have been as high as 5%. However, because this error would have appeared as a slight, consistent offset in our NDVI values, it would have had little affect on the primary conclusions of this paper.

To test the hypothesis that some of the spectral features under examination were independent of physiological effects and resulted from canopy and solar geometry alone, we used the SAIL model of N. Bunnik and W. Verhoef (Verhoef, 1984) as provided by Goel (1988) modified to accommodate narrow-waveband input and output spectra. Using measured canopy LAI and LAD, measured leaf and soil spectra, and calculated solar geometry as inputs, the model calculated the diurnal reflectance spectra at 2-nm wavelength intervals and 20-min time steps.

*Figure 2.* A) Reflectance spectra from a control sunflower canopy upon sudden removal of shade at midday. Figure derived from Gamon et al. (1990). B) Delta reflectance (reflectance at time n minus reflectance at time zero) following shade removal. Derived from data in Figure 2A.
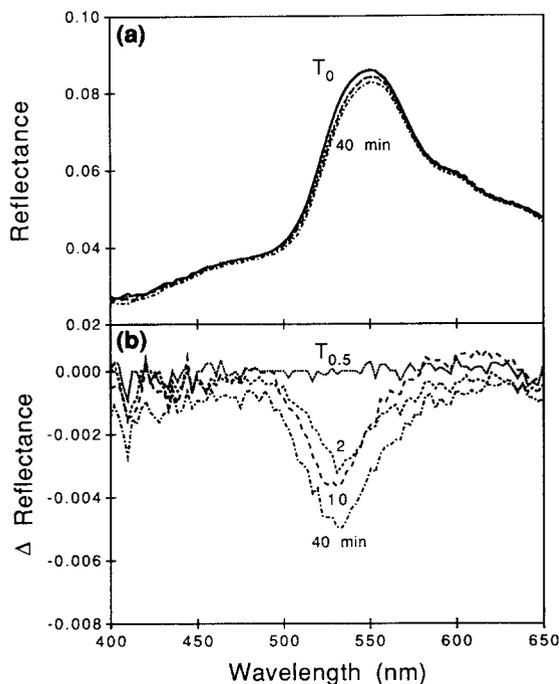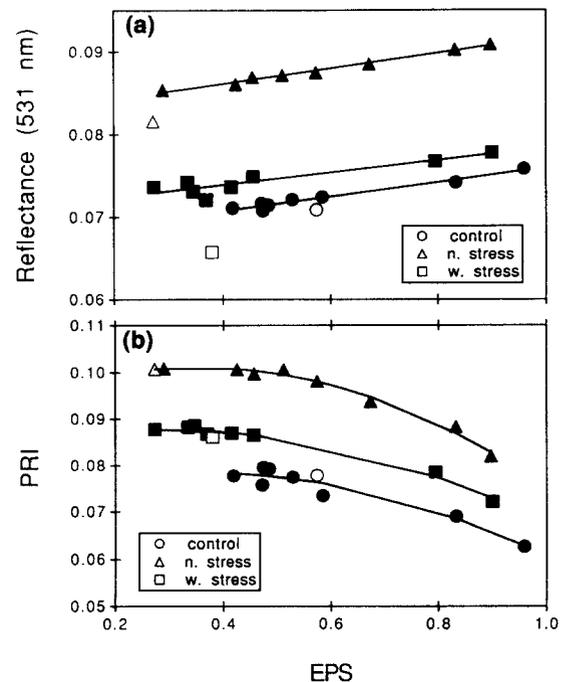
*Figure 3.* A) Reflectance at 531 nm plotted against the epoxidation state (EPS) of the xanthophyll cycle pigments. Solid points represent measurements in the first 20 min after shade removal; open symbols represent measurements 40 min after shade removal. B) PRI [$(R_{550} - R_{531})/(R_{550} + R_{531})$] plotted against EPS. Symbols as in Figure 3A.

# RESULTS

## Shade-Removal Experiments

Sudden transition from low to high light at midday caused a decline in canopy reflectance near 531 nm (Fig. 2). In all treatments, $R_{531}$ and EPS were closely correlated in the first 20 minutes after shade removal (Fig. 3A). By 40 min after high-light exposure, this relationship had begun to break down, probably due to changes in sun angle and leaf orientation. This drift in the $R_{531}$–EPS correlation was especially marked in the water-stress treatment, which had a distinct row structure and exhibited the onset of leaf wilting by 40 min after shade removal.

To eliminate the complicating effects of overlapping spectral features that were unrelated to EPS, we tested reflectance at many wavebands in combination with $R_{531}$ to find a suitable normalized index (PRI). Table 1 presents the Pearson correlation coefficients for ln(1-EPS) and the PRI calculated with $R_{531}$ and several possible reference wavelengths. In the shade-removal experiments, where spectral effects due to changing sun and canopy geometry were minimized, there were many suitable reference wavelengths yielding significant correlations (Table 1). Reexpressing reflectance as the PRI (e.g., with $R_{550}$ as the reference) caused the long-term drift in the reflectance vs. EPS relationships to disappear on all three treatments (Fig. 3B).

## Diurnal Measurements

Figure 4 illustrates diurnal changes in spectral reflectance for control, nitrogen-stress, and water-stress sunflower canopies exposed to naturally changing light regimes. Although diurnal changes in near-infrared reflectance were similar for all three treatments, the temporal changes in visible reflectance varied substantially between treatments. Both the control and nitrogen-stress canopies had midday declines in visible reflectance, and the reflectance change in the green (near 550 nm) was especially noticeable in the nitrogen-stress canopy. This "flattening" of the visible spectrum in the nitrogen-stress canopy was probably related to its lower LAI, allowing increased soil illumination at midday. In the water-stress canopy, there was little diurnal change in green reflectance, but a slight increase in blue reflectance

*Table 1.* Squared Pearson Correlation Coefficients for ln(1-EPS) and PRI Calculated from $R_{531}$ and 14 Selected Reference Wavelengths[a]

| Reference Wavelengths (nm) | ln(1-EPS) | | |
|---|---|---|---|
| | Control | N. Stress | W. Stress |
| 539 | 0.787** | 0.939** | 0.972** |
| 550 | 0.906** | 0.966** | 0.986** |
| 559 | 0.941** | 0.955** | 0.949** |
| 570 | 0.929** | 0.880** | 0.951** |
| 579 | 0.955** | 0.814** | 0.962** |
| 591 | 0.939** | 0.679** | 0.958** |
| 600 | 0.935** | 0.616* | 0.953** |
| 611 | 0.943** | 0.484* | 0.912** |
| 620 | 0.943** | 0.402 | 0.918** |
| 629 | 0.865** | 0.338 | 0.931** |
| 641 | 0.876** | 0.214 | 0.889** |
| 650 | 0.947** | 0.135 | 0.884** |
| 661 | 0.477* | 0.005 | 0.774** |
| 670 | 0.543* | 0.066 | 0.236 |

[a] Significance levels are indicated by superscripts: * $\alpha = 0.05$ and ** $\alpha = 0.01$. Data for shade removal experiments.

*Figure 4.* Spectral reflectance at noon and at 8 a.m. for control (A), nitrogen stress (B), and water stress canopies (C).
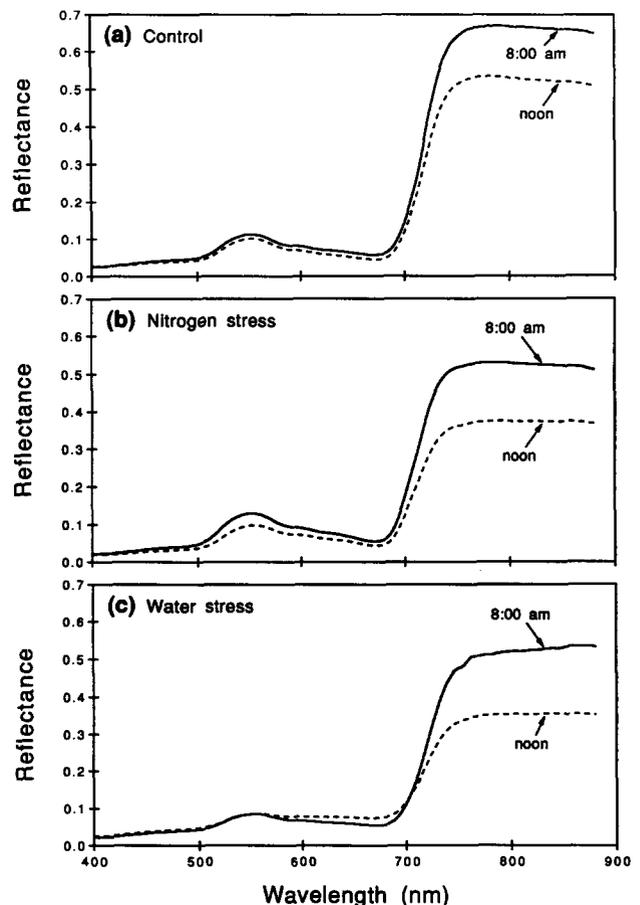
and a large increase in red reflectance. These diurnal patterns were largely attributable to the increased midday soil radiance in this sparse canopy.

Figures 5A and B clearly highlight wavelengths exhibiting large changes in reflectance between early morning and noon by illustrating the ratios and differences of noon and dawn reflectances. The ratio plots emphasize changes in the shape of the spectra, while the difference plots emphasize changes in absolute reflectance values. In each treatment a dynamic spectral feature appears as a slight dip in the difference and ratio plots near 531 nm. The wavelengths of the minima of these dips varied between treatments

Figure 5. A) Spectral reflectance at noon divided by spectral reflectance at 8 a.m. for control, nitrogen stress, and water stress canopies. Derived from data in Figure 4. B) Spectral reflectance at noon minus spectral reflectance at 8 a.m. for control, nitrogen stress, and water stress canopies. Derived from data in Figure 4. C) Spectral reflectance at noon minus spectral reflectance at 8 a.m. for control, nitrogen stress, and water stress canopies modeled with SAIL (see Materials and Methods).
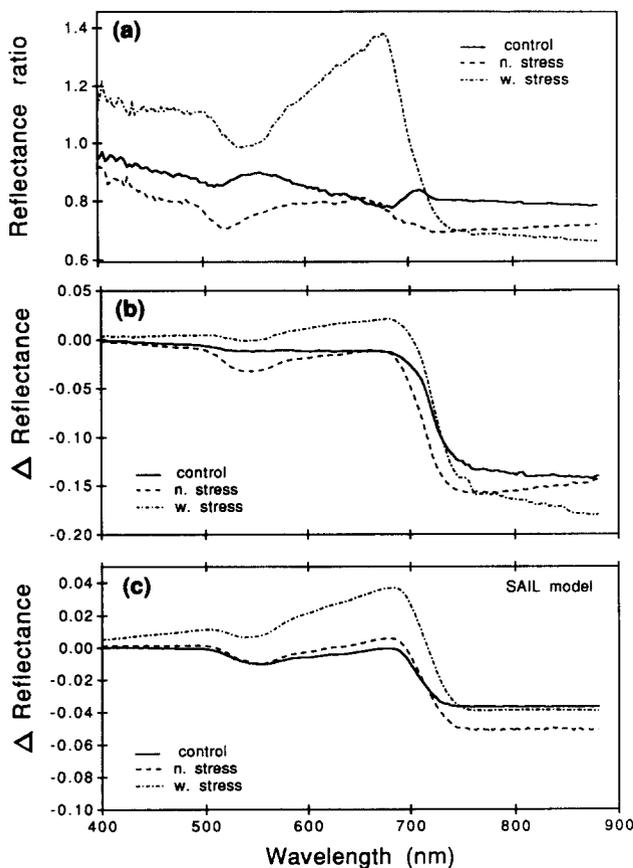


by as much as 30–40 nm, possibly due to overlapping spectral features associated with canopy structure and sun angle.

To test the possibility that diurnal changes in sun angle alone could explain part of the diurnal reflectance changes near 531 nm, we examined the output of the SAIL model (Verhoef, 1984), modified to provide high spectral resolution comparable to that of our spectroradiometers. Although the model did not exactly depict all of the subtle spectral features seen in the field data (Fig. 5B), it recreated some of the major features in the visible region and the visible / near-infrared boundary (the "red edge") (Fig. 5C). Even though the input spectra for the SAIL model included no diurnal variation in leaf spectral characteristics, the delta reflectance plots produced by the model exhibited dips at approximately 550 nm, a slightly longer wavelength than the dips in the actual field data. The model simulations did not consider row structure or diurnally changing leaf angle distributions, and these factors could account for part of the difference between the measured and modeled spectra. Yet this simple exercise clearly demonstrated that changes in canopy absorptance and scattering properties due to sun-angle changes alone can produce large dynamic reflectance features in the visible. Clearly, interpretation of possible physiological reflectance signals in the visible would require normalization for these large spectral features.

Principle components analysis of diurnal reflectance spectra suggested a means of normalizing for overlapping reflectance features unrelated to physiological changes. Using the covariance matrix of 148 diurnal spectra (480–580 nm only) obtained from all three treatments, principle components analysis yielded a first component (PC) associated with changes in "brightness" (the factor score coefficients were weighted similarly for all wavelengths), a second component associated with "greenness" (the factor score coefficients reached a local maximum near 550 nm), and a third component associated with the xanthophyll signal (the factor score coefficients reached a local maximum near 531 nm) (Fig. 6). By employing a difference divided by a sum, the PRI was intentionally formulated to normalize for these overlapping features related to brightness and greenness. The denominator of the PRI tended to normalize for differences in brightness (the first PC), whereas
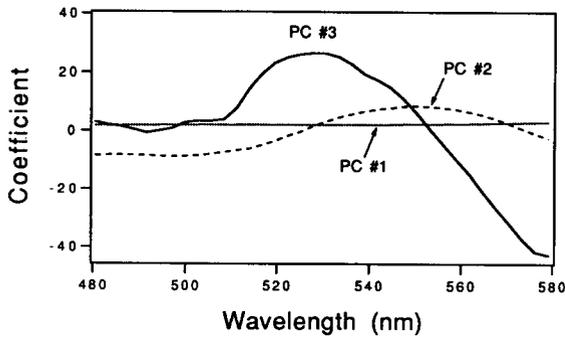
*Figure 6.* Factor score coefficients for principle components (PCs) 1,2, and 3, using the covariance matrix on 148 diurnal reflectance spectra. PCs 1,2, and 3 accounted for approximately 88%, 11%, and 1% of the variance, respectively.

subtraction of $R_{531}$ from $R_{550}$ (the green reflectance peak) tended to normalize for changes in greenness (the second PC).

In the absence of normalization, the diurnal course of $R_{531}$ yielded no clear correlation with either EPS or photosynthetic efficiency (Table 2). However, the PRI, using 550 nm as a reference wavelength, exhibited a diurnal pattern that mirrored EPS and photosynthetic efficiency in both the control and the nitrogen-stress canopies (Fig. 7). In these two treatments, the PRI yielded significant correlations with EPS and photosynthetic efficiency, but not with NDVI (Table 3, Figs. 8a and b). Thus, the diurnal variation in PRI primarily reflected changes in photosynthetic activity and not canopy architecture.

In the water-stress treatment, an unambiguous interpretation of the PRI was complicated by the large diurnal changes in canopy structure associated with severe midday wilting. In this case, the PRI with $R_{550}$ as a reference did not work well as a physiological indicator, but good correlations between PRI and photosynthetic indicators (EPS and efficiency) were obtained when wavelengths slightly longer than 550 nm were used as references (Table 3). However, at longer
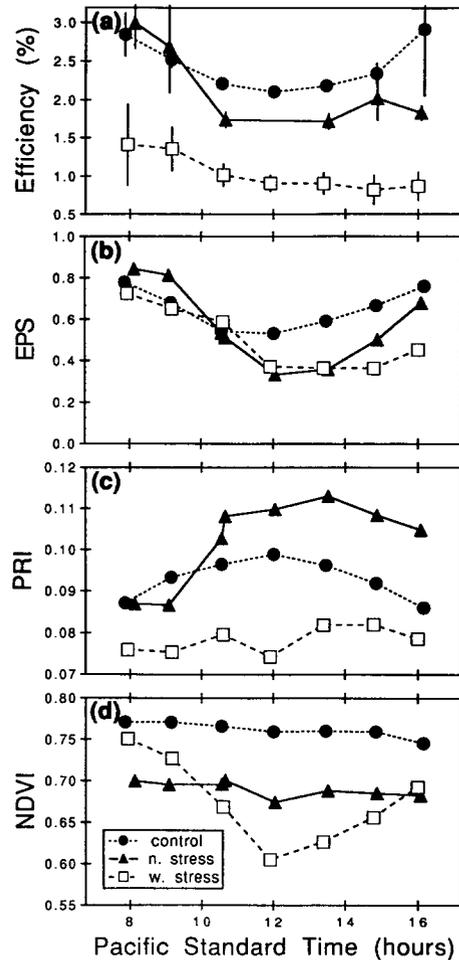


*Figure 7.* Diurnal courses of photosythetic efficiency (A), EPS (B), PRI [$(R_{550} - R_{531})/(R_{550} + R_{531})$] (C), and NDVI (D). Points in 7A are means ± 1 SEM of three to five leaves; points in 7B are means of three to five samples extracted and assayed together. Different sets of leaves were used for these two assays. Points in 7C and 7D are single measurements of approximately 1 m$^2$ of canopy area (including 8–10 plants each).

reference wavelengths, there were also strong correlations between the PRI and NDVI, suggesting an influence of wilting on the PRI when longer reference wavelengths were used. Thus,

*Table 2.* Squared Pearson Correlation Coefficients for EPS, NDVI, Photosynthetic Efficiency, and $R_{531}$[a]

| | Control | | | N. Stress | | | W. Stress | | |
|---|---|---|---|---|---|---|---|---|---|
| | *NDVI* | *Effic.* | *$R_{531}$* | *NDVI* | *Effic.* | *$R_{531}$* | *NDVI* | *Effic.* | *$R_{531}$* |
| EPS | 0.008 | 0.906** | 0.008 | 0.314 | 0.729* | 0.058 | 0.773** | 0.854** | 0.032 |
| NDVI | | 0.028 | 0.616* | | 0.271 | 0.018 | | 0.656* | 0.018 |
| Efficiency | | | 0.013 | | | 0.054 | | | 0.002 |

[a] Significance levels are indicated by superscripts: * $\alpha = 0.05$ and ** $\alpha = 0.01$. Data for diurnal course experiments.

*Table 3.* Squared Pearson Correlation Coefficients for ln(1-EPS), NDVI or Photosynthetic Efficiency vs. PRI Calculated from $R_{531}$ and Selected Reference Wavelengths[a]

| Reference Wavelengths (nm) | Control | | | N. Stress | | | W. Stress | | |
|---|---|---|---|---|---|---|---|---|---|
| | *ln(1-EPS)* | *NDVI* | *Effic.* | *ln(1-EPS)* | *NDVI* | *Effic.* | *ln(1-EPS)* | *NDVI* | *Effic.* |
| 539 | 0.852** | 0.013 | 0.884** | 0.958** | 0.305 | 0.841** | 0.066 | 0.298 | 0.008 |
| 550 | 0.945** | 0.058 | 0.931** | 0.914** | 0.308 | 0.910** | 0.236 | 0.093 | 0.346 |
| 559 | 0.823** | 0.149 | 0.885** | 0.869** | 0.271 | 0.918** | 0.578* | 0.627* | 0.659* |
| 570 | 0.659* | 0.316 | 0.709* | 0.837** | 0.365 | 0.872** | 0.806** | 0.933** | 0.760** |
| 579 | 0.141 | 0.413 | 0.260 | 0.704** | 0.489 | 0.817* | 0.736* | 0.951** | 0.666* |
| 591 | 0.146 | 0.426 | 0.047 | 0.638* | 0.570* | 0.753* | 0.687* | 0.964** | 0.594* |
| 600 | 0.177 | 0.546 | 0.073 | 0.612* | 0.563* | 0.731* | 0.677* | 0.970** | 0.576* |
| 611 | 0.125 | 0.712* | 0.082 | 0.536* | 0.607* | 0.689* | 0.615* | 0.949** | 0.506 |
| 620 | 0.212 | 0.642* | 0.151 | 0.546* | 0.601* | 0.669* | 0.630* | 0.955** | 0.523 |
| 629 | 0.375 | 0.513 | 0.272 | 0.477 | 0.558* | 0.664* | 0.656* | 0.964** | 0.557 |
| 641 | 0.263 | 0.578* | 0.203 | 0.442 | 0.598* | 0.616 | 0.615* | 0.951** | 0.508 |
| 650 | 0.480 | 0.388 | 0.406 | 0.421 | 0.646* | 0.564 | 0.616* | 0.949** | 0.498 |
| 661 | 0.445 | 0.419 | 0.371 | 0.345 | 0.667* | 0.523 | 0.573* | 0.931** | 0.464 |
| 670 | 0.416 | 0.457 | 0.329 | 0.237 | 0.651* | 0.399 | 0.521 | 0.908** | 0.411 |

[a] Significance levels are indicated by superscripts: * $\alpha = 0.05$ and ** $\alpha = 0.01$. Data for diurnal course experiments.

in cases of widely varying canopy structure, variation in the PRI may not be solely attributable to physiological factors.

Unlike the PRI, NDVI failed to track the diurnal changes in EPS and photosynthetic efficiency in either the control or nitrogen-stress treatments (Fig. 7D). The slight noontime NDVI dip in the nitrogen-stress canopy may have been caused by increased midday soil illumination in this nearly closed canopy. In the water-stress treatment, with its low LAI, distinct row structure, and midday leaf wilting, the diurnal variation in NDVI (Fig. 7D) was largely due to changes in radiance from illuminated soil. In this treatment,

NDVI exhibited a weak correlation with photosynthetic efficiency and a slightly better correlation with EPS (Table 2 and Figs. 8c and d). These correlations probably do not represent a direct, physiological connection between NDVI and photosynthetic regulation, but reflect coincident changes in photosynthetic activity and soil illumination.

## Leaf-Level Measurements

To ensure that the PRI was not an artifact of canopy structure, sun angle changes or other non-physiological processes, we also made diurnal re-
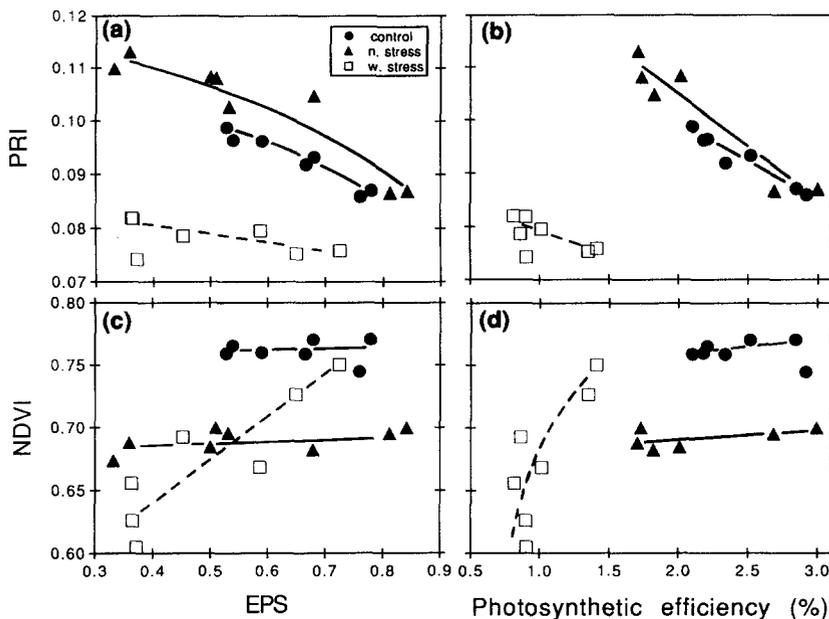


*Figure 8.* PRI $[(R_{550} - R_{531}) / (R_{550} + R_{531})]$ plotted against EPS (A) and photosynthetic efficiency (B). NDVI plotted against EPS (C) and photosynthetic efficiency (D).

flectance measurements on individual leaves. At the leaf level, diurnal changes in PRI roughly mirrored changes in EPS and photosynthetic efficiency (Fig. 9), further supporting the hypothesis that the PRI depicts physiological processes associated with photosynthetic regulation.

Although leaf spectra may exhibit diurnal changes in brightness and greenness (e.g., due to chloroplast movement), they lack the complications associated with interactive effects of sun angle and canopy structure. Thus, 550 nm would not necessarily be the most appropriate reference wavelength for a xanthophyll signal at the leaf level. In measurements of individual leaves, a slightly better correlation between the PRI and EPS or photosynthetic efficiency was obtained with reference wavelengths closer to 570 nm (data not shown).

*Figure 9.* Diurnal course of photosynthetic efficiency (A), EPS (B), and PRI $[(R_{550} - R_{531}) / (R_{550} + R_{531})]$ (C) for individual leaves of similar size and canopy position. A separate set of leaves was used for each assay. All photosynthetic efficiency values are means ($\pm 1$ SEM) of six leaves. EPS values represent single extractions on three combined samples, or the means ($\pm 1$ SEM) of three samples assayed separately (6:30 a.m. and 12:00 noon only). PRI values are the means of two to four leaves.
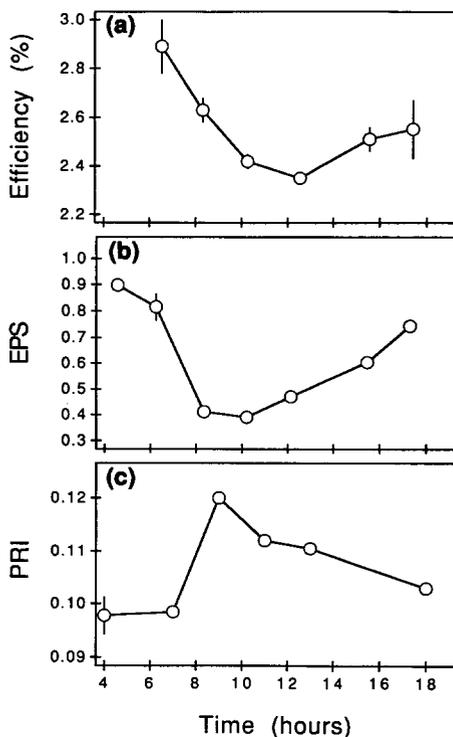


## CONCLUSION

In well-developed sunflower canopies, the physiological reflectance index (PRI), which is based on radiance or reflectance in only two narrow spectral bands, tracked the diurnal changes in photosynthetic activity, measured as changes in EPS or photosynthetic efficiency. However, a single formulation of this index was not readily applicable across all canopy types or in canopies of diurnally changing structure. For example, the PRI (using $R_{550}$ as a reference) did not work well in sunflower canopies under water stress where severe midday wilting and diurnal changes in soil illumination dominated dynamic reflectance features in the visible.

Because the PRI can reduce complications associated with changing sun angle, its best applications might be in monitoring diurnal photosynthetic regulation within a single, well-developed canopy. Alternate formulations of the PRI might lead to a more widely applicable photosynthetic index. Clearly, more work is needed to fully evaluate the utility of the 531 nm reflectance signal in a wide range of canopy types at differing spatial and temporal scales.

Although NDVI is widely used as an indicator of vegetation cover, it is often a poor indicator of real-time photosynthetic fluxes. Furthermore, although NDVI is very sensitive at low cover values, it tends to saturate at high leaf area indices (Sellers, 1987). The value of the PRI may lie in its ability to reveal short-term changes in actual photosynthetic efficiency, particularly in well-developed canopies where NDVI is least sensitive.

The potential applications for narrow-bandwidth reflectance in studies of vegetation physiology are rapidly expanding. NASA has a narrow-bandwidth spectroradiometer (AVIRIS) curently operational from aircraft (Curran and Dungan, 1990). It may be possible to extract similar image-based physiological indices from this new instrument. Other useful applications of the PRI might be in "close-up" remote sensing of leaves, canopies and landscapes. In combination with broad-band indices, narrow-band physiological vegetation indices could improve our ability to monitor vegetation function from the leaf to the landscape scale.

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