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Yellowness index: an application of spectral second derivatives to estimate chlorosis of leaves in stressed vegetation

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Abstract. This paper introduces yellowness index (YI) as a measure for chlorosis of leaves in stressed plants. YI provides a measure of the change in shape of the reflectance spectra between the maximum near 0.55 μm and the minimum near 0.65 μm. Quantitatively, YI is a simple, three-point approximation of the second derivative of the spectra, calculated using a finite divided difference approximation. YI is compared with two vegetation indices based on spectral changes in the region of the red edge, VI and NDVI. For manganese-deficient soybean leaves, YI, VI and NDVI were all closely related to leaf chlorophyll concentrations. YI is calculated from wavelengths in the visible that are thought to be less sensitive to changes in leaf structure or water content. Because YI is an approximation of a spectral second derivative, it should be less sensitive to atmospheric effects than many other vegetation indices. Finally, although the results reported here are promising, they are based on leaf-level observations and must be verified at the canopy level for remote sensing applications. An example of the correlation between NDVI and YI in AVIRIS data is given.

1. Introduction

A variety of spectral measures that relate to chlorophyll content or plant stress have been developed. As leaves become more chlorotic, reflectance increases and the reflectance peak normally centred at about 0.55 μm, broadens towards the red as absorption of incident light by chlorophyll decreases. These changes are perceived visually as a yellowing of the leaf. The earliest measures of chlorosis were derived from visible wavelengths only, and usually took the form of regressions of reflectance at selected wavelengths versus chlorophyll content of leaves (Benedict and Swidler

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1961, Thomas and Oerther 1972, Tsay *et al.* 1982). Kanemasu (1974) related the green/red reflectance ratio to chlorosis with some success.

Although the spectral changes in the visible are readily apparent in spectra of stressed vegetation (Baret *et al.* 1987, Adams *et al.* 1993), the effects are subtle compared with the changes in the red edge: the sharp increase in reflectance between the red and near infrared. The robustness and good signal-to-noise ratio of the red edge, in combination with its sensitivity to vegetation, seem to have provided some of the impetus for the development of vegetative indices based on features of the red edge in preference to the visible wavelength range. The availability of broad-band satellite imagery also contributed to the preference for red edge-based vegetation indices.

The red edge is produced by the combination of strong absorption by chlorophyll in the red region and strong reflectance in the IR due to scattering in the leaf mesophyll and the absence of absorption by pigments (Woolley 1971, Gausman 1985). The resulting contrast in reflectance is unique to vegetation reflectance. It is also the most prominent feature in the reflectance spectrum of vegetation and is responsive to stress and relatively insensitive to noise, a great advantage in terms of aircraft and satellite-based remote sensing. The infrared–red contrast had been well documented by photointerpreters using colour infrared photography (Bawden 1933, Murtha 1978), and was quickly adapted for use with digital imagery when that became available (Tucker 1979).

The more commonly used spectral measures of vegetation, which rely on the region of the red edge, have been used to estimate vegetative biomass, productivity, leaf area index, photosynthetic activity or chlorophyll content (e.g. Jordan 1969, Tucker 1979). The most common of these measures are the various 'vegetation indices'. The simplest is the vegetation index (VI), sometimes called the ratio vegetation index (RVI), which is a ratio of the infrared to red radiation (Jordan 1969, Tucker 1979). Several variations have emerged over the years, notably, the perpendicular vegetation index (PVI) proposed by Richardson and Wiegand (1977), the greeness index of Kauth and Thomas (1976), and the soil adjusted vegetation index (SAVI) of Huete (1988), all of which were introduced to better account for background reflectance from soil. Another measure, the normalized difference vegetation index (NDVI), was proposed by Rouse *et al.* (1974) to minimize the sensitivity to noise. Although superficially different, there appears to be little functional difference among most of these indices (Perry and Lautenschlager 1984).

Two of the most widely used red edge measures are the vegetation index (VI) ($\mathrm{VI} = \mathrm{IR}/\mathrm{Red}$) and the normalized difference vegetation index (NDVI) ($\mathrm{NDVI} = (\mathrm{IR} - \mathrm{Red})/(\mathrm{IR} + \mathrm{Red})$).

In stressed vegetation the absorption efficiency of the chlorophyll decreases and the IR reflectance decreases due to changes in the cell structure of the plant. This leads to a reduction in reflectance in the IR simultaneous with an increase in reflectance in the red. A ratio of the red band to the IR band enhances the sensitivity to stress relative to ratios based on visible bands only, by capitalizing on the opposing response in red and IR bands. Spectral ratios tend to eliminate differences that are due only to illumination variations and provide a more stable measure of the vegetation type as well as a more reliable indicator of stress. VI and NDVI have been applied to broad band measurements as well as narrow band measurements The bands may be chosen to emphasize changes in amplitude of the signal.

Demetriades-Shah *et al.* (1990) noted that, while spectral ratios (e.g. VI and NDVI) reduce the soil contribution in canopy spectral measures, they do not eliminate it. The same can be said for the position of the red edge, which is determined from first derivative spectra. Demetriades-Shah *et al.* (1990) further noted that changes in leaf chlorophyll content (i.e. a change in leaf colour) could be misinterpreted as low cover or low leaf area by canopy VI.

The use of derivative spectra, primarily first derivative spectra, was proposed as a means of removing sources of variability associated with broad band ratios, and to provide more sensitive measures of stress than broad band measurements (Horler *et al.* 1983). Demetriades-Shah *et al.* (1990) proposed the use of second derivative spectra in canopy measurements since second derivative spectra essentially eliminate the effects of soil background, while first derivative spectra do not. A mathematical development of the second derivative and its benefits in canopy remote sensing are given in Li *et al.* (1993), who point out that the second derivative is insensitive to typical soil reflectance. Spectral derivatives are also relatively insensitive to atmospheric effects (Philpot 1991). For example, spectral variations due to Rayleigh scattering (Intensity$\propto \lambda^4$) should be completely eliminated by using the fourth derivative.

Second derivatives are typically computed from high resolution spectra on a point-by-point basis using as many points around the central wavelength as are required for the particular order derivative being computed (e.g. two points are needed for a first derivative, three points for the second, four points for the third, etc.). We believe this to be a rather limiting case. There is no reason, *per se*, why a second derivative could not be taken utilizing points further away from the central wavelength to provide a more integrated measure of spectral curvature. This is, in effect, what Li *et al.* (1993) did in calculating second derivatives from the four broad spectral bands of the Exotech Model 100-A. The choice of points could reasonably depend on the size of the feature one is trying to examine rather than on the resolution of the instrument.

In this paper our primary objective is to estimate leaf colour/chlorosis of nutrient-stressed leaves using a prototype second derivative measure calculated by finite divided difference (Chapra and Canale 1988, Philpot 1991) rather than the more common 'local' derivative method. We also discuss the potential of this type of measure in stress detection and its potential uses in estimating other quantities of interest to remote sensers.

2. Materials and methods

Soybean seedlings (*Glycine max*, cv 'Bragg') were grown hydroponically in 6 l of aerated nutrient solution in an environmentally controlled growth chamber under a mixture of fluorescent and incandescent lighting with 16 hour days (24°C) and 8 hour nights (20°C). Light intensity at plant tops was 680 μmol m^{-2} s^{-1}.

The plant culture solution composition is described in the second experiment of Adams *et al.* (1993). Four treatments with three replications were imposed by adding aliquots of a stock MnCDTA (Mn cyclohexanediaminetetraacetate) solution to give final total concentrations of 0.1, 0.3, 1.0 and 3.0 μMMn^{2+}, respectively, in solution. The treatment levels were chosen to supply insufficient to adequate levels of Mn to the plants. One of the effects of growing soybean in low Mn solutions is the development of mottled, interveinal chlorosis on younger leaves (Grundon 1987). The four treatments yielded leaves with chlorophyll concentrations varying from approximately 5 to 30 μg cm^{-2}. Other details related to plant culture are given in Adams *et al.* (1993).

Plants were harvested 15, 19 and 24 days after seed imbibition. Just prior to harvest, a small (1.71 cm diameter) leaf disc was cut from the centre leaflet on selected trifoliolate leaves at a point on the centre-line, roughly three-quarters of the way from the base to the tip. Chlorophyll was extracted in the dark from the discs using dimethylformamide. Total chlorophyll (tchl) was determined by the method of Inskeep and Bloom (1985) and expressed as tchl cm^{-2} of leaf surface. All other harvested tissue was analysed for Mn and other mineral elements by inductively coupled argon-plasma emission spectrophotometry.

Reflectance of the centre leaflet from two or three different trifoliolates from each replicate pot was measured prior to each harvest using a Spectron† SE590 spectroradiometer referenced against an 18% Kodak† Gray Card. The Spectron SE590 is a compact, portable spectroradiometer that uses a diffraction grating to disperse the incoming light (400 nm–1100 nm) onto a silicon photodiode array. A full spectrum is collected in less than a second. Although there are 256 spectral bands recorded with 2.8 nm separating each sample, the true bandwidth for any sample is about 8 nm. Radiometric sensitivity was poorest in the blue, but was quite good through the rest of the spectrum. Since reflectance relative to a reflectance standard was the object of the measurements, the SE590 was not absolutely calibrated. The instrument automatically measures the dark current, detects the maximum radiance and selects the optimal integration time. The SE590 was found to be stable over time and measurements were repeatable to within about 2% of full scale.

Reflectance was measured on leaves still attached to the plants under artificial light (a mixture of fluorescent and incandescent lamps). The growth chamber provided essentially diffuse illumination and the observations were made at nadir. Leaves were placed flat on a piece of black matte foil mounted in an apparatus that positioned the surface of the leaf 9 cm away from the optics of the spectroradiometer. Reflectance of the black matte foil was approximately 2% and variations in reflectance with wavelengths measured by the Spectron were +/− 1% (data not shown). The field-of-view of the spectroradiometer at 9 cm was 4.41 cm^2. The reflectance spectra were imported into a spreadsheet program for further analysis.

Three parameters were derived from the reflectance spectra: the vegetation index (VI), the normalized difference vegetation index (NDVI) and the yellowness index (YI is described below). The wavelengths used to calculate VI and NDVI were 0.667 μm (red) and 0.774 μm (infrared) (Wessman *et al.* 1993). Prior to computing the parameters, each of the reflectance spectra was passed through a mean value smoothing filter using a 5×1 template (i.e. a five-point moving average). A 5×1 template was chosen because it was narrow enough to minimize damping of features of interest in the spectra and wide enough to remove a good deal of the noise, particularly in the near-infrared. The effective bandwidth is approximately 0.015 μm. Data obtained from the harvest on day 24 are used in this paper.

2.1. *Derivation of yellowness index*

The yellowish appearance of chlorotic leaves is one of the most obvious visual indications of plant stresses that reduce chlorophyll concentrations. Chlorosis is distinctive enough to be easily recognized and consistent enough to serve as a

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† Proprietary or brand names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

semiquantitative visual index of the severity of nutritional and other disorders in crops (Weiss 1943). Chlorosis and yellowness have been known for many years to be related to chlorophyll content (Weiss 1943, Benedict and Swidler 1961).

Gates *et al.* (1965) presented data that showed that the slope of the reflectance spectra of white oak (*Quercus alba*), between the local maximum at 0.55 μm (yellow-green) and the local minimum at 0.68 μm (red), changed as a function of leaf age. The change was presumably due to changes in chlorophyll concentration. Baret *et al.* (1987) defined a measure they called 'red slope' based on the observations of Gates *et al.* (1965), which calculated the slope of the reflectance spectra between two points, 0.58 and 0.66 μm. Although Baret *et al.* (1987) did not relate 'red slope' to leaf chlorophyll content directly, they did relate it to NDVI and the proportion of yellow senescing leaves in the wheat canopy, both of which are influenced by leaf chlorophyll concentrations. Demetriades-Shah *et al.* (1990) proposed several canopy spectral indices, including a second derivative index centred at 0.636 μm that was correlated with leaf chlorophyll content. This measure was calculated using a 'local' derivative method.

As has been noted by other researchers, we observed increases in visible reflectance of chlorotic leaves. In particular, we noted that the increased reflectance at orange-red wavelengths (*ca* 0.62 μm) relative to reflectance at yellow-green wavelengths (*ca* 0.55 μm) were associated with the yellowish appearance of chlorotic leaves. We have also noticed this feature in spectra obtained from chlorotic leaves of plants exposed to other nutrient deficiencies. This shift in reflectance is supported qualitatively in figure 1, which shows reflectance spectra from 0.40 to 0.80 μm from the same trifoliolate leaf from each of the four Mn treatments. The most notable change in reflectance between 0.55 and 0.65 μm, outside of magnitude changes, was a change in the shape of the reflectance spectra from concave up for Mn-sufficient leaves to concave down for Mn-deficient leaves. This change in the shape of the spectra from leaves of differing chlorophyll content prompted our development of the yellowness index. Our mathematical description of yellowness is basically the

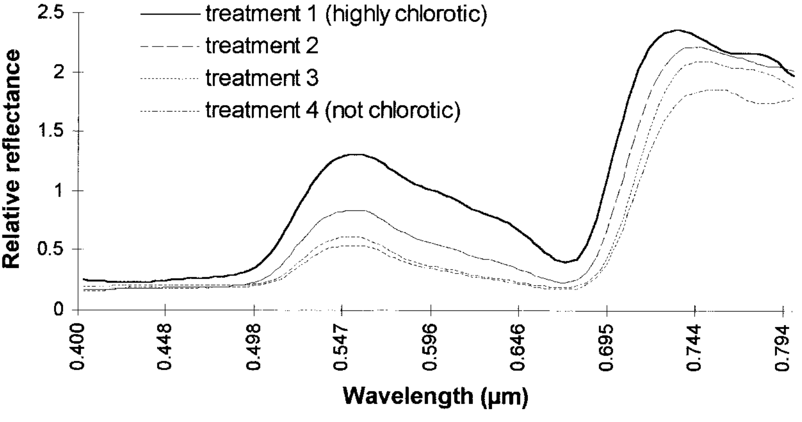


Figure 1. Reflectance spectra from one leaf from each of four Mn treatments. The change in the shape of the reflectance spectrum between 0.55 and 0.65 μm changes from concave up to concave down with increasing stress (Mn deficiency).

centre divided difference finite approximation of the second derivative of the reflectance spectrum (Philpot 1991):

$$YI \propto \frac{R(\lambda_{-1}) - 2R(\lambda_0) + R(\lambda_{+1})}{\Delta\lambda^2} \cong \frac{d^2 R}{d\lambda^2} \tag{1}$$

where $R(\lambda_0)$ is the reflectance at the central waveband, $R(\lambda_{-1})$ and $R(\lambda_{+1})$ are the lower and higher wavelength wavebands, and $\Delta\lambda$ is the spectral distance between wavebands ($\Delta\lambda = \lambda_0 - \lambda_{-1} = \lambda_{+1} - \lambda_0$). This is a gross measure of the spectral shape in the green-red region.

Our choice of wavelengths was based empirically on the spectral changes noted in figure 1. The wavelengths were selected so that the band separation ($\Delta\lambda$) was as large as possible while keeping all three wavebands between the reflectance maximum at *ca* 0.55 μm and the minimum at *ca* 0.68 μm. For the data obtained in this experiment, $R(\lambda_0)$ was centred at 0.624 μm, $R(\lambda_{-1})$ was centred at 0.580 μm, and $R(\lambda_{+1})$ was centred at 0.668 μm. Thus, $\Delta\lambda$ was 0.044 μm. This is only one of many possible combinations of wavebands that can be used. Preliminary work to determine optimal λ_0 and $\Delta\lambda$ for calculating YI for this dataset has shown that the relationship between YI – calculated with different λ_0 and $\Delta\lambda$ other than 0.624 μm and 0.044 μm, respectively – and tchl can vary widely from very weak ($R^2 \approx 0.10$) to closely related ($R^2 \approx 0.96$) (data not shown). The λ_0 and $\Delta\lambda$ selected for this study represent one of the better combinations of wavebands in terms of a strong relationship to tchl (see below).

The yellowness index was calculated as 10^{-1} times the negative of the finite approximation of the second derivative to reduce the range of yellowness index values (when $\Delta\lambda$ is expressed in μm) and to represent increasing yellowness by increasingly positive values. Unlike some vegetation indices, YI is not dimensionless. Its units are relative reflectance units μm^{-2} (RRU μm^{-2}). The magnitude of YI is sensitive to the band separation, $\Delta\lambda$. Should this method prove to be a useful approach, normalization may be advisable in order to remove the sensitivity to $\Delta\lambda$.

2.2. *AVIRIS data*

NDVI and YI were computed for an AVIRIS scene of Jasper Ridge, California, collected on 2 June 1992. YI was computed using equation (1). Each value was the average of three adjacent AVIRIS bands with λ_0 centred at 0.626 μm, λ_{-1} centred at 0.577 μm and λ_{+1} centred at 0.666 μm. An average band separation of 0.044 μm was used for $\Delta\lambda$. NDVI was computed using the two 3-band averages centred on 0.744 μm and 0.666 μm.

3. Results

3.1. *Application to leaf level data*

The relationship between yellowness index (YI) and total chlorophyll in $\mu g\ cm^{-2}$ (hereafter abbreviated tchl) for trifoliolates 1, 2 and 3 (i.e. the first, second and third trifoliolates to emerge) measured on day 24 is nearly linear between tchl concentrations of 5 and 15 $\mu g\ cm^{-2}$ (figure 2). YI falls to a steady value of -3.3 to -4.0 RRU μm^{-2} for green leaves with tchl concentrations above 23 $\mu g\ cm^{-2}$.

The relationships of VI and NDVI to tchl cm^{-2} are shown in figure 3(*a*) and (*b*), respectively. VI varies nearly linearly with increasing tchl cm^{-2}, within the range of tchl cm^{-2} measured in this experiment. The relationship, however, is better described by a low power exponential function. The relationship of NDVI to tchl cm^{-2} is

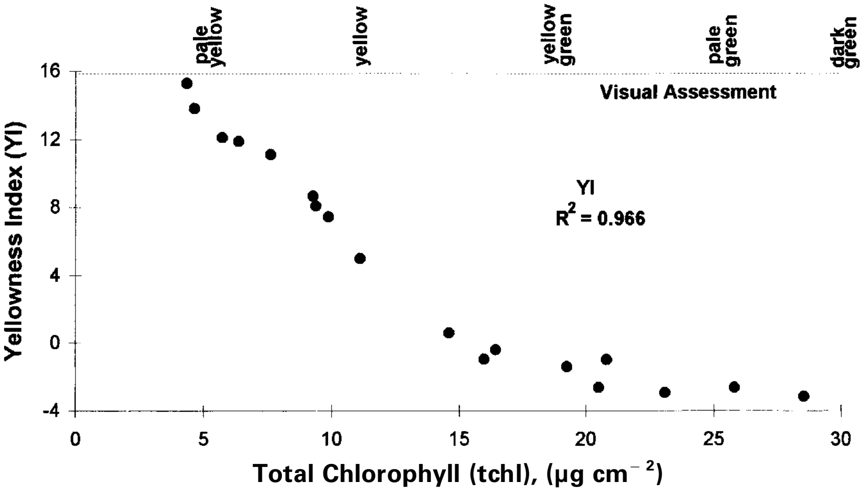
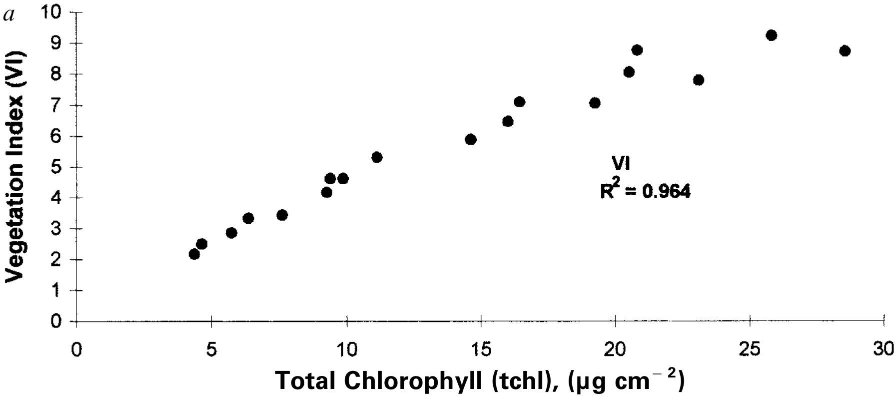


Figure 2. The effect of tchl cm^{-2} on the yellowness index (data taken from trifoliolates 1, 2 and 3 harvested on day 24).



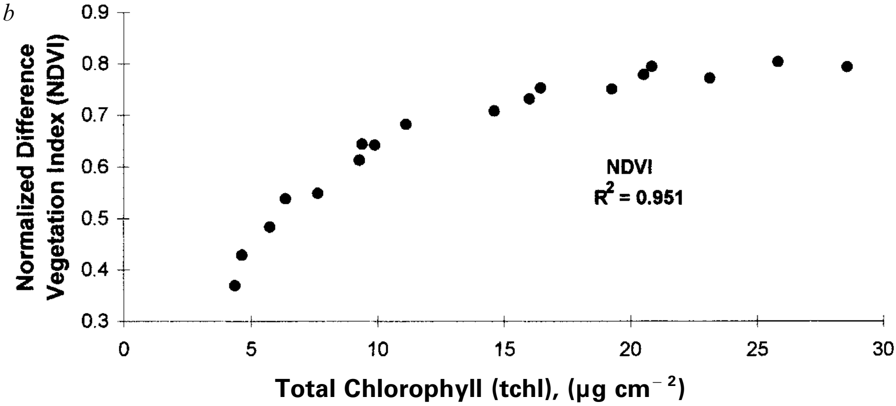


Figure 3. The effect of tchl cm^{-2} on (*a*) VI and (*b*) NDVI (data taken from trifoliolates 1, 2 and 3 harvested on day 24).

clearly curvilinear with increasing tchl, and approaches a maximum value of about 0.83 at about 28 μg cm^{-2}.

All three indices show a strong relationship to tchl cm^{-2} (See figures 2 and 3). Each index was fitted by linear regression to ln(tchl cm^{-2}). Goodness of fit was measured by coefficient of determination, R^2. All three characteristics fitted well with R^2 of 0.966, 0.964 and 0.951 for YI, VI and NDVI, respectively.

For tchl concentrations less than 20 μg cm^{-2}, NDVI showed the highest overall coefficient of determination and had the lowest noise of the three indices. VI was also highly related to tchl, and was also the only measure sensitive to tchl at the highest levels measured in this experiment. Although YI had an R^2 that was marginally higher than the other two indices, the coefficients of determination for the three measures were comparable.

3.2. *Application to AVIRIS data*

It remains to be demonstrated that YI will be an effective measure when using satellite or aircraft data. Lacking the appropriate ground data at this point, it is only possible to compare YI and other vegetation indices. In particular, since both YI and NDVI are highly correlated with total chlorophyll in the laboratory experiments, they should also be correlated with one another. This is, in fact, the case for the laboratory data (figure 4), which show a strong negative relationship between YI and NDVI. If YI is to be useful for remote sensing, one would anticipate the same relationship to be apparent in satellite and aircraft data. To test this, NDVI and YI were computed for an AVIRIS scene of Jasper Ridge, California collected on 2 June 1992 (figure 5(*a*) and (*b*), respectively). The scattergram showing the relationship between YI and NDVI for this scene is shown in figure 6.

YI and NDVI are most strongly correlated over the range of values corresponding to the most highly vegetated regions: $1.5 < \text{YI} < 3.0$; $0.1 < \text{NDVI} < 0.4$. Although the correlation is obvious, there is also a great deal of dispersion of the data about the correlation axis. This is most obvious for high values of NDVI. The dark areas in figure 7 correspond to values of NDVI$>$ 0.4. For these values of NDVI, YI appears to be distinctly bimodal (figure 6): $0.5 < \text{YI} < 1.5$; and $1.5 < \text{YI} < 2.5$. The images corresponding to these two ranges for YI, given NDVI$>$ 0.4 are shown in figure 8(*a*)

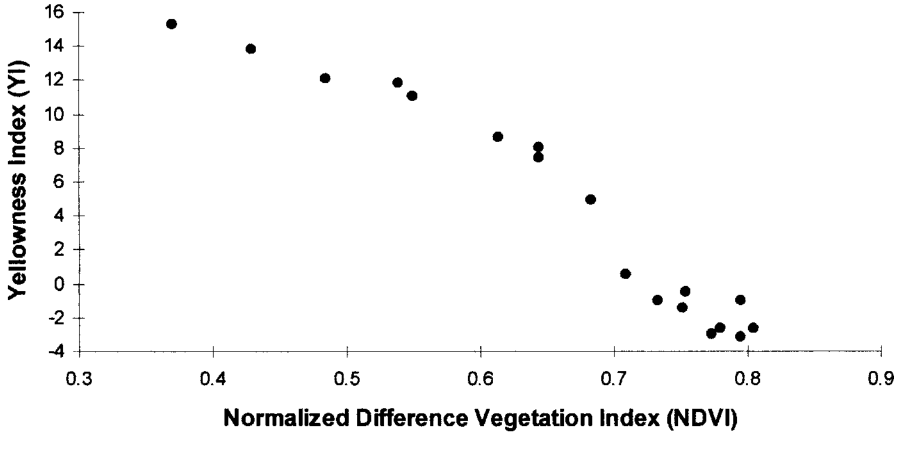
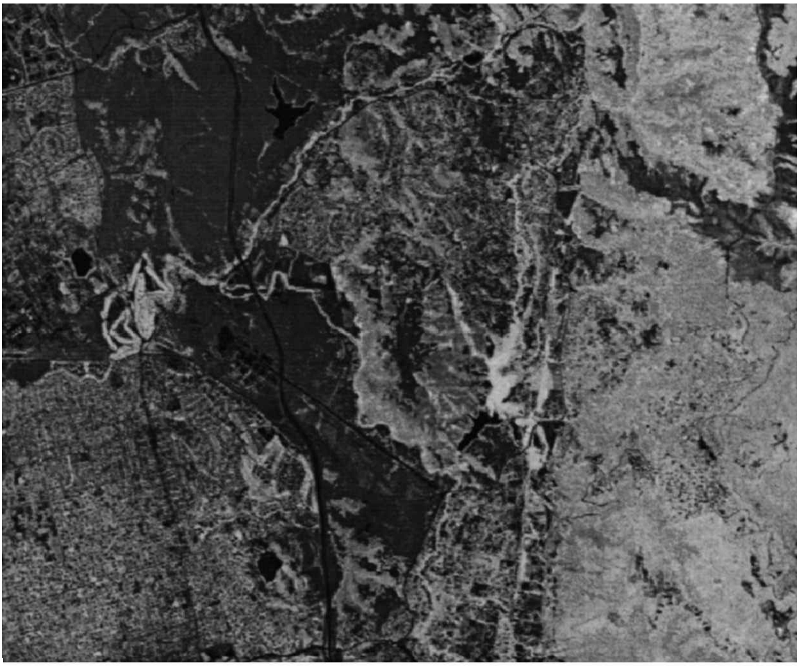
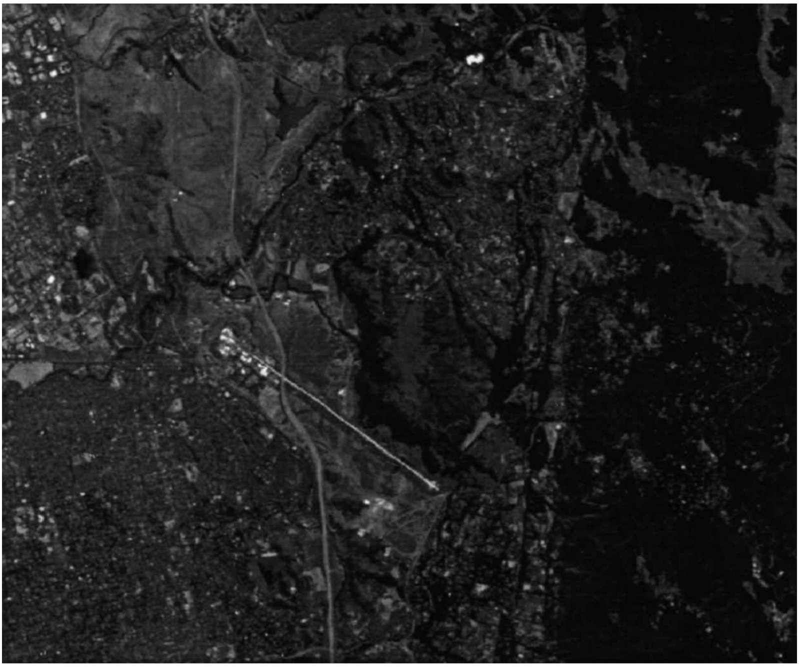


Figure 4. The relationship between YI and NDVI for the data taken from trifolioliates 1, 2 and 3 harvested on day 24.



(*a*)



(*b*)

Figure 5. (*a*) NDVI and (*b*) YI images for the AVIRIS scene of Jasper Ridge, 2 June 1992.

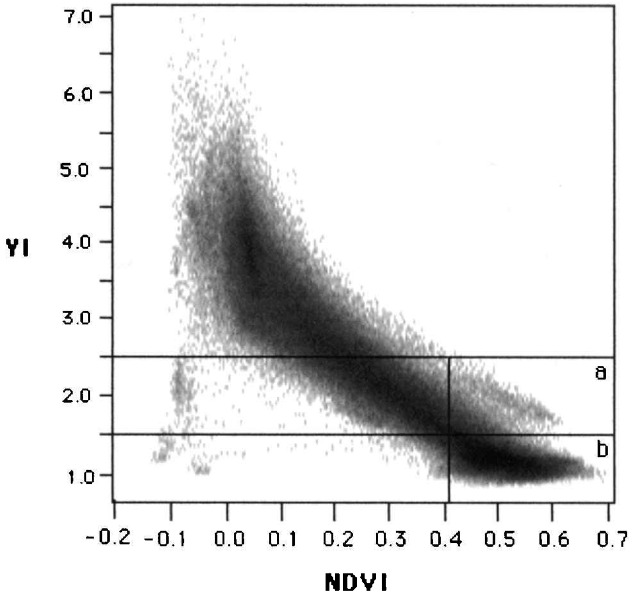


Figure 6. Scattergram illustrating the relationship between the yellowness index (YI) and the normalized difference vegetation index (NDVI) for the AVIRIS scene of Jasper Ridge, 2 June 1992. The rectangle marked 'a' represents areas that would be more chlorotic than areas marked 'b'.

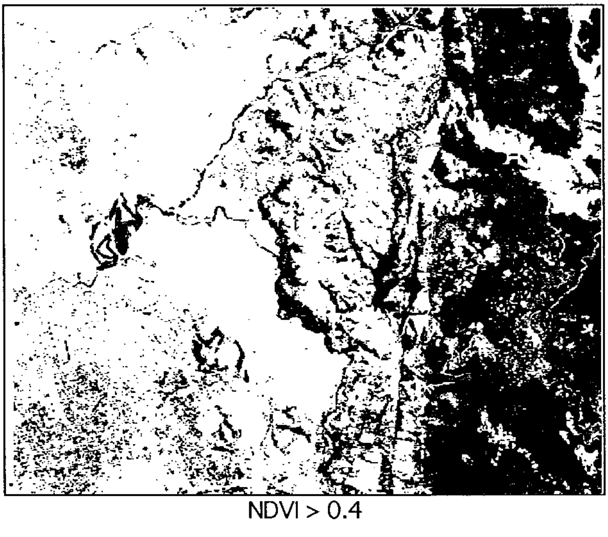
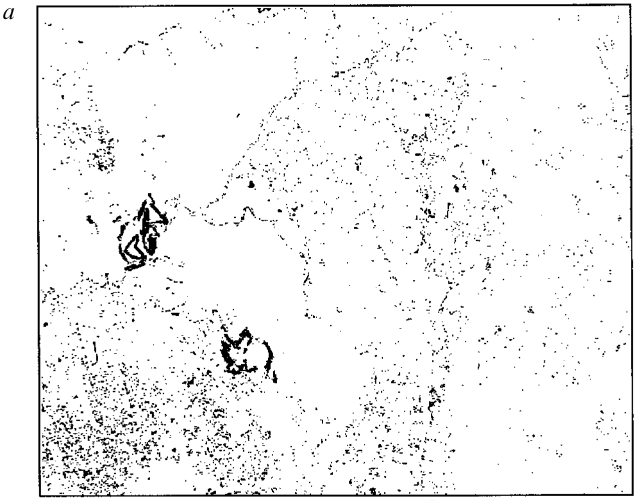
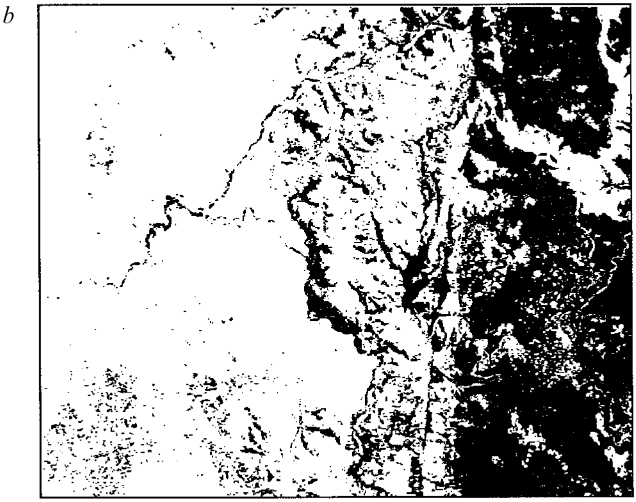


Figure 7. The Jasper Ridge image showing areas (dark) for which NDVI> 0.4.

and (*b*). Dark areas in figure 8(*a*) corresponds to YI values that would indicate vegetation with a more yellow appearance while dark areas in figure 8(*b*) would correspond to greener vegetation. This discrimination is not possible with NDVI alone and suggests that YI contains unique information and is not simply an alternate version of NDVI.



NDVI > 0.4; 1.5 < YI < 2.5



NDVI > 0.4; 0.5 < YI < 1.5

Figure 8. The Jasper Ridge image showing areas (dark) for which NDVI> 0.4 and (*a*) 1.5< YI< 2.5 (corresponds to rectangle 'a' in figure 6), (*b*) 0.5< YI< 1.5 (corresponds to rectangle 'b' in figure 6).

4. Discussion

Red edge measures, such as VI and NDVI, are obviously sensitive to chlorophyll concentrations, but they are also sensitive to changes in cell structure. Since changes in cell structure drive the changes in IR reflectance, it is possible that red edge measures may change independently of pigmentation. YI, on the other hand, should be sensitive principally to pigment concentration and, thus, should not be much affected by changes in cell structure. A similar suggestion was made by Gamon *et al.* (1992), who proposed an index derived from reflectance changes in the visible, the physiological reflectance index (PRI). They showed that PRI was independent of changes in the infrared reflectance over short time periods. The fact that YI, VI and

NDVI are highly related to tchl in this laboratory experiment arises, in part, because NIR reflectance was correlated positively with visible reflectance.

There are several advantages to the yellowness index as a measure of chlorosis. First, YI provides a measure of stress that is a function primarily of changes in pigment absorption, with chlorophyll being the primary pigment driving the change. YI should be a sensitive measure of leaf chlorosis, because YI is sensitive to chlorophyll in a range of chlorophyll concentrations where changes in the visual colour of leaves occur. This characteristic should make YI a useful measure of nutrient deficiencies (as we have shown for manganese (Adams *et al.* 1993)). Second, unlike red edge measures, YI is calculated from wavelengths that are not affected by leaf structure (Maas and Dunlap 1989), and should also be unaffected by leaf water content (Bowman 1989). Thus, when the measurement of chlorosis is the key feature of interest, YI could be used in place of or in addition to the red edge measures. Third, the use of YI in combination with other indices creates the potential to distinguish between responses to stress that are purely structural and those that also involve pigment concentration. The differences between YI and NDVI in the AVIRIS images are an indication that the two measures are not simply redundant but contain significantly different information. Fourth, YI, as an approximation of a second derivative, should be less affected by atmospheric or soil effects than VI or NDVI (Demetriades-Shah *et al.* 1990, Philpot 1991, Li *et al.* 1993). Thus, YI has the potential to be a more consistent measure over a scene with a varying atmosphere or when comparing scenes at different times or at different geographic locations.

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