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Detection and Discrimination of Stress in Bean (*Phaseolus vulgaris* Tendergreen) Caused by Oil Pollution and Waterlogging Using Combined Spectral and Thermal Remote Sensing

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Abstract: Remote sensing of plant stress holds promise for the detection of pollution caused by oil; however, plant stress can be caused by a range of abiotic and biotic factors present to varying degrees within a given environment. Thus, for the accurate application of remedial measures, it is important to be able to detect and discriminate between different causes of plant stress. In oil-rich delta regions of the world, waterlogging is a frequent source of plant stress that has similar physiological effects to those of oil pollution. Hence, this study investigated the dual capabilities of spectral and thermal remote sensing for detecting and discriminating between plant stress caused by a combination of oil pollution and waterlogging. In a glasshouse, plants of pot grown bean (*Phaseolus vulgaris* Tendergreen) were subjected to oil pollution, waterlogging and combined oil and waterlogging treatments. Canopy physiological, spectral and thermal measurements were taken every 2-3 days following treatment to follow the development of stress responses. For plants treated with oil, spectral and thermal responses were evident 6 days before symptoms could be observed visually. However, in waterlogged plants only spectral responses were observed and up to 8 days before visual symptoms. Based on timing and consistency in sensitivity, a narrowband reflectance ratio R_{673}/R_{545} was most efficient in detecting stress symptoms caused by oil and waterlogging. The absolute canopy temperature and derived thermal Index (Ig) were good indicators of developing oil and combined oil and waterlogging stress in bean but were insensitive to waterlogging alone. Thus, this study reports that by combining spectral and thermal remote sensing, plant stress caused by oil pollution can be detected and discriminated from stress caused by waterlogging. The findings justify further research to investigate the wider applicability of this approach and its potential as the basis for an operational monitoring technique for oil pollution.

Key words: Remote sensing, spectral reflectance, thermography, oil pollution, waterlogging, plant stress

INTRODUCTION

Oil pollution is a major cause of environmental degradation and can arise from spills of crude and refined oil in aquatic and terrestrial environments (Ogboghodo *et al*., 2004). Oil pollution has safety, health, economic and environmental implications including long-term soil contamination and destruction of vegetative ecosystems and arable crops and lands. Generally, plant stress can cause physiological and/or biochemical changes that impact the way in which plants interact with light (Liew *et al*., 2008). Earlier studies have observed changes in biochemistry and spectral reflectance of vegetation growing near natural hydrocarbon seeps (Bammel and Birnie, 1994; Yang, 1999) and leaking gas pipelines (Pysek and Pysek, 1989; Smith *et al*., 2000; Smith, 2002). Thus, there may be some potential for bio-detection of oil pollution using hyperspectral remote sensing to measure the changes in vegetation reflectance due to oil-induced stress.

Oil reserves are often found in river delta regions where natural vegetation and agricultural crops are also frequently affected by waterlogging. For example, in the oil-rich deltas at the Western foot of the Andes along the Pacific coast >30% of agricultural land is affected by waterlogging due to irrigation run-off from higher catchments (De La Torre, 1987). Therefore, there is the possibility in such regions that oil pollution arising from exploration and exploitation activities could affect crops either as a lone stress or in combination with waterlogging. It is important to know the particular type of stress acting on a crop before correct remedial

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measures can be applied. Thus, there is the need to develop an approach that can be used to detect and discriminate between oil pollution and waterlogging.

Remote sensing technology has potential in this regard. Indeed, reflectance measurements can be useful for detecting a wide range of vegetation changes associated with various factors affecting plant growth and productivity. It has been shown that hydrocarbon contamination and waterlogging can be detected in plants using changes in reflectance spectra (Anderson and Perry, 1996; Pickerill and Malthus, 1998; Smith *et al*., 2004). However, there is a poor understanding of the capabilities of a mult-sensor approach and more specifically, the contribution to be made from thermal remote sensing with regard to this problem. Furthermore, there is the added difficulty that similar spectral responses result from different causes of stress thus making it challenging to discriminate between different causes. For example, Smith *et al*. (2005) found that in oilseed rape (*Brassica napus*) there was no difference between the spectral reflectance response of plants stressed using elevated concentrations of natural gas and those stressed as a result of 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 (Milton *et al*., 1989; Carter, 1993; Mariotti *et al*., 1996).

Changes in the rate of transpiration by plants can be exploited as an indicator of developing stress (Liew *et al*., 2008) with thermal imaging providing information on the effects of stress on stomatal related parameters (West *et al*., 2005). It is known that oil contaminated soil can indirectly induce water stress in plants. De Jong (1980) observed that oil markedly decreased water uptake by wheat from contaminated soil layers or from deeper water tables. In studying the effects of soil contamination with diesel oil on yellow lupine, Wyszkowski *et al*. (2004) found that as oil penetrates soil it blocks air spaces and thereby decreases fluxes of air and water leading to a decrease in crop yield. This presumably is due to anoxia, decreased nutrient and water uptake and/or a combination of all three. Since, oil contaminated soil can induce water stress in plants, thermal remote sensing techniques are potentially of value as an indicator of oil-induced stress.

Consequently, there is the need to determine whether spectral and/or thermal remote sensing can be used for the detection and discrimination of concomitant oil and waterlogging stress. Thus, this study aimed to investigate the spectral and thermal responses of bean subjected to three stress regimes: oil pollution, waterlogging and combined oil pollution and waterlogging. The objective was to determine whether changes in spectral and thermal properties of plants could be used for early, non-destructive detection and discrimination of each of these two stresses. Bean was chosen as a model species as it is economically important and forms a major source of protein for humans and animals (Shellie-Dessert and Bliss, 1991) particularly in developing countries throughout Central and South America, in Eastern Africa and Nigeria in particular where oil pollution of valuable farmlands is a common occurrence.

MATERIALS AND METHODS

Plant material: The experiment was conducted in a glasshouse (5×3 m) at Lancaster University, UK under semi-natural conditions in November 2008 at day and night temperatures of 26°C (±2°C) and 15°C (±1°C), respectively. A 12 h supplementary photoperiod (06.00-18:00 h) provided by Osram Plantastar 600 W sodium lamps supplied a Photosynthetic Photon Flux Density (PPFD) of 400 μmol m^{-2} sec^{-1} at bench height. Two seeds of dwarf french bean (*Phaseolus vulgaris* Tendergreen) were sown per 2 L pot containing a loam-based compost (John Innes No. 2, J. Arthur Bowers, Lincoln, UK) and placed on capillary matting and watered daily. After 2 weeks, plants were thinned to one per pot and left to continue to establish for a further week prior to treatment.

Plant treatments: Four treatments comprising eight replicate plants were established, being: control, oil, waterlogg and a combined oil and waterlogging treatment. For treatments containing oil, 15 W/40 diesel engine oil (Unipart, Crawley, UK) was applied to the surface of compost as a percentage volume of the Water Holding Capacity (WHC) of the pot soil (field capacity minus oven dry), earlier determined as 0.63 g H_2O/g compost at a density of 0.8 g/cm^3. Application rate was 20% of WHC, being equivalent to 96 g oil/kg soil. The waterlogging stress was achieved by overwatering pots to a depth of 2.5 cm above the soil surface on a daily basis. The control and oil treated plants were watered normally.

Physiological, thermal and spectral measurements: The third trifoliate leaf which was the most dominant from nadir was chosen for physiological measurements which started on day 0 immediately prior to treatment and then every 2-3 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} sec^{-1} and a leaf equilibration time of 3 min prior to recording data.

Canopy thermal images were acquired in the glasshouse using an SC2000 infrared thermal imaging camera (FLIR Systems, West Malling, UK), operating in a waveband from 7.5-13 μm with a thermal sensitivity of 0.07°C at 30°C. The Field of View (FOV) was 24°×18° with a spatial resolution of 1.3 mrad and emissivity set to 0.95. The camera was positioned at nadir 75 cm above the plant canopy and four images of each plant were captured, rotating the pot 90° between image acquisitions. Measurements were made following the procedures of Grant *et al.* (2006) and each frame included wet (sprayed with water) and dry (coated in petroleum jelly) reference leaf surfaces alongside the canopy of interest. The temperature of these references (T_{dry} and T_{wet}) were used with canopy temperature (T_{canopy}) to calculate the thermal index Ig where Ig = $(T_{dry}-T_{canopy})/(T_{canopy}-T_{wet})$ and under any given environmental conditions is theoretically proportional to canopy conductance (Jones, 1999).

Canopy spectral reflectance data were collected in a dark room directly opposite the glasshouse and immediately after physiological and thermal measurements, for each plant in turn, using a GER 1500 Spectroradiometer (Geophysical and Environmental Research Corp., Millbrook, NY). The instrument covers the spectral range 350-1050 nm using 512 bands with a nominal width of 1.5 nm. Spectral measurements were taken with the instrument positioned at nadir 20 cm above the plant canopy, giving a FOV of approximately 3 cm diameter. Artificial illumination was provided by a 500 W halogen lamp mounted 70 cm away from the canopy at a 45° zenith angle. Eight spectral measurements were captured for each plant canopy by making small rotational movements of 45°C to the pot between each measurement. Each canopy spectrum was paired with one obtained from a white spectralon reference panel placed immediately above the canopy, so that a percentage reflectance spectrum could be calculated that incorporated a correction for the reflectance properties of the spectralon panel.

Data analysis: To account for daily fluctuation and variability in glasshouse ambient temperature and humidity during the course of measurements, results for photosynthesis, transpiration and stomatal conductance of treated plants were expressed as a percentage of control on each sampling occasion. The software package ThermaCAM Researcher (FLIR Systems, West Malling, UK) was used to extract data from thermographs by manually selecting the target canopy and wet and dry reference areas of interest from each frame, to provide raw temperature values and for subsequent calculation of Ig.

In order to examine the spectral responses of bean to treatments and to identify wavelengths of high variation, differences between the mean reflectance spectra for control and treated plants were plotted. The Red-Edge Position (REP) and its amplitude were calculated using the methods of Cho and Skidmore (2006) and explored for their physiological significance. Novel and existing spectral indices were evaluated with the aim of assessing their utility for accurate tracking of canopy spectral changes attributable to differences in physiological properties caused by oil pollution and waterlogging. Individual narrow wavebands were extracted from spectra reflectance based on wavebands of high variation between the control and treated plants. Only the wavebands associated with a significant difference ($p<0.05$) were selected. Spectral indices were then generated from the individual narrow wavebands by means of ratioing all possible two-band combinations. Statistical analysis was performed on the spectral indices and other spectral and thermal parameters using ANOVA to determine their ability to detect and distinguish between subtle signs of stress arising from treatments. Post hoc test analyses using Tukey HSD were performed on the ANOVA to determine significant treatment differences by a mean square multiple comparison procedure. All significant differences were at the 0.05 level of confidence. Two important parameters were used in judging the ability of spectral and thermal parameters: timing of response and consistency in response until the end of the experiment.

RESULTS

Physiological responses: Treated plants showed significant declines in photosynthesis, transpiration and stomatal conductance (Fig. 1a-c) with plants treated with oil and combined oil and waterlogging treatments showing greatest decreases in photosynthesis, transpiration and stomatal conductance compared to waterlogged plants only. However, there was no significant difference between oil and combined oil and waterlogged plants throughout the experiment. At 14 days photosynthesis, transpiration and stomatal conductance in waterlogged plants had respectively decreased by 42, 29 and 33% and in oil and combined oil and waterlogged plants by typically 100, 91 and 93%, respectively.

Visual stress observations: Stress symptoms were first observed visually on day 8 for plants treated with oil and combined oil and waterlogging and on day 10 for those exposed to waterlogging alone. Symptoms progressed with time and included leaf chlorosis, rolling and wilting,

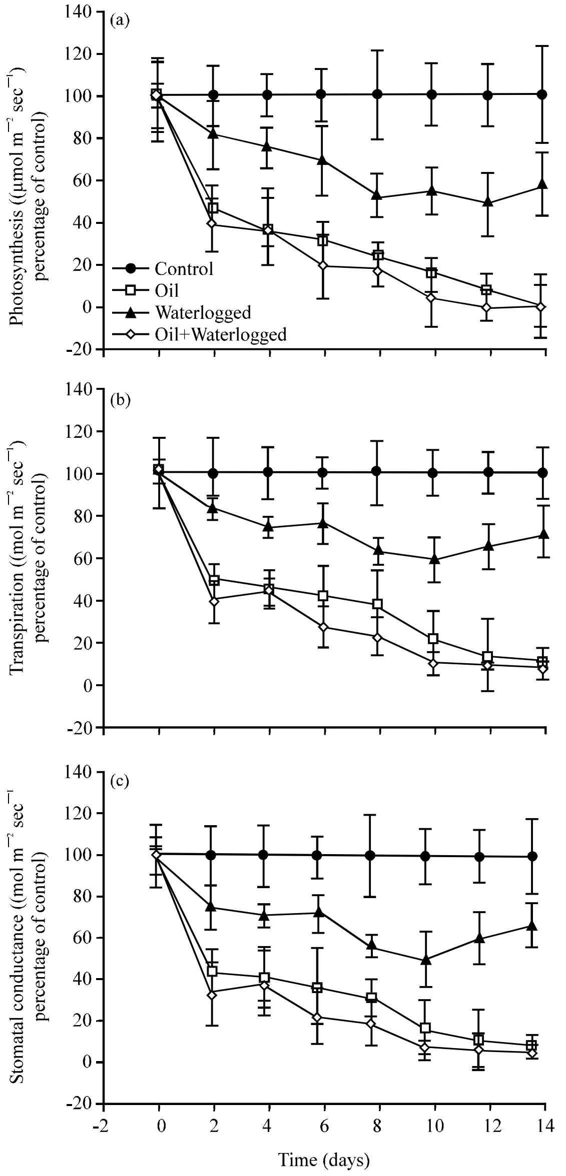


Fig. 1: Effects of oil, waterlogging and combined oil and waterlogging on. a) photosynthesis; b) transpiration and c) stomatal conductance in bean over a 14 days exposure period. Treatments are denoted by the key. Error bars = 1 ×Standard error, n = 8

the thinning of canopies and slower growth. Control plants displayed no visual symptoms of stress during any stage of the experiment.

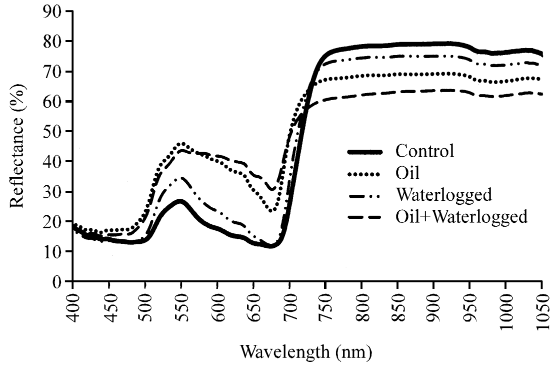


Fig. 2: Mean reflectance spectra for control and treated bean plants 14 days after treatments commenced (n = 64). Treatments are denoted by the key

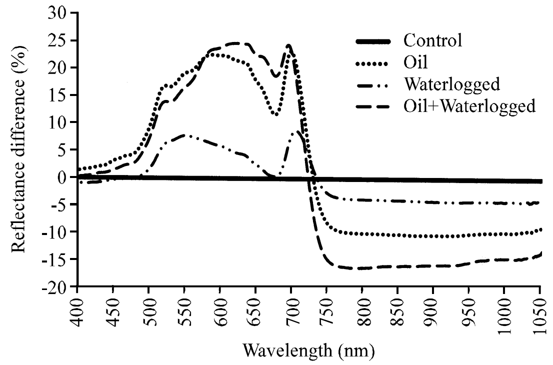


Fig. 3: Differences between mean reflectance spectra for control and treated bean plants 14 days after treatments commenced (n = 64). Treatments are denoted by the key

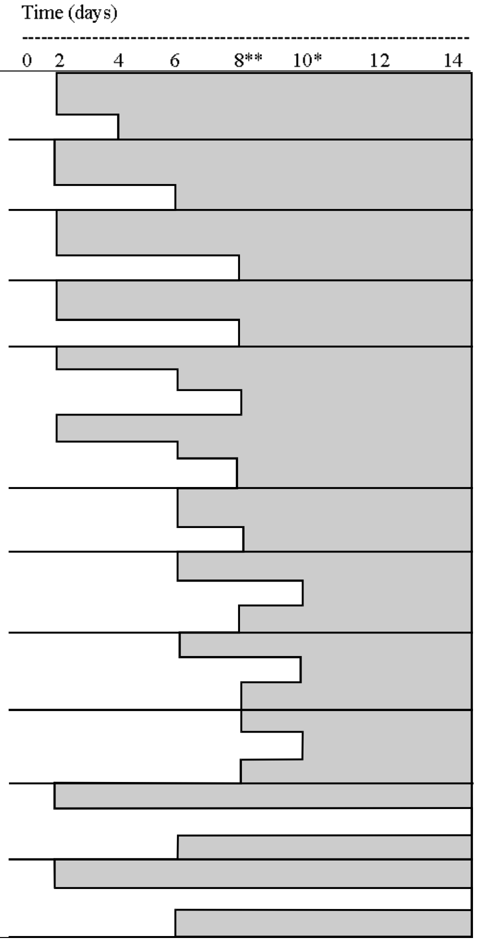
Spectral responses: In observing mean reflectance spectra responses (Fig. 2) it can be seen that there was a general increase in reflectance in the visible region (380-750 nm) and a decrease in the Near Infrared (NIR) region (750-2500 nm) in response to all treatments relative to control. Treatments containing oil produced a larger change in spectral reflectance than that produced by waterlogging alone.

By the end of the experiment, there was a significant increase in reflectance of plants treated with oil and the combination of oil and waterlogging in nearly all wavebands in the visible and red edge regions (Fig. 3). However, a significant increase in reflectance of waterlogged plants was only found in particular wavebands between 536-572 and 698-716 nm. The decrease in NIR reflectance was greatest for plants treated with the combined oil and waterlogging treatment and at the end of the experiment this difference was statistically significant. For those plants treated with oil and to waterlogging alone, the differences in NIR reflectance at the end of the experiment were not statistically significant.

Regarding spectral indices, their order as presented in Table 1 indicates the overall level of sensitivity to

Table 1: Sensitivity of spectral and thermal indices in control and treated plants over the course of the experiment. Unshaded = inconsistency or no significant difference; Shaded = consistent significant differences between treated plants and controls. *Time when stress symptoms were visually observed for the waterlogging treatment alone; **Time when stress symptoms were visually observed for the oil and the combined oil and waterlogging treatment

Spectral indices	--------------------Treatments----------------------	
R_{673}/R_{545}	Control	Oil stress
		Waterlogged
		Oil+Waterlogged
R_{673}/R_{631}	Control	Oil stress
		Waterlogged
		Oil+Waterlogged
R_{545}/R_{445}	Control	Oil stress
		Waterlogged
		Oil+Waterlogged
$(R_{755}-R_{716})/$ $(R_{755}+R_{716})$	Control	Oil stress
		Waterlogged
		Oil+Waterlogged
R_{826}/R_{545}	Control	Oil stress
		Waterlogged
		Oil+Waterlogged
R_{977}/R_{545}	Control	Oil stress
		Waterlogged
		Oil+Waterlogged
R_{631}/R_{445}	Control	Oil stress
		Waterlogged
		Oil+Waterlogged
R_{826}/R_{631}	Control	Oil stress
		Waterlogged
		Oil+Waterlogged
R_{977}/R_{631}	Control	Oil stress
		Waterlogged
		Oil+Waterlogged
REP (nm)	Control	Oil stress
		Waterlogged
		Oil+Waterlogged
Absolute temperature (°C)	Control	Oil stress
		Waterlogged
		Oil+Waterlogged
Ig	Control	Oil stress
		Waterlogged
		Oil+Waterlogged



treatments (n.b. indices that did not respond to at least one treatment type are not shown in Table 1). As can be seen, the ratio R_{673}/R_{545} showed a statistically significant response to oil and waterlogging treatments alone on day 2 of the experiment and a significant response to the combined oil and waterlogging treatment on day 4. As Table 1 shows, several other spectral indices had equally rapid responses to the individual oil and waterlogging treatments as for the R_{673}/R_{545} ratio but slightly slower responses to the combined treatment. The temporal response of R_{673}/R_{545} expressed as a percentage of control is shown in Fig. 4.

For plants exposed to treatments containing oil, the R_{673}/R_{545} ratio increased relative to the controls, initially displaying a gradual change and then a larger exponential response beyond day 10 of the experiment. In plants treated to waterlogging alone the spectral ratio decreased initially then increased relative to the control for the remainder of the experiment but the response was always less than for plants with treatments containing oil.

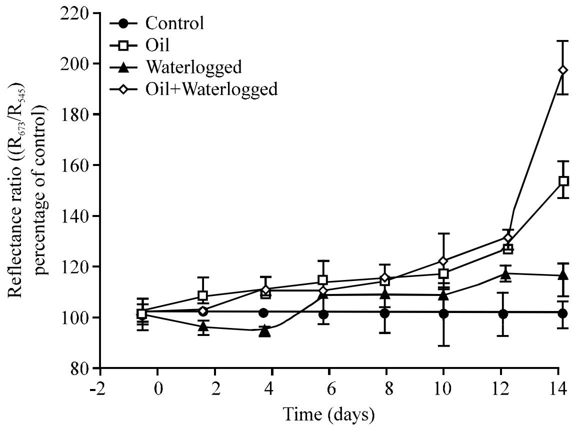


Fig. 4: Temporal response of R_{673}/R_{545} for control and treated bean. Treatments are denoted by the key. Error bars = 1×Standard error; n = 8

Both the treated and control plants showed single peaks in the first derivative of reflectance in the red-edge

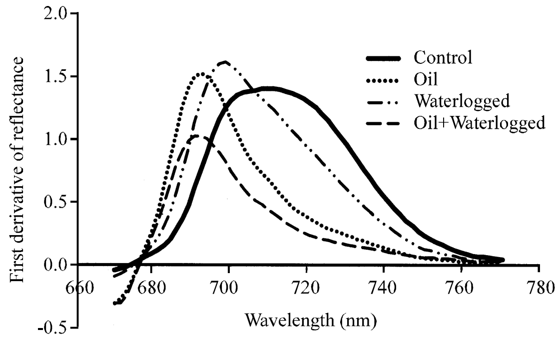


Fig. 5: First derivative of reflectance of control and treated bean 14 days after treatment. Treatments are denoted by the key

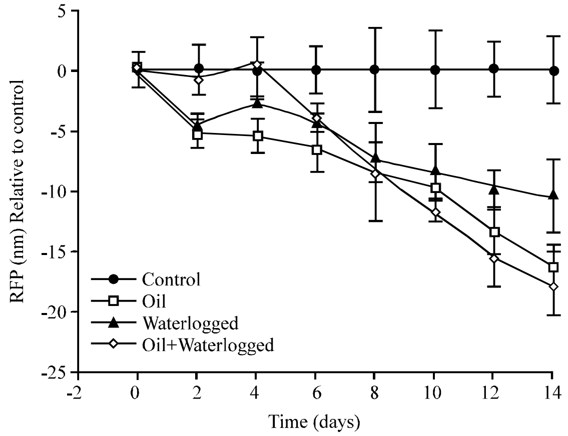


Fig. 6: Temporal change in REP of control and treated bean. Treatments are denoted by the key. Error bars = 1×Standard error, n = 8

region of the spectrum as shown in Fig. 5 for mean spectra at the end of the experiment. There was some variation in the amplitude of the first derivative in the red edge region however such changes were not statistically significant at any point of the experiment.

Figure 6 shows the changes in REP for the different treatments over the course of the experiment. The REP became significantly different to the controls on day 8 for the oil and combined oil and waterlogging treatments and on day 10 for the waterlogging alone. By the end of the experiment, there was a total shift of 5, 12 and 16 nm to shorter wavelengths for the waterlogging, oil and the combined oil and waterlogging treated plants, relative to the control (Fig. 6).

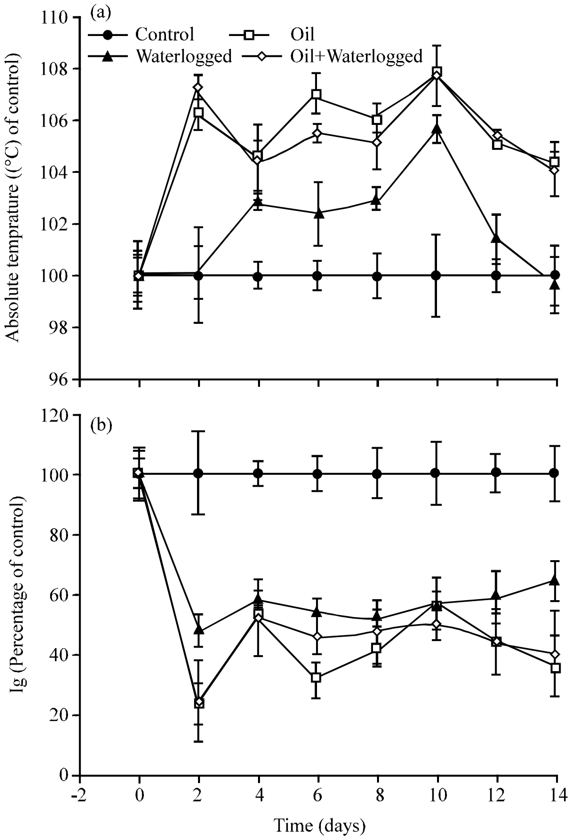


Fig. 7: Temporal changes in: a) canopy absolute temperature and b) thermal Index (Ig) of treated and control plants. Treatments are denoted by the key. Error bars = 1×Standard error; n = 8

Thermal responses: The canopy temperature of treated plants was higher than the controls from day 2 of the experiment onwards for the oil and combined oil and waterlogging treatments (Fig. 7a). Plants exposed to waterlogging stress showed a less systematic response in terms of absolute canopy temperature with significantly higher temperatures than controls only occurring on certain days part way through the experiment.

Treated plants showed a systematic decrease in Ig relative to the controls from day 2 onwards (Fig. 7b). This effect was consistent across all treatments. It was apparent that Ig for waterlogged plants decreased to a lesser extent than that for oil and combined oil and waterlogged treatments. The significance of the changes in canopy absolute temperature and Ig in response to the treatments and the timing of the responses are summarised in Table 1. This demonstrates the consistent response of absolute temperature and Ig to treatments that involved oil but the lack of a consistent response to waterlogging alone.

DISCUSSION

The results demonstrate that whenever oil was present within a treatment, there was a greater impact on plant physiological responses than with the waterlogging treatment alone. Oil can have detrimental effects on plants and through a multitude of different mechanisms such as soil oxygen depletion, reduced water uptake and toxic effects (Noomen *et al*., 2003; Schumacher, 1996; De Jong, 1980; Rowell, 1977; Wyszkowski *et al*., 2004). In the case of oil pollution, soil oxygen is further reduced by an increase in demand for oxygen brought about by the activities of oil-decomposing micro-organisms (Gudin and Syratt, 1975) which may not occur under waterlogged conditions. Furthermore, oil reduces the available nitrogen content of the soil (Sojka *et al*., 1975) which results from consumption of all available nitrogen by bacteria and fungi growing on a hydrocarbon medium thus restricting the uptake of these elements by plants (Xu and Johnson, 1997). These effects are exacerbated by depression in ammonification and nitrification processes triggered by inhibition in the conversion of mineral and organic nitrogen compounds in soil by petroleum derived compounds (Amadi *et al*., 1996). Furthermore, oil can have toxic effects on plants by penetrating into plants/leaf tissue and consequently damaging cellular integrity and preventing leaf and shoot regeneration (Webb, 1994; Pezeshki *et al*., 1995, 2000). This combination of effects from oil may well explain the greater impact of treatments involving oil compared to waterlogging, found in this study.

The results concur with the findings of earlier investigations where waterlogged conditions were found to significantly reduce the photosynthetic rates of a wide range of plant species such as oilseed rape, bentgrass and barley (Zhou and Lin, 1995; Baldock *et al*., 1987; Dormaar, 1988; Yordanova *et al*., 2005). Bradford and Yang (1981) reported that decreased leaf water potential (Ψ) does not always accompany flooding injury; in most cases (Ψ) remains unaffected or increases in flooded plants. This suggests that the decrease in the photosynthetic rate of bean by waterlogging observed in the present study may be as a result of non-stomatal responses such as soil oxygen depletion. Similar observations were made by Bradford (1983) who reported that the photosynthetic rate of flooded tomato plants remained constant or declined at high intercellular CO_2 concentration. The researcher attributed this to non-stomatal (biochemical) factors such as inability for Ribulose-1,5-Bisphosphate (RuBP) regeneration within the Calvin cycle. Indeed, it has been demonstrated that prolonged flooding causes root injuries that restrict photosynthetic capacity by altering the biochemical reactions of photosynthesis (Yordanova *et al*., 2005).

Many investigations have focused on the short term effects of waterlogging on plants. For example, Else *et al.* (2001) found that soil flooding reduced stomatal conductance, transpiration, CO_2 uptake and leaf elongation in *Ricinus communis* within 2-6 h. Zang and Zang (1994) observed in pea that stomata begin to close in the first few hours of flooding with a parallel decrease in transpiration and stomatal conductance. Yordanova *et al.* (2005) investigated the impact of short-term soil flooding on stomatal function and morphology and on leaf gas exchange in barley leaves. They observed decreases in transpiration and stomatal conductance within a short period of time (2-24 h) after the onset of waterlogging. In the present study, observations are based on responses to prolonged waterlogging conditions over the course of 2 weeks. Nevertheless a significant physiological effect was observed on the second sampling occasion, 2 days after the start of the experiment which concurs with the findings of the previous short-term studies.

Stress conditions in plants are known to result in changes in the reflectance spectra of leaves and canopies (Knipling, 1970; Noomen *et al*., 2003; Kempeneers *et al*., 2005) and can be detected before symptoms are observed visually (Carter *et al*., 1996). In the study of Carter *et al*., (1996), herbicide-induced stress was detected 16 days prior to the first visible signs of damage. This finding concurs with the results obtained in the present study where oil, waterlogging and combined oil and waterlogging-induced stress were spectrally detected 6, 8 and 4 days before observation of visual stress symptoms (leaf chlorosis, rolling and wilting), respectively. Likewise, oil-related stress was thermally detected 6 days (for oil treatment alone) and 2 days (for the combined oil and waterlogging treatments) before the observation of visual symptoms.

Substantial changes in spectral reflectance were observed in relation to all of the treatments. In particular, a rapid and significant increase in reflectance was found in the green and red-edge regions. These results are in line with the findings of Anderson and Perry (1996) where reflectance of red maple leaves increased at 550 nm as a result of flooding in wetland areas. Similarly, Smith *et al.* (2004) found a significant increase in reflectance within the regions from 508-654 and 692-742 nm in dwarf bean treated to a waterlogging stress. The green and red-edge are regions of weak absorption by the chlorophylls (Zwiggelaar, 1998). As has been observed earlier, reflectance is more sensitive to high concentrations of pigments at wavelengths where the absorption coefficients of pigments are low (Jacquemoud and Baret, 1990; Yamada and Fujimara, 1991). Hence, the reflectance changes observed indicate that oil and waterlogging caused a small decrease in chlorophyll but that overall concentration remained high, at least in the initial stages of the experiment.

Such observations provide some explanation of why a ratio of reflectance that combined narrow wavebands in the green and red regions (R_{673}/R_{545}) was most sensitive for detecting stress symptoms caused by oil and waterlogging. In this case, the green band appears to be responding rapidly to subtle changes in chlorophyll concentration at the initial stages of treatment while the red band, at the centre of a major chlorophyll absorption feature, remains invariant due to insufficient changes in chlorophyll concentration. Earlier investigations also found that changes in ratio of reflectance in the red and green wavebands (670~680/555~565 nm) was useful in detecting plant stress caused by elevated natural gas in soils due to pipeline leaks (Smith *et al*., 2005). The ratio of reflectance was found to increase in response to stress. Similarly, the researchers found that the ratio of reflectance increased in response to herbicide-induced stress in field grown oilseed rape (*Brassica napus*). Hence, this spectral ratio appears to be a sensitive indicator of plant stress induced by a range of different factors.

Various single stresses have been found to cause minimal reflectance change in the NIR. Smith *et al*. (2004) found a small change in the NIR reflectance in bean and barley that had been waterlogged. This concurs with the results which show no significant reflectance difference in the wavelength ranges 723-1050 nm and between 717-1050 nm in plants treated with oil and waterlogging, respectively. However, plants treated with a combination of oil and waterlogging showed significant reflectance differences in the wavelength ranges 739-1050 nm. This suggests that multiple stresses such as the combination of oil and waterlogging expectedly may have done greater damage to the leaf cellular structure of bean than a single stress alone.

All treatments resulted in plants showing single peaks in the first derivative of reflectance in the red-edge region of the spectrum. In a similar way, single peaks were observed by Smith *et al*. (2004) for bean treated with different stresses such as waterlogging, natural gas and argon. On the contrary, Smith *et al*. (2004) found double peaks in barley treated with the same stresses as bean. It has been suggested that differences between bean and barley in the shape of the peak that defines the red edge may be related to the different leaf structures of monocotyledons and dicotyledons Smith *et al*. (2004). Researchers noted that the internal structures of mono and dicotyledons differ and that in dicotyledons, the upper and lower epidermises are separated by the spongy mesophyll containing many air spaces. The leaf of a monocotyledon is more compact with fewer air spaces (Gausman, 1985). Since, the spongy mesophyll in a leaf of a dicotyledon is more developed with more air spaces than the leaf of monocotyledon, the reflectance of the former is generally higher than that of the latter (Gausman, 1985; Guyot, 1990) and thus, allows more light scattering between the cell walls (Smith *at al*., 2004). Since, the red edge is influenced by low reflectance caused by strong chlorophyll absorption in the red region and high reflectance in the NIR caused by leaf cellular structure, differences in reflectance due to leaf structure may affect the shape of the peak of the red edge in the first derivative in this region (Smith *at al*., 2004).

The REP of bean which is defined by the wavelength of the single peak in the first derivative spectrum, appears to be a stable indicator of stress induced by the three types of treatment but only in the later stages of the experiment. The results show that the REP shifted significantly towards shorter wavelengths for the plants treated with oil and the combined oil and waterlogged treatment on day 8 and for the waterlogged plants on day 10. This concurs with previous findings where the REP shifted towards the shorter wavelengths as plants became stressed (McCoy *et al*., 1989; Reid *et al*., 1988; Crawford, 1986; Cwick *et al*., 1995). Soil oxygen displacement was found to cause inconsistent change in the magnitude of the first derivative at the position of the red edge in bean and barley which either increase or decrease relative to the control (Smith *et al*., 2004). As may have been the case in the study, the change was attributed not only to decreasing amount of total chlorophyll but also to change in the ratio of chlorophyll a to chlorophyll b in the exposed plants. The absolute temperature of bean canopies under all treatments was higher than controls, suggesting that canopy temperature was a useful indicator of stress. On the contrary, Grant *et al*. (2006) detected no significant differences between leaf temperatures of well-watered control grapevines and those subjected to water stress. This was related to greater environmental variation in a glasshouse experiment with relatively large plants. In the study, thermography was undertaken in a dark room where a consistent source of illumination was used and thus may have resolved glasshouse variability. The absolute canopy temperature was not consistently sensitive to waterlogging stress in bean and this can be explained by the smaller response of transpiration and stomatal conductance to waterlogging than to the treatments involving oil.

The Ig of plants treated with oil and combined oil and waterlogging was consistently lower than those of the controls. As stated earlier, Ig is proportional to canopy conductance and thus is likely to be responding to the effects of oil on stomatal function. The Ig was not consistently sensitive to waterlogging in bean which is again explicable in terms of the decreased response of transpiration and stomatal conductance to waterlogging as opposed to treatments involving oil. The lack of response may also be attributed to a possible cooling effect on the plant canopy caused by water evaporating from the waterlogged soil. It is likely that this cooling effect is decreased for the combined oil and waterlogging treatment as the immiscibility between oil and water creates a layer of oil at the surface of the waterlogged soil which limits evaporation.

The results of this study indicate that plant stress caused by oil pollution can be discriminated from plant stress caused by waterlogging by using a combination of thermal and spectral remote sensing. A simple decision-tree approach to this discrimination is demonstrated in Fig. 8. If absolute temperature increases or Ig decreases, this indicates that the stress is caused by oil pollution but if the thermal response does not change and a spectral index such as R_{673}/R_{545} increases then the stress is caused by waterlogging. If there is no thermal or spectral change then there is neither oil pollution nor waterlogging induced stress. In order to implement this type of approach in an operational monitoring programme, there would likely be a need to characterise the spectral and thermal responses of unstressed reference plants within the environment under investigation. This could be achieved using imagery acquired before an event or by identifying canopies within an image which are unaffected by pollution or waterlogging. Furthermore, it would be important to identify the appropriate thresholds to be used at each stage of the decision-tree process and these may vary according to factors such as vegetation species composition and other environmental considerations. Hence, substantial further research is required in order to translate this approach into an operational technique nevertheless the findings of this study provide the evidence that this is feasible in principle.

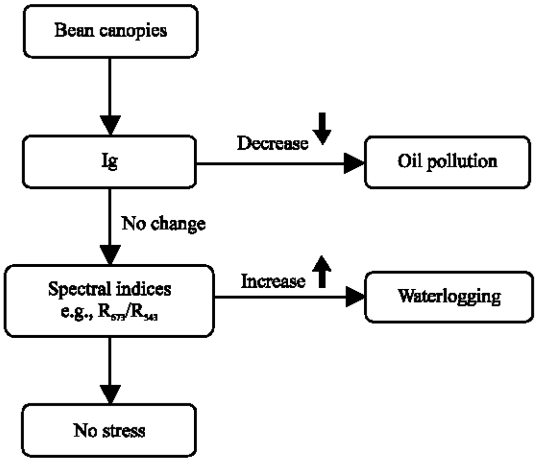


Fig. 8: Flowchart showing the approach for deploying remote sensing measure for discriminating between plant stress caused by oil pollution and waterlogging in bean canopies

CONCLUSION

Combined spectral and thermal remote sensing techniques were able to detect stress caused by oil far in advance of symptoms being observed visually, however only spectral sensing was able to provide a pre-visual indication of stress caused by waterlogging. Among various spectral indices tested for detecting stress symptoms caused by oil and waterlogging, a simple ratio of reflectance that combined narrow wavebands in the green and red regions (R_{673}/R_{545}) was most sensitive to both treatments. While the canopy absolute temperature and thermal index (Ig) were good indicators of developing oil and combined oil and waterlogging stress in bean, they were poor indicators of stress caused by waterlogging alone. Due to the similar physiological effects of oil and waterlogging stress on plants, it may be difficult to discriminate between the two causes of stress using spectral or thermal sensing alone. However, this study suggests that by using a combined dual approach of spectral and thermal remote sensing of plants, the presence of oil pollution can be detected and discriminated from waterlogging induced stress. Further research is needed to test the robustness of this approach on different plant species, across a range of spatial scales and in combination with further significant causes of plant stress such as water deficit.

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