DEVELOPMENT OF ROBUST HYPERSPECTRAL INDICES FOR THE DETECTION OF DEVIATIONS OF NORMAL PLANT STATE

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ABSTRACT

This research was conducted to assess the potential of hyperspectral indices to detect iron deficiency in capital-intensive multi-annual crop systems. A well-defined hyperspectral multi-layer dataset was constructed for a peach orchard in Zaragoza, Spain, consisting of hyperspectral measurements at various monitoring levels (leaf, crown, airborne). Trees were subjected to four different treatments of iron application (0 g / tree, 60 g / tree, 90 g / tree, and 120 g / tree). Ground-based measurements were used to characterise the on-site peach (*Prunus persica* L.) orchard in terms of chlorophyll, dry matter, water content, and leaf area index (*LAI*). Indices were extracted from the spectral profiles by means of band reduction techniques based on logistic regression and narrow-waveband ratioing involving all possible two-band combinations. Physiological interpretations extracted from leaf-level experiments were extrapolated to crown- and airborne level. It was concluded from leaf level measurements that a decrease in leaf chlorophyll concentration resulted due to iron deficiency. The results suggested that spectral bands and narrow waveband ratio vegetation indices, selected via multivariate logistic regression classification, were able to distinguish iron untreated and iron treated trees (*C*-values>0.8). Moreover, the most appropriate indices obtained in this manner fulfilled the expectations by being highly correlated (R^2>0.6) to the measured chlorophyll concentrations. The visible part of the spectrum, mostly dominated by the amount of pigments (e.g. chlorophyll, carotenoids), provided the most discriminative spectral region (505 - 740 nm) in this study. The discriminatory performance of a combined chlorophyll and soil-adjusted vegetation index was compared to the results of the selected vegetation indices to estimate the effects of soil background and *LAI* on those indices.

Keywords: vegetation hyperspectral indices, normal plant state, iron deficiency.

INTRODUCTION

The potential yield of capital-intensive multi-annual crops (e.g., fruit orchards) is seldom realised in reality. Reductions in yield and fruit quality can be caused by physiogenic aspects, pathogens (biotic stress), abiotic stress (e.g., extreme temperature, dryness, high salinities), and improperly managed vegetative production systems (1,2). However, a targeted monitoring and modelling of growth processes in such agricultural production systems potentially could enable early detection and treatment of production limiting factors. Non-destructive techniques, furthermore, are essential for capturing data in a continuous manner, thereby enabling rapid response and minimised unintentional impacts. Remote sensing provides an excellent tool to capture the structure and physiological status of a plant using reflectance patterns (3). The incident energy of the sun is partly reflected, transmitted, and absorbed by plant material. The amount of reflected light depends on a number of leaf-related factors, such as external morphology, internal structure, concentration, and internal distribution of biochemical components, etc. Stress-induced physiological changes could affect these factors, thereby making stress detection at the leaf-level possible at an early stage of sub-optimal photosynthetic functioning. Any opportunity to monitor these factors in an ongoing or periodic remote sensing framework offers the potential to model plant production processes, and therefore also to steer the process by means of adapted management measures.

Carter (4) examined the wavelengths at which leaf reflectance was generally most responsive to stress. He found that the reflectance at visible wavelengths increased consistently due to decreased absorption by pigments with stressed leaves for eight stress agents and among six vascular plant species. The visible reflectance seemed to be most sensitive to stress in the 535-640 nm and 685-700 nm wavelength ranges. The infrared reflectance was comparatively unresponsive to stress, but increased at 1400-2500 nm with severe leaf dehydration and the accompanying decreased water absorption. Similar results were obtained in other studies investigating plant stress (5,6,7,8). The induced iron stress in this study was chosen to validate those findings, because iron catalyses the production of chlorophyll, and varying amounts of iron application should therefore be detectable in the visible region according to the findings of Carter (4). One should note, however, that the airborne hyperspectral images in this study will be more difficult to interpret due to soil background effects, Bidirectional Reflectance Distribution Function (*BRDF*) effects, and atmospheric effects which scatter light and result in an alteration of the vegetative reflectance spectrum.

Even though hyperspectral data can be beneficial to various applications, data volume and dimensionality cause both technical (storage capacity, CPU time for processing, data transfer, etc.) and statistical challenges. Hence, the development and optimisation of band reduction techniques and vegetation indices using only a limited amount of data are of utmost importance. We therefore attempted to generate indices in this study that enable effective detection and quantification of anomalies in the “normal” plant production process at leaf, crown, and airborne level.

The study objectives can be summarised as (i) assessment of the utility of hyperspectral data at the leaf, crown, and airborne level for plant stress detection, (ii) evaluation of the potential to track spectral changes due to differences in biophysical indicators of iron stress, such as leaf chlorophyll $a+b$ (C_{a+b}), dry matter (C_m), water (C_w), and leaf area index (*LAI*), and (iii) to test and generate hyperspectral indices to model the changes of those specific biochemical constituents caused by iron stress.

METHODS

Study area

The peach orchard used for data collection was located in Zaragoza, Spain (41°46’ N, 1°37’ E). A total of 48 trees from 205 were selected for leaf and crown measurements. Those selected peach trees were treated in blocks of 3 trees with different amounts of iron chelate applications (Sequestrene), varying between 0 g / tree, 60 g / tree, 90 g / tree, and 120 g / tree.

Leaf level measurements

Leaf hyperspectral reflectance data were collected for a total of 716 leaves in July 2005 using a plant probe attached to a portable FieldSpec Pro spectroradiometer (9) (Analytical Spectral Devices Inc., Boulder, USA) with a spectral range of 350 - 2500 nm. The sampling interval across the 350 - 1050 nm range is 1.4 nm with a spectral resolution of 3 nm. The sampling interval and the spectral resolution are approximately 2 nm and 10 nm, respectively, for the 1050 - 2500 nm range. A Spectralon (ASDI, Boulder, USA) white reference panel was used to adjust the sensitivity of the instrument detector according to the specific illumination conditions and for reflectance derivation.

Laboratory reflectance and transmittance measurements were collected for a total of 155 leaf samples using a Li-Cor 1800-12 Integrating Sphere (Li-Cor 1800-12S, Li-COR, Inc., Lincoln, Nebraska, USA) coupled to a portable FieldSpec JR spectroradiometer (Analytical Spectral Devices Inc., Boulder, USA) with a spectral range of 350 - 2500 nm. While the sampling interval and spectral resolution across the 350 - 1050 nm are similar to that of the FieldSpec Pro model, the values for the 1050 - 2500 nm range are approximately 2 nm and 30 nm, respectively. Both spectroradiometers were warmed up for 90 minutes prior to measurement to avoid spectral steps at the detector overlap wavelength regions, which occur due to different warm-up rates for the three spectroradiometer arrays. Resultant data were interpolated by the ASDI software to produce values at each nanometer interval.

Chlorophyll was measured for each leaf with a SPAD-502 Minolta Chlorophyll Meter and mean values were calculated per tree to compare with parameters obtained at the crown level. The SPAD measurements were calibrated to obtain chlorophyll concentration by comparing SPAD readings with concentrations derived from destructive chemical analysis in the laboratory for a subset of leaf samples. The result is shown in Figure 1. A coefficient of determination of 0.86 was obtained.

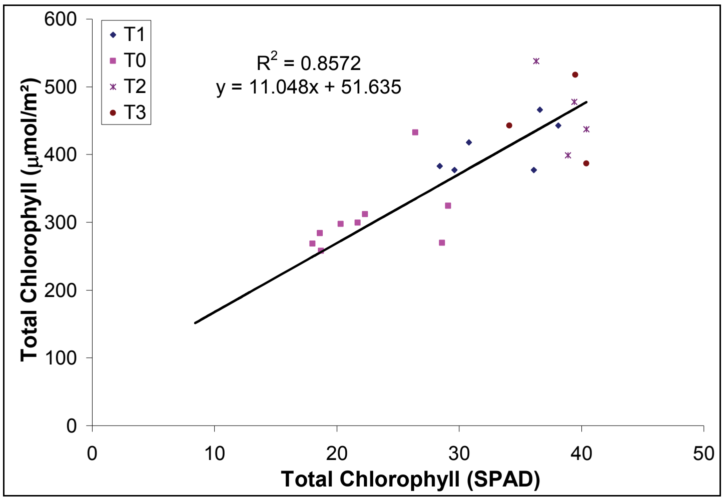

Figure 1: Calibration of the field SPAD values using C_{ab} concentrations derived from chemical analysis in the laboratory. Data of leaves over the whole range of iron chelate applications were taken into account: T0 = 0 g / tree, T1 = 60 g / tree, T2 = 90 g / tree, T3 = 120 g / tree.

Crown level measurements

Crown level hyperspectral measurements were gathered from 48 selected peach trees with the portable FieldSpec JR spectroradiometer specified above. Measurements were collected from a cherry picker, allowing an approximate height of 3.60 m above the trees. A footprint diameter of 159.6 cm was obtained, given a field of view of 25°. Each group of three trees was treated with differing amounts of Sequestrene, resulting in 12 trees without iron application, 12 trees with 60 g / tree, 12 trees with 90 g / tree, and another 12 trees with 120 g Sequestrene / tree.

Leaf area index was estimated for 10 trees with an LAI-2000 (Licor Inc., Nebraska) instrument in addition to the spectral data. The LAI-2000 is a portable instrument that is able to provide immediate LAI estimates by measuring diffuse radiation by means of a fisheye light sensor. The calculations for LAI were performed according to Villalobos et al. (10).

Airborne level measurements

Airborne image data were acquired with the AHS -160 sensor (63 bands; 450 – 2500 nm) on July 12, 2005, under cloud free conditions. Image data were processed by the Flemish Institute for Technological Research using in-house developed software (11) described by Kempeneers et al. (12). The ground resolution of 2.5 m was similar to the average crown diameter, which complicated the tree identification in the image. The peach trees were planted with a row spacing of four metres (8/5 pixels). Image data were processed to tree level by resampling to a resolution of 0.5 m (nearest neighbour). Any given tree, therefore, was covered by a region of interest (ROI) with an integer number of (sub) pixels. Furthermore, the sub-pixels contain exactly the same spectral information as the original pixels due to the use of nearest neighbour resampling. However, a ROI could potentially contain sub-pixels of neighbouring trees due to a combination of imperfect tree positioning, overlapping ROIs, and artifacts due to resampling.

Vegetation indices

A logistic regression-based band selection technique (13) was used due to the non-normality of the data to select the wavebands that were most suitable to detect the spectral changes caused by the application of different amounts of iron chelates. Single wavebands can constitute acceptable indicators of biochemical constituents, but are subject to variability caused by environmental factors, e.g., solar angle and background scattering. Vegetation indices also result in reduction of data dimensionality and therefore might be valuable in terms of data processing and analysis. Moreover, they are able to surpass the limitations of single bands by minimising external factors, resulting in improved correlations with vegetative biochemical constituents. Most of the classical vegetation indices developed to detect stress in plants are based on chlorophyll (14,15,16,17,18,19,6,20) and water content (21,22).

The Normalised Difference Vegetation Index (*NDVI*) (Eq. 1) is probably the most studied vegetation index in literature. It makes use of the characteristic features of vegetative reflectance spectra $R(\lambda)$, namely low reflectance in the red part of the spectrum due to chlorophyll absorption and high reflectance values in the near infrared domain, due to intra-cellular structure.

$$NDVI = \frac{R(NIR) - R(RED)}{R(NIR) + R(RED} \tag{1}$$

However, the most relevant information on the physiological status of a plant is not necessarily related to these two regions. Furthermore, the *NDVI* often is not a good indicator of stress as it is precise for Leaf Area Index (*LAI*), biomass, and chlorophyll determination only at relatively low levels of those factors (23,19). The concept of this vegetation index, however, is potentially useful for the derivation of novel standardised indices with the potential for improved estimation of biophysical parameters. The inclusion of a shortwave infrared (SWIR) spectral band can, for example, provide useful complementary information on the moisture status of the leaf/canopy. Therefore, an attempt was made to identify the most optimal combination of two distinct spectral features. A standardised difference of the measured reflectance values R was calculated for each possible combination of two different wavelengths λ_1 and λ_2 (Eq. 2). This standardised difference was then used as independent variable in a logistic regression analysis. This approach allowed for the selection of an optimal standardised difference vegetation index, as well as being a tool to test existing vegetation indices.

$$SDVI = \frac{R(\lambda_2) - R(\lambda_1)}{R(\lambda_2) + R(\lambda_1)} \tag{2}$$

The same analysis was performed on randomly selected datasets to ensure that the process output remained insensitive to a variation in inputs. These datasets were randomly chosen subsamples from the original dataset.

Soil background and *LAI* have a considerable impact on the vegetation spectrum, making it difficult to use vegetation indices at all sensing levels (leaf, crown, airborne). Much attention has been paid to increasing the robustness of biochemical parameter estimation over the last number of years (24,25,15,26). The influence of the soil background on the spectrum necessitated researchers to develop new soil adjusted indices such as *SAVI* (27) and *TSAVI* (28). Rondeaux et al. (29) developed the "optimised soil adjusted vegetation index", *OSAVI*, based on the near infrared and red reflectance properties of soil and vegetation. Haboudane *et al.* (30) proposed a combination of the chlorophyll index *TCARI* (Transformed Chlorophyll Absorption Ratio Index) (Eq. 4) and *OSAVI* (Eq. 5) to retrieve leaf chlorophyll at canopy level, thereby taking into account the effects of non-photosynthetic materials and *LAI* (Eq. 3). Acceptable results were obtained in that study based on simulated data and including a wide range of leaf area index values. The use of this index therefore was also taken into account in this study, due to the fact that iron stress causes chlorosis, i.e., chlorophyll degradation.

$$Chl = -30.605 \ln \frac{TCARI}{OSAVI} \tag{3}$$

$$TCARI = 3\left((R_{700} - R_{670}) - 0.2(R_{700} - R_{570})\frac{R_{700}}{R_{670}} \right) \quad (4)$$

$$OSAVI = (1 + 0.16)\frac{R_{800} - R_{670}}{R_{800} + R_{670} + 0.16} \quad (5)$$

One potential drawback of developed indices is that band locations and bandwidths vary with sensors. The nearest central wavelengths available in the AHS sensor, presented in Table1, were therefore used to test selected indices.

Table 1: Suggested bands by Haboudane and available band in AHS sensor

Suggested band (Haboudane)	Available band (AHS)	Bandwidth
550 nm	542 nm	28 nm
670 nm	659 nm	28 nm
700 nm	718 nm	28 nm
800 nm	804 nm	28 nm

Discriminatory performance

The ability of the indices to discriminate between spectra of trees with different amounts of iron treatments is represented by *C*-index values. This *C*-index is identical to a widely used measure of diagnostic discrimination, the area under the ‘Receiver-Operator Characteristic’ (ROC) (31). ROC plots are created by plotting the sensitivity values, the true positive fraction (i.e. iron treated tree correctly classified as iron treated) against 1-specificity, the false-positive fraction (i.e. iron untreated tree classified as iron treated tree). A curve that maximises sensitivity for low values of the false-positive fraction is considered a good model and is quantified by calculating the area under the curve (*C*-index). The *C*-index is an interpretable and objective statistical measure to evaluate and compare the discriminatory performance of the different wavelengths or linear combinations of them, and has values usually ranging from 0.5 (random) to 1.0 (perfect discrimination) but can have values below this range indicating a model that is worse than random (32). Values above 0.8 are generally accepted to represent significant discriminative models (33). Wavelengths with *C*-values above 0.8, therefore, were selected in this study due to their ability to discriminate between spectral values of infected and non-infected vegetation.

RESULTS AND DISCUSSION

The most obvious symptom of plants affected by *Fe* deficiency is leaf chlorosis (13). This was confirmed through visual analysis of the measured vegetation spectra. An overview of the spectral signatures collected by the spectroradiometer at leaf (a) and crown levels (b) is given in Figure 2. Blue lines represent spectra measured in trees treated with 60 g of Sequestrene (*Fe*-chelate), while red spectra were obtained by measuring trees lacking *Fe* treatment. Considerable overlap was found in the spectra of these two treatments, but there was a clear dissimilarity observable in the visible part of the spectrum (more specifically between 500 - 700 nm), mostly dominated by pigment concentrations (34). The iron deficient trees seemed to reflect more energy in this spectral domain compared to the iron treated trees, indicating a reduction in pigments available to absorb the energy in these wavelengths for the first case.

This statement was also statistically verified as shown in Figure 3. Results of the per-wavelength logistic regression technique (35) are illustrated here. Based on the fact that *C*-values above 0.8 represent significant discriminative performance between two treatments, it was concluded that a lack of iron applications resulted in differences in the spectral domain between 500 nm and 750 nm. No *C*-index values above 0.8 were obtained for the distinction between 60 g, 90 g and 120 g iron per tree, implying that iron stress is only detectable in extreme circumstances when using single bands of hyperspectral imagery or, alternatively, that no iron stress was present when a minimum of 60 g Sequestrene per tree was applied.

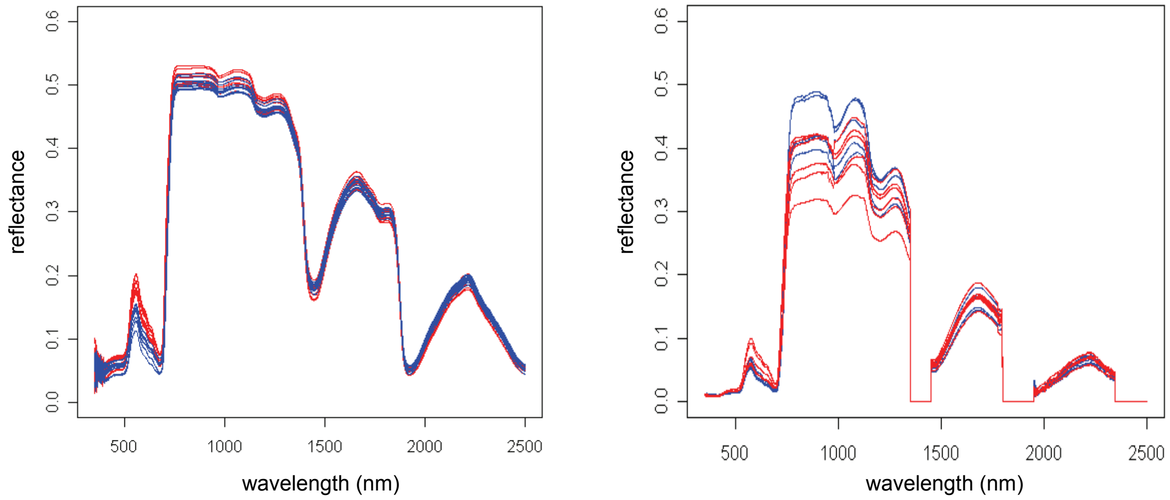

Figure 2: (a) Spectra obtained by measuring leaf reflectance of iron treated trees (blue) and non treated trees (red) and (b) spectra obtained by measuring top of canopy reflectance of iron treated trees (blue) and non treated trees (red).

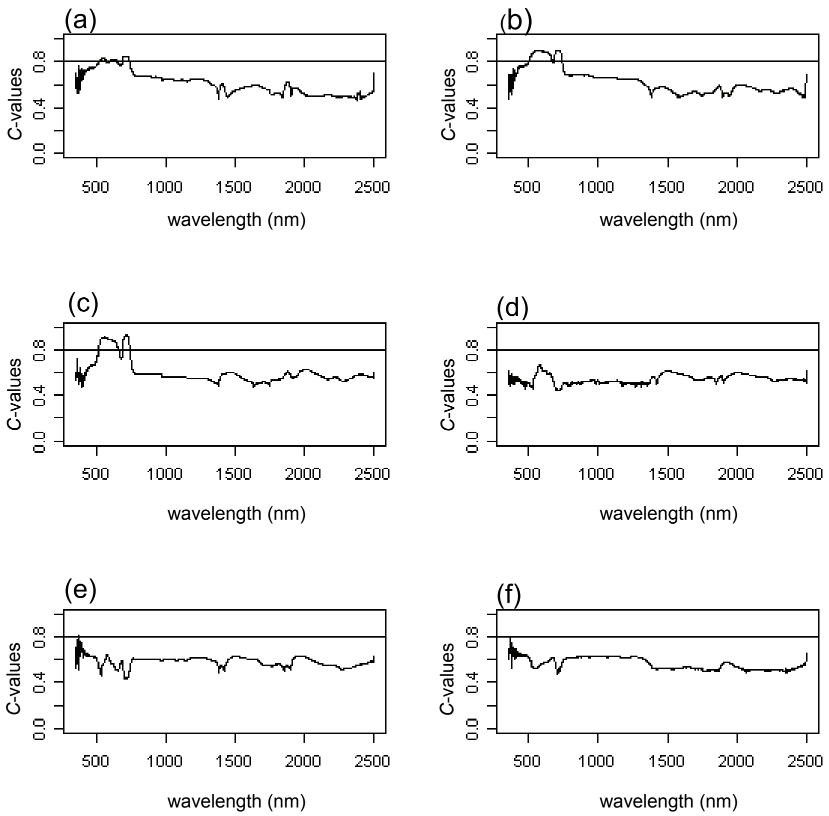

Figure 3: Discriminatory performance of the per-wavelength logistic regression to discriminate between leaf spectra of trees treated with a different amount of Sequestrene: (a) 0 g – 60 g, (b) 0 g - 90 g, (c) 0 g - 120 g, (d) 60 g - 90 g (e) 60 g – 120 g, and (f) 90 g - 120 g .

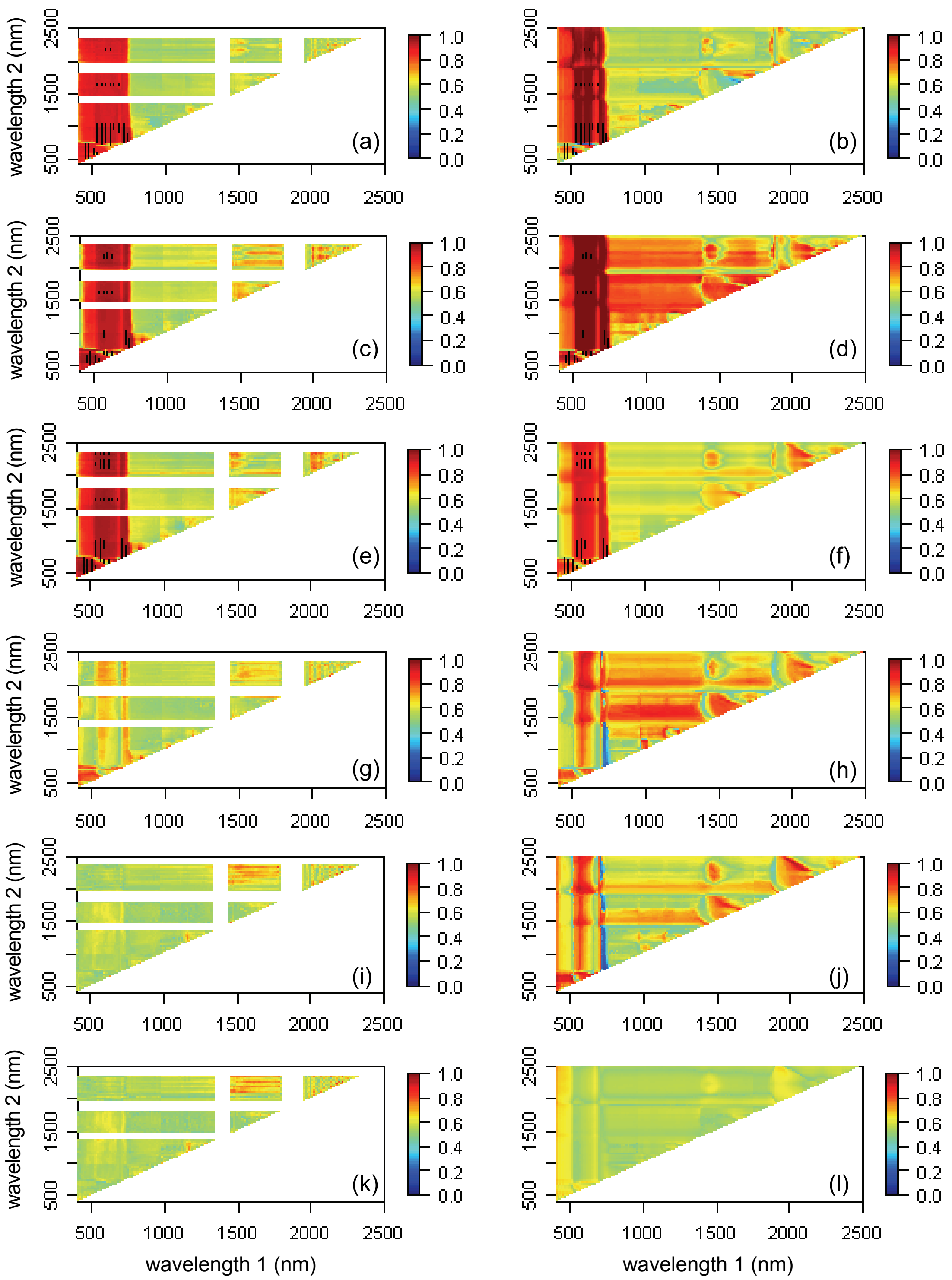

Figure 4: Discriminatory performance (C-value) of logistic regression models with standardised vegetation indices as independent variables at crown (left), leaf (right), and airborne (black dots) level. Images (a) & (b), (c) & (d), (e) & (f), (g) & (h), (i) & (j), and (k) & (l) respectively illustrate the discriminatory performances for trees treated with 0 g and 60 g, 0 g and 90 g, 0 g and 120 g, 60 g and 90 g, 90 g and 120 g, and 60 g and 120 g Sequestrene.

The investigation into vegetation indices corroborated these findings, as shown in Figure 4. The three dimensional graphs shown in Figure 4 represent the discriminatory performance (*C*-value) of logistic regression models at leaf (left), crown (right) and airborne (black dots) level. The *x*- and *y*-axes represent wavelength 1 and wavelength 2, respectively (Eq. 2), while the third dimension represents the *C*-index value visualised via colour-coding. *C*-index values of 0.8 and greater are indicative of good discrimination. All results are shown for leaf and crown level, while for airborne level, due to the limited number of wavebands, only indices with *C*-values above 0.8 were plotted as black dots in each graph. Images (a) and (b) represent the discriminatory performance of the logistic regression model as binary response variables trees lacking any form of iron application and trees treated with 60 g of Sequestrene at leaf and crown level, respectively. Images (c) & (d), (e) & (f), (g) & (h), (i) & (j), and (k) & (l), respectively, illustrate the discriminatory performances of all possible *SDVI*'s for the cases with 0 g and 90 g, 0 g and 120 g, 60 g and 90 g, 90 g and 120 g, and 60 g and 120 g Sequestrene added per tree.

It has been reported in literature that biochemical parameters such as chlorophyll content, water content, and dry matter content can be estimated from hyperspectral imagery via spectral indices (36,37). Figure 5 represents the R^2-values of a simple linear regression between all possible standardised vegetation indices (see Eq.2) and measured (a) chlorophyll, (b) water, and (c) dry matter contents to evaluate relationships between these biochemical parameters and iron deficiency.

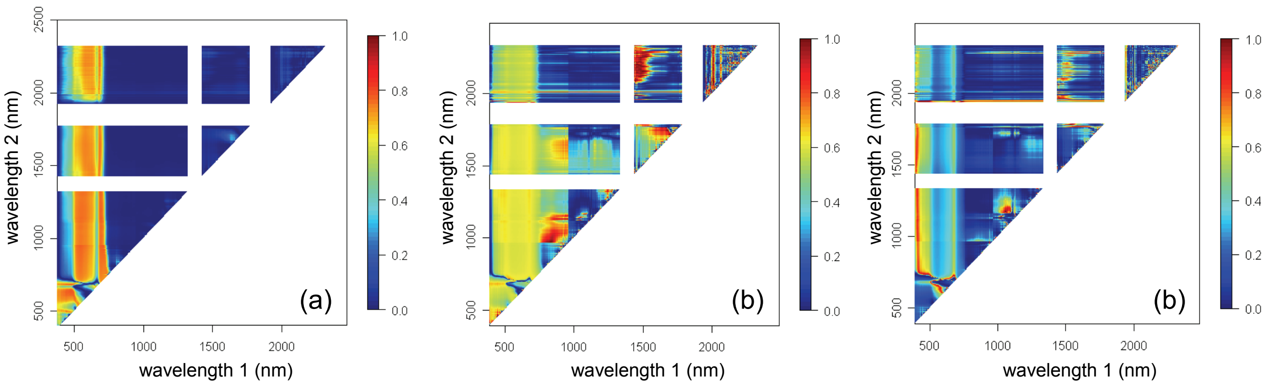

Figure 5: R^2-values of a simple linear regression between all possible standardised vegetation indices and averaged (a) chlorophyll, (b) water, and (c) dry matter content for each tree. Spectral regions with atmospheric disturbances (e.g., water absorption bands) were removed from the image.

From Figures 4 and 5 it can be deduced that the most useful vegetation indices to detect iron stress are those that are closely related to chlorophyll concentration (R^2>0.6, Figure 5), which emphasises the conclusion that relates iron deficiency to chlorosis. Reduced correlations were found between indices predicting iron deficiency and those related to measured water content (R^2 ~0.6), and even lower correlations to dry matter content (R^2~0.4).

The 571 nm waveband was selected from 63 AHS wavebands, as the most useful band when combined with a NIR (948 nm, 975 nm, 1004 nm) or SWIR (1622 nm, 2140 nm, 2152 nm, 2175 nm) waveband for the extraction of chlorophyll amount or merely to discriminate between iron treated and iron untreated trees at leaf, crown, and airborne levels (Table 2). The discriminatory performances of the vegetation indices were lower at crown level than at leaf level, which was attributed to the interaction of background effects. Therefore, the discriminatory performance of the combined index TCARI/OSAVI (Figure 6), which is insensitive to LAI variations (from 0.5 to 8) and minimises soil effects on the crown reflectance (25), was compared to the results of above-mentioned vegetation indices to estimate the effects of soil background and LAI on those indices. The discriminative power of single wavebands and standardised differenced vegetation indices was, however, deemed satisfactory for the detection of iron chlorosis in this study.

TCARI/OSAVI was shown to be a suitable index at leaf level (*C* = 1) and crown level (*C* = 0.93), but exhibited reduced discriminative power at airborne level (*C* = 0.76), since only *C*-values above 0.8 are assumed to represent an acceptable discriminatory performance.

The SDVI based on wavelengths 571 nm and 1622 nm was found to perform the best for predicting iron deficiency at all levels, although the statistical significance between differences was not evaluated. Reduced LAI and soil background effects were found for these indices since *C*-values at crown and airborne level were still relatively high.

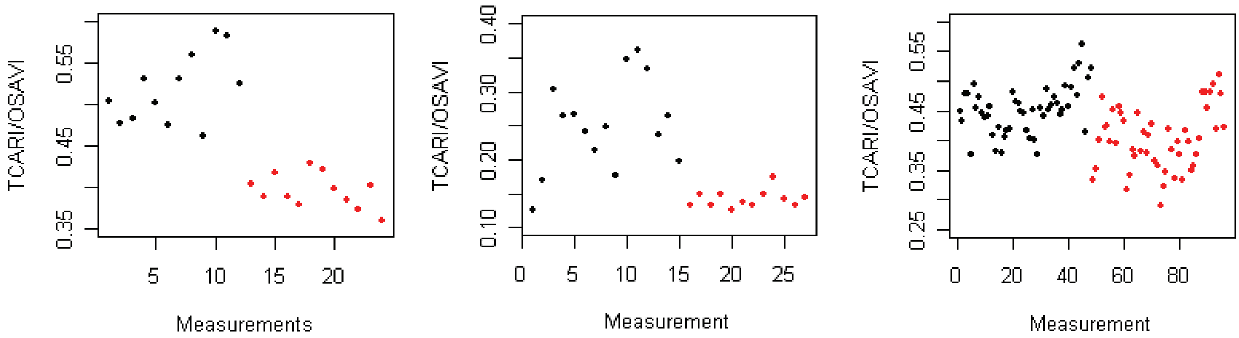

Figure 6: Index values of a combined chlorophyll and soil adjusted vegetation index (TCARI/OSAVI) at each monitoring level (left: leaf level, middle: crown level, right: airborne level). Black dots represent measurements of trees with iron chlorosis while red dots represent measurements of trees with 60 g Sequestrene added per tree.

Table 2: Wavelength combinations which performed best when discriminating between iron treated (e.g. in case 60 g / tree) and iron untreated trees, compared to the TCARI/OSAVI index performance for each monitoring level (leaf, crown and airborne). Index performances are given by C-index values, while biophysical correlations are shown using R^2-values of simple linear regressions.

***C*-Index / R^2**	**Leaf**	**Crown**	**Airborne**	C_{a+b}	C_w	C_m
TCARI/OSAVI	1.00	0.93	0.76	0.73	0.55	0.24
SDVI (571 nm, 948 nm)	1.00	0.94	0.80	0.77	0.59	0.28
SDVI (571 nm, 975 nm)	1.00	0.94	0.81	0.77	0.59	0.28
SDVI (571 nm, 1004 nm)	1.00	0.93	0.81	0.75	0.61	0.29
SDVI (571 nm, 1622 nm)	**1.00**	**0.94**	**0.89**	**0.70**	**0.60**	**0.27**
SDVI (571 nm, 2140 nm)	0.97	0.94	0.83	0.62	0.57	0.23
SDVI (571 nm, 2152 nm)	0.98	0.94	0.82	0.65	0.56	0.22
SDVI (571 nm, 2175 nm)	0.98	0.95	0.84	0.66	0.54	0.21

CONCLUSIONS

This study explored the potential of hyperspectral reflectance data to differentiate between iron deficiency and healthy peach trees at leaf, crown, and airborne level. Logistic regression was used to identify wavelengths or wavelength ratios that best differentiated among the iron treated and untreated trees within the peach orchard. The most appropriate vegetation indices to detect iron stress were found to be closely related to chlorophyll concentration (R^2>0.6, Figure 5), which corroborated the conclusion that iron deficiency is one of the causes of leaf chlorosis. Reduced correlations were found between indices predicting iron deficiency and those related to measured water content (R^2 ~0.6) and dry matter content (R^2~0.4). The combination of two wavebands in a standardised differenced vegetation index format resulted in higher discriminatory power than single wavebands. Waveband 571 nm was selected as the most useful band when combined with a NIR (948 nm, 975 nm, 1004 nm) or SWIR (1622 nm, 2140 nm, 2152 nm, 2175 nm) waveband for application at all monitoring levels. Such waveband combinations proved useful for the extraction of the chlorophyll amount or merely to discriminate between iron treated and iron untreated trees. Tree LAI values were generally high in this study, resulting in minor effects of soil background on crown spectra.

The results suggested that the detection of iron chlorosis using hyperspectral remote sensing has significant potential. This is of importance to the agricultural market, e.g., where an early warning system based on spectral inputs would be an ideal solution to the enforced reduction of pesticide or fertilisation use. Vegetation indices could be easily incorporated in process models, thereby negating the need to collect full-range spectral datasets for monitoring vegetation production systems. Farmers subsequently only have to apply specific chemicals when and where biotic abnormalities are detected in the normal growth pattern of crops. Future at-satellite measurements will enable managers to obtain frequent hyperspectral coverage of large areas, thereby making continuous monitoring of biotic stress possible in capital-intensive crop production systems.

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