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Detection and discrimination of oil and water deficit-induced stress in maize (*Zea mays* L.) using spectral and thermal responses

Ebele J. Emengini[1], George A. Blackburn[2], Julian C. Theobald[3]
[1]*Department of Surveying and Geoinformatics, Faculty of Environmental Sciences, Nnamdi Azikiwe University, P.M.B. 5025, Awka, Anambra State, Nigeria.*
[2]*Lancaster Environment Centre, Lancaster University, Lancaster, LA1 4YQ, UK*
[3]*Marlborough Winery, Pernod Ricard New Zealand, Blenheim, P.O. Box 331, New Zealand*

Abstract: *Oil pollution and water deficit are threat to the environment and can impose serious problems in crop yield and productivity. Early detection and discrimination between these stress agents are important to facilitate timely delivery of remedial measures. Remote sensing technology has this potential but there is poor understanding about the ability of the combined spectral and thermal information for early detection and discrimination between oil and water deficit-induced stress in plants. In order to understand this, in a glasshouse, pot grown maize was treated with oil, water deficit and combined oil and water deficit. Thereafter, leaf thermal, spectral and physiological measurements were taken every 2 to 3 days to monitor the development of stress responses. Our result showed that stress caused by oil pollution can be detected spectrally before visual stress symptoms are observed in maize but it was a poor indicator of water deficit stress. On the contrary, leaf absolute temperature can indicate water deficit stress prior to visual stress symptoms, although it may be difficult to discriminate between oil and water deficit stress using this measure. Based on our findings, we conclude that the combination of hyperspectral and thermal remote sensing has potential in the early detection and discrimination between oil and water deficit stress in maize.*

Keywords – *Oil pollution, Spectral reflectance, thermography, Water deficit, Maize.*

I. Introduction

Under field conditions, plants are exposed to a wide range of abiotic, biotic and anthropogenic stress inducing factors within the growth environment, which consequently alter their physiological and biochemical functioning. Thus, early detection of stress in plant is important to mitigate damage and enhance plant growth and productivity. In regions where oil is extracted and refined, plants are vulnerable to oil pollution due to leakages from pipelines and other facilities. For example, in developing countries such as Nigeria, where oil facilities like pipelines crisscross the country passing through different land surfaces such as swampy and dry terrain, oil pollution regularly affects subsistence crops and natural vegetation growing across a range of hydrological settings from wetlands through to arid environments. Hence, any single plant may be simultaneously exposed to both oil and water stress and we need a means of discriminating between the two.

Indeed, reflectance measurements can be useful for detecting a wide range of vegetation changes associated with various factors affecting plant growth and productivity. However, similar spectral responses result from different stress factors which make it difficult to discriminate between these factors. For example, Smith *et al*. (2005) found that in oilseed rape (*Brassica napus*), there was no difference between the spectral reflectance pattern of plants stressed via elevated concentration of natural gas and those stressed via herbicide application. Likewise, several other studies have suggested that it may not be possible to distinguish between different causes of stress using spectral remote sensing alone (Smith *et al*. 2005, Massoni *et al.* 1996, Carter, 1993).

Recent applications of thermal imaging techniques have shown that water stress can be detected through an increase in leaf temperature as a result of stomatal closure in response to soil drying during a water deficit (Jones 1999, Grant *et al.* 2006). Using such techniques, Olga *et al.* (2007) were able to distinguish between irrigated and non-irrigated grapevine canopies, and even between different deficit irrigation treatments. When leaf or canopy photosynthesis is compromised due to stress, stomatal conductance is expected to decrease because of a decrease in demand for atmospheric CO_2 (Farquhar and Sharkey 1982). If transpiration is restricted due to stomatal closure, leaf temperatures will increase (Nobel 1991, Pezeshki and DeLaune 1993) because of less cooling by transpired water as it evaporates from the leaf surfaces. Thus, changes in leaf temperature may occur as a direct effect of soil water deficit or as an indirect consequence of a decrease in photosynthesis that may result from a range of different types of stress.

Hence, while spectral and thermal sensing individually may be inadequate for discriminating the effects of different types of stress in plants, the combination of the two techniques may hold promise. Thus, this study explores the physiological and/or biochemical basis of spectral and thermal properties of maize for the early detection and discrimination between oil pollution and water deficit. Maize was chosen as model species because in developing countries where oil pollution is often a major problem affecting subsistence agriculture, maize is a common crop type and yields of maize are set to double and surpass that of wheat and rice by 2020 (Pingali 2001).

II. Materials and Methods

2.1 Plant material

Maize (*Zea mays* L.) was grown in a glasshouse (10 x 3m) under natural conditions in February 2009, at day and night temperatures of 26^{o}C (±2^{o}C) and 15^{o}C (±1^{o}C), respectively. A 12 h supplementary photoperiod (06.00 h to 18:00 h) was provided by Osram Plantastar 600W sodium lamps giving a photosynthetic photon flux density (PPFD) of 400 µmol m^{-2}s^{-1} at bench height. Two seedlings of maize (previously pre-germinated for three days on damp tissue paper in darkness) were sown per 2 litre pot containing a loam-based compost (John Innes No.2, J. Arthur Bowers, Lincoln, UK). Pots were placed on capillary matting and watered daily. After two weeks plants were thinned to one per pot and left to continue to establish. During this period, crops continued to be watered daily for a further three weeks, when forty well established plants were selected for treatment.

2.2 Plant treatments

Four treatments comprising ten replicates were established, namely: control, oil, water deficit and the combination of oil and water deficit. For the oil treatment, 15W/40 diesel engine oil (Unipart, Crawley, UK) was applied to the surface of the compost as a percentage volume of the water holding capacity (WHC) of the pot (field capacity minus oven dry), previously determined as 0.63g H_2O/g compost at a density of 0.8g cm^{-3}. Application rates were 20% of WHC, being equivalent to 96g oil/kg soil. Water deficit stress was induced by watering to 25% of the soil WHC on a daily basis. The control and oil treated plants were watered to 80% of soil WHC daily. During the experimental period, pot locations were randomized to minimize possible effects of microclimate differences in the glasshouse on plant development.

2.3 Physiological, thermal and spectral measurements

The seventh fully emerged maize leaf was chosen for all measurements which started on Day 0 immediately prior to treatment and then every 2 to 3 days thereafter. Rates of photosynthesis, transpiration and stomatal conductance were determined using a portable infrared gas analyser (CIRAS-2, PP Systems, Hitchin, UK) with leaf cuvette conditions set to track ambient glasshouse temperature, humidity and ambient CO_2 concentration (38.5 Pa), with a PPFD of 600 µmol m^{-2}s^{-1}, and a leaf equilibration time of 3 minutes prior to recording data. Plants were assessed for any visual signs of stress everyday. Leaf temperatures and reflectance were measured in a dark room immediately after physiological measurements using an SC2000 thermal camera (FLIR Systems, West Malling, UK) and ASD FieldSpec® Pro Spectroradiometer (Boulder, CO 80301 USA) respectively. Thermal measurements were made following the procedures of Grant *et al.* (2006). To fully illuminate the target, a 500W halogen lamp was mounted in a fixed position at nadir 70cm away from each leaf to be measured. For reflectance measurements, the ASD foreoptics were positioned at nadir, 6 cm above each leaf to be measured. An 18^o FOV was used which covered a sample area of leaf of approximately 2cm diameter. Leaves were shifted slightly between measurements to capture spectral variations along each leaf. The ASD sampling interval over the 350-1050 nm range was 1.4 nm with a spectral resolution of 3 nm. Over the 1050-2500 nm range the sampling interval was 2 nm and the spectral resolution between 10 and 12 nm. The instrument interpolated data points to give output reflectance values at 1 nm intervals. Ten spectral measurements were captured per leaf for each of the 10 replicates per treatment. Each leaf spectrum was paired with a spectrum obtained from a spectralon reference panel placed immediately above the leaf, to measure irradiance. Each leaf spectrum was divided by the corresponding reference spectrum in order to calculate a percentage reflectance spectrum, and a correction for the reflectance properties of the Spectralon panel was applied.

2.4 Measurements of leaf pigments and water content

After leaf thermal and spectral properties were measured, pigment concentrations of the same leaves were determined. Ten circular discs, each 10 mm in diameter (equivalent to 0.79 cm^2 leaf disk areas) were punched from five of the ten replicate leaves for each treatment. On the next day of measurements, discs were taken from the other five replicate leaves. The alternate disc collection sequence was maintained until the end of the experiment to ensure that any possible damage to the leaves was minimised. A pilot study confirmed that the disc sampling technique used did not produce any significant differences in physiology or remotely-sensed response compared to leaves where discs were not removed. Immediately after disc removal, five of the leaf samples were frozen at - 50^{o}C for later determination of pigment content. The rest of the samples were immediately weighed to determine fresh mass before they were dried at 80^{o}C until a constant dry mass was obtained. Leaf water content was calculated as the difference between leaf fresh and dry mass and expressed per unit leaf area.

For pigment content determination, the frozen samples were crushed in a few drops of methanol (100 percent pure solvent) with a pinch of calcium carbonate, to form a homogenous slurry. Pigments were extracted from the crushed samples by adding 5 ml of methanol solvent in a centrifuge tube. The tubes were placed in a refrigerator at < 5^{0}C overnight to ensure complete extraction before centrifuging to remove particulates. Three replicate extractions derived from each leaf disc were analysed using a Shimadzu UV mini 1240 UV-VIS spectrophotometer, with measurements of absorbance at 665.2 nm, 652.4 nm and 470 nm. Prior to measurements, blank samples of methanol were measured to calibrate the cuvettes for each wavelength. The analysis procedure was designed to minimise the completion time after removing each leaf sample from the freezer. Thus, the preparation and analysis procedure took approximately 10 minutes per sample, excluding the overnight extraction time. All procedures were carried out under low-light conditions in the laboratory in order to minimise photo-oxidation of pigments.

The concentrations of chlorophyll *a* (chl *a*), chlorophyll *b* (chl *b*), chlorophyll *a* + *b* (chl *a* + *b*) and carotenoids *x* + *c* (cars *x* + *c*) in μg/cm^2 were determined using the equations derived by Lichtenthaler (1987):

Chl $a = 16.72*A_{665.2}) - (9.16*A_{652.4})$ (1)
Chl $b = (34.09*A_{652.4}) - (15.28*A_{665.2})$ (2)
Chl $a + b = (1.44*A_{665.2}) + (24.93*A_{652.4})$ (3)
Cars $x + c = ((1000*A_{470})-(1.63*\text{chlorophyll } a)-(104.96*\text{chlorophyll } b))/221$ (4)

While equation 3 gives the concentration of total chlorophyll, i.e., the sum of chl *a* and chl *b* (hereafter referred to as total chlorophyll), equation 4 gives the concentration of total carotenoids, i.e., the sum of the xanthophylls and β-carotene (*x* + *c*). These equations gave pigment concentrations in micrograms per ml of extract which was converted to concentrations in micrograms per cm^2 of leaf.

2.5Data and statistical analysis

Physiological and biochemical measurements of treated plants were expressed as percentage of control on each measurement occasion in order to account for the effects on absolute values, of daily glasshouse variability in ambient temperature and humidity at the time of measurement. Sensitivity analysis was performed on the physiological and biochemical data using ANOVA to determine when significant responses to the different treatments occurred and whether these responses were consistent throughout the experiment. The average leaf temperatures were extracted from captured thermographs using a software package (ThermaCAM reporter), and thereafter the thermal index (I_G), which under any given environmental conditions is theoretically proportional to stomatal conductance (Jones 1999) was calculated as: $I_G = (T_{dry}-T_{leaf})/(T_{leaf} - T_{wet})$, where T_{dry} = dry references (^{o}C), T_{wet} = wet references (^{o}C), and T_{leaf} = temperature of leaf of interest (^{o}C).

In order to examine the spectral responses of plants to treatments, the differences between the mean reflectance spectra for control and treated plants were plotted in order to identify wavelengths of high variation. T-tests were performed on wavelengths of high variation to determine whether differences in spectral responses were significantly different between treatments. Correlograms were computed to determine the relationships between the measured physiological and biochemical parameters and reflectance in each individual ASD waveband. In order to develop optimal spectral indices, wavebands with the highest correlations were identified in addition to wavebands with minimum correlations. Based on previous studies and theoretical considerations, the sensitivity of a spectral index is improved when wavebands that are responsive to given physiological properties e.g. photosynthetic activities is referenced to nonresponsive wavelengths (Schepers *et al.* 1996). Thus, several simple and normalised difference ratios were developed based on this theory. However, in order not to overlook some other potentially valuable spectral indices, a range of existing spectral indices identified in the literature were also tested.

III. Results

3.1Visual observations

Stress symptoms were first visually observed in plants on day 8 for water deficit (alone) and the combined oil and water deficit treatments and on day 11 for oil pollution treatment (alone). Symptoms worsened with time and included leaf chlorosis, rolling and wilting (Fig. 1), the thinning of canopies and slower growth.

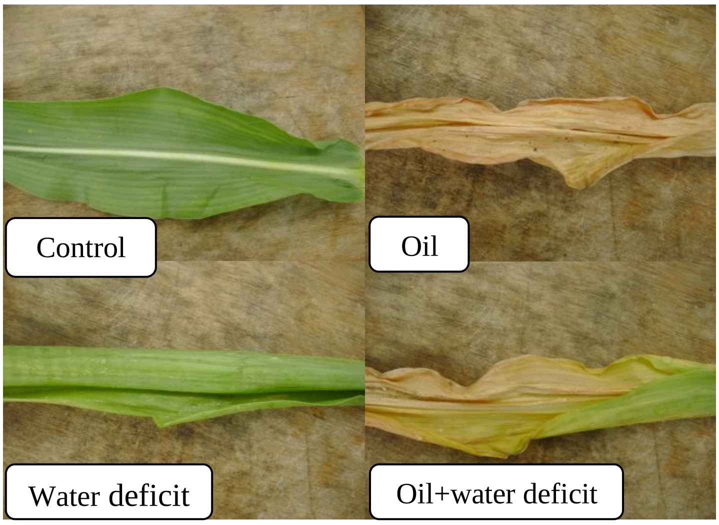

Figure 1. Visual stress symptoms in maize leaves caused by oil pollution, water deficit and combined oil and water deficit at the end of the experiment. No visual stress symptoms were observed in the controls.

3.2Photosynthesis, transpiration, and stomatal conductance

Treatments significantly decreased rates of photosynthesis; transpiration and stomatal conductance of maize (Fig. 2 (a - c)). Whenever water deficit was involved in the treatment, there is a significantly larger reduction in photosynthesis than for oil treatment. Thus, plants treated with water deficit and combined oil and water deficit showed the greatest reduction in photosynthesis, but there was no significant difference in photosynthesis between these two treatments throughout the experiment. Photosynthetic activity ceased on day 8 for the plants treated with water deficit and combined oil and water deficit (100%), while photosynthesis ended on day 18 for the plants treated with oil alone (100%). The rate of transpiration and stomatal conductance showed similar responses to photosynthetic activities. By the end of the experiment, there was a total reduction in transpiration rate of treated plants by 94%, 92% and 66% relative to the controls, for water deficit, the combined oil and water deficit, and oil pollution alone, respectively. For the stomatal conductance, there was a total reduction in stomatal conductance of treated plants by 96%, 96% and 58% relative to the controls, for water deficit, the combined oil and water deficit, and oil pollution alone, respectively.

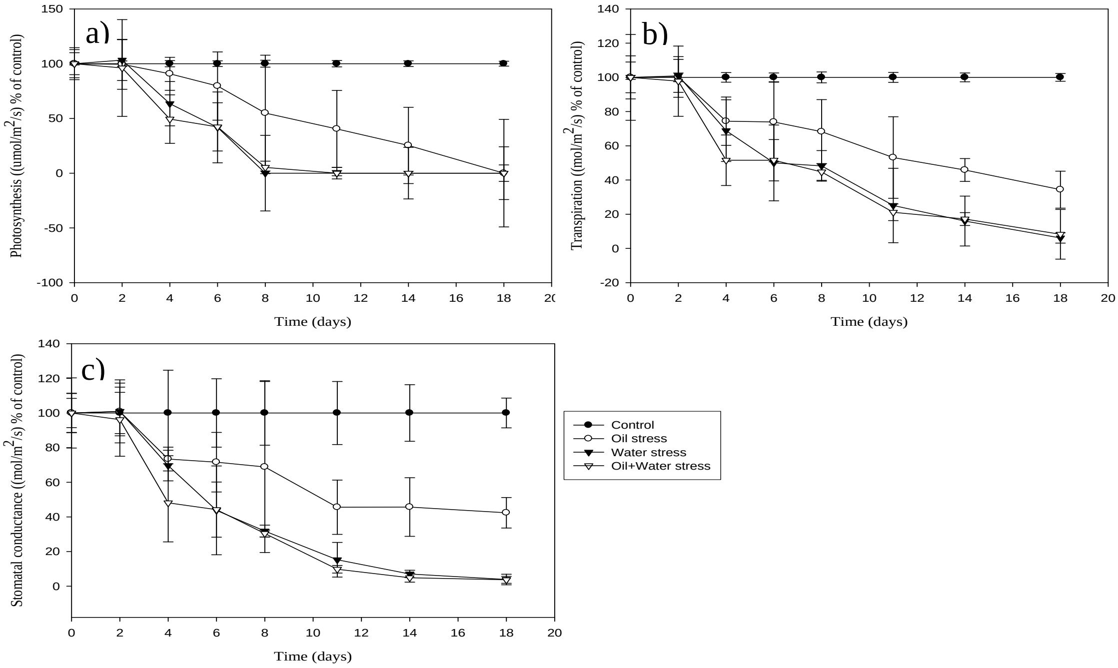

Figure 2. Effects of oil pollution, water deficit and the combined oil and water deficit on photosynthesis, transpiration and stomatal conductance activities of maize over time. Treatments as denoted by the key. Error bars = 1 x SE, n = 10.

3.3Leaf total chlorophyll, carotenoids, and water content

There was general decrease in total chlorophyll content over the course of the experiment in plants treated with oil, as can be seen in Fig. 3a. Before visual stress symptoms were observed, plants treated with oil and combined oil and water deficit showed a significant reduction in total chlorophyll content (on day 6), compared to the controls. However, no significant reduction in total chlorophyll content was observed in plants treated with water deficit throughout the experiment. This implies that significant reduction in total chlorophyll was only observed whenever oil was involved in the treatment. Thus, oil and oil and water deficit treatments showed a reduction in total chlorophyll content, but there was no significant difference between these two treatments throughout the experiment.

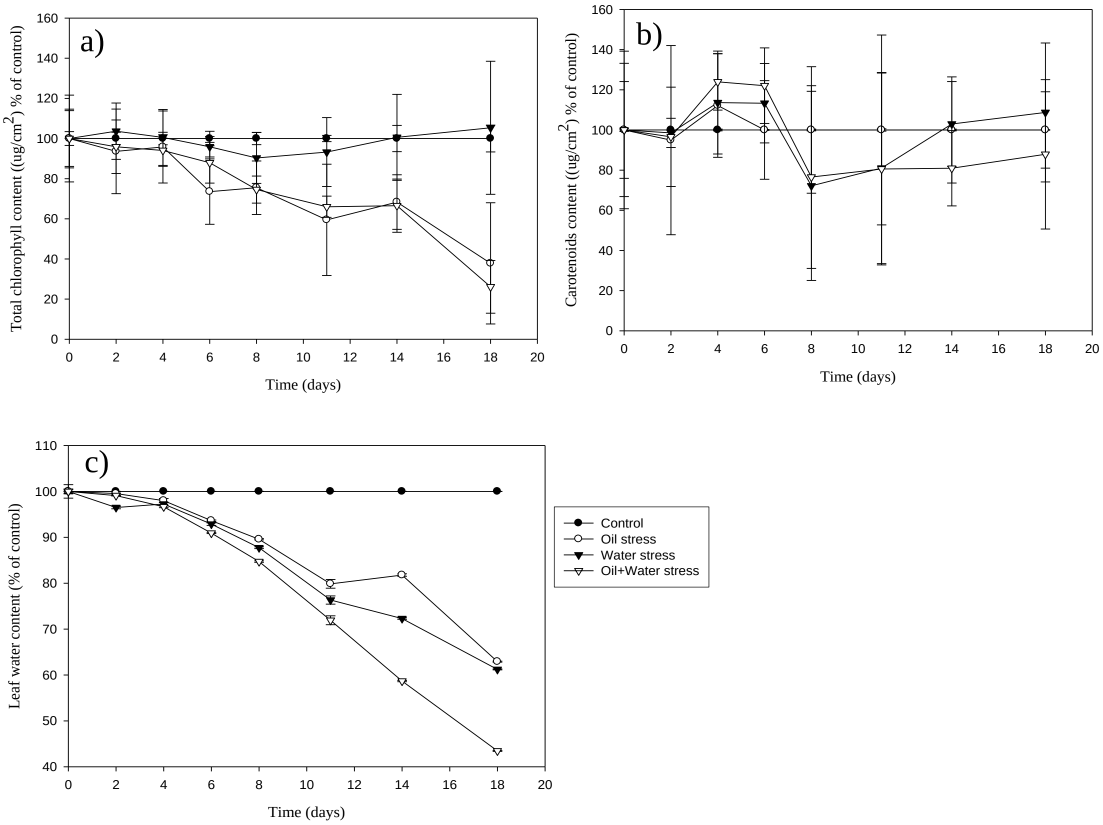

Figure 3. Effects of oil contamination of soil, water deficit and the combined oil and water deficit on total chlorophyll, carotenoid, and water contents of maize over time. Treatments are denoted by the key. Bars = 1 x SE, n = 5.

By the end of the experiment, there was a total reduction in total chlorophyll content of treated plants by approximately 63% and 74% for oil and the combined oil pollution and water deficit, respectively. The carotenoid content of the treated plants did not change systematically throughout the experiment (Fig. 3b). While the carotenoid content of plants treated with water deficit and combined oil pollution and water deficit fluctuated relative to the controls, the carotenoid content of the plants treated with oil pollution alone remained largely unchanged. The carotenoid content of all the treated plants was not significant to the controls at any time during the experiment. The leaf water content of all the treated plants decreased as stress progressed (Fig. 3c). However, the rate of reduction was relatively slow at the early stage of the experiment and faster at the later stage. Thus, the leaf water content of all the treated plants became significantly lower than that of the controls 8 days after treatments. The leaf water content of plants treated with a combination of oil and water deficit reduced at the fastest rate, followed by those treated with water deficit alone and then oil pollution alone. By the end of the experiment, there was a total reduction in leaf water content by 57%, 39% and 38% relative to the controls for plants treated with the combined oil and water deficit, with water deficit alone and with oil alone, respectively.

3.4. Interrelationships between physiological and biochemical variables

A moderate polynomial relationship was found between total chlorophyll and photosynthetic activities of maize leaves (Fig. 4a). The leaf water content also had a moderate polynomial relationship with both transpiration and stomatal conductance (figures 4b and 4c), respectively; however, there was no correlation between the carotenoid and total chlorophyll. The physiological rates were intercorrelated, as expected, as photosynthesis yielded a strong linear relationship with transpiration (R^2 = 0.74) and stomatal conductance (R^2 = 0.91) and there was also a strong linear relationship between transpiration and stomatal conductance (R^2 = 0.90) (data not shown).

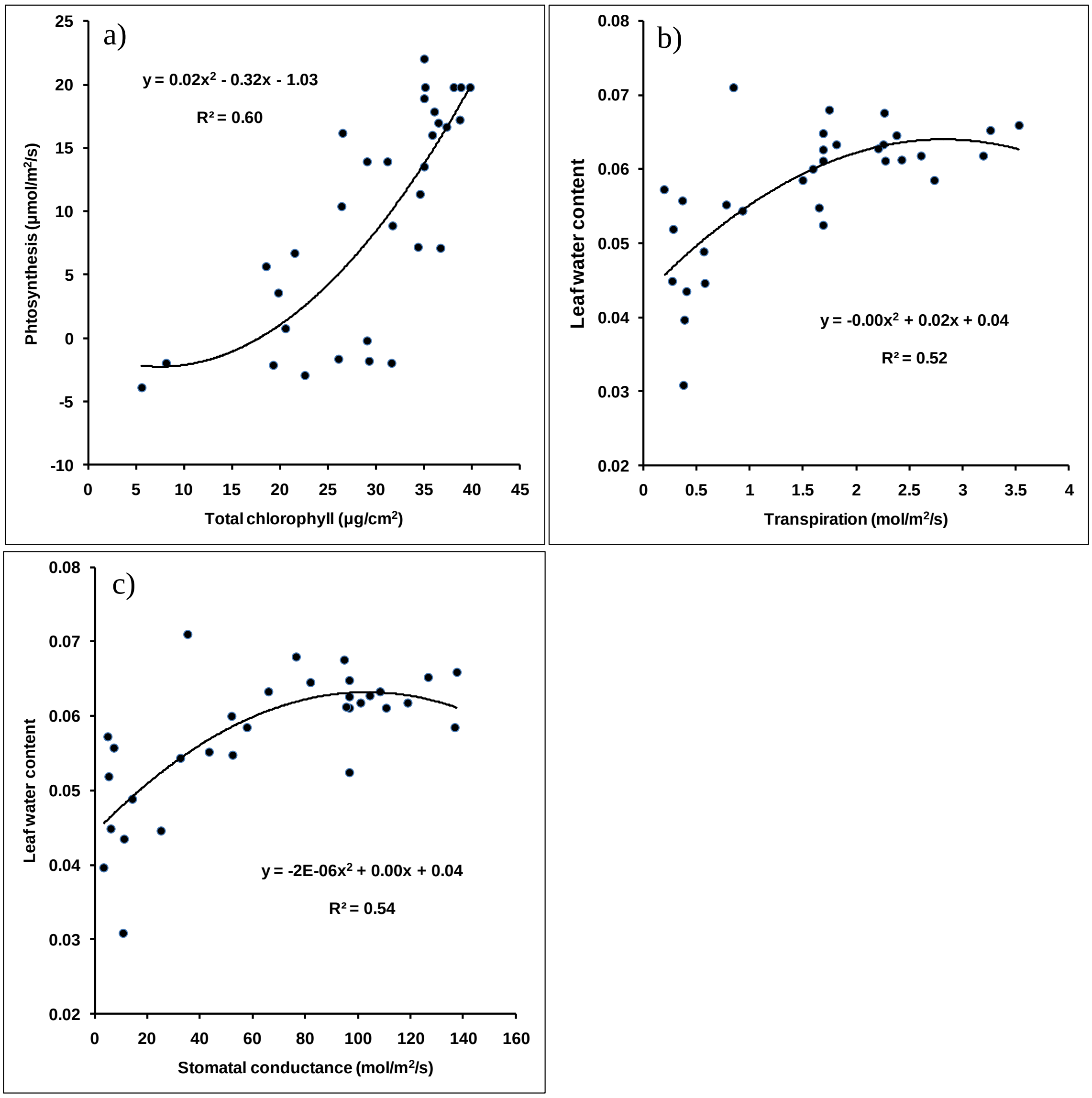

Figure 4. Relationships between physiological and biochemical variables in maize, total chlorophyll content and photosynthetic activities (a), transpiration and leaf water content (b), stomatal conductance and leaf water content, n = 32 (mean values per treatment, per sampling occasion).

3.5Responses of spectral reflectance to treatments

Looking at the mean spectra obtained at the end of the experiment (Fig. 5a), it can be seen that the leaf reflectance at all wavebands was higher in all the treated plants when compared to the controls. In the visible region, spectral reflectance of plants treated with oil and the combined oil and water deficit was higher than those treated with water deficit alone. However, in the NIR and SWIR regions, the reflectance of plants treated with water deficit alone and the combined oil and water pollution were higher than those treated with oil alone. In order to clearly show the magnitude at which the spectral reflectance of the treated plants deviated from the control plants, the difference between the mean spectral reflectance of the treated and control leaves at the end of the experiment was computed and plotted (Fig. 5b). The major reflectance differences were found between 513 and 760nm and 1380 and 1910nm, but the differences varied according to the type of treatment. T-tests were conducted to determine whether differences in spectral reflectance were statistically different between treatments. The results showed that in the regions 513 to 639nm and 680 to 722nm, the spectral reflectance of plants treated with oil alone and the combined oil and water deficit were significantly higher than those treated with water deficit alone ($p < 0.05$). However, in the region 1387 to 1536nm, the spectral reflectance of plants treated with water deficit alone and the combined oil and water deficit were significantly higher than those treated with oil pollution alone ($p < 0.05$).

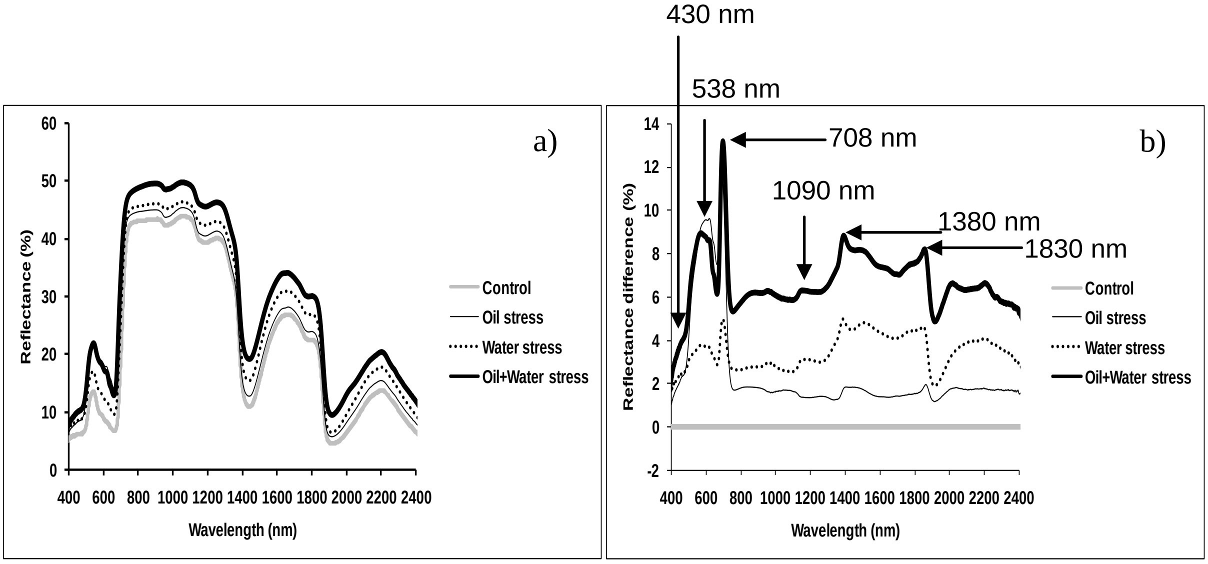

Figure 5. Spectral response to treatments, mean reflectance spectra (a), differences between the mean spectral reflectance (b) of the treated and control maize leaves 18 days after treatment. Treatments as denoted by the key, n = 100.

3.6Relationships between spectral reflectance and physiological/biochemical variables

Using data across all treated and control plants, it was found that there was a strong negative relationship between the photosynthetic activity and spectral reflectance in the visible region. Maximum correlations were found in the green and red regions, precisely at 528nm (R = - 0.71) and 715nm (R = - 0.74) respectively. Across the NIR and SWIR only weak relationships were found for photosynthetic activity. The relationships between transpiration, stomatal conductance, leaf total chlorophyll and reflectance were similar to those for photosynthesis across the spectrum. Carotenoids were largely uncorrelated with reflectance across most wavelengths, though there were some weak relationships in certain regions. The highest correlations were found in the blue region (between 401nm and 488nm) and SWIR (between 1131nm and 2093nm) with the waveband 430nm having the highest correlation (R = - 0.38). Weakest relationship was found in the NIR region at the waveband 736nm. Interestingly, the relationships between leaf water content and reflectance were similar to those for total chlorophyll across the spectrum. Hence, parts of the spectrum in the SWIR that have been found previously to be sensitive to water content variations (e.g. Gao and Goetz, 1994)) were found to be largely uncorrelated with leaf water content in the present study ($R = \leq - 0.29$). However, there was a strong negative relationship between the reflectance and leaf water content in the green and red regions with the largest correlations at 500nm (R = - 0.80) and 726nm (R = - 0.78), respectively. The NIR is highly correlated with leaf water content with the maximum precisely 900nm (R = - 0.73). As we move towards the SWIR, correlations decrease and a minimum correlation was found at 1926nm (R = 0.00).

3.7Relationships between Spectral Indices and Biochemical Variables

TABLE 1 shows the correlations between a number of spectral indices and biochemical variables. Using these results an optimal spectral index which provided the highest correlation with each variable was selected for further analysis. The best indices and their R values were indicated in bold.

Table 1. Summary of the correlation coefficients (R) between the spectral reflectance indices and measured physiological/biochemical parameters.

Biochemicals estimated	**Spectral indices**	**R**	**References**
Total chlorophyll	R_{550}/R_{850}	-0.920	Schepers *et al.*, (1996)
	$(R_{790}-R_{720})/(R_{790}+R_{720})$	0.947	Barnes *et al.*, (2000)

	$(R_{750}-R_{445})/(_{705}+R_{445})$	0.810	Sims and Gamon (2003)
	$(R_{750}-R_{445})/(R_{705}-R_{445})$	0.947	Sims and Gamon (2002)
	$(R_{750}-R_{720})/(R_{700}-R_{670})$	0.940	Le Maire *et al.* (2004)
	R_{708}	-0.930	New
	R_{1330}/R_{708}	0.930	New
	$(R_{1330}-R_{708})/(R_{1330}+R_{708})$	-0.940	New
	R_{800}/R_{708}	0.930	New
	R_{538}	-0.930	New
	R_{1330}/R_{538}	-0.940	New
	$(R_{1330}-R_{538})/(R_{1330}+R_{538})$	**0.949**	New
Carotenoids	R_{800}/R_{470}	0.350	Blackburn (1998a)
	$(R_{800}-R_{470})/(R_{800}+R_{470})$	0.340	Blackburn (1998a)
	R_{430}	-0.380	New
	R_{736}/R_{430}	0.410	New
	$(R_{736}-R_{430})/(R_{736}+R_{430})$	**0.420**	New
	R_{800}/R_{430}	0.400	New
Leaf water content	$(R_{858}-R_{1240})/(R_{858}+R_{1240})$	-0.080	Gao, (1996); Zarco-Tejada *et al.*, (2003b)
	$(R_{858}-R_{1640})/(R_{858}+R_{1640})$	0.010	Fensholt & Sandholt, (2003)
	R_{900}	**-0.730**	New
	R_{1926}/R_{900}	0.060	New
	$(R_{1926}-R_{900})/(R_{1926}+R_{900})$	0.040	New
	R_{800}/R_{900}	0.060	New

Result of regression analysis showed a strong positive linear relationship between normalised difference ratio $(R_{1330}-R_{538})/(R_{1330}-R_{538})$ and total chlorophyll ($R^2 = 0.92$). While there was a poor relationship between the normalised difference ratio $(R_{736}-R_{430})/(R_{736}+R_{430})$ and carotenoid, the individual narrow waveband R_{900} had a moderate relationship with leaf water content ($R^2 = 0.67$).

3.8Temporal responses of optical spectral indices

The optimal chlorophyll index $(R_{1330}-R_{538})/(R_{1330}+R_{538})$ decreased in treated plants as stress progressed. Before visual stress symptoms were observed, the index significantly reduced for plants treated with oil and combined oil and water deficit (on day 6), compared to the controls (see TABLE 2). However, no significant reduction in the index was observed in plants treated with water deficit throughout the experiment. This implies that significant reduction in $(R_{1330}-R_{538})/(R_{1330}+R_{538})$ was only observed whenever oil was involved in the treatment. Thus, oil and oil and water deficit treatments showed a reduction in $(R_{1330}-R_{538})/(R_{1330}+R_{538})$, but there was no significant difference between these two treatments throughout the experiment. By the end of the experiment, there was a total reduction of the index of treated plants by approximately 44% and 42% for oil and the combined oil pollution and water deficit, respectively.

The optimal carotenoid index $(R_{736}-R_{430})/(R_{736}+R_{430})$ decreased in treated plants as stress progressed. Before visual stress symptoms were observed, the index had significantly decreased in plants treated with combined oil and water deficit (on day 6), compared to the controls (see TABLE 2). The index did not decrease systematically in treated plants although this was more pronounced in plants treated with oil alone. No significant change was found between treatments throughout the experiment but only in some days between oil treated plants and the combined oil and water deficit. By the end of the experiment, there was a total reduction of the index of treated plants by approximately 11% for oil and the combined oil pollution and water deficit, and 8% for the water deficit.

The optimal leaf water content index R_{900} increased in treated plants as stress progressed. Before visual stress symptoms were observed, the index significantly increased in plants treated with oil alone (on day 8), compared to the controls (see TABLE 2). Significant increment of the index was observed in plants treated with water deficit and the combined oil and water deficit only at the later stage of the experiment. Greatest increment was found in plants treated with the combined oil and water deficit, followed by oil (alone) and then the water deficit (alone). By the end of the experiment, there was a total increment of the index of treated plants by approximately 20%, 17% and 12% for the combined oil pollution and water deficit, oil pollution alone and water deficit alone, respectively.

Table 2. Results of ANOVA tests demonstrating when there were significant differences between the changes in the spectral and thermal properties of treated and control plants, over the course of the experiment. Unshaded = no significant difference; Shaded = significant difference. *Time when visible stress symptoms were observed in oil treatment alone, **time when visible stress symptoms were observed in water deficit and the combined oil and water deficit treatment.

Stress indices	Treatments		Time (Days)							
			0	2	4	6	8**	11*	14	18
$(R_{1330}-R_{538})/(R_{1330}+R_{538})$	Control	Oil stress				▨	▨	▨	▨	▨
		Water stress								
		Oil+Water stress				▨	▨	▨	▨	▨
$(R_{736}-R_{430})/(R_{736}+R_{430})$	Control	Oil stress			▨			▨	▨	▨
		Water stress						▨	▨	▨
		Oil+Water stress		▨		▨	▨	▨	▨	▨
R_{900}	Control	Oil stress					▨	▨	▨	▨
		Water stress						▨	▨	▨
		Oil+Water stress						▨	▨	▨
Absolute temperature	Control	Oil stress						▨	▨	▨
		Water stress			▨	▨	▨	▨	▨	▨
		Oil+Water stress			▨	▨	▨	▨	▨	▨
I_G	Control	Oil stress			▨			▨	▨	▨
		Water stress			▨		▨	▨	▨	▨
		Oil+Water stress		▨	▨	▨	▨	▨	▨	▨

3.9Thermal responses

As can be seen in Fig. 6a, the absolute leaf temperatures of treated plants increased relative to control. The statistical analysis revealed that before visual stress symptoms were observed, leaf absolute temperatures showed a significant increase (on day 4) in the plants treated with water deficit and combined oil pollution and water deficit, compared to the controls (see TABLE 2). However, for plants treated with oil pollution alone, a significant rise in leaf absolute temperature occurred on the same day as visual stress symptoms. Over the course of the experiment there were no consistent differences between the plants treated with oil, water deficit or their combination.

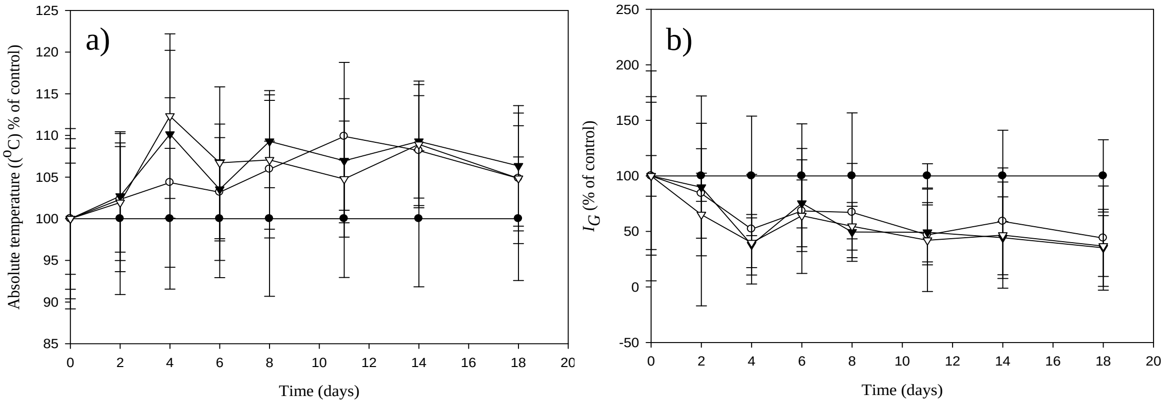

Figure 6. Effects of oil pollution, water deficit and the combined oil and water deficit on the absolute temperature (a); and thermal index (I_G) (b) of maize leaves over time. Treatments are denoted by the key. Bars = 1 x SE, n = 10.

The thermal index (I_G) of the treated plants was significantly reduced by treatments when compared with the control plants (Fig. 6b). The reduction was significant 6 days before the visual stress symptoms were observed in plants treated with the combined oil and water deficit (TABLE 2). For plants treated with water deficit (alone) and oil pollution (alone), a significant reduction in I_G was observed four days before visual stress symptoms, but this difference was not consistent on the following sampling occasion. From the point when visual symptoms were observed, I_G was significantly lower for the plants treated with water deficit (alone) and oil pollution (alone) than the controls, until the end of the experiment. Similar to leaf absolute temperature of treated plants, there were no consistent differences in I_G between the plants treated with oil, water deficit or their combination, over the course of the experiment. A moderate linear relationship was found between the I_G and stomatal conductance as can be seen in Fig. 7.

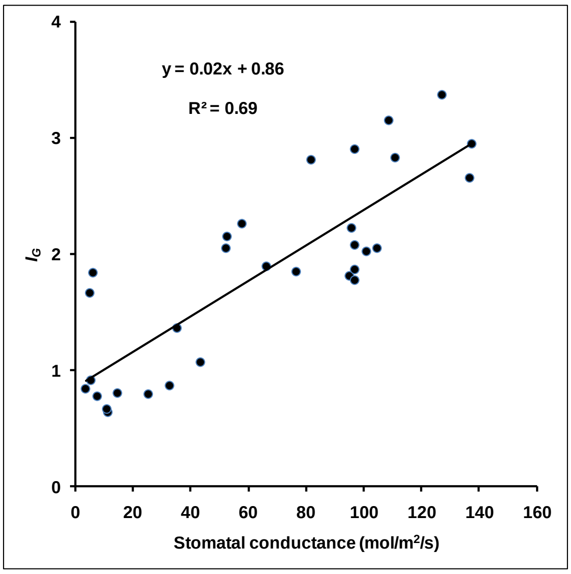

Figure 7. Relationships between the stomatal conductance and thermal index (I_G), n = 32.

IV. Discussion

For all of the physiological variables, plants treated with water deficit alone and the combined oil and water deficit responded at a faster rate than those treated with oil pollution. A recent study found that when irrigation was withheld to induce severe soil drying, gas exchange decreased and then stopped in three Mediterranean cedar species: *Cedrus atlantica*, *C. Brevifolia* and *C. Libani* (Ladjal *et al*. 2007). An empirical study by Ray and Sinclair (1998) found that the overriding factor determining transpirational response in maize (*Zea mays* L.) and soyabean (*Glycine max* L.) to drought stress was soil dryness. A recent study attributed reduced transpiration in plants to soil water limitation as well-irrigated crops can usually supply enough water to the leaves to satisfy transpiration demand (Tilling *et al*. 2007). These suggest that treatments may have reduced soil water needed to sustain transpiration processes and thus, can explain decrease in transpiration rates of plants. When transpiration is restricted due to lack of water, stomata closure is induced resulting in less water evaporating from the leaf surface (Jackson 1986). Not surprisingly similar responses were found for transpiration and stomatal conductance and this is explained by the strong linear relationship found between transpiration and stomatal conductance. Plants treated with oil pollution alone also experienced a reduction in both the transpiration and stomatal conductance. It is known that oil can reduce water uptake by wheat (Jong 1980) and thus, oil may have indirectly caused a reduction in transpiration and stomatal conductance in maize.

The total chlorophyll content of plants treated with oil pollution decreased significantly in contrast with those treated with water deficit alone. A significant change in total chlorophyll content of plants treated with oil occurred before visual stress symptoms were observed. This is in contrast with total chlorophyll content of plants treated with water deficit which at no time showed a significant change. This implies that by quantifying the total chlorophyll content, plant stress caused by oil pollution can be detected early and also could be discriminated from water deficit stress. The reduction in total chlorophyll content of plants treated with oil pollution may possibly be attributed to toxic effect of oil as it destroys cell membranes. Earlier investigation noted that petroleum toxicity in plants can alter leaf pigmentation properties such as chlorophyll (Rosso *et al.* 2005, Larcher 1995). Indeed, previous studies have found that oil can penetrate plants/leaf tissue and consequently, destroy cellular integrity, and prevent leaf and shoot regeneration (Webb 1994, Pezeshki *et al.* 2000, Pezeshki *et al.* 1995). However, several studies have shown that chlorophyll does not always relate to water content. In a temperate forest, no correlation was found between the chlorophyll and water content for five different species (Gond *et al.* 1999). The authors reported that while the chlorophyll concentration decreases in autumn due to the phenological status of the plant in some of the species, the water content remained constant.

In our study, while the photosynthetic activities of plants treated with water deficit reduced significantly, their total chlorophyll content did not change significantly. Our result concurs with the findings of Maracci *et al.* (1991) where maize (*Zea mays* L.) subjected to drought stress experienced some disturbances in the photosynthetic functioning of the plant without a change in the pigment concentration. The authors reported that while the chlorophyll concentration remains unchanged, the net photosynthetic activity of the maize plants decreases with increasing water deficiency. Earlier works also found that the stomatal closure reduces leaf photosynthesis because of restricted entry of CO_2 through stomatal pores (Pezeshki *et al.* 1995, Webb 1994, Pezeshki and DeLaune 1993). Furthermore, water stress may cause closure of leaf stomata and reduction in CO_2 supply (Jackson and Ezra 1995). This evidence can explain our findings concerning the disruption in photosynthetic activities of plants treated with water deficit which may be attributed to stomatal closure and/or accumulation of internal CO_2 rather than a decrease in chlorophyll content. Thus, this suggests that the photosynthetic response of plants treated with water deficit may indicate indirect effects of a reduction in transpiration and stomatal conductance rather than reduction in photosynthetic pigments.

It is known that carotenoids generally decline less quickly than chlorophyll (Sims and Gamon 2002), perhaps due to its role as a photoprotective pigment (Demming-Adams and Adams 1996, Härtel and Grimm 1998). Additionally, it has been found that the concentrations of carotenoids are usually high enough in stressed leaves that absorption in the 400 to 500nm range remains similar to that in healthy leaves (Merzlyak *et al.* 1999). These concepts possibly explain the inconsistency and insignificant change in carotenoid content of treated plants in our study. The few occasions where carotenoid content of treated plants were higher than those of control, may possibly be attributed to the damaging effect of the stresses. Interestingly from our results, there is an indication that carotenoids were able to perform the photoprotective function irrespective of stress type.

The reduction in leaf water content of treated plants was not significant until 8 days after treatment. This concurs with Beaumont (1995) who found that the water content of sunflower leaves did not change much due to moderate water stress since the plant tried to maintain a level compatible with its basic functioning. Past study indicates that a reduction in transpiration helps to conserve available water (Larcher 1995), as does the stomatal conductance. Thus, the insignificant change in leaf water content of the treated plants identified at the early stage of plant stress may be attributed to plant water conservation mechanisms as both transpiration and stomatal conductance are reduced. A significant change in leaf water content did not occur until the later stage of the experiment when plants perhaps could no longer conserve water. At that point, visual stress symptoms caused by oil pollution, water deficit and the combined oil and water were manifest.

The results of the present study indicated that spectral reflectances of treated plants were sensitive to various stresses and this conforms to the findings of numerous authors that used wide range of plant stresses such as waterlogging, natural gas, nutrient stress, heavy metal toxicity and soil oxygen deficiency (Smith *et al.* 2004, Noomen *et al.* 2003, Anderson and Perry 1996, Carter and Miller 1994, Carter 1993, Milton *et al.* 1989, Horler *et al.* 1983, Woolley 1971). It has long been known that stress generally increases reflectance in the visible region due to a decrease in the dominant absorption features such as the photosynthetic pigments. Thus, light reflected by vegetation in the visible region of the spectrum is predominantly influenced by the presence of chlorophyll pigments in the leaf tissues (Haboudane *et al.* 2002). Similar to our findings, Carter (1993) noted that for individual leaves; increased reflectance at visible wavelengths (400 – 700nm) is generally the most consistent response to stress within the 400 – 2500nm range. Expectedly, the relationships between photosynthesis, total chlorophyll and carotenoids of treated plants and reflectance were strongest in the visible region. The sensitivity of other physiological and biochemical variables such as the leaf water content, transpiration and stomatal conductance were expected to be found at the other regions of the spectrum (Ceccato *et al.* 2001). On the contrary, their relationships with reflectance were also found to be strongest in the visible region. The reason for this may be due to interrelationship found between the leaf water content and total chlorophyll and the fact that the total chlorophyll was changing over much wider range (86%) than leaf water content (57%). Thus indirect relationships were observed between water content reflectance in the visible region.

The NIR reflectance is influenced principally by the internal cell structure of the leaf (Tilling *et al.* 2007, Ceccato *et al.* 2001). Well-hydrated, healthy spongy mesophyll cells strongly reflect infrared wavelengths (Gates *et al.* 1965). Leaf turgor is associated with cellular growth and function (Graeff and Claupein 2007). When turgor becomes zero under strong water deficiency, cells collapse and the leaf wilts. Turgor can be maintained by cell wall hardening during the development of a water deficit. While cell wall hardening helps to sustain turgor, it impedes cell growth. Structural changes in the arrangement of the spongy mesophyll structure as described by Ripple (1986) and Boyer *et al.* (1988) may occur as a consequence of a loss of cell turgor pressure and this has implications for leaf reflectance. As the leaf internal structure may have been deteriorated due to reduction in transpiration and stomatal conductance, other factor may have biased the relationship we found between the transpiration/stomatal conductance and reflectance in the NIR and SWIR regions such as the leaf dry matter content. Additionally, the leaf internal structure which Ceccato *et al.* (2001) found to have the greatest influence for reflectance at 1600nm may have added to the weak relationships found in the SWIR.

Similar to our results, Graeff and Claupein (2007), Carter *et al.* (1989), Bowman (1989) and Woolley (1971) found that reflectance tends to increase in the 400 – 1300nm region, when water is lost from a leaf. The reason for the increase of reflectance in the 400 – 1300nm region has been inferred as the changing of the internal structure of the leaf besides water losing (Graeff and Claupein 2007, Sinclair *et al.* 1971, Gausman and

Allen 1973). In the visible wavelengths, absorption by leaf water content is weak and changes in reflectance resulting directly from leaf water loss will not be directly detectable (Danson and Aldakheel 2000). This concurs with our findings as reflectance in the visible region by plants treated with water deficit alone was insignificant when compared to those treated with oil or the combined oil and water deficit. Furthermore, the incidental increase in reflectance in the visible region by plants treated with water deficit may be attributed indirectly to the apparent stomata closure and consequential reduction in CO_2 supply. Similarly, earlier workers noted that closure of leaf stomata and reduction in CO_2 supply may lead to increased visible reflectance (Jackson and Ezra 1995).

For individual leaves, there is normally a negative relationship between the leaf water content and reflectance in the near and middle infrared wavelengths (Aldakheel and Danson 1997, Danson *et al*. 1992). This concurs with our correlation in the NIR region but disagrees with the ones in the visible and the SWIR regions where correlations were strongest and weakest respectively. The authors attributed the strong relationships as a direct function of the absorption characteristics of water, which dominate the spectral response of vegetation in that region. The weak correlation found between reflectance and the leaf water content in the SWIR region may be due to other factors influencing reflectance at that region such as the leaf dry matter and the leaf internal structure (Ceccato *et al.* 2001). An empirical study by Cheng *et al.* (2006) found that at leaf scale, changes in dry matter content produced more errors in water content than other leaf biochemical properties. Studies have reported extensive influences caused by both dry matter content and leaf internal structure parameter on reflectance in the NIR and SWIR regions simulated by the PROSPECT leaf reflectance model (Bacour *et al*. 2002, Ceccato *et al*. 2001). A study by Cheng *et al*. (2006) demonstrated that more significant changes in leaf reflectance are introduced by changes of leaf dry matter than by leaf internal structure. Therefore, the correlation between reflectance and the leaf water content in the SWIR region may have been further complicated by variations in the leaf dry matter content. However, the strong correlation found between reflectance and the leaf water content in the visible region may indirectly be related to the influence of strong absorption by the chlorophylls and carotenoid at that spectral region as discussed earlier.

Based on the spectral indices tested, additional evidence was found about the relationships between reflectance and the measured physiological/biochemical variables. A normalized-difference spectral index $(R_{1330}-R_{538})/(R_{1330}+R_{538})$ that combined a waveband in the green with one in the NIR region had a strong relationship with total chlorophyll content. Several studies have shown similar results where the leaf reflectance values around 580 and 700nm wavelengths were closely related with leaf chlorophyll level (Jacquemoud and Baret 1990, Daughtry *et al*. 2000, Carter and Spiering 2002, Zhao *et al*. 2003). Thus, earlier studies noted that the green-peak and red-edge spectral regions are generally critical for the detection of plant stress (Schepers *et al*. 1996, Carter and Knapp 2001). Furthermore, an empirical study by Zhao *et al*. (2005) found that among other reflectance ratios, the one that combined reflectance value in the green or red regions with one in the NIR had strongest relationship with chlorophyll concentration in cotton (*Gossypium hirsutum* L.). Additionally, the index significantly decreased in plants treated with oil and the combined oil and water deficit before visual stress symptoms were observed when compared with the control. On the contrary, the index did not show significant change in plants treated with water deficit alone when compared with the control.

The weak relationships found in this study between the carotenoid spectral indices and carotenoid concentration concurs with the findings of Blackburn (1998b). While identifying the optimum wavebands for pigment indices using leaves of four different deciduous tree species at different phenological stage, the author found no relationships between carotenoid specific simple/normalised difference ratios and carotenoids concentration. The result was attributed to the effects of convolution of carotenoid absorption maxima with other pigments. This may possibly be the case in the present study as stronger relationship was also found between chlorophylls and reflectance (R = - 0.49) in the same region where carotenoid absorption maxima was found. This may also be responsible for the significant decrease of the carotenoids index $(R_{736}-R_{430})/(R_{736}+R_{430})$ of treated plants when compared with the controls. Previous work noted that chlorophyll has strong absorption peaks not only in the red regions of the spectrum but also in the blue region where its absorption peak overlaps with the absorbance of the carotenoid (Sims and Gamon 2002). However, results from further work undertaken by Blackburn (1998a) show much better relationships between carotenoid specific simple/normalised difference ratios and carotenoid concentration in bracken (*Pteridium aquilinum*) canopy. The author attributed this to increased range of carotenoid concentration per unit area used in the canopy scale experiment compared to the deciduous tree leaves study.

Regarding thermal responses of leaves to treatments, the consistent increase in the absolute temperatures of the treated plants in relation to controls is likely to be due to the reduction in the transpiration and stomatal conductance of treated plants. The early significant difference found between the absolute leaf temperatures of plants treated with water deficit and the combined oil and water deficit treated plants and control plants as presented in table 2 show that, change in absolute temperature of leaf in response to stress may be useful for early detection of water deficit stress in plant. However, the results indicate that absolute leaf temperature may only be useful for detecting plant stress caused by oil pollution after a prolonged period of stress. The inconsistent and insignificant differences found between the absolute temperature of plants subjected to different types of treatment indicates the limitation in this remotely-sensed parameter in predicting accurately the type of stress affecting the plants i.e. it is difficult to discriminate between oil and water deficit stress. Therefore, prior knowledge about the type of stress affecting plant may be required for accurate detection of stress using leaf absolute temperature.

Generally, the results show that the thermal index (I_G) can detect oil pollution and water deficit stress in maize. Similar to the leaf absolute temperature, the consistent decrease in the thermal index (I_G) of treated plants as percentage of control is likely to be responding to the reduction in the transpiration and stomatal conductance of treated plants. The time of response of the I_G to treatments suggests that the I_G may be useful for early detection of stress caused by the combined oil and water deficit. However, this is not the case for plants treated with oil alone and water deficit alone, as their I_G was found to be consistently significant from the control on the same day as visible stress symptoms. Like the leaf absolute temperature, it may be difficult to accurately predict the type of stress affecting plant due to inconsistency and insignificant differences found between the I_G of the plants exposed to different types of treatment. Again, this suggests that there may be the need for prior knowledge of stress affecting plants before accurate discrimination can be achieved using the I_G. The relationships found between the stomatal conductance and the I_G suggests that the index has potentials for stress detection in plants.

In summary, by using spectral reflectance in chlorophyll absorption bands particularly in the regions 513 to 639nm and 680 to 722nm, it was possible to discriminate between oil and water deficit stress in maize as reflectance associated with oil pollution was significantly higher than that associated with water deficit in these wavebands. Also, the water absorption wavebands in region 1387 to 1536nm can be used to discriminate between oil and water deficit stress in maize as reflectance associated with water stress was significantly higher than that associated with oil pollution in this wavebands. Additionally, while the chlorophyll index (R_{1330}-R_{538})/(R_{1330}+R_{538}) can detect oil-related stress but not water deficit stress, the leaf absolute temperature detect water deficit and I_G can detect combined oil and water deficit stress in maize, respectively. This suggests that the combination of hyperspectral and thermal remote sensing can not only detect oil and water deficit stress in maize before visual stress symptoms manifest, but also can effectively discriminate between the two stresses.

V. Conclusion

The results of this study indicate that the concept of measuring leaf spectral reflectance and thermal responses for early detection and discrimination between oil and water deficit stresses in plant is sound. It was demonstrated that hyperspectral remote sensing can accurately measure the chlorophyll concentration in leaves. This study shows that oil pollution adversely affects leaf chlorophyll content and therefore, plant stress caused by oil pollution can be detected remotely. Remote sensing of carotenoid concentration alone is not sufficient both for early detection and discrimination between oil pollution and water deficit stress. However, it can provide additional information about plant stress particularly as carotenoids maintain some degree of stability while chlorophyll content is decreasing. Chlorophyll index (R_{1330}-R_{538})/(R_{1330}+R_{538}) can detect oil related stress before visual stress symptoms are observed but may not be suitable for assessment of stress in maize caused by water deficit alone. This is because plants may show signs of stress including reduced evapotranspiration without experiencing a reduction in chlorophyll content. Interestingly, thermography appears to have some potential in this regard. While changes in leaf absolute temperature can indicate water deficit stress in maize prior to visual stress symptoms, it may be difficult to discriminate between oil and water deficit stress using this measure. Indirect measurement of the stomatal conductance using I_G has potential in pre-visual detection of stress caused by the combined oil and water deficit but, again, this lacks the ability to discriminate between oil and water deficit stress. Thus, our findings suggest that the combination of hyperspectral and thermal remote sensing has potential in the early detection and discrimination between oil and water deficit stress in maize.

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