Hyperspectral and Thermal Remote Sensing of Plant Stress Responses to Oil Pollution

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DECLARATION

I declare that this thesis is my own work, and has not been submitted for the award of any other degree at this institution or elsewhere.

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ABSTRACT

This study investigates the potential use of hyperspectral and thermal remote sensing for the early pre-visual detection and quantification of plant stress caused by oil pollution. Further, it examines the potential for these techniques to discriminate between oil pollution and two typically encountered plant stresses of waterlogging and water deficit. Results show that oil pollution, waterlogging and water deficit significantly decreased the physiological functions of plants and can result in pre-visual changes in spectral and thermal responses. Various spectral indices such as $(R_{755}-R_{716})/(R_{755}+R_{716})$ and R_{800}/R_{606} were efficient for the early detection of oil-induced stress in maize (up to 10 days earlier) and bean (up to 4 days earlier), respectively. These indices and other simple ratios of reflectance such as R_{673}/R_{545} were also sensitive in the early detection (up to 6 days earlier) of stress symptoms caused by waterlogging in bean. The canopy absolute temperature and thermal index (I_G) were good indicators of oil related stress in bean, but were insensitive to waterlogging. Absolute leaf temperature had minimal potential for detecting oil pollution in maize. While the spectral indices lacked ability for the early detection of stress caused by water deficit at the leaf scale in both maize and bean, absolute temperature was effective in this regard irrespective of scale of measurement. Results show that by combining spectral and thermal information, oil pollution can be discriminated from waterlogging or water deficit treatment. This study concludes that hyperspectral and thermal remote sensing have the potential to detect and quantify plant stress caused by oil pollution and it is possible to discriminate between this and other common stresses. However, further work is needed to refine and operationalise the approach, and the problems and challenges associated with this are presented and discussed.

CONTENTS PAGE

LIST OF FIGURES PAGE

LIST OF TABLE

PAGE

Chapter 1

INTRODUCTION

1.1 Background

Oil pollution is noted as one of the major causes of environmental degradation and can arise from spills of crude and refined oil in aquatic and terrestrial environments (Ogboghodo *et al.,* 2004). Possible sources include accidental oil well blow out, loading activities of oil tanks, tank washing activities of ocean going vessels, port and harbour run off from pipeline leaks and road tanker accidents. Equipment failure such as malfunctioning, overloading, corrosion or abrasion of parts has also increased the incidence of oil spills (Nwankwo and Ifeadi, 1986). In recent years, wilful vandalism of oil pipelines, particularly in some locales, has also contributed to the menace. For example, vandalism is a leading cause of oil spills in Nigeria today (Yo-Essien, 2008). The environmental, safety, economic and health implications of oil pollution cannot be over emphasised. Some hundreds of thousands barrels of oil are lost to the environment due to oil spill incidents (Aroh *et al.*, 2010). Available statistics show that, approximately three million, one hundred and twenty one thousand, nine hundred and ten barrels of oil were lost between 1976 and 2005 as a result of oil spills. Many lives have been claimed by oil spill disasters. For example, the Jesse (in Niger Delta) spill incident of 1998 resulted in a fire incident that claimed over a thousand lives and raved the fragile ecosystem (Yo-Essien, 2008). People have contacted various illnesses and diseases through drinking polluted water and eating contaminated food (Aroh *et al.*, 2010, Odu and Offodum, 1986). Furthermore, damage done to fishponds, nets and traps was put at over 2 million naira (Odu and Offodum, 1986).

Contamination of soils with petroleum products is becoming an ever-increasing problem, especially in the light of several breakdowns of oil pipelines and wells reported recently (Wyszkowski *et al.,* 2004). For safety and security reasons, oil facilities such as pipelines are kept constantly under surveillance. This is done in several ways such as foot patrols by appointed officials and intermittent aerial surveillance particularly the critical sections of the pipelines using manual observations from aircraft. The overall aim is to guard the pipeline from damage and to look out for possible leaks. Despite the security and safety measures in place, reports of oil leaks and spills with disastrous effects continue to rise rapidly, especially in some parts of the world. For example, Nigeria which is the largest oil producer in Africa and the sixth largest in the world recorded a total number of 4,835 oil spill incidents between 1976 and 1996 and 2,097 between 1997 and 2001 (Nwilo and Badejo, 2004). In addition, 253, 588, and 419 oil spill incidents were reported in 2006, 2007, and first two quarters of 2008, respectively (Edem, 2008).

The aerial surveillance of oil pipelines and facilities is costly, has flight risks associated with low level aircraft and relies absolutely on the accuracy of the pilot (Smith *et al.*, 2004). Foot patrol is tedious and time consuming and cannot cover a large area. It is also logistically difficult in inaccessible areas and hostile environments. If not detected and stopped early, oil leaks can develop into massive spills, leading to fire outbreak which can be very disastrous. This has safety, health, economic and environmental implications including soil contamination, destruction of vegetative ecosystems and arable crops/lands, contamination of surface and underground water, air pollution and extinction of endangered species. Thus, given the severe limitations and demonstrable ineffectiveness of current surveillance approaches, it is imperative that a technique is developed for frequent, accurate and spatially-comprehensive monitoring and detection of oil pollution.

1.2 Effects of oil pollution on plants: threats and opportunities

Plants are extremely important in the lives of people throughout the world. People depend upon plants to satisfy such basic human needs such as food, clothing, shelter and health care. These needs are growing rapidly because of a growing world population, increasing income and urbanisation. Unfortunately under field conditions, plants are constantly vulnerable to a wide range of biotic, abiotic and anthropogenic stress inducing factors within the growth environment, which consequently alter their physiological and biochemical functioning. In regions of oil exploration and exploitation, oil pollution regularly affects subsistence crops and natural vegetation growing across a range of hydrological settings from wetlands through to arid environments. Previous investigations have found that plants are influenced considerably by hydrocarbon pollution. Thus, identification of the best approaches for monitoring and detecting the menace of oil pollution in the environment remain a subject of growing concern.

Today, there is a growing interest in the study of plant stress caused by various agents through a multitude of different mechanisms, such as soil oxygen depletion, increased carbon dioxide (CO_2), reduced water uptake and toxic effects using remote sensing techniques (e.g. Masoni *et al.*, 1996; Peñuelas *et al.*, 1997; Riedell and Blackmer, 1999; Else *et al.*, 2001; Wyszkowski *et al.*, 2004; Dobrowski *et al.*, 2005; Thomas, 2005; Yordanova *et al.*, 2005; Ladjal *et al.*, 2007; Graeff and Claupein, 2007). The logic behind the approach is that unfavourable growing conditions result in morphological, physiological and/or biochemical changes that impact on the manner with which plants interact with light (Liew *et al.*, 2008). For example, changes have been observed in biochemistry and reflectance in vegetation growing near natural hydrocarbon seeps (Lang *et al.,* 1985, Bammel and Birnie, 1994, Yang *et al.,* 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.

Changes in the rate of transpiration by plants can also 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. Jong (1980) observed that oil markedly decreased water uptake by wheat from contaminated soil layers or from deeper water tables below. 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 the fluxes of air and water, leading to a decrease in crop yield. This presumably is due to anoxia, decreased nutrient and water uptake, 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. In combination, several remotely-sensed spectral and thermal parameters have been identified as viable indicators of plant stress, but their potential in the early detection of oil-induced stress is poorly understood.

In the real world, other natural stress occurring factors such as waterlogging and water deficit affect plants and this can occur separately or concurrently with oil pollution. Land degradation and serious environmental and poverty impacts have been associated with waterlogging (World Bank, 1994). Waterlogging can cause stress in plants by displacing the oxygen in soil by filling the soil spaces with water and thus limiting oxygen supply to roots and preventing carbon dioxide from diffusing away (Smith, 2004a). The principal causes of waterlogging are irrigation without drainage, over-irrigation, low delivery efficiency of the irrigation and malfunctioning of the drainage system (Mirani and Memon, 2001). Waterlogging is a typical problem in many river valleys and delta areas where farmlands are constantly affected. For example, in many river valleys and deltas at the western foot of the Andes along the coast of the Pacific Ocean more than 30% of the agricultural land is affected by waterlogging due to irrigation of the higher-lying lands (De la Torre, 1987). Oils are also found in delta regions and thus, there is the possibility that oil pollution which can arise from exploration and exploitation activities and waterlogging can affect plants in such regions singly or collectively. Thus, there is the need to develop an approach that can be used in discriminating between oil pollution and waterlogging. It has been found that waterlogging can instigate malfunctioning of the root thus, it is expected that such conditions could result in reflectance changes commonly related to plant stress, such as increased reflectance in the chlorophyll and water absorption regions (Carter, 1993; Lichtenhaler *et al.*, 1996). Indeed, some studies have shown that waterlogging can be detected in plants using changes in reflectance spectra (Anderson and Perry, 1996; Pickering and Malthus, 1998; Smith *et al.*, 2004a). However, there is a poor understanding of the capabilities of thermal remote sensing in this context.

It is generally known that water is a vital component for all forms of life but unfortunately, water deficit is identified as one of the major naturally occurring stress factors. In plants, water plays a key role in photosynthesis and the movement of nutrients; as water evaporates from the surface of leaves, it pulls water upwards from the root system thus, transporting nutrients and other solutes to the above ground components of the plant (Audesirk and Audesirk, 1999). When water is in short supply, plants become stressed as the amount of water taken up by the roots is unable to keep up with the rate of evaporation of water from the leaves. Thus, the leaves of the plant begin to wilt as the amount of water present within the leaf tissue decreases. Water stress is typically well developed and negatively affecting the plant before it is detected visually, as visual detection of water stress already indicates high levels of water stress (Griffeth III, 2009). Therefore, there is the need for early detection of stress caused by water deficit in order to facilitate timely delivery of remedial measures which can enhance plant growth and productivity. Also, since water deficit is an important biotic stress agent that can affect plants singly or concurrently with other stresses such as oil pollution, therefore, there is the need to develop an approach that can be used in discriminating between them.

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 have been shown to be sensitive to different forms of plant stress, there is little evidence with respect to oil pollution. Moreover, with these water-related stresses being commonplace, it is likely that oil-induced stress will occur in combination with water-related stress. Yet, little work has been done in the use of remote sensing technology for detecting, quantifying and discriminating between these stresses.

1.3 Research aims and objectives

Remote sensing technology has been identified as a useful tool for monitoring vast areas of land surface and it is also viable in ecological studies such as in monitoring plant health status. For early detection and accurate monitoring of oil pollution, there is the need to develop a system that is sensitive to physiological changes in plants prior to visual stress observation. Thus, this study investigated the potential of hyperspectral reflectance and thermal information for detecting and quantifying plant stress induced by oil pollution. Furthermore, it examined the potential of these remote sensing techniques for discriminating between oil-induced stress in plants and other stresses caused by waterlogging and water deficit. In order to achieve this aim, the study was motivated by the following four scientific questions:

- What is the optimum remotely-sensed index for early detection of oil-induced stress in plants at lethal and sub-lethal levels?
- What is the optimum set of spectral and thermal responses that can be used for early, non-destructive quantification and discrimination between oil pollution and waterlogging stress in plants?
- What is the optimum set of spectral and thermal responses that can be used for early, non-destructive quantification and discrimination between oil pollution and water deficit stress in plants?
- How consistent are the spectral and thermal responses of plants to oil and water deficit stress between species and across leaf and canopy scales?

1.4 Research outline

The thesis commences with a literature review as presented in chapter 2. The details about the effect of oil on soils and plant are discussed. Specific reference is made on the use of remote sensing techniques for monitoring the effects of a wide range of stress factors that affect plant, and to provide the conceptual basis for developing techniques for remote detection of oil-induced stress. Generally, the chapter aims to understand the background theory and general discussion going on in this area of study and to identify gaps that would ultimately be covered. Chapter 3 presents the general methodology adopted in this study. Chapter 4 starts with a pilot study with the aim of testing overall feasibility, logistics and some of the proposed experimental designs. Primarily, the impacts of oil pollution on the physiological, optical, and thermal properties of maize (*Zea mays* L.) are investigated in this chapter. In chapter 5, the spectral and thermal response of stress in bean (*Phaseolus vulgaris 'Tendergreen'*) canopies caused by oil pollution and waterlogging are explored with the aim of identifying the optimum set of responses that could be used for early, non-destructive quantification and discrimination between the two stresses. Chapter 6 exploits spectral and thermal responses of maize leaves for early detection and discrimination of stress caused by oil pollution and water deficit. In chapter 7, the spectral and thermal responses of bean for early detection and discrimination of stress caused by oil pollution and water deficit are explored with the aim of determining whether the responses translated from leaf to canopy scale. Finally, chapter 8 summarises the main conclusions of this study and presents a synthesis of the whole thesis and suggestions for possible areas for further investigations.

Chapter 2

LITERATURE REVIEW

2.1 Introduction

Recent studies have identified remote sensing as a valuable tool for detecting oil spills in the environment. Remote sensing applications in spill detection have mostly been in the marine environment using a variety of sensors operating across the optical to microwave domains. Consequently, there is a considerable body of literature in this area. However, spill monitoring and detection in the terrestrial environment has received inadequate attention. However, to address some of the needs of agricultural, ecological and environmental sectors, earlier and on-going studies have led to quantitative estimation of the biochemical, biophysical, and physiological properties of plants using various remote sensing techniques. Information about these properties is generally useful in predicting the health status of vegetation. The emergence of hyperspectral remote sensing technology has further promoted applications in this area. The high spectral resolution data provided by hyperspectral remote sensing systems has created an opportunity for remote sensing of vegetation stress caused by various environmental factors in a way that was not possible using traditional broad band multispectral data. Environmental stressors are diverse in nature and range from biotic to abiotic factors. The focus of this review is on the use of remote sensing technologies for monitoring, and discriminating the effects of these factors on plant, and to provide the conceptual basis for developing techniques for remote detection of oil-induced stress.

2.2 Plants

Plants growing in a particular place play an essential role to humans and their environment. Plants are very crucial for a sustainable ecosystem, as they coexist and live inter-dependably with humans and animals. They provide a necessary habitat for wildlife populations and are the ultimate sources of metabolic energy for fauna. The Iowa Department of Transportation (2007) noted that 25 percent of all prescriptions written annually in the United States contain chemicals from plants and that many important drugs are yet to be discovered. In addition, about 98 percent of plant species are yet to be tested for their medical potential. Plants are good sources of some industrial products, they aid in erosion control and enhance both air and water qualities. They positively influence regional climate and plant communities form the basis for many important recreational activities.

2.2.1 Plant stress

Plant stress describes any unfavourable condition and environmental constraints that are faced by plants. Osmond *et al.* (1987) reasoned that plant stress has general connotations rather than a precise definition. Thus, while attempting to make plant stress a measurable and meaningful term, their study defined it as any factor that decreases plant growth and reproduction below the genotype's potential. Similarly, Jackson (1986) defined plant stress as any disturbance that adversely influences vegetation growth. Potentially, adverse environmental conditions affect plant growth and development and trigger a wide range of responses, from altered gene expression and modifications in cellular metabolism to changes in growth rate and crop yields (Kacperska, 2004).

Plant fitness depends on acquiring sufficient resources for growth and reproduction. However, an optimal environment for plant growth varies with plant species and growing stage (Hashimoto, 1989) and there is an understanding that environmental stress may retard plant growth yet improve its quality. For example, Lichtenthaler (1998) suggested that a mild stress may activate cell metabolism and increase the physiological activity of the plant, without causing any damaging effects even at a long duration. On the other hand, high stress will cause damage to the plant and induce early senescence and finally death if the stressor is not removed (Smith, 2002). An optimal environmental condition for plant growth is not defined because, as environmental conditions vary, so the adaptability of various plant species to change varies.

Plants are constantly threatened by either nature or humans or both. Table 2.0 illustrates examples of natural and anthropogenic stress factors. Crude petroleum, petroleum by-products and heavy metals are the most prevalent industrial pollutants (Rosso *et al.*, 2005). Previous investigations have found that hydrocarbon influences the soil and vegetation around hydrocarbon seepage (Noomen *et al.*, 2003). Displacement of soil oxygen by natural gas leaking from pipelines into the soil was the main damaging effect on plant growth (Smith, 2002). Van Der Meijde *et al.*, (2004) found that fields directly above the gas pipeline show significant increase in vegetation stress possibly due to gas leaks. This is because one of the major environmental problems related to pipelines is the leakage of hydrocarbons into the environment.

Table 2.0 List of natural and anthropogenic stresses acting on terrestrial vegetation.

I. Natural stress factors:

- high irradiance (photoinhibition, photooxidation)
- heat (increased temperature)
- low temperature (chilling)
- sudden and late frost
- water shortage (desiccation problems)
- natural mineral deficiency (e.g. nitrogen shortage)
- long rainy periods
- insects
- viral, fungal, and bacterial pathogens

II. Anthropogenic stress factors:

- herbicides, pesticides, fungicides
- air pollutants (e.g., SO_2, NO, NO_2, NOx)
- ozone (O_3) and photochemical smog
- formation of highly reactive oxygen species
- ($1O_2$, radicals O_2- and OH•, H_2O_2)
- photooxidants (e.g. peroxyacylnitrates)
- acid rain, acid fog, acid morning dew
- acid pH of soil and water
- mineral deficiency of the soil, often induced by acid rain
- oversupply of nitrogen (dry and wet NO_3- deposits)
- heavy metal load (lead, cadmium, etc.)
- spills from petroleum
- overproduction of NH_4+ in breeding stations (uncoupling of electron transport)
- increased UV radiation (UV-B and UV-A)
- increased CO_2, global climate change

Adapted from Lichtenthaler (1998)

Hydrocarbons can establish locally anomalous zones that favour the development of a diverse array of chemical and mineralogical changes (Van Der Meijde *et al.*, 2004). Thus, any vegetation present in these zones is likely to be influenced by the hostile and polluted environment. Furthermore, Godwin *et al.*, (1990) found restricted growth and reproduction, and decreased number of individuals of plants subjected to natural gas leakage into the surrounding soil. Plant stress creates all manner of visible and invisible stress conditions such as etiolating, wilting, leaf colouring, stomatal closure, poor crop yield, and early senescence. Smith *et al.* (2005) recorded visible evidence in vegetation change around gas leaks. Unfortunately, stress conditions cannot be completely avoided due to the nature of their causative factors. However, they could ultimately be mitigated if detected on time.

2.3 Impact of oil on soils and plants

Oil is known to exert adverse effects on soil properties and plant communities (Osuji and Nwoye, 2007). Crude oil in soil makes the soil condition unsatisfactory for plant growth (De Jong, 1980), due to the reduction in the level of available plant nutrients or a rise in toxic levels of certain elements such as iron and zinc (Udo and Fayemi, 1995). Beyond 3% concentration, oil has been reported to be increasingly deleterious to soil biota and crop growth (Baker, 1976; Amadi *et al.*, 1993; Osuji *et al.*, 2005). Crude oil is composed of the following elements or compounds: Carbon – 84%, hydrogen – 14%, sulphur – 1 to 3% (hydrogen sulfides, sulfides, disulfides, elemental sulphur), nitrogen – less than 1% (basic compounds with amine groups), oxygen (O_2) – less than 1% (found in organic compounds such as CO_2, phenols, ketones, carboxylic acids), metals – less than 1% (nickel, iron, vanadium, copper, arsenic), salts – less than 1% (sodium chloride, magnesium chloride, calcium chloride) (Freudenrich, 2008).These compounds are largely responsible for changed fertility of soil (Tyczkowski, 1993; Iwanow *et al.*, 1994) and properties which can result to damage of organisms such as plants growing therein (Figure 2.0). Soil fertility may be defined as the capacity of the soil to support the growth of plants on sustained basis under given conditions of climate and other relevant properties of land (Aina and Adedipe, 1991). Loss of soil fertility and other forms of soil degradation are major problems associated with agricultural productivity in the oil producing areas of Nigeria (Osuji and Nwoye, 2007) perhaps, due to the frequent occurrence of oil spills in the environment. A study conducted for NEST/FORD FOUNDATION in the Niger Delta, NDES (1999) reported that soil fertility loss and declining crop yield were found to be indirect sources of pressure on natural resources and community structure, especially among the poor.

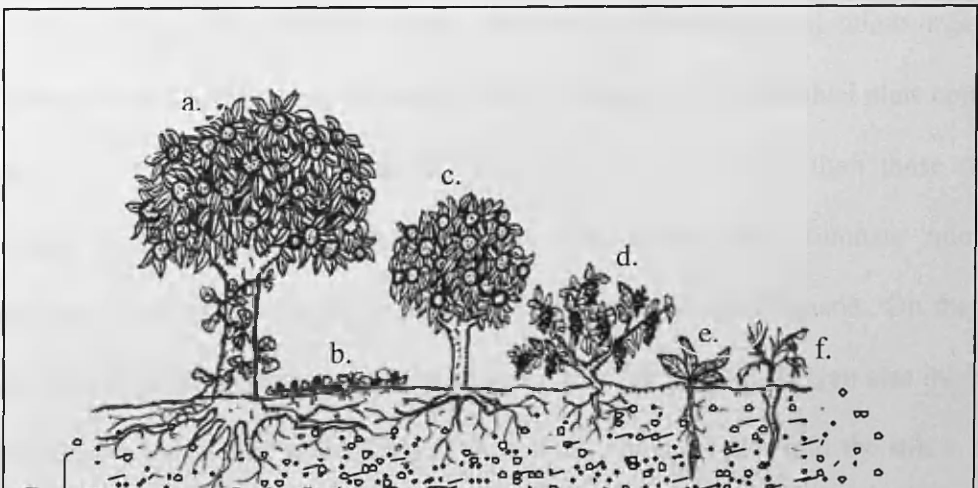

Figure 2.0 Schematic of plant canopies (a-f) and soil structure (g).

2.3.1 Effects of oil on soil

Oil can change the mineralogy of the soil and can displace soil air, including the oxygen. Indeed, previous studies noted that oil leads to depletion of oxygen or insufficient aeration in the soil (Rowell, 1977; De Song, 1980; Schumacher, 1996; Noomen *et al.*, 2003) and prevents water from entering the soil layers (Wyszkowski *et al.*, 2004). Soil fertility is influenced by the activity of bacteria and fungi, thus, oxygen deficit in the soil gives rise to changes in the reduction-oxidation potential and soil pH. The pH of the oil-impacted soils was found to be significantly lower than the uncontaminated soils (Osuji and Nwoye, 2007). This was attributed to possible disruption of leaching of basic salts which are responsible for raising pH in non-contaminated soils. In general, these activities create imbalances in the metabolic functions of plant organisms, thereby introducing stress, as their normal growth and general health condition are disrupted. 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). Lee and Banks (1993) found that the microbial plate counts in petroleum contaminated vegetated soil were significantly higher than those of un-vegetated contaminated soil. This indicates that plant roots stimulate microbial populations in polluted soils which promote degradation of contaminants. On the other hand, as the microbial population in the soil increases demand for oxygen also increases. Overall, soil aeration can be depleted if the rate at which oil gets into the soil is faster than the rate the oil is degraded by microbes.

Furthermore, when oil covers the soil surface, oxygen movement into the soil is restricted which can lead to more anaerobic soil conditions (Ranwell, 1968; Cowell, 1969). Apparently, CO_2 increases with decrease in O_2 thus, depletion of O_2 in the soil as a result of effects of hydrocarbon and activities of microbial will invariably lead to increased concentration of CO_2 in the soil (Hillel, 1998). Accumulation of CO_2 in the soil may affect the water permeability of roots more directly than O_2 deficiency and a buildup of inhibitory concentrations of ethylene in anaerobic soils may affect plant growth (de Wit, 1978; Trought and Drew, 1980a). Soil O_2 depletion can disrupt root metabolism which, in turn, can affect the hormone balance of the shoot (Trought and Drew, 1980b). A number of factors such as soil type, soil organic matter, size fraction of soil mineral matter (Figure 2.0) and soil texture play significant roles in the fate of hydrocarbon in the soil and have extensively been reviewed elsewhere (Pezeshki *et al.*, 2000). Generally, oil has adversely affected soil drainage. Earlier studies found that oil reduced water infiltration (Toogood, 1977; Everett, 1978) in mineral soils and this was attributed to a decrease in soil permeability resulting from the formation of hydrophobic films on soil particles. Similarly, Gill *et al.* (1992) reported that fresh crude oil showed a coagulatory effect on the soil, binding the soil particles into a water impregnable soil block which seriously impair water drainage and oxygen diffusion. Gassed soil deteriorates soil drainage so that the soil constantly puddled (Schollenberger, 1930; Hoeks, 1972). Godwin *et al.* (1990) also found that the soil drainage was decreased in the vicinity of gas wells and that puddles formed at the surface.

Oil reduces the available nitrogen content of the soil (Sojka *et al.*, 1975; Jong, 1980) which results from consumption of all available nitrogen by bacteria and fungi growing on a hydrocarbon medium in soil thus, restricting the uptake of these elements by plants (Małachowska-Jutsz *et al.,* 1997; Xu and Johnson, 1997). These activities are caused by a depression in ammonification and nitrification processes triggered by inhibition in conversion of mineral and organic nitrogen compounds in soil by petroleum derived compounds (Iwanow *et al.,* 1994; Amadi *et al.,* 1996). Oil degrading or hydrocarbon-utilizing microbes such as *Azobacter* spp. have been reported to become more abundant while nitrifying bacteria such as *Nitrosomonas* spp. become reduced in number (Odu *et al*., 1985) in oil contaminated soil. Osuji and Nwoye (2007) suggest that the process of nitrification might have reduced following the incidence of oil spillage which has led to reduction in the concentration of nitrate-nitrogen (NO_3-N) in oil contaminated sites.

The physical, chemical and geological characteristics of soil play significant roles in the degree of its vulnerability to an oil spill (Gundlach and Hayes, 1978). In some areas, oil may sink and/or be buried rapidly, making clean up difficult while in some areas, most of the oil will not adhere to, nor penetrate into the compacted soil. For example, among the shoreline type, salt marsh and mangrove forest are the most vulnerable to oil spill while the exposed rocky headland is the least. Oil may persist for years in salt marsh and mangrove forest areas making cleaning of oil in these areas a challenging task. On the contrary, the exposed rocky headland areas may require no clean-up, as wave reflection keeps most of the oil offshore (Gundlach and Hayes, 1978).

Furthermore, contamination of soil with refinery products modifies the structure (Figure 2.0) and appearance of the soil and deteriorates its biochemical and physicochemical properties (Tyczkowski, 1993; Kucharski and Wyszkowska, 2001; Wyszkowska *et al.,* 2002; Wyszkowski *et al.,* 2004). Schollenberger (1930) and Hoeks (1972) found that the gassed soil was darker than the ungassed soil, and the normal structure of the soil was lost. Several studies have indicated that soil polluted by petroleum-based products loses its biological activity and may not recover it over ten years (Sparrow and Sparrow, 1988; Racine, 1993; Wyszkowska *et al.,* 2001). A recent study noted that the greasy texture of hydrocarbons, in excessive amount in the soil, is responsible for the prevailing amounts of organic carbon over those of nitrogen in soil (Wyszkowski *et al.,* 2004). Partial coating of soil surfaces by the hydrophobic hydrocarbons might reduce the water holding capacity of the soil due to some significant reduction in the binding property of clay (Osuji and Nwoye, 2007). Usually, such partial coats lead to a breakdown of soil structure and the dispersion of soil particles, which reduce percolation and retention of water. Osuji *et al.* (2006b) found that soils develop severe and persistent water repellency following contamination with crude oil. The coupling effects of this and exhaustion of oxygen in the soil can increase the microbial activity and thus interfere with the plant-soil-water relationship (Esenowo *et al.*, 2006). This can affect plants general growth and productivity.

2.3.2 Effects of oil on plants

Studies show that plants are important productive resources but very vulnerable in the event of an oil spill (West *et al.,* 2005). They are highly susceptible to oil exposure and this may kill them within a few weeks to several months (Omosun *et al.*, 2008). Thus, they are considered number one priority in oil spill response assignments. It has been discovered that very often, it is difficult to get rid of the oil from the environment once contaminated; hence lots of damage is done as oil persist therein for many years (Gundlach & Hayes, 1978). Both heavy metals and petroleum oils are known to cause stress in plants (Mendelssohn *et al.,* 2001). The adverse effects of oil pollution on economic plants have been reported (Odu, 1981; Isirimah *et al.*, 1989; Amadi *et al.*, 1993; Anoliefo and Okoloko, 2000). At high concentrations of oil in soil, most plants species suffered serious depression in growth (Udo and Fayami, 1975; Amakiri and Onoteghara, 1984). This condition has been attributed to poor soil conditions, dehydration and impaired nutrient uptake by the roots, created by the presence of crude oil (Anoliefo *et al.*, 2003).

Oil spills directly or indirectly contaminate plants in several ways. Oil can enter the soil and create unfavourable conditions (explained in section 2.3.1) for plant growth and survival (De Jong, 1980; Günther *et al.*, 1996). For example, Edema *et al.* (2009) noted that crude oil reduced phosphate, sulphate and nitrate ionic concentrations in soils and thus, oil spillage could make vital plant nutrients unavailable to plants (Odu, 1981; Anoliefo *et al.,* 2003). Also, it was found that oil markedly reduced water uptake by wheat from contaminated layers or below such layers (Jong, 1980) and that water absorption may be inhibited after long periods of anaerobis (Smith, 2002). On the other hand, plants can be directly affected through physical contact with oil, for example, through coating of plant foliage (Pezeshki *et al.*, 2000), especially when plant canopies grow over the land surface (as labeled b. and e. in Figure 2.0). Coating of plant leaves by oil causes stomatal closure and consequently, an increase in leaf temperature because of blocked transpiration pathways (Pezeshki and DeLaune, 1993). However, it is not clear whether similar thermal effects occur in plants that are indirectly exposed through oil contamination of soil.

Stomatal closure also reduces leaf photosynthesis because of restricted entry of CO_2 through stomatal pores (Pezeshki and DeLaune, 1993; Webb, 1994; Pezeshki *et al.*, 1995). Other workers have mentioned the effects of crude oil on the growth and physiology of different plants (Cook and Westlake, 1974; Terge, 1984; Gill *et al.*, 1992; Pezeshki and DeLaune, 1993; Quinones-Aquilar *et al.*, 2003). Previous studies have mentioned that the crude oil penetrates the pore spaces of terrestrial vegetation (Bossert and Bartha, 1984) and subsequently impedes photosynthesis and other physiological processes of the plant (Odu, 1977, 1981). Through physical contact, refined and light oil in particular, can penetrate into plants/leaf tissue and consequently, destroy cellular integrity, and prevent leaf and shoot regeneration (Webb, 1994; Pezeshki *et al.*, 1995; Pezeshki *et al.*, 2000). The adverse effects of petroleum and its compounds on plant growth have earlier been reported by Gill *et al.* (1992). Also, the inhibition of plant growth by harmful metallic ions present in petroleum was reported by Winter *et al.* (1976).

It has been found that oil penetrating and accumulating in plants can cause damage to cell membranes and leakage of cell content (Baker, 1970). Consequently, it has been observed that oil affects germination, plant height, grain yield, and dry matter content of crops especially when pollution is heavy (Ogboghodo *et al.*, 2004). A recent study noted that soils contaminated with crude oil contain polycyclic aromatic hydrocarbons (PAH) and heavy metals that are toxic to plants (Edema *et al.*, 2009). Crude oil is phytotoxic because it creates unsatisfactory conditions for plant growth ranging from heavy metal toxicity to inhibited aeration of the soil. Edema *et al.* (2009) also found that the nature of crude oil and its components was responsible for the low number of plant families encountered in the field. Toxicity symptoms observed in plants exposed to oil pollution include chlorosis, necrosis, stunted growth, suppression of leaves, enormous reduction in biomass to stomatal abnormalities (Baker, 1970).

In some salt-tolerant plants, petroleum hydrocarbons may damage root membranes, thereby adversely affecting the ionic balance of the plants and their ability to tolerate salinity (Gilfillan *et al.*, 1989). Further investigations have found that the growth of cereal in oil polluted soil was inhibited, with leaves undergoing chlorosis and general plant dehydration (Udo and Fayemi, 1975). Oxygen is generally obtained from the soil and is required for correct functioning of plant roots (Smith, 2002). It is necessary for aerobic respiration and the supply of metabolic energy, which is used for the production of new root cells for growth and for the uptake of nutrients from the soil (de Wit, 1978). Drew and Sisworo (1979) found significant effects on the normal functioning of waterlogged barley due to mild oxygen depletion from the soil. Therefore absence or insufficient oxygen in soil caused by oil pollution can lead to plant death.

Spartina alterniflora is an important coastal salt-marsh species and is particularly susceptible to coastal oil slicks thus; considerable attention has been drawn towards investigating their response to oil pollution as illustrated in Pezeshki *et al.* (2000). Several studies found that accumulation of high levels of crude oils in the soil resulted in the death of *Spartina alterniflora* (Krebs and Tanner, 1981; Alexander and Webb, 1987). A similar study using the same species found that leaves died after about 40 days of contamination (Pezeshki *et al.*, 1995). Overall, oil pollution reduces plant transpiration and carbon fixation and increases plant mortality (Baker, 1970; Pezeshki and Delaune, 1993). However, the extent of damage highly depends on a number of factors for example; season of spill, soil type, oil type and these were extensively discussed in Pezeshki *et al.* (2000). Overall, plant stress whether directly or indirectly induced by oil pollution can cause harmful effects on vegetation leading to growth inhibition, early senescence, chlorosis, dehydration, and death.

In order to minimise the impacts of oil pollution in the environment and to ensure timely response, recovery and possible bioremediation measures; its early detection through remotely-sensed response of vegetation becomes of paramount importance. Fortunately, stress condition in plants is visible in the spectra (Knipling, 1970; Noomen *et al.*, 2003; Kempeneers *et al.*, 2005) thus, making remote sensing a valuable tool for early detection of plant stress (Rosso *et al.,* 2005).

2.4 Remote sensing of plant stress

Remote sensing is broadly defined as the science of acquiring information about an object with a device without being in physical contact with it. In general, the process requires measuring the interactions between matter and electromagnetic radiation to identify properties and processes of the object of interest. These interactions are controlled by the physical, chemical and biological characteristics of the object (Liew *et al.,* 2008) which, in turn, control its remotely-sensed response. Incident radiation (I) on a plant leaf is either reflected (R), absorbed (A), or transmitted (T), as illustrated in Figure 2.1, and their relative proportions vary with the wavelength of radiation. The absorbed energy may be subsequently emitted by the object. Remote sensing systems record the reflected and emitted energy which, when processed appropriately, can reveal information about the object measured.

Figure 2.1 Interaction of incident electromagnetic radiation with plant leaf.

2.4.1 The spectral reflectance of plants

The spectral 'signature' of plants is defined by the reflectance or absorption of electro-magnetic radiation in the visible, near-infrared (NIR) and short-wave infrared (SWIR) wavebands. The 'signature' is formed when the intensity of light energy coming from the plant is plotted over a range of wavelengths; the connected points produce a curve hence its spectral 'signature' (Figure 2.2). Plants have generally low reflectance in the visible region and high reflectance in the NIR and lower reflectance in the SWIR. However, while this typical 'signature' is characteristic of healthy leaves and canopies, the spectral reflectance of plants can vary considerably depending upon a wide range of factors.

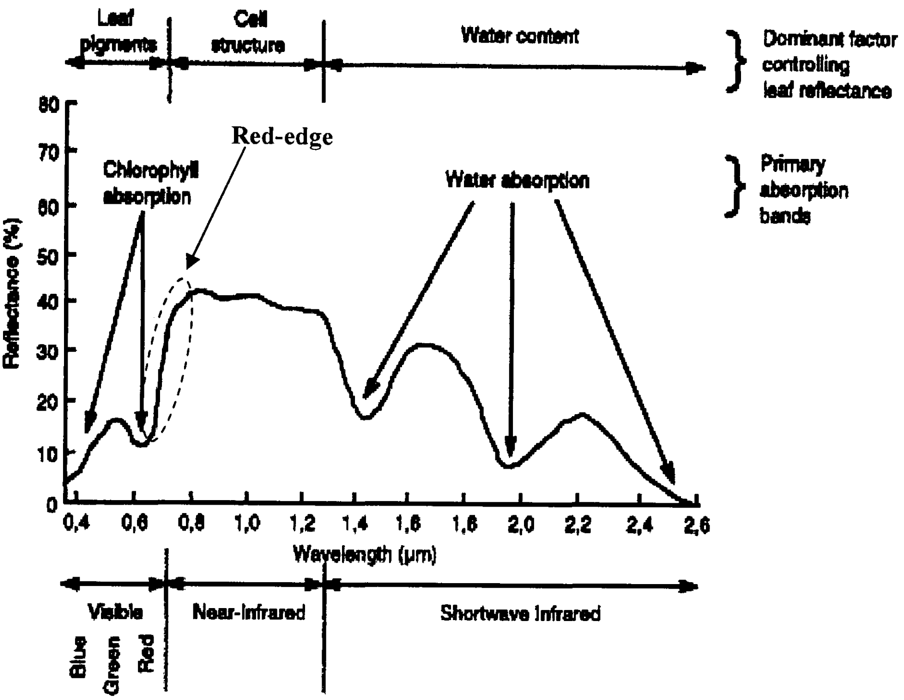

Figure 2.2 Typical reflectance characteristics of leaves. Adapted from Hoffer (1978).

Leaf reflectance in the visible region is predominantly influenced by chlorophylls and, to a varying extent, other photosynthetic and photoprotective pigments (Woolley, 1971; Wessman, 1990; Volgelmann, 1993; Fourty *et al.,* 1996; Ustin, *et al.*, 1999, 2004; Asner, 2004; Baltzer and Thomas, 2005; Liew *et al.*, 2008). These pigments absorb light strongly in the visible wavelengths and thus create low reflectance. In the NIR and SWIR, leaf cell structure (Slaton *et al.,* 2001) and water content in the tissues (Buschmann and Lichtenthaler, 1988) are the dominant factors, respectively. Chlorophylls which are of two forms (chlorophyll *a* and *b*) have a dominant control upon the amount of solar radiation that a leaf absorbs (Smith, 2002; Blackburn, 2007). Most pigments absorb in the blue region centered around 445 nm but only chlorophyll absorbs in the red centered around 645 nm (Gates *et al.,* 1965). There is high reflectance in the NIR due to light scattering of the leaf cell structure and non absorption of chlorophylls. The structure of the leaf, with many air-water interfaces, makes a very strong scattering medium that causes high reflectance and transmittance in any region where absorbance is low (Woolley, 1971). A summary of the major features responsible for absorption/ reflectance of certain wavelengths that has been derived from Berry and Ritter (1997), Zwiggelaar (1998), Smith (2002), Blackburn (2007) is given in Table 2.1.

Table 2.1 Absorption features of plant spectra.

Controlling factor	Waveband/wavelengths (nm)	Spectral effect
Chlorophyll a	435, 670-680, 740	Strong absorption
Chlorophyll b	480, 600-650	Strong absorption
α-carotenoid	420, 440, 470	Strong absorption
ß-carotenoid	425, 450, 480	Strong absorption
anthocyanins	400-550	absorption
chlorophyll a & b	550	strong reflectance/weak absorption
lutein	425, 445, 475	absorption
violaxanthin	425, 450, 475	absorption
water	970	weak absorption
water, CO_2	1450, 1944	strong absorption
water, oxygen	760	strong absorption

Adapted from Zwiggelaar (1998), Smith (2002), Blackburn (2007).

2.4.1.1 Controls on canopy-scale reflectance of plants

Single leaf reflectance can be very misleading for predicting reflectance at the canopy scale (Moran *et al.,* 2004). This is because other non-green materials such as the senescent leaves and litter, bark, wood, and dry reproductive structures do occur in the majority of plant canopies, and can contribute significantly to canopy reflectance (Blackburn, 1993). Other controlling factors are canopy specific such as senescent vegetation; phenology, soil background, and canopy geometry (plant architecture, Leaf-Area-Index (LAI), Leaf Angle Distribution (LAD) and viewing geometry specific such as solar zenith-, sensor look-, and relative solar azimuth angles (Milton and Wardley, 1987; Kasischke *et al.*, 2004; Moran *et al.,* 2004; Blackburn, 2007). Although stress senescent detection is closely related to chlorophyll degradation (Goetz *et al.*, 1983; Horler *et al.,* 1983), live and dry vegetation amounts within two canopies of the same total biomass amount may vary, thus can create change in canopy reflectance (Blackburn, 1993). Indeed, studies have shown that plants at different developmental stages alter the type of canopy element presented to the sensor (Peterson, 1992; Peterson and Nilson, 1993).

Apart from the effects of soil and litter background, physiological stress can cause wilting of canopy elements which can change reflectance of the canopy as more soil and less vegetation is seen by the sensor (Collins, 1978). Recent studies have given evidence about the biophysical sources of variability in canopy reflectance and bidirectional reflectance function (BRF) variations due to observing geometry (Jacquemoud, 1993; Asner, 1998; Gastellu-Etchegorry *et al.,* 1999). The majority of these factors influence measurements mostly in field conditions particularly from space-borne sensors. However, this study investigates the reflectance of stressed vegetation at the plant scale within a controlled environment and thus, not all the factors may affect canopy scale reflectance measurements.

2.4.2 Diagnostic indicators of plant stress

2.4.2.1 Visible reflectance

The visible region ranges from 0.4-0.7μm (400-700nm), which is an extremely small portion of the electromagnetic spectrum but this corresponds to the spectral sensitivity of the human eye. The blue, green and red colours are ascribed to the approximate ranges of 0.4-0.5μm (400-500nm), 0.5-0.6μm (500-600nm) and 0.6-0.7μm (600-700nm) respectively. Several studies have recorded that visible reflectance increases consistently in various plant species in response to stress induced by a range of different stressors (Carter and Miller, 1994; Carter *et al.*, 1996).

Spectral measurements by Smith *et al.* (2004) showed that vegetation exposed to high concentrations of natural gas in the soil had significantly increased reflectance in the visible and decreased reflectance in the infrared. Several researchers identified similar responses to a wide range of plant stresses such as waterlogging, nutrient stress, heavy metal toxicity and soil oxygen deficiency (Woolley, 1971; Horler *et al.*, 1983; Milton *et al.,* 1989; Carter, 1993; Carter and Miller, 1994; Anderson and Perry, 1996; Noomen *et al.*, 2003). In response to a number of different stressors, plants exhibit a decrease in the production of chlorophyll and other biochemical constituents, which leads to a decrease in their absorption capacity and therefore an increase in reflectance in the visible region. Sensitivity analysis of leaf spectral reflectance to leaf characteristics performed by Ceccato *et al.* (2002) using the new version of the PROSPECT model (Jacquemoud, *et al.*, 2000) shows that chlorophyll content had a major influence (followed by leaf internal structure) over reflectance values between 400 and 710nm compared to other pigments.

As the visible region is characterized by high absorption coefficients for pigments, reflectance in this region is more sensitive to lower pigment concentrations. For example, Blackburn (1998a, 1998b) and Sari *et al.* (2005) noted that reflectance at wavelengths corresponding to the centre of the major absorption features are most sensitive to low pigment concentrations as found in early immature and later senescent leaves and canopies with low leaf area and canopy cover. An empirical study by Rosso *et al.* (2005) showed that highly contaminated plants reflected incident radiation in the deep absorption features of the visible spectrum such as 670nm.

2.4.2.2 Red-edge region

The region of the reflectance red-edge has been used as a means of identifying stress in plants. The red-edge adjoins the red end of the visible portion of the spectrum. It is an area where there is change in reflectance between wavelengths 690 and 750nm which characterises the boundary between dominance by the strong absorption of red light by chlorophyll and the high scattering of radiation in the leaf mesophyll (Smith *et al.*, 2004). At this region, reflectance rises rapidly leading to a plateau of high reflectance in the near-infrared, where pigments no longer absorb radiation (Blackburn, 2007). Horler *et al.* (1983) also stated that the red-edge is the sharp rise in reflectance of green vegetation between 670 and 780nm.

There is further suggestion that the red-edge region of the spectrum is considered a unique parameter for detecting stress in plant. The reflectance of stressed plants often shows a shift of the 'red edge' position towards shorter wavelengths (Noomen *et al.*, 2003). Red-edge shifts measured in airborne imaging spectrometer data have been proposed useful to provide an early indicator of vegetation stress. Evidence is given in Rock *et al.* (1988) where a shift in red-edge towards the blue, of approximately 5nm was detected when measuring severe foliage stress on spruce trees due to air pollution. The shift which was attributed to decline in chlorophyll in the pine needles was detected before visual symptoms became apparent.

A small number of investigations have looked specifically at the effects of hydrocarbon pollution on the reflectance red-edge of vegetation. Investigations by Bammel and Birnie (1994) discovered a consistent and significant blue shift of the green peak and red trough positions of sagebrush spectra and concluded that the red-edge is the most reliable indicator of hydrocarbon-induced vegetation stress. A large body of literature exists that generally shows a decrease in chlorophyll in natural vegetation due to stress, resulting in a shift to shorter wavelength of the red-edge. However, spectroscopic analysis by Yang *et al.* (1999) showed that the red-edge position of wheat spectra taken from areas of well known hydrocarbon microseepage has shifted 7nm to longer wavelengths.

To explain the situation, it is important to note that it is generally accepted that the position and shift of the red-edge is related to leaf and canopy chlorophyll concentration. Hence, a decrease or increase in chlorophyll results in red-edge shift towards either the shorter or longer wavelengths, respectively. In the case of Yang *et al.* (1999), it was suspected that hydrocarbons might have served as nutrients during the short growing season of wheat, which however needs further investigation. Evidence from previous studies shows that red-edge inflection point (λp) (the peak in the first derivative of reflectance that can be used to describe changes due to stress) ranges between 700 and 745nm. Jago and Curran (1996) found two first derivative maxima within the red-edge with peaks at approximately 693 and 709nm, while studying grassland canopies at a site contaminated with oil. However, the potential for exploiting the position of these peaks and other red edge features such as the distance between the peaks, and the magnitude or area of the red edge for plant stress detection have not been explored.

2.4.2.3 Near infrared (NIR) region

The NIR waveband ranges between 700 and about 1000nm. The region is characterised by high reflectance primarily due to light scattering by leaf tissue or cellular structure (Gausman *et al.,* 1970). Ceccato *et al.* (2002) found that the leaf internal structure accounts for 70-80% of reflectance variations in the NIR whereas the leaf dry matter accounts for the remaining variations (30-20%). Leaf reflectance is very high in the NIR at ~800nm (Lenk *et al.*, 2007) and a decrease of the reflectance at 800nm may be taken as an indicator of reduced aerial interspaces in the mesophyll of leaves under stress conditions (Gausman and Quisenberry, 1990; Buschmann *et al.*, 1991). A body of literature has recently been developed through experimental studies, which show substantial evidence of high and low reflectance in non-stressed and stressed plants respectively within this region (Noomen *et al.*, 2003; Smith *et al.*, 2004; Kempeneers *et al.*, 2005; Rosso *et al.*, 2005; Smith *et al.*, 2005;). Within these empirical studies, different problems were simulated given different scenarios. These include utilisation of different types of plant species, which were subjected to a range of stressors including water and nitrogen stress, water logging, shading, gas and heavy metal at varying levels of contamination. A similarity within this range of studies lies in the use of a ground based sensor – the spectroradiometer to measure the spectral reflectance characteristics of the experimental plants.

Treating plants of *Salicornia virginicia* with two metals – cadmium and vanadium, at different levels of contamination, Rosso *et al.* (2005) found that reflectance differences in the near infrared (NIR) portion followed a similar progression as the symptom expression; in contrast to visible wavelengths, towards a reduction in reflectance with stress. A reduction in intercellular spaces produces less light scattering and less reflectance (Rosso *et al.*, 2005). Water stress influences reflectance in the NIR region because of changes in mesophyll structure (Bowman, 1989). However, leaf structural characteristics have more influence in NIR reflectance than at short wave infrared, whereas water content has a strong control on reflectance at short wave infrared (SWIR) (Woolley, 1971; Ceccato *et al.*, 2001). It is worth noting that absorption of radiation by water does not have a large direct influence on reflectance in the NIR but it does have an important indirect effect due to its influence on leaf cellular structure which varies considerably as water content varies. Further evidence had been given in Ustin *et al.* (1999) and Jacquemoud *et al.* (1996); that NIR reflectance is strongly determined by the structural characteristics of leaf parenchyma, fractions of air spaces and air-water interfaces.

2.4.2.4 Shortwave infrared (SWIR) region

The SWIR ranges between 1300 and 2500 nm and is characterised by light absorption by the leaf water. Tucker, (1980) and Gausman, (1985) show that SWIR is heavily influenced by water in plant tissue. Bowman (1989) indicated that water stress influences reflectance at the SWIR region because of a reduction of water content. A study by Fourty and Baret (1997) showed that the wavelengths at 1530 and 1720nm seem to be most appropriate for assessing vegetation water. Also, the radiative transfer model PROSPECT (Jacquemoud *et al.,* 2000) as a function of chlorophyll a & b concentration, C_w, C_m, and N was very efficient for estimation of vegetation water content at leaf level. In an attempt to detect vegetation leaf water content using reflectance in the optical domain, Ceccato *et al.* (2001) found that parameters such as the Equivalent water thickness (C_w) are not the only parameters responsible for significant reflectance variations within the SWIR range. Other controlling factors include the internal structure (N) and the dry matter content (C_m). The N and C_m affect reflectance at wavelength range from 700 to 2500 nm, while C_w affects the wavelength range from 900 to 2500 nm. While C_w accounts for 86.7% of the reflectance variation in the SWIR, N and C_m account for only 5.8% and 7.5% respectively. Thus, the SWIR reflectance value alone is not suitable for retrieving vegetation water content at leaf scale. Although C_w is the dominant factor, the study suggests that combination of information from both NIR (820nm) and SWIR (1600nm) is necessary for accurate estimation of vegetation water content at leaf scale from optical observations.

Ceccato *et al.* (2001) explained several indices proposed to measure vegetation stress due to water stress such as Crop Water Stress Index (CWSI), the Stress Index (SI), and the Water Deficit Index (WDI). These indices assumed that differences between the air and surface temperatures were related to plant water content and to water stress. Other indices, such as the moisture stress indices that combine satellite-based information on the relationship between Normalised Difference Vegetation Index (NDVI), surface temperature, and air temperature, in association with production efficiency models, have been developed (Goetz *et al.,* 1999). These indices do not provide a very accurate way for estimation of water stress because vegetation status is not a direct measurement of water content and many species may show signs of reduced evapotranspiration without experiencing a reduction in water content (Ceccato *et al.,* 2001).

2.4.2.5 Spectral and derivative indices

Several researchers have developed a wide range of spectral indices and wavelength regions that are feasible in detecting stress in a wide range of plant species (Carter, 1994a; Tarpley *et al.,* 2000; Read *et al.,* 2002; Sims and Gamon, 2002; Smith *et al.,* 2005; Campell *et al.,* 2007). Spectral indices based on reflectance spectroscopy offer the possibility for estimation of leaf pigment content. The indices commonly use reflectance ratios derived from dividing leaf reflectance at stress-sensitive wavelengths by that at stress insensitive wavelengths (Liew *et al.,* 2008). The idea for using this approach is to eliminate the effects of leaf internal reflections and thus, provide stronger quantitative relationships with chlorophyll content (Carter and Miller, 1994). A diverse range of spectral indices that combine reflectance in wavebands of different spectral regions have been employed for plant stress detection and includes simple ratios of reflectance and normalised difference ratios.

For example, in studying plant spectral responses to gas leaks and other stresses, Smith *et al.* (2005) calculated a reflectance ratio by combining wavebands in the visible region near 560nm and 670nm. The study found that in contrast to the control or the shade-stressed plants, the ratio increased in gas- and herbicide-stressed plants. They suggested that an increase in the ratio $R_{670\text{-}680}/R_{555\text{-}565}$ could be used to detect plant stress caused by elevated natural gas in soils due to leaks. Tarpley *et al.* (2000) suggested that simple reflectance ratios that combine leaf reflectance values at 700nm or 716nm and 755-920nm could improve precision and accuracy in predicting cotton leaf nitrogen concentration. Read *et al.* (2002) found strong associations between leaf constituents such as chlorophyll, carotenoids and nitrogen and simple ratios of reflectance at the wavelengths 415/695nm, 415/685nm, and 415/710nm, respectively. They found that reflectance at waveband 415nm appeared to be a more stable spectral feature under nitrogen stress, as compared with more pronounced changes along the reflectance red edge at 630nm - 690nm.

Zhao *et al.* (2005) found high correlation between the reflectance ratios of R_{551}/R_{915} and R_{708}/R_{915} and chlorophyll concentrations in field-grown cotton. They also found the same relationship at a single wavelength of 551nm or 707nm and high linear correlation between nitrogen concentrations and a spectral reflectance ratio of R_{517}/R_{413}. Sims and Gamon (2002) and le Maire *et al.* (2004) enhanced spectral indices by incorporating waveband in the blue region to correct for specular reflectance. This resulted to more accurate estimation of leaf chlorophyll concentrations. Many other spectral indices derived not only from spectral reflectance but also from derivative spectroscopy have been found useful for studying plant damage. For example, derivatives ratios such as D_{715}/D_{705}, D_{REP}/D_{714}, D_{744}, D_{705}, or D_{745} (where D represents the amplitude of the first derivative at specific wavelength and D_{REP} is the amplitude of the first derivative at the wavelength of the maximum amplitude in the red edge region) were sensitive to stress and reflect the differences in the shape of the first derivative curve among damage levels (Entcheva, 2000). In their experimental study, Campell *et al.* (2007) found that D_{715}/D_{705} consistently performed well as they exhibited high values for the unstressed condition and significantly lower values as vegetation stress increased.

From the foregoing discussion, it is clear that change in plant reflectance spectra at specific regions, red-edge features, ratios of narrowband reflectance and derivatives are valuable indicators of stress. However, the optimal index to monitor plant stress response to oil pollution is not known. Besides, the potentials of other red-edge features such as the position of the double features, the distance between them and the magnitude or area of the red-edge for plant stress detection have not fully been explored.

2.4.3 Optical remote sensing techniques

Optical remote sensing techniques use data from sensors that collect radiation in the reflected solar spectrum (about 350 to 2500nm). Optical remote sensing instruments can be operated from different platforms such as ground-based, air-borne or space-borne, each with various strengths and weaknesses. Basically, at field and laboratory scales, the methodology or approach that could be applied at a larger scale for various plant stress monitoring applications could be developed. For example, a variety of narrow band spectral reflectance features have been shown to be related to changes in vegetation condition and amount through laboratory and field studies (Treitz and Howarth, 1999). In addition, results from laboratory scale studies can provide the basis for operational applications of vegetation stress monitoring. However, aside from scale or platform definitions, optical remote sensing for vegetation stress monitoring has been more commonly categorized according to spectral resolution.

2.4.3.1 Multispectral and hyperspectral remote sensing

Multispectral sensors collect data in a few broad spectral bands that cover important regions of the reflected solar spectrum and have been applied for a wide variety of environmental applications (Okin and Roberts, 2004). Van Der Meer *et al.* (2002) noted that the laboratory and field scale spectra of vegetation stress have been studied in detail, but the resolution of broad-band instruments such as the Landsat Thematic Mapper (TM) or Multispectral Scanner (MSS) is not sufficiently high for comparison with laboratory or field spectra. This means that the broad bandwidth cannot characterize all the absorption features that respond to vegetation stress, regardless of the type of enhancements employed or the type of information extraction method applied (Van Der Meer *et al.*, 2002). For this reason, a frequent use of multispectral remote sensing systems is with vegetation indices.

Van Der Meer *et al.* (2002) note that vegetation indices are quantitative measurements, based on digital values, which attempt to measure biomass or vegetative vigour and the most popular and widely used is NDVI. The index combines two channels in a ratio or difference i.e. (NIR-RED)/(NIR+RED) which allows response to vegetation growth to be distinguished from the background signals. Some of the inherent limitations associated with NDVI are adequately provided in Okin and Roberts (2004) and Van Der Meer *et al.* (2002). For vegetated landscapes, attention has been directed towards increased spectral sampling because of great spectral variability, in the 0.7μm to

2.5μm range (Curran, 2001). A detailed description of the band-ratio strategy is given in Van Der Meer *et al.* (2002).

Multispectral sensors feature a combination of limited number of spectral bands with planes, helicopters or satellites as their platforms. With satellites, it is possible to acquire high spatial resolution images at a very wide coverage and on regular basis which makes it cost effective. However, satellite data are known to be adversely affected by cloud cover, atmospheric attenuation and scattering which necessitate some corrections. In addition, fixed satellite orbits impose some limitations as they create inflexibility in timing of data acquisition. For example, when high cloud cover for a given region coincides with time the satellite orbits that region, it will be impossible to acquire clear images for that region. The visible and infrared regions are affected in particular and are very critical for vegetation monitoring. Hence, satellite-based multispectral systems have been proved very useful in regions where there are relatively clear skies, but can be very limited in regions with frequent cloud cover. Using data from a feasibility study from 1990, Steven *et al.* (1997) found that in UK, the number of days with less than 2 oktas cloud cover between June and September sampled by the SPOT (orbiting 11 times every 26 days) and Landsat (orbiting once every 16 days), systems were between 2 and 9 days.

Multispectral remote sensing technologies have well-known applications in vegetation studies, for example in the mapping of physical and structural features of vegetative ecosystems and in forest surveys (Treitz and Howarth, 1999). In addition, it has offered opportunity for successful monitoring of deforestation and desertification through quantification and estimation of vegetative ecosystems. With multispectral remote sensing such as the Landsat Thematic Mapper, it is possible to quantify vegetation biophysical properties such as Leaf Area Index (LAI) using spectral indices derived from their broad wavebands (Asner, 1998; Treitz and Howarth, 1999; Blackburn, 2007). Table 2.2 illustrates some characteristics of airborne and spaceborne multispectral systems.

Table 2.2 Characteristics of multispectral remote sensing systems (adapted from NERC Earth Observation data centre; Satellite Imaging Corp., and Natural Resources Canada).

Sensor	Spatial resolution	Spectral resolution	Temporal resolution (days)	Number of bands	Launch date	Application
AVHRR/3 (Advanced very high resolution radiometer)	4.4 km (GAC) 1.1 km (LAC)	580 - 12500	2 x per day	5	May, 1998	For determining cloud cover and surface temperature, and for vegetation monitoring.
MODIS (Moderate Resolution Imaging Spectroradiometer)	250m, 500m, & 1km	400 - 14,400	1-2	36	Dec. 18, 1999 (Terra), May 4, 2002 (Aqua)	For understanding of global dynamics and processes occurring on the land, in the oceans, and in the lower atmosphere.
IKONOS	0.82 m - 1m (pan.), 3.2 m 4.2 m (MS)	450 – 900	3	5 (panc, blue, green, red, and NIR)	Sept. 24, 1999	Urban and rural mapping of natural resources and of natural disasters, tax mapping, agriculture and forestry analysis, mining, engineering, construction, and change detection.
Landsat - 7 ETM +	30 m, 60 m (for thermal IR channel) and 15 m in pan. band	450 – 2350	16	7 plus panchromatic band 8	April 15, 1999	For global change research and applications in agriculture, geology, forestry, regional planning, education and national security.
SPOT – 5	10 m 2.5 - 5 m 20 m	500 - 890 (VIS & NIR) 480 - 710 (pan.) 1,580 - 1,750 (SWIR)	2 - 3	3 1	May 3, 2002	For applications such as medium-scale mapping (at 1:25 000 and 1:10 000 locally), urban and rural planning, oil and gas exploration, and natural disaster management.
Quickbird	0.61 m (pan.) 2.44 m (VIS & NIR)	450 - 900 (panchro.) 450 - 900 (VIS & NIR)	1 - 3.5	1 4	Oct. 18, 2001	For analyses of changes in land usage, agricultural and forest climates.
Worldview - 1	0.55 m	VIS - NIR (400 - 900)	1.7	1 panchromatic	Sept. 18, 2007	Provides highly detailed imagery for precise map creation, change detection and in-depth image analysis.
Worldview - 2	0.55m (pan.) 1.8 m (MS)	400 – 1050	1.1	1 pan. 8 (MS : 4 Standard colours (VIS & NIR) and 4	Mid 2009	For enhanced spectral analysis, mapping and monitoring applications, land-use planning, disaster relief, exploration, defense and intelligence, and visualization and simulation environments.

				new colours(coastal, yellow, red-edge & NIR)		
Geo-Eye – 1 Geo-Eye - 2	0.4 m (pan) 1.65 m (MS 0.25 m	Pan. and multispec.	< 3		Aug. 22, 2008 2010 - 2011	For various applications in field of defence, National and Homeland security, air and marine transportation, oil and gas, energy, mining, mapping and location-based services, state and local government, insurance and risk management, agriculture and natural resources and environmental monitoring.
FORMOSAT - 2	2 m (pan.) 8 m(MS)	450 – 900	Daily	5	May 21, 2004	For land distribution, natural resources research, forestry, environmental protection, disaster prevention, rescue work, and other applications.
CBERS - 2	20 m - 260 m	0,63 - 0,69 µm (red) 0,77 - 0,89 µm (infrared)	5	2	Oct. 21, 2003	Satellite images from CBERS-2 are used in important areas, as deforestation and fire control in the Amazon Region, water resources monitoring, urban growth, soil occupation, education and several other applications.
CARTOSAT - 1	2.5 m	400 - 700 (VIS region only)	5	2 panchromatic	May 5, 2005	For cartographic application in India and for large scale mapping applications and stimulate newer applications in the urban and rural development, land and water resources management, disaster assessment, land cover change detection, relief planning and management, environmental impact assessment and various other Geographical Information Systems (GIS) applications.
ASTER (Advanced spaceborne thermal emission and reflection radiometer)	15 (VNIR) 30 m (SWIR) 90 m (TIR)		16	14: 1-3 (VNIR); 4-9 (SWIR); 10-14 (TIR)	Dec. 18, 1999	Monitors cloud cover, glaciers, land temperature, land use, natural disasters, sea ice, snow cover and vegetation patterns.
ALOS	2.5 m (pan.) 10 m (MS)	420 – 890	2	10	Jan. 24, 2006	For digital elevation mapping (DEMs), precise land coverage observation, and for day-and-night and all-weather land observation and enables precise land coverage observation and can collect enough data by itself for mapping on a scale of 25,000:1, without relying on points of reference on the ground.
Z/I Imaging DMC	12 micrometer	400 - 580, 550 - 650, 590 - 675, 740 – 850 (MS) 400-900 (pan)	revisit rate is dependent upon flight scheduling	4 multi-spectral 1 band panchro.		

LEICA ADS 40	6.5micrometer	430-490,535-585,610-660,835-885	“	Successfully used in agricultural studies conducted in Japan.
Geovantage Geo Scanner			“	For remote sensing requirements of forestry, agriculture, urban planning and environmental management.
Applanix Emerge DSS			“	Rapidly assigns a geographic coordinate to each pixel without needing to be on the ground in the area and thus, used to help manage, analyze, and assess damage due to any type of disaster.

The main advantage of airborne remote sensing is that the effects of cloud cover, atmospheric attenuation and scattering can be controlled or avoided. Data can be acquired when the skies are clear and at any desired temporal frequency on a repetitive basis thus, leading to a cost effective means of monitoring the environment. The system provides several advantages over satellite systems as they are simple, reliable and inexpensive (Campbell, 1996). However, airborne systems have a more limited spatial coverage than satellite systems, which offer the potential of complete global coverage. In addition, there are inherent risks associated with low level flights required for monitoring leaks from oil pipeline as the accuracy of information depends solely on the pilot.

However, the major consideration in the choice of appropriate remote sensing system for vegetation stress monitoring is the spectral resolution. As information about the general health status of vegetation is often embedded in narrow spectral features, a high spectral resolution is required. The spectral resolution, which is the ability of a sensor to resolve spectral features, is controlled by the bandwidth, spectral sampling interval and number of bands. In principle, the higher the spectral resolution, the greater the chances of gathering useful information for better understanding of plant health status. Biochemical constituents relate to and invariably provide accurate information about physiological characteristics and thus, allow assessment of vegetation condition. Many biochemicals have fine spectral features which cannot be sampled using the broad bandwidths of some optical remote sensing systems (Clark, 1999; Yang *et al.*, 2000; Curran, 2001; Broge and Mortensen, 2002; Van Der Meer *et al.*, 2002). This is because they use average spectral information over broadband widths resulting in loss of critical information available in specific narrow bands (Blackburn, 1998; Thenkabail *et al.,* 2000).

Overall, it is clear how spectral resolution can be important in determining the ability of a remote sensing system to monitor vegetation stress.

Spatial resolution specifies the smallest object that could be detected by a sensor. There are several remote sensing systems of very high spatial resolution of 1m or less (Table 2.2) but they have a limited spectral resolution. High spatial resolution data have primary applications in managing forest inventory related to assessing stock levels and classification of vegetation types (Wulder, 1998; Wulder *et al.,* 2000). Indeed high spatial resolution data are extremely useful for refining stress detection methods by allowing us to discriminate between different vegetation types and therefore constrain our predictions. However, there is growing evidence that for mapping of vegetation condition associated with health and nutrition, and biological invasion (pest, diseases, and weeds), a sensor that can measure in several hundreds of narrow bands is required, usually with a bandwidth of 10nm or less (Filella and Peňuelas, 1994; Yang *et al.,* 2000; Bronge and Mortensen, 2002; Asner and Vitousek, 2005; Liew *et al.,* 2008). Unfortunately, due to technical constraints, satellite remote sensing systems are unable to offer both high spatial and high spectral resolution but airborne systems do have this capacity.

In reviewing hyperspectral techniques for estimating biophysical parameters of forest ecosystems, Treitz and Howarth (1999) provide characteristics of several imaging spectrometers that can acquire contiguous spectra over land and water surfaces. These are presented in Table 2.3.

Table 2.3 Characteristics of selected hyperspectral imaging spectrometers.

Sensor	No. of bands	Spectral coverage (nm)	Spatial resolution (m)	Band width (nm)	Period of operation	Platform
CASI	288	385-905	25cm-1.5m	10	since 1989	Airborne
AVIRIS	224	380-2500	20m	9.4-16.0	since 1987	Airborne
SFSI	240	1200-2400	4	10	since 1994	Airborne
Probe-1	128	400-2450	1-10m	Nov-18	since 1994	Airborne
Hymap	126	50-2500	03-Oct	15-20	since 1999	Airborne
Hyperion	242	400-2500	30	10	since 2000	Space-borne

CASI – Compact Airborne Spectrographic Imager
AVIRIS – Airborne Visible Infrared Imaging Spectrometer
SFSI – Shortwave Infrared Full Spectrum Imager
(Adapted from NERC Earth Observation data centre and Treitz and Howarth, 1999)

2.4.4 Thermal infrared imaging techniques

The common target and overall aim for remote sensing of plant stress remains early detection of stress with an interest to achieve timely response and treatment. Although remote sensing research has traditionally focused on reflectance measurements in the visible and NIR in order to fulfil this aim, there is significant potential for using the techniques of thermography in this context.

Theoretically, all objects that possess heat energy that are above 0 k emit electromagnetic radiation continuously, as a result of random particle motion (Asner, 2004). In the context of plant organisms, the temperature and emission of thermal radiation is linked to the stomatal conductance, which is controlled by a complex regularity network that integrates environmental and developmental factors (Fan *et* *al.,* 2004; Chaerle *et al.,* 2005; Li *et al.,* 2006). Disturbance to the processes of transpiration can be exploited as cues for plant stresses (Liew *et al.,* 2008) and the thermal imaging technique provides information on the effect of stressor on stomatal related parameters (West *et al.,* 2005).

Past studies show that the thermal dynamics of vegetation, involving changes in leaf or canopy temperatures are good indicators of vegetation stress. Water deficit in plant induces stomatal closure and as a result restricts transpiration processes which ultimately could lead to less water evaporation from the leaf surface (Ceccato *et al.*, 2001). Thus, this brings about less cooling effects through latent heat loss and consequently an increase in leaf temperature (Jackson, 1986).

Evidence shows that it is feasible to employ thermal imaging techniques for plant stress detection because their thermal properties in the use of captured light energy possibly changes upon stress (Buschmann, 1999). One way of employing thermal techniques for plant stress detection is by use of thermography. The operational principle of thermography as a passive imaging system for detecting the long-wave (thermal) radiation emitted by the subject is as an indicator of leaf temperature (Chaerle *et al.,* 2007). Jones (1999) indicated that thermography visualizes leaf surface temperature, and has equally been pronounced as a proxy for transpiration and stomatal conductance. The technique can monitor the event of water stress as decreased transpirational cooling from stomatal closure leading to an increase in leaf temperature (Jones, 1999; Jones, 2004; Grant *et al.,* 2007).

Thermography has been successfully used at the laboratory scale to reveal stress situations that affect stomatal conductance (Jones, 2004). Stomatal conductance is one of the key factors that determine plant yield (West *et al.,* 2005); hence it is an acceptable parameter for measuring stress condition. Surface temperature control offered by the transpiration process helps us to interpret different thermal signals exhibited by plants with respect to stress response. Thus, the difference in the thermal signals as imaged by thermography could provide reliable information about the health status of plants. There are instances where the initial rise in leaf temperature corresponds to plant resistance to biotic stress, otherwise called hypersensitive response. Hypersensitive reaction of tobacco to tobacco mosaic virus results in initial rise in leaf temperature caused by stomatal closure (Chaerle *et al.*, 2007), resulting from accumulation of salicylic acid (Chaerle *et al.*, 1999). After an initial rapid thermal expansion over a given period of time, the thermal signal gradually declines. This gives additional support and offers strong evidence about the potentials of thermal imaging techniques as a viable tool in early detection of plants stress – particularly pathogen-induced. Besides disease induced stress, Jones (2004) noted that most applications of thermal imaging systems are related to monitoring plant responses to water deficit stress.

Apart from biotic stress, few of the abiotic-induced stress effects on plant thermal response have been studied. In a comparative study, Carter *et al.* (1996) found no significant difference between plant canopy temperature subjected to herbicide-induced stress and unstressed canopy. As explained in section 2.4, there is a view that temperature increases when leaves are coated with oil (Pezeshki *et al.*, 2000) due to blockage of transpiration pathways (Pezeshki and DeLaune, 1993). However, the thermal response of plants indirectly exposed to oil pollution, through soil contamination is not known.

2.4.5 Synthetic Aperture Radar (SAR) imaging techniques

Radars are active sensors which operate in the microwave region of the electromagnetic spectrum (wavelengths in the order of millimeters to centimeters). SAR imaging has potential for large area coverage and is noted to have all weather and cloud cover penetration capabilities, and thus, is valuable in areas that are prone to frequent cloud cover. The European Space Agency (ESA) (2007) indicates that the microwave capability offered by the ERS series means that observation is not limited by weather or light conditions as are optical data. The agency provides an overview of the wide range of applications of Earth Resources Satellite (ERS) SAR data. These ranges of practical application of the earth observation system have been classified under oceanic and land environments and have also been noted as an emergency application technique.

For example, on the oceans, most of the illegal or accidental anthropogenic spills, as well as natural seepage from oil deposits, are clearly visible on radar images. Ships can be detected and tracked from their wakes. Ice monitoring, mapping of the topography of the ocean floor and provision of input data (such as ocean waves and their direction of displacement) for wave forecasting and marine climatology are achievable. Major areas of application of SAR images include:

(i) mapping and monitoring landuse/landcover and for forestry changes and agriculture studies for monitoring crop development.

(ii) enhancement of geological or geomorphological features.

(iii) supports georeferencing of other satellite imagery to high precision, and in regular updating of thematic maps.

(iv) helps to optimize response initiatives and assess damages after flooding.

(v) interferometric SAR can be used under suitable conditions, to derive elevation models or to detect small surface movements, in the order of a few centimeters, caused by earthquakes, landslides or glacier advancement.

Monitoring the scale of global crop production and trade has been identified as an area in which SAR data may be able to assist. In addition, these systems provide information for mapping forest extent and type, particularly in tropical areas which have not previously been mapped due to almost continuous cloud cover. It is a unique source of data, and in conjunction with other remotely sensed data it can be used to map forest damage, the encroachment of agriculture onto forested areas unsuitable for development, and in general to provide inventories of timber areas.

It is worth noting that despite many advantages of SAR system, it has some inherent limitations especially in the context of vegetation stress monitoring. There is a lack of evidence that it can be used in this context as many of the available microwave sensors lack spatial resolution to be practical for plant stress monitoring. They are more responsive to change in vegetation structure than function thus, can only be of relevance for severe or later stages of stress especially when plant death must have occurred.

2.4.6 LiDAR imaging techniques

One emerging technology that is gaining rapid attention in remote sensing of vegetation particularly at canopy scale is LiDAR (Light Detection and Ranging). LiDAR is an active system; based on an artificial radiation source that operates in the near-infrared. Vegetation has high reflectance and transmittance at this region;

allowing a strong return from the forest canopy as well as from the forest floor (Kasischke *et al.*, 2004). The technology provides horizontal and vertical information at high spatial resolutions and vertical accuracies (Lim *et al.,* 2003). LiDAR has the capability of measuring the geometrical structure of plants which is the most important factor that influences the reflectance of plants at canopy scale. For example, Riano *et al.* (2004) demonstrated the possibility of measuring canopy LAI from LiDAR imagery.

Thus, while LiDAR imagery alone is probably insufficient for monitoring plant stress, its combination with hyperspectral imagery is very promising, in this respect. For example, one notable area of LiDAR data application which has improved the accuracy of pigment estimates at the stand scale is in extraction of spectral information from tree crowns, while extraneous spectral information from canopy gaps are removed (Blackburn, 2002). The study noted that this was possible by applying spatial filters created from the canopy surface elevation models derived from the LiDAR data to imaging spectrometer data from forests. Again, with imaging LiDAR, it is possible to quantify total canopy chlorophyll content; by using the measured canopy LAI to scale-up estimates of foliar chlorophyll concentration derived from hyperspectral data (Solberg *et al.*, 2005). Blackburn (2007) suggests that the combination of LiDAR and hyperspectral imaging technique in studying the geometrical structure of heterogeneous canopies remain a possibility which needs further investigation.

2.5 Conclusion

Plant stress can be caused by various biotic and abiotic factors. Oil pollution is an abiotic factor that can affect plants. Plants can be affected directly through physical contamination with oil or indirectly through soil pollution. Various remote sensing techniques have been identified as valuable tools for estimating and mapping plant biochemical and biophysical properties, in order to understand the health status of plants.

In the context of hyperspectral remote sensing, several approaches have been found to be useful for plant stress detection both at early and later stages. These include: the use of characteristics of spectral reflectance in the visible, NIR and SWIR regions, the characteristics of the red edge such as the position, selection of diagnostic individual narrow wavebands, and a plethora of spectral reflectance- and derivative-based ratios. However, the optimal spectral indicator for monitoring plant stress induced by oil pollution is not known. The potential of thermal imaging techniques for detection of plant stress, particularly abiotic-induced stresses other than water deficit, have not been extensively studied. The literature suggests that increased leaf temperature is one of the possible effects of physically-induced oil pollution on plants but it is not known if the same effect occurs when plants are polluted indirectly through soil contamination. In summary, there is strong evidence that hyperspectral and thermal remote sensing techniques hold considerable potential for monitoring plant stress, but the specific case of detecting and quantifying stress induced by oil pollution requires further investigation.

Chapter 3

METHODOLOGY

3.1 Introduction

This chapter deals with the general methodology adopted in this study. It covers the range of plant materials used and various treatments applied. Various measurements undertaken including the instrumentation and measurement procedures used are presented. The methods and procedures used to analyse the data are explained and key criteria for evaluating the information found are identified.

3.2 Plant material

With the exception of a field based pilot study (reported in Chapter 4 below), all experiments were carried out in a glasshouse (10 x 3m) at the Lancaster Environment Centre, Lancaster University, UK. Day and night temperatures were typically 26°C (±2°C) and 15°C (±1°C) respectively, and a 12 h supplementary photoperiod (06.00 h to 18:00 h) was provided by Osram Plantastar 600W sodium lamps to give a photosynthetic photon flux density (PPFD) of 400 μmol $m^{-2}s^{-1}$ at bench height. Maize (*Zea mays* L.) and French dwarf bean (*Phaseolus vulgaris 'Tendergreen'*) were the model plant species chosen for this study. Two seedlings of maize (previously pre-germinated for three days on damp tissue paper in darkness) or bean, were sown per 2 L pot containing a loam-based compost (John Innes No.2, J. Arthur Bowers, Lincoln, UK). Pots were placed on capillary matting that was watered daily to ensure that soils were kept moist and to prevent waterlogging and possible nutrient leaching that may have arisen by overhead watering. Plants were thinned to one per pot after two weeks and left to continue to establish for a further week before treatments commenced. Initial 'zero time' measurements were taken for all plants immediately before first treatment application. Measurements were repeated every 2 to 3 days thereafter.

3.3 Plant treatments

In all experiments, the control received no treatment. For the oil treatment, 15W/40 diesel engine oil (Unipart, Crawley, UK) was applied to the soil surface and allowed to penetrate down through the pore spaces. In each case, the dosage was determined based on a percentage volume of the soil water holding capacity (WHC) of the pot (field capacity minus oven dry), previously determined as 0.63g H_2O g^{-1} compost at a density of 0.8g cm^{-3}. Application rates were 20% of WHC, being equivalent to 96g oil kg^{-1} soil. Waterlogging stress was instigated by flooding the pots with water to a depth of 2.5cm above the soil surface twice a day. A 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. This was to ensure that plants received equal volumes of water, to avoid totally displacing oil treatments where present, and to prevent occurrence of incidental waterlogging where not required. During the experimental period, pots were randomized and periodically rotated around benches to minimize possible effects of differences in glasshouse microclimate on plant development.

3.4 Physiological measurements

In order to understand the physiological responses of plants to the stress treatments, the same leaf on each plant was monitored throughout the experiment. The sixth or seventh fully emerged maize leaf and third trifoliate bean leaf (which was the most dominant from nadir at the start of the experiment in bean), were chosen for physiological measurements. All measurements 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). The leaf cuvette conditions were 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 in the cuvette prior to recording data. At the same time plants were visually inspected for any visual signs of stress.

3.5 Thermal imaging

Thermographs for individual leaves (for leaf scale measurements) and canopies (for canopy scale measurements) were acquired in the glasshouse (unless otherwise stated) using an SC2000 thermal camera (FLIR Systems, West Malling, UK). The thermal camera operates in a waveband from 7.5 to 13 μm with a thermal sensitivity of 0.07°C at 30°C. The field of view (FOV) was 24° x 18°, the spatial resolution 1.3 mrad and emissivity was set to 0.95. Measurements were made following the procedure of Grant *et al.* (2006). At each time of measurement, two leaves were cut off from the reserved maize plants and placed close to the leaf of interest in order to act as wet and dry reference surfaces. The wet references were regularly sprayed with water to keep them moist while the dry references were covered in petroleum jelly to inhibit water (and therefore heat) loss. The acquired thermal images were recorded on a portable disk and later downloaded to a PC for analysis.

3.6 Spectral measurements

Leaf and/or canopy spectral reflectance data were collected in a dark room directly opposite the glasshouse and immediately after physiological and thermal measurements, using a field portable GER 1500 spectroradiometer or an ASD FieldSpec® Pro Spectroradiometer (Boulder, CO 80301 USA). The GER 1500 uses a diffraction grating with a silicon diode array that has 512 discrete detectors that provides the capability to read 512 spectral bands. Thus, it scans the spectrum at approximately 1.5 nm intervals and covers a portion of the Ultraviolet (UV), the Visible, and the Near-infrared (NIR) wavelengths from 350 nm to 1050 nm. 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.

The spectral measurements were carried out in a dark laboratory room in order to ensure stable and uniform illumination conditions (Mutanga *et al.,* 2003; Vaiphasa *et al.,* 2005). To minimise the effects of background spectral variations, each pot was placed on a fixed black tray directly under the sensor (Gong and Heald, 2002; Mutanga *et al.,* 2003). Before leaf spectral measurements were taken, the leaves were clipped onto a flat low-reflectance surface. The low reflectance was provided to minimise the effects of a background spectra on the sample spectrum (Gong *et al.,* 2002).

To fully illuminate the target, a 500W halogen lamp was mounted at a fixed position away from each leaf or plant canopy to be measured. Where an ASD FieldSpec® Pro Spectroradiometer was used for reflectance measurements, the ASD foreoptics were positioned at nadir, 6cm above each leaf and plant canopy to be measured. An 18^0 FOV was used which covered a sample area 2cm diameter on the surface of individual leaves and approximately the same diameter at the top surface of the plant canopies sampled. Prior to scanning, the lamp was switched on for 20 minutes to eliminate spectral changes in the lamp as it warmed up (Smith *et al.*, 2004a). Ten spectral measurements were captured per leaf or canopy for each of the 10 replicates per treatment. Additionally, spectra were taken randomly by concentrating around the centre of the leaf or canopy and avoiding outer boundaries. Leaves were slightly shifted between measurements to capture spectral variations within each leaf. In order to capture spectral variation within canopies, small adjustments were made to the position and rotation of the pot between each spectral measurement. Prior to spectral measurement, a reference measurement was first made using a white Spectralon panel (Labsphere, North Sutton, New Hampshire, USA) placed in the same position as the leaf or canopy. In each case, the time between reference and target measurements never exceeded one minute. The leaf spectral reflectance (R) was computed by dividing the radiation reflected from the leaf or canopy (It) by that reflected from the white spectralon reference panel (Ir) and applying a correction (k) for the spectral reflectance properties of the panel, as no perfectly reflecting panel exist in practice (Milton, 1987).

$$\text{Thus, } \%\, R = \frac{It}{Ir} \text{ x } k \text{ x } 100 \qquad (1)$$

3.7 Measurement of leaf pigments and water content

After leaf and/or canopy 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 (for maize) and 6 mm in diameter equivalent to 0.28 cm^2 leaf disk areas (for bean) 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^oC for later determination of pigment content. The rest of the samples were immediately weighed to determine fresh mass before they were dried at 80^oC 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 100% methanol with a pinch of calcium carbonate, to form a homogenous slurry. Pigments were extracted from the crushed samples by adding 5 ml of 100% methanol in a centrifuge tube. The tubes were placed in a refrigerator at $< 5^0C$ overnight to ensure complete extraction before centrifuging to remove particulates. The samples were spun for 2 minutes at 30,000 revolutions per minute (rpm). 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*, chlorophyll *b*, chlorophyll *a* + *b* and carotenoids *x* + *c* (cars) in μg cm^{-2} were determined using the equations derived by Lichtenthaler (1987):

$$\text{Chlorophyll } a = 16.72{*}A_{665.2}) - (9.16{*}A_{652.4}) \quad (2)$$

$$\text{Chlorophyll } b = (34.09{*}A_{652.4}) - (15.28{*}A_{665.2}) \quad (3)$$

$$\text{Chlorophyll } a + b = (1.44{*}A_{665.2}) + (24.93{*}A_{652.4}) \quad (4)$$

$$\text{Cars} = ((1000{*}A_{470})\text{-}(1.63{*}\text{chlorophyll } a)\text{-}(104.96{*}\text{chlorophyll } b))/221 \quad (5)$$

While equation 3 gives the concentration of total chlorophyll, i.e., the sum of chlorophyll *a* and chlorophyll *b* (*a* + *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 contents in micrograms per cm^2 of leaf.

3.8 Data analysis

3.8.1 Physiological analysis

In order to ascertain and quantify the effects of treatments on plant physiological properties, the photosynthetic rate, transpiration and stomatal conductance for each sample was measured on each day of assessment. Physiological 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.

3.8.2 Thermal imaging analysis

Thermal images were analysed using ThermaCAM Researcher 2001 software (FLIR Systems, West Malling, UK). Polygon areas were selected from the image covering the wet and dry leaf references as well as the target leaf or canopy of interest to measure their thermal characteristics. The minimum, maximum, and average leaf or canopy temperatures were extracted for each replicates of control and treated plants. The thermal index (I_G) was calculated from leaf or canopy temperatures as:

$$I_G = (T_{dry} - T_{leaf})/(T_{leaf} - T_{wet}) \text{ (Grant \textit{et al.}, 2006)} \quad (6)$$

Where, T_{dry} = temperature of the dry reference

T_{leaf} = temperature of the leaf or canopy of interest

T_{wet} = temperature of the wet reference

The I_G is theoretically proportional to stomatal conductance (g_s) under any environmental conditions (Jones, 1999). The outputs were transferred to Microsoft Excel spreadsheets in order to determine treatment means and standard errors. Finally, leaf or canopy mean temperature and I_G (% of control) values were plotted against time in order to observe the effects of treatments on thermal responses.

3.8.3 Spectral data analysis

The spectral data in GER1500 .sig files were imported into Microsoft Excel for processing using a Visual Basic macro. Individual reflectance spectra were displayed and visually assessed to eliminate erroneous data. Differences between the initial spectral reflectance of control and treatments were computed. These were used to normalise subsequent spectral reflectance of treatments. This was to ensure a meaningful comparison between changes in spectral reflectance of control and treated plants. In order to examine the effect of treatments on plant spectral properties, the mean reflectance spectra of control and treatments were plotted against wavelength. However, wavelengths shorter than 400 nm were not analysed due to excessive noise. Differences between the mean reflectance spectra of treatments and control were plotted in order to identify wavelengths of high variation between the treatments and control.

Derivative spectroscopy concerns the rate of change of reflectance with wavelength (Smith *et al.,* 2004a). The derivative analysis was undertaken in order to (i) discriminate between overlapping bands, (ii) locate the position of the primary red-edge wavelength associated with leaf damage (Miller *et al.,* 1990; Martin, 1994; Smith *et al.,* 2005) and (iii) identify other red-edge features that may indicate stress in leaves. Derivative analysis can enhance absorption features that might be masked by interfering background absorptions (Elvidge, 1990; Dawson and Curran, 1998) and leaf background effects. Thus, derivatives can provide a more sensitive analysis than using original reflectance spectra (Smith *et al.,* 2004b). A first derivative spectrum displays the variation with wavelength in the slope of the original reflectance spectrum (Blackburn, 2007b). Thus, the first derivative was calculated using the ratio of difference between original spectral reflectance values in two individual narrow wavebands that span a window of three adjacent wavebands. The resulting derivative was smoothed using a three-band window moving average across the spectrum. This procedure eliminated the effects of noise and at the same time minimised the loss of fine spectral detail.

The red-edge region is the region of occurrence of multiple peaks. The red-edge position (REP) is conventionally marked by the wavelength corresponding to the maximum amplitude of the first derivative within the region of the red-edge. Limitations associated with this method and other methods used in determining the REP were identified by Cho and Skidmore (2006). This led to the development of a new approach called the linear extrapolation technique. The model defines the REP as the wavelength that corresponds to the intersection of two straight lines extrapolated through two points on the far-red and two points on the NIR flanks of the first derivative reflectance spectrum of the red-edge region. Thus:

$$\text{REP} = \frac{-(c1 - c2)}{(m1 - m2)} \tag{7}$$

where c1 and c2, and m1 and m2 are the intercepts and slopes of the far-red and NIR lines respectively. Afterwards, the amplitude which gives the degree of height of the REP, in other words, the first derivative reflectance of the REP was recorded.

Besides the REP and its amplitude, other red-edge attributes analysed include (i) the positions of the first and second of the double peaks and (ii) the distance between the positions of the double peaks in the second derivative reflectance red-edge region. Thus, the second derivative was calculated by taking the ratio of difference between first derivative reflectance values in two individual narrow wavebands that span a window of three adjacent wavebands. Essentially, the positions of the double peaks correspond to points where the second derivative curves have a value of zero (Cho and Skidmore, 2006). Thus, the position of the first of the double peaks was calculated as the wavelength that corresponds to the first data point of zero value in the second derivative curve in the red-edge region. Similarly, the position of the second peak was calculated as the wavelength that corresponds to the second data point of zero value in the second derivative curve in the red-edge region. The distance between the wavelength positions of the double peaks was then calculated.

Various individual narrow wavebands and spectral indices were also used to analyse stress effects on leaf spectral reflectance. These were chosen based on systematic selection of bands (using a single waveband per region of the spectrum) and systematic combination of wavebands across the entire wavelength range. In addition, wavebands at which reflectance variation between treatments and controls were high were analysed. The entire reflectance spectrum was considered because subtle differences arising from physiological stress do not appear only at specific regions such as the red-edge but are distributed across the spectrum. The limitation of not investigating all combinations of wavebands across the entire spectrum was due to the significant computation time for performing the sensitivity analysis.

Spectral indices proposed by several studies as being useful for plant stress detection were investigated (Bonham-Carter, 1988; Miller *et al.,* 1990; Miller *et al.*, 1991; Vogelman *et al.,* 1993; Carter, 1994; Belanger *et al.,* 1995). These indices calculate ratios of bands, or properties mainly in region of the red-edge (Kempeneers *et al.*, 2005).

3.8.4 Statistical and sensitivity analysis

Statistical analysis was performed using Analysis of Variance (ANOVA) to ascertain which of the stress indicators was optimal for early detection, prediction and quantification of stress arising from treatments. The criteria used to judge their sensitivity to stress were time of detection and consistency in detection during the remainder of the experiment. Time of detection was particularly considered in order to determine whether stress arising from treatments could be detected by hyperspectral and/or thermal sensing before symptoms became apparent to an unaided eye. However, both factors would help to establish reliability and general sensitivity for each of the stress indicators. All significant differences were at the 0.05 level of confidence unless otherwise stated. The hypothesis tested using ANOVA was that there is no significant effect of treatments on plant physiological, spectral and thermal properties. Post hoc test analyses using Tukey HSD were performed on ANOVA to determine significant treatment differences by a mean square multiple comparison procedure. This helps to ascertain the sensitivity of an indicator to various treatments. Regression analysis was conducted to ascertain relationships between remotely-sensed stress indicators and physiological parameters.

Where biochemical measurements were made, the measurements of treated plants were expressed as percentage of control on each measurement occasion. Sensitivity analysis was performed on the biochemical data using ANOVA to determine when significant responses to the different treatments occurred and whether these responses were consistent throughout the experiment.

T-tests were performed on wavelengths of high spectral 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 a given physiological property e.g. photosynthetic rate, are 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 (Tarpley *et al.*, 2000; Read *et al.*, 2002) were also tested.

Chapter 4*

PRE-VISUAL DETECTION OF OIL-INDUCED STRESS IN MAIZE (*Zea mays*, L.) USING LEAF SPECTRAL AND THERMAL RESPONSES

4.1 Introduction

Many studies have shown that pollution-induced stress has a negative effect on the physiological functioning of plants. For example, it has been demonstrated that leakage of natural gas into the soil caused restricted plant growth and reproduction as well as decreased the number of individuals (Godwin *et al.,* 1990). However, changes in plant growth rate or species composition and plant community structure are relatively slow to respond to gas leaks and thus are generally inadequate as early stress indicators (Mendelssohn *et al.,* 2001). Alternatively, visual observations of plants may provide timely indications of the symptoms of plant stress. For example, visual stress symptoms in the form of shoot and leaf chlorosis and to some extent, thinner canopies were observed in *Salicornia virginica* 13 days after high levels of cadmium treatment (Rosso *et al.,* 2005). Similarly, severe effects of cadmium on *Spartina alterniflora* leaf expansion were observed 5 days after treatment (Mendelssohn *et al.,* 2001). Shoot mortality, stunting and a moderate chlorosis were exhibited by *Salicornia virginica* 19 days after vanadium treatment and growth was inhibited in *S. virginica* 10 and 32 days after being contaminated with 'Escravos' and 'Alba' petroleum, respectively (Rosso *et al.,* 2005). These studies give evidence that it can take a considerable time for plants to show visible stress symptoms induced by pollution and that this time varies according to the type

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of pollutant. Thus, relying solely on visual manifestation of stress for early detection of stress may not be adequate.

Stress conditions in plants are known to result in changes in the reflectance spectra of leaves and canopies (Knipling, 1970; Noomen *et al.,* 2003; Kempenneers *et al.,* 2005) that can be detected before symptoms can be observed visually (Carter *et al.,* 1996). In the latter study, herbicide-induced stress was detected 16 days prior to the first visible signs of damage. Alterations in plant biochemistry and cellular composition imposed by environmental stressors produce changes in the reflectance characteristics that can be detected using remote sensors (Rosso *et al.,* 2005). Indeed, numerous studies have found a significant increase in visible reflectance and decrease in near-infrared reflectance in response to various stresses (Carter, 1993; Carter and Miller, 1994; Carter *et al.,* 1996; De Oliveira, *et al.,* 1997; Rosso *et al.,* 2005). In contrast, no significant reflectance changes were found in younger leaves of plants contaminated with heavy metal at sub lethal levels (Mendelssohn *et al.,* 2001). This was attributed to the immaturity of the leaves, as spectral reflectance was measured in the youngest fully expanded leaves, which were usually robust and healthy.

A large and growing body of literature has used changes in thermal properties of leaves or canopies to monitor stress, particularly water deficit. Plant temperature is a valuable index for detection of plant and canopy water status (Ehrler, 1973; Jackson *et al.,* 1977). Thus, it is worthwhile to investigate whether the thermal properties of leaf could serve as additional way of detecting stress induced by oil pollution. In some cases, combined spectral and thermal techniques have been employed to provide rapid identification of crop growth status (Al-Abbas *et al.,* 1974; Thomas and Gausman, 1977; Schepers *et al.,* 1996; Gitelson *et al.,* 2003; Raun *et al.*, 2005). Hence, the fusion of spectral and thermal remote sensing techniques is potentially of value in the context of oil pollution.

This chapter deals with measurements and analysis of physiological, spectral and thermal properties of maize (*Zea mays* L.) contaminated with different levels of oil. 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). Additionally, maize is an important crop which, in its different processed forms, makes a large contribution towards feeding the world's populace and its livestock. Its by-products are used in the manufacture of diverse commodities including ethanol, glue, soap, paint, insecticides, toothpaste, shaving cream, rubber tyres, rayon and moulded plastics. Oil pollution is known to affect farmlands which are likely to incorporate maize, which is widely cultivated throughout the world, and a greater weight of it is produced each year than any other grain.

Measurements were undertaken at various times before and after visual stress symptoms were seen. The time when significant changes in reflectance spectra and thermal properties first occurred was compared with the time of first visible stress symptoms. Several spectral and thermal indices have been developed as viable stress indicators, but the indices that are most sensitive to oil-induced stress in maize are not known. In this study, the sensitivity of a number of stress indicators shall be compared in order to discover which is optimal. Attempt is made to quantify the effect of refined oil on the photosynthesis, transpiration and stomatal conductance activities of maize. The physiological properties are correlated with spectral and thermal properties in order to understand their relationships. The specific objectives are:

i) To determine the efficacy of spectral and thermal properties of plants as indicators for oil pollution.
ii) To determine an optimal remotely-sensed index for early detection of oil-induced stress in plants.

4.2 Pilot study

In order to improve the quality and efficiency of this experiment, a feasibility study was first undertaken to test logistics and gather useful information prior to the actual experiments. There were key issues from the proposed experimental design that needed clarification before undertaking further experiments. These include: (i) working out appropriate dosage for each treatment level as standardised lethal and sub lethal levels of oil contamination are not known; (ii) assessing the appropriate duration of experiments; and (iii) developing methods of data analysis. Overall, there was the need to gain insight and understanding about the basic, technicalities and operational principles of some of the techniques that would be used. Apart from clarifying the above methodological issues, the key aim of the experimental study was to investigate the effects of heavy refined oil product (engine oil) on the spectral reflectance properties of two plant species, with two specific objectives:

(i) to examine how oil pollution at varying levels affect the spectral properties of individual plant species.
(ii) to examine how different plant species respond to oil pollution.

4.2.1 Plant materials and treatments

A deciduous shrub called forsythia (*Forsythia suspensa*) and ornamental fountain grass (*Pennisetum alopecuroides*) plant species were used for the experiment. These plants were used for the pilot study because of their availability at the time of the experiment. Plants were up to six months old before the experiment studies begun on the 14th August, 2007 and ended on the 11th September, 2007. Four treatments, each comprised of two replicates were established for each of the plant species. These include the control and three dose levels of oil treatment. Systematically, 20%, 40% and 60% of soil WHC were chosen to represent low, medium, and high dose levels respectively. Pots were kept outdoors under natural and uniform environmental condition except when plants were taken into a dark room for spectral measurements. The plants were watered on a regular basis.

4.2.2 Spectral measurements and analysis

Plants were transferred in their pots from outside to a laboratory for measurements. This was to control the influence of other factors on the spectra not related to plant vigour, such as change in illumination angle, atmospheric effects (Luther and Carrol, 1999; Mutanga *et al.,* 2003; Vaiphasa, *et al.,* 2005) and areas of shadow (Blackburn, 2007). The relatively dense canopy structure formed by the plants also controlled the effects of even more controlling factors such as soil/litter surface reflectance, % canopy ground coverage, and presence of non-leaf elements (Blackburn, 2007). A field portable GER 1500 spectroradiometer was used for all reflectance measurements. The specifications of the instrument and every other procedure taken were given in chapter 3, section 3.5. However, the sensor was mounted in a fixed position at about 1.5 m above the canopy at the nadir position with a standard 4^{0} field of view fore-optic. Spectral measurements were undertaken every four to five days and development of stress symptoms was visually observed every week.

Wavelengths considered for analysis were based on systematic selection of different spectral regions that is, the blue (400-500nm), green (500-600nm), red (600-700nm), near infrared (700-800nm) and far infrared (800-900nm). With respect to these spectral regions, the wavebands at which the reflectance difference between the treated plants and controls was high were selected for statistical analysis. This was to ascertain whether change in their spectral reflectances were statistically different. The hypotheses tested were:

(i) There is no significant difference between changes in spectral reflectance of plants treated with oil at different doses.

(ii) There is no significant difference between change in spectral reflectance of different plant species (i.e. grass and forsythia) treated with oil.

ANOVA comparisons were used to test the first hypothesis. Where the spectral reflectance of control and treated plants was statistically different, further analysis was carried out using Post hoc multiple comparisons to ascertain which samples were different. The second hypothesis was tested using Wilcoxon signed-rank test. Although, scale level of measurement was used for data acquisition, the Wilcoxon signed-rank test was used because the sample size is small and they are also related.

4.2.3 Results of pilot study

4.2.3.1 Visual stress symptoms

Treated plants of both grass and forsythia were visually affected by oil pollution as shown in Figures 4.0 and 4.1 respectively. A variety of visible stress symptoms ranging from stunting, leaf chlorosis and shoot mortality were generally observed in all treated plants as summarised in Table 4.0. While stress symptoms were observed in grass one week after oil treatments, the forsythia showed stress symptoms after two weeks. However, the control plants flourished throughout the experimental period.

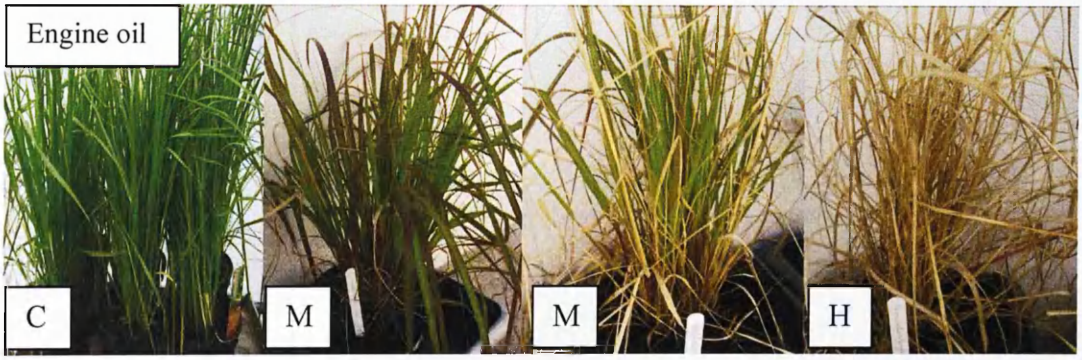

Figure 4.0 Visual symptoms of grass according to treatment levels of engine oil. C = control, L = low, M = medium, H = high.

Figure 4.1 Visual symptoms of forsythia 28 days after treatments with engine oil at varied doses. C = control, L = low, M = medium, H = high.

Table 4.0 Visual stress symptoms of grass and forsythia contaminated with engine oil at varied doses. C = Control, L = Low, M = Medium, H = High.

Plant specie	Treatments	Visual stress symptom				
		Day 0	Day 7	Day 14	Day 21	Day 28
Grass	**L**	same as above	growth rate declining while some leaves were dehydrating	few leaves were dehydrated	few leaves were partially dehydrated while some change to reddish brown	few leaves were partially dehydrated while some change to reddish brown
	M	same as above	growth rate declining while some leaves are dehydrating	same as low but involves more number of leaves	same as low but with an increased rate	same as low but with an increased rate
	H	same as above	same as low and medium but at relatively higher rate	almost all leaves were affected	leaves completely dehydrated	plant death
Forsythia	**L**	same as above	same as above	still green	very few leaves appeared pale	very few leaves appeared pale while others remained green
	M	same as above	same as above	chlorosis affecting very few number of leaves	similar symptoms as low	similar symptoms as low but affecting more number of leaves
	H	same as above	same as above	same as the medium but with an increased rate	similar symptoms as low but involves larger number of leaves	some of the leaves wilted and appeared pale and reddish brown, shoot mortality occurred

4.2.3.2 Spectral response to stress

The spectral reflectance of treated plants generally increased in the visible and decreased in the NIR region of the spectrum relative to control. Figures 4.2 and 4.3 show the mean reflectance of the treated plants and controls on the final day of the experiment. The pattern of reflectance changes generally follows the dose level except in forsythia where medium dose level had highest reflectance in the NIR (Figure 4.3).

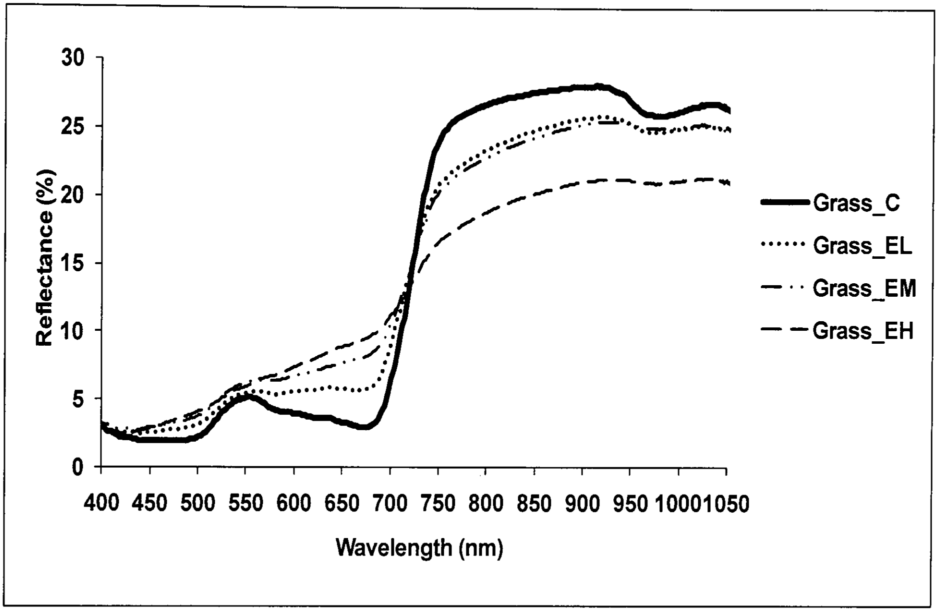

Figure 4.2 Mean reflectance spectra of engine oil treatments and control in grass 28 days after treatments commenced. C = control, EL = engine oil low dose, EM = engine oil medium dose, EH = engine oil high dose.

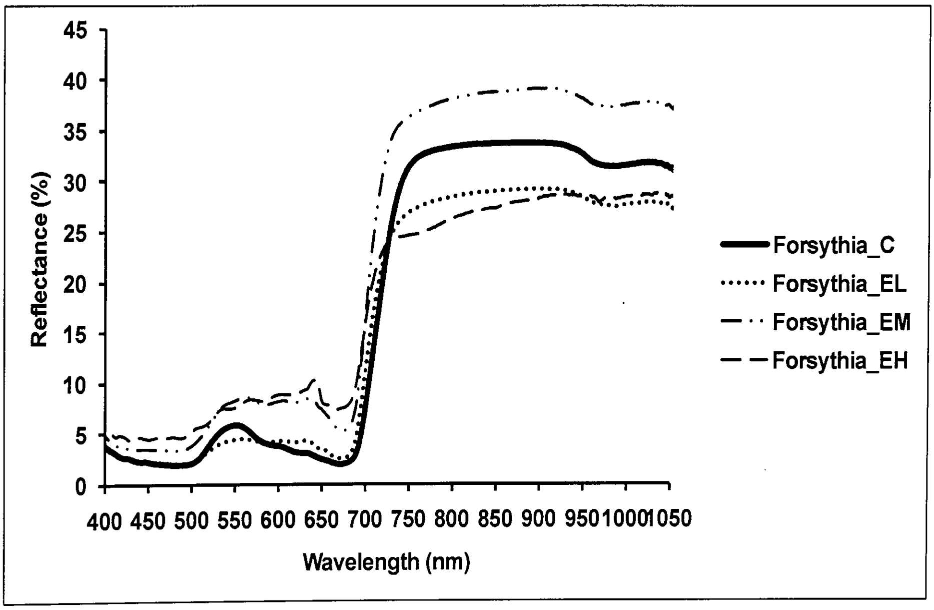

Figure 4.3 Mean reflectance spectra of engine oil treatments and control in forsythia 28 days after treatments commenced. C = control, EL = engine oil low dose, EM = engine oil medium dose, EH = engine oil high dose.

Table 4.1 shows a summary of ANOVA testing for significant differences between the spectral reflectance of control and treated plants. Grasses treated with high dose of oil showed a significant change in reflectance in the blue region. In the same region (blue), high treatment doses significantly affected forsythia's spectral reflectance. In the green region, high treatment doses significantly affected the spectral reflectance of grass. Similarly, medium and high treatment doses had a significant effect on forsythia in the green region. In the red region, medium and high treatment doses significantly affected the spectral reflectance of grass and forsythia. In the NIR, high doses significantly affected grass spectral reflectance. However, medium and high doses had no significant effect on forsythia spectral reflectance in the same region (NIR). At longer wavelengths in the NIR, all treatment doses had a significant effect on grass spectral reflectance unlike in forsythia where no significant difference was found at all treatment doses.

Table 4.1 ANOVA showing significant difference in spectral reflectance changes of grass and forsythia treated with oil at different treatment doses. In the wavelength column, subscripts $_G$ and $_F$ refer to grass and forsythia respectively.

Wavelength (nm)	Treatments	Plant species	
		Grass	Forsythia
494.7_G, 401.2_F	Low	0.951	0.972
	Medium	0.541	0.681
	High	0.001*	0.000*
598.6_G, 550.8_F	Low	1.000	0.022*
	Medium	0.621	0.837
	High	0.001*	0.000*
681.1_G, 698.6_F	Low	0.087	0.159
	Medium	0.000*	0.000*
	High	0.000*	0.000*
700.2_G, 798.5_F	Low	0.887	0.246
	Medium	0.300	0.832
	High	0.001*	0.899
800.1_G, 877.7_F	Low	0.000*	0.068
	Medium	0.000*	0.237
	High	0.000*	0.976

n = 20, $_G$ = Grass, $_F$ = Forsythia, * = significant difference at 0.05.

There is a significant difference between changes in spectral reflectance of grass and forsythia treated with engine oil at different levels at all waveband regions. Except in the NIR where the ANOVA test showed no significant difference between changes in spectral reflectance of forsythia treated with oil at different levels. Thus, the hypothesis that there is no significant difference between changes in spectral reflectance of plants treated with oil at different doses was rejected at all wavelengths for the grass and accepted in the NIR for the forsythia only. A Wilcoxon signed-rank test showed no significant difference ($p = 0.109 > 0.05$) between changes in spectral reflectance of grass and forsythia treated with engine oil. Thus, the hypothesis that there is no significant difference between change in spectral reflectance of different plant species (i.e. grass and forsythia) treated with oil was accepted.

4.2.3.3 Discussion

Oil can flow through plant growing medium when spilled onto the soil surface. Oil reached the plant root zone as it flowed through the soil substrates, given that excesses were collected on the plastic bowls placed under the pots. The estimated dose for each treatment level needed review. The treatment doses adopted for the medium (40%) and low (20%) levels appeared to exhibit similar effects particularly on the spectra. Thus, it suggests that the low treatment be reduced by 10%. In addition, plant response to oil doses varied with species and therefore, there was the need to determine oil treatment doses for each species used in subsequent experiments. Timing the experiment of this kind was not straightforward as several factors such as plant type or specie, type of pollutant and level of pollution influence it. Plants have different levels of sensitivity to stress, but can generally respond quickly to high dose of pollution and slowly if contaminated at a sub lethal level. On average, it can take couple of weeks for a potted plant to reach mortality level from the time of stress initiation. Visual observation and spectral reflectance features have potential for monitoring oil pollution. Consequently, this potential shall be explored further in subsequent experiments.

4.2.3.4 Conclusion

The visible region appears to be most sensitive to oil pollution for both plant species. This is expected as it is the region of strong chlorophyll absorption. Since chlorophyll is responsible for light absorption particularly in the red, a higher reflectance exhibited by the polluted plants in this region implies a decrease in chlorophyll content. The low dose level (measured as 20% of soil WHC) showed similar spectral effects as the medium dose (measured as 40% of soil WHC). Thus, in the next experiment, the low dose treatment will be reduced by 10% in order to simulate more accurately a sub lethal level of oil pollution. Plant response to oil pollution varied with species and therefore, there may be the need to determine oil treatment doses for each species used in subsequent experiments. Based on the stress symptoms observed visually, forsythia appears to be more resistant to oil pollution than grass. This could possibly be attributed to its strong root system that may have stored sufficient resources needed to sustain plant growth. However, from the progression of stress observed in forsythia, one can predict that mortality will occur if the experiment is continued for a longer period than that used in the present experiment. This suggests that irrespective of level of oil pollution, duration of exposure could also count as an important factor for assessing plant damage by oil pollution. Thus, in subsequent experiments, measurements will continue until it becomes impossible to take further measurements as a result of plant mortality.

Overall, spectral reflectance, particularly in the visible region, appears to be a potential indicator of oil stress that could be applicable across different plant species. This pilot study indicates that the use of spectral reflectance as an indicator of oil-induced stress is worthy of further investigation, that may be focused particularly on identifying appropriate analytical methods for quantifying spectral changes that are most sensitive. The subsequent experiments shall exclusively, be undertaken in a glasshouse and available resources will allow the use of maize for the experiment.

4.3 Methods

This section describes distinctive approaches that were used to investigate the efficacy of spectral and thermal responses for early detection of oil-induced stress in maize. In addition to the use of information and understanding that were gathered from the pilot study, the methodology previously described in chapter 3 was adopted in this experiment.

Four treatments comprised of eight replicates were randomly selected from fifty established plants. Each treatment represents the control, low, medium, and high doses of oil pollution. The pots were placed in plastic trays and labelled accordingly. The doses were chosen in order to subject the plants to lethal and sub lethal levels since oil pollution occurs at varied intensity. It was intended that the low and the high dose levels represent the lethal and sub lethal levels respectively while the medium dose stands as an intermediate level. The doses were determined by calculating 10%, 30%, and 50% of the average soil WHC (see chapter 3, section 3.2) to represent low, medium, and high levels respectively. Liquid fertilizer was applied at intervals after about one week when plant growth had increased in order to avoid nutrient deficiency.

The GER 1500 spectroradiometer (already discussed in chapter 3, section 3.5) was mounted in a fixed position, at nadir, 15 cm above each leaf blade to be measured. An 8^o fore-optic was used which covered an instantaneous field of view approximately 2cm diameter centered upon the midrib of the leaf. The 500W halogen lamp was mounted at a zenith angle of 45^o and at a distance of 70cm from the leaf. Leaf pigments and water content were not measured in this experiment. A summary of the individual narrow wavebands, spectral indices and normalised difference ratios analysed is given in Table 4.2.

Table 4.2 Individual narrow wavebands and spectra indices used for spectra analysis.

Individual narrow waveband	Spectral region	Spectral ratios	Band combination
450	Blue	530/440	Blue/Green
550	Green	685/440	Blue/Red
650	Red	740/440	Blue/NIR
705	Far-red	685/530	Green/Red
706	Far-red	740/530	Green/NIR
708	Far-red	760/695	Red/NIR
710	NIR	750/700	Far-red/NIR
711	NIR	715/705	Far-red/NIR
712	NIR	740/685	Red/NIR
714	NIR	755/716	Far-red/NIR
716	NIR	(920-655)/(920+655)	Red/NIR
717	NIR	(755-716)/(755+716)	NIR/NIR
750	NIR		
850	NIR		
950	NIR		

4.4 Results

4.4.1 Photosynthesis

Oil treatments at all levels reduced the photosynthetic activities of maize (Figure 4.4.). The relative reductions followed dose levels with photosynthesis decreasing as stress increases. The net gas exchange was not affected at low dose level until after 6 days of treatment while the medium and high levels were both affected after 2 days. The statistical significance of the differences between the photosynthetic activities of the different dose levels and the controls is given in Table 4.3.

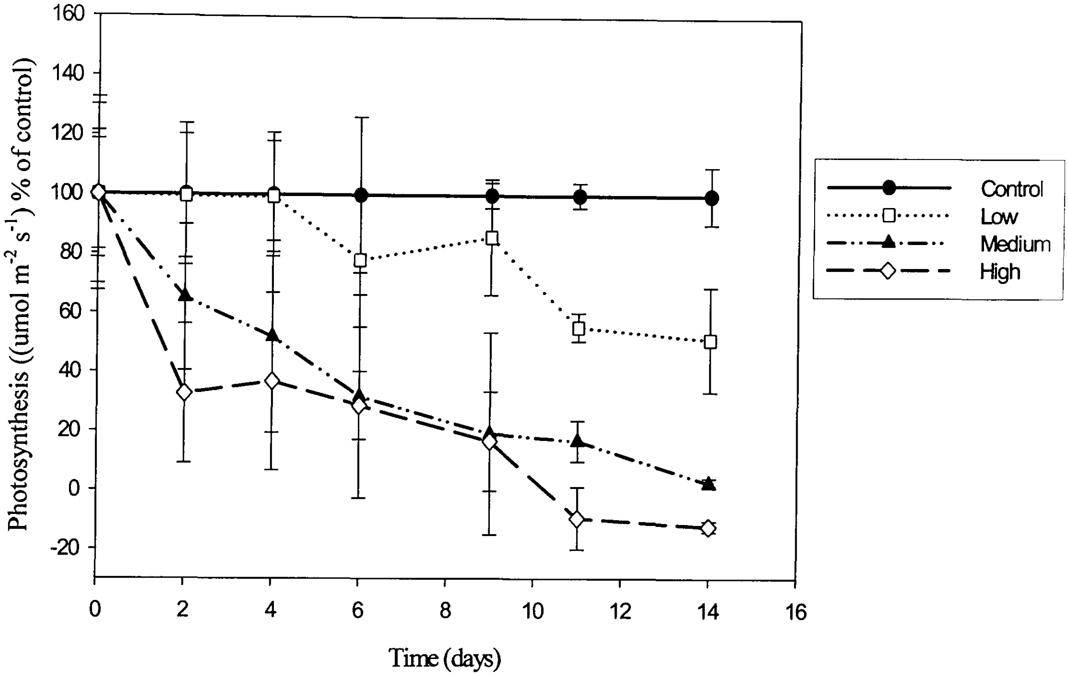

Figure 4.4 Effects of treatment on photosynthesis in maize over the course of the experiment. Treatments are denoted by the key. Error bars = 1 x SD, n = 8.

Table 4.3 Statistics showing the significance of the differences in photosynthetic activity between the different dose levels and controls.

Parameter	Treatment		Mean difference (µmol m^{-2} s^{-1})	Sig.
Photosynthesis (µmol m^{-2} s^{-1})	Control	Low	1.67	.51
		Medium	7.54*	.00
		High	9.51*	.00
	Low	Control	-1.67	.51
		Medium	5.88*	.00
		High	7.85*	.00
	Medium	Control	-7.54*	.00
		Low	-5.88*	.00
		High	1.97	.36
	High	Control	-9.51*	.00
		Low	-7.85*	.00
		Medium	-1.97	.36

*The mean difference is significant at 0.05, no. of leaves measured per sample n = 8.

4.4.2 Transpiration

The rate of transpiration increased for the low dose level a few days after treatment and gradually declined as stress progressed (Figure 4.5). On the contrary, the medium and high dose levels decreased from an early stage and this continued to the later stages of the experiment. The statistical significance of the differences between transpiration rates of the different dose levels and the control are given in Table 4.4.

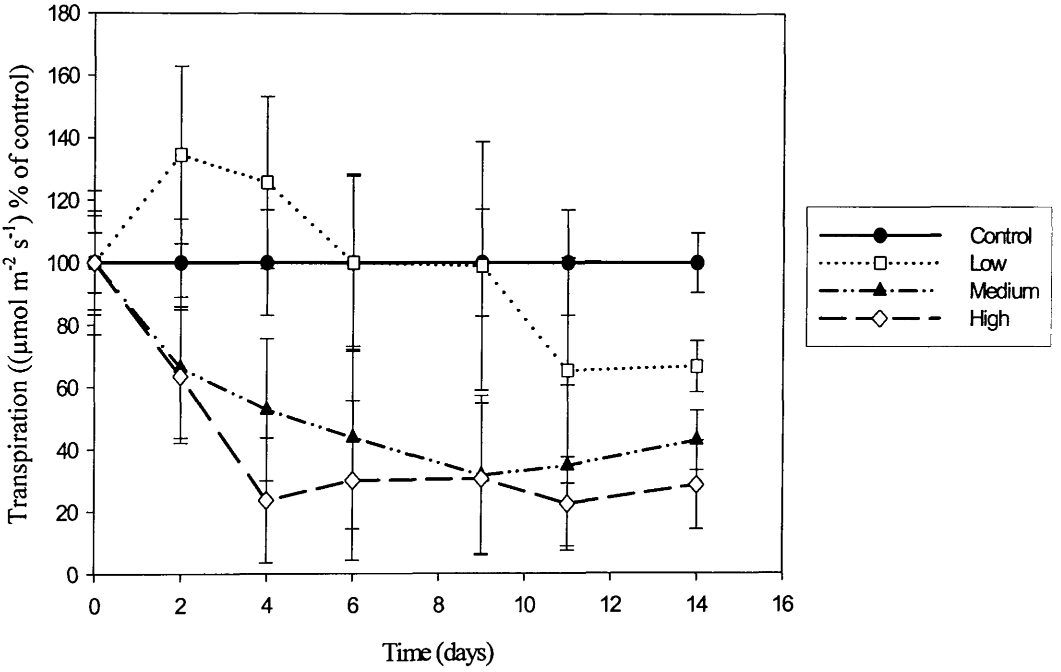

Figure 4.5 Effects of treatment on transpiration in maize over the course of the experiment. Treatments are denoted by the key. Error bars = 1 x SD, n = 8.

Table 4.4 Statistics showing significance of the differences in transpiration rates between the different dose levels and controls.

Parameter	Treatment		Mean difference (μmol m^{-2} s^{-1})	Sig.
Transpiration (μmol m^{-2} s^{-1})	Control	Low	-.12	.75
		Medium	.69*	.00
		High	.84*	.00
	Low	Control	.12	.75
		Medium	.80*	.00
		High	.96*	.00
	Medium	Control	-.69*	.00
		Low	-.80*	.00
		High	.15	.59
	High	Control	-.84*	.00
		Low	-.96*	.00
		Medium	-.15	.59

*The mean difference is significant at 0.05, no. of leaves measured per sample n = 8.

4.4.3 Stomatal conductance

There was a general decrease in stomatal conductance which typically followed dose levels (Figure 4.6). Although the low level increased slightly at the early stage of experiment, it eventually decreased at a later stage. While the high dose level had a continuous decrease in stomatal conductance from the onset until the end of the experiment, the medium followed similar trend but appeared to increase slightly towards the end. High and medium dose levels had similar, rapid rate of response when compared with low dose plants which responded much more slowly. The significant difference between changes in stomatal conductance of different dose levels and control is given in Table 4.5.

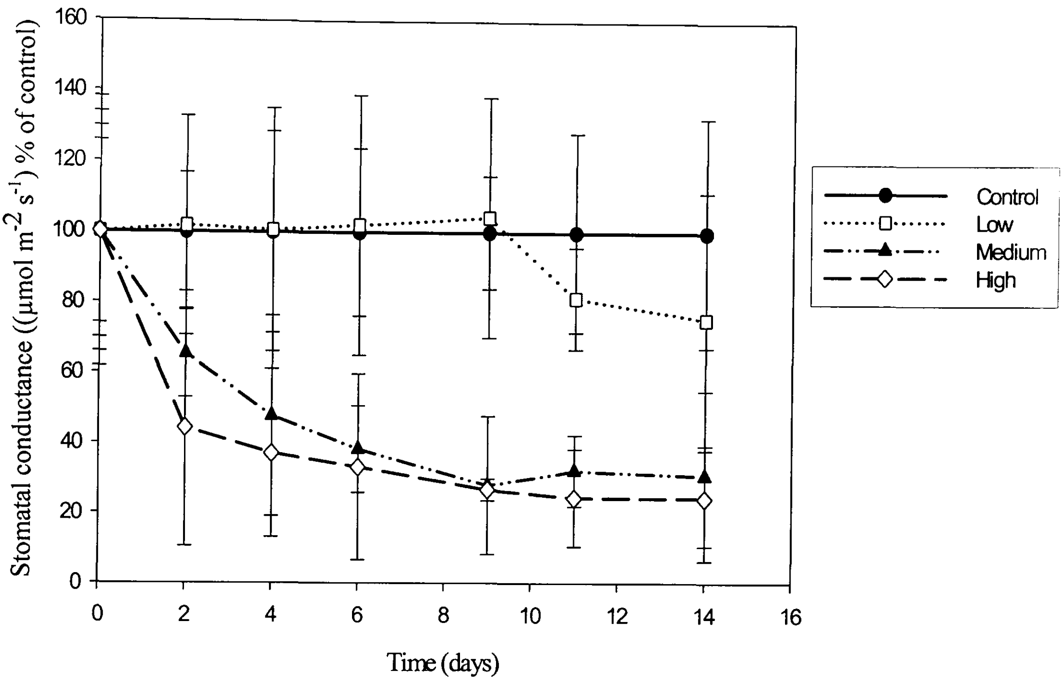

Figure 4.6 Effects of treatment on stomatal conductance in maize over the course of the experiment. Treatments are denoted by the key. Error bars = 1 x SD, n = 8.

Table 4.5 Statistics showing significance of the differences in stomatal conductance between different dose levels and controls.

Parameter	Treatment		Mean difference (µmol m^{-2} s^{-1})	Sig.
Stomatal conductance (µmol m^{-2} s^{-1})	Control	Low	-14.16	.19
		Medium	43.68*	.00
		High	55.80*	.00
	Low	Control	14.16	.19
		Medium	57.84*	.00
		High	69.95*	.00
	Medium	Control	-43.68*	.00
		Low	-57.84*	.00
		High	12.11	.32
	High	Control	-55.80*	.00
		Low	-69.95*	.00
		Medium	-12.11	.32

*The mean difference is significant at 0.05, no. of leaves measured per sample n = 8.

4.4.4 Visual stress observations

Expectedly, treatments caused different visible stress symptoms such as leaf chlorosis, rolling and wilting, the thinning of canopies and slower growth in maize and these increased with dosage and duration of stress (Figure 4.7). About five days after oil stress initiation, the controls and treated plants did not show any signs of stress visually. The plants flourished and appeared healthy and green. On the sixth day, there was slight chlorosis on some of the leaves of the high dose level plants. By the eleventh day, the medium and high levels showed symptoms of stress such as shoot and leaf chlorosis, thinner canopies and to some extent growth reduction. The low dose plants exhibited a moderate leaf chlorosis after fourteen days while medium and high showed severe wilting and general mortality at that stage. The control plants did not show any visual symptoms of stress at any time throughout the experimental period.

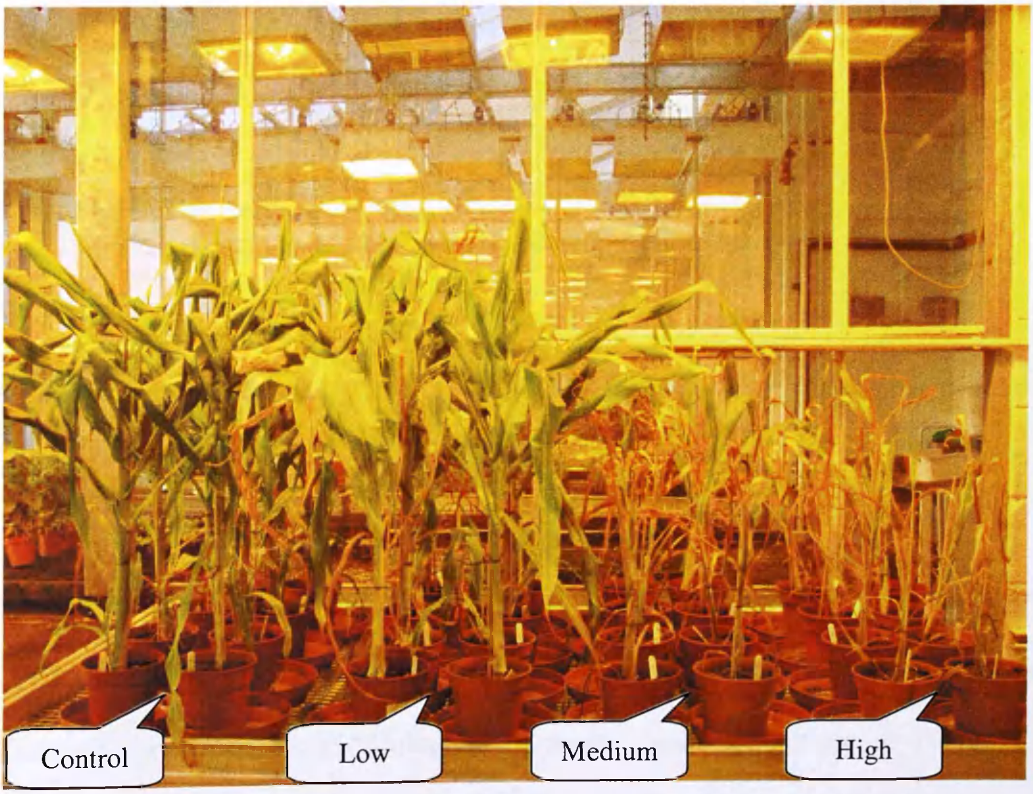

Figure 4.7 Visual stress symptoms of maize according to dose levels 14 days after treatment.

4.4.5 Spectral reflectance

There was a general increase in leaf reflectance in the visible region and a decrease in the NIR in response to oil pollution relative to control (Figure 4.8). The magnitude of the response followed dose levels with the highest increment in the visible range (560 – 700 nm) for the high dose level, followed by medium and then low, and vice-versa in the NIR. Differences between the mean spectral reflectance of controls and treated leaves were highest in the red and far-red region of the spectrum. The greatest reflectance difference was found around 750 nm while the blue, green, and NIR had minimal spectral differences.

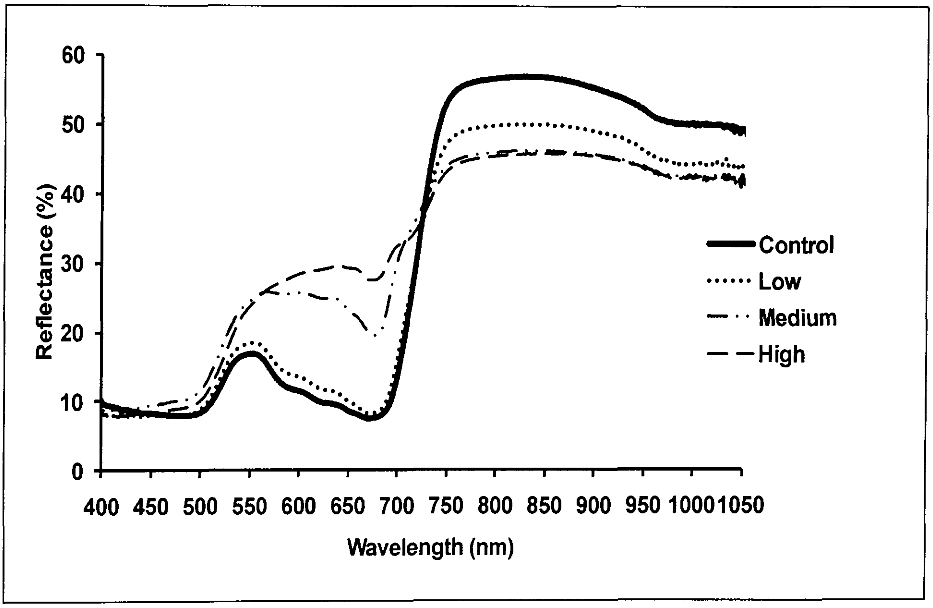

Figure 4.8 Mean reflectance spectra of control and treated maize 14 days after treatment. Dose level of treatments are denoted by the key, n = 80.

There was a strong relationship between the physiological parameters and reflectance around 700nm, particularly at approximately 705nm (Figure 4.9 a-c). The relationship between the physiological parameters and reflectance at 705nm are asymptotic which suggest that they saturate at a point. Thus, the sensitivity of 705 nm wavelength would struggle to predict changes in physiological parameters beyond 1 mol m^{-2} s^{-1}. The temporal change in reflectance at 705nm is shown in Figure 4.10. Reflectance at 705nm increases with dose level and duration of pollution and responds very quickly (within 2 days) to the high dose treatment, with slightly slower responses to the lower doses when compared to the control.

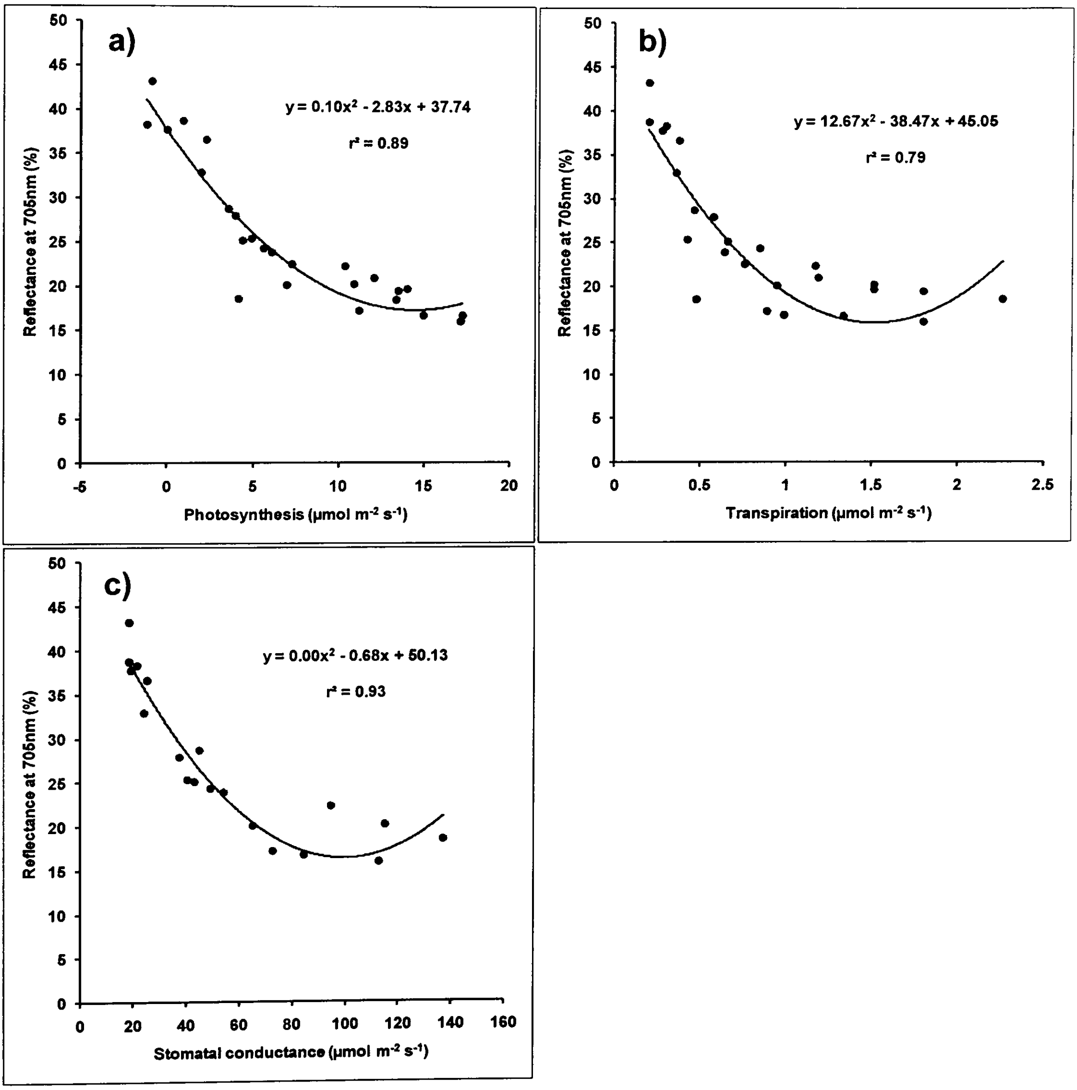

Figure 4.9 Relationship between reflectance and measured physiological properties: a) R705 and photosynthesis; b) R705 and transpiration; c) R705 and stomatal conductance.

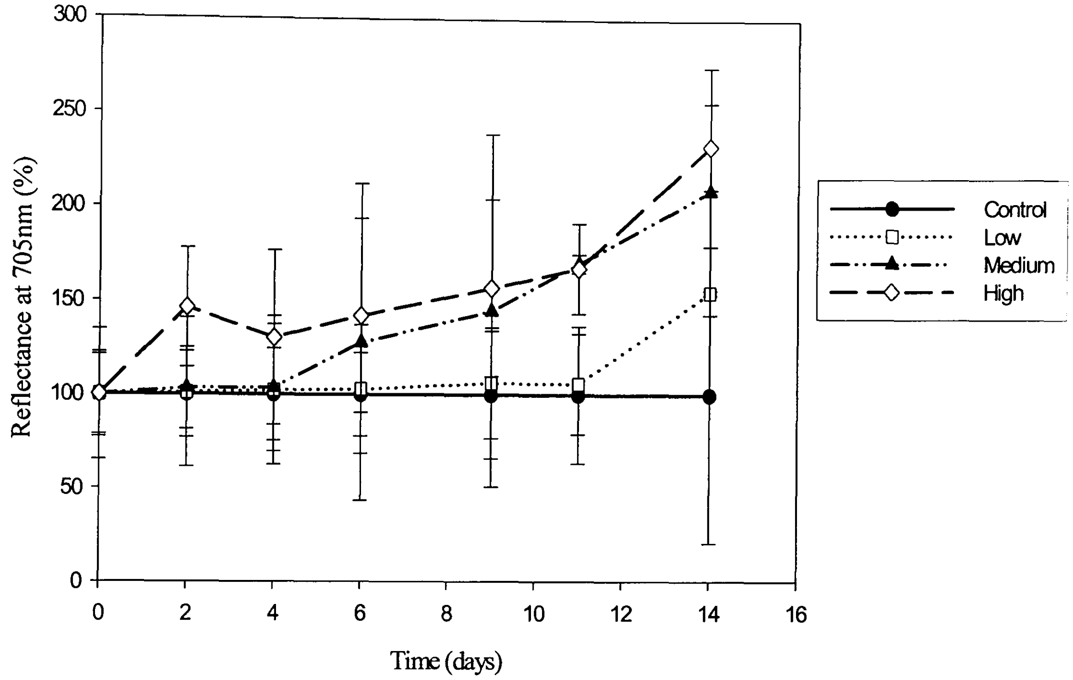

Figure 4.10 Temporal change in mean reflectance spectra of treatments at varied dose levels and control in maize at approximately 705 nm.

Table 4.6 illustrates the results of the sensitivity analysis performed on the reflectance values of selected individual narrow wavebands and on the simple and normalised-difference spectral indices. Individual narrow wavebands such as R_{450} and R_{550} did not show consistency in their response except at the later stage. The sensitivities at R_{450} and R_{550} were consistent after 9 days of treatment in only high dose level and in medium and high dose levels respectively. At R_{650}, the sensitivity was consistent at the early stage (2 days after treatment) to high dose level. After 6 days of treatment, the waveband showed a consistent significant difference between spectral reflectance of the controls and the medium dose levels. However R_{650} was insensitive to low level treatment throughout the experiment. R_{705} and R_{710} were very consistent in their sensitivity to oil pollution at all dose levels. Both wavebands showed a significant difference between the controls and high dose levels only 2 days after treatments and were sensitive to medium and low dose levels after 6 and 11 days respectively. Individual narrow wavebands in the regions R_{706}–R_{708} and R_{711}–R_{717} were consistent and performed in a similar manner. These wavelengths displayed a significant change in reflectance after 6 days for the high dose level and after 11 days for the medium and low levels. R_{750}, R_{850} and R_{950} did not show a consistent significant change in spectral reflectance at any dose level.

Table 4.6 Sensitivity analysis of selected individual narrow wavebands and spectral indices across varied dose levels of oil pollution over time. Unshaded areas depict either inconsistency or consistent but not significant while shaded areas depict a significant difference that occurs after treatment and remains consistent across at least two sample days, until the end of the experiment. *, **, ***Time when visual stress symptoms were observed in low, medium and high dose levels, respectively.

Wavelengths (nm)	Treatments		Time (Days)						
			0	2	4	6***	9	11**	14*
R_{450}	Control	Low							
		Medium							
		High					▨	▨	▨
R_{550}	Control	Low							
		Medium					▨	▨	▨
		High					▨	▨	▨
R_{650}	Control	Low							
		Medium				▨	▨	▨	▨
		High		▨	▨	▨	▨	▨	▨
R_{705}	Control	Low						▨	▨
		Medium				▨	▨	▨	▨
		High		▨	▨	▨	▨	▨	▨
R_{706}	Control	Low						▨	▨
		Medium						▨	▨
		High				▨	▨	▨	▨
R_{708}	Control	Low						▨	▨
		Medium						▨	▨
		High				▨	▨	▨	▨
R_{710}	Control	Low						▨	▨
		Medium				▨	▨	▨	▨
		High		▨	▨	▨	▨	▨	▨
R_{711}	Control	Low						▨	▨
		Medium						▨	▨
		High				▨	▨	▨	▨

R_{712}	Control	Low						▨	▨
		Medium						▨	▨
		High				▨	▨	▨	▨
R_{714}	Control	Low						▨	▨
		Medium						▨	▨
		High				▨	▨	▨	▨
R_{716}	Control	Low						▨	▨
		Medium						▨	▨
		High				▨	▨	▨	▨
R_{717}	Control	Low						▨	▨
		Medium						▨	▨
		High				▨	▨	▨	▨
R_{750}	Control	Low							
		Medium							
		High							
R_{850}	Control	Low							
		Medium							
		High							
R_{950}	Control	Low							
		Medium							
		High							
R_{530}/R_{440}	Control	Low					▨	▨	▨
		Medium							
		High							
R_{685}/R_{440}	Control	Low							
		Medium		▨	▨	▨	▨	▨	▨
		High		▨	▨	▨	▨	▨	▨
R_{740}/R_{440}	Control	Low							
		Medium				▨	▨	▨	▨
		High				▨	▨	▨	▨
R_{685}/R_{530}	Control	Low							
		Medium				▨	▨	▨	▨
		High				▨	▨	▨	▨
R_{740}/R_{530}	Control	Low				▨	▨	▨	▨
		Medium			▨	▨	▨	▨	▨
		High		▨	▨	▨	▨	▨	▨
R_{760}/R_{695}	Control	Low					▨	▨	▨
		Medium		▨	▨	▨	▨	▨	▨
		High		▨	▨	▨	▨	▨	▨
R_{750}/R_{700}	Control	Low					▨	▨	▨
		Medium		▨	▨	▨	▨	▨	▨
		High		▨	▨	▨	▨	▨	▨
R_{715}/R_{705}	Control	Low					▨	▨	▨
		Medium			▨	▨	▨	▨	▨
		High		▨	▨	▨	▨	▨	▨
R_{740}/R_{685}	Control	Low						▨	▨
		Medium			▨	▨	▨	▨	▨
		High		▨	▨	▨	▨	▨	▨
R_{755}/R_{716}	Control	Low				▨	▨	▨	▨
		Medium		▨	▨	▨	▨	▨	▨
		High		▨	▨	▨	▨	▨	▨
$(R_{920}-R_{655})/$	Control	Low							

$(R_{920}+R_{655})$		Medium			▨	▨	▨	▨	▨
		High		▨	▨	▨	▨	▨	▨
$(R_{755}-R_{716})/$ $(R_{755}+R_{716})$	Control	Low			▨	▨	▨	▨	▨
		Medium		▨	▨	▨	▨	▨	▨
		High		▨	▨	▨	▨	▨	▨

The simple spectral index R_{530}/R_{440} lacked consistent sensitivity in medium and high dose levels. However, it showed a consistent significant difference between spectral reflectance of the controls and the low dose level at the later stage. R_{685}/R_{440} was consistent in sensitivity throughout the experiment. It was sensitive to both medium and high levels after 2 days of treatment but was not sensitive to the low dose level. Both R_{740}/R_{440} and R_{685}/R_{530} were not consistent in sensitivity until after 6 days when they became significantly different to the medium and high dose levels but not to the low dose level. The result showed that R_{740}/R_{530} was very consistent in sensitivity with significant difference between spectral reflectance of the control and the high, medium, and low dose levels after 2, 4, and 6 days of treatments respectively. R_{760}/R_{695}, R_{750}/R_{700}, and R_{715}/R_{705} had similar responses, with consistent sensitivity to high and medium dose levels after 2 days of stress initiation and after 9 days for the low level, except R_{715}/R_{705} that showed consistent sensitivity to medium dose level after 4 days. R_{740}/R_{685} showed consistent significant difference between the controls and high, medium, and low dose levels after 2, 4, and 11 days respectively.

R_{755}/R_{716} was consistent throughout the experiment and showed significant change in spectral reflectance 2 days after treatments in high and medium levels and 6 days in low. Thus, it is most sensitive to all dose levels when compared with other simple spectral indices and individual narrow wavebands that were tested. The normalised difference spectral ratio $(R_{920}-R_{655})/(R_{920}+R_{655})$ showed consistent sensitivity to medium and high dose levels only. It showed significant difference between spectral reflectance of the controls and the high and medium dose levels 2 and 4 days after treatments respectively. Similarly, $(R_{755}-R_{716})/(R_{755}+R_{716})$ also had consistent sensitivity throughout the experiment and showed a significant difference between spectral reflectance of the controls and the high and medium dose levels 2 days after stress initiation and 4 days to the low dose level. When the sensitivity of the normalised-difference spectral index $(R_{755}-R_{716})/(R_{755}+R_{716})$ was compared with that of other tested spectral indices including the simple spectral index R_{755}/R_{716}, $(R_{755}-R_{716})/(R_{755}+R_{716})$ was found to be the most sensitive index in monitoring maize response to refined oil pollution. Moreover the index $(R_{755}-R_{716})/(R_{755}+R_{716})$ has a strong polynomial relationship with photosynthesis (Figure 4. 11).

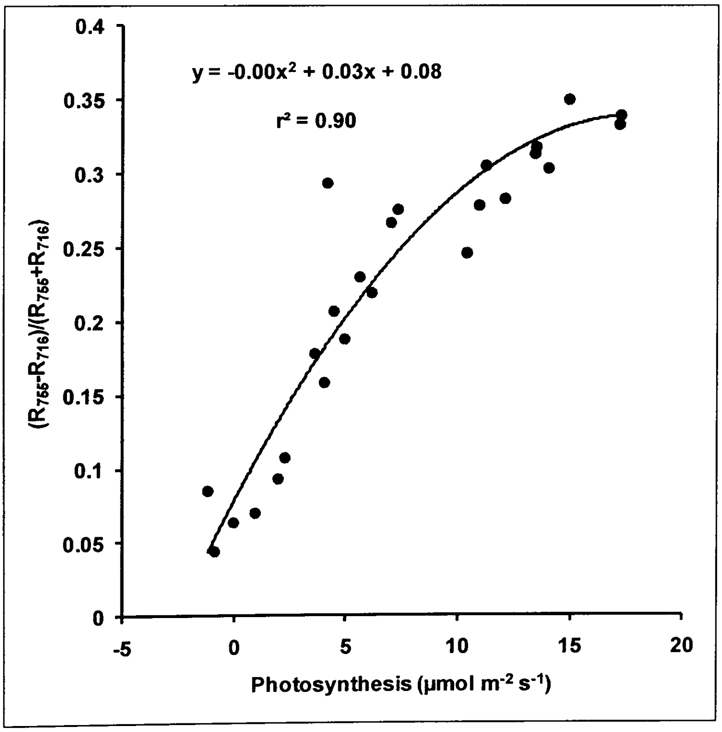

Figure 4.11 Relationship between photosynthesis and index $(R_{755}-R_{716})/(R_{755}+R_{716})$.

The REP was consistent in sensitivity to all levels of pollution (Table 4.7.). It displayed a significant change in reflectance 2 days after treatments for the high and medium dose levels and 9 days after for the low level. The amplitude was consistent in sensitivity but showed no significant difference between reflectance of the controls and the dose levels (data not shown). The sensitivity of the first of the double peaks was consistent only to the low dose level 6 days after treatments.

Table 4.7 Sensitivity analysis of the red-edge features across varied dose levels of oil pollution over time. Unshaded areas depict either inconsistency or consistency but not significant while shaded areas depict a significant difference that occurs after treatment and remains consistent across at least two sample days, until the end of the experiment. *, **, ***Time when visual stress symptoms were observed in low, medium and high dose levels, respectively.

			Time (Days)						
Red-edge features	Treatments		0	2	4	6***	9	11**	14*
REP	Control	Low					▨	▨	▨
		Medium		▨	▨	▨	▨	▨	▨
		High		▨	▨	▨	▨	▨	▨
Amplitude	Control	Low							
		Medium							
		High							
Position of the first of the double peaks (nm)	Control	Low				▨	▨	▨	▨
		Medium		▨	▨	▨	▨	▨	▨
		High		▨	▨	▨	▨	▨	▨
Position of the second of the double peaks (nm)	Control	Low							
		Medium							
		High							
Distance between the positions of the double peaks (nm)	Control	Low				▨	▨	▨	▨
		Medium				▨	▨	▨	▨
		High				▨	▨	▨	▨

The position of the second of the double peaks was consistently sensitive to low dose level throughout the experiment and 4 days after treatments to high and medium levels. However, there was no significant difference between the positions of the second of the double peaks of the controls and the dose levels. The distance between the positions of the double peaks was consistent in sensitivity to low dose level 6 days after treatments when there was a significant difference between the distance separating the positions of the double peaks of the controls and the dose level. The distance between the positions of the double peaks was not consistent to high and medium dose levels.

Two features known as the 'double peaks' were found within the red-edge region of the first derivative in both control and treated plants (Figure 4.12a-d). A change in steepness or sharpness of the peaks of the two features was observed over time. The change introduces either a shift of the red-edge to the longer wavelengths or to the shorter wavelengths. At the early stage of the experiment, there was a gentle peak in the first of the double features and a steep peak in the second feature. As time progresses, the shape of the red-edge changes as the first of the double features increased in steepness while gradually shifting towards the shorter wavelengths in both treated and control plants. The extent of the red-edge region was computed and interestingly, it was found that the time (after day 11) the extent of the red-edge region of the controls had a rapid decrease corresponded with the time their REP shifted to longer wavelengths. These observations explain that the extent of the red-edge region in both stressed and unstressed plants generally decreased with time, as the leaves mature.

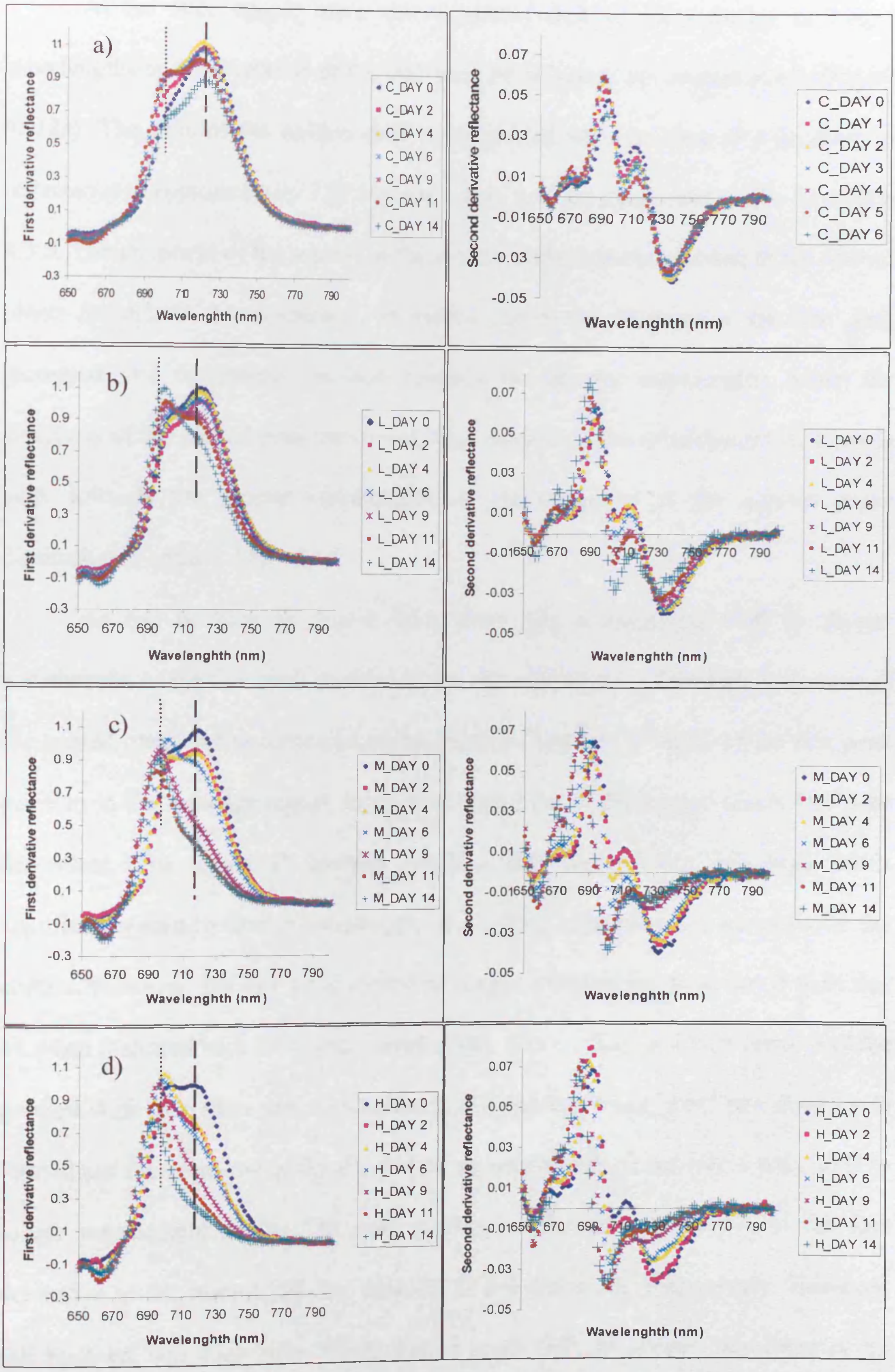

Figure 4.12 Mean first (left) and corresponding second (right) derivative reflectance curves showing temporal change in the shape of the red-edge and steepness of the double features found in the red-edge region: a) control; b) low; c) medium; d) high. Dot and dash lines depict first and second peaks respectively.

At the later stages, there was a sudden shift of the red-edge to longer wavelengths as the steepness of the first peak decreased in the control plants (Figure 4. 12a). The time of the sudden shift corresponded with the time of a decrease in reflectance at approximately 750 nm (see Figure 4.10) as mentioned earlier in section 4.3.6. The steepness of the second of the double peaks was not affected in the control plants throughout the experiment. In treated plants, the steepness of the first peak increased and consistently shifted towards the shorter wavelengths while the steepness of the second peak decreased. This resulted to the development of a single peak towards the shorter wavelengths as the steepness of the second peaks diminishes (Figure 4. 12b - d).

As can be seen in Figure 4.13, there was a consistent shift to shorter wavelengths of the first peak position in the red-edge region of the first derivative of the treated plants when compared to the controls. The rate of shifts of the first peak position in the red-edge region of the first derivative of the treated plants was dose dependent. The statistical analysis revealed that the medium and high levels significantly shift to shorter wavelengths from day 2 onwards when compared to the control. However, the low level shifted to longer wavelengths from day 6 until day 14 when it shifted back to shorter wavelengths. The medium and high levels had the greatest shift thus, there was no significant difference between these two dose levels throughout the experiment. By the end of the experiment, there was a total shift to longer wavelengths in the first peak position in the red-edge region of the first derivative of the control and low dose by 12 nm and 5 nm, respectively. However, the medium and high dose levels had a total shift to shorter wavelengths by approximately 4 nm and 6 nm, respectively.

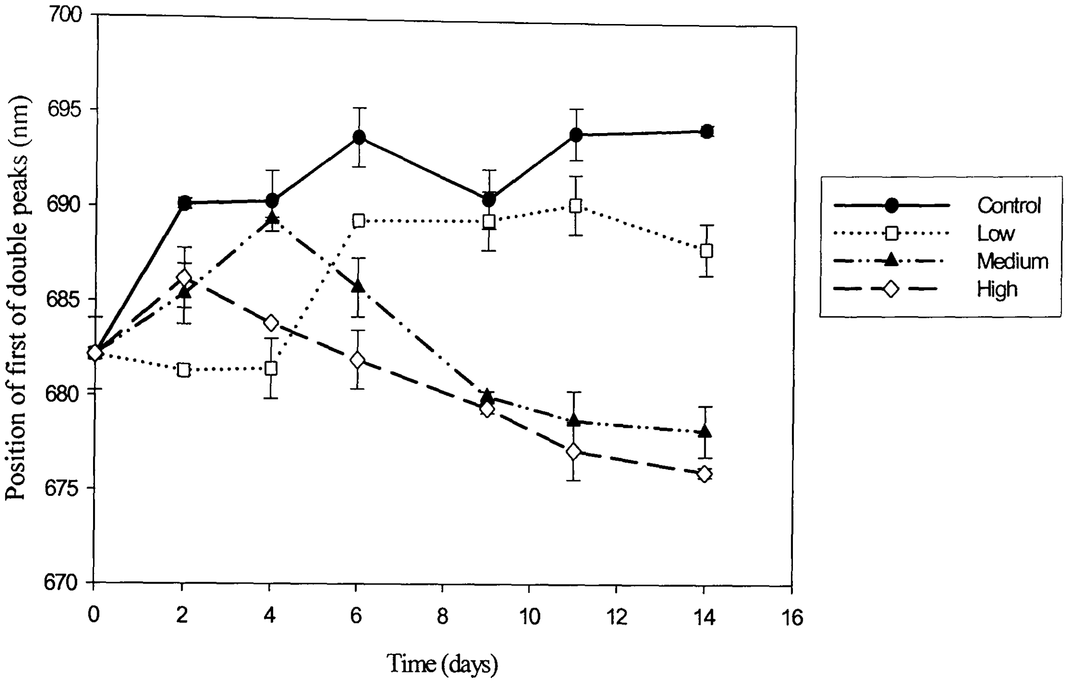

Figure 4.13 Temporal change in position of first peak in red-edge region of the first derivative in maize. Treatments are denoted by the key, n = 8.

The shifts in the position of the second peak of the double features were not consistent in all treatments, compared to the controls (data not shown). The second of the double features was not found in treatments except at the early stage. As stress progressed, the feature diminished and thus, may not be an effective technique for early detection of oil-induced stress. Furthermore, the position of the feature was prominent in the controls throughout the experiment and there was no significant difference in the position of second of the double peaks in the controls and treatments at any time ($p = 0.05$).

The REP generally shifts towards the shorter wavelength in both control and treated plants (Figure 4. 14.). At the end of the experiment, there was a total shift of 7nm, 22nm, 28nm, and 30nm to shorter wavelengths for the controls and the low, medium, and high dose levels, respectively. The trend of shift followed order of treatment and was consistent in all dose levels except in control where there was a sudden shift to longer wavelengths at later stage. Again, this corresponds with the time of sudden shift of the red-edge to longer wavelengths as the steepness of the first peak decreased in first derivative reflectance and the time when there was decrease in reflectance spectra at approximately 705 nm as mentioned earlier.

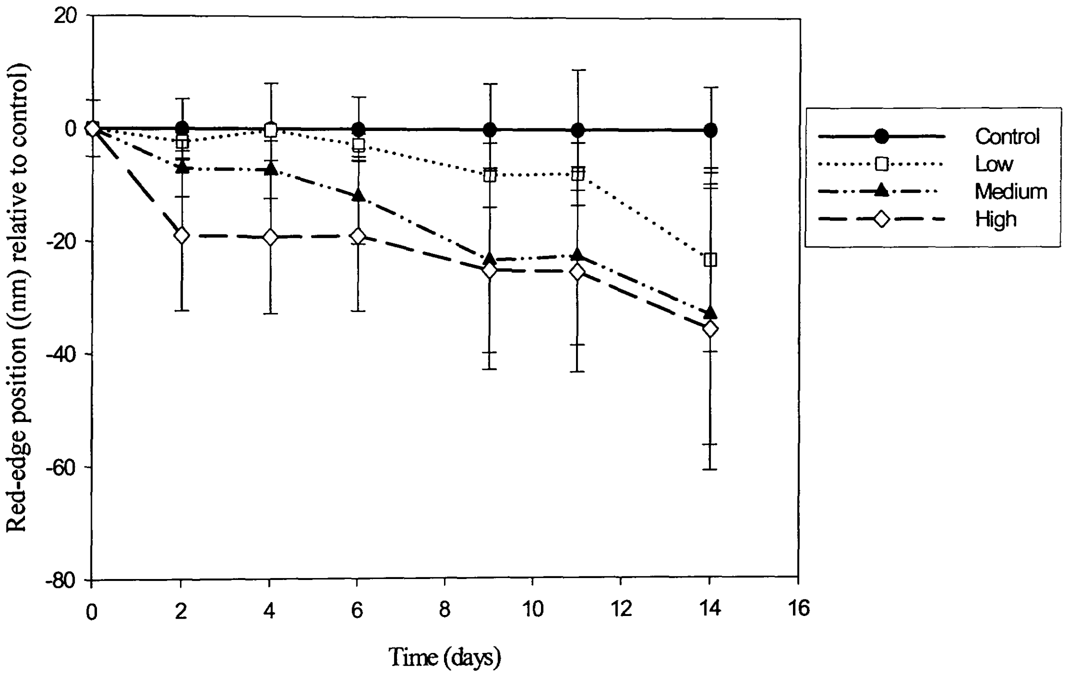

Figure 4.14 Temporal change in REP of control and treated maize. Treatments are denoted by the key. Error bars = 1 x SD, n = 8.

There was a significant difference in the REP of the control and the medium and high dose levels throughout the experiment (n = 80, p = 0.000 < 0.05) and no significant difference for the low dose level (n = 80, p = 0.596 > 0.05) until after 9 days of treatment (n = 80, p = 0.006 < 0.05). Statistics showed a strong relationship between the REP and measured physiological properties (Figure 4. 15.). There was a decrease in the amplitude of the red edge position of treated plants as dose level increased. The differences in amplitude of the red edge position of the control and treated plants were not significant at any time ($n = 80$, $p = 1.000 > 0.05$) of the experiment.

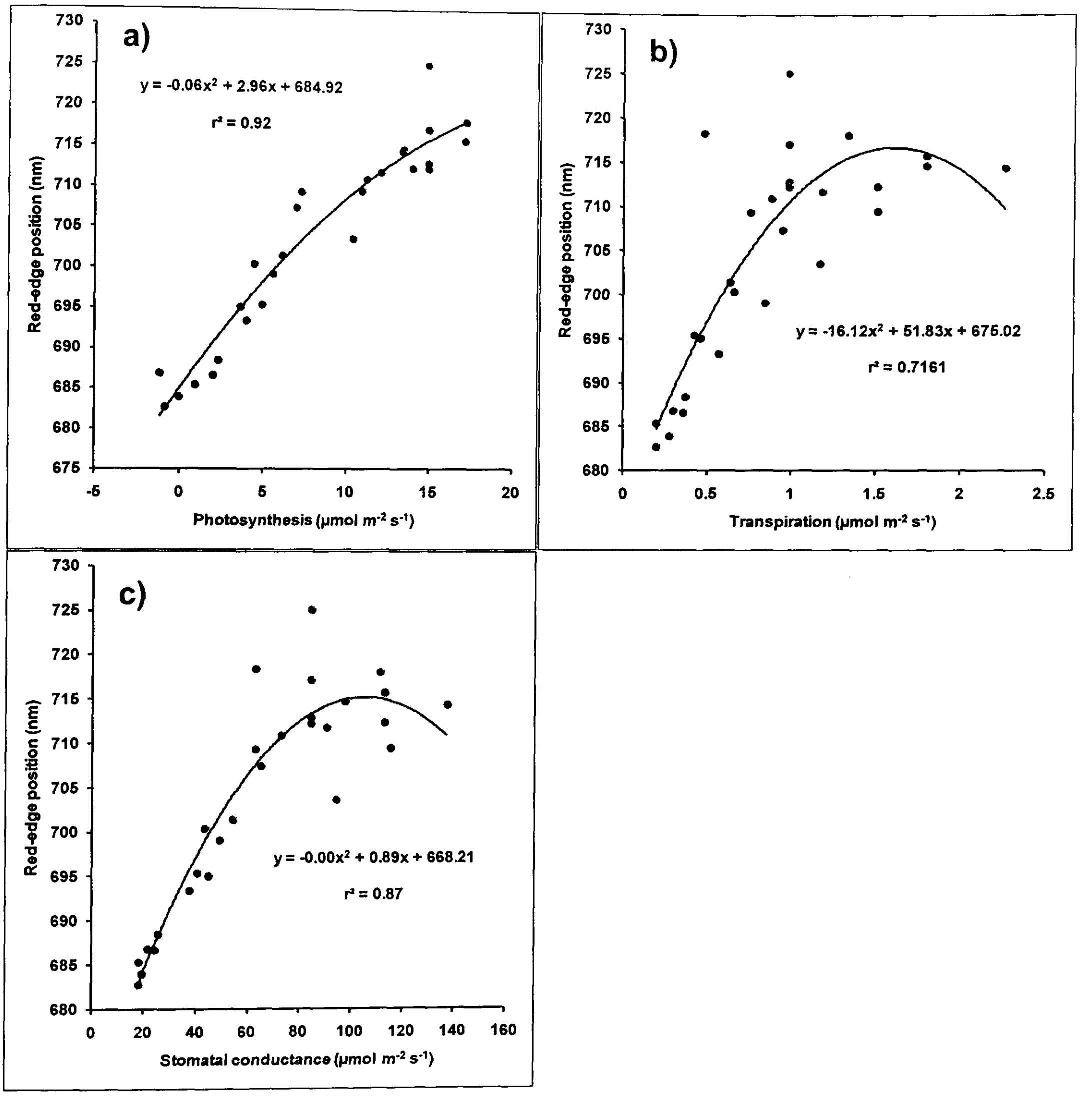

Figure 4.15 Relationship between the REP and measured physiological properties: a) photosynthesis; b) transpiration; c) stomatal conductance.

4.4.6 Thermal imaging

The average leaf temperature of the control and treated plants fluctuated throughout the experiment. The average temperature of the treatments did not follow a definite pattern through the experiment. The temperature continued to rise and fall relative to control (Figure 4.16.). Statistics did not show significant differences between the average temperature of the controls and treatments (n = 56, p = 0.999, 0.248, 0.782 > 0.05). The thermal index (I_G) of treated plants consistently fell below the control plant throughout the experimental period (Figure 4.17.). There was a moderate linear relationship between I_G and stomatal conductance (Figure 4. 18).

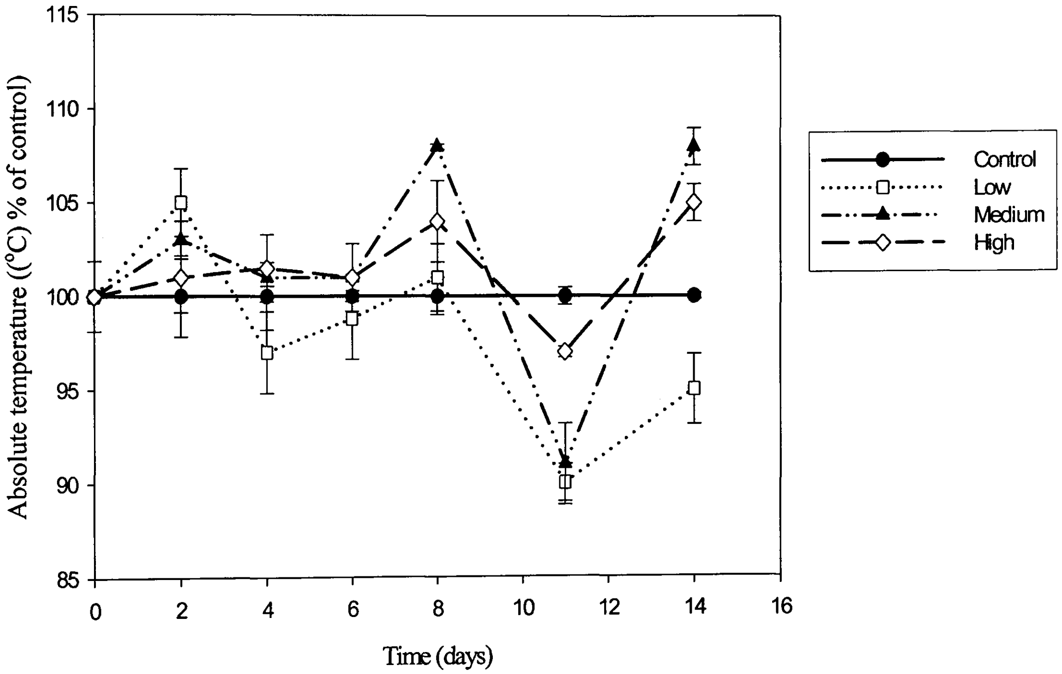

Figure 4.16 Temporal changes in leaf absolute temperature of treated and control plants. Treatments are denoted by the key. Error bars = 1 x SD, n = 8.

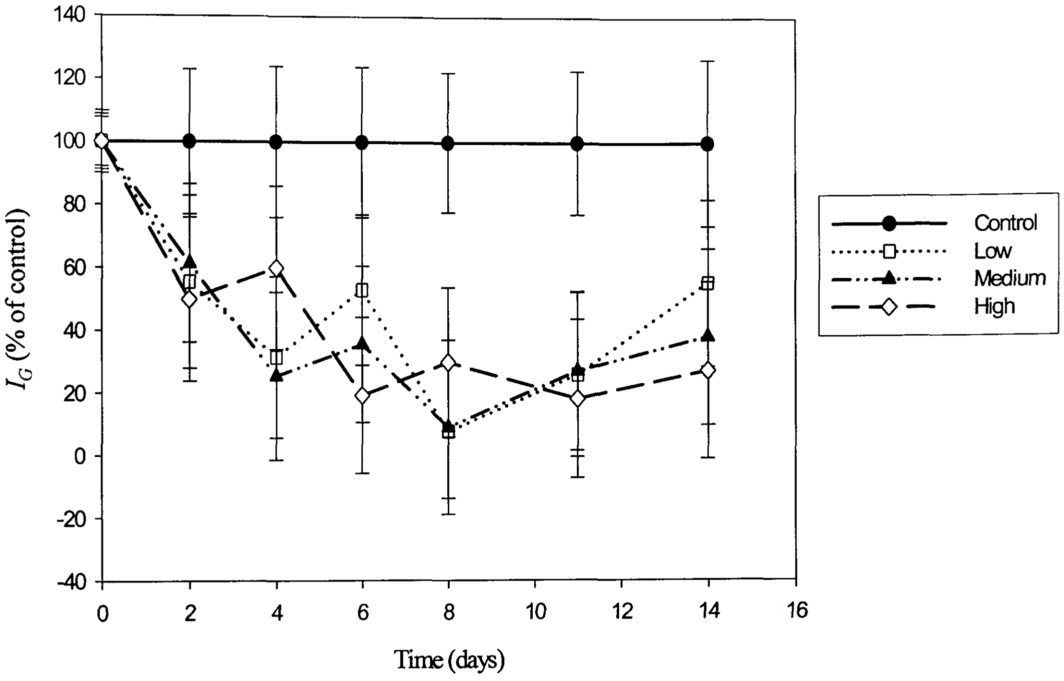

Figure 4.17 Temporal changes of thermal index (I_G) of treated and control plants. Treatments are denoted by the key. Error bars = 1 x SD, n = 8.

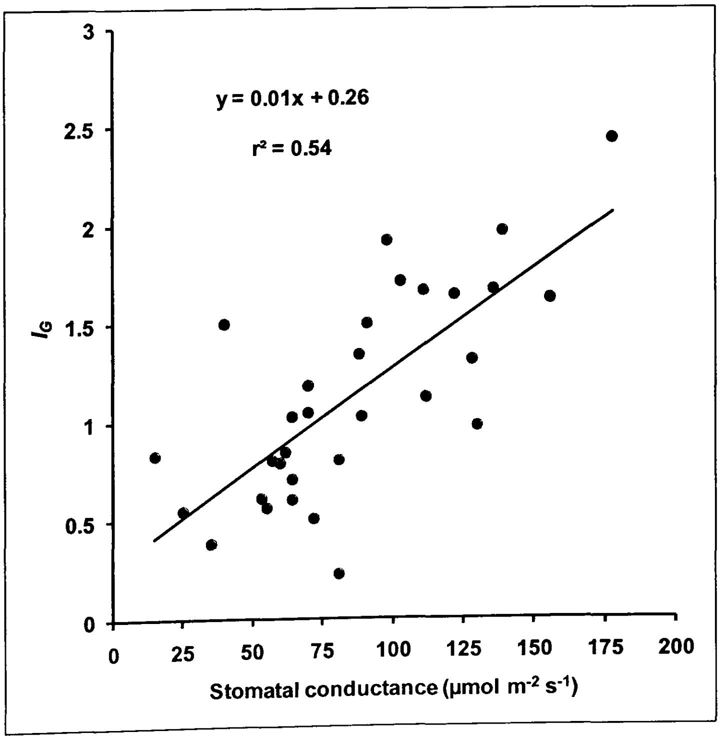

Figure 4.18 Relationship between thermal index (I_G) and stomatal conductance.

4.5 Discussion

Visible stress symptoms such as leaf and stem chlorosis, dryness, and growth impairment were observed in all the dose levels of pollution. For samples exposed to high level of pollutants, symptoms were first observed after 6 days. For samples exposed to a medium or low level, symptoms were first observed after 11 and 14 days, respectively. Earlier studies using a wide range of plant species and stresses discovered the first visual signs at different times such as 6, 7, 8, 14, 15, 30 days after inducement (Schollenberger, 1930; Arthur *et al.,* 1985; Pysek and Pysek, 1989; Ketel, 1996; Smith *et al.*, 2004a; Smith *et al.*, 2005). These variations suggest that the time of first visible stress symptom is a function of plant species, type and degree of stress. Symptoms at all dose levels started mildly by affecting only a few leaves and gradually becoming severe by spreading over all the leaves. The visible stress symptoms progressed in a way similar to that observed in oilseed rape leaves affected by natural gas elevation in the soil and other stresses (Smith *et al.,* 2005).

There was a general and significant change in the spectral reflectance of treated plants. Generally, the reflectance spectra increased in the visible and decreased in the NIR regions of the spectrum. A decrease in the NIR reflectance is similar to the results of Pickerill and Malthus (1998) and Smith *et al.* (2005). Pickerill and Malthus (1998) found that the NIR reflectance was lower for wheat crops growing over the leaks from rural aqueducts than the surrounding canopy due to the reduced plant biomass and the presence of standing water and wetter soil. However, Smith *et al.* (2004a) found that argon-treated barley showed a significant increase in the NIR. It is known that a number of factors such as the size of the cells, the number of cell layers and the thickness of the leaf mesophyll influence NIR reflectance. Maize and barley presumably have a similar leaf internal structure as monocotyledons, thus, reflectance differences exhibited by the two plant species may be associated with the extent of damage in the leaf internal structure. The effects of the stressors on the leaf internal structure may have varied due to their different chemical compositions. Differences in the NIR response could also be related to their different surface characteristics such as hairs/waxes and moisture content. The leaf of a monocotyledon is more compacted with fewer air spaces (Gausman, 1985) consequently, these have lower NIR reflectance. In this case, the air spaces may have further been closed-up by oil if oil is being transported from the roots to the leaves or the cellular turgor and leaf structure may have deteriorated due to indirect effects of the oil on the plant water relations. In a field experiment that investigated the physical and chemical effects of oils on mangrove, it was found that the concentrations of hydrocarbons in leaves increased with increasing oil application to the sediments, although the effects varied in the different species (Suprayogi and Murray, 1999).

Significant spectral reflectance change was found mainly in the red-edge region of the spectrum particularly across 650nm to 720nm. A study by Carter (1993) found that increased reflectance in the 685 to 700nm wavelengths range was constantly sensitive to different stresses across species. Changes in the spectral reflectance were not significant towards the longer wavelengths of the near-infrared, particularly at the early stage and as dose levels decreased. Carter (1993) found that the infrared reflectance shorter than 1400 nm was comparatively unresponsive to stress. This suggests that the spectral reflectance at the longer wavelengths may not be a good diagnostic measure for monitoring oil pollution in leaves. Individual narrow wavebands around R_{700} were more consistent in sensitivity than those around R_{650} and the shorter wavelengths. While wavebands around 700 nm could show significant changes in spectral reflectance at all dose levels, those around 650 nm only responded significantly to higher dose levels. Similarly, the blue (R_{450}) and green (R_{550}) only responded significantly to higher dose levels of pollution at the later stages of the experiment. The NIR (R_{750}, R_{850}, R_{950}) did not perform very well in their response to oil pollution irrespective of the dose level.

The red and green waveband ratio R_{685}/R_{530} significantly increased as stress progressed and was sensitive to medium and high treatments at later stages of the experiment but not to the low dose level. This observation was similar to findings of Smith *et al.* (2005) where the ratio increased rapidly in the gas and herbicide-stressed plants. This could be related to increases in reflectance in the strong chlorophyll absorption region due to a decrease in pigment contents and high reflectance in the green region resulting from a weaker absorption of the pigments. A stable and high sensitivity shown by the simple ratios that ranged between $R_{715-760}$ and $R_{695} - R_{716}$ concurred with the findings of (Tarpley *et al.*, 2000). Tarpley *et al.* (2000) noted that a combination of the red-edge measure with a waveband of high reflectance in the NIR region could improve precision and accuracy in predicting cotton leaf nitrogen concentrations. The normalised difference ratio $(R_{755}-R_{716})/(R_{755}+R_{716})$ that combined these two wavebands was highly sensitive in terms of temporal change and consistency in sensitivity. This index showed a significant change 2 days after treatments in high and medium dose levels whereas stress symptoms were visually shown only 6 and 11 days after for the high and medium dose levels respectively. While this index showed changes after 4 days for the low dose level, stress symptoms were seen visually some 14 days after stress initiation. Coops *et al.* (2003); Goel *et al.* (2003); Ferri *et al.* (2004); Zhao *et al.* (2005) when working on a single crop type/species recognised the superiority and efficiency of a normalised difference ratio that employs just two narrow wavebands.

A consistent and significant shift of the REP to shorter wavelengths in treated plants showed that this was a reliable spectral parameter for early detection of oil-induced stress. The shift of the REP was strongly related to a decrease in photosynthesis and thus, chlorophyll contents and other biochemical concentrations. Rock *et al.* (1988) showed that the REP of foliage stress of spruce trees caused by air pollution shifted to shorter wavelengths. Other physiological factors like the stomatal conductance and transpiration may have influenced the REP in some way given the correlation found in the regression analysis. A study showed that the REP is dependent not only on chlorophyll content, but also on additional effects such as leaf developmental stage, leaf layering or stacking and leaf water content (Horler *et al.*, 1983). Early shift of the REP to shorter wavelengths after contamination indicates its potentials for early stress detection. Interestingly, this might not be true in all cases because the REP of the controls also made early shifts to shorter wavelengths. However, as stress persists, the control suddenly shifted to longer wavelength while treated plants maintained shifts to shorter wavelength.

Similarly, Smith *et al.* (2004a) found that the position of the red-edge moved to longer wavelengths for control bean plants as they matured, but not for treated plants. Leaf developmental stage is likely to be a suitable argument in case of various shifts of the REP in control given variation in plant age during the period of spectral measurements. Plant leaves in their early immature and later senescent phases are associated with low concentrations of pigments (Blackburn, 2007). Furthermore, past studies showed that the red-edge shifts associated with phenological crop development were towards longer wavelengths as chlorophyll concentration increased with crop maturity (Horler *et al.*, 1983; Miller *et al.*, 1991).

The results indicate that the REP is a valuable technique not only for early stress detection at varied levels of oil pollution but also for long term stress monitoring owing to its continuous and significant shifts to shorter wavelengths found at the early and later stages in treated plants. Several studies found a shift to shorter wavelengths of the REP by natural vegetation with low chlorophyll content due to long term stress (Lang *et al.*, 1985a, 1985b; Crawford, 1986; Reid, 1988; McCoy *et al.*, 1989; Cwick *et al.*, 1995; De Oliveria and Crosta, 1996).

The amplitude of the first derivative reflectance in the region of the red edge showed no significant increase or decrease in treated plants. Although there was the tendency for the amplitude to be either minimally changed or to decrease, with only low treatment showing pronounced increment at later stages of the experiment. Smith *et al.* (2004a) found similar results where changes in the amplitude of the first derivative at the position of the red-edge was not consistent, and could either increase or decrease relative to the control. The inconsistency could be related to a variation in steepness of the double peaks which either increases or decreases. Change in steepness of one of the double peaks tends to affect the other in the opposite way. This was observed particularly in treated plants where the steepness of the first of the double peaks increased with a decrease in the second peak. It is recognized that the absorption features of pigments and other biochemical constituents overlap (Blackburn, 2007), and variation in amplitude may have resulted from change in the ratio of chlorophyll *a* and *b* contents of the leaf.

An increase in the steepness of the first of the double peaks also causes a shift of the red-edge to shorter wavelengths. This may be attributed to a possible narrowing of the strong chlorophyll absorption feature due to a decreased amount of chlorophyll. When the steepness of the second peak increased, there tends to be shift of the red-edge to longer wavelengths. This was observed in control plants at the later stages of the experiment which corresponds with the time when there was a sudden shift of the REP to longer wavelengths and a sharp decrease in reflectance at high chlorophyll absorption wavelengths. This implies that the positions of the double features could serve as possible indicators of oil stress. Llewellyn and Curran (1999) found that the dominance of the shorter wavelength feature indicated grasslands with high levels of soil contamination whereas the longer wavelength feature indicated lower levels of contamination. Thus, dominance of the first peak means low chlorophyll content whereas dominance of the second peak corresponds with high chlorophyll levels (Lamb *et al.,* 2002). These findings help in understanding the behavioral pattern of the first and second of the double features in first derivative recorded in the present experiment. The positions of the first of the double peaks performed as well as the distance between the double features and both were superior to visual observations as early stress indicators.

Leaf temperature fluctuated as stress progressed irrespective of dose level and did not differ significantly between treated and control plants. Similarly, a field experimental study of herbicide-induced stress in a mixed stand of 5 year old loblolly pine (*Pinus taeda L.*) and slash pine (*Pinus elliottii Engelm*) did not show a significant difference in canopy temperatures between the treated and control plots (Carter *et al.*, 1996). The study attributed this to a coupling of leaf temperatures with air temperature, and an equalization of temperatures among treatments due to wind and environmental moisture. Grant *et al.* (2006) detected no significant differences between the leaf temperature of grapevine subjected to water stress and those well fed with water. This was related to greater environmental variation inevitable in an experiment with relatively large plants across a greenhouse. This may well explain the inconsistency in thermal responses observed in this study.

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. This implies that I_G is a more sensitive parameter for quantifying plant stress induced by oil pollution than ordinary leaf temperature. Theoretically, I_G is expected to be linearly related to stomatal conductance (Jones, 1999) and this was the case in the present study. Tilling *et al.* (2007) found that nitrogen treatments had no effect on canopy temperature of field grown wheat. Generally, the influence of nitrogen treatments on canopy temperature was minor compared with the effect of water treatment. Several workers have successfully applied techniques of thermography to monitor water stress, across a wide range of species in controlled environments and field conditions (Leinonen and Jones, 2004; Cohen *et al.*, 2005; Grant *et al.*, 2007; Möller *et al.*, 2007;). Thus, most applications of thermal imaging have related to monitoring plant responses to water deficit stress (Jones, 2004). Greater levels of confidence have been established about thermal techniques for acquiring accurate information about plant water status than any other stresses. With regard to the present experiment, to explore if the inconsistency in absolute temperature of treated plants was mainly due to effects of variations in environmental conditions and/or instrumental error or mere insensitivity to oil pollution, there is the need to measure and compare plant thermal responses to both water and oil-induced stress within a more constrained environment. This could be achieved by taking measurements in a dark room where only an artificial source of illumination is provided.

From these results, it has been shown that spectral reflectance is a more sensitive and reliable parameter for detection of refined oil-induced stress in maize than the visual observations and thermal responses. Significant changes in spectral reflectance were detected at all dose levels before visual stress signs were seen. While there was consistent and significant changes in spectral reflectance particularly around 700 nm, changes in absolute temperature were neither consistent nor significant. However, the I_G showed potential in detecting oil pollution in maize.

4.6 Conclusion

There was a very strong positive relationship between reflectance spectra and the physiological parameters. These include: a strong positive linear relationship between the reflectance at several individual narrow wavebands and photosynthesis, a strong positive linear relationship between $(R_{755}-R_{716})/(R_{755}+R_{716})$ and photosynthesis, and a strong positive linear relationship between the REP and photosynthesis. These results suggested that the spectral reflectance of leaves has potential in detection of oil pollution. A stronger positive linear relationship between the last two factors (i.e. REP and $(R_{755}-R_{716})/(R_{755}+R_{716})$) and photosynthesis can be valuable indicators for early detection of oil pollution irrespective of the intensity of pollution. Results from thermal response suggest that while the absolute leaf temperature has minimal potential for detecting oil pollution in plants, thermal index I_G is promising. While the REP was superior to visual observations and other red-edge features, the normalised-difference spectral indices that combines a waveband in the red-edge with one of high reflectance in the NIR region: $(R_{755}-R_{716})/(R_{755}+R_{715})$ performed best comparatively to all the tested diagnostic stress indicators viz the individual narrow wavebands, the simple ratios, the red-edge features, and the visible stress symptoms.

In terms of time and consistency, $(R_{755}-R_{716})/(R_{755}+R_{716})$ was found to be optimal for early detection of oil-induced stress at varied levels of pollution. Therefore, its application could enhance precision and accuracy for early detection of oil pollution via plant stress responses. Further studies plan to test the capability of this approach for early detection and discrimination between oil- and water related stress such as waterlogging and water deficit in plants as both are important naturally occurring stress factors. Thus, the next chapter deals with detection and discrimination of stress in bean (*Phaseolus vulgaris 'Tendergreen'*) caused by oil pollution and waterlogging using spectral and thermal remote sensing.

Chapter 5[*]

DETECTION AND DISCRIMINATION OF STRESS IN BEAN (*Phaseolus vulgaris 'Tendergreen'*) CAUSED BY OIL POLLUTION AND WATERLOGGING USING SPECTRAL AND THERMAL RESPONSES

5.1 Introduction

Waterlogging is known as one of the important natural stresses affecting plants. It can cause stress in plants by displacing the oxygen in soil by filling the soil spaces with water and thus limiting oxygen supply to roots and preventing carbon dioxide from diffusing away (Smith, 2004a). Gases such as O_2 and CO_2 diffuse very slowly in water (Gibbs and Greenway, 2003) thus; replacement of these gases from the surface is slower. Removal of gaseous products produced in the waterlogged soil will also be slower through the water and there may be a build-up of toxic chemicals that could have an effect on the plants (Smith, 2004a). For example, Godwin and Mercer (1983) noted that ethylene concentrations are known to increase in waterlogged soils and this has deleterious effects on plant growth causing inhibition of root growth, allowing the invasion of decay organisms. Since a major function of roots is supplying plants with water and nutrients (Lynch, 1995), waterlogging has a subsequent effect on the above-ground parts of a plant as they are unable to obtain enough water and nutrients through the roots.

A number of studies have sought to understand the effects of waterlogging in plants. Manabu *et al.* (1999) found that the growth of tropical forage legumes called *Urb. cv. Siratro.* decreased with long periods of waterlogging treatment when

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compared with controls. While studying the effects of waterlogging on root systems of soybean Morita *et al.* (2004) discovered that waterlogging prohibits the growth of the taproot and its lateral roots. It has been noted that few plants will survive prolonged periods in ground saturated with water unless they have special roots that are adapted to acquire oxygen in waterlogged conditions (The Royal Horticulture Society, 2009).

Since waterlogging can instigate malfunctioning of the root, it is expected that such conditions could result in reflectance changes commonly related to plant stress, such as increased reflectance in the chlorophyll and water absorption regions (Carter, 1993; Lichtenhaler *et al.*, 1996). Indeed, some studies have shown that waterlogging can be detected in plants using changes in reflectance spectra. Anderson and Perry (1996) found that flooded trees in wetland areas showed elevated reflectance at 550 nm and in the NIR at 770 nm when compared to non-flooded trees. Pickering and Malthus (1998) worked on a small leak from an aqueduct, which showed severe waterlogging of the soil and vegetation within the area was stunted, yellow and sparse. The centre of the leak had a higher visible reflectance and lower NIR reflectance compared to the surrounding unstressed vegetation. Smith *et al.* (2004a) found that soil oxygen displacement by waterlogging caused a significant increase in reflectance in the visible between 508- 654nm and in the red-edge region between 692-742nm with little change in the NIR in bean. The REP of the waterlogged bean shifted towards shorter wavelengths compared to the controls.

Although there is evidence in the effectiveness of spectral reflectance for detecting plant stress caused by waterlogging, there is a poor understanding of the capabilities of spectral and thermal remote sensing for discriminating between oil pollution and waterlogging stresses. Furthermore, in the previous chapter (4) spectral and thermal responses of plants were identified that are of value for early detection of stress caused by oil pollution alone. It is now important to determine whether remote sensing can be used for the detection and discrimination of concomitant oil and waterlogging stresses. Thus, this chapter investigates the spectral and thermal responses of bean (*Phaseolus vulgaris 'Tendergreen'*) plants subjected to three stress regimes: oil pollution, waterlogging and the combination of oil pollution and waterlogging. Bean is used as model specie as it is economically important and forms a major source of protein particularly in developing countries like Nigeria where oil pollution of farmlands is common. It also provided a compact, dense canopy which is amenable to growth, manipulation and measurement at the canopy scale in laboratory conditions. The crop is widely grown in other parts of the world such as countries of Central and South America, and Central and East Africa where animal protein is limited and beans are consumed in large quantities (Shellie-Dessert and Bliss, 1991).

The objective was to identify the optimum set of responses which could be used for early, non-destructive quantification and discrimination of each of these two stresses.

5.2. Methods

The methodology described in chapter 3 was adopted in this experiment except that canopy thermal images were acquired in a darkroom (provided with an artificial illumination (see chapter 3, section 3.5) mounted in a fixed position at nadir 70cm away from each canopy to be measured), with the camera positioned at nadir

75cm above the plant canopy. Thirty two well established plants were selected for treatment. Four treatments, comprised of eight replicates, were established namely: control, oil, waterlogging and a combination of oil and waterlogging.

A GER 1500 Spectroradiometer (Geophysical & Environmental Research Corp., Millbrook, NY) already described in chapter three (section 3.5) was used to acquire reflectance spectra of treated and control plants. In this experiment, the instrument was positioned at nadir 20cm above the plant canopy, giving a FOV of approximately 3cm diameter and the light source was at a 45° zenith angle. Eight spectral measurements were captured for each plant canopy by making small movements to the position and rotation of the pot between each measurement. Leaf pigments and water content were not measured in this experiment.

Spectral indices were generated from the individual narrow wavebands by means of ratioing all possible two-band combinations. The optimal index found in the results of chapter four was also added into the analysis.

5.3 Results

5.3.1 Visual stress observations

Stress symptoms were first visually observed in plants on day 8 for oil and the combined oil and waterlogging treatments and on day 10 for waterlogging treatment (alone). Symptoms worsened with time and included leaf chlorosis, rolling and wilting, the thinning of canopies and slower growth (figure 5.0). The control plants did not show visual stress symptoms.

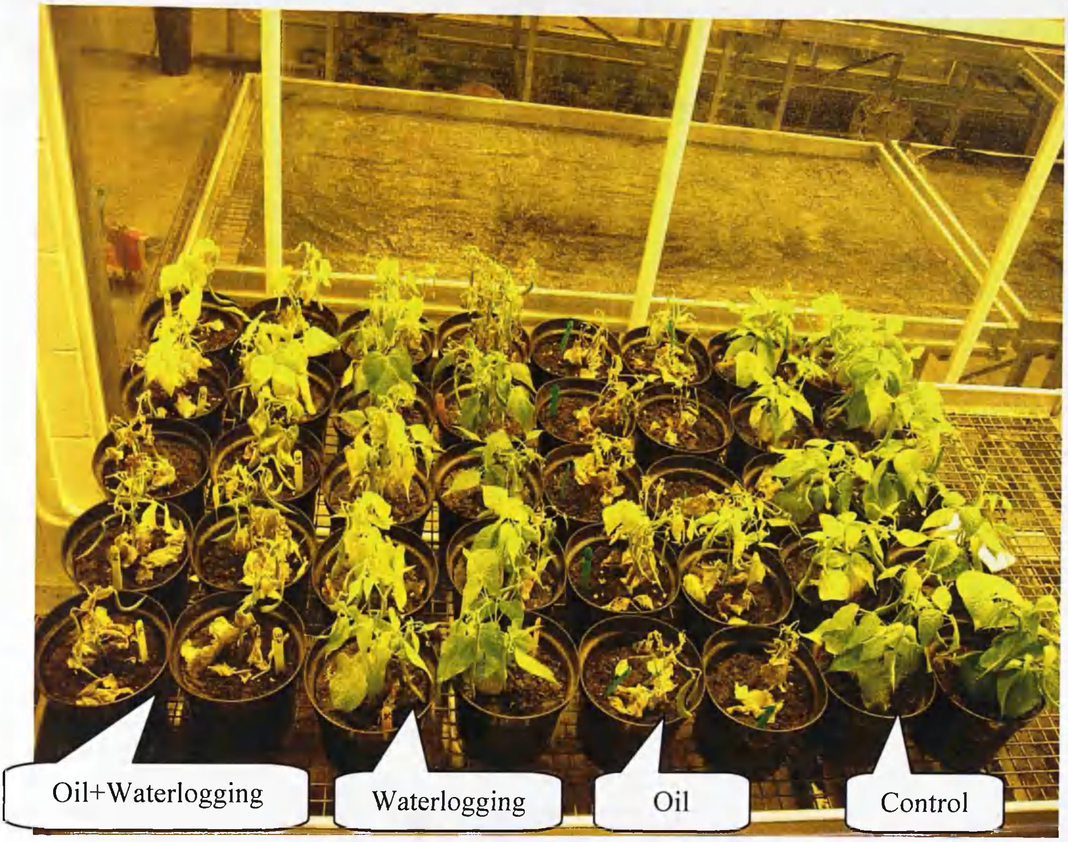

Figure 5.0 Visual stress symptoms in bean caused by oil pollution, waterlogging and combined oil and waterlogging at the end of the experiment. No visual stress symptoms were observed in the controls.

5.3.2 Photosynthesis

Treated plants showed a decline in photosynthetic activity as can be seen in Figure 5.1. The statistical analysis revealed that from day 2 onwards, all of the treatments showed a reduction in photosynthesis, compared to the controls. Whenever oil was involved in the treatment, there was a significantly larger reduction in photosynthesis than for waterlogging alone. Thus, oil and oil and waterlogging treatments showed the greatest reduction in photosynthesis, but there was no significant difference between these two treatments throughout the experiment. By the end of the experiment, there was a total reduction in the photosynthetic activities of treated plants by 42% for waterlogging and 100% for oil and the combination of oil and waterlogging, relative to the controls.

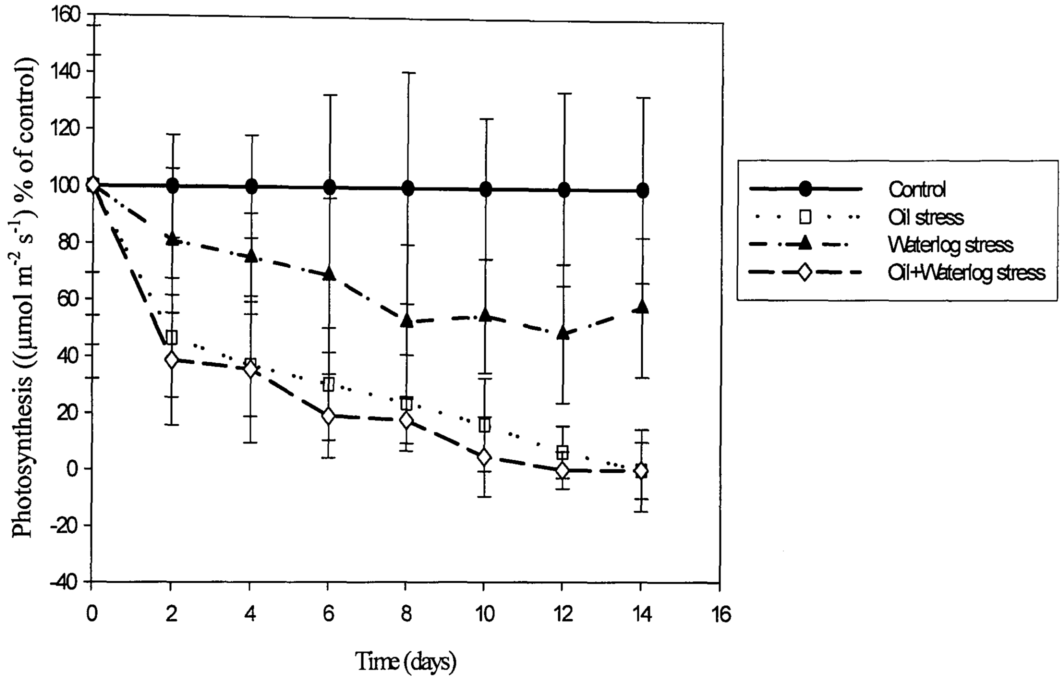

Figure 5.1 Effects of treatment on photosynthesis in bean over the course of the experiment. Treatments are denoted by the key. Error bars = 1 x SD, n = 8.

5.3.3 Transpiration

As shown in Figure 5.2, the rate of transpiration for all treated plants decreased relative to controls, showing similar responses to photosynthetic activities. From day 2 onwards, all of the treatments showed a reduction in transpiration, compared to the controls. Again, whenever oil was involved in the treatment, there was a significantly larger reduction in transpiration than for waterlogging alone. Thus, oil and oil and waterlogging treatments showed the greatest reduction in transpiration, but there was no significant difference between these two treatments throughout the experiment. By the end of the experiment, there was a total reduction in transpiration rate of treated plants by approximately 29%, 88%, and 93% for waterlogging, oil and the combination of oil and waterlogging, relative to the controls, respectively.

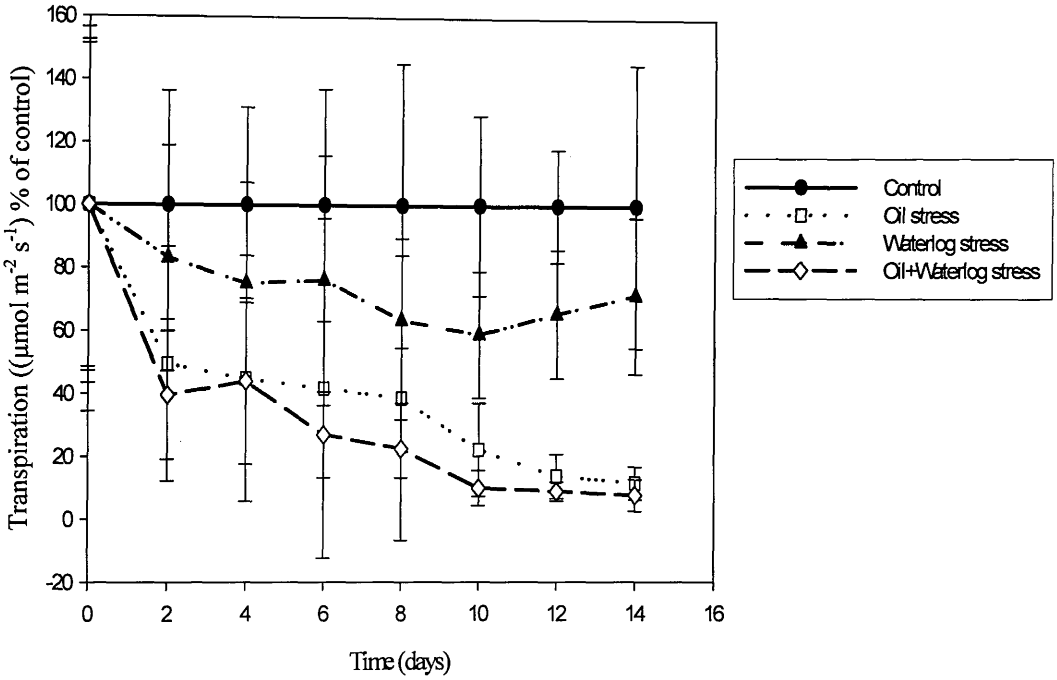

Figure 5.2 Effects of treatment on transpiration in bean over the course of the experiment. Treatments are denoted by the key. Error bars = 1 x SD, n = 8.

5.3.4 Stomatal conductance

There was a general decrease in stomatal conductance of treated plants as can be seen in Figure 5.3. Again, from day 2 onwards, all of the treatments showed a reduction in stomatal conductance, compared to the controls. Similarly, whenever oil was involved in the treatment, there was a significantly larger reduction in stomatal conductance than for waterlogging alone. Thus, oil and oil and waterlogging treatments showed the greatest reduction in stomatal conductance, but there was no significant difference between these two treatments throughout the experiment. By the end of the experiment, there was a total reduction in stomatal conductance of treated plant by 33%, 92%, and 94% for waterlogging, oil and the combination of oil and waterlogging, relative to the controls, respectively.

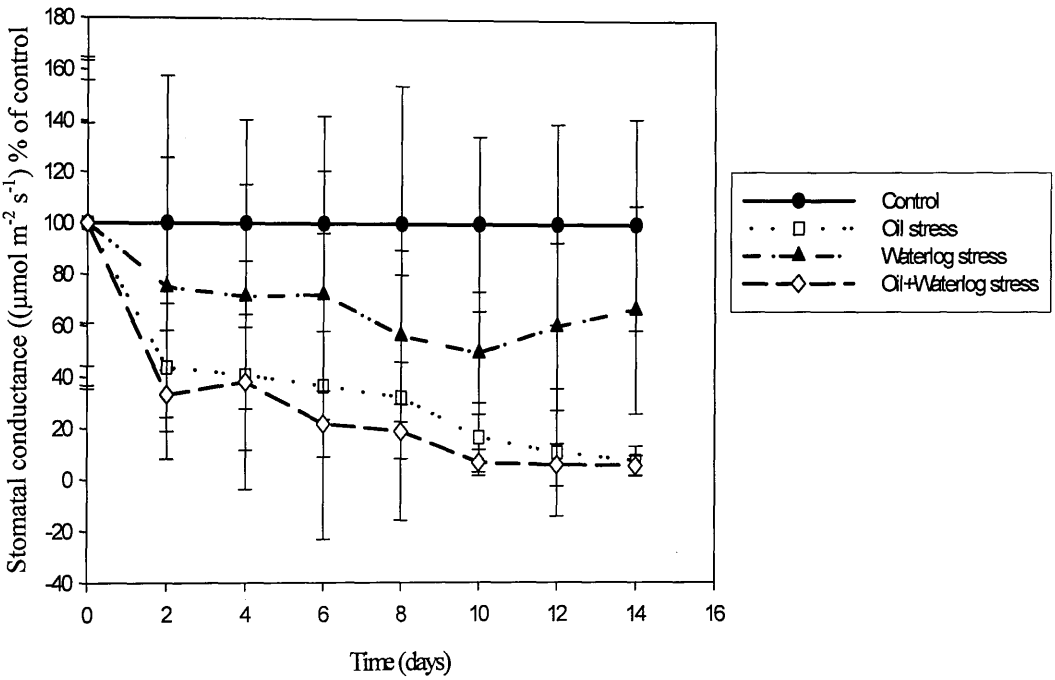

Figure 5.3 Effects of treatment on stomatal conductance in bean over the course of the experiment. Treatments are denoted by the key. Error bars = 1 x SD, n = 8.

5.3.5 Spectral Reflectance

5.3.5.1 Visible and NIR reflectance

Looking at mean spectra obtained at day 14 (Figure 5.4), it can be seen that there was a general increase in reflectance in the visible region and a decrease in the NIR in response to all treatments relative to the control. Oil treated plants showed the highest increment in the visible region except between 570 to 700 nm, where plants treated with the combination of oil and waterlogging showed the highest increment.

While a significant increase in reflectance of waterlogged plants were found in a few wavebands such as between 536 nm to 572 nm and between 698 nm to 716 nm, a significant increase in reflectance of plants treated with oil and the combination of oil and waterlogging was found in nearly all wavebands in the visible and red edge regions. The reduction in NIR reflectance was greatest for plants treated with the combination of oil and waterlogging, and at day 14 this difference was statistically significant. For those plants treated with oil and with waterlogging, the differences in NIR reflectance at the end of the experiment were not statistically significant.

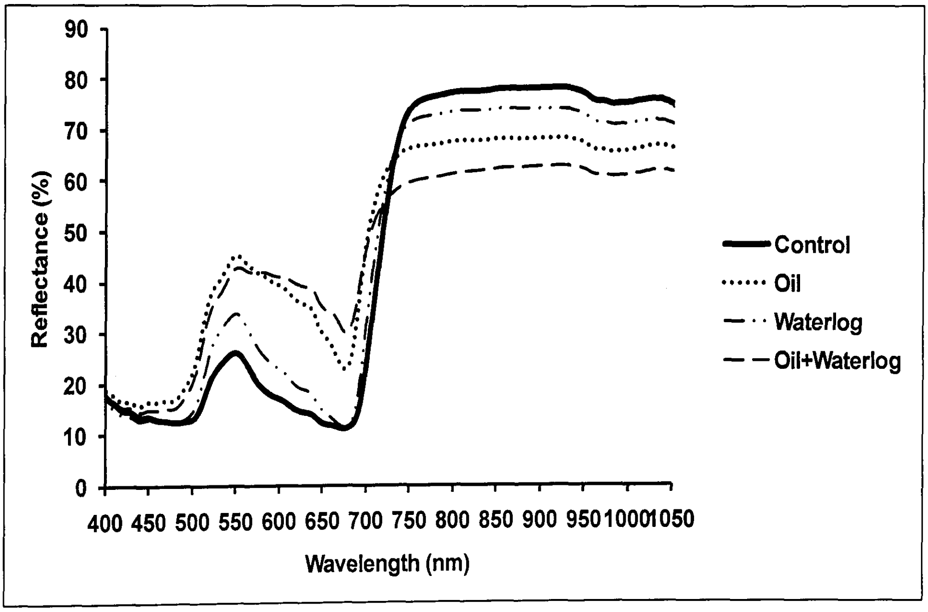

Figure 5.4 Mean reflectance spectra of control and treated bean 14 days after treatment. Treatments are denoted by the key, n = 80.

5.3.5.2 Spectral indices

In order to identify optimal spectral indices for early and consistent detection of plant responses to the treatments, ANOVA was performed using reflectance in individual narrow wavebands, simple ratios and normalized difference ratios of narrow wavebands, for each day of the experiment. The results of this analysis are shown in Table 5.0, where the order of the indices presented in the table indicates the overall level of performance. As can be seen the ratio R_{673}/R_{545} showed a statistically significant response to oil and waterlogging treatments on day 2 of the experiment, and a significant response to the combination of oil and waterlogging on day 4. Several other indices showed equally rapid responses to the individual oil and waterlogging treatments but slightly slower responses to the combined treatment.

Table 5.0 Sensitivity analysis of novel and existing spectral indices in control and treated plants over time. Unshaded = no significant difference; Shaded = significant difference. *Time when visible stress symptoms were observed in waterlogging treatment alone, **time when visible stress symptoms were observed in oil and the combined oil and waterlogging treatment.

Wavelengths (nm)	Treatments	Time (Days)							
		0	2	4	6	8**	10*	12	14
R_{673}/R_{545}	Control Oil stress		▨	▨	▨	▨	▨	▨	▨
	Waterlog stress		▨	▨	▨	▨	▨	▨	▨
	Oil+Waterlog stress			▨	▨	▨	▨	▨	▨
R_{673}/R_{631}	Control Oil stress		▨	▨	▨	▨	▨	▨	▨
	Waterlog stress		▨	▨	▨	▨	▨	▨	▨
	Oil+Waterlog stress				▨	▨	▨	▨	▨
R_{545}/R_{445}	Control Oil stress		▨	▨	▨	▨	▨	▨	▨
	Waterlog stress		▨	▨	▨	▨	▨	▨	▨
	Oil+Waterlog stress					▨	▨	▨	▨
$(R_{755}-R_{716})/(R_{755}+R_{716})$	Control Oil stress		▨	▨	▨	▨	▨	▨	▨
	Waterlog stress		▨	▨	▨	▨	▨	▨	▨
	Oil+Waterlog stress					▨	▨	▨	▨
R_{826}/R_{545}	Control Oil stress		▨	▨	▨	▨	▨	▨	▨
	Waterlog stress				▨	▨	▨	▨	▨
	Oil+Waterlog stress					▨	▨	▨	▨
R_{977}/R_{545}	Control Oil stress		▨	▨	▨	▨	▨	▨	▨
	Waterlog stress				▨	▨	▨	▨	▨
	Oil+Waterlog stress					▨	▨	▨	▨
R_{631}/R_{445}	Control Oil stress				▨	▨	▨	▨	▨
	Waterlog stress				▨	▨	▨	▨	▨
	Oil+Waterlog stress					▨	▨	▨	▨
R_{826}/R_{631}	Control Oil stress				▨	▨	▨	▨	▨
	Waterlog stress						▨	▨	▨
	Oil+Waterlog stress					▨	▨	▨	▨

R_{977}/R_{631}	Control Oil stress				▨	▨	▨	▨	▨
	Waterlog stress						▨	▨	▨
	Oil+Waterlog stress					▨	▨	▨	▨
R_{673}/R_{445}	Control Oil stress						▨	▨	▨
	Waterlog stress								
	Oil+Waterlog stress						▨	▨	▨
R_{631}/R_{545}	Control Oil stress							▨	▨
	Waterlog stress								
	Oil+Waterlog stress						▨	▨	▨
R_{631}	Control Oil stress							▨	▨
	Waterlog stress								
	Oil+Waterlog stress						▨	▨	▨
R_{826}/R_{445}	Control Oil stress							▨	▨
	Waterlog stress								
	Oil+Waterlog stress							▨	▨
R_{977}/R_{445}	Control Oil stress							▨	▨
	Waterlog stress								
	Oil+Waterlog stress							▨	▨
R_{826}/R_{673}	Control Oil stress							▨	▨
	Waterlog stress								
	Oil+Waterlog stress							▨	▨
R_{977}/R_{673}	Control Oil stress							▨	▨
	Waterlog stress								
	Oil+Waterlog stress							▨	▨
R_{673}	Control Oil stress								
	Waterlog stress								
	Oil+Waterlog stress							▨	▨
R_{977}/R_{826}	Control Oil stress								
	Waterlog stress								
	Oil+Waterlog stress								
R_{445}	Control Oil stress								
	Waterlog stress								
	Oil+Waterlog stress								
R_{545}	Control Oil stress								
	Waterlog stress								
	Oil+Waterlog stress								
R_{826}	Control Oil stress								
	Waterlog stress								
	Oil+Waterlog stress								
R_{977}	Control Oil stress								
	Waterlog stress								
	Oil+Waterlog stress								

5.3.5.3 Red-edge features

Both the treated and control plants showed single peaks in the first derivative of reflectance in the red-edge region of the spectrum, as shown in Figure 5.5 for mean spectra at the end of the experiment. Figure 5.6 shows the changes in REP for the different treatments over the course of the experiment. As shown in Table 5.1 the differences in REP become significantly different 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 nm, 12 nm, and 16 nm to shorter wavelengths for the waterlogging, oil, and the combined oil and waterlogging treated plants, relative to the control (Figure 5.6). There was some variation in the amplitude of the first derivative in the red edge region, as shown in Figure 5.5. However, such changes were not statistically significant at any point of the experiment (Table 5.1).

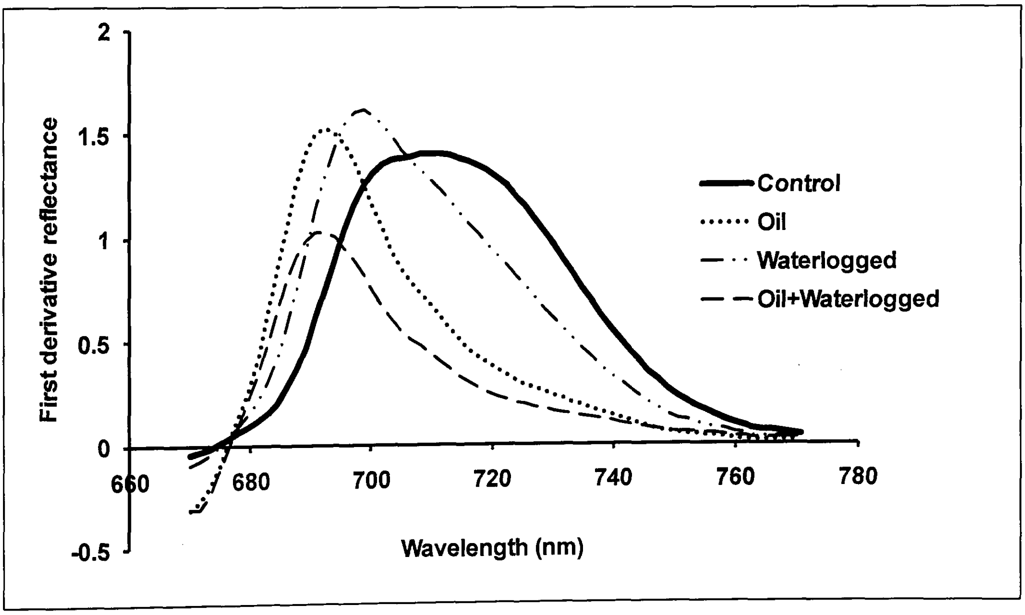

Figure 5.5 First derivative of reflectance of control and treated bean 14 days after treatment. Treatments are denoted by the key.

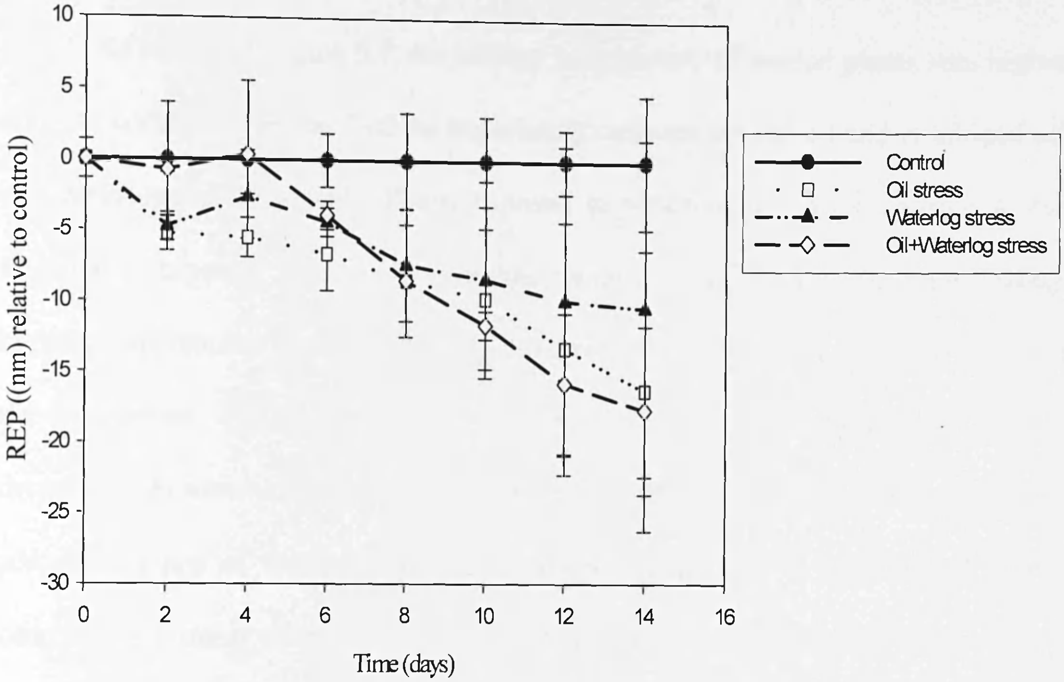

Figure 5.6 Temporal change in REP of control and treated bean. Treatments are denoted by the key. Error bars = 1 x SD, n = 8.

Table 5.1 Sensitivity analysis of the red-edge features of control and treated plants over time. Unshaded = no significant difference; Shaded = significant difference. *Time when visible stress symptoms were observed in waterlogging treatment alone, **time when visible stress symptoms were observed in oil and the combined oil and waterlogging treatment.

Red-edge features	Treatments	Time (Days)							
		0	2	4	6	8**	10*	12	14
REP (nm)	Control Oil stress					▨	▨	▨	▨
	Waterlog stress						▨	▨	▨
	Oil+Waterlog stress					▨	▨	▨	▨
Amplitude	Control Oil stress								
	Waterlog stress								
	Oil+Waterlog stress								

5.3.6 Thermal imaging

As shown in Figure 5.7, 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. 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. Figure 5.8 shows that the treated plants showed a systematic decrease in I_G relative to the controls from day 2 onwards. This effect was consistent across all types of treatment. It was apparent that I_G of the waterlogged plants decrease to a lesser extent than that of the oil and combined oil and waterlogging treatments. The sensitivities of absolute temperature of the canopy and I_G that occurred in bean due to oil, waterlogging and combined oil and waterlogging stress and the timing of the responses is given in Table 5.2. This demonstrates the consistent sensitivity of temperature and I_G to treatments that involved oil, but the lack of a consistent response to waterlogging alone.

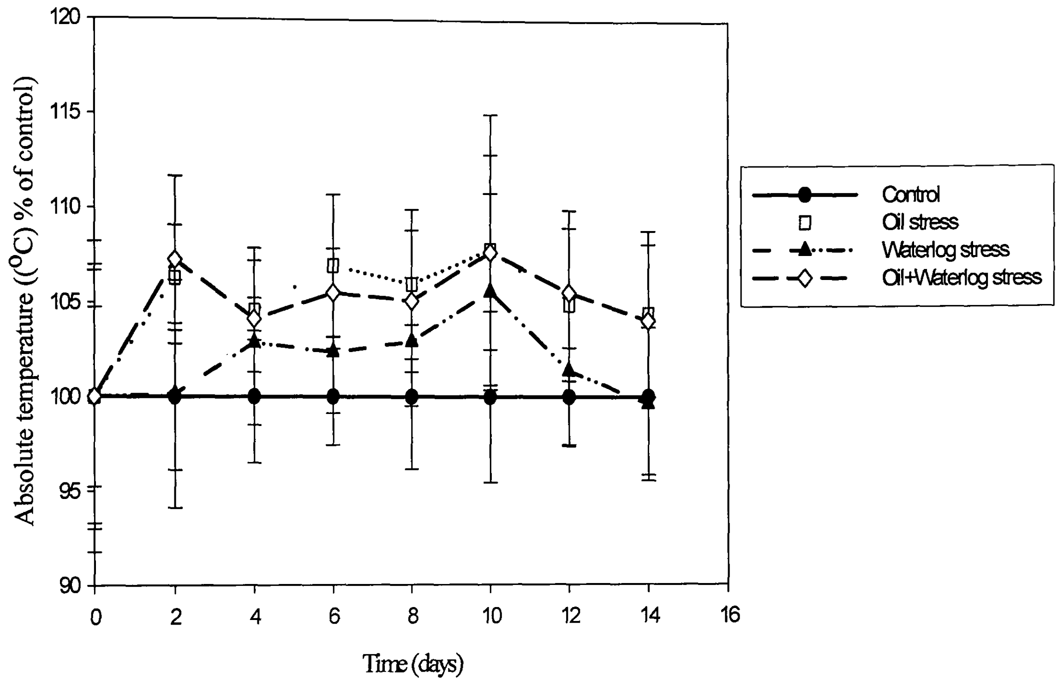

Figure 5.7 Temporal changes in canopy absolute temperature of treated and control plants. Treatments are denoted by the key. Error bars = 1 x SD, n = 8.

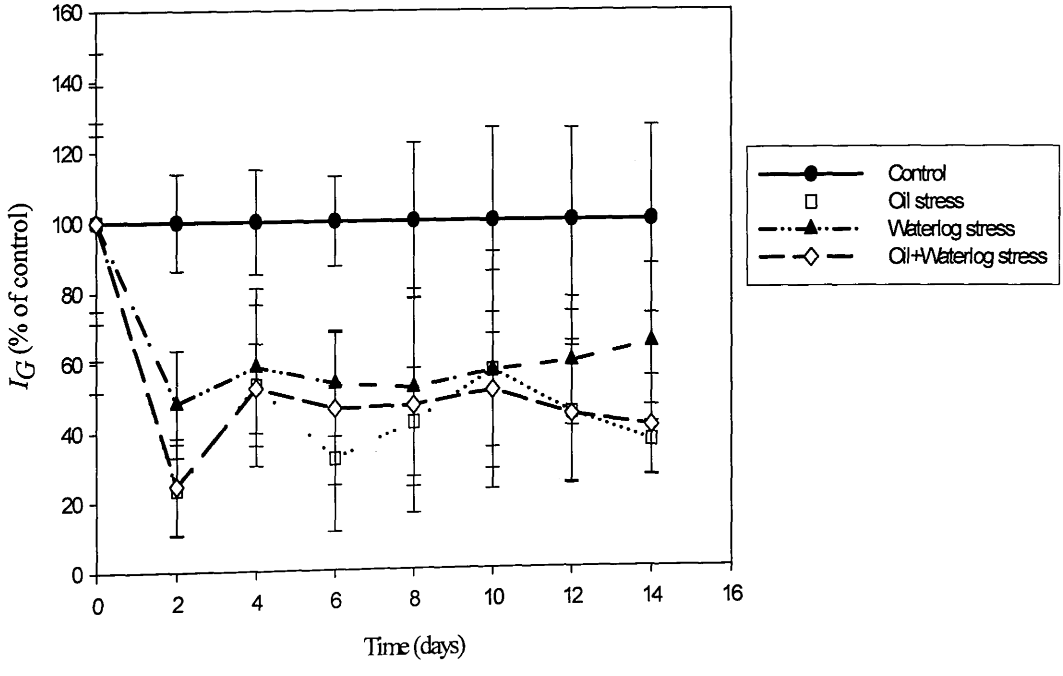

Figure 5.8 Temporal changes of thermal index (I_G) of treated and control plants. Treatments are denoted by the key. Error bars = 1 x SD, n = 8.

Table 5.2 Sensitivity analysis of the thermal properties of control and treated plants over time. Unshaded = no significant difference; Shaded = significant difference. *Time when visible stress symptoms were observed in waterlogging treatment alone, **time when visible stress symptoms were observed in oil and the combined oil and waterlogging treatment.

Thermography	Treatments	Time (Days)							
		0	2	4	6	8**	10*	12	14
Absolute temperature (^{O}C)	Control Oil stress		▨	▨	▨	▨	▨	▨	▨
	Waterlog stress						▨	▨	▨
	Oil+Waterlog stress		▨	▨	▨	▨	▨	▨	▨
I_G	Control Oil stress		▨	▨	▨	▨	▨	▨	▨
	Waterlog stress								
	Oil+Waterlog stress		▨	▨	▨	▨	▨	▨	▨

5.4 Discussion

All treatments significantly reduced the photosynthetic activity, transpiration and stomatal conductance of bean and these reductions were greatest when oil was involved in the treatment. While previous work has not investigated the combination of oil and waterlogging stresses, the findings in this study are in accordance with studies that have investigated the effects of waterlogging on plant physiology. Several studies have found that waterlogged conditions significantly reduce the photosynthetic rates of a wide range of plant species such as oilseed rape, bentgrass and barley (Baldock *et al.*, 1987; Dormaar, 1988; Zhou and Lin 1995; Yordanova *et al.*, 2005). A recent study has reported that the more common response to flooding is partial stomata closure within the first few hours of treatment (Yordanova *et al.*, 2005) but, the response of stomata after a prolonged exposure to waterlogging remains uncertain. Bradford and Yang (1981) reported that decreased leaf water potential (Ψ) does not always accompany flooding injury; even in most cases (Ψ) remains unaffected or increases in flooded plants. This suggests that a decrease in the photosynthetic rate of bean by waterlogging treatment observed in the present experiment may be as a result of non-stomatal factors, 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 concentrations. The author attributed this to non-stomatal (biochemical) factors, such as an inability for Ribulose-1,5-bisphosphate (RuBP) regeneration in 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 on waterlogging have focused on the short term effects 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) found that in pea plants, stomata begin to close in the first few hours of flooding with a parallel decrease in transpiration and stomatal conductance (Jackson and Hall, 1987). 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. The study found that flooding of barley plants for a short time (2 - 24 h) decreased transpiration and stomatal conductance. The result obtained in this study were based on the response of prolonged waterlogging conditions of up to 2 weeks rather than a short-term effect, nevertheless a significant physiological effect was observed on the second sampling occasion, 2 days after the start of the experiment.

In the present study, whenever oil is present in the treatment, there was a greater impact on plant physiological rates than with the waterlogging treatment alone. As indicated in chapter 4, oil pollution can have detrimental effects on plants, through a multitude of different mechanisms, such as soil oxygen depletion, reduced water uptake and toxic effects (Rowell, 1977; De Song 1980; Jong, 1980; Schumacher, 1996; Noomen *et al.*, 2003; 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 in waterlogged conditions. Furthermore, oil reduces the available nitrogen content of the soil (Sojka *et al.*, 1975; Jong, 1980) 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 (Małachowska-Jutsz *et al.,* 1997; 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 (Iwanow *et al.,* 1994; Amadi *et al.,* 1996). Finally, studies have shown that 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; Pezeshki *et al.*, 2000). This combination of effects from oil may well explain the greater impact of treatments involving oil than the waterlogging, found in the present study.

Substantial changes in spectral reflectance were observed in relation to all of the treatments used in the present experiment. Waterlogging produced a significant increase in reflectance in the visible in a region centred on 550nm and a second region centred on 715nm. This concurs with the findings of Anderson and Perry (1996) where reflectance of trees was elevated at 550 nm as a result of flooding in wetland areas. 550 nm and 715nm are regions of weak absorption by total chlorophyll (Zwiggelaar, 1998). As has been observed previously, 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 waterlogging caused a small decrease in chlorophyll but that overall concentrations remained high.

Furthermore, the wavelength ranges (centred on 550 and 715nm) where there was an increase in reflectance in waterlogging treated plants falls within the region (from 508 to 654 nm and 692 to 742 nm) where Smith *et al.* (2004a) found a significant increase in reflectance in dwarf bean (*Phaseolus vulgaris*) treated with waterlogging. However, in barley Smith *et al.* (2004a) found that waterlogging stress caused a significant decrease in reflectance across a wider wavelength range from 496 to 744 nm. The differences in the spectral responses of bean and barley could be attributed to their different genetic, biochemical or structural characteristics, as dicotyledon and monocotyledon species, respectively. In the present experiment it was found that the changes in spectral reflectance of bean treated with oil and the combination of oil and waterlogging occurred over a broad region within the visible spectrum. This was similar to the findings of Smith *et al.* (2004a) for barley exposed to waterlogging. This suggests that wavelength ranges 493 to 534 nm and 573 to 697 nm may serve as good indicators for discriminating between bean and barley when stressed with waterlogging and for discriminating between stresses induced by oil and waterlogging in bean. However, this needs further investigation probably on diverse plant species under varied environmental stress conditions; when the stability and dynamics of these spectral regions can be ascertained.

Various single stresses have been found to cause minimal reflectance change in the NIR. Smith *et al.* (2004a) found a small change in the NIR reflectance in bean and barley treated with waterlogging. In the present study there was no significant reflectance difference in the wavelength ranges 723 to 1050 nm and between 717 to 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 to 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 just a single stress factor.

Results from the sensitivity analysis indicated that narrowband spectral ratio indices were more sensitive in discriminating subtle signs of stress arising from oil pollution, waterlogging and the combined treatment, when compared with red-edge features and thermal stress indices. Based on consistency and time of detection, a simple reflectance ratio R_{673}/R_{545} that combined wavebands in the red and green regions performed best. This ratio exploits those regions of the visible which correspond with the absorption maxima and minima of chlorophyll.

In the present experiment 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.* (2004a) for bean treated with different stresses such as waterlogging, natural gas and argon. On the contrary, Smith *et al.* (2004a) 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 *at al.*, 2004a). The study noted that the internal structure of mono- and dicotyledons differs 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 compacted with fewer air spaces

(Gausman, 1985). Since the spongy mesophyll in a leaf of dicotyledons is more developed with many air spaces than the leaf of monocotyledons, their reflectance is generally higher than those of monocotyledons (Gausman, 1985; Guyot, 1990) and thus, allows more light scattering between the cell walls (Smith *at al.*, 2004a). 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.*, 2004a).

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 in bean, but only in the later stages of impact. In the present study, the REP shifted significantly towards shorter wavelengths for the plants treated with oil and the combination of oil and waterlogging on day 8 and for the waterlogged plants on day 10. This concurs with the previous findings presented in chapter 4 where the REP of maize treated with oil shifted towards the shorter wavelengths. Previous investigations have found that the REP shifted towards the shorter wavelengths as plants became stressed (Lang *et al.,* 1985a, 1985b; Crawford, 1986; Reid, 1988; McCoy *et al.,* 1989; Cwick *et al.,* 1995; De Oliveria and Crosta, 1996). The amplitude of the first derivative of reflectance for the treated plants was at no time significantly different to that of the control plants. 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.*, 2004a). As may have been the case in the present study, the change was attributed not only to the 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 the canopy of bean under all treatments was higher than the controls. This differs from the findings in chapter 4, where the leaf temperature of maize treated with oil fluctuated as stress progressed and did not differ significantly from that of control plants. It was suggested in chapter 4 that the inconsistency may have resulted from irregularities in the ambient temperature of the glasshouse that occurred at different times of measurement. Previous investigations of plant stress detection in the field using changes in canopy temperatures (as discussed in section 4.5) have experienced some limitations due to the effects of variation in air temperature, wind and environmental moisture. Greater environmental variation inevitable in an experiment with relatively large plants across a greenhouse was another identified setback. In the present study, thermography was undertaken in a more controlled environment (dark room) where a consistent source of illumination was used, ensuring that leaf temperature was a useful indicator of stress.

The sensitivity of the absolute canopy temperature of bean was significant soon after oil treatment and about 6 days after the combined oil and waterlogged treatments. Poor immiscibility between oil and water may have delayed the downward flux of oil and penetration into the plant root zone which may have delayed the effect of the combined oil and waterlogged stress in bean. 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 I_G of plants treated with oil and combined oil and waterlogging was consistently lower than those of the controls. As stated earlier, the I_G is theoretically proportional to the stomatal conductance thus, is likely to be responding to the reduction in stomatal conductance caused by oil. In chapter 4, the I_G was identified as a potentially valuable index for early detection of oil pollution in maize. Similarly to the absolute canopy temperature, the I_G changed significantly soon after oil treatment and 6 days after the combined oil and waterlogged treatments. Again, the poor immiscibility between oil and water may have caused the slower response to the combined oil and waterlogged stress. The I_G was not consistently sensitive to waterlogging in bean, which is again explicable in terms of the reduced response of transpiration and stomatal conductance to waterlogging as opposed to treatments involving oil.

5.5 Conclusion

The spectral reflectance and thermal properties of bean effectively distinguished subtle signs of stress induced by oil pollution and waterlogging. There was a significant increase in reflectance across the visible region for plants treated with oil and combined oil and waterlogging. However, for plants treated with waterlogging alone, there was only a significant increase in reflectance in two specific regions centred on 550nm and 715nm. Hence, it was deduced that these waveband regions could serve as good indices for discriminating between stress symptoms arising from oil or combined oil and waterlogging and those arising from waterlogging alone. NIR reflectance could be used to discriminate between stress induced in bean by single and multiple factors as it was found that the combined oil and waterlogging treatment caused a significant decrease in NIR reflectance while the individual oil and waterlogging treatments did not invoke such a response.

Among various spectral and thermal 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. The REP was sensitive to oil and waterlogged induced stress in bean but only at later stages of impact. While the canopy absolute temperature and the thermal index (I_G) were good indicators of developing oil and combined oil and waterlogging stress in bean, they were poor indicators of stress caused by waterlogging. Thus, by combining spectral and thermal information, oil-induced stress could be discriminated from waterlogging. In addition to waterlogging, the other major form of water-related stress which plants experience is that of water deficit. In the next chapter, we will investigate the performances and stability of thermal and spectral remote sensing for distinguishing between oil pollution and water deficit.

Chapter 6*

EXPLOITING SPECTRAL AND THERMAL RESPONSES OF MAIZE (*Zea mays* L.) FOR EARLY DETECTION AND DISCRIMINATION OF STRESSES CAUSED BY OIL POLLUTION AND WATER DEFICIT

6.1 Introduction

Water is essential for plant metabolism, and any limitation in its availability affects almost all plant functions, including the assimilation and partitioning of carbon (Deng *et al.*, 1990; Onillon *et al.*, 1995). Under field conditions, crops 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. Oil pollution has been noted as one of the major causes of environmental degradation and can arise from spills of crude and refined oil in aquatic and terrestrial environments (Ogboghodo *et al.*, 2004). 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 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.

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Recent studies have shown that petroleum toxicity conditions in plants are known to alter leaf pigmentation properties and cause changes in the reflectance spectrum (Rosso *et al.,* 2005) that can be detected before symptoms can be observed visually (Carter *et al.,* 1996). 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 stresses 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 (Carter, 1993; Massoni *et al.*, 1996; Smith *et al.*, 2005).

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. Indeed, as reported in Chapter 5, it was found that spectral and thermal sensing can effectively distinguish between stress induced by oil pollution and waterlogging in bean (*Phaseolus vulgaris 'Tendergreen'*). The combined effect of oil pollution and water deficit (the more widespread form of water-related stress) now needs to be addressed. Hence, the objective of the present study was to explore the physiological/biochemical basis of thermal and spectral properties of maize crops for the early detection and discrimination between oil contamination and water deficit.

6.2 Methods

In this experiment, four treatments comprising ten replicates were established, namely: control, oil, water deficit and the combination of oil and water deficit. Canopy thermal images were acquired in a darkroom (with an artificial illumination provided by a halogen lamp (see chapter 3, section 3.5). The light source was mounted in a fixed position at nadir 70cm away from each leaf to be measured. The camera was positioned at nadir 75cm above the plant canopy. An ASD FieldSpec® Pro Spectroradiometer (Boulder, CO 80301 USA) already described in chapter 3, section 3.5 was used for all reflectance measurements. Ten spectral measurements were captured per leaf for each of the 10 replicates per treatment.

6.3 Results

6.3.1 Physiological and biochemical responses to treatments

6.3.1.1 Visual stress symptoms

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, the thinning of canopies and slower growth (Figure 6.0). The control plants did not show visual stress symptoms but had fully matured by the end of the experiment.

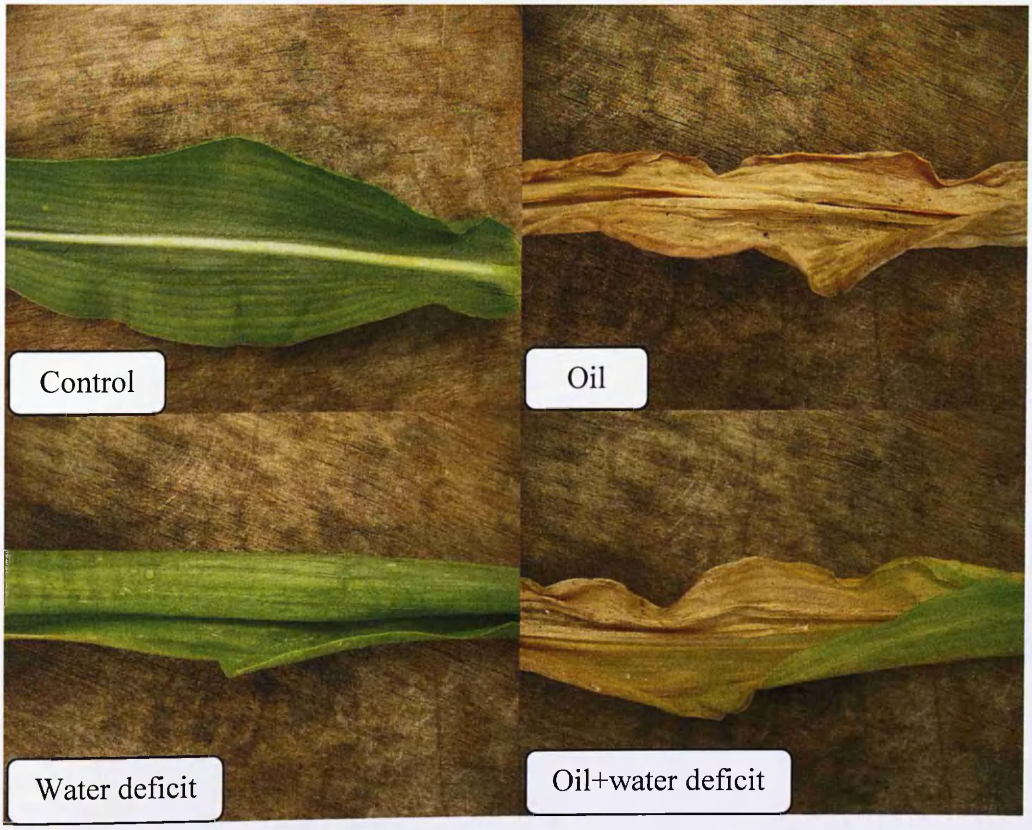

Figure 6.0 Visual stress symptoms in maize leaves caused by oil pollution, water deficit and combined oil and water deficit at the end of the experiment. No visual stress symptoms were observed in the controls.

6.3.1.2 Photosynthesis

Treated plants showed a decline in photosynthetic activity as can be seen in Figure 6.1. The statistical analysis revealed that before visual stress symptoms were observed, photosynthesis showed a significant reduction (on day 4) in the plants treated with water deficit and combined oil pollution and water deficit, compared to the controls (see Table 6.0 on page 141). However, for plants treated with oil pollution alone, a significant reduction in photosynthesis occurred on the same day as visual stress symptoms. Whenever water deficit was involved in the treatment, there is a significantly larger reduction in photosynthesis than for oil treatment alone. 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, while photosynthesis ended on day 18 for the plants treated with oil alone.

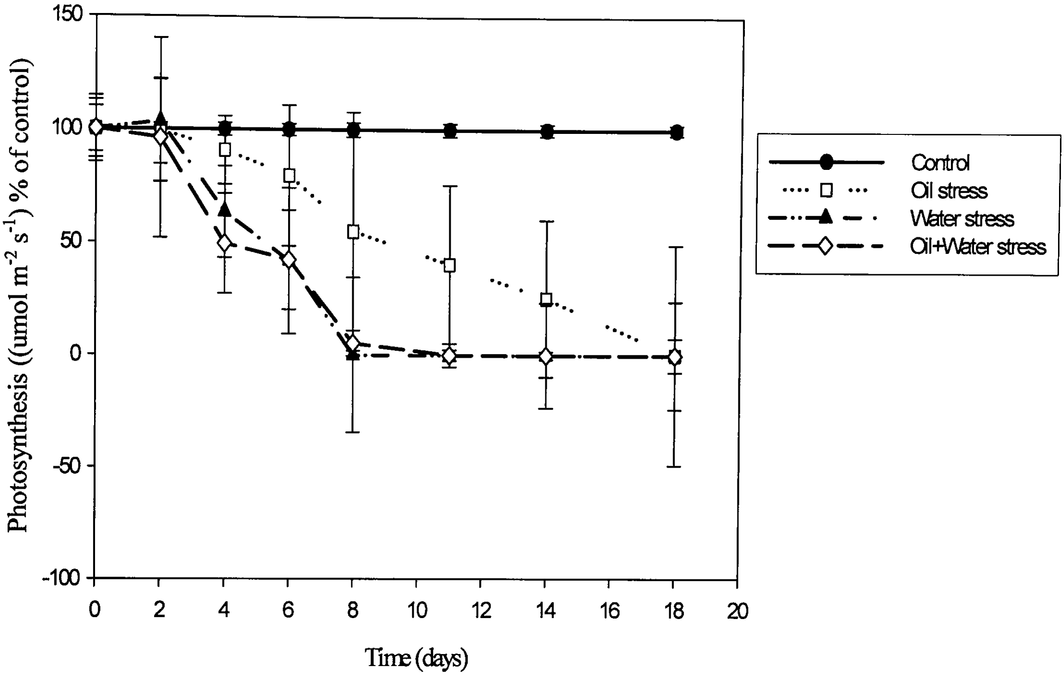

Figure 6.1 Effects of oil contamination of soil, water deficit and combined oil contamination and water deficit on photosynthetic activities of maize over time. Treatments are denoted by the key. Bars = 1 x SE, n = 10.

6.3.1.3 Transpiration

As shown in Figure 6.2, the rate of transpiration for all treated plants decreased relative to the controls, showing similar responses to photosynthetic activities. Before visual stress symptoms were observed, all of the treatments showed a significant reduction in transpiration, compared to the controls (see Table 6.0 on page 141). Again, whenever water was involved in the treatment, there was a significantly larger reduction in transpiration than for oil treatment alone. Thus, water and oil and water deficit treatments showed relatively the greatest reduction in transpiration, but there was no significant difference between these two treatments throughout the experiment. 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.

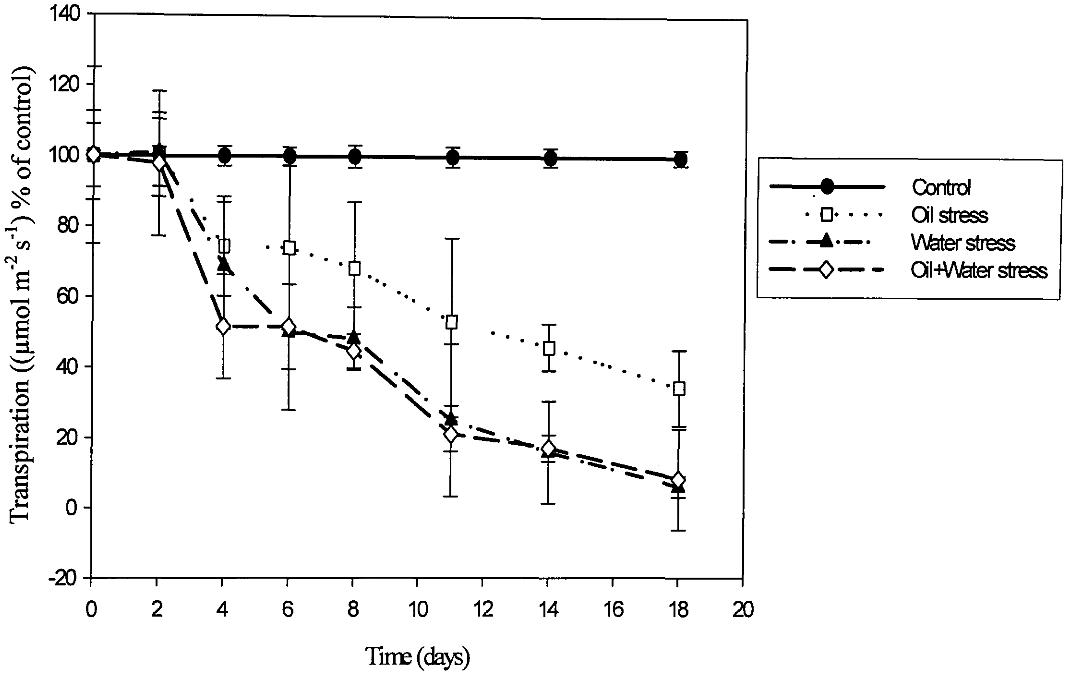

Figure 6.2 Effects of oil contamination, water deficit and the combined oil and water deficit on transpiration of maize, over time. Treatments are denoted by the key. Bars = 1 x SE, n = 10.

6.3.1.4 Stomatal conductance

There was a general decrease in stomatal conductance of treated plants as can be seen in Figure 6.3. Again, before visual stress symptoms were observed, all of the treatments showed a significant reduction in stomatal conductance, compared to the controls (see Table 6.0 on page 141). Similarly, whenever water was involved in the treatment, there is a significantly larger reduction in stomatal conductance than for oil treatment alone. Thus, water and oil and water deficit treatments showed the greatest reduction in stomatal conductance, but there was no significant difference between these two treatments throughout the experiment. By the end of the experiment, 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.

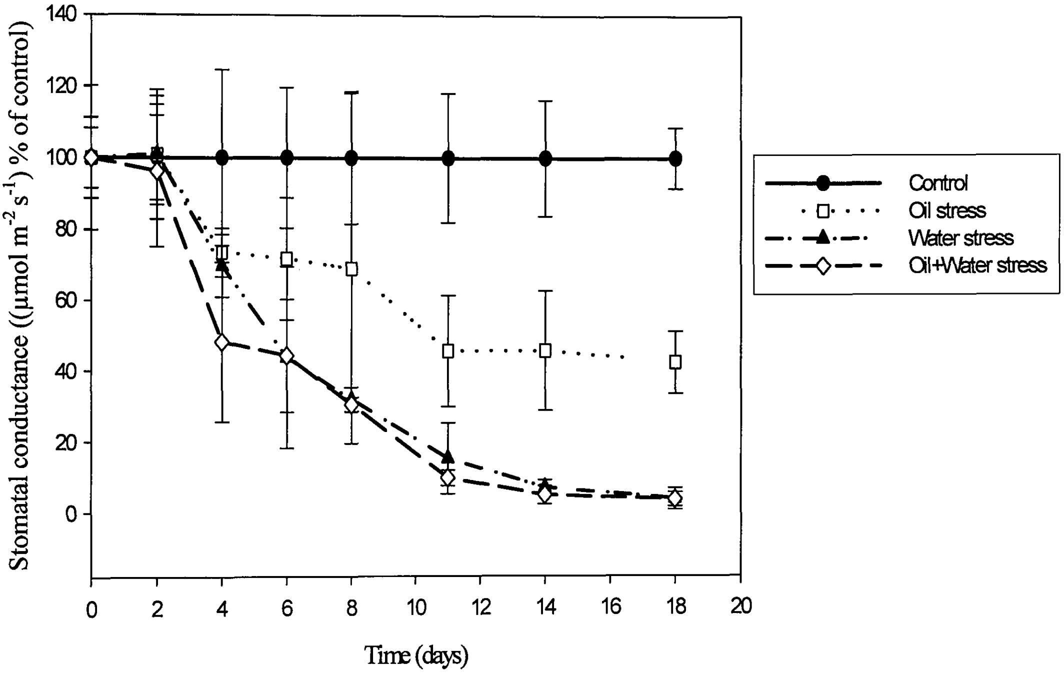

Figure 6.3 Effects of oil contamination, water deficit and the combined oil and water deficit on stomatal conductance of maize, over time. Treatments are denoted by the key. Bars = 1 x SE, n = 10.

Table 6.0 Results of ANOVA tests demonstrating when there were significant differences in the physiological and biochemical properties between the 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.

		Time (Days)							
Properties	Treatments	0	2	4	6	8**	11*	14	18
Photosynthesis (μmol $m^{-2} s^{-1}$)	Control Oil stress						▨	▨	▨
	Water stress			▨	▨	▨	▨	▨	▨
	Oil+Water stress			▨	▨	▨	▨	▨	▨
Transpiration (μmol $m^{-2} s^{-1}$)	Control Oil stress					▨	▨	▨	▨
	Water stress			▨	▨	▨	▨	▨	▨
	Oil+Water stress			▨	▨	▨	▨	▨	▨
Stomatal conductance (μmol $m^{-2} s^{-1}$)	Control Oil stress					▨	▨	▨	▨
	Water stress			▨	▨	▨	▨	▨	▨
	Oil+Water stress			▨	▨	▨	▨	▨	▨
Total chlorophyll (μg cm^{-2})	Control Oil stress				▨	▨	▨	▨	▨
	Water stress								
	Oil+Water stress				▨	▨	▨	▨	▨
Carotenoids (μg cm^{-2})	Control Oil stress								
	Water stress								
	Oil+Water stress								
Leaf water content (g)	Control Oil stress					▨	▨	▨	▨
	Water stress					▨	▨	▨	▨
	Oil+Water stress					▨	▨	▨	▨

6.3.1.5 Leaf total chlorophyll

There was a general decrease in total chlorophyll content over the course of the experiment in plants treated with oil, as can be seen in Figure 6.4. 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 (see Table 6.0). However, no significant reduction in total chlorophyll content was observed in plants treated with water deficit throughout the experiment. This implies that the 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. 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.

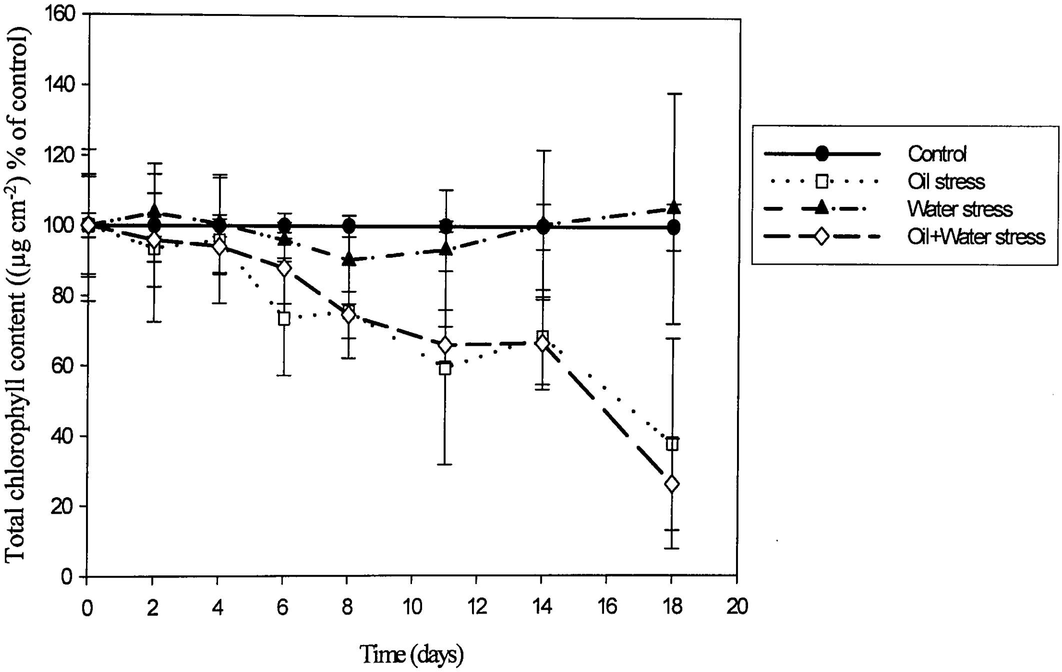

Figure 6.4 Effects of oil contamination of soil, water deficit and combination of oil and water deficit on total chlorophyll contents of maize. Treatments are denoted by the key. Bars = 1 x SE, n = 5.

6.3.1.6 Carotenoids

The carotenoid content of the treated plants did not change systematically through the experiment (Figure 6.5). 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 (Table 6.0).

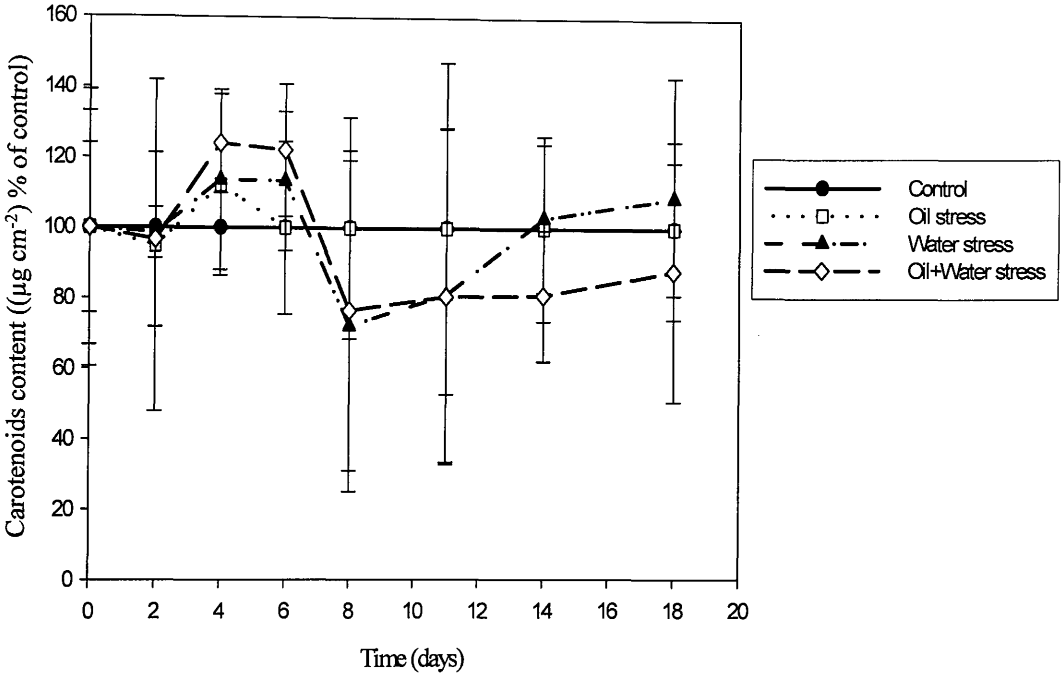

Figure 6.5 Effects of oil contamination, water deficit and the combined oil and water deficit on carotenoid content of maize. Treatments are denoted by the key. Bars = 1 x SE, n = 5.

6.3.1.7 Leaf water content

The leaf water content of all the treated plants decreased as stress progressed (Figure 6.6). 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 (see Table 6.0). 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.

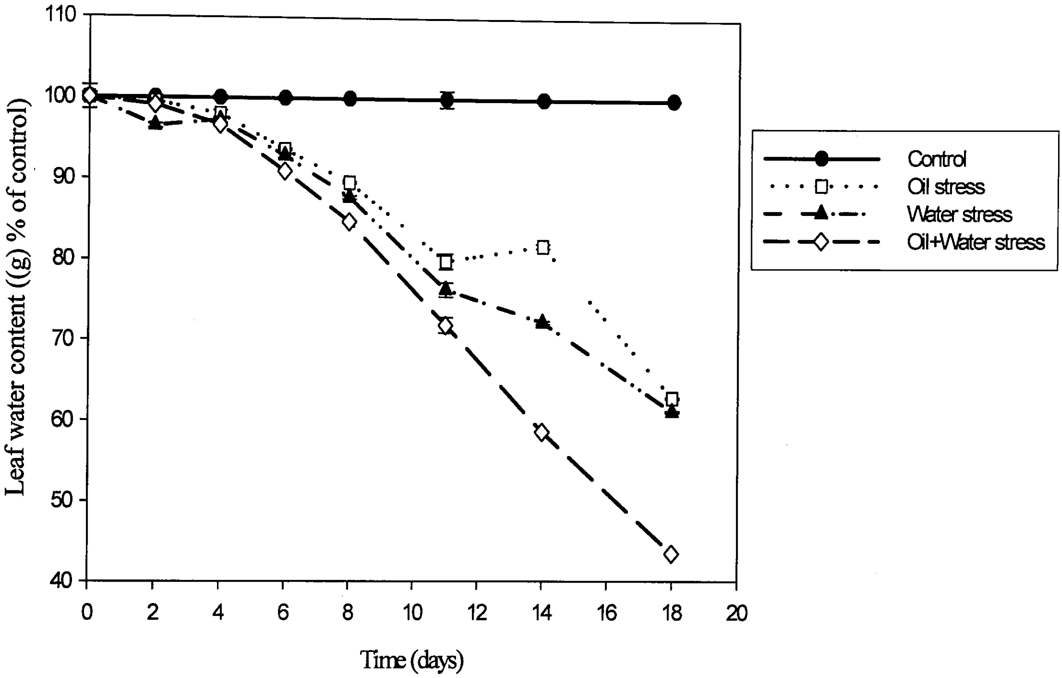

Figure 6.6 Effects of oil contamination, water deficit and the combined oil and water deficit on leaf water content of maize over time. Treatments are denoted by the key. Bars = 1 x SE, n = 5.

6.3.2 Interrelationships between physiological and biochemical variables

A moderate polynomial relationship was found between total chlorophyll and photosynthetic activities of maize leaves (Figure 6.7). The leaf water content also had a moderate polynomial relationship with both transpiration and stomatal conductance (see Figures 6.8 and 6.9), 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).

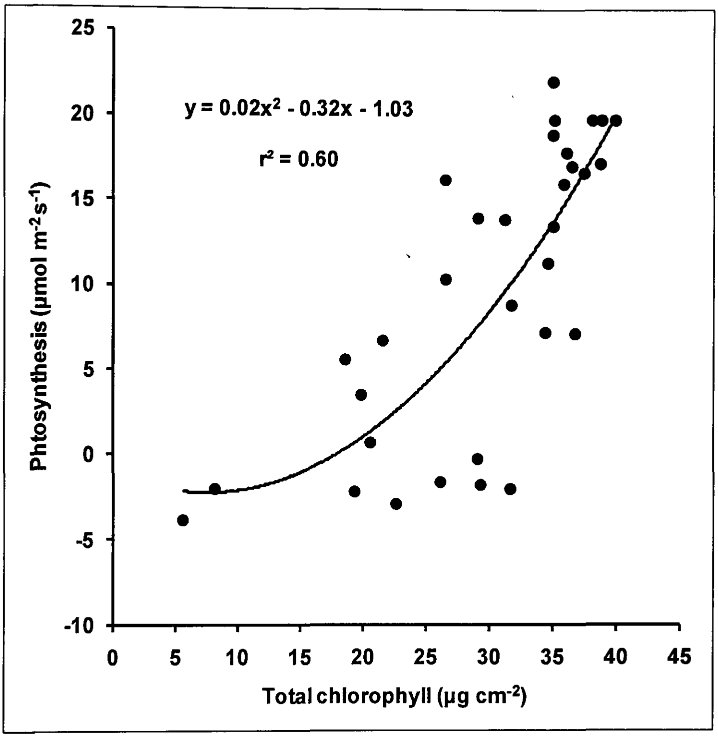

Figure 6.7 Relationships between total chlorophyll content and photosynthetic activities of maize, n = 32 (mean values per treatment, per sampling occasion).

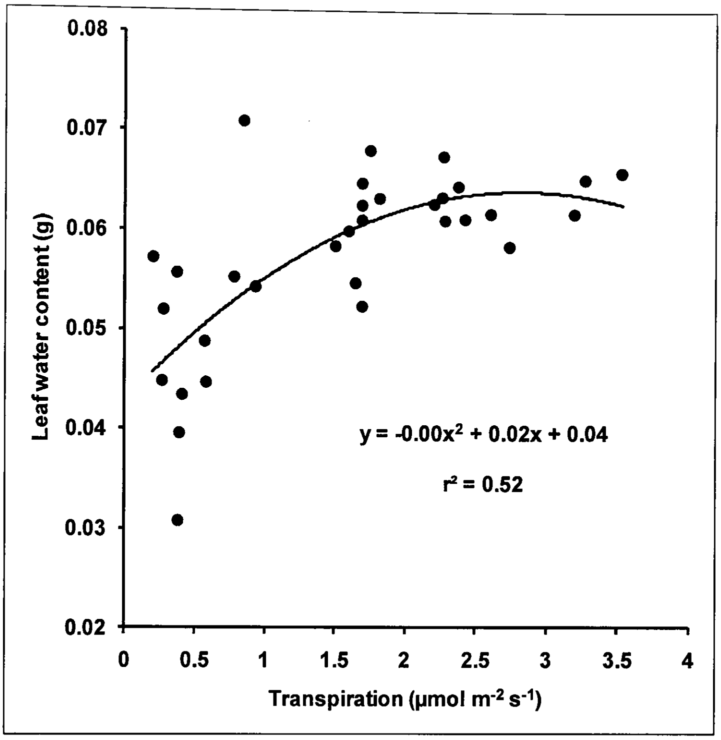

Figure 6.8 Relationships between transpiration and leaf water content of maize, n = 32.

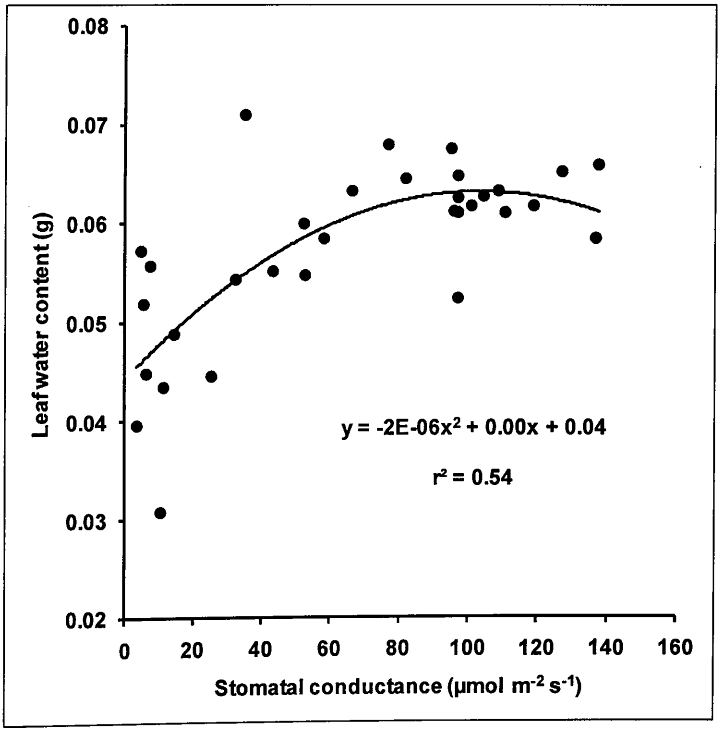

Figure 6.9 Relationships between stomatal conductance and leaf water content of maize, n = 32.

6.3.3 Responses of spectral reflectance to treatments

Looking at the mean spectra obtained at the end of the experiment (Figure 6.10), 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. 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 ($p < 0.05$).

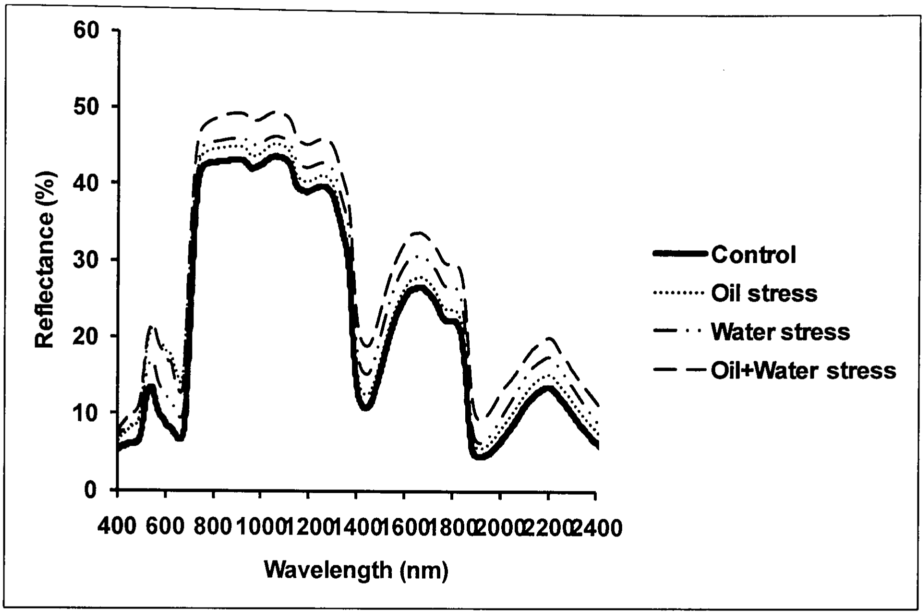

Figure 6.10 Mean reflectance spectra of treated and control leaves 18 days after treatment. Treatments as denoted by the key, n = 100.

6.3.3.1 Relationships between spectral reflectance and physiological and 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 (Figure 6.11). 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.

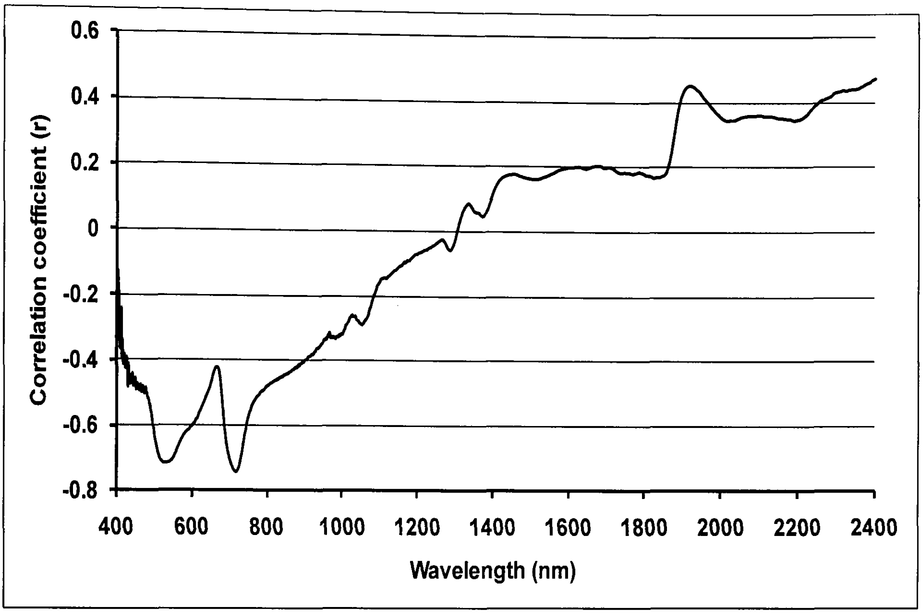

Figure 6.11 Correlogram showing the variation with wavelength in the correlation between the photosynthetic activity of maize and spectral reflectance, n = 32.

The relationships between transpiration and reflectance were similar to those for photosynthesis across the spectrum (Figure 6.12). There was a strong negative relationship between the transpiration rate and spectral reflectance in the visible region. Maximum correlations were found in the green and red regions, precisely at 520nm (r = - 0.69) and 715nm (r = - 0.71), respectively. Across the NIR and SWIR only weak relationships were found for transpiration.

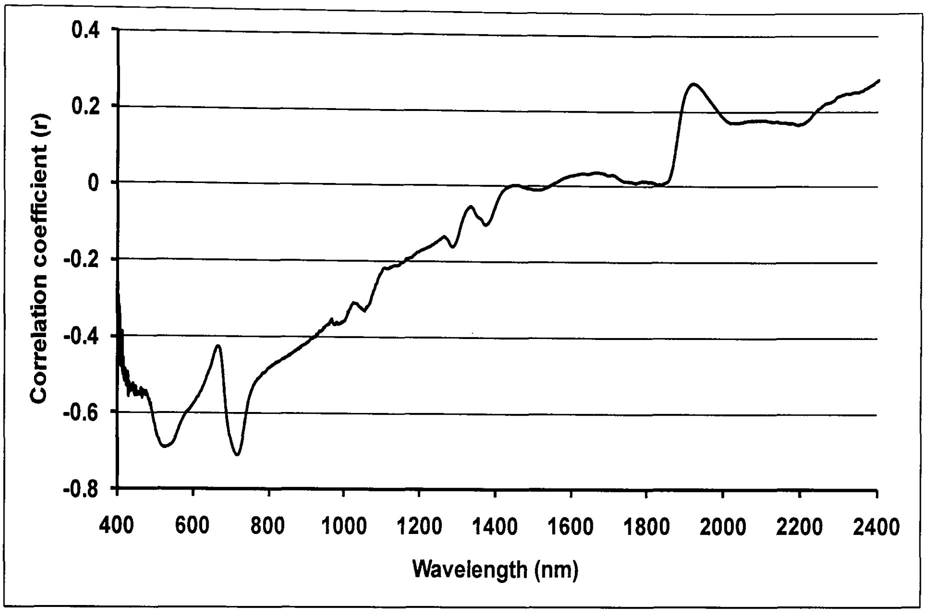

Figure 6.12 Correlogram showing the variation with wavelength in the correlation between the transpiration rate of maize and spectral reflectance, n = 32.

The relationships between stomatal conductance and reflectance were similar to those for photosynthesis and transpiration across the spectrum (Figure 6.13). There was a strong negative relationship between the stomatal conductance and spectral reflectance in the visible region. Maximum correlations were found in the green and red regions, precisely at 524nm (r = - 0.67) and 715nm (r = - 0.71), respectively. Across the NIR and SWIR only weak relationships were found for stomatal conductance.

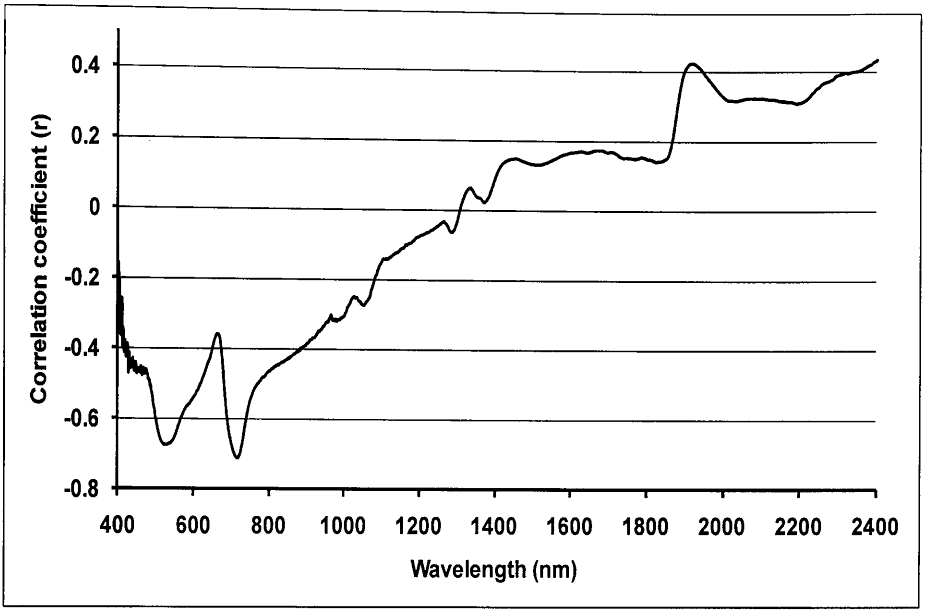

Figure 6.13 Correlogram showing the variation with wavelength in the correlation between the stomatal conductance of maize and spectral reflectance, n = 32.

As can be seen in Figure 6.14, there was a strong negative relationship between the leaf total chlorophyll content and spectral reflectance in the visible region. Again, maximum correlations were found in the green and red regions, precisely at 538nm (r = - 0.93) and 708nm (r = - 0.93), respectively. A weak relationship was found across the NIR and SWIR.

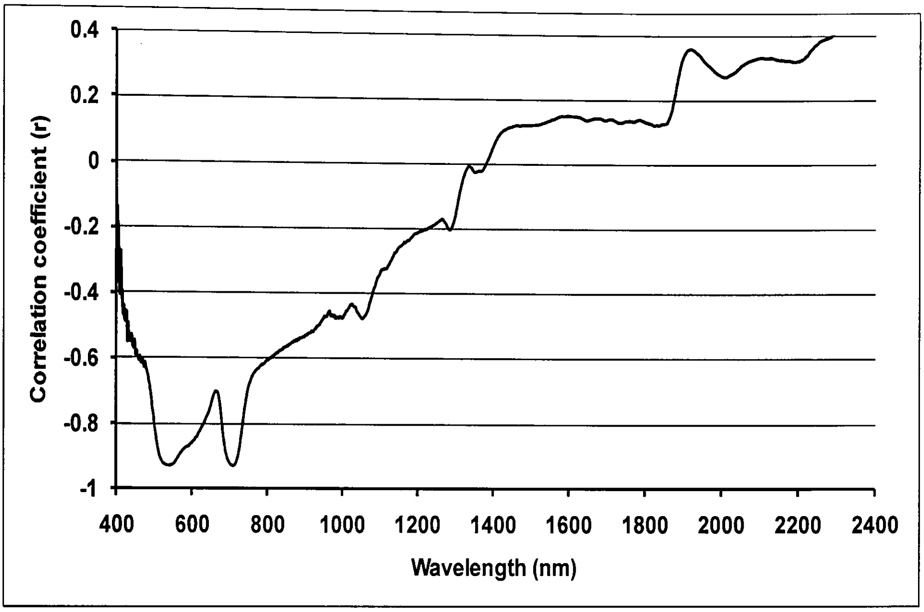

Figure 6.14 Correlogram showing the variation with wavelength in the correlation between the leaf chlorophyll content of maize and spectral reflectance, n = 32.

Carotenoids were largely uncorrelated with reflectance across most wavelengths, though there were some weak relationships in certain regions (Figure 6.15). 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). The weakest relationship was found in the NIR region at the waveband 736nm.

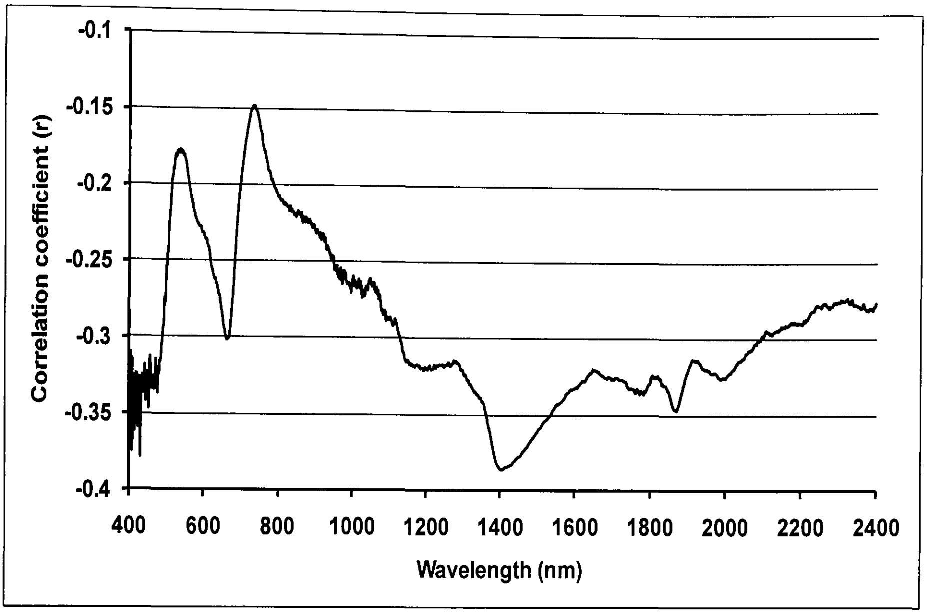

Figure 6.15 Correlogram showing the variation with wavelength in the correlation between the leaf carotenoid content of maize and spectral reflectance, n = 32.

Interestingly, the relationships between leaf water content and reflectance were similar to those for total chlorophyll across the spectrum (Figure 6.16). 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 = ≤ - 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).

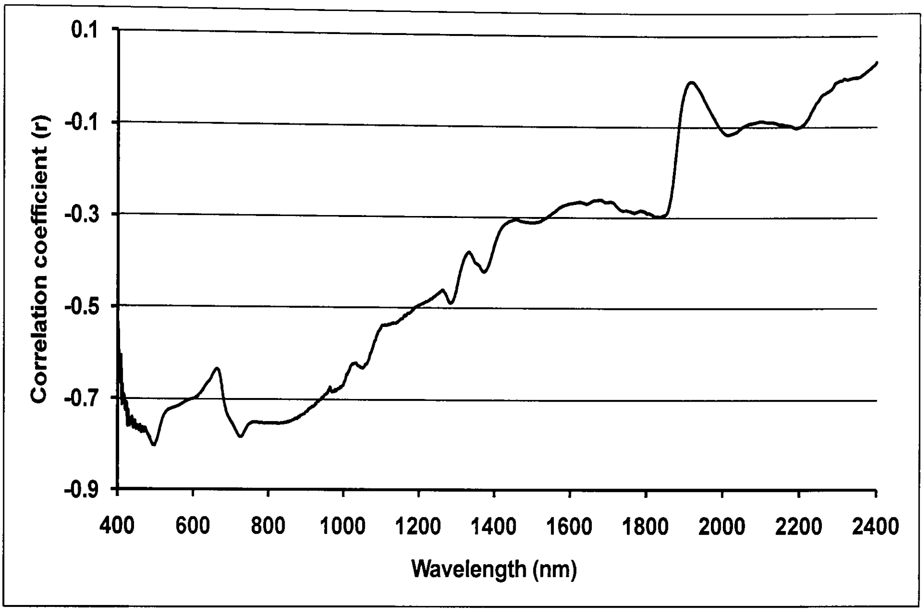

Figure 6.16 Correlogram showing the variation with wavelength in the correlation between the leaf water content of maize and spectral reflectance, n = 32.

6.3.3.2 Relationships between spectral indices and biochemical variables

Table 6.1 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 are indicated in bold.

Table 6.1 Summary of the correlation coefficients (r) between the spectral reflectance indices and measured physiological/biochemical parameters.

Biochemicals estimated	Spectral indices	r	References
Total chlorophyll ($\mu g\ cm^{-2}$)	$(R_{755}-R_{716})/(R_{755}+R_{716})$	0.940	From chapter 4
	R_{673}/R_{545}		From chapter 5
	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 ($\mu g\ cm^{-2}$)	R_{800}/R_{470}	0.350	Blackburn (1998)
	$(R_{800}-R_{470})/(R_{800}+R_{470})$	0.340	Blackburn (1998)
	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 (g)	$(R_{858}-R_{1240})/(R_{858}+R_{1240})$	-0.080	Gao, (1996); Zarco-Tejada *et al.*, (2003)
	$(R_{858}-R_{1640})/(R_{858}+R_{1640})$	0.010	Fensholt and 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

Correlations are significant at $p < 0.05$.

As can be seen in Figure 6.17, there was a strong positive linear relationship between normalised difference ratio $(R_{1330}-R_{538})/(R_{1330}-R_{538})$ and total chlorophyll. While there was a poor relationship between the normalised difference ratio $(R_{736}-R_{430})/(R_{736}+R_{430})$ and carotenoid (figure 6.18), the individual narrow waveband R_{900} had a moderate relationship with leaf water content (Figure 6.19).

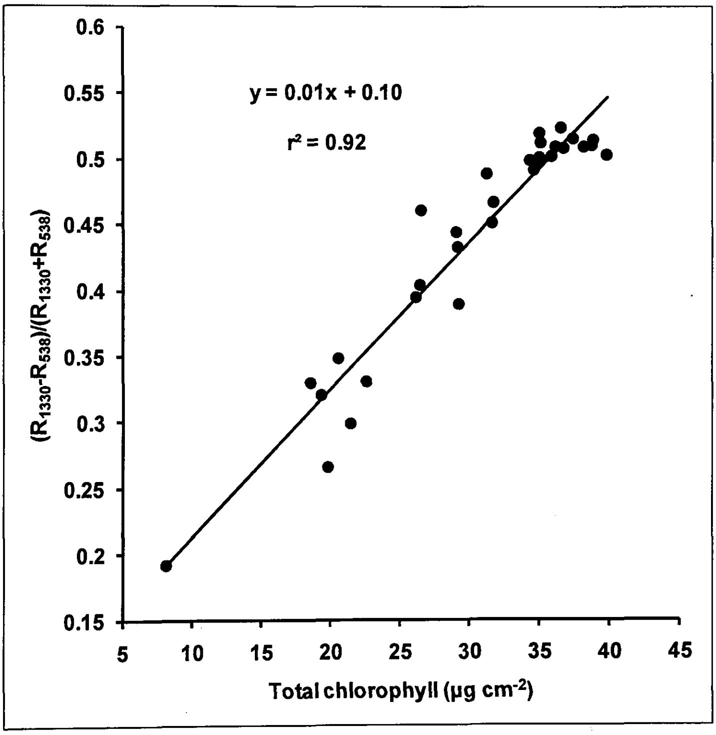

Figure 6.17 Relationships between $(R_{1330}-R_{538})/(R_{1330}+R_{538})$ and total chlorophyll content of maize, n = 32.

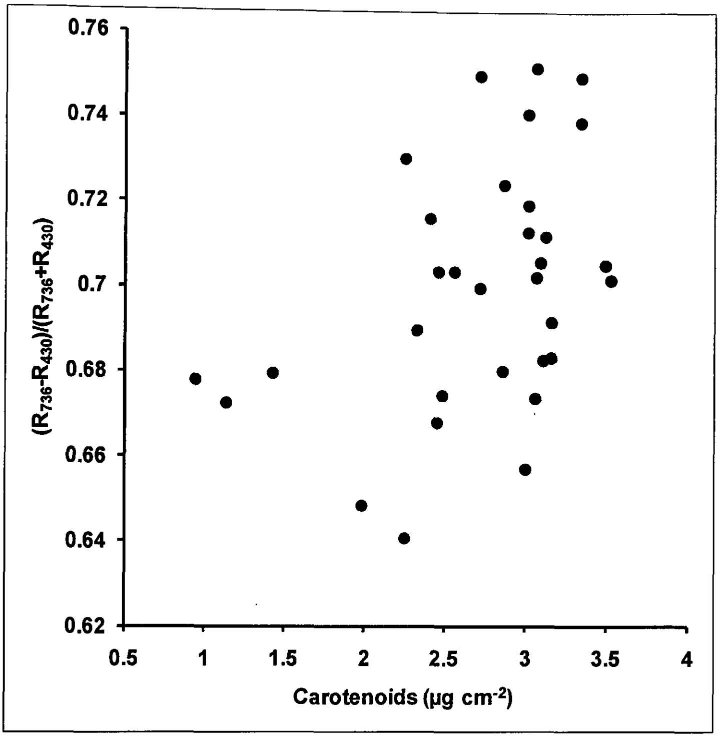

Figure 6.18 Relationships between $(R_{736}-R_{430})/(R_{736}+R_{430})$ and carotenoid content of maize, n = 32.

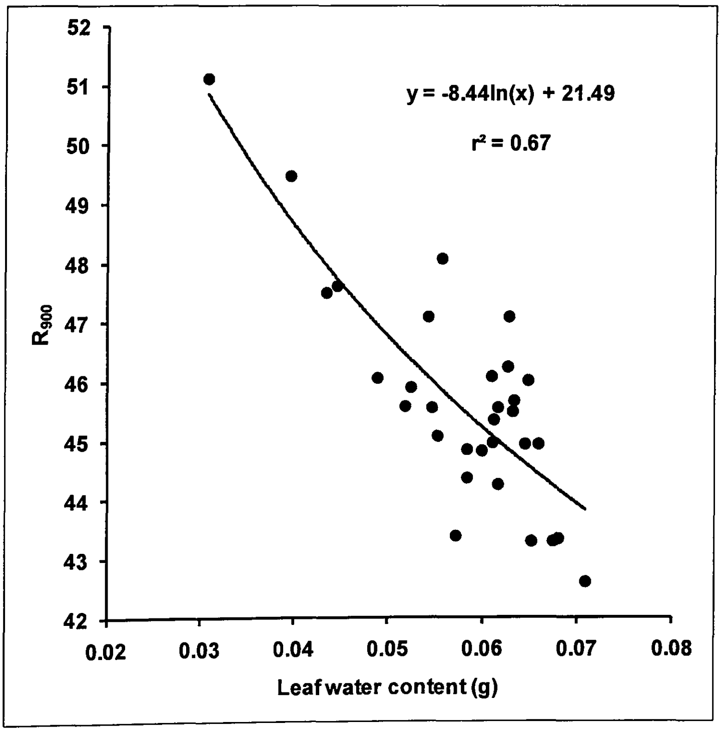

Figure 6.19 Relationships between R_{900} and leaf water content of maize, n = 32.

6.3.3.3 Temporal response of optimal spectral indices

As can be seen in Figure 6.20, 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 6.2). However, no significant reduction in the index was observed in plants treated with water deficit throughout the experiment. This implies that a 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.

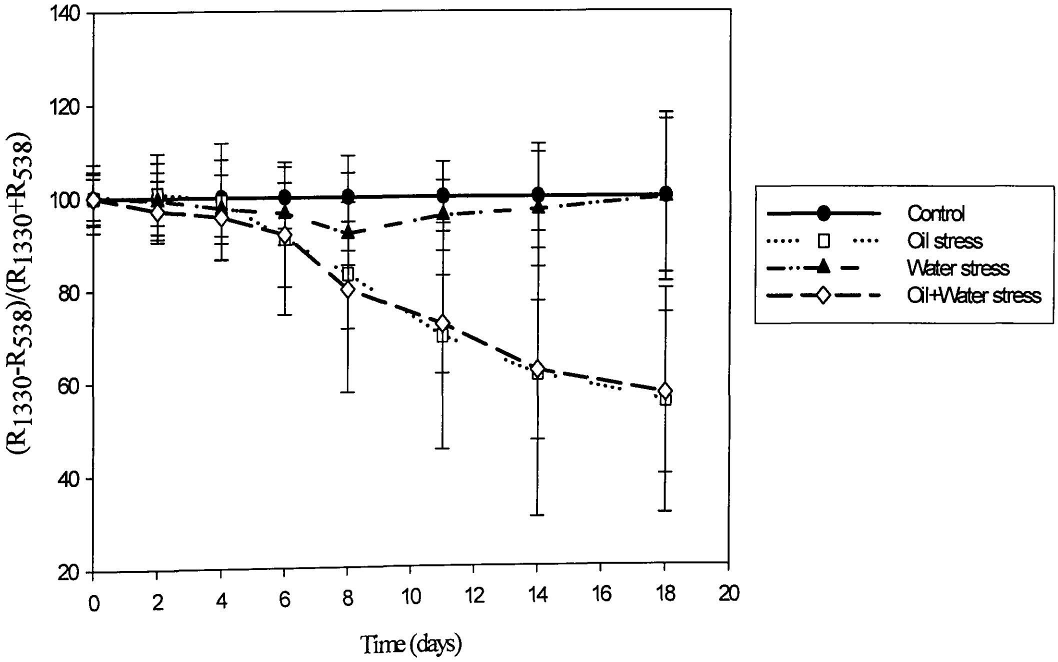

Figure 6.20 Change in $(R_{1330}-R_{538})/(R_{1330}+R_{538})$ with time. Treatments are denoted by the key. Bars = 1 x SE, n = 10.

The optimal carotenoid index $(R_{736}-R_{430})/(R_{736}+R_{430})$ decreased in treated plants as stress progressed (Figure 6.21). 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 6.2). 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.

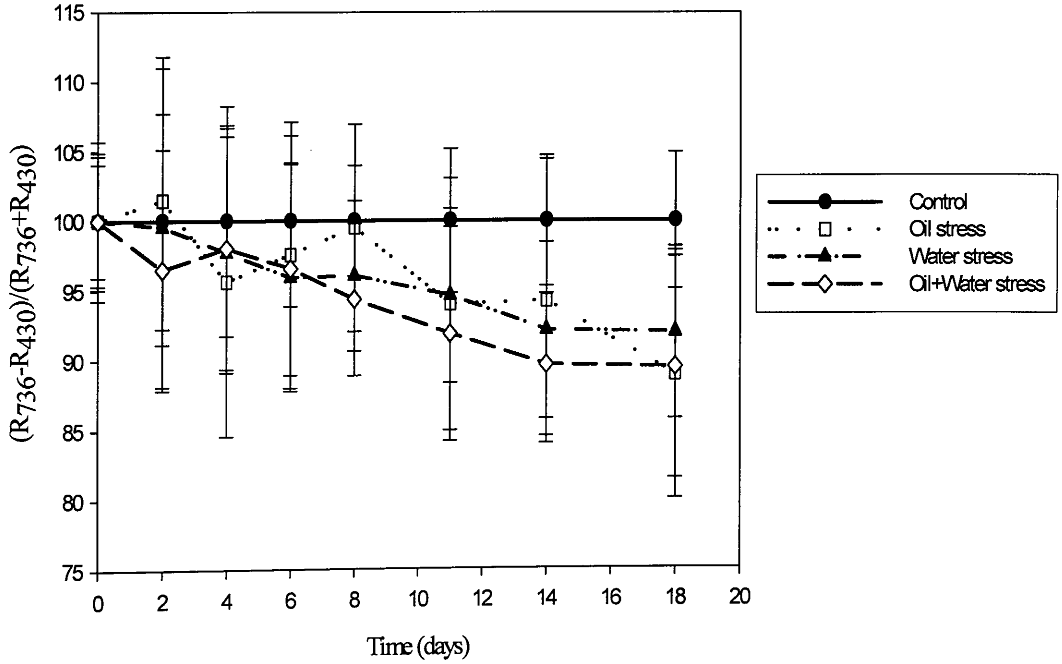

Figure 6.21 Change in $(R_{736}-R_{430})/(R_{736}+R_{430})$ with time. Treatments are denoted by the key. Bars = 1 x SE, n = 10.

As can seen in Figure 6.22, 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 6.2). A 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. The highest 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.

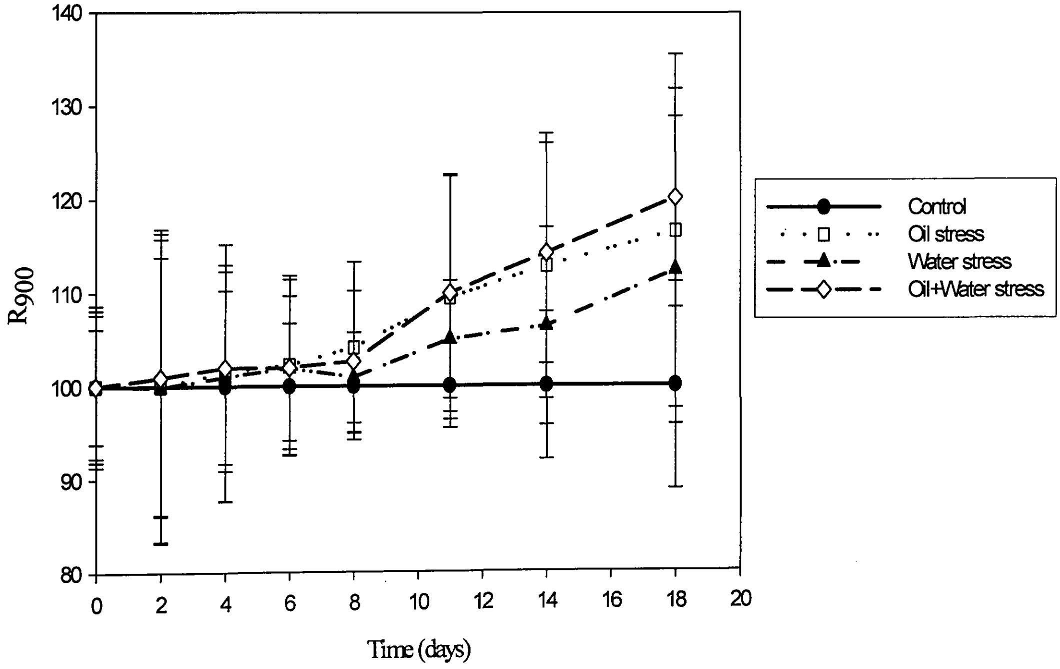

Figure 6.22 Change in R_{900} with time. Treatments are denoted by the key. Bars = 1 x SE, n = 10.

Table 6.2 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 (^{o}C)	Control	Oil stress						▨	▨	▨
		Water stress			▨	▨	▨	▨	▨	▨
		Oil+Water stress			▨	▨	▨	▨	▨	▨
I_G	Control	Oil stress			▨			▨	▨	▨
		Water stress			▨		▨	▨	▨	▨
		Oil+Water stress		▨	▨	▨	▨	▨	▨	▨

6.3.4 Thermography

As can be seen in Figure 6.23, the absolute leaf temperatures of treated plants increased relative to the 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 6.2). 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.

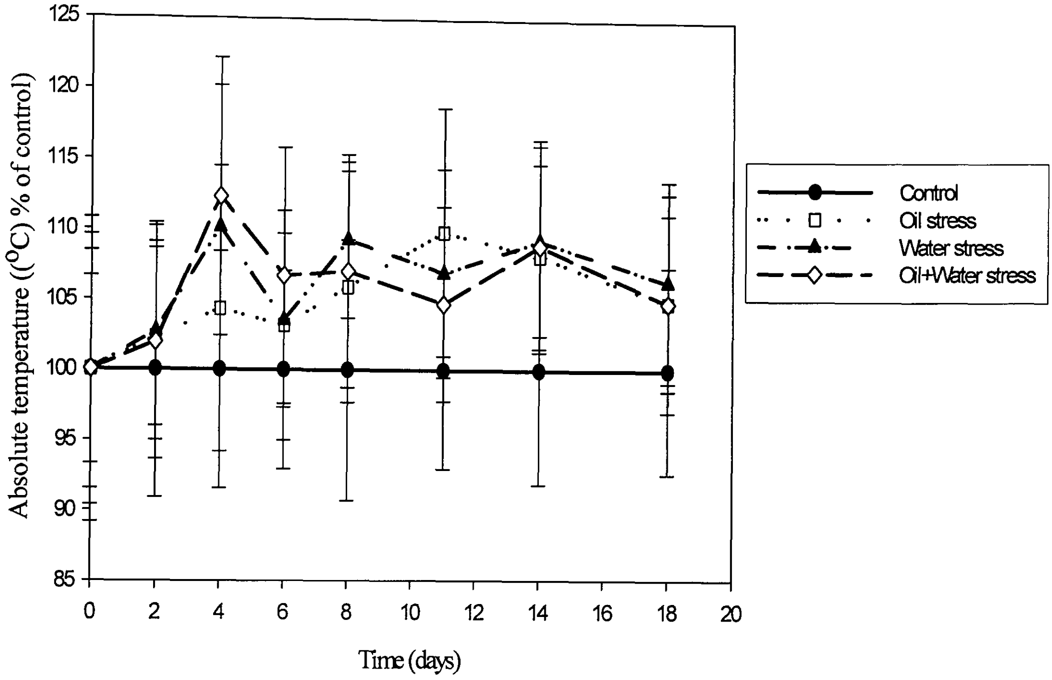

Figure 6.23 Effects of oil contamination, water deficit and the combined oil and water deficit on the absolute temperature of maize leaves over time. Treatments are denoted by the key. Bars = 1 x SE, n = 10.

The thermal index (I_G) of the treated plants was significantly reduced by treatments when compared with the control plants (Figure 6.24). The reduction was significant 6 days before the visual stress symptoms were observed in plants treated with the combined oil and water deficit (Table 6.2). 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 Figure 6.25.

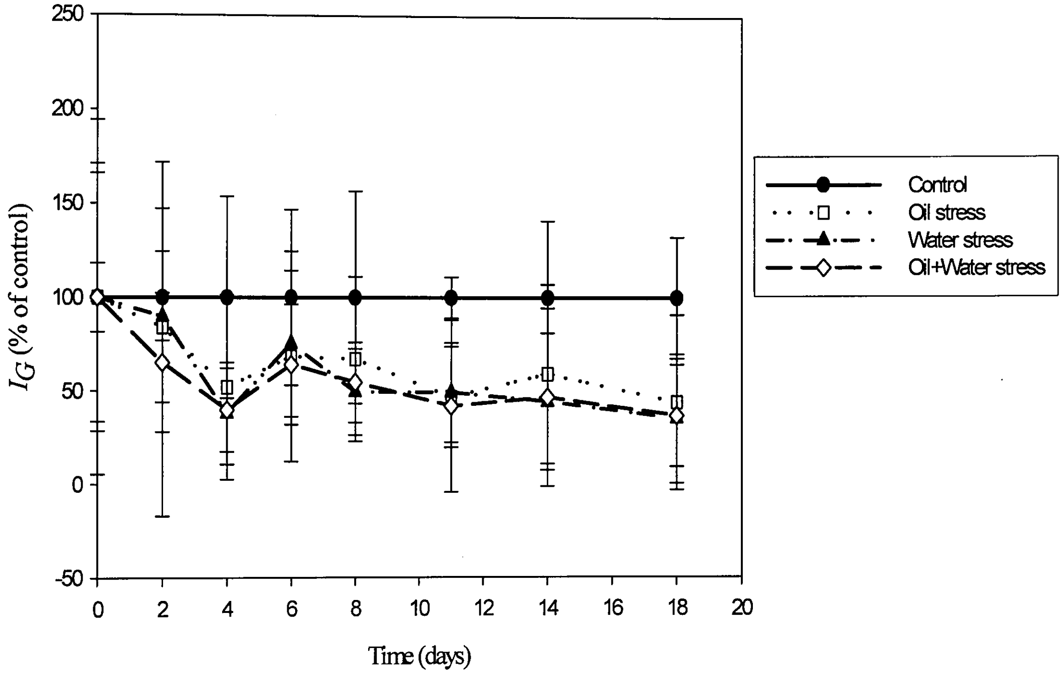

Figure 6.24 Effects of oil contamination, water deficit and the combined oil and water deficit on the thermal index (I_G) of maize leaves over time. Treatments are denoted by the key. Bars = 1 x SE, n = 10.

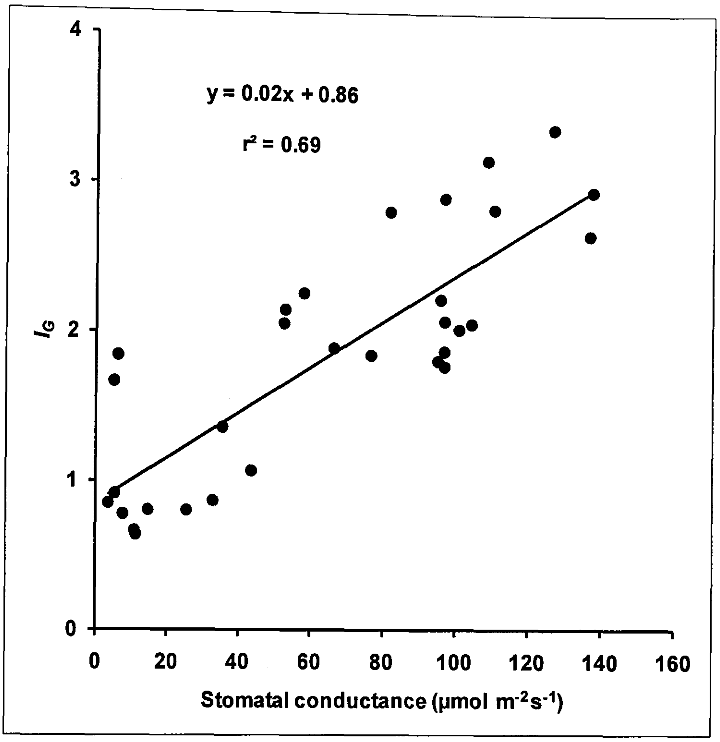

Figure 6.25 Relationships between the stomatal conductance and thermal index (I_G), n = 32.

6.4 Discussion

A wide range of plant stresses have been reported to cause visible stress symptoms such as leaf chlorosis, etiolation, wilting, thinning of canopies and decreased growth in plants (Rosso *et al.*, 2005; Smith *et al.*, 2005). In the present study, while similar visual stress symptoms were observed in treated plants, no visual stress symptoms were observed in control plants. Similar observations were also made in previous studies presented in earlier chapters. The visual stress symptoms started mildly by affecting a few leaves and then gradually increased to affect the whole plant. The result of the present study showed that physiological responses (that is, photosynthetic activities, transpiration and stomatal conductance) of maize plants were similar irrespective of the type of treatment.

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). In the present study, treatments may have reduced soil water needed to sustain transpiration processes and thus, can explain the 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 in the present study and this is explained by the strong linear relationship found between transpiration and stomatal conductance.

Maize 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. It was observed that the physiological properties of plants treated with oil alone reduced at a slower rate than those treated with water deficit and the combined oil and water deficit. The different method of stress application used can explain this situation. For oil treatment, a single application was made while water deficit stress was instigated by continuously decreasing the amount of water plants may require for growth. Consequently, oil treatment affected plant physiology at a slower rate compared to water deficit which rapidly decreases plant physiology.

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 may possibly be attributed to the toxic effects of oil as it destroys cell membranes. 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.*, 1995; Pezeshki *et al.*, 2000). Earlier work has also noted that changes in chlorophyll content can be caused not only by water stress but also by the phenological status of the plant, atmospheric pollution, nutrient deficiency, toxicity, plant diseases, and radiation stress (Larcher, 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). It was 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 (Gond *et al.*, 1999).

In the present study, while the photosynthetic activities of plants treated with water deficit reduced significantly, their total chlorophyll content did not change significantly. The result obtained in this study 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. Maracci *et al.* (1991) 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 and DeLaune, 1993; Webb, 1994; Pezeshki *et al.*, 1995). Furthermore, water stress may cause closure of leaf stomata and a reduction in CO_2 supply (Jackson and Ezra 1995). This evidence can explain the 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 a 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 the present study. The few occasions where carotenoid content of treated plants were higher than those of the control, may possibly be attributed to the damaging effect of the stresses.

Interestingly from the 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 studies indicate that a reduction in transpiration helps to conserve available water (Larcher, 1995), as does the stomatal conductance as explained earlier in this section. 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 studies that used a wide range of plant stresses such as water logging, natural gas, nutrient stress, heavy metal toxicity and soil oxygen deficiency (Woolley, 1971; Horler *et al.,* 1983; Milton *et al.,* 1989; Carter, 1993; Carter and Miller, 1994; Anderson and Perry, 1996; Noomen *et al.*, 2003; Smith *et al.*, 2004). 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 the result of this study, 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 the interrelationship found between the leaf water content and total chlorophyll and the fact that the total chlorophyll was changing over a 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 (Ceccato *et al.*, 2001; Tilling *et al.*, 2007). 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, the 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 deteriorated due to a reduction in transpiration and stomatal conductance, other factors may have biased the relationship that was 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 the results found in the present study, Woolley (1971), Bowman (1989), Carter *et al.* (1989) and Graeff and Claupein (2007) also 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 loss (Sinclair *et al.*, 1971; Gausman and Allen, 1973; Graeff and Claupein, 2007). 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 the results of this study 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 a 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 (Danson *et al.*, 1992; Aldakheel and Danson, 1997). 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. These studies 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 the 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 (Ceccato *et al.*, 2001; Bacour *et al.*, 2002). A study by Cheng *et al.*, (2006) demonstrated that more significant changes in leaf reflectance are introduced by changes in 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 previously in this section.

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 indices $(R_{1330}-R_{538})/(R_{1330}+R_{538})$ 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 values in the green or red regions with one in the NIR had the strongest relationship with chlorophyll concentrations 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 the present 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 a stronger relationship was also found between chlorophylls and reflectance (R = - 0.49) in the same region where carotenoid absorption maxima was found (see figure 6.14). 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 an increased range of carotenoid concentrations 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 the 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 6.3 show that, a change in the absolute temperature of the 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 the leaf absolute temperature. The response in absolute temperature to treatments in this study differ from that found in chapter four where no significant difference was found between the leaf absolute temperature of the treated plants and the control. One possible explanation to this is that, unlike in chapter four where, environmental variation may have influenced absolute leaf temperatures (which is inevitable in an experiment with relatively large plants distributed across a greenhouse), in this study, thermal measurements were undertaken in a more confined environment (dark room) where environmental variation was minimal.

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 and significantly different 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 the 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 in this chapter was similar to that found in chapter four, although an exponential relationship was found in the present study. The difference between the form of the relationship may be attributed to the use of larger dataset, incorporating a wider range of values in this study compared to the previous study.

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 the regions 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 can 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.

6.5 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. Hyperspectral remote sensing 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.

However, results show that chlorophyll index $(R_{1330}-R_{538})/(R_{1330}+R_{538})$ can detect oil related stress before visual stress symptoms are observed.

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, the 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. The results obtained in this study were based entirely on single leaf measurements of leaves that experienced rapid and severe responses to stress. Therefore, in the next chapter, the robustness of this approach shall be tested on a different species both at leaf and canopy scales.

Chapter 7*

ASSESSING THE PERFORMANCE AND STABILITY OF SPECTRAL AND THERMAL RESPONSES IN BEAN (*Phaseolus vulgaris 'Tendergreen'*) TREATED WITH OIL AND WATER DEFICIT AT LEAF AND CANOPY SCALES

7.1 Introduction

In chapter 6, it was found 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. Thus, there is the need to test the robustness and extendibility of this technique using different plant species and measuring both at leaf and canopy scales. The leaf is a basic and often predominant element in a plant and thus, the estimation of its biochemical contents is very meaningful in ecological studies (Shi *et al.*, 2005). Several studies have noted that the absolute and relative concentrations of pigments dictate the photosynthetic potential of a leaf and provide valuable information about the physiological status of plants (Blackburn, 1998a; Sims and Gamon, 2002; Gitelson *et al.*, 2003). On the other hand, plant canopies are structured to maximize canopy photosynthesis under a given irradiance regime (Monsi and Saeki, 1953). Essentially, the plant canopy plays an important role in the exchange of water, energy and greenhouse gases between vegetation and the atmosphere (Blackburn, 1998b). These processes are dependant on leaf biochemistry such as chlorophyll, nitrogen concentrations and leaf hydration state (Asner, 1998). Thus, information about leaf biochemistry could help predict these processes at the

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canopy scale (Sobhan, 2007). Additionally, previous investigations have noted that canopy reflectance primarily depends on foliar spectral properties (Gates *et al.*, 1965; Boochs *et al.*, 1990; Yoder and Pettigrew-Crosby, 1995; Blackburn, 1998b).

Scaling of leaf optical properties to the canopy level is not straightforward due to a number of factors. There is non-uniformity in the distribution of chemical constituents across a given leaf surface and, in turn, across various leaves within a plant canopy (Yoder and Pettigrew-Crosby, 1995). This is due to the organisation of cells and organelles as most proteins and all chlorophylls are packed into chloroplast that migrate and clump, depending on the light environment (Yoder and Pettigrew-Crosby, 1995). Furthermore, the non-uniformity can lead to differential absorbance and reflectance across a leaf surface just as non-uniform vegetation results in variations in optical properties across a landscape. There are several other factors which control canopy reflectance including the LAI, soil background, canopy structure and/or architecture. Interestingly, most of these factors can be controlled in the laboratory; yet, most laboratory studies about the use of optical reflectance in response to specific stressors are limited to the leaf scale. Currently, there is a poor understanding as to whether plant stress detected at the leaf scale can translate to the canopy. Accurate quantitative estimates of biochemical properties of vegetation canopies are important applications of remote sensing for terrestrial ecology (Gao and Goetz, 1995). In real systems, most ecological applications of remote sensing are at a large scale where data are acquired at the canopy level. For this reason, further work is needed that extends remote sensing of plant stress from leaf scale measurements to the canopy.

The previous chapter (6) demonstrated the potential of the spectral and thermal responses of leaves for early detection and discrimination between oil and water deficit stress in maize. In order to understand whether this approach can become useful in ecological studies, there is the need to ensure that the approach is generalisable across scales and species. Therefore, this study investigates the relative merits of spectral and thermal approaches for early detection and discrimination between oil and water deficit stress in bean (*Phaseolus vulgaris 'Tendergreen'*) at both leaf and canopy scales.

This study aimed to assess and compare the stability of spectral and thermal properties of plants for detecting oil and water deficit stress, irrespective of other possible factors that may influence these changes at the canopy scale. The objective was to investigate whether spectral and thermal features of plants would transpose from leaf to canopy in their response to oil and water deficit stress. The comparison was made based on the sensitivities and temporal changes of remotely-sensed responses at leaf and canopy scales.

7.2 Methods

In this experiment, four treatments comprising ten replicates were established, namely: control, oil, water deficit and combination of oil and water deficit. Leaf and canopy thermal images were acquired in a darkroom (provided with an artificial illumination (see chapter 3, section 3.5) mounted in a fixed position at nadir 70cm away from each leaf and canopy to be measured). The camera was positioned at nadir 75cm above the plant leaf and canopy. Reflectance measurements were made using an ASD FieldSpec® Pro Spectroradiometer (Boulder, CO 80301 USA; described in chapter 3, section 3.5.). Ten spectral measurements were captured per leaf and plant canopy for each of the 10 replicates per treatment.

7.3 Results

7.3.1 Physiological and biochemical responses to treatments

7.3.1.1 Visual stress symptoms

Stress symptoms were first visually observed in plants on day 6 for water deficit (alone) and the combined oil and water deficit treatments and on day 9 for oil pollution treatment (alone). Visually, the growth and development in the bean were adversely affected by all treatments. Symptoms worsened with time and included leaf chlorosis (Figure 7.0), wilting and the thinning of canopies (Figure 7.1). No visual stress symptoms were observed in control plants and they had fully matured by the end of the experiment.

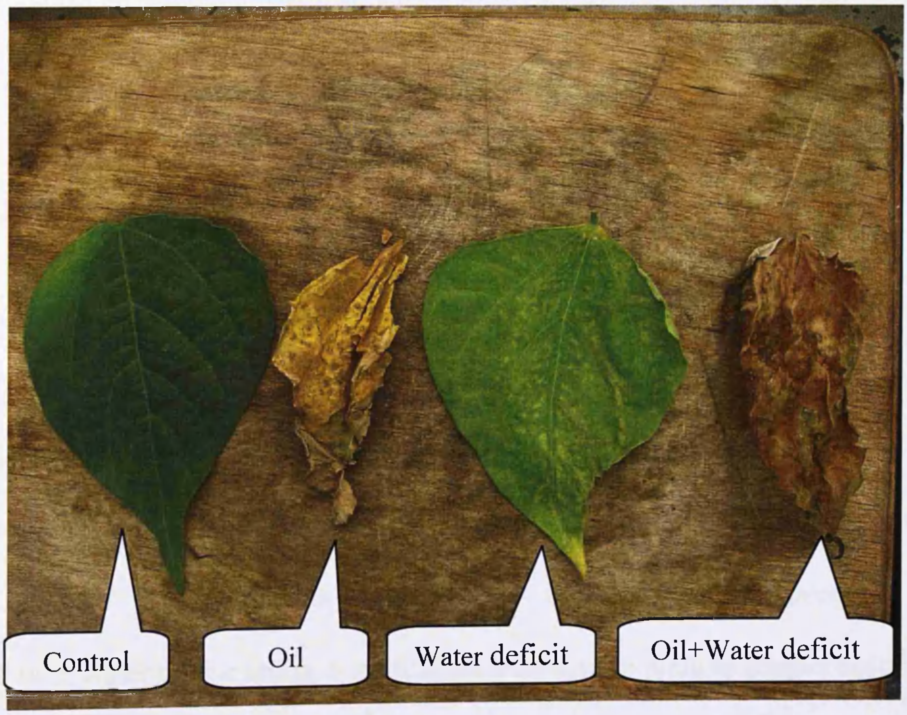

Figure 7.0 Visual stress symptoms in bean leaves caused by oil pollution, water deficit and combined oil and water deficit at the end of the experiment. No visual stress symptoms were observed in the controls.

Figure 7.1 Visual stress symptoms in bean canopies 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.

7.3.1.2 Photosynthesis

Treated plants showed a decline in photosynthetic activity as can be seen in Figure 7.2. The statistical analysis revealed that before stress symptoms were observed visually, photosynthesis showed a significant reduction (on day 4 and 6) in the plants treated with the combined oil pollution and water deficit and oil pollution alone compared to the controls, respectively (see Table 7.0). However, for plants treated with water deficit alone, a significant reduction in photosynthesis occurred on the same day as visual stress symptoms. Whenever oil pollution was involved in the treatment, there was a significantly larger reduction in photosynthesis than for water deficit treatment alone. Thus, plants treated with oil 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 9 for the plants treated with oil and combined oil and water deficit, while photosynthesis ended on day 16 for the plants treated with water deficit alone.

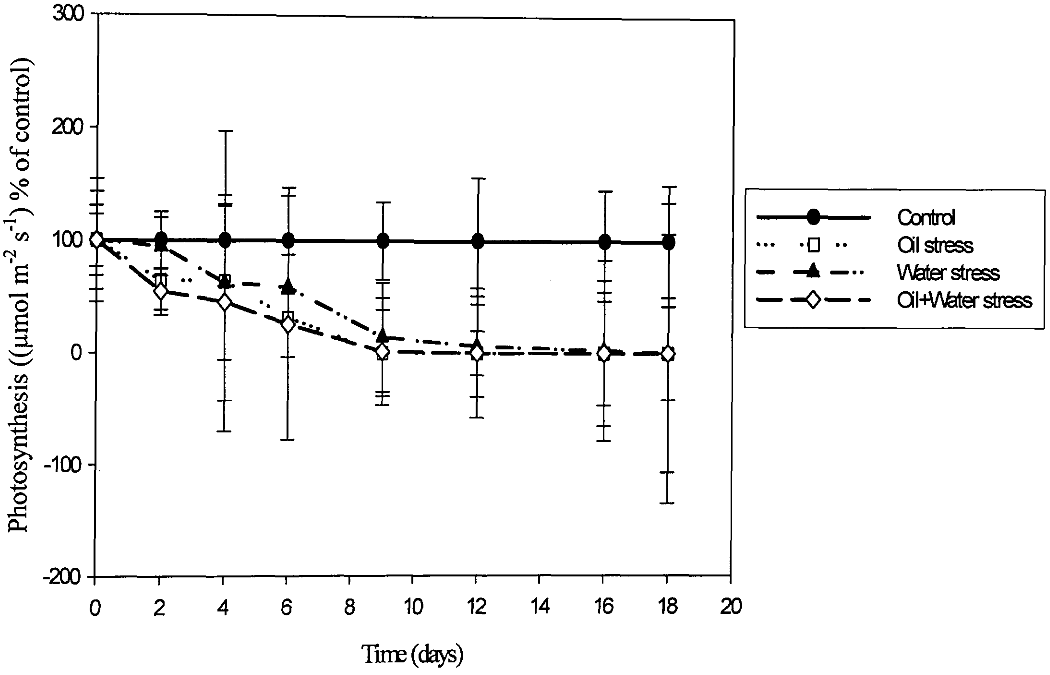

Figure 7.2 Effects of oil contamination of soil, water deficit and combined oil contamination and water deficit on photosynthetic activities of bean over the course of the experiment. Treatments are denoted by the key. Error bars = 1 x SD, n = 10.

7.3.1.3 Transpiration

As shown in Figure 7.3, the rate of transpiration for all treated plants decreased relative to controls, showing similar responses to photosynthetic activities. Before visual stress symptoms were observed, all of the treatments showed a significant reduction in transpiration, compared to the controls (see Table 7.0). All treatments showed similar responses in transpiration and there was no significant difference between treatments throughout the experiment. By the end of the experiment, there was a total reduction in transpiration rate of treated plants by approximately 90%, 88% and 84% for water deficit, oil and the combination of oil and water deficit, relative to the controls, respectively.

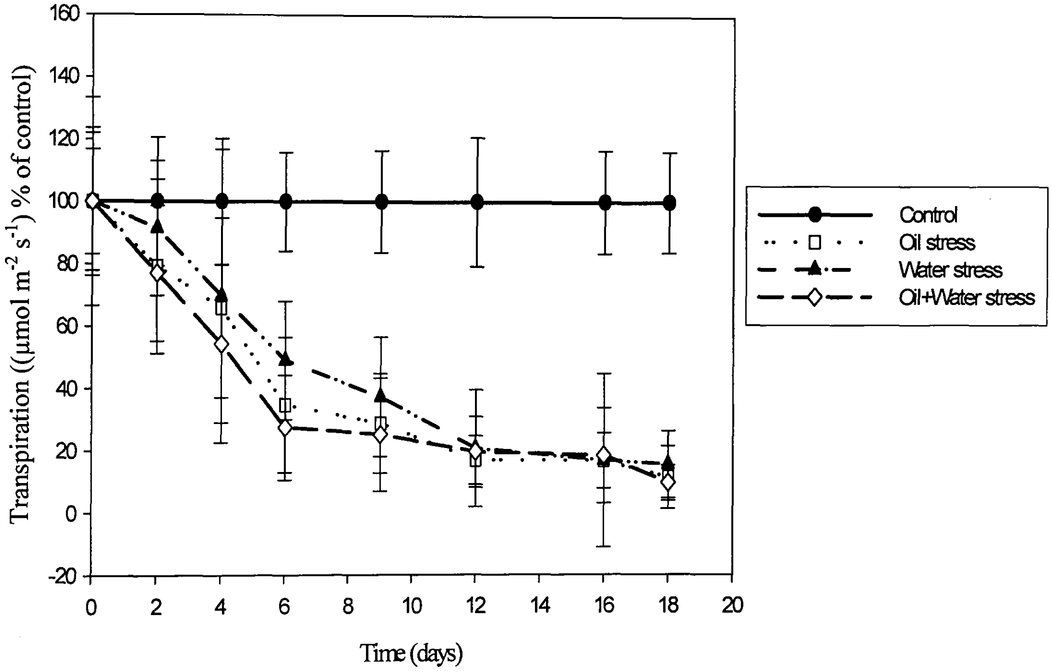

Figure 7.3 Effects of oil contamination, water deficit and the combined oil and water deficit on transpiration of bean, over time. Treatments are denoted by the key. Bars = 1 x SD, n = 10.

7.3.1.4 Stomatal conductance

There was a general decrease in stomatal conductance of treated plants as can be seen in Figure 7.4. Again, before visual stress symptoms were observed, all of the treatments showed a significant reduction in stomatal conductance, compared to the controls (see Table 7.0). All treatments showed similar responses in stomatal conductance and there was no significant difference between treatments throughout the experiment. By the end of the experiment, there was a total reduction in stomatal conductance of treated plants by approximately 98% 93% and 91%, for water deficit, oil and the combination of oil and water deficit, relative to the controls, respectively.

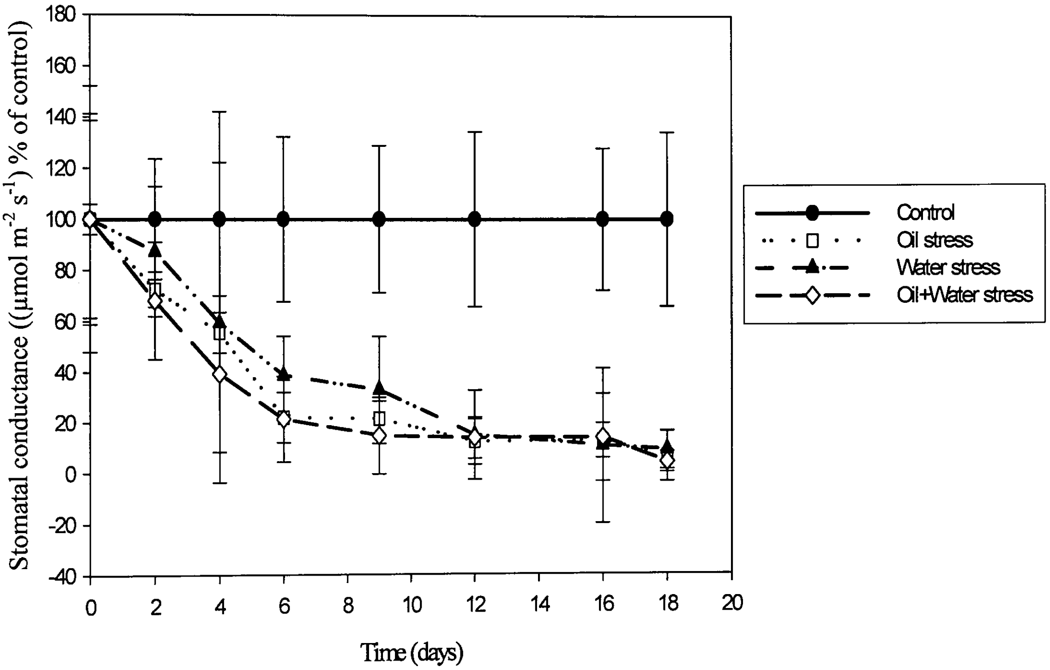

Figure 7.4 Effects of oil contamination, water deficit and the combined oil and water deficit on stomatal conductance of bean, over time. Treatments are denoted by the key. Bars = 1 x SD, n = 10.

Table 7.0 Results of ANOVA tests demonstrating when there were significant differences in the physiological and biochemical properties between the 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.

Properties	Treatments	Time (Days)							
		0	2	4	6**	9*	12	16	18
Photosynthesis (μmol m^{-2} s^{-1})	Control Oil stress				▨	▨	▨	▨	▨
	Water stress				▨	▨	▨	▨	▨
	Oil+Water stress				▨	▨	▨	▨	▨
Transpiration (μmol m^{-2} s^{-1})	Control Oil stress			▨	▨	▨	▨	▨	▨
	Water stress			▨	▨	▨	▨	▨	▨
	Oil+Water stress			▨	▨	▨	▨	▨	▨
Stomatal conductance (μmol m^{-2} s^{-1})	Control Oil stress			▨	▨	▨	▨	▨	▨
	Water stress			▨	▨	▨	▨	▨	▨
	Oil+Water stress			▨	▨	▨	▨	▨	▨
Total chlorophyll (μg cm^{-2})	Control Oil stress			▨	▨	▨	▨	▨	▨
	Water stress						▨	▨	▨
	Oil+Water stress			▨	▨	▨	▨	▨	▨
Carotenoids (μg cm^{-2})	Control Oil stress								
	Water stress								
	Oil+Water stress								
Leaf water content (g)	Control Oil stress							▨	▨
	Water stress						▨	▨	▨
	Oil+Water stress			▨			▨	▨	▨

7.3.1.5 Leaf total chlorophyll

There was a general decrease in total chlorophyll content of treated plants as can be seen in Figure 7.5. 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 4), compared to the controls (see Table 7.1). However, no significant reduction in total chlorophyll content was observed in plants treated with water deficit until the later stage of the experiment when visual stress symptoms have manifested. This implies that oil treatment had a greater impact on total chlorophyll content of plants than water deficit treatment. Thus, there was no significant difference between oil and combined oil and water deficit treatments throughout the experiment. By the end of the experiment, there was a total reduction in total chlorophyll content of treated plants by 57%, 51% and 31% for the combination of oil and water deficit, oil and water deficit alone, relative to the controls, respectively.

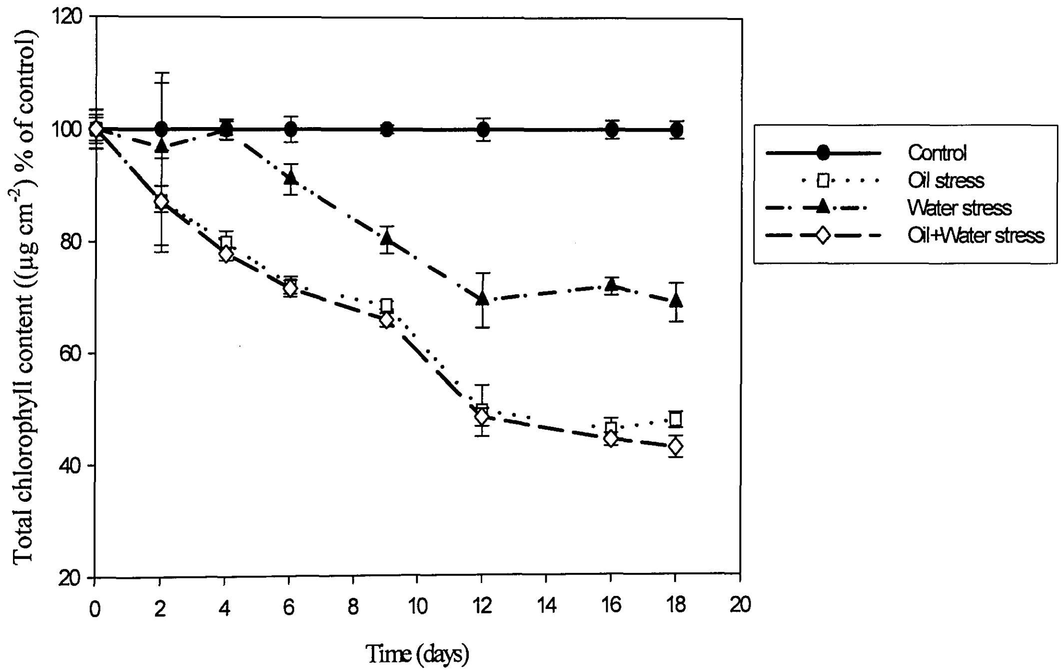

Figure 7.5 Effects of oil contamination of soil, water deficit and combination of oil and water deficit on total chlorophyll contents of bean. Treatments are denoted by the key. Bars = 1 x SD, n = 5.

7.3.1.6 Carotenoids

The carotenoid content of the treated plants did not change systematically through the experiment (Figure 7.6). The carotenoid content of all the treated plants fluctuated relative to the controls. The carotenoid content of all the treated plants was not significantly different to the controls at any time during the experiment (Table 7.0).

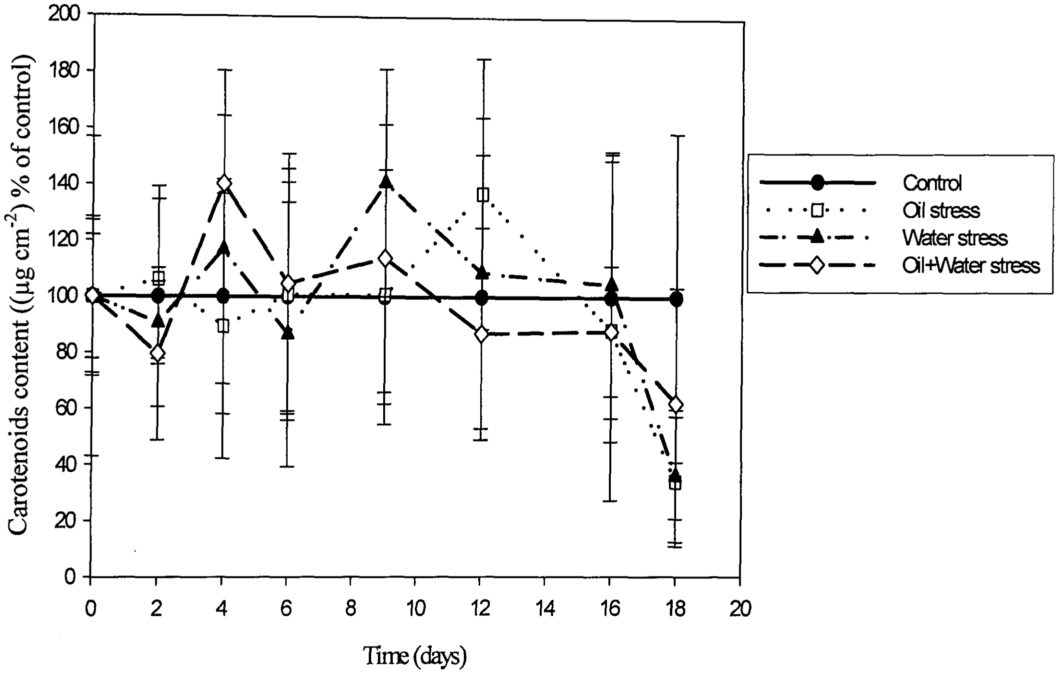

Figure 7.6 Effects of oil contamination, water deficit and the combined oil and water deficit on carotenoid content of bean. Treatments are denoted by the key. Bars = 1 x SD, n = 5.

7.3.1.7 Leaf water content

The leaf water content of all the treated plants decreased as stress progressed (Figure 7.7). However, the rate of reduction was relatively slow at the early stage of the experiment and faster at the later stages. There was a significant reduction in leaf water content of plants treated with water deficit and the combined oil and water deficit on day 12 and on day 16 for oil treatment, relative to controls (see Table 6.1). 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 71%, 50% and 49% relative to the controls for plants treated with the combined oil and water deficit, with water deficit alone and with oil alone, respectively.

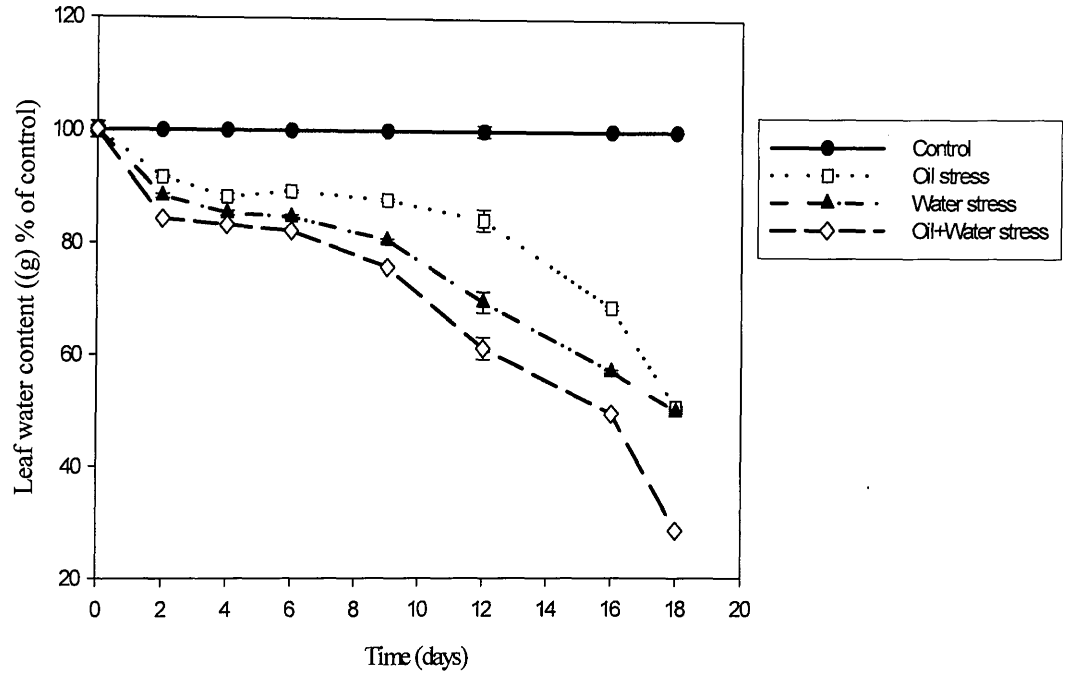

Figure 7.7 Figure 6.5 Effects of oil contamination, water deficit and the combined oil and water deficit on leaf water content of bean over time. Treatments are denoted by the key. Bars = 1 x SD, n = 5.

7.3.2 Interrelationships between physiological and biochemical variables

A strong polynomial relationship was found between total chlorophyll and photosynthetic activities of bean leaves (Figure 7.8). The leaf water content also had a strong logarithmic relationship with both transpiration and stomatal conductance (see Figures 7.9 and 7.10), respectively; however, there was no correlation between the carotenoid and total chlorophyll concentrations. The physiological rates were intercorrelated, as expected, as photosynthesis yielded a strong linear relationship with transpiration (R^2 = 0.75) and stomatal conductance (R^2 = 0.85) and there was also a strong linear relationship between transpiration and stomatal conductance (R^2 = 0.89) (data not shown).

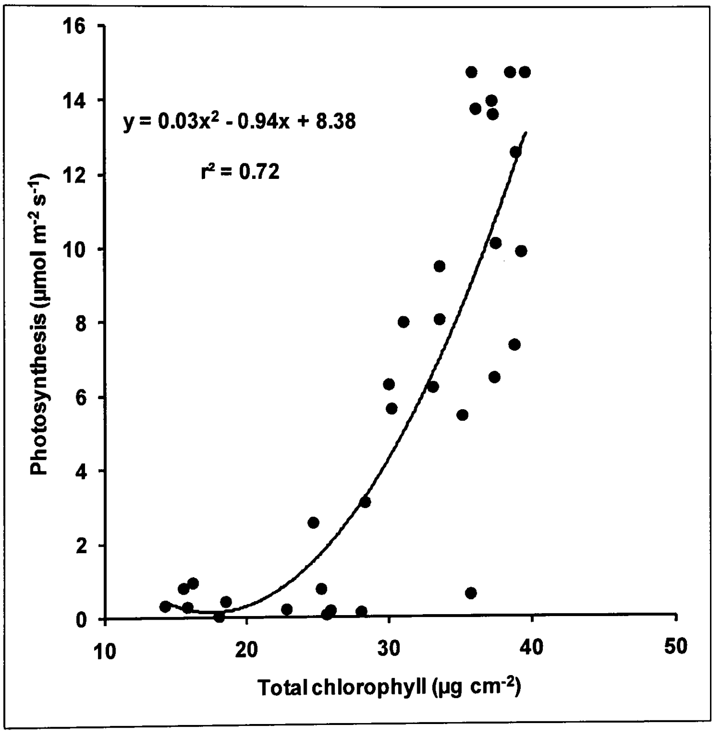

Figure 7.8 Relationships between total chlorophyll content and photosynthetic activities of bean, n = 32 (mean values per treatment, per sampling occasion).

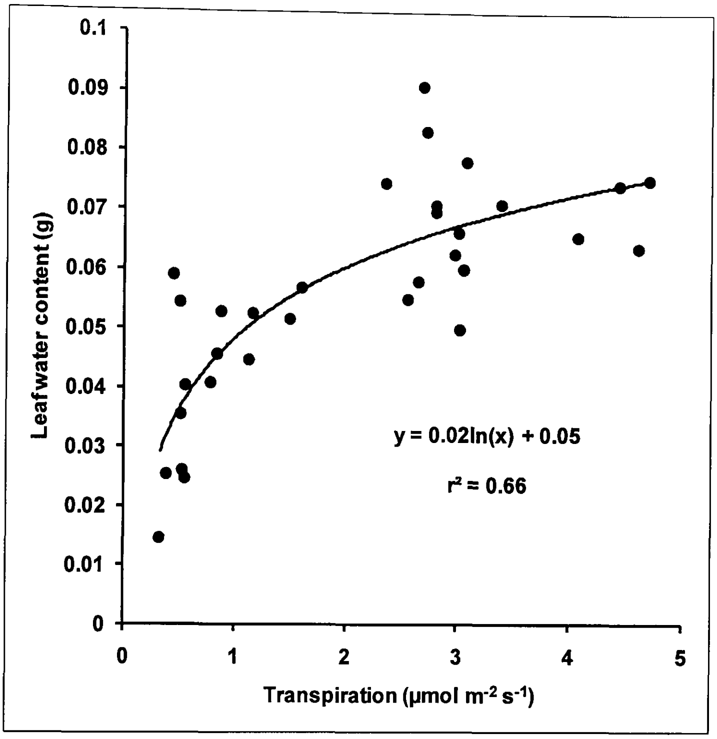

Figure 7.9 Relationships between transpiration and leaf water content of bean, n = 32.

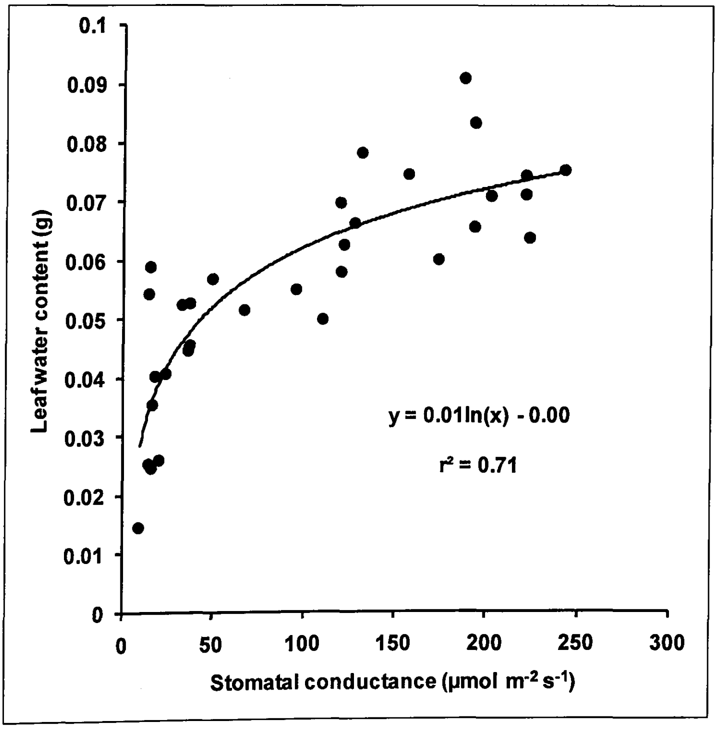

Figure 7.10 Relationships between stomatal conductance and leaf water content of bean, n = 32.

7.3.3 Responses of spectral reflectance to treatments

Looking at the mean spectra obtained at the end of the experiment (Figures 7.11 and 7.12), it can be seen that for all treatments, the leaf and canopy reflectance was higher in the visible and SWIR regions and lower in the NIR when compared with the controls, except in the SWIR where canopy reflectance of plants treated with water deficit alone was not significantly higher than the controls. Also in the visible region, the leaf and canopy reflectance spectra of plants treated with water deficit alone were not distinctly higher than the controls.

Major reflectance differences were found across the whole spectrum except in the visible region where there was no distinct spectral reflectance difference between plants treated with water deficit (alone) and the controls. T-tests were conducted to determine whether differences in spectral reflectance were statistically significant between treatments and controls. The results showed that there was a significant difference in reflectance of each of the stresses in relation to the controls across the spectrum except in the region between 601nm – 700nm where the spectral reflectance of plants treated with water deficit alone did not significantly differ from the controls ($p < 0.05$).

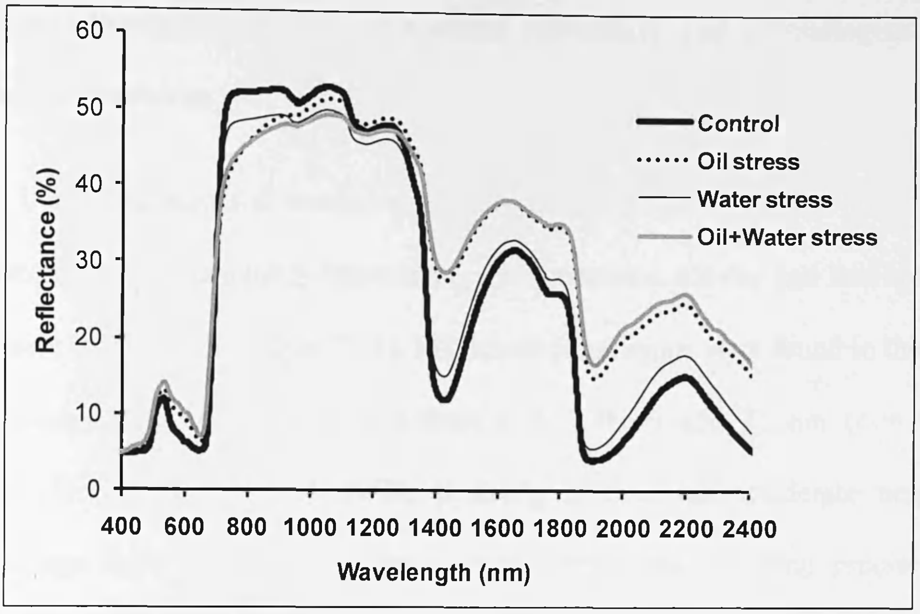

Figure 7.11 Mean reflectance spectra of treated and control bean leaves 18 days after treatment. Treatments are denoted by the key, n = 100.

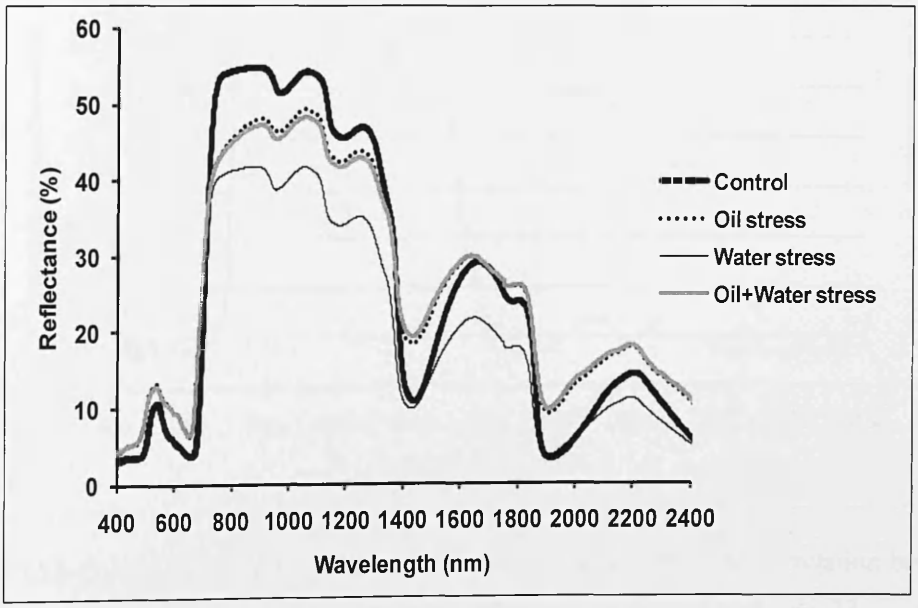

Figure 7.12 Mean reflectance spectra of treated and control bean canopies 18 days after treatment. Note: Oil stress spectral is hidden by the combination of oil and water stress spectral. Treatments are denoted by the key, n = 100.

7.3.3.1 Relationships between leaf spectral reflectance and physiological and biochemical variables

Using data across all treated and control plants, it was found that there was a moderate negative relationship between the photosynthetic activity and leaf spectral reflectance in the visible (Figure 7.13). Maximum correlations were found in the blue and red-edge regions, precisely at 469nm (r = - 0.69) and 753nm (r = 0.79) respectively. In the NIR and SWIR, a strong positive and moderate negative relationships were observed, with the highest correlations occurring precisely at 858nm (r = 0.83) and 2106nm (r = - 0.68), respectively.

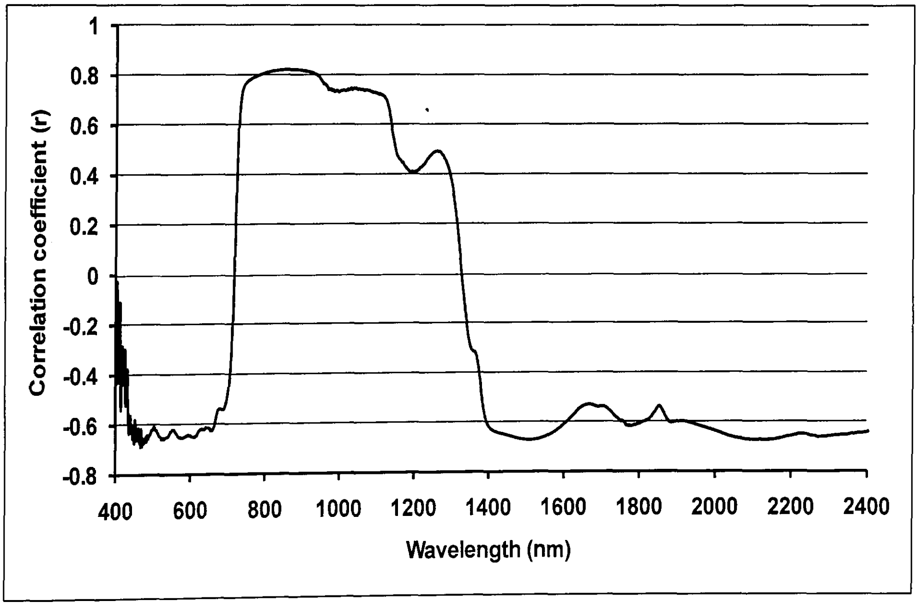

Figure 7.13 Correlogram showing the variation with wavelength in the correlation between the photosynthetic activity of bean and spectral reflectance at the leaf scale, n = 32.

The relationships between transpiration and leaf reflectance were similar to those for photosynthesis across the spectrum. There was a moderate negative relationship between the transpiration rate and leaf spectral reflectance in the visible (Figure 7.14). Maximum correlations were found in the blue and red-edge regions, precisely at 473nm (r = - 0.64) and 745nm (r = 0.72), respectively. In the NIR and SWIR, a strong positive and moderate negative relationships were observed, with the highest correlations occurring precisely at 1041nm (r = 0.77) and 1510nm or 2138nm (r = - 0.68), respectively.

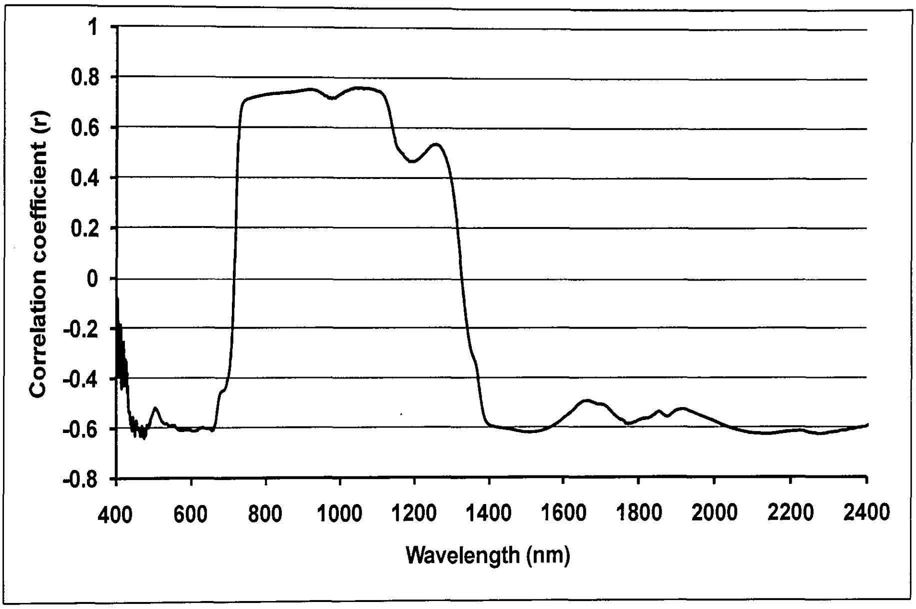

Figure 7.14 Correlogram showing the variation with wavelength in the correlation between the transpiration rate of bean and spectral reflectance at the leaf scale, n = 32.

The relationship between stomatal conductance and leaf reflectance were similar to those for photosynthesis and transpiration across the spectrum. There was a moderate negative relationship between the stomatal conductance and leaf spectral reflectance in the visible (Figure 7.15). Maximum correlations were found in the blue and red-edge regions, precisely at 465nm (r = - 0.61) and 755nm (r = 0.72), respectively. In the NIR and SWIR, strong positive and moderate negative relationships were observed, with the highest correlations occurring precisely at 890nm (r = 0.75) and 2271nm (r = - 0.61), respectively.

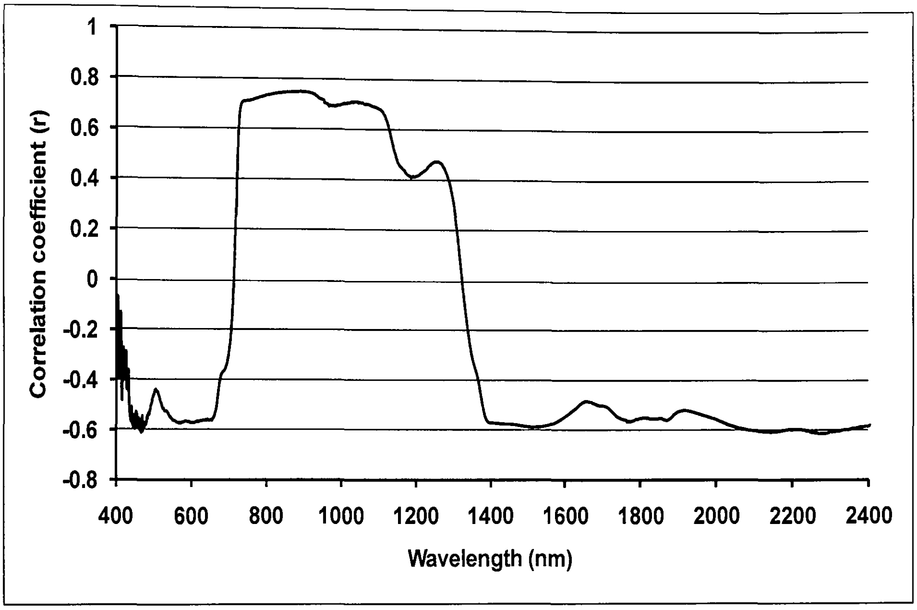

Figure 7.15 Correlogram showing the variation with wavelength in the correlation between the stomatal conductance of bean and spectral reflectance at the leaf scale, n = 32.

As can be seen in Figure 7.16, there was a strong negative relationship between the total chlorophyll content and leaf reflectance in the visible spectrum. Maximum correlations were found in the green and red regions, precisely at 576nm (r = - 0.83) and 606nm (r = - 0.83), respectively. In the NIR and SWIR, strong positive and moderate negative relationships were observed, with the highest correlations occurring precisely at 778nm (r = 0.83) and 1496nm (r = - 0.80), respectively.

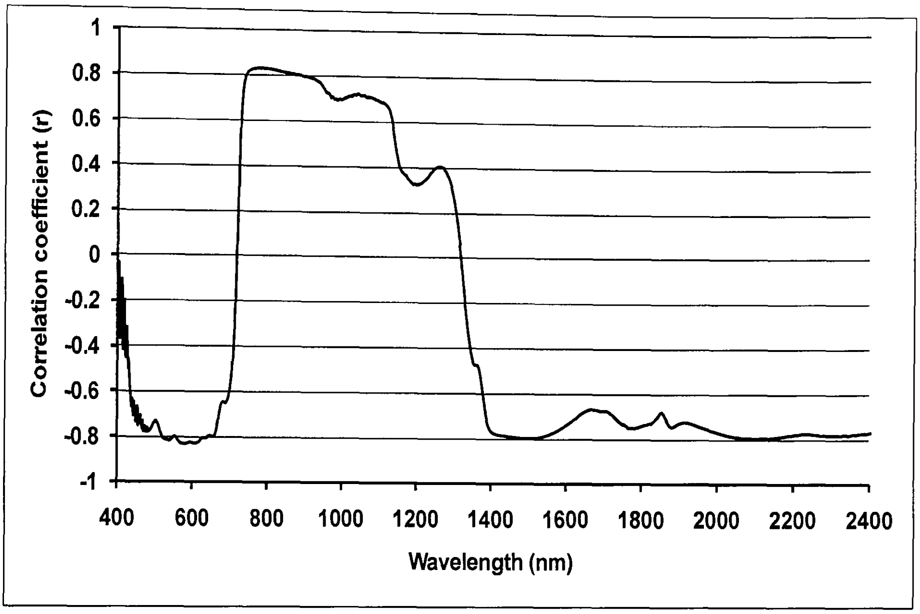

Figure 7.16 Correlogram showing the variation with wavelength in the correlation between the leaf chlorophyll content of bean and spectral reflectance at the leaf scale, n = 32.

Carotenoids were largely uncorrelated with leaf reflectance across wavelengths, though there were some weak relationships in certain regions (Figure 7.17). The best correlations were found in the visible region between 488nm and 520nm and NIR (between 746nm and 1336nm) with the waveband 513nm having the highest correlation (r = 0.31).

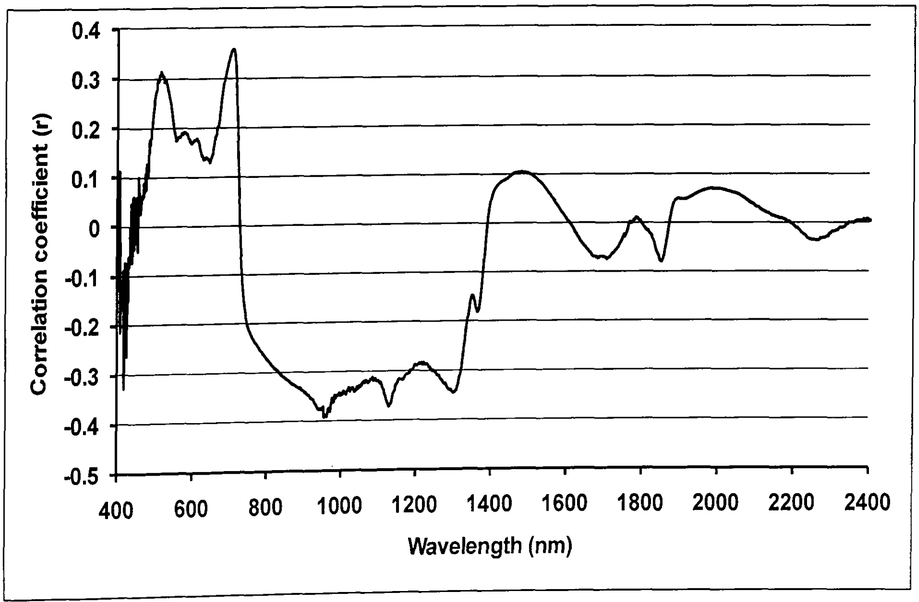

Figure 7.17 Correlogram showing the variation with wavelength in the correlation between the leaf carotenoid content of bean and spectral reflectance at the leaf scale, n = 32.

For the leaf water content, a strong negative relationship was found between the leaf water content and leaf spectral reflectance in the visible region (Figure 7.18). Maximum correlations were found in the visible region (between 432nm and 700nm) precisely at 481nm (r = - 0.70). In the NIR, the leaf water content and spectral reflectance correlated best at 865nm (r = 0.81). A maximum correlation was found in the SWIR, precisely at 1498nm or 2098nm (r = - 0.67).

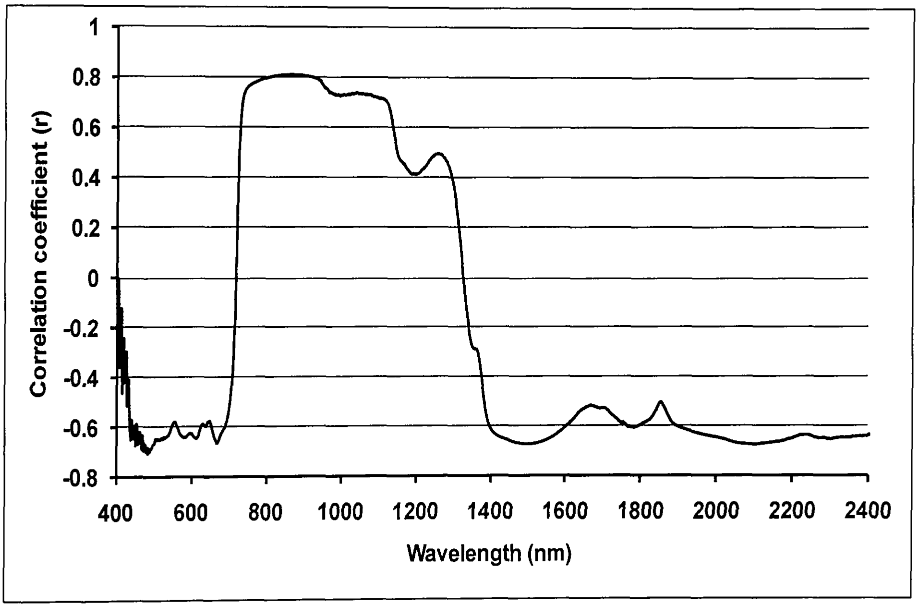

Figure 7.18 Correlogram showing the variation with wavelength in the correlation between the leaf water content of bean and spectral reflectance at the leaf scale, n = 32.

7.3.3.2 Relationships between spectral indices and biochemical variables

Table 7.1 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 are indicated in bold.

Table 7.1 Summary of the correlation coefficients (r) between the leaf spectral reflectance indices and measured physiological/biochemical parameters.

Biochemicals estimated	Spectral indices	r	References
Total chlorophyll (μg cm^{-2})	$(R_{755}-R_{716})/(R_{755}+R_{716})$	0.68	From chapter 4
	R_{673}/R_{545}	-0.36	From chapter 5
	$(R_{1330}-R_{538})/(R_{1330}+R_{538})$	0.82	From chapter 6
	R_{550}/R_{850}	-0.85	Schepers *et al.*, (1996)
	$(R_{790}-R_{720})/(R_{790}+R_{720})$	0.60	Barnes *et al.*, (2000)
	$(R_{750}-R_{445})/(_{705}+R_{445})$	0.79	Sims and Gamon (2003)
	$(R_{750}-R_{445})/(R_{705}-R_{445})$	0.72	Sims and Gamon (2002)
	$(R_{750}-R_{720})/(R_{700}-R_{670})$	0.72	Le Maire *et al.* (2004)
	R_{606}	-0.83	New
	R_{716}/R_{606}	-0.84	New
	R_{1316}/R_{606}	-0.89	New
	$(R_{1316}-R_{606})/(R_{1316}+R_{606})$	0.86	New
	R_{800}/R_{606}	**-0.90**	New
	R_{576}	-0.83	New
	R_{716}/R_{576}	0.85	New
	$(R_{1316}-R_{576})/(R_{1316}+R_{576})$	0.86	New
	R_{800}/R_{576}	0.87	New
Carotenoids (μg cm^{-2})	$(R_{736}-R_{430})/(R_{736}+R_{430})$	-0.06	From chapter 6
	R_{800}/R_{470}	-0.17	Blackburn (1998)
	$(R_{800}-R_{470})/(R_{800}+R_{470})$	-0.16	Blackburn (1998)
	R_{415}/R_{685}	-0.36	Read *et al.*, (2002)
	R_{520}	0.30	New
	R_{726}/R_{520}	-0.31	New
	$(R_{726}-R_{520})/(R_{726}+R_{520})$	-0.26	New
	R_{800}/R_{520}	**-0.36**	New
Leaf water content (g)	R_{900}	0.80	From chapter 6
	$(R_{858}-R_{1240})/(R_{858}+R_{1240})$	0.67	Gao, (1996); Zarco-Tejada *et al.*, (2003)
	$(R_{858}-R_{1640})/(R_{858}+R_{1640})$	0.75	Fensholt and Sandholt,

		(2003)
R_{865}	**0.81**	New
R_{2098}	-0.67	New
R_{1498}	-0.67	New
R_{1323}/R_{865}	-0.67	New
R_{1323}/R_{2098}	0.74	New
R_{1323}/R_{1498}	0.76	New

Correlations are significant at $p < 0.05$.

As can be seen in Figure 7.19, there was a strong positive curvilinear relationship between simple ratio R_{800}-R_{606} and total chlorophyll. While there was a poor relationship between simple ratio R_{800}/R_{520} and carotenoids (Figure 7.20), the individual narrow waveband R_{865} had a moderate relationship with leaf water content (Figure 7.21).

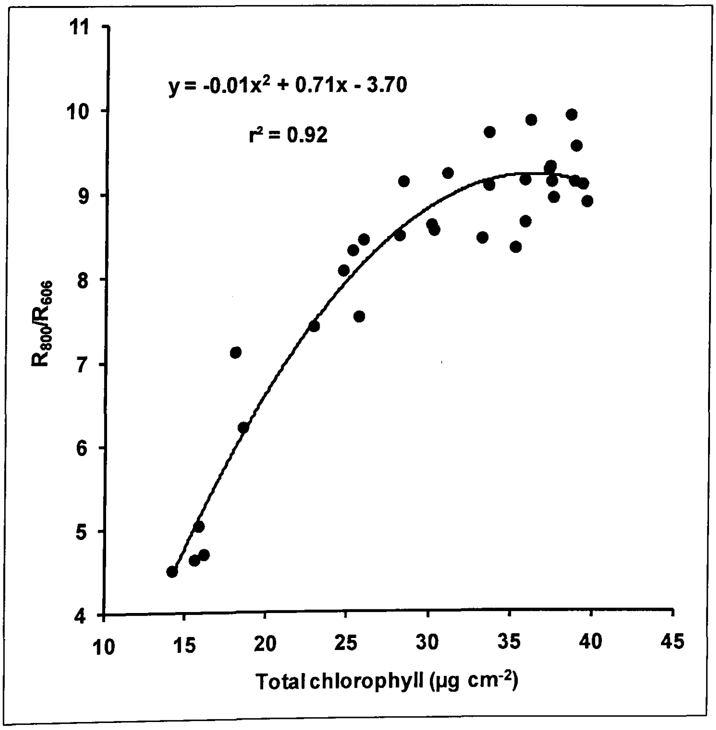

Figure 7.19 Relationships between leaf chlorophyll index R_{800}/R_{606} and total chlorophyll content of bean at leaf scale, n = 32.

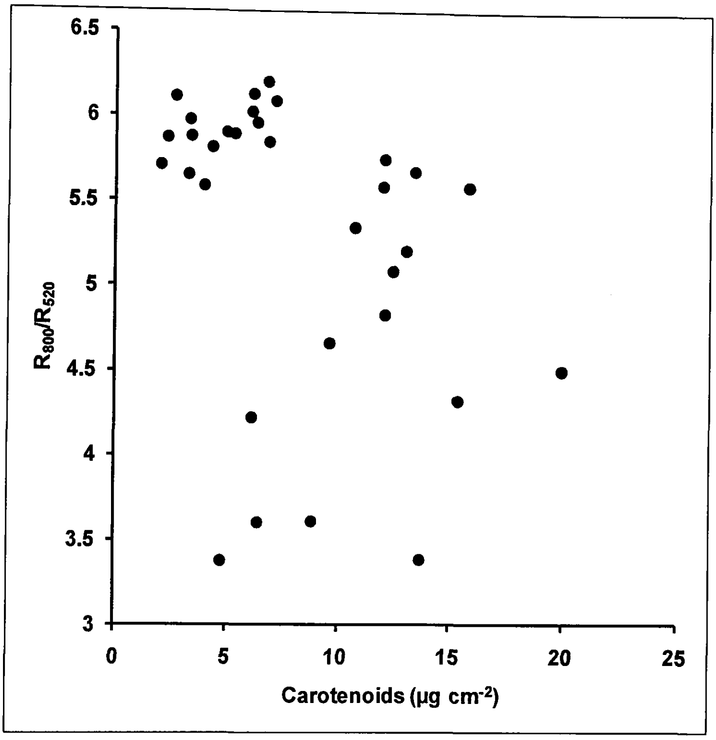

Figure 7.20 Relationships between leaf carotenoids index R_{800}/R_{520} and carotenoid content of bean at leaf scale, n = 32.

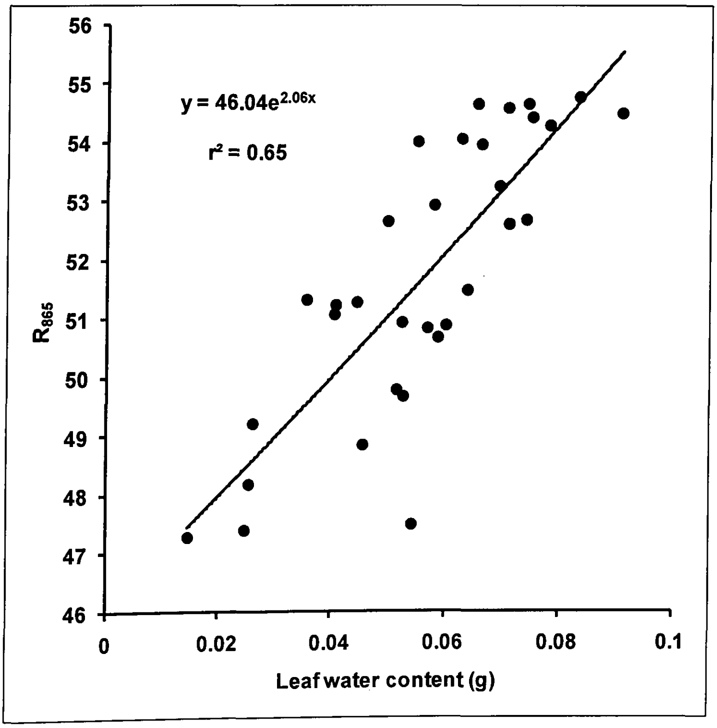

Figure 7.21 Relationships between leaf water content index R_{865} and water content of bean at leaf scale, n = 32.

7.3.3.3 Temporal response of optimal spectral indices

Having identified, in the previous section, the spectral indices which had the highest correlations with biochemical concentrations at the leaf scale, the temporal responses of those spectra indices to different treatments were examined at both leaf and canopy scales. The temporal changes in the optimal chlorophyll spectral index R_{800}/R_{606} at leaf and canopy scales are shown in Figures 7.22 and 7.23 respectively. As can be seen in Figure 7.22, at the leaf scale the index R_{800}/R_{606} decreased in treated plants as stress progressed. Before visual stress symptoms were observed, the index significantly decreased for plants treated with combined oil and water deficit (on day 2), compared to the controls (see Table 7.2). However, while a significant reduction in the index was observed in plants treated with oil pollution alone on the same day as visual stress symptoms (on day 9), reduction of the index in plants treated with water deficit alone was not significant throughout the experiment, compared to the controls. This implies that significant reduction in R_{800}/R_{606} was consistently observed only whenever oil was involved in the treatment. By the end of the experiment, there was a total reduction of the index of treated plants by 47%, 40% and 12% for the combined oil and water deficit, oil pollution and water deficit, respectively.

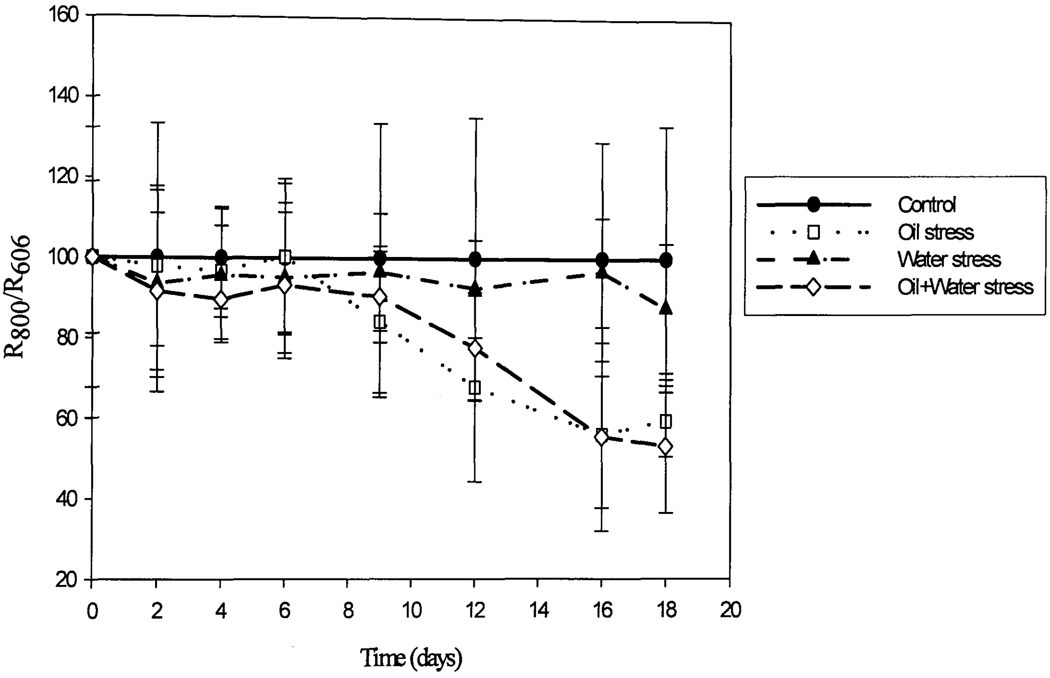

Figure 7.22 Change in simple reflectance ratio R_{800}/R_{606} of bean leaves. Treatments are denoted by the key. Bars = 1 x SD, n = 100.

Table 7.2 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**	9*	12	16	18
Leaf_R_{800}/R_{606}	Control Oil stress					▨	▨	▨	▨
	Water stress								
	Oil+Water stress		▨	▨	▨	▨	▨	▨	▨
Canopy_R_{800}/R_{606}	Control Oil stress							▨	▨
	Water stress							▨	▨
	Oil+Water stress							▨	▨
Leaf_R_{800}/R_{520}	Control Oil stress					▨	▨	▨	▨
	Water stress							▨	▨
	Oil+Water stress					▨	▨	▨	▨
Canopy_R_{800}/R_{520}	Control Oil stress				▨	▨	▨	▨	▨
	Water stress				▨	▨	▨	▨	▨
	Oil+Water stress				▨	▨	▨	▨	▨
Leaf_R_{865}	Control Oil stress					▨	▨	▨	▨
	Water stress								▨
	Oil+Water stress					▨	▨	▨	▨
Canopy_R_{865}	Control Oil stress				▨	▨	▨	▨	▨
	Water stress			▨	▨	▨	▨	▨	▨
	Oil+Water stress				▨	▨	▨	▨	▨
Leaf absolute temperature (^{O}C)	Control Oil stress		▨	▨	▨	▨	▨	▨	▨
	Water stress			▨	▨	▨	▨	▨	▨
	Oil+Water stress		▨	▨	▨	▨	▨	▨	▨
Canopy absolute temperature (^{O}C)	Control Oil stress		▨	▨	▨	▨	▨	▨	▨
	Water stress		▨	▨	▨	▨	▨	▨	▨
	Oil+Water stress		▨	▨	▨	▨	▨	▨	▨
Leaf I_G	Control Oil stress				▨	▨	▨	▨	▨
	Water stress				▨	▨	▨	▨	▨
	Oil+Water stress				▨	▨	▨	▨	▨
Canopy I_G	Control Oil stress		▨	▨	▨	▨	▨	▨	▨
	Water stress		▨	▨	▨	▨	▨	▨	▨
	Oil+Water stress		▨	▨	▨	▨	▨	▨	▨

At the canopy scale, it was observed that change in the chlorophyll spectral index R_{800}/R_{606} differed from that at the leaf scale. As can be seen in Figure 7.23, there was inconsistency in the change of the ratio for all the treated plants at the early stage of the experiment. However, at a later stage, while the ratio increased in plants treated with water deficit alone, the oil and combined oil and water deficit treatments decreased rapidly, relative to the control. The response is reflected in Table 7.3, where the statistical analysis showed a significant reduction in the ratio for plants treated with oil and the combined oil and water deficit, relative to the control. The ratio increased significantly in plants treated with water deficit alone, relative to the control. By the end of the experiment, there was a total reduction of the index of treated plants by 30% for oil and the combined oil and total increment for water deficit by 4%.

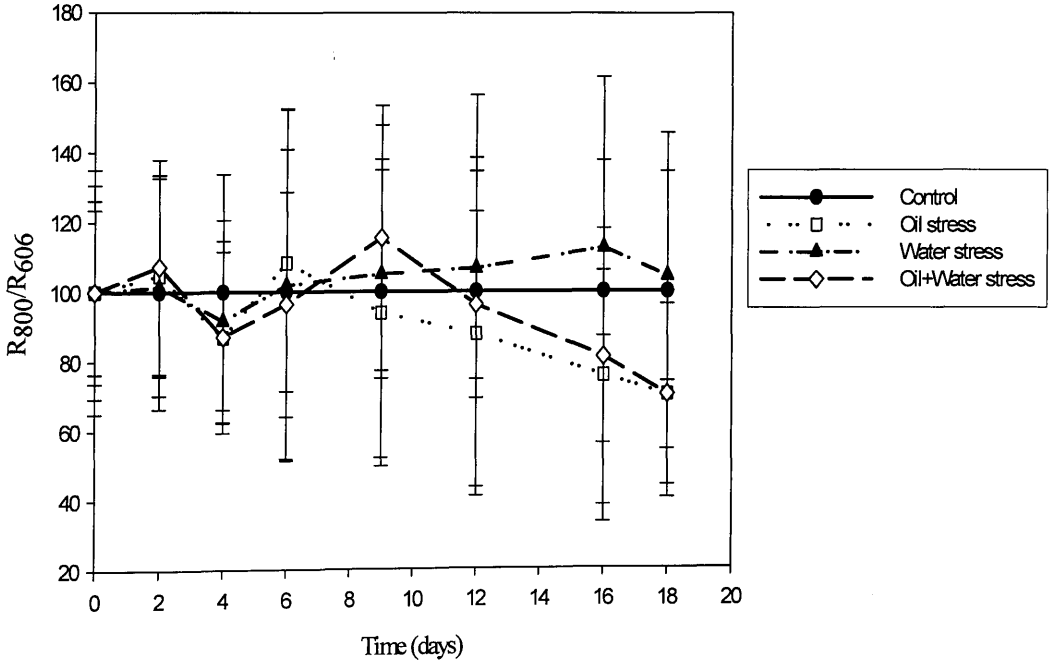

Figure 7.23 Change in simple reflectance ratio R_{800}/R_{606} of bean canopy. Treatments are denoted by the key. Bars = 1 x SD, n = 100.

At the leaf scale, the optimal carotenoid spectral index R_{800}/R_{520} decreased in treated plants as stress progressed (Figure 7.24). Before visual stress symptoms were observed, the index had significantly decreased in plants treated with oil and combined oil and water deficit (on day 4 and 2, respectively), compared to the controls (see Table 7.3). However, the reduction was not consistent in these treated plants until day 9 and remained so until the end of the experiment. The ratio did not change significantly from the control for plants treated with water deficit alone until 16 days after treatment. By the end of the experiment, there was a total reduction of the index of treated plants by 25%, 20% and 6% for the combined oil and water deficit, oil pollution and water deficit, respectively.

On the contrary, the ratio of all the treated plants increased relative to the control at the canopy scale, except on day 4 where the ratio decreased (Figure 7.25). Before visual stress symptoms were observed, the index had significantly increased in plants treated with combined oil and water deficit and oil pollution alone (on days 2 and 6, respectively), compared to the controls (see Table 7.3). The ratio was not consistent in plants treated with combined oil and water deficit until day 6 when the ratio increased in all the treated plants. By the end of the experiment, there was a total increment of the index of treated plants by 26%, 11% and 7% for water deficit alone, the combined oil and water deficit, and oil pollution alone, respectively.

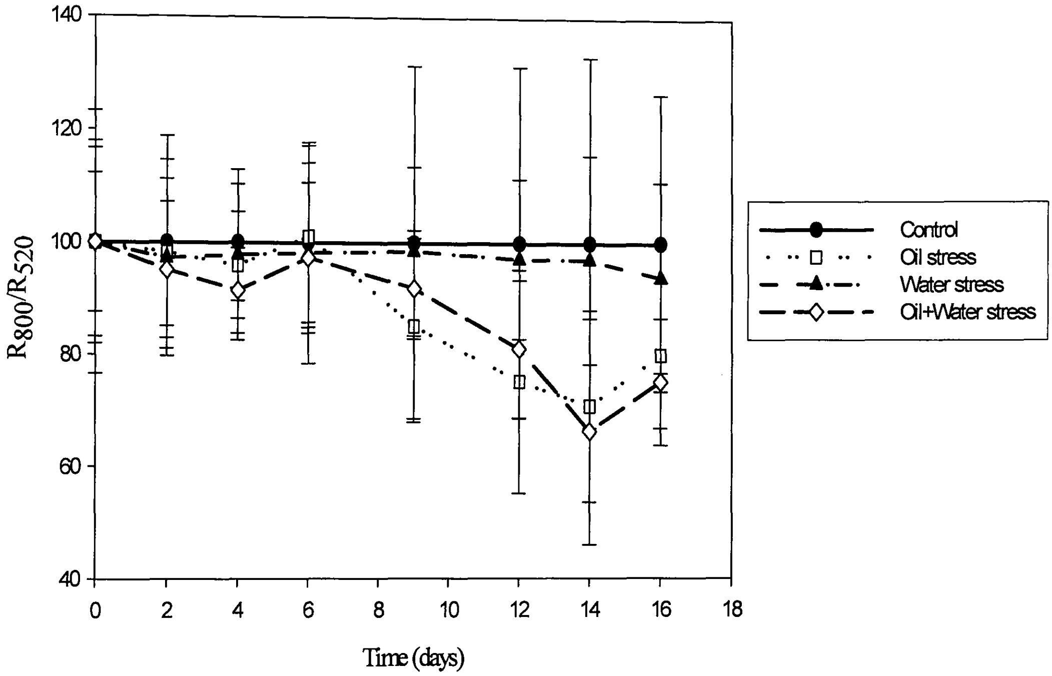

Figure 7.24 Change in simple ratio R_{800}/R_{520} of bean leaves. Treatments are denoted by the key. Bars = 1 x SD, n = 100.

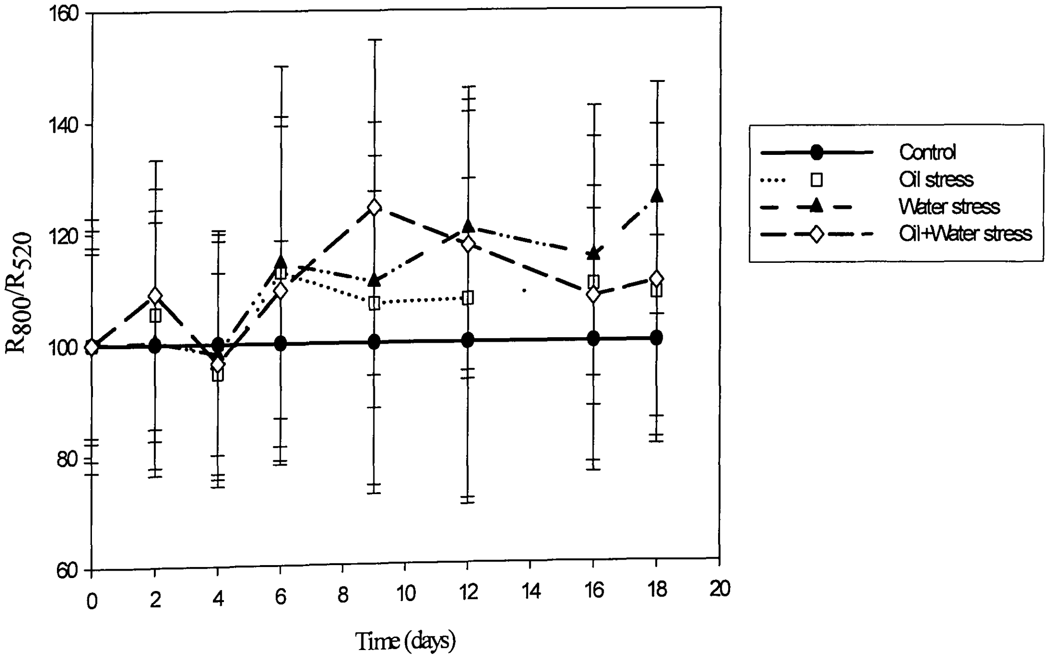

Figure 7.25 Change in simple reflectance ratio R_{800}/R_{520} of bean canopies. Treatments are denoted by the key. Bars = 1 x SD, n = 100.

As can be seen in Figure 7.26, at the leaf scale the optimal leaf water content spectral index R_{865} reduced in treated plants as stress progressed, except on day 2 when the index increased in all the treated plants. Before visual stress symptoms were observed, the index significantly reduced in plants treated with water deficit alone (on day 2), compared to the controls (see Table 7.3), although the reduction was not consistent throughout the experiment. Statistical analysis showed that there was a significant reduction in the index for plants treated with oil and combined oil and water deficit (on day 9) which remained consistent until the end of the experiment. The rate of reduction was similar in all the treated plants thus, no significant difference was found between the treatments. By the end of the experiment, there was a total reduction of the index of treated plants by 10%, 8% and 6% for the combined oil and water deficit, oil pollution alone and water deficit alone, respectively.

Similarly, at the canopy scale, the index decreased in all the treated plants as can be seen in Figure 7.27. Statistical analysis showed that before visual stress symptoms were observed, the index had reduced (on day 4) in plants treated with water deficit alone (Table 7.3). A significant reduction of the index was observed in plants treated with oil and the combined oil and water deficit only on the same day as visual stress symptoms. By the end of the experiment, there was a total reduction of the index of treated plants by 27%, 18% and 17% for water deficit, the combined oil and water deficit alone and oil pollution alone, respectively.

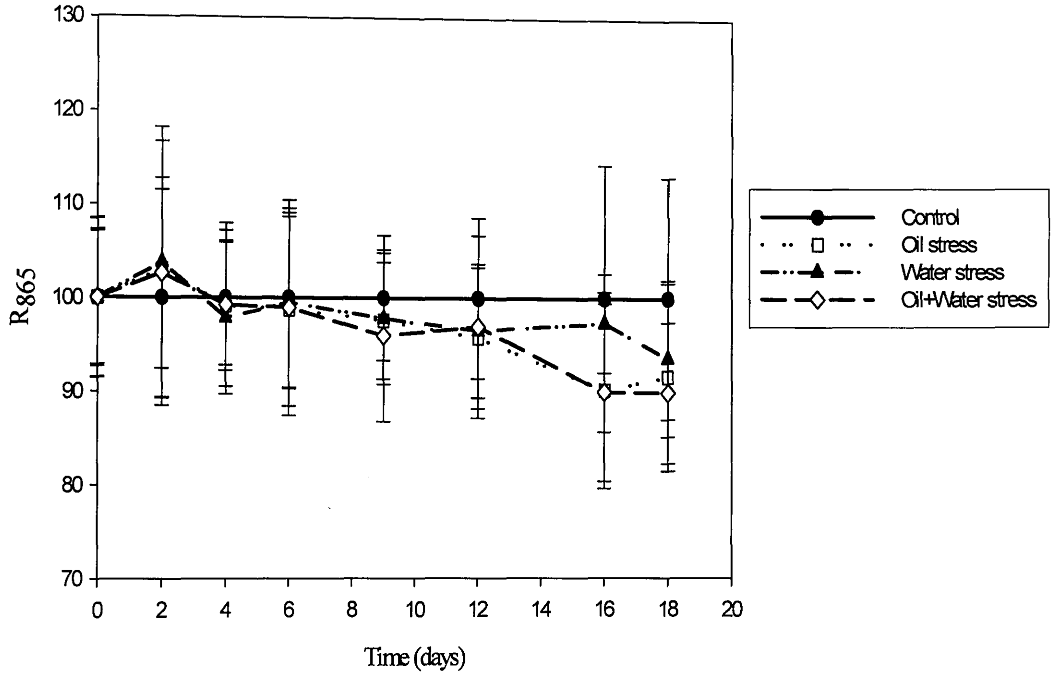

Figure 7.26 Change in mean reflectance of individual narrow waveband R_{865} of bean leaves. Treatments are denoted by the key. Bars = 1 x SD, n = 100.

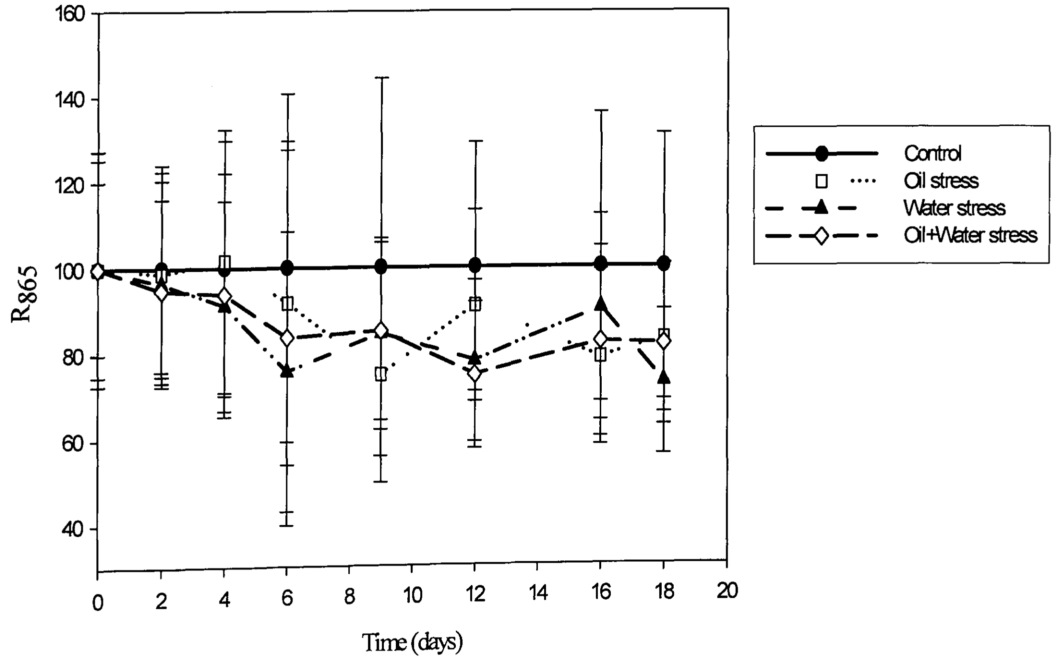

Figure 7.27 Change in mean reflectance of individual narrow waveband R_{865} of bean canopy. Treatments are denoted by the key. Bars = 1 x SD, n = 100.

7.3.4 Thermography

As shown in Figure 7.28, the absolute temperatures of treated plants increased relative to the controls, at the leaf scale. The statistical analysis revealed that before visual stress symptoms were observed, leaf absolute temperatures showed a significant increase (on day 2) in the plants treated with oil and combined oil and water deficit, compared to the controls (see Table 7.3). For plants treated with water deficit alone, a significant rise in leaf absolute temperature occurred on day 4, before visual stress symptoms were observed. Over the course of the experiment there were no consistent differences between the plants treated with oil, water deficit or their combination. The response of canopy temperature was similar to the absolute leaf temperature as can be seen from Figure 7.29. The statistical analysis also yielded the same results at both scales, except for plants treated with water deficit alone which had a significant rise in canopy temperature on day 2, before visual stress symptoms were observed (Table 7.3). Like the leaf absolute temperature, there were no consistent differences between the plants canopies treated with oil, water deficit or their combination over the course of the experiment.

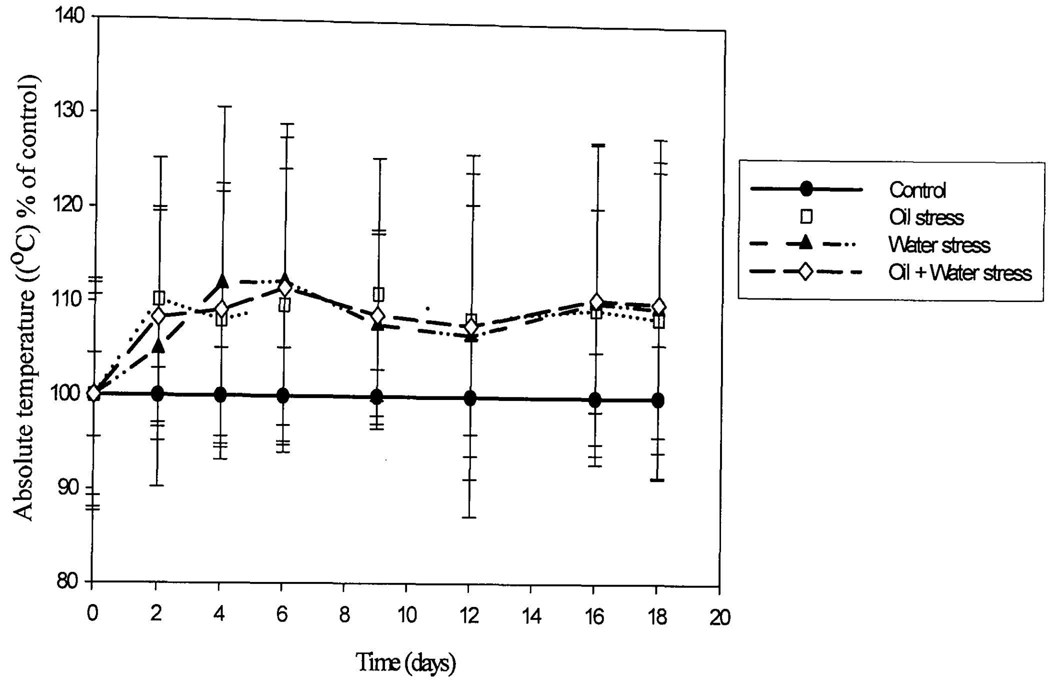

Figure 7.28 Effects of oil contamination of soil, water deficit and the combined oil and water deficit on the absolute temperature of bean leaves over time. Treatments are denoted by the key. Bars = 1 x SE, n = 10.

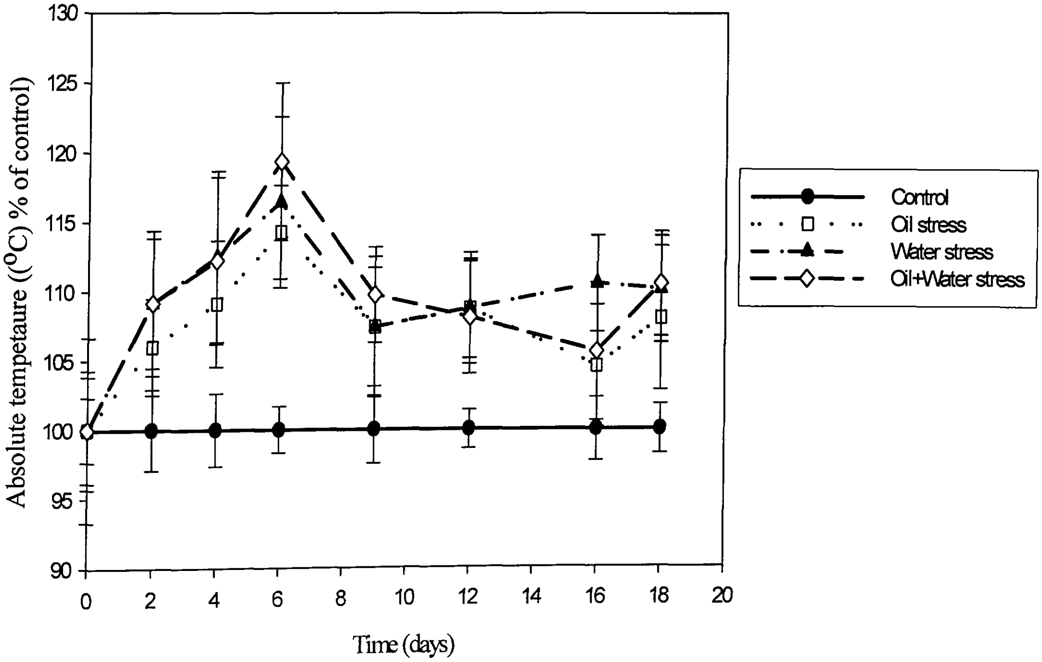

Figure 7.29 Effects of oil contamination of soil, water deficit and combination of oil and water deficit on the absolute temperature of bean canopy over time. Treatments are denoted by the key. Bars = 1 x SE, n = 10.

The thermal index (I_G) of the treated plants was significantly reduced by treatments when compared with the control plants, at the leaf scale (Figure 7.30). The reduction was significant 3 days before the visual stress symptoms were observed in plants treated with oil alone (Table 7.3). For plants treated with water deficit (alone) and the combined oil and water deficit, a significant reduction of I_G of the leaves was observed on the same day as visual stress symptoms. Similar to leaf absolute temperature of treated plants, there were no consistent differences in leaf I_G between the plants treated with oil, water deficit or their combination, over the course of the experiment. The responses of the I_G of plant canopies were similar to the I_G of leaves (Figure 7.31). From the statistics, the major difference found was that the reduction was significant at an earlier stage for the canopies (day 2) after treatment (Table 7.3). A strong curvilinear relationship was found between the I_G and stomatal conductance at the leaf scale, as can be seen in Figure 7.32.

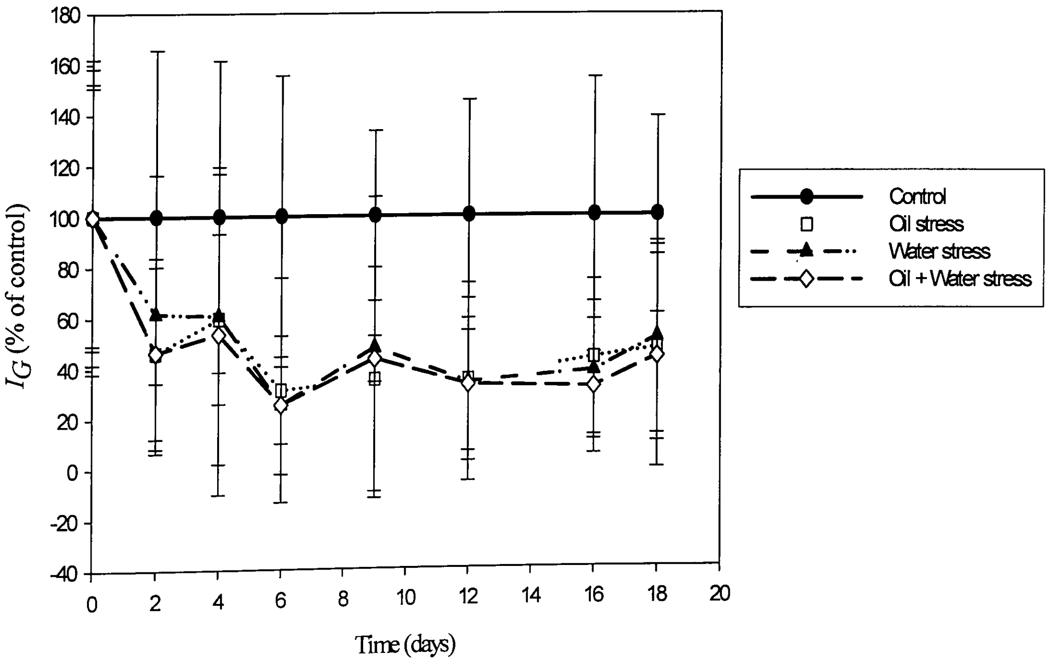

Figure 7.30 Effects of oil contamination of soil, water deficit and combination of oil and water deficit on the thermal index (I_G) of bean leaves over time. Treatments are denoted by the key. Bars = 1 x SE, n = 10.

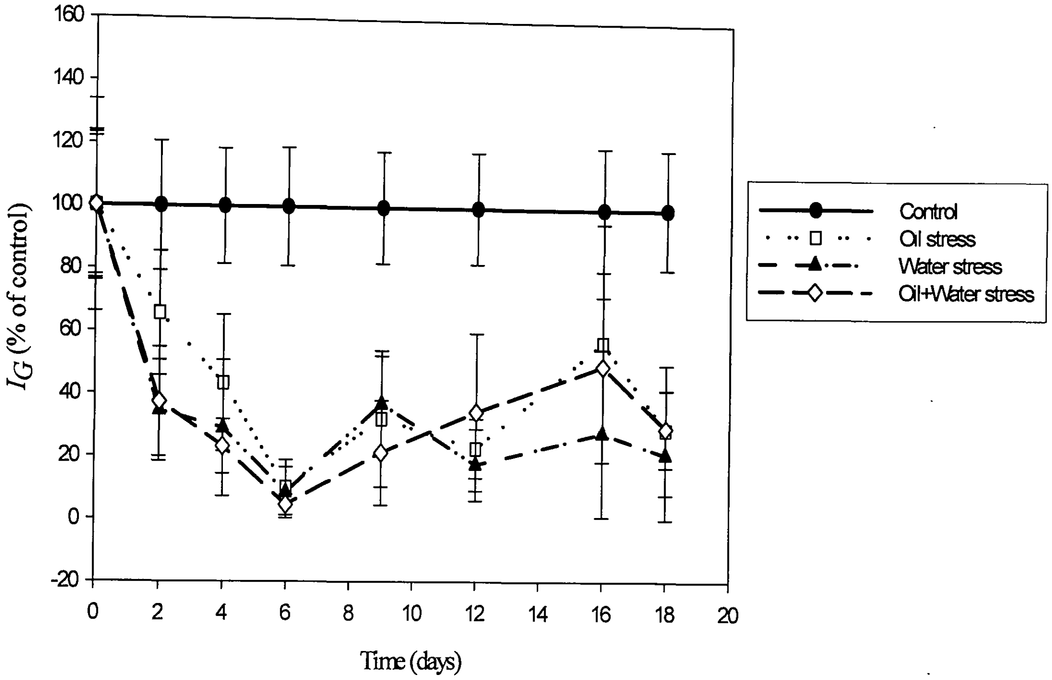

Figure 7.31 Effects of oil contamination of soil, water deficit and combination of oil and water deficit on the thermal index (I_G) of bean canopy over time. Treatments are denoted by the key. Bars = 1 x SE, n = 10.

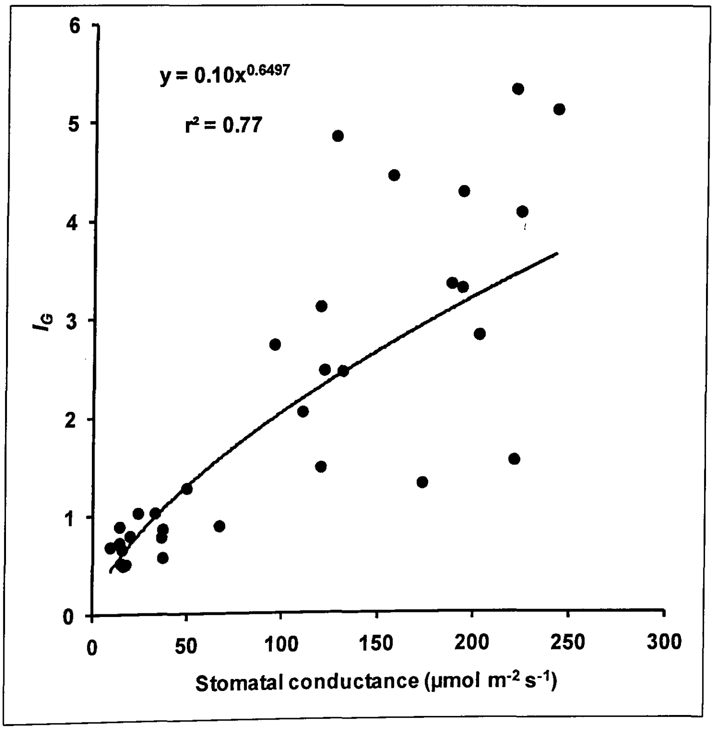

Figure 7.32 Relationships between the stomatal conductance and thermal index (I_G) at the leaf scale, n = 32.

7.4 Discussion

Similar to findings in previous chapters, treatments adversely affected bean growth and development. While treatments caused leaf chlorosis and wilting in bean plants, no visual stress symptoms were observed in the controls. Symptoms in all treated plants started mildly by affecting only a few leaves and then gradually spread over all the leaves. As reported in previous chapters, a wide range of plant stresses have been found to cause various visible stress symptoms (Rosso *et al.*, 2005; Smith *et al.*, 2005).

The photosynthetic activities, transpiration and stomatal conductance of bean leaves were adversely affected by all treatments. The effects of oil pollution on plants including soil oxygen depletion, reduced water uptake and toxic effects have been documented and discussed in previous chapters. Indeed, studies found that accumulation of oil in the soil lead to the death of *Spartina alterniflora* plants (Krebs and Tanner, 1981; Alexander and Webb, 1987) and that the leaves of the same plant died after some days of oil contamination (Pezeshki *et al.*, 1995). Also, it has been found that oil pollution reduces plant transpiration and carbon fixation which increases plant mortality (Pezeshki and Delaune, 1993). Furthermore, 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). When soil oxygen required for the correct functioning of plant roots (Smith, 2002) is depleted due to oil pollution (Noomen *et al.*, 2003), plant growth is inhibited and leaves undergo chlorosis, dehydration and death. This can explain the reduction in photosynthetic activities of plants treated with oil pollution in the present study.

It has been noted that accumulation of oil in the soil can increase the CO_2 concentration in the soil (Hillel, 1998) and can also reduce water uptake by plants (Jong, 1980). Work by Smith (2002) noted that water absorption by plants may be inhibited after long periods of anaerobis and thus, can reduce transpiration and instigate stomatal closure. Furthermore, it has been found that stomatal closure restricts entry of CO_2 into plant leaves and consequently reduces leaf photosynthesis (Webb, 1994; Pezeshki *et al.*, 1995). Reduction in transpiration has also been attributed to soil water limitation (Tilling *et al.*, 2007). Thus, this evidence and the strong positive relationships found between these variables in the present study, can explain the reduction in physiological properties of treated plants found in response to both oil and water treatments in this investigation.

Treatments significantly reduced the total foliar chlorophyll content although a greater impact was found in plants treated with oil and the combined oil and water deficit than water deficit treatment alone. As an important photosynthetic pigment, reduction in total chlorophyll concentration may further explain the reduction in photosynthetic activities of treated plants as a strong positive correlation was found between the two variables. In chapter 5, it was found that whenever oil was present in the treatment, there was a greater impact on bean physiological rates than with the waterlogging treatment alone. This is similar to the result of the present study where treatments involving oil had greater impact on bean physiological rates and total chlorophyll contents than with the water deficit treatment alone. This was possibly attributed to a combination of effects from oil such as toxicity, soil oxygen depletion and reduced water uptake.

However, in chapter 6, it was found that whenever water deficit was present in the treatment, there was a greater impact on maize physiological rates than with the oil treatment alone. In maize and sunflower, it was found that soil drying results in the increase of synthesis of abscisic acid (ABA) which moves in the transpiration stream to the shoots to inhibit stomatal opening and leaf growth (Zhang *et al.*, 1987; Zhang and Davies, 1989, 1990a). An increase in ABA quantitatively accounts for the reduction in stomatal conductance and restriction of leaf growth (Zhang and Davies, 1990a, 1990b). The concentration of ABA was also found to increase in the roots of two cultivars of *Phaseolus vulgaris* L. (cv. Cacahuate-72 and Michoacan-12A3) in the first 10cm of unwatered soil (Trejo and Davies, 1991). Furthermore, the increase progressed to deeper roots in accordance with soil dehydration. This concurs with the early findings by Walton *et al.* (1976) where, dehydration increased the ABA concentration in roots of *Phaseolus vulgaris* L. Trejo and Davies (1991) used large soil columns to promote a gradual drying of the soil from the top to the bottom. Drying of the soil caused stomata closure in *Phaseolus vulgaris* L. even though there was no reduction in total water potential (Ψ_w) or turgor potential (Ψ_p) of the shoots because the roots of the plants had reached about 50cm in depth by the time the first 10 or 20cm of the soil showed a significant reduction in water content. Even though the first layers of soil showed a considerable reduction in water content, the roots at 50cm, where there is plenty of water available, can supply enough water to the aerial part of the plant to keep the (Ψ_w) in the leaves at a considerable value to that of well-watered plants (Davies *et al.*, 1990). These findings suggest that ABA concentration can increase in plants due to soil dehydration irrespective of species.

Therefore, the discrepancies found between the physiological responses of maize and bean to water deficit treatments in the present experiment and that reported in chapter 6, may be attributable to the use of the same pot size and soil volume in both experiments. Maize plants grew much larger, developing larger roots and stems than bean. Thus, in chapter 6, the multiple effects of plant size including leaf size, roots and stems may have increased demand for water needed for growth by maize compared to the bean plants used in chapter 5 and in the present study. In addition, water deficit treatment may have also reduced the total water potential (Ψ_w) or turgor potential (Ψ_p) of the maize shoots as the roots were deeper and stronger than those of bean. Indeed, while reviewing cellular and molecular responses to water deficit, and their influence on plant dehydration tolerance, workers found that the responses of plants to drought vary greatly depending on species and stress severity (Mullet and Whitsitt, 1996).

The photoprotective function of carotenoids as explained in chapter 6, may possibly explain the inconsistency and insignificant changes in carotenoid content of treated plants found in the present study. Thus, despite the large variations in reflectance in response to treatment, leaf carotenoid content was largely uncorrelated with reflectance across the whole spectrum. The reduction in leaf water content of treated plants was not significant until 12 days after treatment for both water deficit and combined oil and water deficit and 16 days for the oil pollution alone. As explained in chapter 6, reduction in transpiration helps to conserve available water in plants (Larcher, 1995), as does the stomatal conductance. Thus, the insignificant change in leaf water content of the treated plants found at the early stage of plant stress in the present study may be attributed to the reduction in both transpiration and stomatal conductance at this stage.

An earlier study found that the water content per unit area of sunflower did not change much due to moderate water stress since the plant tried to maintain a level compatible with its basic functioning (Beaumont, 1995). An empirical study by Trejo and Davies (1991) found an early reduction in stomatal conductance in young seedlings of two cultivars of *Phaseolus vulgaris* L. (cv. Cacahuate-72 and Michoacan-12A3) in response to soil drying when the water supply to the soil was withheld. It was noted that the stomata of these plants started to close before any leaf water deficit could be detected. The cultivar Cacahuate-72 showed a significant reduction in stomatal conductance by day 3, while the cultivar Michoacan-12A3 showed a significant reduction in this variable only by day 5 after treatment. This is similar to the result of the present study where a reduction in stomatal conductance became significant 4 days after treatment with water deficit alone and 2 days after oil and combined oil and water deficit treatments.

Substantial changes in spectral reflectance were observed in relation to all of the treatments used in the present experiment. The results show that treatments increased leaf and canopy reflectance both in the visible and SWIR (except in the regions between 601 and 700nm where the spectral reflectance of plants treated with water deficit alone was not significant compared with the control) and decreased in the NIR for all treatments. The result of the present study is similar to the findings of previous workers who investigated the spectral responses of a wide range of plant species to different stressors. Smith *et al.* (2004a) found that the reflectance spectra of vegetation exposed to high concentrations of natural gas in the soil increased in the visible and decreased in the near infrared.

As noted earlier, while the visible region is principally influenced by the photosynthetic pigments, the NIR and SWIR are heavily influenced by the internal cell structure of the leaf and water in plant tissue respectively (Gausman *et al.,* 1970; Gausman, 1985; Bowman, 1989; Ceccato *et al.*, 2001; Ceccato *et al.,* 2002; Tilling *et al.*, 2007). In the present study a strong negative relationship was found between the total chlorophyll and visible reflectance. The most pronounced reflectance difference found between leaf and canopy scales was that water deficit impacted the NIR more at the canopy scale than at the leaf scale and vice versa in the SWIR. This suggests that factors such as variation in leaf age as typically found in a plant canopy and leaf wilting may have also affected the internal structure of canopies resulting in a greater change in NIR reflectance found at the canopy scale. However, such changes appear not to have affected canopy SWIR reflectance, which remains low as the plant tissues continue to contain sufficient water content to absorb most incident SWIR radiation.

As discussed in chapter 6, Aldakheel and Danson (1997) and Danson *et al.* (1992) noted that for individual leaves, there is normally a negative relationship between the leaf water content and reflectance in the near and SWIR wavelengths. This concurs with our correlation in the SWIR region but disagrees with the finding in the NIR regions where leaf water content correlated positively with reflectance. The strong negative relationships were attributed to water absorption, which dominates the spectral response of vegetation in those regions. A strong positive relationship between NIR reflectance and leaf water content found in the present study may be attributed to leaf structural changes. It has been noted earlier that NIR is strongly affected by the size of the cells, the number of cell layers and the thickness of the leaf mesophyll. In dicotyledons, the upper and lower epidermises are separated by the spongy mesophyll containing many spaces (Smith *et al.*, 2004a). Leaves of dicotyledons generally have higher reflectance than monocotyledons because the spongy mesophyll is more developed (Gausman, 1985; Guyot, 1990) and allows more light scattering between the cell walls (Smith *et al.*, 2004a). In the present study, treatments may have damaged the spongy mesophyll thus, reducing light scattering which may have caused lower NIR reflectance in treated plants.

Thus, a strong positive relationship was found between the leaf water content and reflectance in the NIR region.

The response of leaf and canopy absolute temperature to treatments in the present study concurs with that in chapter 6. The consistent increase in 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 increase in leaf and canopy absolute temperatures of the treated plants were significant before visual stress symptoms were observed suggesting that it can be useful in the early detection of oil pollution, the combined oil and water deficit and water deficit. As explained in chapter 6, the inconsistent and insignificant differences found between the leaf 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. The results show that the thermal index (I_G) can detect oil pollution and the combined oil and water deficit in bean. Similar to the leaf absolute temperature, the consistent decrease in the thermal index (I_G) of treated plants as a percentage of control is likely to be responding to the reduction in the transpiration and stomatal conductance of treated plants. While the I_G significantly decreased in plants treated with oil pollution alone before visual stress symptoms were observed, the combined oil and water deficit and water deficit were observed the same day as visual stress symptoms. This suggests that in the present study, the index I_G can be useful in the early detection of oil pollution in bean. However, it may be difficult to discriminate between oil pollution and water deficit using I_G due to inconsistency and insignificant differences found between the indexes of plants subjected to different types of treatment.

In order to understand whether spectral and thermal properties of plants translate from leaf to canopy scale, the temporal responses, sensitivity and relationships between the optimal indices and biochemical properties were examined at both the leaf and canopy scales. The total chlorophyll index R_{800}/R_{606} was consistently sensitive to oil and combined oil and water deficit and detected the combined oil and water deficit stress prior to visual stress symptoms. While the index was sensitive to oil pollution on the same day as visual stress symptoms, no consistent sensitivity was observed for water deficit. This was not the case at the canopy scale as the index was consistently sensitive to all treatments but only at the later stages of the experiment. This suggests that while the index has potential for early detection of stress caused by combined oil and water deficit at the leaf scale, it can only translate at the canopy scale at the later stage of oil pollution, water deficit and combined oil and water deficit. While a strong positive relationship was found between the index and total chlorophyll content at the leaf scale ($r^2 = 0.92$), a moderate relationship was found at the canopy scale ($r^2 = 0.66$).

At the leaf scale, the carotenoids spectral index was sensitive to oil and the combined oil and water deficit before visual stress symptoms were observed. Although the sensitivity was not consistent until after visual stress symptoms were observed. The same observation was made at the canopy scale except that oil pollution was detected prior to visual stress symptoms. A better relationship (although weak) was found between the index and carotenoids content at the canopy scale than at the leaf scale. Based on these findings, it would have been possible to translate the use of this spectral index from leaf to canopy scale but the major difference found between the temporal response of the index at the leaf and canopy scales hampers this possibility. The differences in the responses of this index to treatment at the leaf and canopy scales could be attributable to a multitude of possibly interacting effects related to leaf age, leaf and canopy structure and differential carotenoid concentrations throughout the canopy.

The leaf water content spectral index R_{865} showed similar sensitivity to oil and combined oil and water deficit at the leaf scale although not before stress symptoms were observed. The index was not consistently sensitive to water deficit alone. At the canopy scale, the index was consistently sensitive to all treatments and responded before visual stress symptoms were observed for oil pollution (alone) and water deficit (alone). This suggests that the sensitivity of the index improved at the canopy scale. Based on the results of the present study, the responses of both the leaf absolute temperature and I_G to treatments of oil, water deficit and the combined oil and water deficit can translate from leaf to canopy scale, and, indeed, in some cases their performance improved at the canopy scale.

7.5 Conclusion

The present study confirms that hyperspectral and thermal remote sensing have potential for early detection and discrimination between oil and water deficit stress in plants. From the results of the present study, the absolute temperature was optimal for early detection of oil pollution, water deficit and the combination of oil and water deficit stress in bean at the leaf scale. In terms of consistency and time of detection, the absolute temperature performed best as it detected oil pollution, the combined oil and water deficit and water deficit stresses 7, 4 and 2 days before visual stress symptoms were observed, respectively. However, it was difficult to discriminate between the oil and water stresses using this index. As found in chapter

6, for maize leaves absolute temperature was also optimal index for early detection of water deficit and the combined oil and water deficit.

The spectral indices R_{800}/R_{606} and R_{865} detected oil-related stress but did not detect water deficit at the leaf scale. This suggests that these spectral indices have the ability to discriminate between oil pollution and water deficit stress. This finding concurs with that in chapter 6, where the spectral index $(R_{1330}-R_{538})/(R_{1330}+R_{538})$ detected oil pollution and the combined oil and water deficit in maize at the leaf scale but was unresponsive to water deficit stress alone. In the present chapter, it was found that the spectral index R_{800}/R_{606} was sensitive to oil-related stress at both the leaf and canopy scale but changes were only detectable at the canopy scale at more advanced stages of stress. On the contrary, other indices such as R_{800}/R_{520}, R_{865}, absolute temperature and I_G, were able to detect stress earlier when measured at the canopy scale than when measured at the leaf scale. Overall, the study has demonstrated that the optimal remotely-sensed index for detection of oil and water-related stresses varies according to the plant species under investigation and the effectiveness of any particular approach varies between leaf and canopy scales.

Chapter 8

CONCLUSIONS AND FUTURE WORK

8.1 Conclusions

This chapter gives a summary of the research findings, a discussion of the overall contribution of the thesis in the context of existing works, and makes suggestions for future research priorities. For the accurate monitoring of plant stress caused by oil pollution, there is a need to develop an approach that is sensitive to physiological changes prior to visual stress observation. Such an approach needs to have the ability to discriminate between oil pollution and other possible concomitant stresses such as waterlogging and water deficit. Thus, this study had the primary objective of investigating the potential value of hyperspectral reflectance and thermal imaging to detect and quantify plant stress caused by oil pollution along with the ability to discriminate between different stresses. In order to achieve this aim, four sets of laboratory experiments were undertaken which tackled four major research questions, as reported in chapters 4, 5, 6 and 7. The four questions are answered in turn here, using the evidence provided in this study:

8.1.1 What is the optimum remotely-sensed index for early detection of oil-induced stress in plants at lethal and sub-lethal levels?

- There was a significant change in spectral reflectance at lethal and sub lethal levels of oil pollution and as early as 4, 9, and 10 days in high, medium and low treatments, respectively before visual stress symptoms were seen (e.g. as in Table 4.6).

- The simple ratios using combinations of narrow wavebands that ranged between $R_{715-760}$ and $R_{695} - R_{716}$ were stable and highly sensitive to lethal and sub lethal levels of oil stress in maize.

- A normalised-difference spectral index that combined a waveband in the red-edge with one of high reflectance in the NIR region: $(R_{755}\text{-}R_{716})/(R_{755}+R_{715})$ was optimal in pre-visual detection of oil pollution in maize at lethal and sub-lethal levels (see Table 4.6).
- There was a strong positive linear relationship between $(R_{755}\text{-}R_{716})/(R_{755}+R_{716})$ and photosynthesis (see Figure 4.11).
- Absolute leaf temperature has minimal potential for detecting oil pollution in maize (see Figure 4.16).
- This study concludes that the application of hyperspectral remote sensing using $(R_{755}\text{-}R_{716})/(R_{755}+R_{716})$ can enhance precision and accuracy for the early detection of oil pollution via plant stress response. This indicates that by detecting plant stress, hyperspectral remote sensing has considerable potential for the timely detection of oil pollution in the environment.

8.1.2 What is the optimum set of spectral and thermal responses that can be used for early, non-destructive quantification and discrimination between oil pollution and waterlogging stress in plants?

- The spectral reflectance and thermal properties of bean canopies effectively distinguished between subtle signs of stress induced by oil pollution and waterlogging.

- There was a significant increase in reflectance across the visible region for plants treated with oil and a combined oil and waterlogging treatment (see Figure 5.4).
- For plants exposed to waterlogging alone, a significant increase in reflectance in two specific regions centred on 550nm and 715nm was observed (see Figure 5.4).
- The study suggests that these waveband regions could serve as good indices for discriminating between stress symptoms arising from oil or a combined oil and waterlogging treatment and those arising from waterlogging alone.
- NIR reflectance could be used to discriminate between stress induced in bean by single and multiple factors as it was found that the combined oil and waterlogging treatment caused a significant decrease in NIR reflectance while the individual oil and waterlogging treatments did not invoke such a response (see Figure 5.4).
- A simple ratio of reflectance that combined narrow wavebands in the green and red regions (R_{673}/R_{545}) was most sensitive in the early detection of stress symptoms caused by oil and waterlogging (e.g. as in Table 5.0).
- The canopy absolute temperature and the thermal index (I_G) were good indicators of developing oil and combined oil and waterlogging stress in bean, and poor indicators of stress caused by waterlogging (see Figure 5.7).
- The study concludes that by combining spectral and thermal information, oil-induced stress could be discriminated from a combined waterlogging stress effect.

8.1.3 What is the optimum set of spectral and thermal responses that can be used for early, non-destructive quantification of and discrimination between oil pollution and water deficit stress?

- Hyperspectral remote sensing can accurately measure the pigment concentration in plants.
- Oil pollution adversely affects chlorophyll contents in plants and therefore, plant stress caused by oil pollution can be detected remotely (e.g. as in Table 6.0).
- Remote sensing of carotenoid concentration alone is not sufficient for early detection or 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 decreases due to stress (e.g. as in Table 6.0).
- Hyperspectral remote sensing may not be suitable for early detection of stress in maize caused by water deficit alone.
- The spectral index $(R_{1330}-R_{538})/(R_{1330}+R_{538})$ was optimal for the early detection of stress caused by oil pollution in maize (e.g. as in Table 6.1).
- The leaf absolute temperature was optimal for early detection of stress caused by water deficit in maize (e.g. as in Table 6.2).
- The leaf absolute temperature and I_G lack the ability to discriminate between a combined oil and water deficit stress.
- Thus, the combination of hyperspectral and thermal remote sensing has potential in the early stress detection and discrimination between oil and water deficit stress in maize.

8.1.4 How consistent are the spectral and thermal responses of plants to oil and water deficit stress between species and across leaf and canopy scales?

- This investigation confirms that hyperspectral and thermal remote sensing have potential for the early detection and discrimination between oil and water deficit stress in plants.
- Absolute temperature was optimal for early detection of oil pollution, water deficit and the combination of oil and water deficit stress in bean at the leaf scale (e.g. as in Table 7.2) while spectral index $(R_{1330}-R_{538})/(R_{1330}+R_{538})$ was optimal in the early detection of oil stress in maize at the same scale (e.g. as in Table 6.1)..
- Spectral indices detected oil-related pollution in both maize and bean at the leaf scale but did not detect water deficit (e.g. as in Table 6.1 & 7.2, respectively). Thus, spectral reflectance has the ability to discriminate between oil pollution and water deficit stress in both species.
- Similar to the maize species, it was difficult to discriminate between oil and water deficit stress in bean using leaf thermal features (see Figure 7.2).
- The spectral index R_{800}/R_{606} was sensitive to oil-related stress at both leaf and canopy scales, although in the latter, changes were only detectable at more advanced stages of stress (see Figure 7.2).
- Other indices such as R_{800}/R_{520}, R_{865}, absolute temperature and I_G, were able to detect stress earlier when measured at the canopy scale than when measured at the leaf scale (see Figure 7.2).
- The study concludes that the optimal remotely-sensed index for detection and discrimination between oil and water-related stresses varies according to the plant species under investigation and the effectiveness of any particular

approach varies between leaf and canopy scales. However, this can be surmounted by using a combination of spectral and thermal remote sensing.

8.2 Synthesis of results

Oil pollution, waterlogging and water deficit can cause stresses in plants which are detrimental to their physiological function and which result in changes in spectral and thermal responses (Figure 8.0). There was a strong relationship between physiological parameters and spectral indices. The form of these relationships was similar for different physiological parameters, being asymptotic on most occasions. Thus, spectral indices would have limitations in predicting changes to physiological parameters beyond certain thresholds, such as 1 mol m^{-2} s^{-1} for transpiration, indicating that the approach has some limitations. However, the thermal index (I_G) showed a linear response to stomatal conductance, and this was consistent across species. Thus, this relationship provides a means to support remote sensing strategies for the early detection and discrimination of different types of stress in plants.

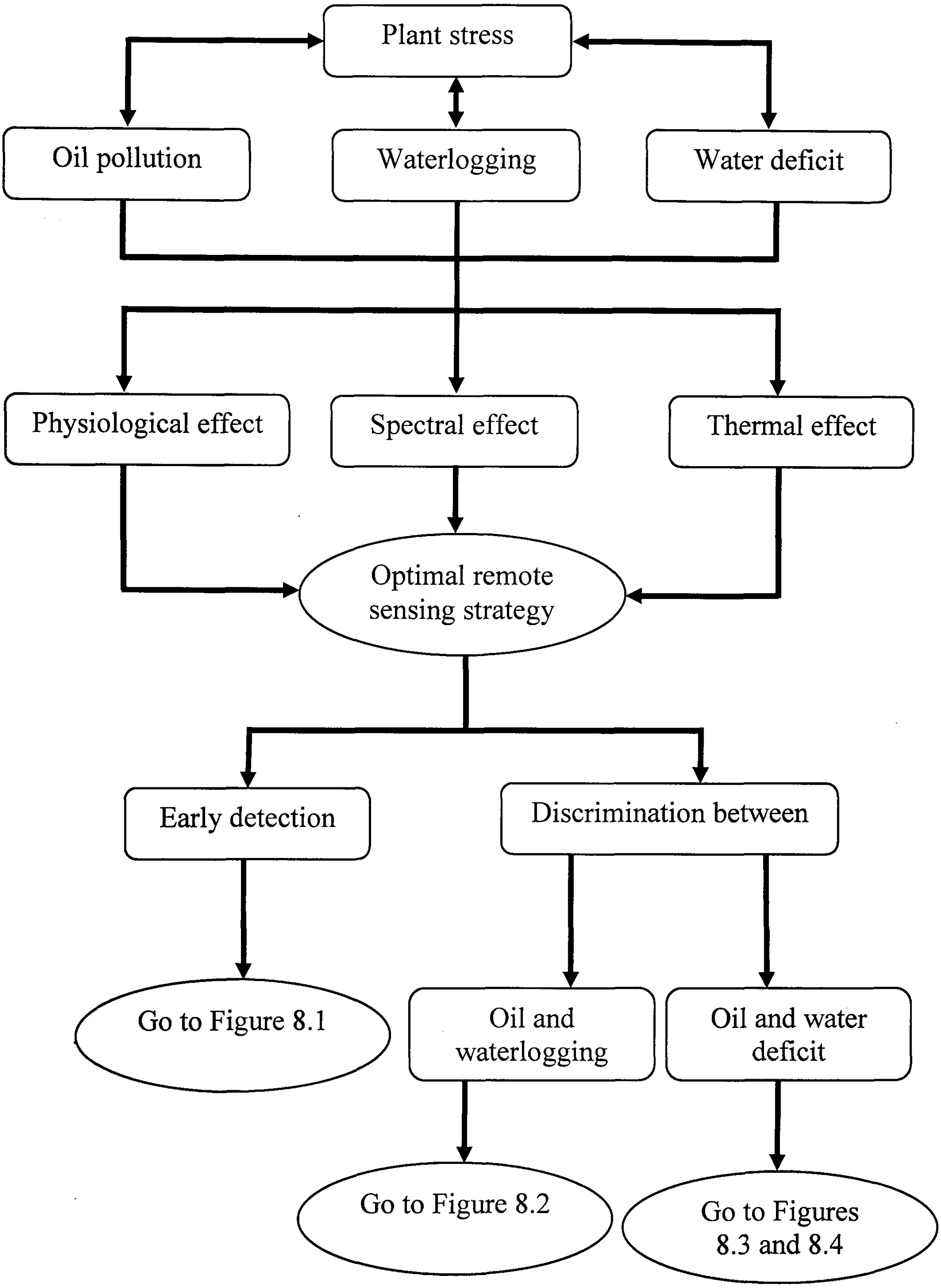

Figure 8.0 Schematic overview of hyperspectral and thermal remote sensing of plant stress responses to oil pollution, waterlogging and water deficit.

8.2.1 Early detection of stress factors

Figure 8.1 summarises the optimal approaches for the early detection of individual stresses.

Spectral indices were optimal in pre-visual detection of oil pollution in maize and bean leaves. In particular, a normalised-difference spectral index $(R_{755}-R_{716})/(R_{755}+R_{716})$ was optimal in maize and other normalised-difference spectral indices such as $(R_{1330}-R_{538})/(R_{1330}+R_{538})$ also worked well. The spectral index $(R_{755}-R_{716})/(R_{755}+R_{716})$ performed well in bean, although other indices such as R_{800}/R_{606} performed slightly better. The absolute leaf temperature had minimal potential for detecting oil pollution in maize. However, canopy absolute temperature and the thermal index (I_G) were good indicators of oil related stress in bean.

Spectral indices were effective for the early detection of waterlogging stress in bean canopies. The spectral index $(R_{755}-R_{716})/(R_{755}+R_{716})$ that was sensitive to oil pollution in maize and bean was also sensitive to waterlogging in bean, which indicates that this index is responding to the stress symptoms in plants caused by anaerobic conditions within the soil generated by different causes. Other simple ratios of reflectance such as R_{673}/R_{545}, R_{673}/R_{631}, and R_{545}/R_{445} were useful for the early detection of waterlogging in bean, however, canopy absolute temperature and the thermal index (I_G) were insensitive to waterlogging.

Spectral indices lacked the ability for early detection of stress caused by water deficit at the leaf scale in both maize and bean but have some potential in bean at the canopy scale. Likewise, the I_G was sensitive to water deficit in bean canopies. However, the absolute temperature was sensitive to water deficit irrespective of species or scale of measurement.

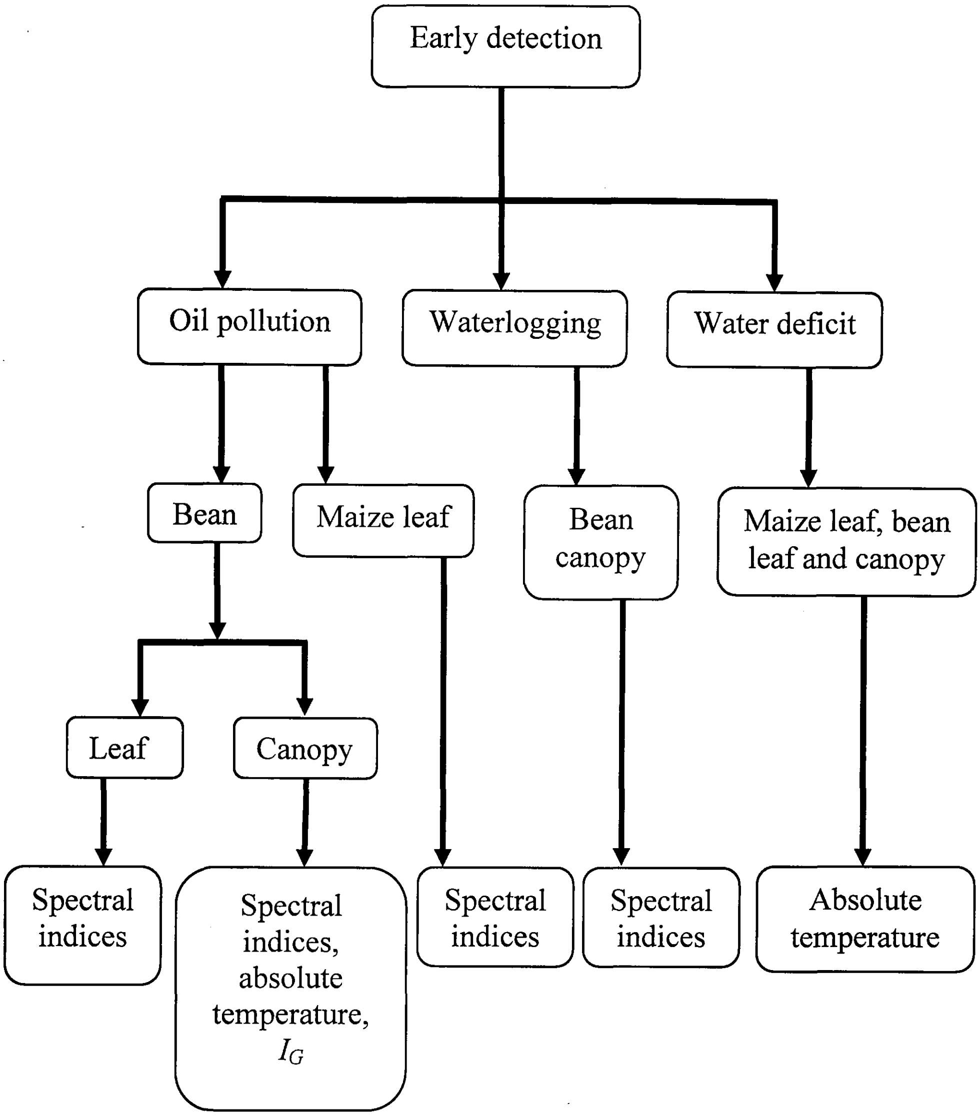

Figure 8.1 Optimal approaches for the early detection of plant stress caused by individual agents (oil pollution, waterlogging and water deficit) based on the most rapidly responding spectral or thermal index sensitive to the stress.

8.2.2 Discrimination of different stresses

This study has demonstrated that remote sensing approaches could be deployed for discriminating between oil pollution, waterlogging and water deficit via plant stress responses. In all cases, a combination of spectral and thermal indices was useful for discriminating between different stresses. As Figure 8.2 demonstrates, to discriminate between oil pollution and waterlogging in bean canopies, a combination of I_G and spectral information could be used. If the I_G decreases, then this indicates that the stress is caused by oil pollution but if the I_G does not change and a spectral index such as R_{673}/R_{543} increases, then the stress is caused by waterlogging. If there is no change in the spectral index, then there is neither oil pollution nor waterlogging stress. As figures 8.3 and 8.4 demonstrate, to discriminate between oil pollution and water deficit a combination of spectral indices and absolute temperature can be used. Indicators of oil-induced stress are spectral indices such as $(R_{755}-R_{716})/(R_{755}+R_{716})$ or $(R_{1330}-R_{538})/(R_{1330}+R_{538})$ for maize leaves, and R_{800}/R_{606} for bean leaves. If these indices do not change and the absolute temperature increases, then the stress is caused by water deficit. If there is no change in the absolute temperature, then there is neither oil pollution nor water deficit stress.

At the canopy scale for beans, a NIR narrow waveband R_{865} responded faster to water deficit stress than it did for oil pollution. This suggests that water deficit damaged plant cellular and canopy structure more rapidly than the oil pollution and thus, may potentially be a good indicator for discriminating between the two stresses. Since the spectral indices, absolute temperature and I_G were sensitive to oil pollution and water-related stress, additional information, for example concerning changes in plant geometrical structure, may be required to improve a way of discriminating between oil pollution and water deficit stress at canopy scale.

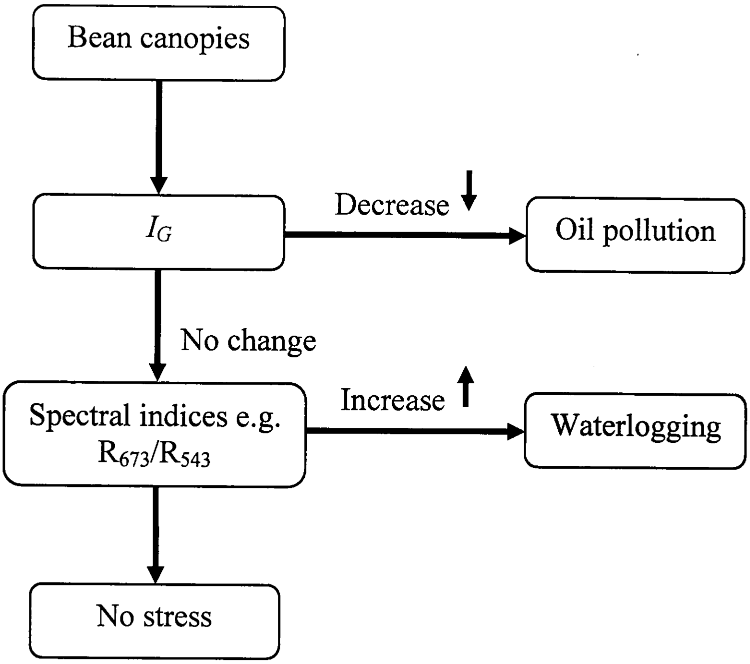

Figure 8.2 Flowchart showing the approach for deploying remote sensing measures for discriminating between plant stress caused by oil pollution and waterlogging in bean canopies.

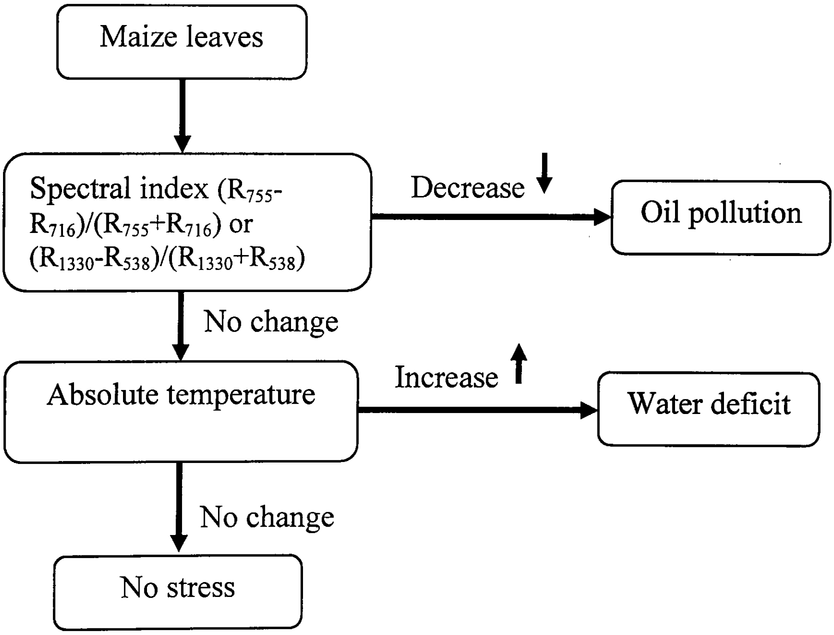

Figure 8.3 Flowchart showing the approach for deploying remote sensing measures for discriminating between plant stress caused by oil pollution and water deficit in maize leaves.

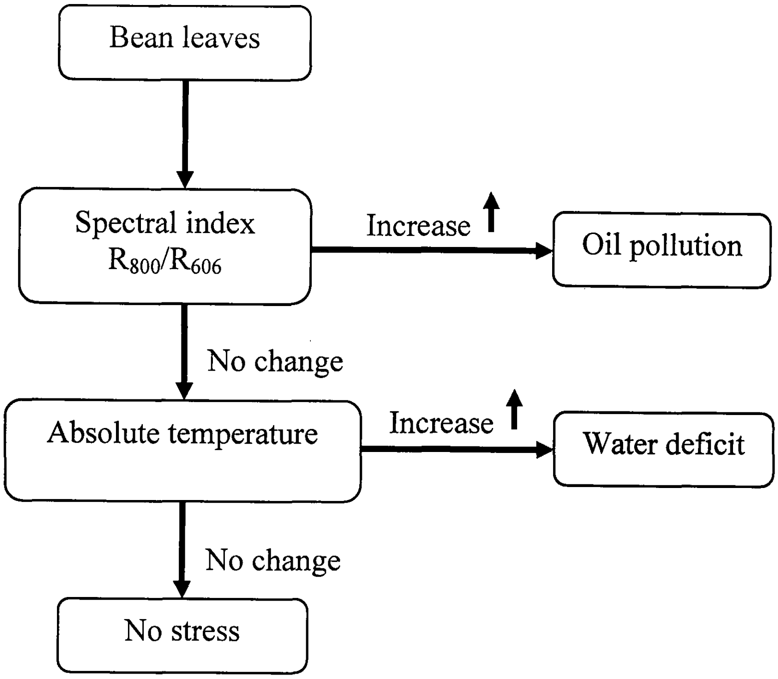

Figure 8.4 Flowchart showing the approach for deploying remote sensing measures for discriminating between plant stress caused by oil pollution and waterlogging in bean leaves.

8.3 Summary of contributions

The first contribution of the thesis is the application of hyperspectral and thermal remote sensing for the detection and non-destructive quantification of plant stress caused by oil pollution. Secondly, it has demonstrated that hyperspectral and thermal remote sensing can detect and discriminate between oil pollution, waterlogging and water deficit, which are stress agents that can affect plants either individually or simultaneously. In most cases, the combination of these remote sensing techniques enhanced the accuracy for discriminating between oil pollution and waterlogging or water deficit stress. Since it is possible to detect oil pollution before visual signs of stress are observed in plants, this implies that detection of anomalous oil concentrations cannot only help to minimise the risks associated with oil pollution in the environment but can also lead to discovery of micro-seepage and related oil reservoirs. The use of this technique could help to speed up and improve response to an oil spill and could significantly reduce the environmental impact and severity of a spill. It can also help to prioritise effort in clean-up operations in the event of an oil spill and can provide information about protection priorities for the affected areas which is one of the most important elements of contingency plans.

8.4 Limitations of the study

While this study has shown evidence about the possibility to detect and discriminate between plant stresses caused by oil pollution, waterlogging and water deficit under controlled environment, there may be issues and problems associated with their field-based application. For example, the pot-based experiments did not include continuous oiling of plants that may happen in the field during oil spillage. Instead, the study adopted one-time oiling treatment which may not be considered truly representative of conditions that plants would encounter during oil spill events. Thus, it may be difficult to translate the general responses of these plants to oil stress in the present experiments as such stress condition may occur at varying intensity and duration in field situations. Additionally, other stress factors such as nutrient deficiency may also be affecting plants growing in the field at the same time with the stresses used in this study.

A slower spectral and thermal response of plants to stress may be expected in field situation because of possible dilution of treatments. Plant spectral and thermal responses of pot-based experiment may also vary from the responses obtained in field situation due to variation in soil structure on which they grow in the field. For pot-based experiments, plants were grown on the same soil type (compost), but in the field, plants usually grow on different soil types. In chapter 2, section 2.3.1, it was noted that the physical, chemical and geological characteristics of soil play significant roles in the degree of its vulnerability to an oil spill (Gundlach and Hayes, 1978). Similarly, Pezeshki *et al.* (2000) noted that factors such as soil type, soil organic matter, size fraction of soil mineral matter and soil texture play significant roles in the fate of hydrocarbon in the soil. While it may be easier for oil to penetrate rapidly within a given soil type (e.g. fine, coarse-grained sand, mixed sand and gravel, sheltered rocky and tidal flat and salt marsh and mangrove forest), most of the oil will not adhere to, nor penetrate into a compacted soil type.

Furthermore, this study used single crop species and thus, their responses to stress may not have adequately represented the type of stress response plant would encounter under field condition. Based on these limitations, this study proposes the following future works.

8.5 Future research directions

This research has provided a basis for the study of plant stress caused by oil pollution. It has also shown that oil, waterlogging and water deficit stress in plant can be detected, quantified and discriminated using hyperspectral and thermal remote sensing. Based on the nature of, and findings in this thesis, the following proposals are made:

- There is a need to test this approach under field conditions since the results of this study were obtained based exclusively on research in the laboratory. This will help to establish whether subtle spectral and thermal features relating to leaf physiological and biochemical changes in laboratory spectrometry and thermography are detectable in spectra and thermography of leaves and plant canopies in the field situation. It may be worthwhile to investigate if the same treatment dose of stresses will have the same effect on plants growing in different pot sizes. This will help to ascertain whether plants stress responses to various stresses could translate from pot-based laboratory experiment to field situation.

- Under field conditions, factors such as the complexity of the canopy structure, soil background, atmospheric, and illumination variation due to sensor-sun geometry have a considerable and specific impact on the vegetation spectrum (Delalieux *et al.*, 2008). However, it is possible to minimise these effects by combining single-band reflectances into a vegetation index that is sensitive to the plant canopy and not to the soil (Leblon, 2010). Ratioing allows removal of the disturbances affecting, in the same way, reflectances in each band. Indeed, work remains to be done to scale this approach to larger scale remote sensing applications. Atmospheric disturbances affect space-borne reflectance measurements; however, this can be overcome by calibrating the remote sensing imagery to reflectance percentage of the target being measured. Furthermore, other environmental and meteorological factors such as wind speed, humidity, cloud, ambient temperature and irradiance that may affect thermal measurements in the field can be overcome by using wet and dry references (as demonstrated in this study). In particular, in order to improve the ability to discriminate between oil and water-related stresses at canopy scale, the potential for collecting additional information on vegetation canopies such as the structure and geometry using LiDAR imagery could be explored.

- In order to operationalise the techniques developed in this study, high spatial, spectral and temporal resolution airborne or satellite remote sensing is required. High spatial resolutions in the order of one metre or less would be required to be able to locate small features such as oil pipelines. If the spectral resolution of the sensor is high in the visible, NIR and SWIR then, subtle differences arising from oil pollution and other causes of plant stress such as waterlogging and water deficit could be discriminated. This is because information about the general health status of plants is often embedded in narrow spectral features. With high temporal resolutions, it may be possible to detect oil, waterlogging and water deficit stress in plants before stress symptoms are seen. With high resolution remote sensing imagery, it may be possible to capture the spatial variations of stress indices proposed in this study, map oil, waterlogging and water deficit stresses and develop time series of spectral and thermal responses of plants to these stresses.
- For safety reasons, this study used 15W/40 diesel engine oil (Unipart, Crawley, UK) (which is not highly flammable) in all experiments to model oil stress in plants. Thus, there may be the need to use crude and/or other refined oil products typically stored and transported through pipelines (e.g petrol and diesel), to confirm consistency in spectral responses.
- It is worthwhile to investigate the potential of this approach for the early detection, non-destructive quantification and discrimination between oil pollution and nutrient deficiency in plants, as some form of nutrient deficiency is prevalent in almost all ecosystems.
- While single crop species have been used in this study, it is important to investigate the possibility of applying the remotely-sensed approaches for

monitoring natural vegetation communities. Hence, the use of a mixture of plant species at different growing stages to closely mimic natural vegetation communities is proposed for future investigations.

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