



The University of
Nottingham

**DETERMINATION AND MONITORING OF VEGETATION
STRESS USING HYPERSPECTRAL REMOTE SENSING**

By

Sani Yahaya

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ABSTRACT

Stress causes crops to grow below their potential and this affects the vitality and physiological functioning of the plants at all levels leading to reduction in yield. Remote sensing of vegetation is regarded as a valuable tool for the detection and discrimination of stress, especially over large or sensitive regions.

The main aim of the research carried out is to assess the potential of remote sensing to detect CO₂ leakage from CCS repositories. Further to this, the capability of remote sensing to discriminate between stresses with similar mode of action is explored. Two stress factors were selected for study: (1) elevated concentrations of soil CO₂ in the plant root zone and; (2) herbicide, applied at sub-lethal levels. To understand the effects of soil CO₂ and herbicide stress on vegetation reflectance, field experiments were carried out on maize (2009) and barley (2010) to investigate the effects of elevated soil CO₂ concentrations and of different levels of herbicide treatments on vegetation growth and canopy reflectance using hyperspectral remote sensing techniques.

The findings from this study shows that the average canopy reflectance response of maize and barley to CO₂ and herbicide stress were increased reflectance in the visible and decrease in near infra-red region as well as changes in the position and shape of the red-edge. The red-edge first-derivative for barley treated with CO₂ were composed of maximum peaks between 716 and 730nm and smaller peaks at 699 and 759nm, the control had peaks at 727 and 730 nm, with similar smaller peaks. Barley treated with herbicide had early peaks (a day after treatment) at 697, 715 and 717nm with a shoulder at 759nm, as the experiment progressed (16 days after treatment) the stress became apparent and the peak remained

stationary at 730nm, the magnitude decreased to 712nm at late treatment period (35 days after treatment). The control had single peak at 726nm. CO₂ treated maize had double peaks at 718 and 730nm, with secondary peaks at 707 and 794nm. Maize treated with herbicide had maximum peaks at 716 and 723nm, with the shoulder at 759 nm; the peaks were similar with the control plots but decreased in magnitude. The main differences between the treatments were in the shape and positions of the peaks that identify the red-edge. The canopy reflectances of the plants were further analysed using the blue (400-550nm) and red (550-750nm). In these regions the main feature of concern is chlorophyll content. The analysis showed that the band depths of controls plants were deeper compared to the stressed plants which is dependent on the stress and crop type.

Other vegetation indices used in this study were the Chlorophyll Normalized Difference Index (*Chl NDI*), the Pigment Specific Simple Ratio for chlorophyll *a* and *b* (*PSSRa* and *PSSRb*) and the Physiological Reflectance Index (*PRI*). The results show that they were promising indicators of early stress detection, some indices performed better than others depending on the stress type, species and duration of stress. *Chl NDI* was sensitive to high soil CO₂ concentration in maize and barley, sub-lethal herbicide treatment at 10% - 40% level in barley and was insensitive to both low CO₂ in the barley and maize as well as 10% herbicide treatment in maize. *PSSRa* was a good indicator of early CO₂ stress in maize and high CO₂ in barley as well as 10- 40% herbicide treatments. *PSSRb* could detect high CO₂ level in maize and barley and all levels (5- 40%) of herbicide treatments. *PRI* was insensitive to 5% herbicide treatment in barley but sensitive to high CO₂ in maize at early stage of the experiment.

This study has demonstrated that remote sensing approach could be deployed for discriminating between different stressors using their red-edge first-derivative peaks, band depths and vegetation indices.

LIST OF PUBLICATIONS

1. Sani, Y., Steven, M. D., Foody, G., 2012. Detection of CO₂ leakage from Carbon Capture and Storage facilities using remote sensing. Abstract accepted in: *American Geophysical Union Annual Conference, Dec 3-7, California- USA*
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<http://www.asprs.org/a/publications/proceedings/Milwaukee2011>
3. Sani, Y., Steven, M. D., Foody, G., 2011. Application of hyperspectral remote Sensing technique to detect CO₂ stress in Barley plant. In: *Proceedings of Remote Sensing and Photogrammetry Society Annual Conference, Sept 13-15, Bournemouth University, United Kingdom.*
4. Sani, Y., Steven, M. D., Foody, G., 2010. The effects of CO₂ and herbicide induced stress on the spectral reflectance of Maize (Poster). In: *Proceedings of the American Society for Photogrammetry and Remote Sensing Annual conference, April 26-30, San Diego, California-USA. pp. 69*
5. Sani, Y., Steven, M. D., Foody, G., 2010. Investigating the spectral reflectance of Maize induced with stresses (Poster). In: *book of abstract for ASD and IEEE GRS; Art, Science and Applications of Reflectance Spectroscopy Symposium, Feb 23- 25, Boulder Colorado-USA. pp. 3.*
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LIST OF CONTENTS

Abstract	i
List of publications	iv
Acknowledgement	vi
List of contents	vii
CHAPTER ONE: INTRODUCTION	
1.1 Scientific context	1
1.2 Mitigation options for atmospheric CO ₂ emission	2
1.3 Detection and monitoring of CO ₂ leaks	8
1.4 Stress monitoring by remote sensing	9
1.5 Discriminating stress- can it be done?	11
1.6 Research aim	13
1.7 Research objectives	13
1.8 Research hypotheses	14
1.9 Thesis outline	14
CHAPTER TWO: LITERATURE REVIEW	
2.1 Introduction	16
2.2 Impacts of leakage from CCS	16
2.3 Stress in plants	18
2.3.1 C3 and C4 plants metabolism and their response to stress	22
2.4 Plant response to root stress	24
2.5 Plant response to soil gases	25
2.5.1 Soil enrichment of CO ₂	25
2.5.1.1 Natural analogues	25
2.5.1.2 Experimental studies of soil CO ₂ enrichment	27
2.5.1.3 The effects of elevated CO ₂ on soil fauna	30
2.5.2 Atmospheric CO ₂ enrichment	30
2.6 Remote sensing applications	33
2.6.1 Remote sensing of vegetation	35
2.6.1.1 Visible reflectance	36
2.6.1.2 Red-edge region	37

2.6.1.3	Near infrared (NIR) region-----	38
2.6.1.4	Shortwave infrared (SWIR) region-----	38
2.6.2	Hyperspectral remote sensing of vegetation -----	40
2.7	Conclusion-----	41

CHAPTER THREE: FIELD DATA METHODS

3.1	Introduction-----	44
3.2	The Artificial Soil Gassing and response detection (ASGARD) Facility-----	44
3.2.1	Site description-----	45
3.2.2	Design of gas injection system-----	46
3.2.3	Experimental Plots layout-----	47
3.2.3.1	Plot infrastructure-----	48
3.3	Gas measurement-----	48
3.3.1	Routine gas monitoring-----	48
3.3.2	Gas concentration mapping -----	49
3.3.3	Plants and treatments-----	50
3.3.3.1	Experiment one-----	50
3.3.3.2	Experiment two-----	51
3.4	Sampling techniques-----	52
3.5	Canopy reflectance measurement-----	53
3.6	Plant growth and biomass measurement-----	55
3.7	Determination of soil pH-----	55
3.8	Chlorophyll content-----	55
3.9	Climatic condition-----	56
3.10	Data analysis-----	56
3.10.1	Spectral data processing-----	57
3.10.2	Derivative spectroscopy-----	57
3.10.3	Techniques for extracting the red edge position from hyperspectral data-----	58
3.10.3.1	Maximum first derivative spectrum-----	58
3.10.3.2	Linear four-point interpolation technique-----	59
3.10.3.3	The inverted Gaussian technique-----	59

3.10.3.4	Higher order curve fitting technique-----	60
3.10.3.5	Linear extrapolation technique-----	61
3.10.4	Vegetation Indices-----	63
3.10.5	Continuum removal analysis-----	64
3.10.5.1	Spectral region of focus for continuum removal analysis-----	65
3.11	Statistical analysis-----	66
3.12	Conclusion-----	66

CHAPTER FOUR: Spectral and physiological responses of maize (*Zea mays*) to elevated soil CO₂ and herbicide stress.

4.1	Introduction-----	67
4.2	Results -----	68
4.2.1	Visible stress symptoms -----	68
4.2.2	Plant growth and biomass-----	70
4.2.3	Soil pH analysis-----	77
4.2.4	Chlorophyll content-----	79
4.2.5	Canopy spectral reflectance-----	80
4.2.5.1	Canopy reflectance differences between CO ₂ treatment and control-----	82
4.2.5.2	Canopy reflectance differences between herbicide treatment and control -----	86
4.2.6	Derivative analysis-----	89
4.2.7	Red Edge Position-----	92
4.2.8	Spectral vegetation Indices-----	95
4.2.8.1	Chlorophyll Normalised Difference Index (Chl NDI)-----	95
4.2.8.2	Pigment Specific Simple Ratios (PSSRa and PSSRb)-----	97
4.2.8.3	Physiological Reflectance Index (PRI)-----	100
4.2.8.4	Continuum removal-----	101
4.3	Conclusion-----	114

CHAPTER FIVE: Remote sensing of barley (*Hordeum*

***vulgare v Concerto*) stressed with CO₂ and herbicide.**

5.1	Introduction-----	118
5.2	Data analysis-----	119
5.3	Results-----	120
5.3.1	Visible stress symptoms -----	120
5.3.2	Biomass Analysis-----	121
5.3.3	Soil pH analysis -----	128
5.3.4	Chlorophyll analysis -----	129
5.3.5	Canopy reflectance measurement -----	131
5.3.6	First derivatives reflectance peaks -----	138
5.3.7	Temporal change in red-edge position -----	141
5.3.8	Vegetation indices-----	143
5.3.9	Continuum removal analysis-----	149
5.4	Comparison of stress responses in maize (C4) and Barley (C3) using hyperspectral remote sensing-----	162
5.4.1	Visible stress symptoms-----	163
5.5	Conclusion-----	166

CHAPTER SIX: SUMMARY, DISSCUSSION AND FUTURE WORK

6.1	Introduction-----	168
6.2	Summary and discussion-----	169
6.2.1	Spectral and physiological responses of maize (<i>Zea mays</i>) to lelevated soil CO ₂ and herbicide stress-----	169
6.2.2	Remote sensing of barley (<i>Hordeum vulgare v Concerto</i>) stressed with CO ₂ and herbicide-----	172
6.3	Discussion-----	177
6.3.1	Detection of CO ₂ leaks by remote sensing-----	181
6.3.2	Synthesis of experimental findings-----	182
6.4	Limitations of the study-----	186
6.5	Conclusion-----	187
6.6	Future research directions-----	190
	REFERENCES -----	191

List of Figures

Figure 1-1:	Rate of historical change in atmospheric CO ₂ -----	2
Figure 1-2:	Illustrative example of the potential global contribution of CCS based on an alternative integrated assessment model (MiniCAM)-----	6
Figure 1-3:	Geologic storage and related projects around the world-----	7
Figure 2-1:	General concept of the phase sequences and responses induced in plants by stress exposure -----	21
Figure 2-2:	Tree kill on the shore of Horseshoe Lake, Mammoth Mountain, California-----	27
Figure 2-3:	Typical reflectance characteristics of leaves-----	35
Figure 3-1:	Artificial Soil Gassing and Response Detection Site (ASGARD) showing some infrastructures-----	47
Figure 3-2:	Illustration of plot infrastructure-----	48
Figure 3-3:	Sampling frame used for measurements -----	53
Figure 4-1:	Visible symptoms of stress in maize leaves due to CO ₂ and herbicide-----	69
Figure 4-2:	CO ₂ gassed maize cob showing distorted maize cob and shriveled grain formation as a result of stress and control plots maize cobs-----	69
Figure 4-3:	Herbicide treated maize cobs showing incomplete grain formation caused by stress and control with full grain cob and three immature maize cobs -----	69
Figure 4-4:	Temporal change in maize height in the control, centre and edge of CO ₂ plots-----	71
Figure 4-5:	Temporal change in maize height in herbicide and the control plots-----	71
Figure 4-6:	Mean number of maize cobs in CO ₂ and herbicide treated plots with their control-----	72
Figure 4-7:	Mean number of tillers in CO ₂ and herbicide treated plots with their control-----	73
Figure 4-8:	Mean number of maize grains in CO ₂ and herbicide treated plots with their control-----	73
Figure 4-9:	Mean fresh and dry weight of maize leaves in CO ₂ and herbicide treated plots with their control-----	73
Figure 4-10:	Mean fresh and dry weight of maize stem in CO ₂ and herbicide treated plots with their control-----	74
Figure 4-11:	Change in soil pH following CO ₂ injection in maize --	77

Figure 4-12:	Change in soil pH following herbicide application in maize crop-----	77
Figure 4-13:	Average chlorophyll content for Low, high gas, herbicide treatment and their controls-----	79
Figure 4-14:	Reflectance spectra of maize grown in gas control plots-----	80
Figure 4-15:	Reflectance spectra of maize grown in low gas concentration zone-----	80
Figure 4-16:	Reflectance spectrum of maize grown in high gas concentration zone-----	81
Figure 4-17:	Reflectance spectra of maize grown in herbicide control plots-----	81
Figure 4-18:	Reflectance spectra of maize grown in herbicide treated plots-----	81
Figure 4-19a-g:	Reflectance difference between control, high and low gas region for maize grown in gassed plots on respective dates-----	83-85
Figure 4-20a-g:	Reflectance difference between control and herbicide treated maize on various dates-----	86-88
Figure 4-21:	First derivative of reflectance peaks of maize grown on control plots-----	89
Figure 4-22:	First derivative reflectance peaks of maize grown on low CO ₂ zone-----	90
Figure 4-23:	First derivative reflectance peaks of maize grown on high CO ₂ zone-----	90
Figure 4-24:	First derivative reflectance peaks of maize grown on herbicide control plots -----	91
Figure 4-25:	First derivative reflectance peaks of maize grown on herbicide treated plots-----	91
Figure 4-26:	Temporal change in red edge position over time for maize control and CO ₂ treatments -----	93
Figure 4-27:	Temporal change in red edge position for maize control and herbicide treatment -----	94
Figure 4-28:	Relationship between red edge position and chlorophyll content-----	94
Figure 4-29:	Temporal change in chlorophyll Normalised Difference Index (Chl NDI) for maize crop treated with CO ₂ and corresponding control plots -----	97
Figure 4-30:	Temporal change in chlorophyll Normalised Difference Index (Chl NDI) for crop treated with herbicide and corresponding control plots	97

Figure 4-31:	Temporal change in Pigment Specific Simple Ratio for chlorophyll a (PSSRa) in CO ₂ and corresponding control plots-----	98
Figure 4-32:	Temporal change in Pigment Specific Simple Ratio for chlorophyll a (PSSRa) in herbicide treated and corresponding control plots-----	99
Figure 4-33:	Temporal change in Pigment Specific Simple Ratio for chlorophyll b (PSSRb) in CO ₂ and control plots ---	99
Figure 4-34:	Temporal change in Pigment Specific Simple Ratio for chlorophyll b (PSSRb) in herbicide treated and control plots -----	99
Figure 4-35:	Temporal change in Physiological Reflectance Index (PRI) in CO ₂ plots and control plots -----	101
Figure 4-36:	Temporal change in Physiological Reflectance Index (PRI) in herbicide treatment and control plots -----	101
Figure 4-37:	A diagram showing original reflectance measured on 4 th June 2010 and the spectral regions (continuum line) where continuum removal was applied -----	102
Figure 4-38:a-e	Mean continuum removed reflectance at 400 - 550nm for maize crop grown on CO ₂ control, low and high gassed zone measured during the experiment-----	103-105
Figure 4-39:a-e	Mean continuum removed reflectance at 550nm-750nm for maize crop grown on CO ₂ control, low and high gassed zone measured during the experiment-----	106-108
Figure 4-40a-e:	Mean continuum removed reflectance at 400-550nm for maize crop grown on herbicide treated maize crop and its control-----	109-111
Figure 4-41a-e:	Mean continuum removed reflectance at 550-750nm for herbicide treated maize crop and its control-----	112-114
Figure 5-1:	Vertical view of barley showing control plot and stress in the plot centre-----	120
Figure 5-2:	40% herbicide and 20% herbicide treatment-----	121
Figure 5-3:	10% herbicide and 5% herbicide treatment-----	121
Figure 5-4:	Mean number of barley plants in the control and CO ₂ gassed plots -----	122
Figure 5-5:	Mean length of barley plants in the control and CO ₂ gassed plots -----	123
Figure 5-6:	Total number of barley tillers in the control and CO ₂ gassed plots -----	123
Figure 5-7:	Total number of barley grains in the control and CO ₂	124

gassed plots

Figure 5-8:	Fresh and dry weight of barley ears in the control and CO ₂ gassed plots -----	124
Figure 5-9:	Fresh and dry weight of barley stems in the control and CO ₂ gassed plots -----	125
Figure 5-10:	Mean number of barley plants in control with herbicide dose-----	126
Figure 5-11:	Mean length of tillers in control with herbicide dose rate-----	126
Figure 5-12:	Total number of tillers in control with herbicide dose rate-----	126
Figure 5-13:	Number of barley grains in control with herbicide dose rate-----	127
Figure 5-14:	Fresh and dry weight of barley ears in control with herbicide dose rate-----	127
Figure 5-15:	Fresh and dry weight of barley stems in control with herbicide dose rate-----	127
Figure 5-16:	Change in soil pH following CO ₂ injection in barley crop-----	129
Figure 5-17:	Average chlorophyll content for control, low and high CO ₂ zones-----	130
Figure 5-18:	Average chlorophyll content for control and herbicide treated plots-----	130
Figure 5-19:	Reflectance spectra of barley grown on CO ₂ control plots-----	132
Figure 5-20:	Reflectance spectra of barley grown on low CO ₂ zones-----	132
Figure 5-21:	Reflectance spectra of barley grown on high CO ₂ zones-----	132
Figure 5-22:	Reflectance spectra of barley grown on herbicide control plots-----	133
Figure 5-23:	Reflectance spectra of barley grown on 5% herbicide treated plots-----	133
Figure 5-24:	Reflectance spectra of barley grown on 10% herbicide treated plots-----	133
Figure 5-25:	Reflectance spectra of barley grown on 20% herbicide treated plots-----	134
Figure 5-26:	Reflectance spectra of barley grown on 40% herbicide treated plots-----	136

Figure 5-27:a-e: Reflectance difference between control, high and low gas regions for barley grown on gassed plots on the given dates-----	134-136
Figure 5-28:a-e: Reflectance difference between control and the different levels of herbicide treatment-----	136-138
Figure 5-29: First derivative peaks of barley grown on control plots-----	138
Figure 5-30: First derivative peaks of barley grown on low gassed zone-----	139
Figure 5-31: First derivative peaks of barley grown on high gassed zone-----	139
Figure 5-32: First derivative peaks of barley grown on herbicide experimental plots measured on 10 th of June 2010, a day after application of herbicide-----	139
Figure 5-33: First derivative peaks of barley grown on the herbicide experimental plots measured on 21 st of June 2010 (13 days after application of herbicide)---	140
Figure 5-34: First derivative peaks of barley grown on herbicide experimental plots measured on 9 th of July 2010 (30 days after application of herbicide)-----	140
Figure 5-35: Temporal change in red edge position for gassed plots-----	144
Figure 5-36: Temporal change in red edge position for herbicide treatment-----	142
Figure 5-37: Temporal change in Chlorophyll Normalised Difference Index (Chl NDI) for barley crop grown on CO ₂ and control plots -----	143
Figure 5-38: Temporal change in Chlorophyll Normalised Difference Index (<i>Chl NDI</i>) for barley crop grown on herbicide and control plots -----	144
Figure 5-39: Temporal change in Pigment Specific Simple Ratio for chlorophyll a (PSSRa) in barley grown on CO ₂ and control plots -----	145
Figure 5-40: Temporal change in Pigment Specific Simple Ratio for chlorophyll a (PSSRa) for barley grown on herbicide treated and control plots -----	146
Figure 5-41: Temporal change in Pigment Specific Simple Ratio for chlorophyll b (PSSRb) for barley grown on CO ₂ and control plots -----	146
Figure 5-42: Temporal change in Pigment Specific Simple Ratio for chlorophyll b (PSSRb) for barley grown on herbicide treated and control plots	146
Figure 5-43: Temporal change in Physiological Reflectance Index	147

(PRI) for barley grown on CO₂ and control plots

Figure 5-44:	Temporal change in Physiological Reflectance Index (PRI) for barley grown on herbicide treated and control plots -----	148
Figure 5-45:a-e:	Continuum removed mean reflectance at 400 - 550nm for barley crop growing on CO ₂ control, low and high gassed zone measured during the experiment-----	149-152
Figure 5-46:a-e:	Mean Continuum removed reflectance at 550 - 750nm for barley crop growing on CO ₂ control, low and high gassed zone measured during the experiment-----	152-154
Figure 5-47:a-f:	Mean Continuum removed reflectance at 400 - 550nm for barley crop growing on control and different levels of herbicide treatment measured during the experiment-----	154-157
Figure 5-48:a-f:	Mean Continuum removed reflectance at 400 - 550nm for barley crop growing on control and different levels of herbicide treatment measured during the experiment-----	157-160

List of Tables

Table 2-1: List of natural and anthropogenic stress factors acting on terrestrial vegetation-----	19
Table 2-2: Absorption features of vegetation spectra -----	36
Table 4-1: Average maize crop characteristics in the control, centre and plot edge measured in the field at t=33 for CO ₂ experiment-----	70
Table 4-2 Average maize crop characteristics of the control and herbicide treated plots measured in the field at t=33-----	71
Table 4-3 Summary of two-way repeated measures ANOVA to determine the effect of elevated soil CO ₂ on plant height, tiller number plant ⁻¹ , cob number plant ⁻¹ and leaf chlorophyll content-----	76
Table 5-1 Summary of indices used in the study and detectable day of stress in maize and barley-----	164

List of abbreviations

CCS	Carbon Capture and Storage
REP	Red-edge position
HRS	Hyperspectral remote sensing
ChI NDI	Chlorophyll Normalised Difference Index
PSSRa	Pigment Specific Simple Ration for chlorophyll a
PSSRb	Pigment Specific Simple Ration for chlorophyll b
PRI	Physiological Reflectance Index
CO ₂	Carbon-dioxide
O ₂	Oxygen
CH ₄	Methane
Nm	Nanometer
Cm	Centimeter
m	Meter
NIR	Near Infra-red
SWIR	Shortwave Infra-red
EOR	Enhanced oil recovery

CHAPTER ONE

INTRODUCTION

1.1 Scientific context.

Global warming is considered a major threat all over the world due to its consequences such as sea level rise, threats to agricultural production and loss of biodiversity (IPCC, 2006, Male *et al.*, 2010, Lakarraju *et al.*, 2010). This problem is driven by increasing greenhouse gasses; one of the major greenhouse gases is carbon-dioxide (CO₂). The rate at which the concentration of atmospheric CO₂ is rising is alarming and demands urgent attention by policy makers, researchers and industrialists (Klausma, 2003). There has been a steady ascent of atmospheric CO₂ concentration from 280 parts per million (ppm) prior to the industrial revolution to about 380 ppm in 2005 (Keeling and Whorf 2005) and this is expected to increase at an average rate of 1.5 ppm per year (IPCC, 2005).

In 2010 the annual average was 389.78 ppm (Mauna Loa observatory report, Hawaii, 2010). Figure 1-1 shows the rate of historical change in atmospheric CO₂ concentration. A large rise in demand for fossil fuels is also projected which could result in an increase in atmospheric CO₂ concentration to three times pre-industrial levels by 2100 and an increase in global mean temperature of up to 5.8°C (IPCC, 2007a).

The increase in temperature is compelled by greenhouse gases. Foremost are CO₂ emissions released as a result of burning fossil fuels and biomass as fuel and from several industrial processes such as ethanol and cement manufacturing industries, as well as deforestation and change in land-use. Methane (CH₄) formed from livestock wastes, landfill sites, coal production

and natural gas together with nitrous oxide (N₂O) and fluorinated gasses also contribute. CO₂ is the major contributor to climate change accounting for 76.7 %, methane 14.3 %, fluorinated gasses 1.1 % and N₂O 7.9 % (IPCC, 2007a).

The threats caused by global warming have culminated in the set-up of several bodies such as IPCC whose main responsibility is addressing scientific problems associated with climate change issues in order to proffer ways of managing the risks through adaptation and/or mitigation options.

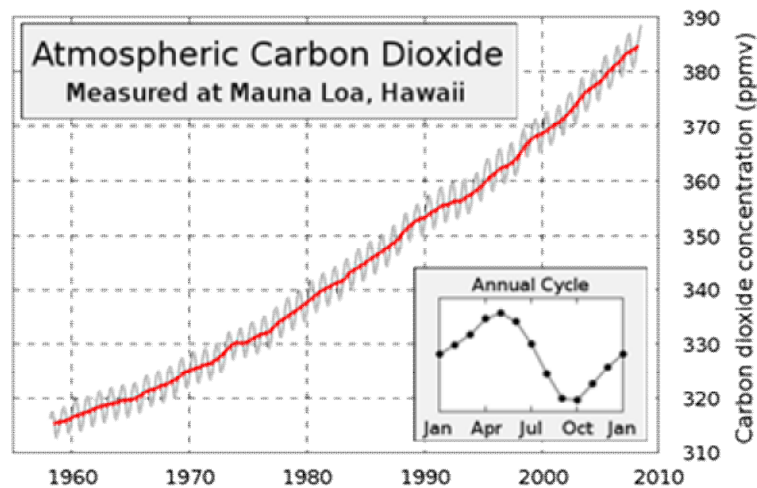


Figure1-1. Rate of historical change in atmospheric CO₂ (Source: Mauna Loa observatory Hawaii, USA.)

1.2 Mitigation options for atmospheric CO₂ emission.

For centuries fossil fuels have provided a cheap and reliable source of energy to humanity, but the continued release of large amount of greenhouse gases poses the threat of global warming (Mills, 2011).

In order to alleviate the increase in atmospheric CO₂, many alternatives have been proposed. These include increasing the efficiency of fuel

conversion processes (Turkenburg, 1997), switching to lower carbon alternatives such as from coal to natural gas (Herzog, 2001, 2004), increasing the use of renewable energy sources (Silver *et al.*, 2004), or nuclear power, enhancing the absorption of atmospheric CO₂ by natural systems (Roxburg *et al.*, 2006), and CO₂ capture and storage (CCS), (Holloway, 1997, Bruant *et al.*, 2002, Mills, 2011).

The effectiveness of any of the above mentioned mitigation strategies will depend on the cost, risk, performance and availability of the technology (IPCC, 2007b). Some of these alternatives may be cheap in the short-term but expensive in the long-run due to high cost of maintenance, making them unaffordable to the poor people who constitute a large percentage of the world population. Invariably these strategies do not address the untenable amount of fossil fuel powered infrastructure and the CO₂ already released into the atmosphere. An immediate switch from fossil fuels as a major source of energy supply is not feasible for now, due to the long life-span of infrastructure; such a move could have negative effects on economies (IEA GHG report, 2008).

One of the emerging technologies considered vital in reducing the rise in atmospheric CO₂ concentration is Carbon Capture and Storage (CCS). CCS could contribute about a quarter of the reduction in emissions needed to control global warming (Marland *et al.*, 2005, Defra, 2006) potentially more than other alternatives such as renewable energy sources, enhancing the absorption of atmospheric CO₂ by natural systems, nuclear power, energy efficiency and coal to gas substitution (IPCC, 2007b). Figure 1-2 illustrates these scenarios.

Carbon Capture and Storage (CCS) involves the confinement of the CO₂ generated from the burning of fossil fuels from industrial processes, and other greenhouse emission facilities and separating it from other component of gases. This is then transported to a safe storage location where it will be stored away from the atmosphere for long term isolation.

There are several suggestions for the long-term storage of CO₂, including deposition into water column in the deep ocean bed and injecting into geological formations, also known as CCS. There are three main options for geological storage of CO₂: depleted oil and gas reservoirs, deep saline aquifers and unmineable coal beds (Freund *et al.*, 2003, IPCC, 2005).

The execution of CCS is faced with several challenges. The level of awareness of the public with regards to CCS is relatively low. There is great uncertainty in the population about the importance of capturing CO₂ which has resulted in a major challenge to its implementation (Oltra *et al.*, 2010, Pollak *et al.*, 2011, De Best-Waldhober *et al.*, 2011).

Another major challenge is the possibility of leakage, as a result of several factors. Even though the probability of the leak is small (Al-Traboulsi *et al.*, 2012b) it is worthy of investigation due to the causes of leakage, such as failure from injection well resulting in minor seepage, leakage through undetected faults, fractures as well as during transportation of captured CO₂. It is important to know that leaks may also be caused by pipeline failure, engineering/construction default, or failure of seals at pipeline joints (for details refer to section 2.2). Therefore, early leak detection from geological storage sites is important to assure the public of its safety. One way of doing this may be through remote sensing of the environment

within the CO₂ sequestration sites using the stress response of vegetation as a proxy indicator (Steven *et al.*, 2010). The methods of detection and monitoring of CO₂ will be discussed more fully in section 1.3. However, the main focus of this thesis is CO₂ leak detection using remote sensing as CCS can play a major role in mitigating climate change issues. CCS involves deep burial of CO₂ in geologic formations, depleted oil and gas reservoirs, deep saline aquifers and unmineable coal beds as discussed in section 1.2. However, leaks are unlikely but possible. Leak detection is therefore necessary in the event of any occurrence for the purpose of safety of the environment and the entire ecosystem (plants and animals) as well as for accounting to know the quantity of CO₂ leaked and the cost and benefits of its early detection.

Various methods have been proposed for leakage detection such as the use of flux towers, laser based instruments, and detection of abnormal soil CO₂ levels using sampling equipment (further details can be found in section 1.3).

Monitoring of CO₂ leaks above the sequestration field is a basic requirement to show that CO₂ is effectively been stored. This could be done by using the spectral signatures of plants growing on/around the storage site. A healthy signature shows that the storage facility is not being compromised and assures the public of its safety and that of the ecosystem in general.

Remote sensing can be regarded as one of the ways forward because of the detailed information it can provide in terms of spatial, temporal, and spectral properties of the vegetation on CO₂ sequestered sites. It can be

used for long term monitoring and detection of several changes in vegetation (Pickles and Cover, 2005). Analyses of plant spectral signatures using hyperspectral remote sensing technique have been found to be a useful tool in vegetation stress study (Noomen, 2007).

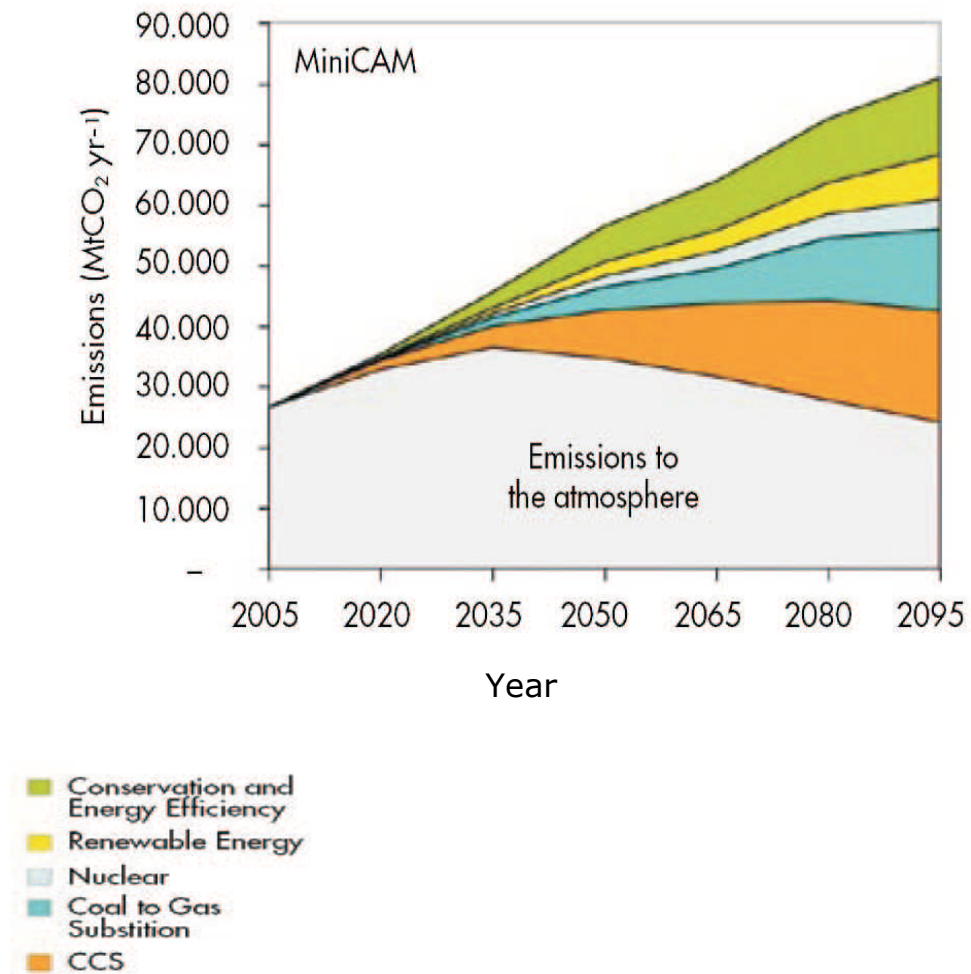


Figure1-2. Illustrative example of the potential global contribution of CCS based on an alternative integrated assessment model (MiniCAM) from the IPCC Special Report on Carbon Dioxide Capture and Storage, 2007b.

Figure 1-2 shows an example of modelled predictions of the contributions made by several methods to reduce overall atmospheric CO₂ emissions. CCS plays a significant role in mitigating climate change. Within the period

modelled the contribution made by CCS is about a quarter of the total provided by a range of alternative methods.

Currently, there are a number of existing CCS projects around the world. Prominent amongst them are: the Sleipner project in the North Sea and the CO₂-EOR projects at Weyburn in Saskatchewan, Canada and Rangely, in Colorado, USA (Mills, 2011). The Sleipner project commenced in 1996 and it has stored approximately 1 million tonnes of CO₂ annually. The total storage capacity through the life time of this facility is 20 million tonnes. 5000 tonnes of CO₂ is stored daily in the Weyburn project which began operation in 2000; it has a total storage capacity of 20 million tonnes. The Rangely Project has stored about 23 million tonnes of CO₂ from its inception in 1986 (Mills, 2011).

Figure 1-3 shows some existing CCS projects around the world and proposals in place for commencement.

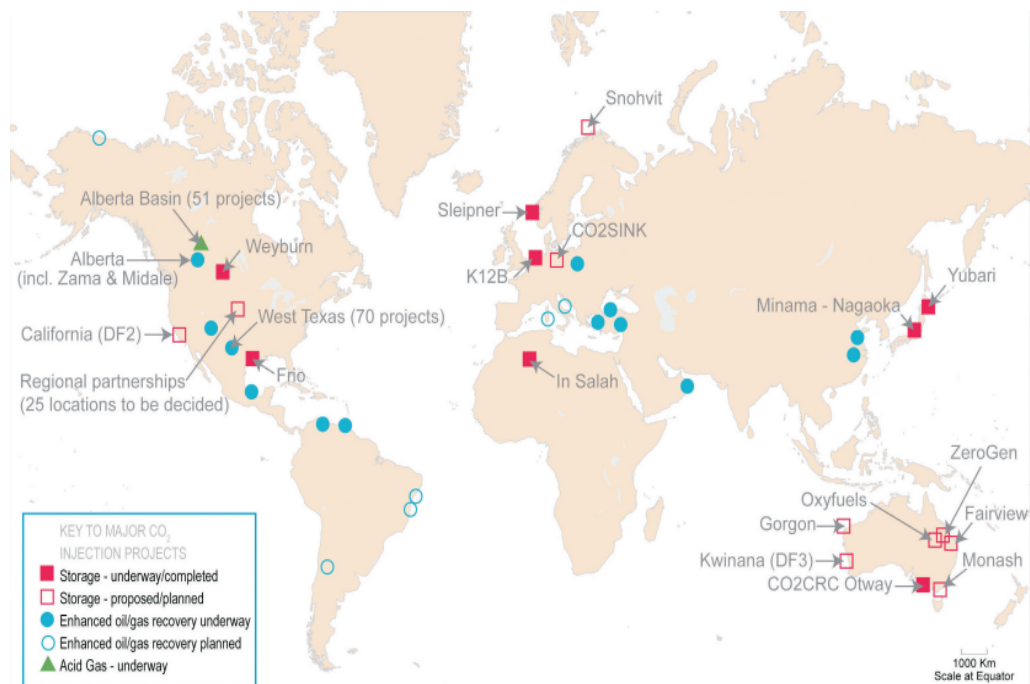


Figure1-3. Geologic storage and related projects around the world (IPCC, 2005)

Figure 1-3 shows existing and proposed geologic storage facilities around the world to address climate change issues. Most are research development or demonstration projects. Several are part of industrial facilities in commercial operation.

1.3 Detection and monitoring of CO₂ leaks.

The current knowledge with regards to detection, monitoring, verification and reporting of the exact leakage rate and consequences associated with it is very minimal (IPCC, 2005). Therefore, the deployment of CCS as a mitigation strategy for climate change related issues will require adequate understanding and experience.

Due to the nature and effects of CO₂ leak from storage facilities, the need for an efficient and effective method of surveillance is paramount in order to curb the attendant consequences. Sampling of the soil and air can be carried out on the surface whilst any changes deep underground can be monitored by geochemical sampling of production fluids, tracking reservoir pressure, detecting sound (seismic), electromagnetic, gravity or density changes within the rock formations (IPCC, 2007a, Mills, 2011).

One alternative approach may be monitoring of atmospheric CO₂ concentration within the storage vicinity using several techniques (such as fixed monitoring using flux towers and laser based equipment). The detection of elevated atmospheric CO₂ could be problematic because of dilution as it passes from the ground to the atmosphere (Leuning *et al.*, 2008, Pollak and McCoy, 2011) which makes it difficult to distinguish between CO₂ fluxes from industrial processes near geologic storage sites.

Barr *et al.* (2011) carried out a study at the Zero Emission Research and Technology (ZERT) facility in Bozeman Montana, USA to detect above and below ground elevated CO₂ concentration with the aid of two laser based instruments. In this study it was found that the CO₂ leak detectable was 0.3 t CO₂ day⁻¹. However, the above ground CO₂ was more affected by rainfall events than underground. This result indicates that rainfall did not have much effect on the concentration of soil CO₂ and that it is easier to measure soil CO₂. The possible explanation for the difference in the above ground measurements is the natural variability in atmospheric CO₂ concentration, (Klausman, 2011), daily variation in solar insolation, soil moisture and temperature (Burba and Anderson, 2010).

Monitoring of soil CO₂ level can also be carried out at intervals using sampling instruments to determine abnormal concentration due to leaks. One technique that has been applied is to study the effects of elevated concentration of soil CO₂ on canopy reflectance, plant growth and development (Smith *et al.*, 2005a, 2005b, Noomen *et al.*, 2009). The main limitation of this technique is that it could be time consuming, especially when dealing with large area as it will require sampling of several points.

1.4 Stress monitoring by remote sensing.

Stress has many effects on plants; the severity depends on a variety of factors such as the condition and time of plants exposure to it, growth stage and duration. Remote sensing has great potential for stress studies and a large body of literature exists on response of vegetation to stress (Huang *et al.*, 1997a, 1997b, Blackburn and Steele, 1999, Carter and Knapp 2001, Richardson *et al.*, 2002, Steddom *et al.*, 2005, Christian *et al.*, 2011).

In this thesis the term stress will be used in association with elevated CO₂ concentration in the soil and sub-lethal herbicide application to plants. The resultant effect is stress on the vegetation. To fully study the effects of stress on plants and its influence on spectral reflectance, the vegetation needs to be subjected to stress in a controlled condition, this will enable measurements to be undertaken and temporal changes observed. For accuracy and comparison purpose the data acquired needs to be normalised with a control that has no stress effects as a result of any inducing agents. Laboratory controlled experiments at leaf level could be used for such studies; this can be scaled-up to canopy level and then the field which is the natural plant growth environment. This study was carried out in the field at the Artificial Soil Gassing and Response Detection (ASGARD) facility, University of Nottingham, United Kingdom. This facility has been designed such that CO₂ and other gases can be injected into the soil to enable the study of response of plants to any changes (refer to section 3.2 for details).

Spectral reflectance measurements by Smith *et al.* (2004a) showed that vegetation exposed to high concentrations of natural gas in the soil had significantly increased reflectance in the visible region and decreased reflectance in the near-infrared. Several researchers have identified similar responses to a wide range of plant stresses such as water logging, nutrient stress, heavy metal toxicity and soil oxygen deficiency (Horler *et al.*, 1983, Milton *et al.*, 1989, Carter, 1993, Carter and Miller, 1994, Noomen *et al.*, 2008). 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 (Zhang *et al.*, 2008, Moorthy *et al.*, 2008).

Spectral indices and wavelength regions, such as the red edge which is a transition zone between the red and near-infrared have been used for detecting stress in a wide range of plant species (Carter, 1994, Sims and Gamon, 2002, Smith *et al.*, 2005b, Noomen, 2007). 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 ratio, such as Normalised Difference Vegetation Indices (NDVI) and Soil Adjusted Vegetation Indices (SAVI) based on reflectance spectroscopy. These 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).

1.5 Discriminating stress- can it be done?

Remote sensing has been used as a tool for the monitoring of health status of vegetation over time in order to identify the cause(s) and find ways to mitigate the effects on plants (Evans *et al.*, 2002, Lakkaraju *et al.*, 2010).

Stress discrimination using remote sensing is a major problem (Male *et al.*, 2010), because there could be several causes of stress affecting the terrestrial ecosystem at the same time. It is therefore important to find ways and methods of distinguishing between the causes of different stress types and/or different levels of stress.

Several studies have shown that similar spectral responses may result from different stress effects which make it difficult to discriminate between the

causative factors. Smith *et al.* (2005b) found that in oilseed rape (*Brassica napus*) there was no significant difference between the spectral reflectance of plant leaves stressed via elevated concentration of natural gas and those stressed via herbicide application. Likewise, other studies have suggested that the use of remote sensing alone cannot distinguish between different causes of stress (Carter, 1993, Smith *et al.*, 2005b).

There are different types of stresses which are likely to affect the terrestrial ecosystem (refer to Table 2-1 for details). The nature and response of plants to stresses may differ due to species, cultivar, and stress type as well as the development stage of the plant at the time of stress (Zaid *et al.* 2003).

Plants could be referred to as either C3 or C4 based on photosynthesis and atmospheric CO₂ utilisation, as such their tolerance to stress could vary. The level of atmospheric CO₂ concentration at any point in time could limit C3 photosynthesis as opposed to C4 plants which tend to be almost saturated with CO₂.

Retardation in growth rate has been found in vegetation subjected to elevated soil CO₂ but the actual way in which this occurs have not been fully understood (Bruant *et al.*, 2002). Most likely, the CO₂ displaces oxygen to the roots of the plant which is vital for the root functions of water and nutrient uptake (Noomen *et al.*, 2008). The resultant effects may be stunted growth, discoloration and death (Smith *et al.*, 2004b).

In this study the elevated soil CO₂ was largely confined to the root zone so that any effect on the plant is likely to be noticed first in the root function before other parts of the plant.

To test whether stress induced by soil CO₂ can be distinguished from other stresses, an alternative source of stress was studied in parallel with soil gassing. Herbicide (Glyphosate) at sub-lethal dose was selected as its mode of action on the plant has similarities to that of CO₂ in that it primarily affects the plant roots. The effects usually start from the root upward by blocking the biosynthesis pathway and accumulate in areas of active growth called meristems, leading to lack of protein synthesis (Ashton and Crafts, 1981, Adcock *et al.*, 1990). Once the root is affected, respiration, nutrient and water uptake become major problems leading to dehydration, desiccation, asphyxiation and ultimately death of the plant.

1.6 Research aim.

The primary aim of this study is to assess the potential of remote sensing to detect CO₂ leakage from CCS repositories. Further to this, the capability of remote sensing to discriminate between stresses with similar mode of action is explored.

1.7 Research objectives.

The research objectives are:

1. To further the understanding of the impacts of CO₂ stress on crop growth and development.
2. To compare stress responses in a C3 and a C4 crop using hyperspectral remote sensing.
3. To explore the capability of hyperspectral remote sensing in detecting CO₂ stress responses with a contrasting stress that affects the roots of crops.

4. To evaluate the effectiveness of a range of hyperspectral analysis techniques in stress detection and discrimination.

1.8 Research hypotheses.

The research hypotheses to be tested in this thesis are:

1. The exposure of plants to elevated soil CO₂ concentration significantly affects their survival, growth and development relative to an unstressed site.
2. Remote sensing can discriminate between stresses with a similar mode of action (i.e. root stressors) using plants spectral response.

These hypotheses will be tested using analysis of variance (ANOVA), with the results evaluated at the conventional 0.05 level of statistical significance (Field, 2012, Al-Traboulsi *et al.*, 2012b)

1.9 Thesis outline.

The outline of this thesis is as follows:

Two major field based experiments form the basis of this thesis. These experiments were conducted in 2009 and 2010 respectively on maize (*Zea mays*) and barley (*Hordeum vulgare v Concerto*). Both experiments deal with the effects of elevated soil CO₂ and different levels of herbicide treatment on vegetation growth and reflectance.

Chapter one describes the background to the study and introduces the research aims, objectives and thesis outline. In chapter two a detailed literature review is presented on remote sensing of vegetation as well as CO₂ stress and how this impacts on the vegetation. While chapter three focuses on the general methodology used in both experiments, in terms of

measurement techniques, sampling methods and experimental designs. Chapter four describes the effects of CO₂ and herbicide stress on the spectral reflectance and physiological properties of maize, in terms of the effects of low and high CO₂ concentrations on maize reflectance, growth and morphology as well as discriminating CO₂ stress from herbicide treatment. Chapter five deals with remote sensing of barley stressed with CO₂ and different levels of herbicide stress, with the aim of investigating whether these stressors can be discriminated from one another using canopy reflectance and other plant physiological parameters. In chapter six, a summary of the results is presented and the possibilities of using remote sensing for the detection, monitoring and discrimination of stresses are discussed.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction.

This chapter will provide a general overview of the risks of leakage from CCS facilities, stress concept, effects and response of plants to stress, culminating in a review of carbon dioxide enrichment in the soil and the atmosphere and their effects on the growth and development of plants. Remote sensing applications with specific reference to vegetation are discussed.

2.2 Impacts of leakage from CCS.

Leakage from CCS facilities could occur in several ways. Failure from injection wells (e.g. failure of geological storage cap) resulting in unexpected and gradual leakage through undetected faults, fractures or wells (IPCC, 2005). Minor seeps of gas may also diffuse through the storage media and up to the surface causing an increase in CO₂ concentration (Klausman, 2003).

There is also the potential risk of leakage during transportation of captured CO₂ (e.g. by pipelines, rail and road tankers, or marine tankers) from production sites to storage locations (Steven *et al.*, 2010). These leaks may arise from pipeline failure, engineering/construction default, or failure of seals at pipeline joints. Whatever is the cause of the leaks there could be attendant consequences (Mazzoldi *et al.*, 2008).

Leakage of CO₂ from CCS may have several effects on the ecosystem; it may cause a rise in the soil CO₂ concentration, perhaps to as high as 100% or it may diffuse through the soil to cause a moderate rise in the natural levels of CO₂ in the soil atmosphere (Klausma, 2003). This situation will

have effects on both the vegetation and animal life living within the soil (Steven *et al.*, 2010).

The environmental impacts of elevated concentration of CO₂ in the shallow sub-surface and soil include: stress on the plants and animals and contamination of ground water.

Soil CO₂ concentration of between 2-8% can cause vegetation stress (Airgas, 2002, Male *et al.*, 2010). This can be attributed either to the displacement of O₂ which is important for root respiration or the direct effect of the elevated CO₂ (Al-Traboulsi *et al.*, 2012a, Smith *et al.*, 2004a). The occurrence of leaks will result in the excess CO₂ in the top layers of soils stressing the vegetation above the sequestration sites and its environs, which could manifest in the form of changes in the plants spectral signatures (Male *et al.*, 2010).

Atmospheric CO₂ enrichment following leakage is likely to happen regardless of the manner of leakage as CO₂ diffuses through the soil to the atmosphere. This is seen in areas of natural carbon dioxide springs (NCDS) where soil above the spring contains high concentration of CO₂ but there is also enrichment of CO₂ in the air above the leak. This could lead to a range of responses such as change in colour of the vegetation and death, where atmospheric concentration of CO₂ is between 2000-8000 ppm there can be changes in species composition due to competition between different species (Lichtenthaler, 1998) and where atmospheric concentration is raised moderately (2-3 times normal atmospheric concentration) vegetation growth is enhanced (Kaligarić, 2001).

Concentrations of CO₂ above 2% in the atmosphere are toxic to human health resulting in problems to respiratory system; its persistence could lead to death (Pfanzer *et al.*, 2004).

2.3 Stress in plants.

Plants respond to stress in several ways such as changes in leaf area, leaf pigments and shape. Some are direct consequences of stress, while others are plant adaptations, and some may be either (Zhao *et al.*, 2005, Campbell *et al.*, 2007).

Significant leaks from CCS facilities could result in plant stress. This can manifest in different forms, such as abnormal growth and development, change in leaf colour, decrease in chlorophyll and other pigments and decrease in yield (Hoeks, 1972, Read *et al.*, 2002, Sims and Gamon, 2002, Smith *et al.*, 2005b).

Plants can also adapt to different environmental conditions to mitigate the effects of stress, such as high/low light growth conditions at any particular point in time (Lichtenthaler, 1996, 1998). Such adjustments could be in the form of modifications in number and density of stomata, size and thickness of leaves. This helps the plants become tolerant to stress. However, if the stress coping mechanism is overworked and the stressor is not removed, this can lead to serious damage and death (Larcher, 1987). There are several stress factors acting on terrestrial vegetation (Table 2-1). The mode in which they affect plants differs, and one or more stresses may be acting on plants at the same time.

Table 2-1. Natural and Anthropogenic Stress Factors 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, herbivores
- Viral, fungal, and bacterial pathogens
- Wind, flood and other natural disasters

II. Anthropogenic stress factors:

- Herbicides, pesticides, fungicides
- Air pollutants (e.g., SO₂, NO, NO₂, NO_x)
- Ozone (O₃) and photochemical smog
- Formation of highly reactive oxygen species
- 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 NO₃⁻ deposits), competition
- Heavy metal load (lead, cadmium, etc.)
- Overproduction of NH₄⁺ in breeding stations
(Uncoupling of electron transport)
- Increased UV radiation (UV-B and UV-A)
- Increased CO₂, global climate change

Lichtenthaler (1998)

Some of the factors listed above have direct link with the present study, depending on the magnitude (e.g. increased CO₂, global climate change, acid pH of soil and water, herbicides, pesticides, fungicides, air pollutants). Others may have indirect effects that interact with plant responses to soil CO₂ (e.g. sudden and late frost, water shortage, long rainy periods, insects, herbivores, viral, fungal, bacterial pathogens and mineral

deficiency of the soil). However, the list is not exhaustive and does not include some key factors, such as soil gases which is the focus of this study.

There is an understanding that in some species environmental stress may improve plants growth due to growth stimulation or relative competitive advantage. For example, Lichtenthaler (1998) suggested that a mild stress may trigger cell metabolism and boost the physiological action of the plant, without resulting in effects that will cause damage to the plant even at a long duration. On the other hand, elevated and prolonged stress can cause damage to the plant and induce early senescence and death if the stressor is not removed (Smith, 2002). According to Hansen *et al.* (2002) the optimal environmental condition for plant growth is difficult to come by because it varies, as does the adaptability of various plant species to change.

In the absence of stress, the plant is said to be in a stable condition physiologically and chemically in relation to the amount of light, water, and mineral supply conditions. However, when stress begins to manifest, this standard condition is disrupted leading to a reduction in vitality from the normal condition.

The stress concept, originally developed by Hans Selye in 1936 categorised the plant's stress responses into four different phases: Response phase, restitution phase, end phase and regeneration phase (Figure 2-2).

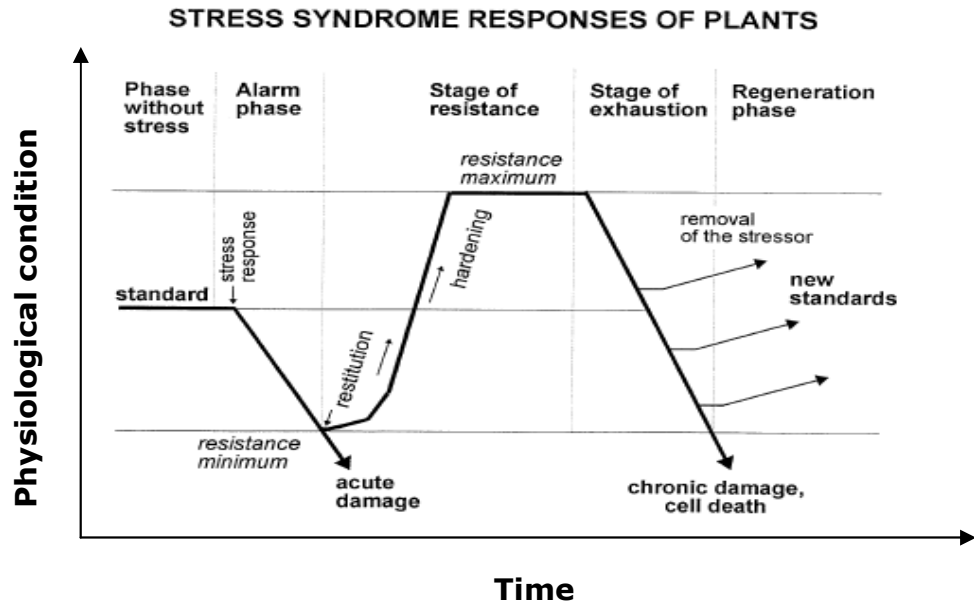


Figure 2-1. General concept of the phase sequences and responses induced in plants by stress exposure (Lichtenthaler, 1996).

As discussed by Lichtenthaler (1996), at the beginning of stress the plant is subjected to malfunction of vital process of growth and development leading to decline in vitality, photosynthesis, transport of metabolites, and/or uptake and translocation of ions. Plants with little or no tolerance will not survive this stage, leading to rapid death and senescence.

However, those plants that are able to withstand this condition, either through high resistance to stress or adaptation will have to undergo some repair processes in order to cope with the stress so as to establish a new physiological standard.

The end phase is characterised by progressive loss in vitality caused by prolonged stress and inability of the plant to with-stand the stress thereby weakening the plant's capacity to cope with the stress. This will cause severe damage and finally cell death. However, when the stressors are removed at the right time before the senescence processes becomes dominant, the plants will regenerate.

At the regeneration phase, the plant is said to recover partially or in full if the stressor is removed and the damage is not too high, this will determine to which new physiological standard, within the range of resistance minimum and maximum, the plants will move into.

2.3.1 C3 and C4 plants metabolism and their response to stress.

The response of plants to stress may differ due to their utilisation of atmospheric CO₂ (Carmon-Silva *et al.*, 2008). They have different photosynthetic pathways. The two most common ones are C3 and C4; the names come from the nature of the carbon compound formed (Brown, 1999). C3 is formed from 3-carbon molecule while C4 has 4 carbon molecules as the first product of photosynthesis. This is common in monocotyledons.

In C3 plants the conversion of light energy to chemical energy which is used to fix CO₂ and to synthesise carbon compound is usually carried out by single chloroplasts in the leaf mesophyll cells (Flexas and Medrano, 2002). The carbon from CO₂ is fixed into stable organic products.

The C4 photosynthetic path way is more complex and is usually carried out by two distinct chloroplast types. The leaves of C4 plants have extensive vascularisation with a ring of bundle sheath cells surrounding each vein and an outer ring of mesophyll, this anatomy is essential in their photosynthesis (Carmon-Silva *et al.*, 2007).

The main difference between C3 and C4 photosynthesis is the existence of compartmentalisation of activities into two specialised cells and chloroplast in C4 plants (Carmon-Silva *et al.*, 2007). The fixation of atmospheric CO₂ in C4 is a two step process: CO₂ is first fixed at the mesophyll cells by phosphoenolpyruvate carboxylase (PEPC) to form a four carbon compound and later converted to malate or separate; then it diffuses to the inner ring

of the bundle sheath and later decarboxylated in the chloroplast (Leakey, 2006).

C3 plants can survive at low light intensities and temperatures. They are mostly found in regions where the rate of solar intensity/radiation is low due to cloud cover and temperate/wet areas. They have the capability to let in more CO₂ through their stomata in the event of excessive level of water in the plants roots there by making the pathway for their metabolism energy efficient (Flexas *et al.*, 2004). They constitute more than 95% of Earth's plant species (Ehlinger and Monson, 1993). Their photosynthesis is limited by present day atmospheric CO₂ concentrations, while C4 photosynthesis is nearly CO₂ saturated.

C4 plants are mostly found in regions with high temperature and dry environment. They are more tolerant to low levels of water, and their water use efficiency is high (Furbank and Taylor, 1995). They can conserve water by reducing canopy and leaf transpiration (Ghannoum, 2009). Studies have demonstrated that with an increase in atmospheric CO₂ concentration plant biomass of C4 plants increases (Wall *et al.*, 2001, LeCain *et al.*, 2003, Leakey, 2006). Water stress is also ameliorated due to reduced stomatal conductance and improved soil moisture (Leakey, 2004, Ghannoum, 2009).

C3 plants have generally been found to respond more in terms of utilisation of atmospheric CO₂, while C4 plants show little response (Ghannoum *et al.*, 2000, Derner *et al.*, 2003) but others have found a more significant response by C4 crops (Rogers *et al.*, 1994, Leakey, 2006). These differences may be due to soil properties, environmental conditions, and response to atmospheric CO₂ variations (Moscatelli *et al.*, 2001). Kimball *et al.* (1993) and Morgan, (1987) found that there was no significant difference between C3 and C4 crops in terms of stomatal conductivity.

2.4 Plant response to root stress.

Stress can affect any part of the plants, depending on the nature and type. The sensitivity of plants to any kind of stress can be attributed to differences in biochemical mechanisms (Lichenthaler, 1998, Steven *et al.*, 2010).

Root stresses are thought to have a major influence on plant roots before cascading to other parts of the plants. This has a direct impact on the water and mineral uptake of the plant. Some plants are resistant to water stress caused by flooding (Zhang *et al.*, 1995). Others are less prone (Pickering and Malthus, 1998). The presence of aerenchyma provides a gaseous transport route in some plants (e.g. rice) which makes them to be more tolerant to excess amount of water in the root zone (Bergfeld *et al.*, 2006).

Heavy metals in soils can also have some damaging effects on plants, including: reduced growth and visible symptoms such as wilting and changes in leaf colour (Cupers *et al.*, 2011), damage to cell membrane, chloroplast pigments and nucleic acids (Apel and Hirt, 2004). However, the degree of damage or tolerance is species/varieties dependent (Sharma and Dietz, 2009), e.g. sun flower was found to be resistant to metal stress (Cadmium and Zinc) due to the presence of an efficient defence mechanism, especially during growth and ripening (Nehnevajova *et al.*, 2012).

Plant response to salinity stress in the soil is usually first experienced in the roots (Liu *et al.*, 2012). This can be in the form of ion toxicity, reduction in growth, water deficit, nutritional imbalance, cellular damage and possibly death of the plants (Muns, 2005, Sahar *et al.*, 2007). Liu *et al.* (2012) conducted a study to determine the tolerance levels of two plant species:

seashore paspalum (*Paspalum vaginatum*) and centipedegrass (*Eremochloa ophiuroides*). These plants were grown on soils watered with 300mM sodium chloride (NaCl) solution for 20 days. At the end of the experiment there was a decrease in root viability and water uptake ability caused by damage to cellular membrane due to change in protein levels. This was more pronounced in the centipedegrass which showed more sensitivity and less tolerance to salinity stress.

2.5 Plant response to soil gases.

2.5.1 Soil enrichment of CO₂.

Several studies have been conducted on enrichment of CO₂ in the soil and its effects on the flora and fauna community, some are on natural analogues (Sorrey *et al.*, 2000, Kaligalic, 2001, Rodriguez *et al.*, 2005), some on land fill sites (Chan *et al.*, 1997), while others are laboratory based experiments (Bunnell *et al.*, 2002, Boru *et al.*, 2003, Pfanzen *et al.*, 2004, Macek *et al.*, 2005)

2.5.1.1 Natural analogues.

One of the major effects of elevated soil CO₂ on the vegetation is stress, which usually manifests as changes in the plant's vitality. Complete die-off can occur if the CO₂ level is very high. One example of such occurred at Mammoth Mountain California, USA in 1989 after an earthquake (Figure 2-2). It was discovered that a large volume of CO₂ was seeping from beneath the volcano which caused death of several species of trees covering an area of about 50 ha. The total rate of gas emission was in the region of 300 t day⁻¹ (Gerlach *et al.*, 1998). The root of the trees were killed (Figure 2-2) as a result of elevated soil CO₂ concentration between 20 and 95% which impaired the roots O₂ uptake thereby interfering with the plants natural

respiration process, nutrient and water uptake (Sorey *et al.*, 2000, Martini and Silver, 2002).

Other examples of sudden CO₂ emissions from volcanic activities which have resulted in loss of lives and damage to the ecosystem occurred in 1984 and 1986 in which the release of CO₂ at volcanic crater lakes in Lake Monoun and Lake Nyos, in Cameroon, West Africa resulted in the deaths of 1700 and 37 people respectively due to the release of about 1.24 million tonnes of CO₂ (IEA GHG report, 2008).

Natural carbon dioxide springs (NCDS) have also been another conduit for migration of CO₂. Due to the prolonged nature of such leaks the vegetation present may have been able to adapt to higher levels of CO₂. The concentration of CO₂ in the soil profile could rise to as high as 100% but the gas may also be contaminated with methane, nitrogen, and sulphurous compounds (Pfanz *et al.*, 2004, Steven *et al.*, 2010). Although soil CO₂ concentration can be high in NCDS areas, most studies have been on the effects of the resulting increased atmospheric concentration. This can reach 5000 ppm at a height of 50 cm above the surface as the gas diffuses from the soil (Pfanz *et al.*, 2004). In Radenci, Slovenia atmospheric CO₂ concentration above 8000 ppm resulted in complete loss of vegetation and between 2000 and 8000 ppm there was a change in the vegetation species around the NCDS owing to competition (Kaligarić, 2001).



Figure 2-2. Trees killed on the shore of Horseshoe Lake, Mammoth Mountain, California (IEA Greenhouse Gas Research and Development Programme- IEA GHG report, 2008).

Other analogues include landfill and leakage from natural gas pipelines. The main components of landfill gases are methane (55-60%), carbon dioxide (~40%) and traces of oxygen. In terms of global greenhouse gas emission, landfills contribute about 3% of the annual total (Couth *et al.*, 2011). Leakage from natural gas pipelines can cause the soil CO₂ to be elevated while the O₂ is depleted due to oxidation of the natural gas by methanotrophic bacteria (Steven *et al.* 2010).

Some key differences between leaks from CCS facilities and the various analogues discussed are: the possibility of presence of impurities in natural analogues (Steven *et al.*, 2010); the fact that natural analogues are usually long lasting, so that the local environment has time to adapt to changes; and possibly differences in geology as the volcanic areas that generate analogues are unlikely to be used as repositories for CCS.

2.5.1.2 Experimental studies of soil CO₂ enrichment.

Some laboratory studies of soil CO₂ enrichment have also been conducted using different plant species and CO₂ concentrations. The scale of study, specie composition and cultivar (Zaid *et al.*, 2003) and the length of time

the experiment was under taken and at what point of the plant growth stage the treatment was applied (Huang *et al.*, 1994) could be responsible for the type of effects this can have on plants.

Pearce and Sjogersten (2009) investigated the effects of elevated soil CO₂ concentration on vegetation biomass and microbial community biomass, respiration and carbon utilisation in temperate grassland. In this study turfs comprised of *lolium perenne*, *Festuca rubra*, *Festuca rubra commutate* and *Poa pratensis* were subjected to soil CO₂ concentration ranging between 6.2-14.5%. The gassing resulted in reduced above and below ground biomass over time. There was no significant difference in the microbial biomass of carbon utilisation but a trend towards reduced microbial respiration was apparent. In a different study, Soybean (*Glycine max*) subjected to carbon dioxide concentrations ranging from 15% to 50% showed a reduction in leaf "greenness" as well as root and shoot growth, whereas rice (*Oryza sativa*) was not affected by these concentrations (Boru *et al.*, 2003). The resistance of rice was attributed to the presence of aerenchyma which provides gaseous transport route in the plant that enables them to withstand flooding (Bergfeld *et al.*, 2006).

Soybeans and rice are both C₃ crops. In contrast, this present work used a combination of C₃ (Barley) and C₄ (Maize). They were chosen because of differences in photosynthetic path-ways and utilisation of atmospheric CO₂.

Daily injection of CO₂ at the rate of 1 l min⁻¹ on sorghum resulted in reduced root, shoot growth and dry matter yield as well as suppression of iron (Fe) uptake which led to chlorosis, this is possibly due to an increase in pH caused by the production of high HCO₃⁻ while phosphorous uptake was reduced by 10% in that with no treatment. Dry matter yield also reduced, with greatest decrease from 15.2 g pot⁻¹ to 2.8 g pot⁻¹ in soils

with lower moisture content (Matocha and Mostaghimi, 1988). With an increase in CO₂ concentration level to 5 l min⁻¹ the roots of peas (*Pisum sativum*) decreased, compared to the controls; 6.5% CO₂ caused an 80% reduction in root lengths after 13 days. However with up to 0.5% soil CO₂ concentration there was a 7% increase of growth (Stolwijk and Thimann, 1957). A similar result was reported by Bouma *et al.*, 1997 where 2% soil CO₂, a level that is normally found naturally in soils, did not affect root respiration of bean (*Phaseolus vulgaris*). Geisler (1967) studied peas (*Pisum sativum*) and barley (*Hordeum vulgare*) treated with up to 2% soil CO₂ and found stimulatory effect on root growth. These studies have shown that soil CO₂ within the normal level (0-2%) (Pfanzen *et al.*, 2004) could be beneficial to plants while above this rate could be detrimental to plant growth and development.

Arthur *et al.* (1985) found that CO₂ concentrations higher than 18% reduced the growth of tomato plants after seven days of root CO₂ exposure. When the CO₂ concentration was doubled, plants were severely stressed and died off.

High CO₂, (20-60%) could lead to a shallow root system due to sinking of the CO₂ down the soil profile and reduction in the amount of oxygen needed for root respiration (Chang *et al.*, 1991). The uptake of water in sunflower (*Helianthus annuus* L.) roots was inhibited by 40% after three minutes with increasing CO₂ level in soil (Glinka and Reinhold, 1962). The possible reason could be the alteration in the permeability of the osmotic barriers. Zhang *et al.* (1995) indicated that nodule biomass and activities in *Leucaena leucocephala* were reduced significantly by flooding or fumigation with different concentrations of CO₂. It was also found that reducing the soil O₂ level to 10% while increasing the CO₂ level by same level in volume reduced the respiration rates for roots of *Agave deserti*, *Ferocactus*

acanthodes, and *Opuntia ficusindica* (Palta and Nobel 1989, Nobel and Palta, 1989).

2.5.1.3 The effects of elevated CO₂ on soil fauna.

The effects of elevated CO₂ do not only manifest in plants, but may cascade to the soil fauna such as earth worms, millipedes, centipedes and insects. Behavioural reactions ranging from changes in movement to paralysis have been found in soil invertebrates subjected to 10-59% CO₂ concentration and lethal effects occurred in some species treated with between 11-50% CO₂ (Sustr and Simek 1996).

A comparison of soil fauna in two landfill sites in Hong Kong with reference to control sites was made using 4-11% CO₂ concentration. Gross animal density in the landfill sites was three to five times higher than the reference sites. The inference from this study is that landfill sites are unique environments in which the cover soil possesses a high landfill gas content (including CO₂) but that this may not be a limiting factor suppressing the bioactivity of plants, microbes and animals (Chang *et al.*, 1997). The major difference between the two studies is that while Sustr and Simek (1996) studied the effects of a sudden influx of CO₂ on soil invertebrates. Chang *et al.* (1997) were interested in the long term effects of elevated soil CO₂ on soil organisms. Hence the invertebrates would have adapted to the high concentration of CO₂. In the case of leakage from CO₂ storage facilities the effects are more likely to be similar to those of the first study since the soil will be subjected to a sudden increase in CO₂ concentration.

2.5.2 Atmospheric CO₂ enrichment.

Unlike elevated soil CO₂ which could be detrimental to plant growth and development, several studies have demonstrated that atmospheric CO₂

enrichment can be beneficial to plants by enhancing their overall productivity (Kimball *et al.*, 1993, Moscatelli *et al.*, 2001, Morgan *et al.*, 2004). Most studies on the atmospheric effects of CO₂ have been undertaken in open-top chambers (OTCs), which are unable to enclose large plots and have the undesired effect of altering the micro-climates around the experimental plots. An alternative approach to this is Free Air Carbon Dioxide Enrichment (FACE) systems which can deliver consistent and uniform amount of elevated atmospheric CO₂ concentration to large plots (25-30m diameter) of an intact ecosystem without any alteration in the plant's microclimate (Nowak *et al.*, 2004). Different types of plants respond differently to high level of atmospheric CO₂ concentration, dependent on environmental conditions (e.g. nutrients, water, solar radiation, and temperature e.t.c) and growth environment (Norby *et al.*, 1999, Silberbush *et al.*, 2003, Torbert *et al.*, 2004).

Increase in atmospheric CO₂ concentration has been found to lead to an increase in photosynthesis, plant growth, yield and water use efficiency and a decrease in transpiration rate and stomatal conductance (Roger *et al.*, 1999, Kimball *et al.*, 2002).

Soybean grown in FACE resulted in an increase in primary production (17-18%) and total yield of 15% (Morgan *et al.*, 2005). Increase in chlorophyll concentration in soybean has also been reported (Vu *et al.*, 2001). In contrast no increase was noticed in 3 weeks old soybean (Sicher *et al.*, 1995) when 100% NH₄⁺ N solution was applied, resulting in a decrease in chlorophyll, which could be attributed to toxic effects of NH₄⁺ N on the leaves which manifested as chlorosis and destabilisation of the chloroplast membrane structure (Tan *et al.*, 2000).

Fitter *et al.* (1996) found an increase in root biomass in grassland grown at ambient plus 250 ppm CO₂ over a period of two years, although there was no increase in above ground production. Similarly Dilustros *et al.* (2002) found that atmospheric CO₂ enrichment (ambient + 350 ppm) resulted in higher length density of roots near the soil surface in a scrub oak ecosystem.

It is important to note that the response of plants could vary due to differences in growth condition such as plants grown on diverse soil types, or the contrast between pot grown and natural vegetation communities where competition for essential nutrients is bound to occur (Edwards *et al.*, 2003).

The effects of CO₂ leaks on the surface ecosystem and humans can be of concern. Therefore, it is important to investigate the leakage route, spread and environmental consequences in order to proffer ways of mitigating them. Several leakage monitoring techniques have been suggested (refer to section 1.3 for details).

Remote sensing systems could provide information on the spatial extent of these leaks (e.g. using airborne systems) with a view to assessing the areas involved (De Jong, 1996, Bateson *et al.*, 2008, Steven *et al.*, 2010). McGonigle *et al.* (2008) measured CO₂ flux (170 Mgd⁻¹) from a volcanic plume at La Fossa crater; Vulcano, Italy using an unmanned aerial vehicle (UAV) with an infra-red spectrometer. The spatial resolution was high covering large area. A limiting factor was the fact that the plume is usually mixed with other gases such as SO₂. This requires further processing to separate the gases using technique such as the ratio of CO₂/SO₂.

2.6 Remote sensing applications.

Remote sensing has been applied in a variety of vegetation studies. The type of application depends on the purpose and usage. Such applications include: Crop yield estimation, vegetation cover, natural resources management, habitat assessment, biomass information, canopy characteristics and vegetation dynamics (Jiyul *et al.*, 2003, Prasad *et al.*, 2006, 2007, Shamseddin and Adeeb, 2012, Esfelder *et al.*, 2012).

Data for vegetation monitoring can be acquired either by airborne or satellite sensor systems, such as Landsat Thematic Mapper (Landsat TM) or SPOT (Satellite Pour l'observation de la Terre). Laboratory/field based equipment such as spectroradiometers can also provide detailed information (high spectral resolution) about objects using their spectral signatures. Spectroradiometers measure the reflectance of targets in many narrow bands, which is why monitoring vegetation stress requires high spectral resolution as it provides information on salient features within the spectral bands so that the health status of plants can be better understood (Yang *et al.*, 2004).

With remote sensing, areas that are not easily accessible can be monitored over time. The changes in landscape features (e.g. topography, slope, aspect, change in vegetation and land-use/land-cover) can be tracked due to coverage and consistency in data collection (Baccini *et al.*, 2008). Protected areas, such as forests, game reserves, endangered plants and animal species can be monitored to avoid extinction (Field *et al.*, 2008). Natural disasters (e.g. earthquakes, floods, desertification, landslides and mudflow e.t.c.) can be mitigated with remote sensing if properly managed. Historical data can provide information on likely future occurrence (Hirata *et al.*, 2001, Liu *et al.*, 2003). However, there are some shortcomings which could arise in the selection of sensor/type of remote sensing system

used. Using satellites enables wide areas to be covered on a regular basis, with the shortcomings that interference by clouds causes loss of data and atmospheric attenuation and scattering requires further processing and corrections for the information to be useful to the intending user (Du *et al.*, 2004, Franke and Menze, 2007).

The timing of the data acquisition is restricted to specific periods (temporal resolution); this can cause some problems in the advent of any impeding factors such as cloud cover at the time of passage of the satellite, as clarity of information from the imagery could be affected (Laudien *et al.*, 2004). The visible and infrared regions which are vital in vegetation monitoring can be affected.

Airborne systems are cost effective and simple, they could be used at anytime (depending on weather conditions), and not constrained by cloud cover; any area of interest can be flown as desired with frequency (Shafri *et al.*, 2012). However, the coverage is limited when compared to satellite systems.

In vegetation stress monitoring one of the most important considerations is the spectral resolution of the remote sensing system in use. The information required in assessing the health status of plants can be embedded in the narrow spectral bands. This is a function of number of bands, bandwidth and spectral sampling intervals (George, 1998, Yang *et al.*, 1998, 1999).

Hyperspectral remote sensing is a useful tool in vegetation stress studies (Lakkaraju *et al.*, 2010). It is non-destructive and sensitive to small variations in signals, and provides a quick way to monitor vegetation status over time (George, 1998, Shafri *et al.*, 2012).

2.6.1 Remote sensing of vegetation.

Remote sensing is considered a valuable tool for the determination and monitoring of vegetation status over time (Pinter *et al.*, 2003). However, in studying the response of plants to any change in the environment, their spectral reflectance characteristics are important. When radiation interacts with a plant leaf it may be reflected, absorbed or transmitted, depending on the chemical constituents and the physical structure of the leaf (Carter, 1991, Miller *et al.*, 1990, Gitelson and Merzlyak 1996, Male *et al.*, 2010).

Figure 2-3 shows some of the dominating factors controlling leaf reflectance and the primary absorption bands. The relative proportions of reflection, absorption and transmission vary with wavelength (Blackburn, 2007). Some features responsible for absorption and reflectance in vegetation spectra are shown in table 2-2.

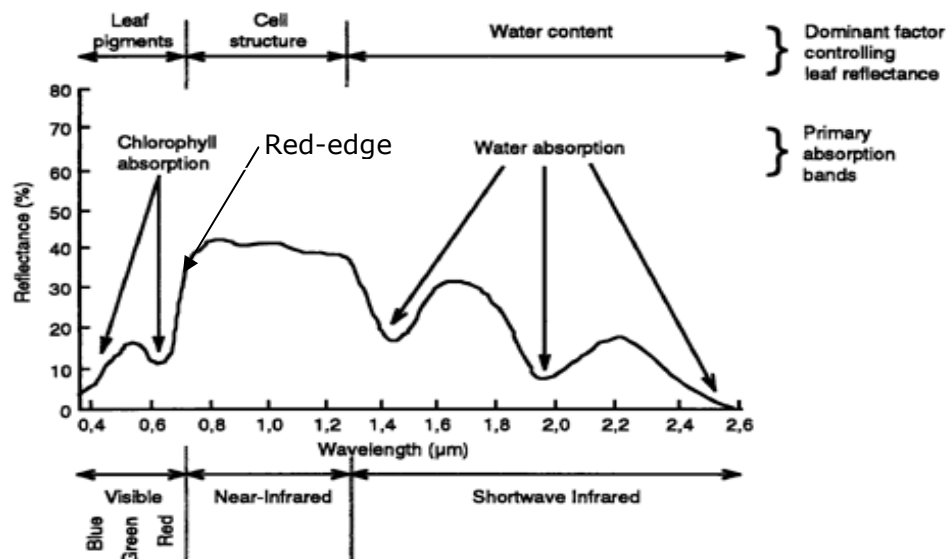


Figure 2-3. Typical reflectance characteristics of leaves. Adapted from Hoffer (1978).

Table 2-2. Absorption features of vegetation spectra. (Adapted from Smith (2002) and Blackburn (2007)).

Contributing factor	Wave lengths (nm)	Interaction/Process
Chlorophyll a	435, 670-680, 740	Strong absorption
Chlorophyll b	480, 600-650	Strong absorption
α -carotenoid	420, 440, 470	Strong absorption
β -carotenoid	425, 450, 480	Strong absorption
Anthocyanins	400-550	Absorption
Lutein	425, 445, 475	Absorption
Violaxanthin	425, 450, 475	Absorption
Chlorophyll a & b	550	Strong reflectance
Water, oxygen	760	Strong absorption
Water	970	Weak absorption
Water, CO ₂	1450, 1944	Strong absorption

2.6.1.1 Visible reflectance.

The visible region ranges from 0.4-0.7 μm (400-700 nm), it is an extremely small portion of the electromagnetic spectrum but this corresponds to the spectral sensitivity of the human eye (Figure 2-3). The blue, green and red colours are ascribed to the approximate ranges of 0.4-0.7 μm (400-500 nm), 0.5-0.6 μm (500-600 nm) and 0.6-0.7 μm (600-700 nm) respectively. Reflectance in this region is mostly affected by absorption by chlorophyll and to a varying extent, other photosynthetic and photoprotective pigments (Blackburn, 1998, Kochubey and Kazantsev, 2007, Liew *et al.*, 2008). Low reflectance in the visible region is caused by the absorption of light by these pigments (Carter, 1993, Noomen *et al.*, 2006, Noomen and Skidmore, 2009).

Several studies have reported that visible reflectance increases consistently in various plant species in response to stress induced by a range of different stressors such as soil oxygen deficiency, heavy metal toxicity,

waterlogging and nutrient stress (Milton *et al.*, 1989a, 1989b, Carter, 1993, Carter and Miller, 1994, Mariotti *et al.*, 1996, Smith *et al.*, 2004a). The increase in reflectance in this region of the spectrum can be attributed to less photosynthesis caused by stress.

2.6.1.2 Red-edge region.

The red-edge region is found within wavelengths 690 and 750 nm, where change in reflectance is prominent and is dominated by the strong absorption of red light by chlorophyll and high scattering of radiation in the leaf mesophyll (Dawson and Curran, 1998, Smith *et al.*, 2004b). In this region, reflectance rises rapidly leading to a plateau of high reflectance in the near-infrared, where pigments no longer absorb radiation (Blackburn, 2007).

The red-edge adjoins the red end of the visible portion of the spectrum as shown in Figure 2-3. Red-edge position has been applied in the study of biomass and estimation of chlorophyll contents in vegetation (Gaussman, 1974, Lichtenthaler, 1998), while some studies have found that this position is less prone to the effects of soil background and atmosphere (Demetriades-Shah *et al.*, 1990, Clevers, 1999, Clevers *et al.*, 2000). Strong correlation has been reported between red-edge position, chlorophyll concentration and leaf area index (Boochs *et al.*, 1990, Curran *et al.*, 1995, Fillella and Penuelas, 1994).

The red-edge region of the reflectance spectrum has been used as a means of identifying stress in plants. The reflectance of stressed plants often shows a shift of the 'red-edge' position towards shorter wavelengths (Noomen *et al.*, 2007). Red-edge shifts measured in airborne imaging spectrometer data have been proposed as useful in providing an early indication of vegetation stress. Evidence is given in Rock *et al.* (1988) in a study conducted to determine the effects of air pollution on spruce trees

before visual symptoms became apparent, a shift in red-edge towards the blue, of approximately 5nm was noticed. It was concluded that this shift may have been caused by deterioration in chlorophyll in the pine needle.

2.6.1.3 Near-infrared (NIR) region.

The NIR waveband ranges between 700 and about 1000 nm. The region is characterised by high reflectance primarily due to light scattering by leaf tissue or cellular structure (Gausman, 1974). Ceccato *et al.* (2001) 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. Leaf reflectance is very high in the NIR at ~800 nm and a decrease of the reflectance at 800 nm may be taken as an indicator of reduced interspaces in the mesophyll of leaves under stress conditions (Buschmann *et al.*, 1993). Some studies have shown substantial evidence of reduced NIR reflectance in stressed plants (Smith *et al.*, 2004a, Smith *et al.*, 2005a, Noomen *et al.*, 2007). 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 (Adams *et al.*, 2000, Osborne *et al.*, 2002a, 2002b, Noomen *et al.*, 2003).

2.6.1.4 Shortwave infrared (SWIR) region.

The SWIR ranges between 1000 and 2500 nm and is characterised by radiation absorption by the leaf water (Figure 2-3). Tucker (1980) showed that the SWIR is heavily influenced by water in plant tissue. Bowman (1989) indicated that water stress influences reflectance in the SWIR region because of a reduction of water content. A study by Baret *et al.* (1987) showed that the wavelengths at 1530 and 1720 nm were the most appropriate for assessing vegetation water as they are heavily influenced by water in plant tissue.

Ceccato *et al.* (2001) found that variables such as the equivalent water thickness are not the only parameters responsible for significant reflectance variations within the SWIR range. Other controlling factors include the internal structure and the dry matter content. The internal structure and the dry matter content affect reflectance in the wavelength range from 700 to 2500 nm, while equivalent water thickness affects the wavelength range from 900 to 2500 nm. This accounted for 86.7% of the reflectance variation in the SWIR, internal structure and the dry matter content accounting 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 equivalent water thickness is the dominant factor, the study suggests that combination of information from both NIR (820 nm) and SWIR (1600 nm) is necessary for accurate estimation of vegetation water content at leaf scale from optical observations.

Ceccato *et al.* (2001) proposed some indices 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.*, 1990). These indices do not provide a very accurate way for estimation of water stress because vegetation status can be affected by several factors and water content alone cannot provide adequate information on plant's vitality and some plants may show signs of reduced evapotranspiration without experiencing a reduction in water content (Ceccato *et al.*, 2001).

2.6.2 Hyperspectral remote sensing of vegetation.

Hyperspectral remote sensing (HRS) enables the acquisition of data about objects in many narrow contiguous bands throughout the electromagnetic spectrum (EMS). HRS data sets are usually composed of several hundreds bands (100-200 or more) with narrow bandwidths (5-10nm), (Liew *et al.*, 2008, Bronge and Mortensen, 2002). Examples of such hyperspectral imagers are Compact Airborne Spectrographic Imager (CASI), Airborne Visible Infrared Imaging Spectrometer (AVIRIS), Shortwave Infrared Full Spectrum Imager (SFSI) and Hymap and satellite based sensors such as Hyperion.

The capabilities of HRS in monitoring vegetation stress are vast (Ren *et al.*, 2008, Blackburn, 2007). HRS (airborne) spectral data have high resolution (spectral and spatial) as well as temporal resolution. Shafri *et al.* (2012) used airborne hyperspectral remote sensing in Malaysia for the detection and mapping of diseased oil palm with vegetation indices and red-edge techniques.

In a study by Male *et al.* (2010) at the Zero Emissions Research and Technology (ZERT) field in Bozeman, Montana, hyperspectral sensing was used to detect CO₂ leaks from a sequestration field. Stress was observed in the spectral signatures of the plants within 1 m of the well 4-5 days after injection. As the experiment progressed, 10 days later the spatial distribution of vegetation stress showed that the areas close to the injection well which had high CO₂ flux were the most affected, the severity decreased farther away. The study concluded that HRS technique can quantify the amount of stress acting on the terrestrial ecosystem at a time, but cannot discriminate between the causes.

Identification of signs of stress at sub-visual levels can be carried out with HRS technique (Smith *et al.*, 2004b, Noomen *et al.*, 2008). With hyperspectral scanning imager, Keith *et al.* (2009) was able to detect CO₂ leaks.

HRS has been reported to be useful in detection of stress caused by disease and insects (Lawrence and Labus, 2003, Samson *et al.*, 2003), plant leaf chlorophyll estimation (Datt, 1998), quantification of vegetation stress (Carter, 1994, Rock *et al.*, 1998) and biochemical content of plants (Lewis *et al.*, 2001).

2.7 Conclusion.

Plant stress can be caused by various biotic and abiotic factors; the effects could be detrimental to the overall growth and development of plants as well as the environment. Stresses symptoms may include stunted growth, wilting, reduce shoot growth and chlorosis. Changes in the chlorophyll concentration can be detected as change in the spectral characteristics of leaves in the visible region of the wavelength. Various remote sensing techniques have been identified as valuable tools for estimating plant biochemical and biophysical properties, in order to understand the health status of plants. Hyperspectral remote sensing technique has been used for detection of plant stress both at early and later stages using several approaches.

The effects of CO₂ leaks from CCS will depend on the concentration reached in both the atmosphere and the soil. If the leakage causes a rise in the soil CO₂ concentration, several researches have reported that levels of CO₂ above 2% can cause decrease in root and shoot growth, water absorption, respiration rate and nutrient deficiency and uptake. If high

enough this could lead to death of the plant (Matocha and Mostaghimi, 1998, Bunnell, 2002, Rodrigues, 2005).

The majority of the studies done on CO₂ leak detection and its effects are laboratory based at leaf reflectance level (Gausman, 1974, Gitelson and Mezlyak, 1996, 1998, Smith *et al.*, 2004a, Ren *et al.*, 2008, Noomen *et al.*, 2009). There are currently few studies on the effects of elevated soil CO₂ concentration on the ecosystem at a field scale (Steven *et al.*, 2010). The consensus among many researchers is that hyperspectral remote sensing can detect stress but it cannot distinguish between the causes (Male *et al.*, 2010; Smith *et al.*, 2005b).

Stress discrimination has also been studied, but most of these studies used a single stress factor with different concentrations on the same or different plants with the view to distinguishing between the treatments levels (Arthur *et al.*, 1985, Bouma *et al.*, 1997, Boru *et al.*, 2003, Mutanga *et al.*, 2003). These experiments can be modified and controlled and variables changed and/or adjusted. It is known that scaling from the leaf level to canopies in the field could be a complex process which can introduce additional structural, physical and biological elements. The operational environment can also play a significant role in the scaling; as any major changes (such as nutrient, soil moisture, competition and temperature) can affect the plant's growth and development.

In contrast this study used two stressors on two crops, maize (C4) and barley (C3) with different photosynthetic path ways. These stressors (CO₂ and herbicide) are thought to affect the roots of plants before having any impact on other parts.

The detection of CO₂ leaks at canopy level in the field is important as this represents the natural growth environment. If any leaks were to occur this

could undermine the main objective of CCS and the public perception will be negative which can affect the prospect of any CCS project (Pollak and McCoy, 2011).

An example of field site for studying CO₂ leak is the Zero Emission Research and Technology Centre (ZERT) at Montana State University, USA where CO₂ can be injected into the soil to study its effects on the vegetation (Rouse *et al.*, 2010, Male *et al.*, 2010). The Artificial Soil Gassing and Response Detection Site (ASGARD) facility at the University of Nottingham is also a facility where experiments can be carried out on the effects of elevated CO₂ concentration on the vegetation and soil microbial communities (Pearce and Sjogersten, 2009, Al-Traboulsi *et al.*, 2012a).

In the light of this, the study will investigate the impacts of CO₂ and herbicide stress on plants, response of the crops to varying concentrations of the treatment and to find out if these can be discriminated using hyperspectral remote sensing techniques.

CHAPTER THREE

FIELD DATA METHODS

3.1 Introduction.

This chapter explains the general methods adopted in this study. The investigation is field based as it provides a more realistic environment for stress studies in vegetation and allows canopy scale measurements of reflectance spectra. The ASGARD system described below is a facility that allows studies to be carried out in the field while retaining a high degree of experimental control. The following sections cover a description of the site and plot infrastructure used for the study, gas measurements procedures and sampling techniques adopted including the instrumentation, plants and treatments applied in the experiments, canopy spectra reflectance, plant growth, biomass, soil pH level and chlorophyll measurements as well as the data processing and analysis methods employed in the study.

3.2 The Artificial Soil Gassing and Response Detection (ASGARD) facility.

3.2.1 Site description.

This research was carried out at the Artificial Soil Gassing and Response Detection (ASGARD) facility developed at the Sutton Bonington Campus of University of Nottingham, United Kingdom (52.2°N, 1.2°W), this was initially a pasture land, but in 2006 it was converted to a field site for the study of the effects of leakage from underground CO₂ storage on plants growth and development as well as on the soil ecology (West *et al.*, 2009).

The geology of the area is characterised by sand and gravel-rich terrace deposits, surrounded by sheets of lithologically variable head. Lithologically, these deposits are characterised by moderately well-consolidated sand with abundant rounded polymict gravel, derived from

Triassic sandstones and pebble-beds. These sand and gravel deposits are dissected and highly degraded, as much of their material has been remobilised through periglacial processes and recent weathering (Ford, 2006). The resulting head deposits incorporate varying amounts of red clay from the Mercia Mudstones Group, and showing a wide range of grain sizes, degrees of sorting and levels of consolidation.

A consistent thickness of approximately 0.3m of dark brown sandy topsoil with a reasonably sharp base with the undifferentiated head is interpreted to be present over much of the site (Ford, 2006). This latter unit varies considerably in thickness, ranging from 0m in the West of the site to approximately 0.3m in the East and is composed of red-brown, slightly clayey, gravelly silty sand.

A relatively persistent horizon of gravelly head occurs in the West and North of the site, which is 0.15m thick, occurring at regular depth of the 0.3m to 0.6m beneath the ground surface (Ford, 2006, West *et al.*, 2009). This unit is typically associated with the base of the overlying undifferentiated head, and is characterized by abundant medium to coarse gravelly sand. Sandy head occurs in the central and northern parts of the site and is characterized by comparatively well sorted red-brown or light red sand and silt sand with occasional fine to medium gravel.

Clayey head is present across much of the site, occurring at a relatively low level in the succession and is characterised by red-brown silty, clayey sand with occasional gravel (Ford, 2006). Locally, thin red clay laminae (up to 2cm thick) are present. The increased clay content associated with this unit may be due to the relatively proximity to rockhead and varies dramatically in thickness, ranging from over 1m in the south-west of the site, to less than 0.2m in the north of the site. The topsoil ranges from 0.2m-0.4m

depth underlain with deposits of gravel, sand and clay to a depth of 1-1.2m (Ford, 2006).

3.2.2 Design of gas injection system.

Carbon dioxide is stored in 2 x 200L cryogenic vessels (BOC, Derby, UK) that are refilled as required from a road tanker as shown in Figure 3-1. The CO₂ is delivered via a single inlet mass flow controller (Alicat, Tucson, USA) to 16 individual mass flow controllers (Alicat, 0.1-10l min⁻¹) that regulate the gas flow to individual experimental plots. The mass flow controllers are controlled and the system data logged by a PC-based control system (TVC, Great Yarmouth, UK). For the studies described in this thesis the gas injection rate to each plot was 1 l min⁻¹. The depth of the gas injection was restricted to 0.6m to mitigate the effects of lithological variation on the geology of the site which could cause excessive gas migration.

The gas injection tubing is inserted into the ground 65cm from the North edge of the plot and at an angle of 45° such that the end of the tubing is positioned 60cm below the centre of the plot. The tubing is sealed at the end but has twenty six 5mm holes drilled in the end 21cm of the tube to release the gas.

Vertical plastic sampling tubes (100mm long, 19mm internal diameter) are installed permanently into the plots to enable measurements of soil gas concentration to be taken. The bottom of each sampling tube is at a depth of 30cm. The tubing is sealed at the bottom but 14 equally spaced holes (4.5mm diameter) drilled in the lower 15cm enable diffusion of gas from the soil into the tube so as to attain equilibrium with the soil gas. The top end of the tube is sealed with a bung containing a plastic on/off valve which is used when taking measurements of the soil CO₂ concentration and subsequently locked to prevent dilution with atmospheric CO₂. Two tubes

are installed at 15 and 70cm from the centre of each gassed plot on a diagonal line from the centre and towards the North East of each plot. One tube is installed at 15cm from the centre of each control plot.

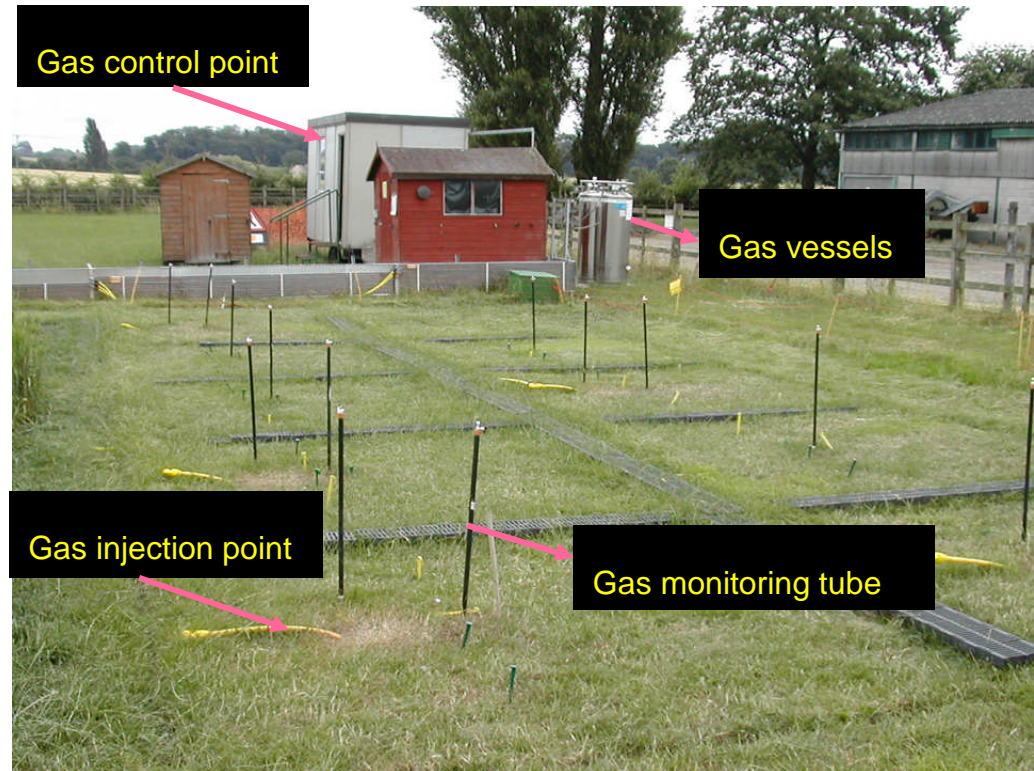


Figure 3-1. Artificial Soil Gassing and Response Detection (ASGARD) showing some of the infrastructure. (Source: [www.CO₂storage.org](http://www.CO2storage.org) .UKCCSC).

3.2.3 Experimental Plots layout.

In 2009 eight plots (each 2.5 x 2.5 m) with 0.5 m pathways between, were laid out within the experimental area to enable CO₂ gas to be delivered to maize (Figure 3-1). Four of these plots were continually injected with CO₂ throughout the duration of the experiment while four were left as controls without any CO₂. (Refer to appendix 1 for the diagram). Further away (20m) from these plots, eight plots were also rotovated, four were treated with herbicide and four controls. (Refer to appendix 1 for the diagram). The control plots for both experiments were randomly distributed and sometimes adjacent to the gassed or herbicide plots.

In 2010, the same numbers of experimental plots were used for a CO₂ gas experiment on barley. However, about 250 meters away from the ASGARD facility twenty blocks of plots (each 1.6 x 2.5 m) were laid out for the corresponding herbicide experiment. (Refer to appendix 2 for the diagram).

3.2.3.1 Plot infrastructure.

Figure 3-2 shows a gassed plot infrastructure. However, the Bartz root camera tube was not used in these particular studies. The blue shaded area at the edge of the plots is a discard area giving a 25cm space around the plots to reduce edge effects on the growth of the plant canopy. Such effects can be caused by exposure to wind and greater light intensity at the edge of the plots and a greater tendency for bird attack.

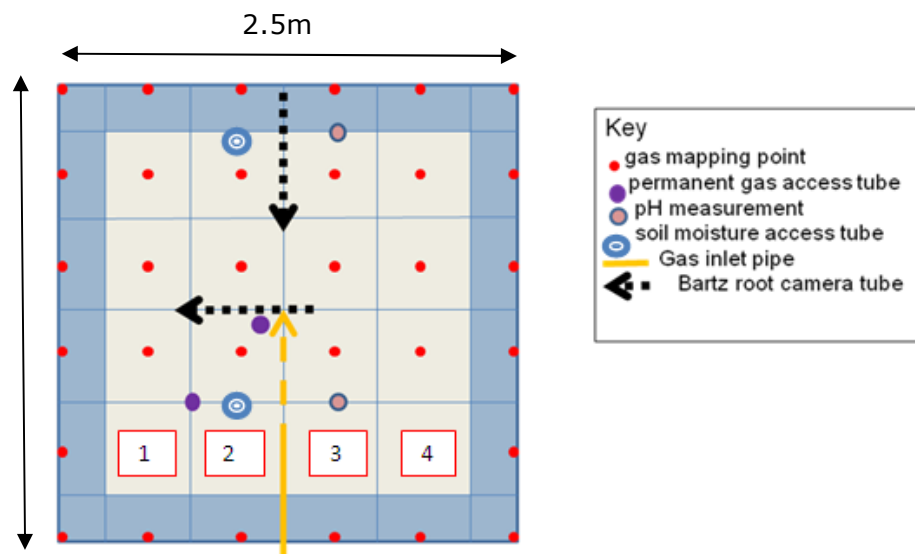


Figure 3-2. Illustration of plot infrastructure viewed from the north, the numbers 1-4 represents the points where spectral measurements were taken during the experiment. (Source: www.ieaghg.org).

3.3 Gas measurement.

3.3.1 Routine gas monitoring.

Gas concentrations in the soil were measured by means of vertical plastic tubes which are 30cm deep into the soil and perforated at the bottom and installed at a distance of 15cm and 70cm from the centre of each gas plot

as shown in Figure 3-1. Soil CO₂ and O₂ concentrations were measured three times weekly from the start of each experiment until the end using a GA2000 Landfill Gas Analyser (Geotechnical Instruments, Warwickshire, UK). The analyser extracts a sample at a flow rate of 300 ml min⁻¹ and measures CO₂ concentration in the range of 0-100% and O₂ in the range 0-25%. To obtain measurements, the sample tube of the GA2000 was attached to the valve of the sample tube and soil air was extracted and analysed by the instrument. The response time used for both gasses was 30 seconds to minimise for the effects of dilution with atmospheric gasses.

Because the gas is released at a single point beneath the plot centre, there is a CO₂ gradient across each gased plot. The gassing strategy aims to achieve a maximal value in the plot centre; the gradient is then used in this study as a means of investigating dose-response relationships. Gassing at the rate of 1 l min⁻¹ is sufficient to generate values of 50-80% in the plot centre (varying with the weather, and to some extent, with the individual plot).

The data acquired in this study from the gas measurements were used to estimate seasonal average gas concentration. Refer to appendix 3 for samples of data and calculations.

3.3.2 Gas concentration mapping.

Barholing was used in this study to measure the rate of dispersion of CO₂ and O₂ throughout the plots. A Safeway searcher bar (Peter Wood, Sheffield, UK) was inserted into the soil to a depth of 30cm at 36 intersection points on a 50cm grid across each plot. The bar was then removed and the GA2000 Landfill Gas Analyser probe inserted into the hole and measurements of CO₂ and O₂ taken.

Barholing measurements were carried out once at the end of each growing season on 22nd September, 2009 on and 17th August, 2010 for both maize and barley experiments. The measurements were used with the seasonal average from the 70cm fixed tube to estimate the average gas concentration at the measurement points which represents the dose applied in this study (See appendix 3 for details).

3.3.3 Plants and treatments.

Two separate experiments were carried out in 2009 and 2010 on maize and barley respectively using CO₂ and herbicide as stressors. The reason for choosing these stressors is because they both act on the root system of plants, CO₂ displaces oxygen which is essential for roots respiration and development and the herbicide (Glyphosate) used in this study also acts on the root to inhibit growth and development of plants resulting in stress (Smith *et al.*, 2005b).

3.3.3.1 Experiment one.

In 2009 dwarf sweet corn variety (*Zea mays L. cv. F1 Swift*; Sutton Seeds, Devon, UK) were initially sown in Levington F2S compost in modular trays in a glasshouse on 5th May, the seedlings germinated on 10th May and were moved outside during the day from 19th May in order to adapt to the surrounding environment. They were later transplanted to the field plots on 3rd June to eight experimental plots. The seedlings were planted at a spacing of 50cm between rows and 25cm between each plant within the row. After transplanting, fertiliser (NPK, 25:5:5) was applied at the rate of 10g per 50cm square, (this is equivalent to 250g plot⁻¹). Netting was placed above the seedlings to avoid damage by birds. In addition, electric fence was erected round the experimental plots to further prevent intrusion by rabbits and other animals. Soil CO₂ was injected continually from 16

July 2009, (72 days after sowing) to four plots at a rate of 1 l min⁻¹, with four plots as controls. CO₂ Injection to the four plots was terminated on 15th September, 2009 when the crop had fully matured and was ready for harvest; the visible stress symptoms were obvious at this stage.

A separate set of eight plots were established 20m away from the CO₂ experiment. In these plots *Roundup Energy* herbicide (Monsanto Imagine UK; active ingredient 450g l⁻¹ Glyphosate) was applied on the same day as the CO₂ injection started in ASGARD field plots. The herbicide application rate was 0.4 l ha⁻¹ in 74.6 ml of water, which is equivalent to 10% of the lethal dose. This application was designed to stress but not to kill the maize plants; three plots were treated with the herbicide and three left as controls. The reduction in the number of herbicide treated and control plots were caused by poor crop establishment which resulted in the need to transplant some of the seedlings to make up six good plots.

3.3.3.2 Experiment two.

In 2010 barley (*Hordeum vulgare v Concerto*) was sown at a density of 250 seeds m⁻² (Thousand Grain Weight (TGW) 50g) in 20 rows at row spacing of 12.5cm into the same eight plots of the ASGARD facility on 8th April, 2010. All plots received an initial treatment of seed bed fertiliser, NPK 12:11:18 at 333.33 kg ha⁻¹, and then at the seedling three-leaf stage, Nitram 34.5% N (Growhow, Cheshire, UK) was applied at 319 kg ha⁻¹. The plants germinated on April 21. Following germination and crop development CO₂ was delivered continually from 3rd June (62 days after sowing) to four plots at a rate of 1 l min⁻¹, with four plots as controls. Gassing was terminated on 10th August 2010 when the crops were fully matured and ready for harvest.

Twenty blocks of plots which were set up in an open field about 250 meters from the ASGARD facility in the same campus of the University of Nottingham were used for the herbicide experiment (See appendix 4). These plots were machine drilled with barley at a rate of 250 seeds m⁻² on April 11th. The same rate of fertiliser as that used on the CO₂ treated plots was applied. Sixteen randomly selected plots were treated with herbicide while four plots were left as controls with no herbicide treatment. Glyphogan (Makhteshim-Agan UK Ltd) containing 360g l⁻¹ of glyphosate was applied on June 3rd, 2010 at four different levels of concentration (four plots per treatment) at the rate of 0.15, 0.3, 0.6 and 1.2 l ha⁻¹ in 200 l ha⁻¹ of water. These rates are equivalent to 5, 10, 20 and 40% sub-lethal doses diluted to give the normal rate of spray coverage. This was designed to provide a range of levels of stress to the barley crop.

3.4 Sampling technique.

Due to the CO₂ gradient across the plots, the sampling unit used in this study was actually a subplot of 0.5m². All plants measurements are averages for the subplots and gas values are estimate for the same area. To enable easy replication of sampling throughout the experiment, a sampling frame was prepared and used throughout the duration of the experiment. The frame is 2.5 x 2.5m and is divided into sixteen 0.5 x 0.5m squares. The grid is identified with numbers and letters to provide easy identification of sample locations as shown in Figure 3-3.

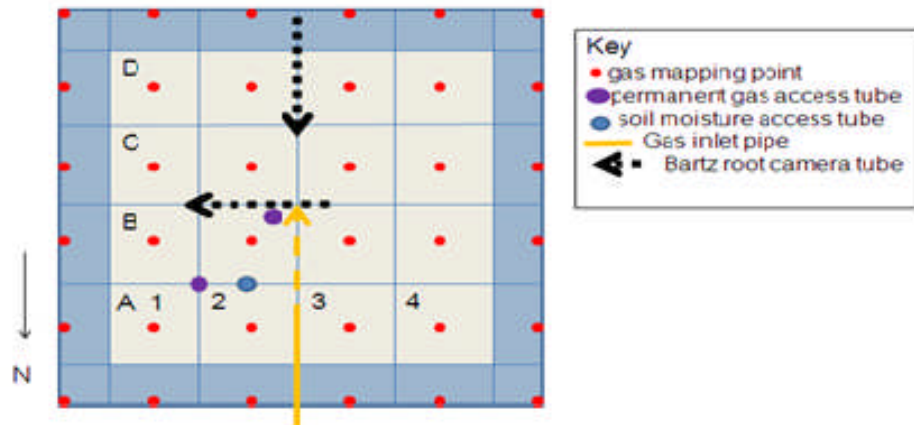


Figure 3-3. Sampling frame used for measurements.

The sampling areas for plant measurements are shown as blue squares represented by letters A-D and 1-4. While the ones for soil gas mapping measurements are shown as red dots. The letters and numbers are for sample identification.

3.5 Canopy reflectance measurement.

Spectral measurements were made weekly along a transect of subplots A1 to A4 as shown in Figure 3-3 at an interval of 50cm across each plot. A total of 392 and 640 spectral measurements were made in 2009 and 2010 respectively (Refer to appendix 4 for sample). Care was taken to ensure measurements were done at the same spot on each occasion, using tiny pegs flagged at each point as the permanent marker. The scans were made between 350 and 2500nm with an ASD Fieldspec FR spectroradiometer (ASD, Boulder, USA) fitted with a fibre optic probe having a 23° field of view. The sensor was held at a height of 1.23m above ground level viewing an area of 50cm diameter. The sampling interval over the 350–1050nm range is 1.4nm with a resolution of 3nm.

Over the 1050–2500nm range the sampling interval is about 2nm and the spectral resolution is between 10 and 12nm. After each set of four

measurements on one plot, the spectral reflectance measurements were calibrated by taking a measurement above a white halon panel (spectralon). The results were then interpolated by the ASD software to produce readings at every 1nm. Measurements were taken between 10:30am and 1:30pm (BST). Conditions varied from cloud free to overcast skies but care was taken to avoid scans when clouds were passing overhead. Quality checks on the data were carried out while on the field by ensuring that noisy data sets were discarded and measurements re-taken on the spot, as well as at a later stage to reduce and/or eliminate errors that could be associated with the data.

In both the 2009 and 2010 CO₂ studies, each control reflectance represents an average of sixteen spectral measurements made over the crop at 50, 100, 150 and 200cm along the transect in the four control plots. In the gassed plots, the low CO₂ concentration zone (1.6-13.4%) represents an average of eight spectral measurements at 50 and 200cm (locations A1 and A4) along the transect in the four gassed plots, while the high CO₂ concentration zone (3.5-27.1%) represents an average of eight spectral measurements at 100 and 150cm (locations A2 and A3) along the transect in the same plots (See Figure 3-3).

In the 2009 herbicide study there was an average of twelve spectral measurements for both herbicide treated plots and controls measured in the three treated plots and three control plots. In 2010 averages of sixteen spectral measurements for all herbicide levels and controls were used, this represents four control plots and four plots for each of the levels of herbicide treatment.

3.6 Plant growth and biomass measurement.

In both experiments and years the heights of plants were measured weekly. At the end of the experiment the crops were harvested in 0.5m² subplots (Figure 3-3), according to the sampling frame and transported in polythene bags to the laboratory for the measurement of the length of tillers, total number of plants, total number of tillers, total number of grains, fresh weight of ears and stems, fresh weight of the leaves, stems and number of cobs. All these materials were dried in an oven at 100°C for four days and reweighed to determine the dry weight. These measurements were done for both the CO₂ and herbicide treatments to determine any variations after the experiment.

3.7 Determination of soil pH.

Soil pH measurements were done before the start of the experiment and at the end with a bench pH meter (Accumet, AP5. Fisher scientific, UK) using a standard procedure accredited by United Kingdom Accreditation Service (UKAS) and Environmental Agency's Monitoring Certificate Scheme (MCERTS), (Laboratory guidelines School of Geography University of Nottingham, United Kingdom) this was to determine if there were changes as a result of the CO₂ injection. Refer to appendix 5 for detailed procedure.

3.8 Chlorophyll content.

In 2009 chlorophyll content of the leaves were measured weekly in the field using a SPAD meter (Minolta SPAD, UK); six SPAD readings per leaf of four upper leaves in the same measurements subplots as used for spectral scanning were taken and averaged. A calibration of the SPAD values to determine chlorophyll content was carried out in the laboratory. Refer to appendix 6 for details.

In 2010 Chlorophyll extraction and analysis was carried out using the standard method of Bruinsma (1963). Refer to appendix 6 for details of the procedure. Chlorophyll samples were extracted from the leaves of known area (1cm²) within the 0.5m² subplots used for measurement as discussed in section 3.4. The leaves were stored in polythene bags at 4°C in the dark to prevent chlorophyll break down, until chlorophyll extraction was carried out. The extraction and analysis was usually done within 24 hours after the sampling (Legood, 1993).

3.9 Climatic conditions.

The climatic conditions were measured at Sutton Bonnington meteorological station which is about 400m from ASGARD site. The mean daily as well as cumulative air temperature, total rainfall and solar irradiance for the two experimental periods i.e. 2009 and 2010 were compared to illustrate the pattern of change during the experiment. Refer to appendix 7 for details of data and representation. The two seasons were actually quite similar. Accumulated temperatures were slightly higher in 2009, although most of the difference was in January/February before the crops were sown. 2009 was also about 10% wetter. Solar radiation was virtually identical. The main period of interest is about day 100 (early April) to day 200 (early July).

3.10 Data analysis.

A range of techniques were applied in analysing the spectra data from the experiments: Derivative analysis, red-edge position, vegetation indices and continuum removal. These techniques were tested for their ability to determine and/or distinguish between the stress type and/or level of stress applied in the studies carried out.

3.10.1 Spectral data processing.

The spectral data from the ASD Fieldspec FR spectroradiometer were converted to ASCII files (see Appendix 4 for sample) then imported into Microsoft Excel; this is then used to calculate the average spectra for individual plots/treatments, standard deviation, derivative spectra and smoothing of the spectra. Individual reflectance spectra were displayed on Microsoft Excel and plotted against wavelength. In order to examine the effects of treatments on plant spectral properties, the mean reflectance spectra of control and treated plots were plotted against wavelength and analysed using different techniques listed in section 3.10.2.

The raw spectrum was first smoothed using the 5 point weighted moving average defined by Smith *et al.* (2004a). This procedure helps to reduce noise and at the same time minimises the loss of fine spectral detail in the derivative.

3.10.2 Derivative spectroscopy.

Derivative spectroscopy concerns the rate of change of reflectance with wavelength (Smith *et al.*, 2004a). The derivative analysis was undertaken in order to (i) locate the position of the primary red-edge wavelength (Miller *et al.*, 1990, Smith *et al.*, 2005b) and (ii) identify other red-edge features that may indicate stress in plants such as oil seed rape, maize and barley (Smith, 2002). Derivative analysis can enhance absorption features that might be masked by interfering background absorptions (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, 2007). Thus, the first derivative was calculated by dividing the difference

between successive spectral values by the wavelength interval separating them.

The red-edge region (Refer to figure 2-3) which is considered a vital portion of the spectrum for stress detection was extracted; this is a region of occurrence of derivative peaks.

3.10.3 Techniques for extracting the red edge position from hyperspectral data.

Several methods have been proposed in the existing literature for the calculation of the red-edge position (REP). Below are some of the widely used methods:

3.10.3.1 Maximum first derivative spectrum.

The maximum first derivative method calculates the REP by the wavelength of the maximum first derivative of the reflectance spectrum in the region of the red edge. This is calculated using a first-difference transformation of the reflectance spectrum (Dawson and Curran, 1998) which can be derived from:

$$D_{\lambda(i)} = (R_{\lambda(j+1)} - R_{\lambda(j)}) / \Delta\lambda \quad (3.1)$$

Where $D_{\lambda(i)}$ is the first derivative of reflectance at a wavelength i , midway between wavebands j and $j+1$, $R_{\lambda(j)}$ is the reflectance at the j wavelength, $R_{\lambda(j+1)}$ is the reflectance at the $j+1$ waveband, and $\Delta\lambda$ is the difference in wavelengths between j and $j+1$.

The accuracy of the REP using the maximum first derivative method may be limited by the continuity and spectral resolution of the reflectance spectrum (Dawson and Curran, 1998, Cho and Skidmore, 2006).

3.10.3.2 Linear four-point interpolation technique.

The linear four-point interpolation method (Guyot and Baret, 1988) assumes that the reflectance curve at the red edge can be simplified to a straight line centred near the midpoint between the reflectance in the NIR at about 780nm and the reflectance minimum of the chlorophyll absorption feature at about 670nm. It is calculated using four wavelengths (670, 700, 740 and 780nm), and the REP is determined by using the equations below:

(i) Calculations of the reflectance at the inflexion point (R_{re}):

$$R_{re} = (R_{670} + R_{780}) / 2 \quad (3.2)$$

Where R is reflectance.

(ii) Calculation of red edge position (REP):

$$REP = 700 + 40 (R_{re} - R_{700}) / (R_{740} - R_{700}) \quad (3.3)$$

700 and 40 are constants resulting from interpolation in the 700–740nm interval.

According to Shafri *et al.* (2006) this method can easily be affected by soil background noise. Furthermore, a 10nm over-estimation of the REP compared with the first derivative method was reported by Dawson and Curran (1998), while Mutanga and Skidmore (2007) commented on the inappropriateness of using this technique where the REP has double peaks.

3.10.3.3 The inverted Gaussian technique.

The inverted Gaussian technique uses a least-squares procedure to fit a normal curve to the reflectance red edge in the 660-780nm wavelengths (Miller *et al.*, 1990, Dawson and Curran, 1998, Pu *et al.*, 2003). The estimated red edge is then the ascending edge in the normal curve.

This is represented by the below equation:

$$R(\lambda)=R_s - (R_s- R_o) \exp [(- (\lambda_0 - \lambda)^2 /2\sigma)] \quad (3.4)$$

Where R_s is the maximum or shoulder spectral reflectance, R_o and λ_0 are the minimum reflectance and corresponding wavelength, and σ is the Gaussian function variance.

The REP is then defined as:

$$REP= \lambda_0 + \sigma \quad (3.5)$$

Pu *et al.* (2003) in a study to estimate forest LAI found out that the correlation with LAI was high for Linear interpolation method and Polynomial fitting, and low for the Gaussian method. This indicates that the Gaussian method cannot be appropriate for the estimation of LAI with high accuracy. In another investigation to determine the REP in multi and hyperspectral data sets using supervised and unsupervised computations, Pierce (2002) found that the Gaussian method is satisfactory for supervised, but not for unsupervised REP computation. Furthermore, this method is computationally complex and not suitable for canopy spectra (Dawson and Curran, 1998)

3.10.3.4 Higher order curve fitting technique.

The higher order curve fitting technique method locates the REP as the maximum first derivative of the reflectance spectrum in the region of the red edge using higher order curve fitting techniques such as third-order polynomials and cubic spline to fit the spectrum (Demetriades-Shah *et al.*, 1990). This is then used to compute the maximum of the derivative in the range of interest. This technique is computational complex and very demanding.

Dawson and Curran (1988) assessed the capability of Inverted Gaussian technique and linear techniques using different chlorophyll concentrations and found that the red edge position for 50mg m⁻² of chlorophyll was 707nm for the Inverted Gaussian and 715nm for linear technique. This made it difficult to ascertain which method was best and therefore casts doubt as to the exact REP position. Further to this, they also found that the REP for linear method at 50mg m⁻² chlorophyll concentration was similar to that at 350mg m⁻² using the Gaussian methods. The conclusion to be drawn from this is that the choice of the wavelength parameters in the methods chosen can affect the results.

3.10.3.5 Linear extrapolation technique.

Cho and Skidmore (2006) also developed a technique which is based on linear extrapolation of two straight lines (Equations 3.6 and 3.7) through two points on the far-red (680 to 700nm) and two points on the NIR (725 to 760nm) of the first derivative, after which intersection of the lines is calculated to determine the REP (Equation 3.8)

The linear relationship between leaf chlorophyll content and REP determined by this method yielded higher coefficient of determination ($R^2 = 0.75$) compared with the First Derivative, (0.50) Linear Interpolation (0.60), Inverted Gaussian (0.61) or 3rd Order Polynomial Fitting (0.62) (Cho and Skidmore, 2006).

$$\text{Far-red line: FDR} = m_1\lambda + c_1 \quad (3.6)$$

$$\text{NIR line: FDR} = m_2\lambda + c_2 \quad (3.7)$$

Where m and c are the slope and intercept of the lines and λ is the wavelength at intercept (Cho and Skidmore, 2006). At the intersection, the

two lines have equal λ (wavelength) and FDR values. Therefore, the REP which is the wavelength at the intersection is calculated as:

$$REP = - (c_1 - c_2) / (m_1 - m_2) \quad (3.8)$$

The present study is field based and is expected that there will be variability in leaf chlorophyll content as the experiment progressed due to the stresses applied to the crops. The REP calculation technique used in this study was the linear extrapolation technique (Cho and Skidmore, 2006).

The linear extrapolation technique was selected because it has proven to be useful in REP extraction from hyperspectral data and has the potential for explaining variations in leaf chlorophyll content while minimizing the effects of leaf and canopy biophysical parameters such as leaf area index (LAI), leaf inclination distribution and leaf dry matter content (Cho and Skidmore, 2006) when compared with other methods like linear interpolation, Gaussian and polynomial fitting techniques. It is simple to implement as only four spectral bands are required for the extrapolation and can be used to extract REP from wider bandwidth spectra.

The discontinuity in REP data as a result of double peak feature, which is the case in this study, can be mitigated by this technique. It has also been applied under different conditions using canopy reflectance models such as PROSPECT and SAILH models (Cho and Skidmore, 2006).

3.10.4 Vegetation Indices.

Spectral indices proposed by several studies as being useful for plant stress detection were also investigated.

In this study, the Chlorophyll Normalized Difference Index (*ChINDI*) (Richardson *et al.*, 2002), the Pigment Specific Simple Ratio for chlorophyll-a (*PSSRa*) and chlorophyll-b (*PSSRb*) (Blackburn, 1998) and the Physiological Reflectance Index (*PRI*) (Gamon *et al.*, 1992) were calculated to determine the change in chlorophyll content over time. These indices were chosen due to their sensitivity to chlorophyll, which is useful in stress studies. They have also been used by Lakarraju *et al.* (2010) in a similar study of the effects of elevated CO₂ concentration on plants.

Mathematically these indices are represented below:

$$ChINDI = \frac{(R_{750} - R_{705})}{(R_{750} + R_{705})} \quad (3.9)$$

Where R_{750} and R_{705} are spectral reflectance values at 750 and 705 nm respectively.

$$PSSRa = R_{800} / R_{675} \quad (3.10)$$

$$PSSRb = R_{800} / R_{650} \quad (3.11)$$

Where R_{800} , R_{675} and R_{650} are spectral reflectance values at 800, 675 and 650 nm respectively.

$$PRI = ((R_{570} - R_{531})) / (R_{570} + R_{531}) \quad (3.12)$$

Where R_{570} , and R_{531} are the spectral reflectance values at 570 and 531nm respectively.

3.10.5 Continuum removal analysis.

According to Kokaly and Clark (1999), continuum removal emphasises absorption features by referring spectral values to an estimate of pre-absorption based on a convex hull over the part of the spectrum to be analysed. The continuum reflectance ($R'_{(\lambda)}$) is obtained by dividing the reflectance value $R_{(\lambda)}$ for each wavelength in the absorption pit by the reflectance level of the continuum line (convex hull) at the corresponding wavelength (Mutanga *et al.*, 2004).

$$R'_{(\lambda)} = R_{(\lambda)} / R_{C(\lambda)} \quad (3.13)$$

Band depth (BD) at each wavelength in the absorption feature was calculated after continuum removal was applied by subtracting the continuum removed reflectance (CRR) i.e. $R'_{(\lambda)}$ from 1 (Kokaly and Clark, 1999)

$$BD = 1 - CRR \quad (3.14)$$

The band depth was normalised by dividing the BD at a certain wavelength by the maximum band depth for that absorption feature (Kokaly and Clark, 1999).

$$NBD = BD / BD_{max} \quad (3.15)$$

Continuum removal was applied to two absorption features of the spectrum, the blue (400-550nm) and red (550-750nm) bands. In these regions the main feature of concern is chlorophyll content. This method was used by Noomen *et al.* (2006) to study the effects of natural gas, methane and ethane on maize leaf reflectance. In contrast, this study was conducted using canopy reflectance. Band depth analysis was carried out

for each treatment over time to determine which wavelengths best discriminate the treatments.

3.10.5.1 Spectral region of focus for continuum removal analysis.

The red and blue region were chosen because when compared to other absorption features in the SWIR region they are not usually affected by foliar water effects (Mutanga *et al.* 2003, Noomen *et al.*, 2006). Further to this, in the study carried out the region was masked and not clear enough (very noisy) for further analysis due to the effect of atmospheric water vapour and there was inadequate solar radiation to make meaningful measurements. The visible region is also where you expect to find stress effects because this is where all the photosynthetic pigments are absorbed. There are several metrics available for use in continuum removal analysis such as band depth analysis (BDA), maximum band depth (BD_{max}), band depth normalised to area (BDNA) and width at half height of the peak of the absorption curve (Dx). However, in the present study, band depth analysis was used due to its lower sensitivity to fractional canopy coverage compared to other metrics (Kokaly and Clark, 1999). This technique has also been applied to spectroscopic data in field and laboratory spectral measurements to determine their correlation with biochemical parameters (Kokaly 2001, Pu *et al.*, 2003, Mutanga and Skidmore 2004 and 2007, Kokaly *et al.*, 2009, Sykioti *et al.*, 2011), as well as in mapping the distribution of minerals by comparing remotely sensed absorption band shapes with those in a reference library (Clark and Roush 1984). However, there is currently very few application of band depth analysis using fresh canopies (Mutanga *et al.*, 2005)

3.11 Statistical analysis

Statistical analysis was performed using SPSS 16.0. Analysis of Variance (ANOVA) was used to ascertain which of the stress indicator(s) was optimal for early detection of stress arising from the treatments applied. The criteria used were time/date of detection and consistency during the remainder of the experiment. Early detection was particularly considered in order to determine whether stress arising from treatments could be detected by hyperspectral remote sensing technique before visible symptoms. However, both time and consistency would help to establish reliability and general sensitivity to each of the stress indicators. All tests used the 0.05 level of significance. Post hoc test analysis using Tukey HSD were performed on ANOVA to determine the significant differences arising from the treatments compared to controls. This helps to ascertain the sensitivity of an indicator to various treatments.

Where physiological measurements were made, the measurements of treated plants were also compared with the control on each measurement occasion.

3.12 Conclusion.

The methods of data acquisition, sampling techniques, processing and analysis were discussed in this chapter. The choices of data analysis methods were based on the literature and their expected sensitivity to stress; these methods were applied to two different crops at several levels of two types of stress with a view to determine their appropriateness in the studies undertaken.

CHAPTER FOUR

Spectral and physiological responses of maize (*Zea mays L*) to elevated soil CO₂ and herbicide stress

4.1 Introduction.

Under field conditions, crops are exposed to a wide range of biotic and abiotic stresses within the growth environment, which consequently alter their physiological and biochemical functioning (Levitt, 1980, Lichtenthaler, 1998, Liew *et al.*, 2008). Stress caused by any factor can be detrimental to plants and therefore have negative effects on their growth and development (Jensen, 2000). For example, it was demonstrated that leakage of natural gas into the soil caused restricted plant growth of vegetation cover 15 to 30 days after stress inducement (Pysek and Pysek, 1989).

This chapter deals with the analysis of canopy reflectance and physiological properties of Maize (*zea mays*) stressed with elevated concentration of soil CO₂ and herbicide. Maize is a common crop grown in many parts of the world; it plays a significant role in feeding both human and 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 (FAO, 2012, USAID, 2012). It is referred to as a C4 crop in terms of its photosynthetic activity and response to atmospheric CO₂ (Flexas *et al.*, 2004). C4 crops constitute less than 1% of the Earth's plant species and are mostly found in regions with high temperature and dry environment. They are tolerant to low levels of water, and their water use efficiency is high (Ghannoum, 2009).

The experiment was conducted in ASGARD site between 5th May and 15th September 2009; it began with the sowing of maize in green house after

which the seedlings were transplanted on to the field plots, and fertilisers were applied during the plant growth. CO₂ was injected into the field plots and dilute herbicide was applied in a parallel trial (Refer to section 3.2.3 for addition information), following which spectral measurements were taken weekly throughout the duration of the experiment (Refer to section 3.5 for details). As the experiment progressed, the height of the plants, number of tillers, leaves and cobs were taken on weekly basis (Details are given in section 3.6). Chlorophyll contents of the leaves were measured using a SPAD meter (See section 3.8 for details). At maturity the crops were harvested and taken to the laboratory for further analysis.

4.2 Results.

4.2.1 Visible stress symptoms.

The first signs of visible stress symptoms were noticed 16 days after soil CO₂ injection and 25 days after herbicide treatment. This was in the form of yellowing of leaves, which was more severe in the CO₂ experiment, and reduction in maize height growth compared to the control plants, especially in the centre of the gassed plots where the concentration of gas was high. As the experiment progressed the leaves became pale, curvy, dry and wilted. In the herbicide treated plots the leaves showed yellowing in the veins and edges; this was gradual and later spread across the entire leaf as the experiment progressed as shown in Figure 4-1.

After the harvest on 15th September, 2009 (day 62 of both treatments) some symptoms were also noticed in the maize cobs; in the middle of the CO₂ plots the maize cobs were distorted and shrivelled where grain formation had occurred, thereby resulting in low grain numbers in the cob (Figure 4-2). The effects of the herbicide treatment on the maize cobs were different from the CO₂ treatment, although there was distortion in the cobs,

the manifestations of stress were incomplete grain formation and immature maize cobs, and this is depicted in Figure 4-3.



Figure 4-1. Visible symptoms of stress in maize leaves due to CO₂ (left) and herbicide (right).



Figure 4-2. CO₂ gassed maize cob (left) showing distorted maize cob and shriveled grain formation as a result of stress and control plots maize cobs (right).



Figure 4-3. Herbicide treated maize cobs (left) showing incomplete grain formation caused by stress and control with full grain cob and three immature maize cobs (right).

4.2.2 Plant growth and biomass.

Table 4-1 shows the CO₂ experiment plant height, number of leaves, tillers, primary cobs and immature cobs of maize measured in the field at t=33, (where t, represents day after treatment). At this point the stresses on the maize crops were at maximum. The plant heights in the high CO₂ regions at the plot centre were only 60% of the controls while at the plot edge it was 86% of control. The number of leaves in the plot centre was 50% of the control and plot edge. While the number of tillers and maize primary cobs were about 60% and 75% of the control plots respectively. There was an increase of 50% in the number of immature cobs in the plot centre, which showed the effects of CO₂ stress on the crop.

For the herbicide experiment the number of primary cobs and immature cobs in the maize treated with herbicide was approximately 50% of the control plots as shown in Table 4-2. There was a significant difference compared with the control plants when tested with ANOVA (*p-value 0.012; 0.007; ($\sigma \leq 0.05$); n=16*) in the plant height and the number of tillers, the number of leaves was the same.

Table 4-1. Average maize crop characteristics in the control, centre and plot edge measured in the field at t=33 for CO₂ experiment

Location	Plant height (cm)	Number of leaves	Number of tillers	Number of Primary cobs	Number of immature cobs
Control	120.5	10	5	4	2
Edge	104	10	4	3	2
Centre	75	5	2	1	4

Table 4-2. Average maize crop characteristics of the control and herbicide treated plots measured in the field at t=33.

Location	Plant height (cm)	Number of leaves	Number of tillers	Number of Primary cobs	Number of immature cobs
Control	88	7	3	3	2
Treated	72.5	7	2	1.5	4

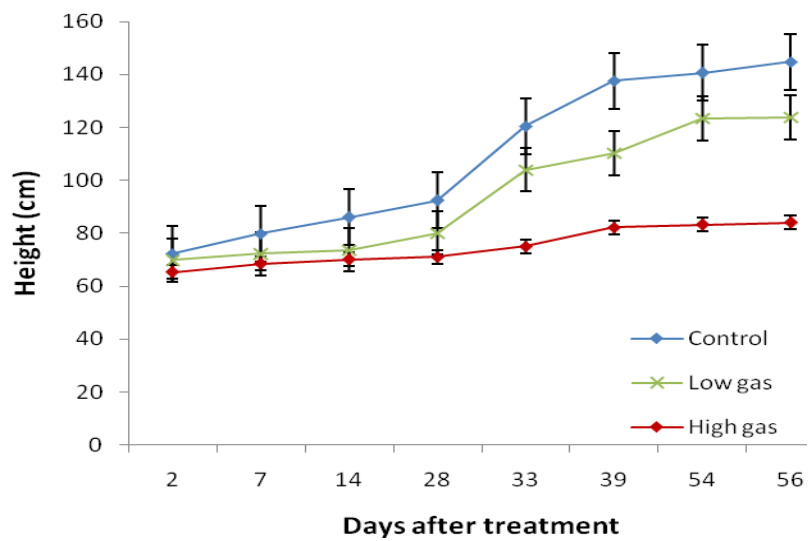


Figure 4-4. Temporal change in maize height in the control, centre (high gas and edge (low gas) of CO₂ plots.

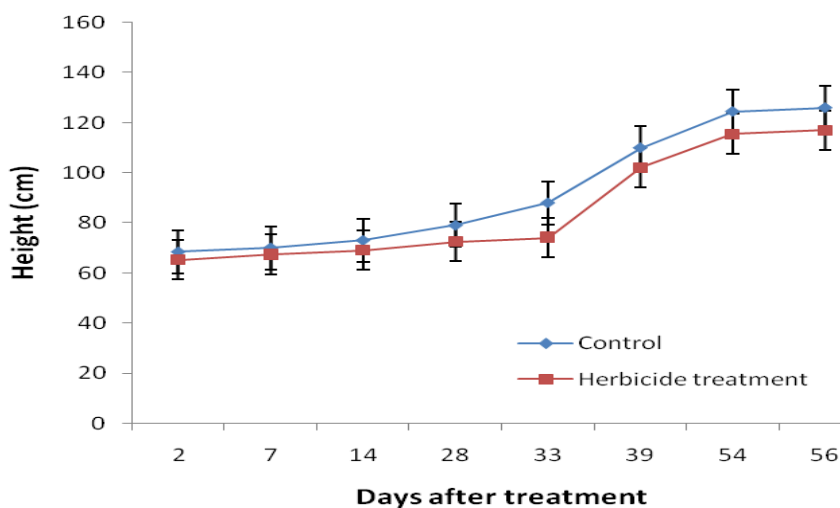


Figure 4-5. Temporal change in maize height growing on herbicide and control plots in the field.

Temporal change in maize height since the gas injection commenced is shown in Figure 4-4. During the first week (t=2 to t=7) after the gas injection, the heights of the plants growing on gassed plots were not significantly different using ANOVA (*p-value* 0.052; 0.075; ($\sigma \leq 0.05$); n=16). At t=14, the difference in the height of maize plant between low and high gas zone compared with control became significant when tested with ANOVA (*p-value* 0.030 ($\sigma \leq 0.05$); n=16). From t=14 until t=56 the plants growing in the high gas zone were 25 to 40% shorter than the control plants on each measurement day. The plants growing in the low gas zone were 10 to 15% shorter than control plants. From Figure 4-5 the plants growing on herbicide treated plots showed no significant difference from t=2 until t=56, (ANOVA (*p-value* 0.072; 0.281; 0.274; 0.466; 0.739; 0.161; 0.578; 0.401 ($\sigma \leq 0.05$); n=30) in height compared to control plants throughout the experiment. Although individual dates may not show significant difference, there is clearly a consistent drop in maize height.

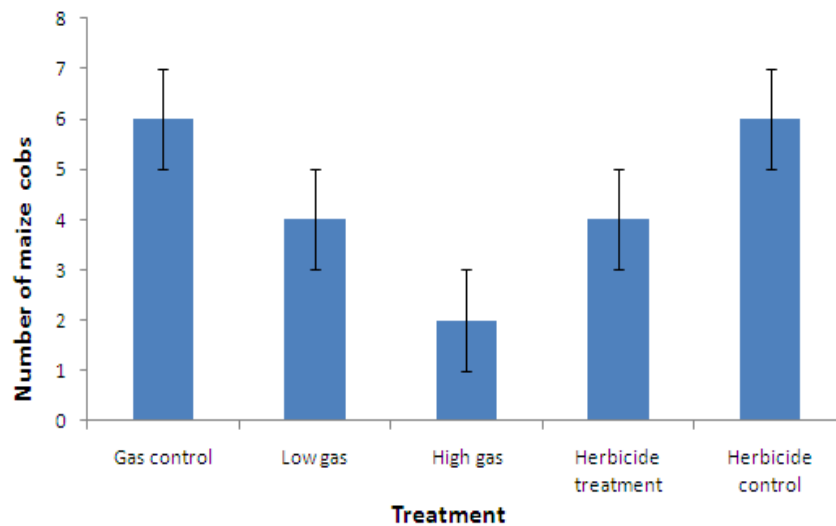


Figure 4-6. Mean number of maize cobs in CO₂ and herbicide treated plots with their control.

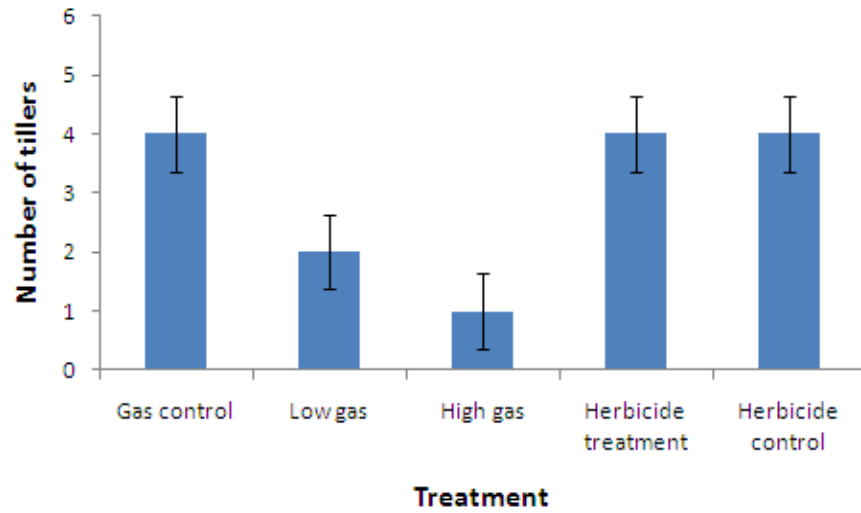


Figure 4-7. Mean number of tillers in CO₂ and herbicide treated plots with their control.

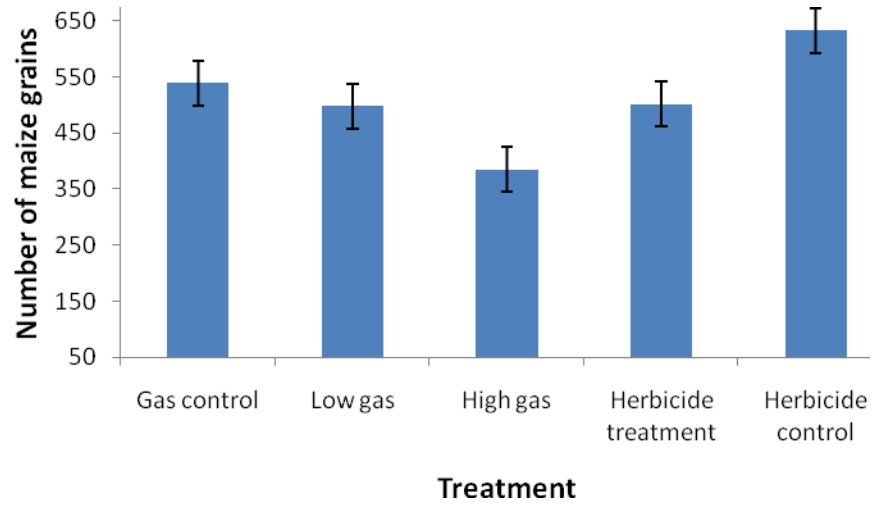


Figure 4-8. Mean number of maize grains in CO₂ and herbicide treated plots with their control.

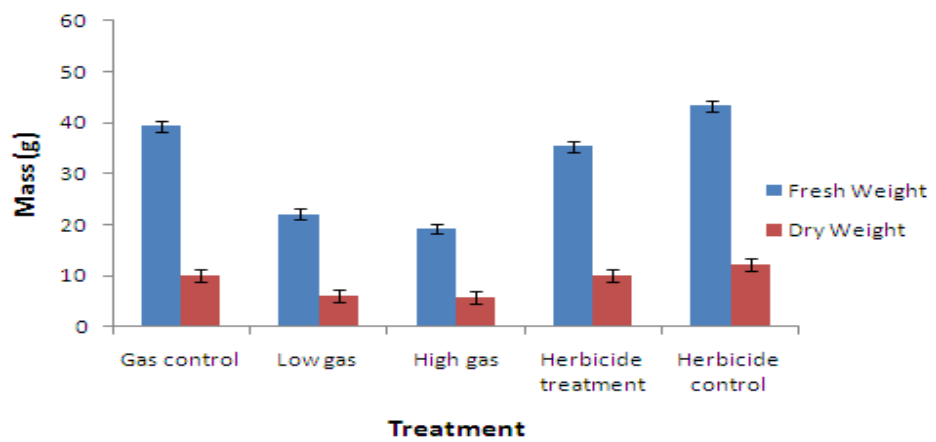


Figure 4-9. Mean fresh and dry weight of maize leaves in CO₂ and herbicide treated plots with their control.

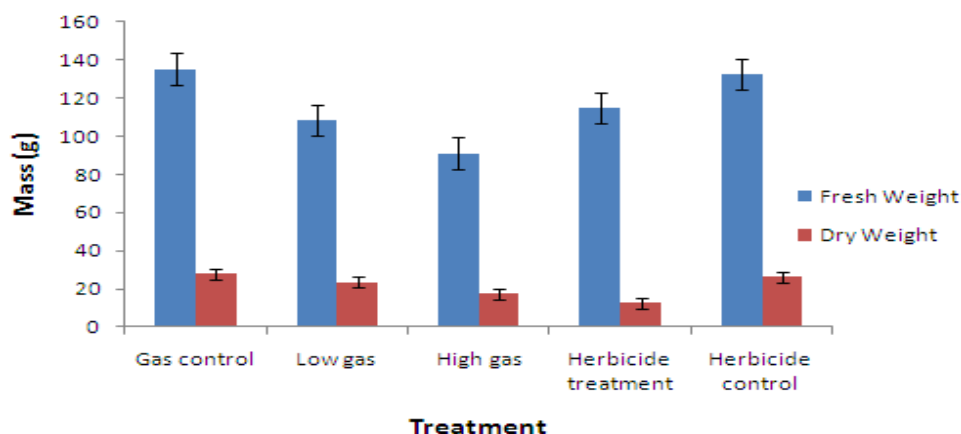


Figure 4-10. Mean fresh and dry weight of maize stem in CO₂ and herbicide treated plots with their control.

The results of biomass analysis carried out for both CO₂ and herbicide stress are shown in Figures 4-6 to 4-10. The error bars in the Figures represents standard error in measurement. These Figures indicate that the mean number of maize cobs growing in the region of high gas concentration shows a decrease of about 50% compared to the control plants while for the low gas region the decrease was about 30% as shown in Figure 4-6.

The difference between maize growing on herbicide treated plots with the control was about 30% decrease. In terms of numbers of tillers the high and low gas zones showed a difference of 65 and 50% respectively compared to control plots as depicted in Figure 4-7. The herbicide treated plots and control showed no significant difference when tested with ANOVA from t=2 to t=56 (*p-value* 0.056; 0.377; 0.286; 0.612; 0.853; 0.415; 0.230; 0.516 ($\sigma \leq 0.05$); n=30). The gas control plots had higher number of maize grains compared to the high and low gas zone, the decrease ranged between 15-45% for high, low gas zone and herbicide treated plots, this is depicted in Figures 4-8. There was a decrease of between 70-80% in dry weight of maize leaves and stems compared to control, for both CO₂ and herbicide stress, as shown in Figures 4-9 and 4-10 respectively. This study has shown that the effect of elevated CO₂ concentration and

herbicide stress manifested in the maize crop as a reduction in biomass content when compared with control plots, with more effects observed in the region of high concentration of CO₂.

The effects of treatment and location within the gassed plots on plant height, tiller number plant⁻¹ total cob number plant⁻¹ and chlorophyll content were tested using repeated measures ANOVA. CO₂ treatment was the main effect (independent factor) with two levels, gassed and control; plant location (50, 100, 150 and 200 cm) from plot centre) and date were analysed as repeated measures. Interaction effects were also tested i.e. CO₂ × location, CO₂ × date, date × location, CO₂ × date × location.

Table 4-3. Summary of two-way repeated measures ANOVA to determine the effect of elevated soil CO₂ on plant height, tiller number plant⁻¹, cob number plant⁻¹ and leaf chlorophyll content (n=4, 50 gassed plants and 50 control).

Variable	Source	F	P
Plant height	CO ₂	2.80	0.28
	Date	1.30	0.002
	Location	1.30	0.32
	CO ₂ * Date	6.80	0.04
	CO ₂ * Location	0.40	0.68
	Date* Location	2.00	0.15
	CO ₂ *Date*Location	4.00	0.04
Tiller number Plant ⁻¹	CO ₂	0.10	0.11
	Date	19.10	0.009
	Location	0.79	0.44
	CO ₂ * Date	1.30	0.32
	CO ₂ * Location	3.00	0.03
	Date* Location	1.80	0.04
	CO ₂ *Date*Location	3.10	0.008
Total cob number Plant ⁻¹	CO ₂	6.98	0.045
	Date	59.90	0.008
	Location	3.60	0.003
	CO ₂ * Date	6.40	0.002
	CO ₂ * Location	2.20	0.12
	Date* Location	1.40	0.22
	CO ₂ *Date*Location	1.60	0.18
Chlorophyll content	CO ₂	11.20	0.002
	Date	22.20	0.038
	Location	5.89	0.043
	CO ₂ * Date	7.01	0.003
	CO ₂ * Location	7.40	0.002
	Date* Location	3.90	0.009
	CO ₂ *Date*Location	4.90	0.012

Plant height, tiller number plant⁻¹ total cob number plant⁻¹ and chlorophyll content were all lower in the gassed plots. The impact was more near the point of injection where the concentration of CO₂ was higher; this corresponds to the area within 100-150 cm in diameter. The development of severe chlorosis was a reflection of the progressive decline in chlorophyll content in the gassed plots due to stress. The number of cobs plant⁻¹ continued to increase in the control plots, but decreased in gassed plots as

the experiment progressed. The CO₂ × location, CO₂ × date, CO₂ × date × location interactions were all significant for plant height and total cob number plant⁻¹.

4.2.3 Soil pH analysis.

Soil samples were taken from both the CO₂ treated and herbicide experiment on 10th April, 2009 before gas injection and herbicide application and on 28th October, 2009 at the end of the season. The samples were taken at 10, 30, and 80cm depths for the controls, gassed and herbicide treated plots to determine any variation (Refer to appendix 5 for additional information on soil pH measurement).

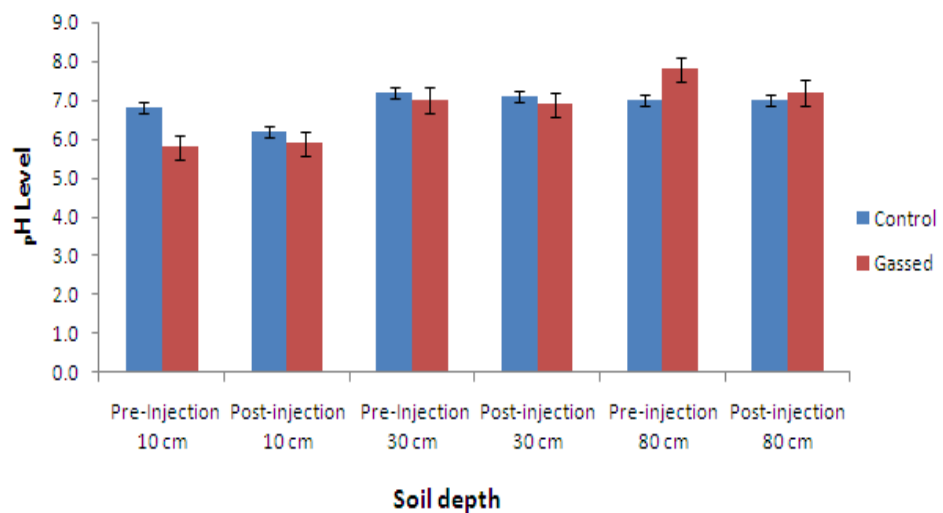


Figure 4-11. Change in soil pH following CO₂ injection in maize crop.

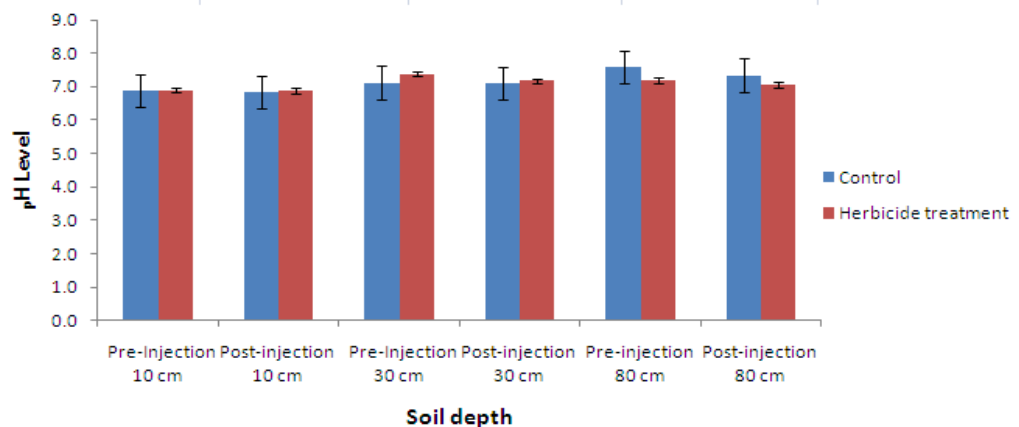


Figure 4-12. Change in soil pH following herbicide application in maize crop.

The change in soil pH levels as a result of CO₂ injection and herbicide treatment are shown in Figures 4-11 and 4-12. The average pH values before injection for the control plots at the depth of 10, 30, and 80cm were 6.8, 7.2, and 7.0 respectively. At post-injection a difference of 0.6, 0.1 and 0 were observed at the measured depths. The pre-injection soil pH value for the gassed plots at the same depths were 5.8, 7 and 7.8 which resulted in a difference of 0.1, 0.1 and 0.6 post-injection pH at the various measured depths. These results show that at each of the depth the soil pH decreased, relative to control plots although the difference compared to the control was not significant when tested with ANOVA (*p-value* 0.143 ($\sigma \leq 0.05$); n=144). In contrast Schumacher (1996) found that high CO₂ concentration in soil and low oxygen can cause changes in soil pH and redox potential.

The herbicide treated maize experiment also showed decrease in soil pH at the soil depths analysed relative to control plots as depicted in Figure 4-9. The difference was not significant (ANOVA, *p-value* 0.231 ($\sigma \leq 0.05$); n=144) compared to the CO₂ experiment. The control plots had a post injection soil pH difference of 0.06, 0.02, and 0.25 at 10, 30 and 80cm depths which were relatively small compared to the CO₂ controls. The difference between the pre-injection and post-injection soil pH for the herbicide treated plots were 0.03, 0.22 and 0.14 at the measured soil depths.

The result showed that there were differences in soil pH between the treatments types as well as the pre and post injection. These differences could be linked to the sinking of CO₂ down the soil or the effects of fertiliser applied during the growing season.

4.2.4 Chlorophyll contents.

Chlorophyll contents of the leaves were measure in the field using a SPAD meter (Minolta SPAD, UK); (Refer to section 3.8 for details)

In the gassed plots there was a decrease in chlorophyll content of about 20 and 50% for low and high gas zone respectively when compared to the controls, while for the herbicide experiment the decrease was 50%. Compared to maize crops grown on controls, both treatments showed decrease in chlorophyll content as depicted in Figure 4-13. These decreases were manifestations of both stresses, as reduction in chlorophyll content has been linked to stress and malfunction of the physiological status of plants (Curran, 1998).

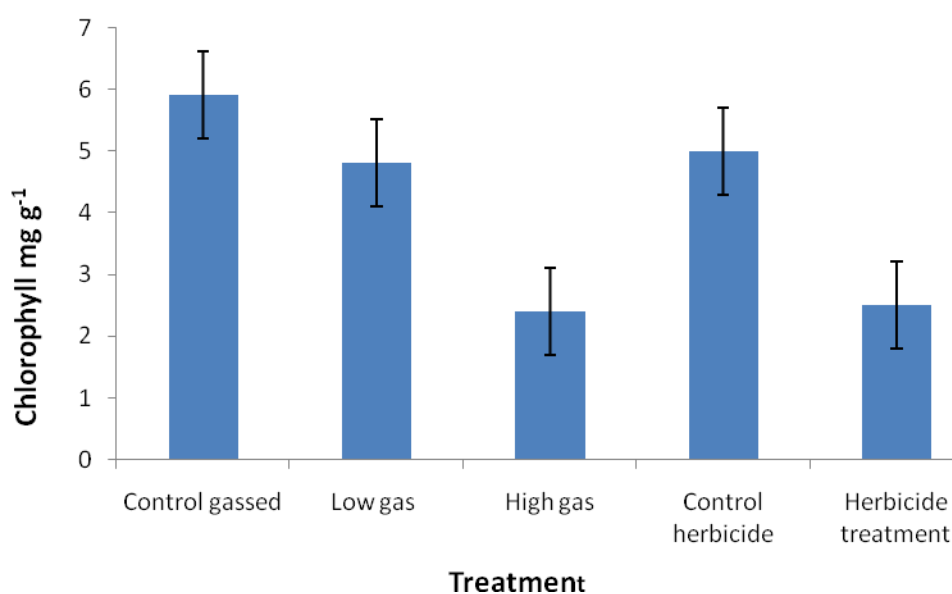


Figure 4-13. Average chlorophyll content for Low, high gas , herbicide treatment and their controls.

4.2.5 Canopy spectral reflectance.

Changes in spectral reflectance have been associated with response of plants to stress (Macek *et al.*, 2005, Blackburn, 2007, Zhang *et al.*, 2008).

Canopy reflectance were measured on days 2, 7, 14, 33, 39, 54, 56 where day zero is the start date (15th July, 2009) of CO₂ injection and herbicide application. For additional information on reflectance measurement techniques, refer to sections 3.3.

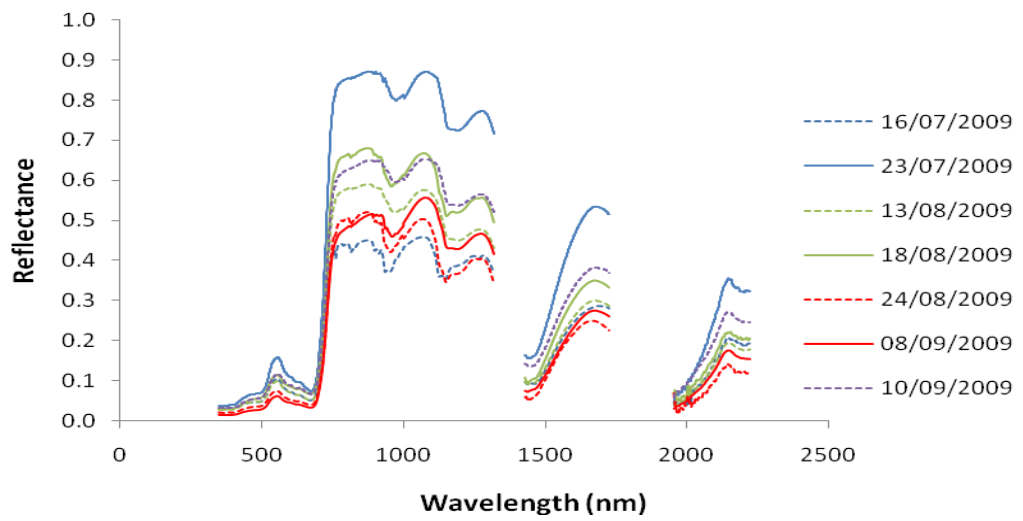


Figure 4-14. Reflectance spectra of maize grown on gas control plots.

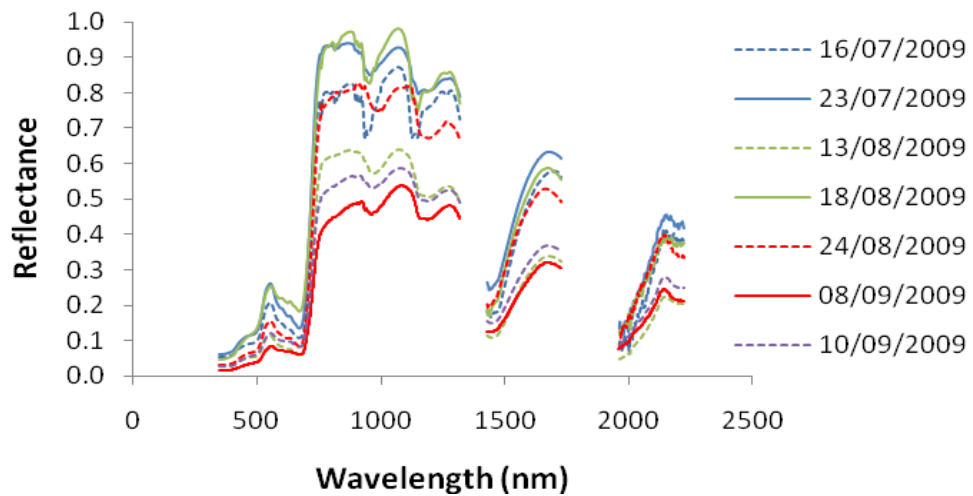


Figure 4-15. Reflectance spectra of maize grown on low gas concentration zone.

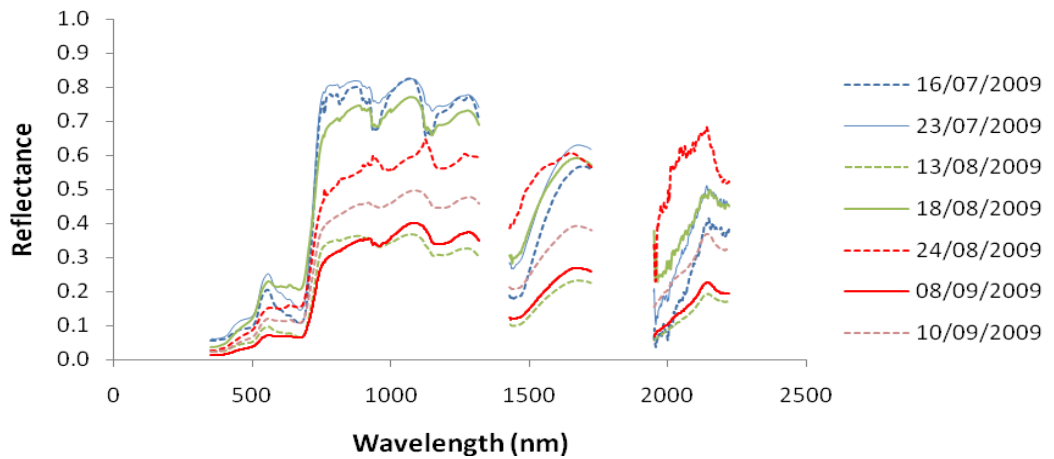


Figure 4-16. Reflectance spectrum of maize grown on high gas concentration zone.

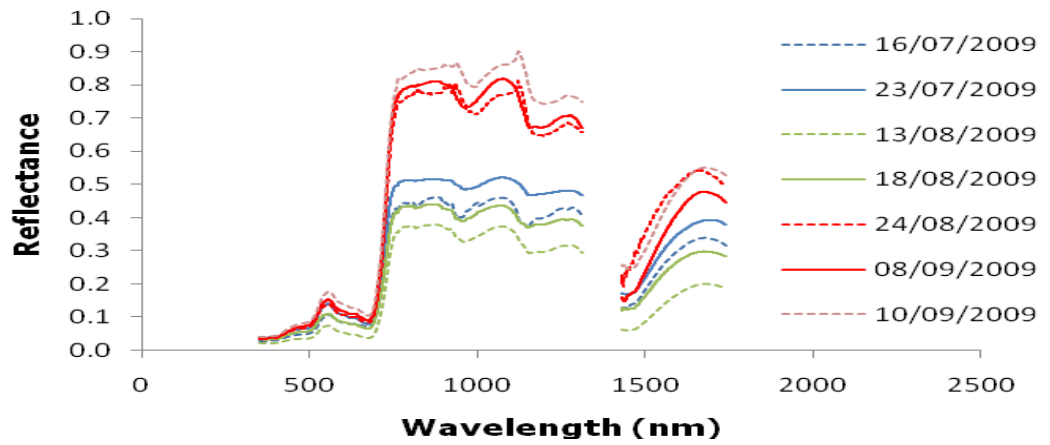


Figure 4-17. Reflectance spectra of maize grown on herbicide control plots.

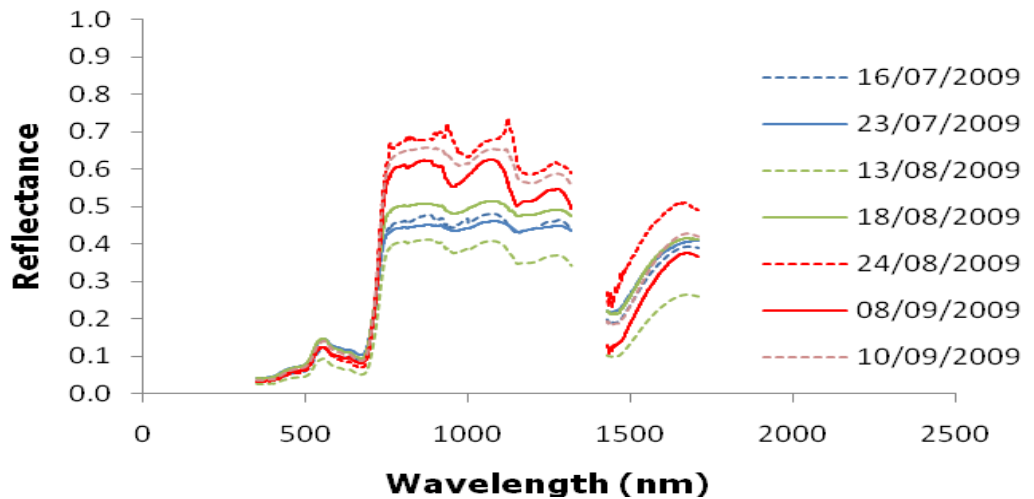


Figure 4-18. Reflectance spectra of maize grown on herbicide treated plots.

Reflectance spectra of maize crop stressed with CO₂ and herbicide as well as their respective controls measured throughout the duration of the experiment (16th July, 2009 – 10th September, 2009) are shown in Figures 4-14 to 4-18; each spectral curve for the control plots for each date is an average of sixteen measurements while the high and low gas concentration curves represent an average of eight measurements corresponding to the plot centre and edge respectively. The gaps in the spectral are regions affected by atmospheric water vapour where there is not enough energy in the solar radiation spectrum to make meaningful measurements.

4.2.5.1 Canopy reflectance differences between CO₂ treatment and control.

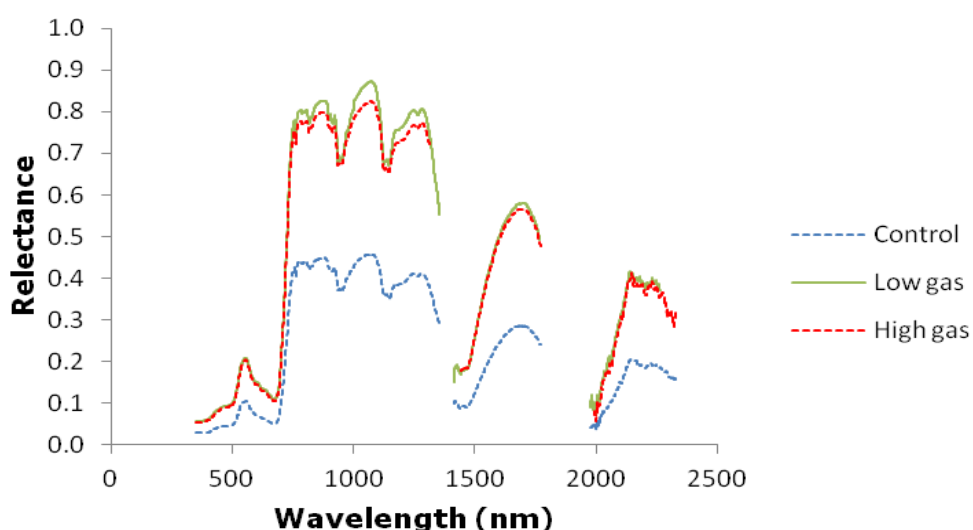
The means of the reflectance between the two treatments level of soil CO₂ (low and high) were tested to determine whether they were significantly different at each wavelength. This was statistically tested using one-way analysis of variance (ANOVA). The statistical tests were done at different periods after treatments with CO₂ in order to assess the spectral reflectance difference(s) between treatments at different stages of plants' physiological status.

The mean reflectance difference between low and high CO₂ zone compared to control throughout the duration of the experiment are shown in Figures 14-19a-g. These Figures depicts differences in wavelengths in the visible region compared to control. The ANOVA test for the spectral ranges was calculated using the average spectral reflectance within the range. When tested the results showed significant differences between the treatments relative to control in the visible region (400-750nm) of the spectrum, statistically significant bands occurred between 450 nm and 535 nm and also between 574 nm and 700 nm (Refer to appendix 8 and 9) except for Figures 14-19c and the last two dates of measurement shown in Figures

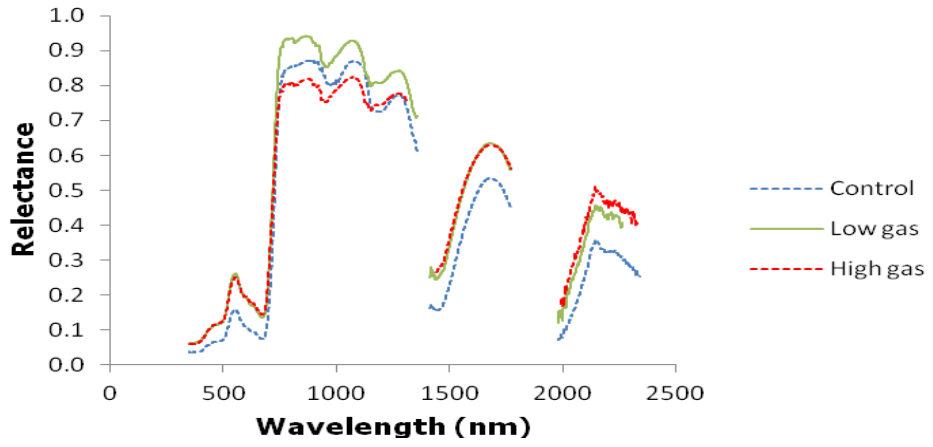
14-19f and g. However, it is important to note that the difference between the levels of CO₂ treatments (high and low) in the visible region were not significant for virtually all the measurement dates as shown in Figures 4-19a-g.

In the NIR region there was significant difference between control and the treatment levels in all the spectral measurement dates using ANOVA (Refer to appendix 10 for details) and well as between the treatment levels. Channels that showed much significant difference were between 1010 and 1370 nm. The large differences in the near-infrared can be visually recognized in Figures 4-19d and e.

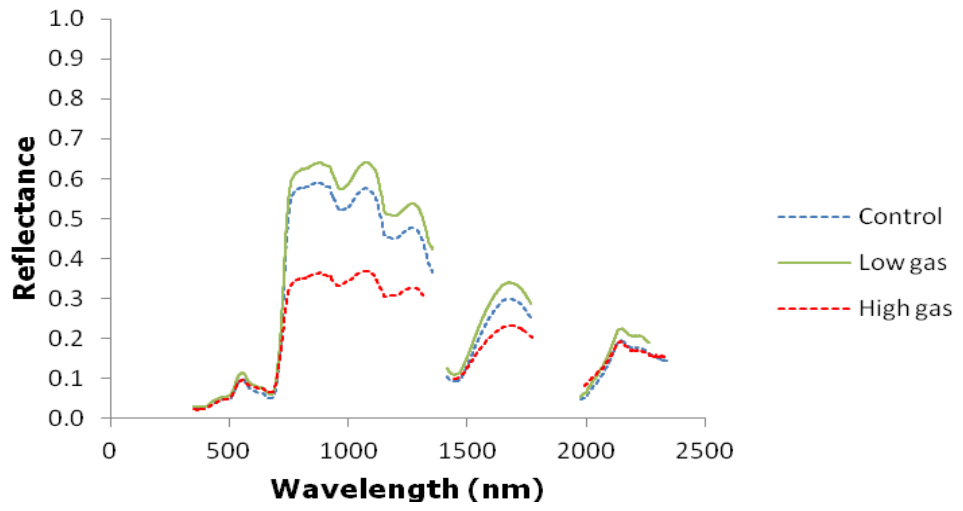
While in the SWIR region as shown in Figures 14-19a-e there was significant difference in all the dates at wavelengths 1500, 1605, 1680, 1760, 2064, 2200, 2320 and 2315 nm with ANOVA (Refer to appendix 11 for details), except for Figures 14-19f and g, at this point the crops were fully matured and virtually turned yellow.



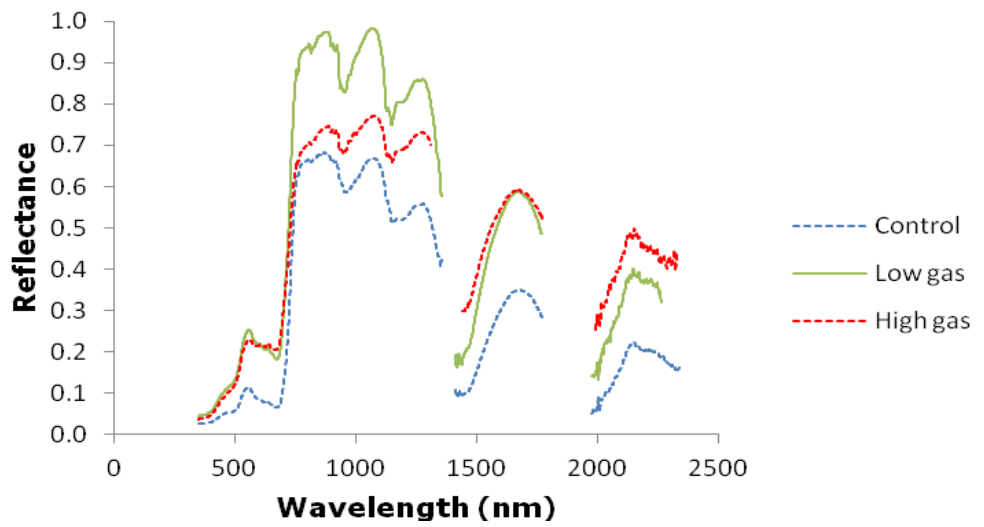
A. (16/07/2009).



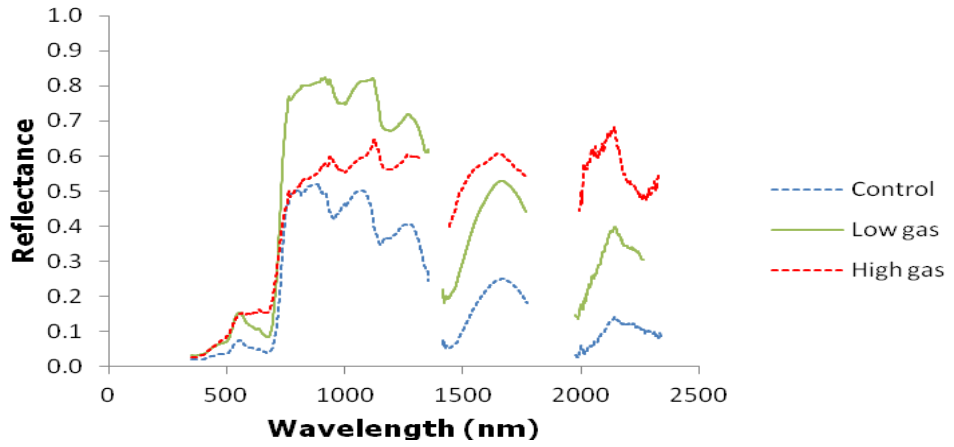
B. (23/07/2009).



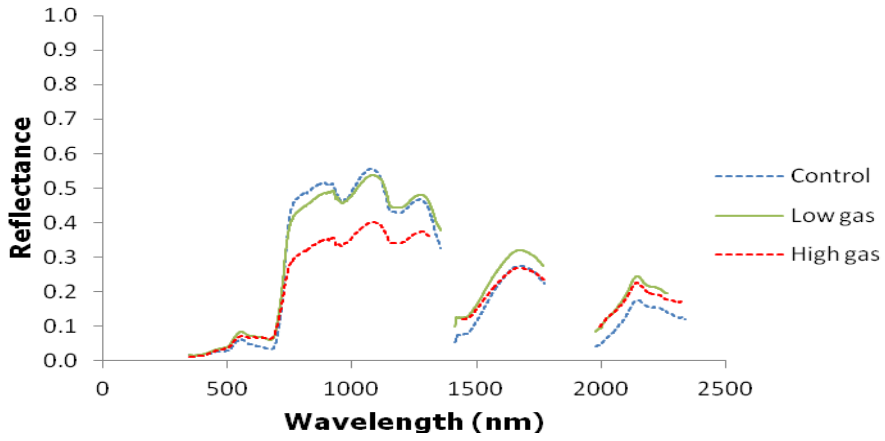
C. (13/08/2009).



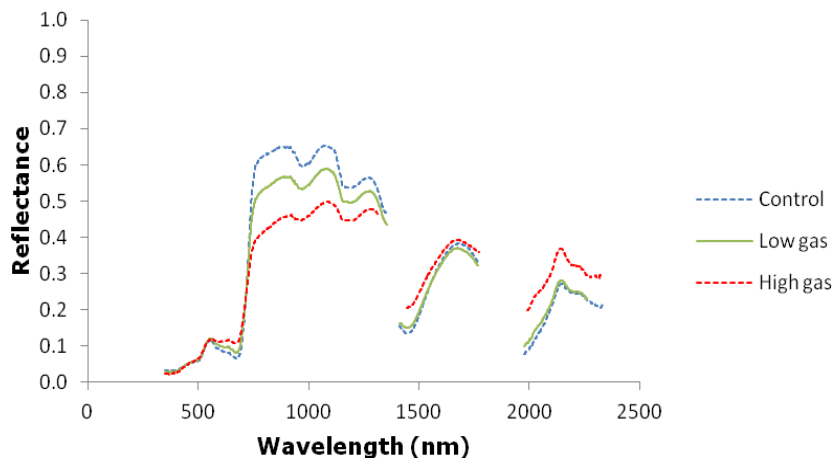
D. (18/08/2009).



E. (24/08/2009).



F. (08/09/2009).

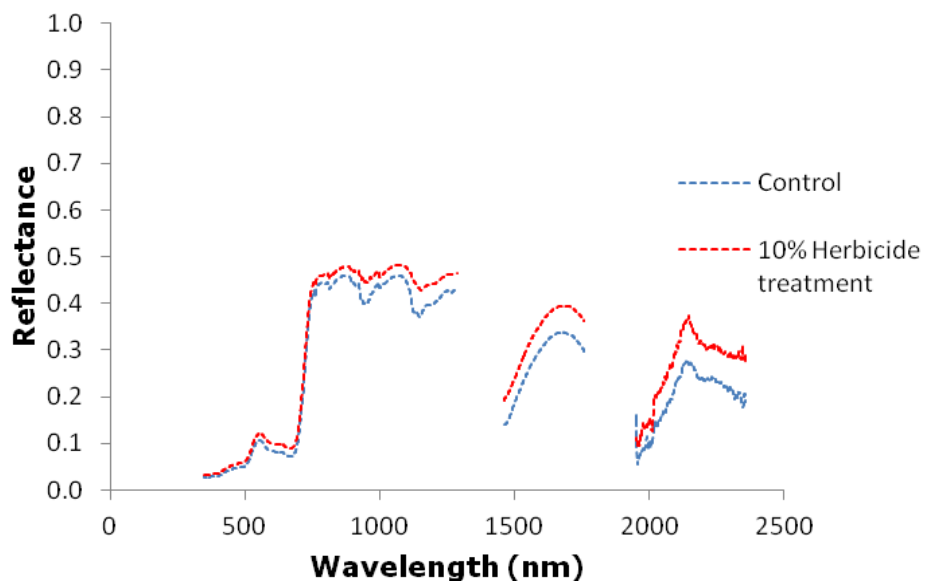


G. (10/09/2009).

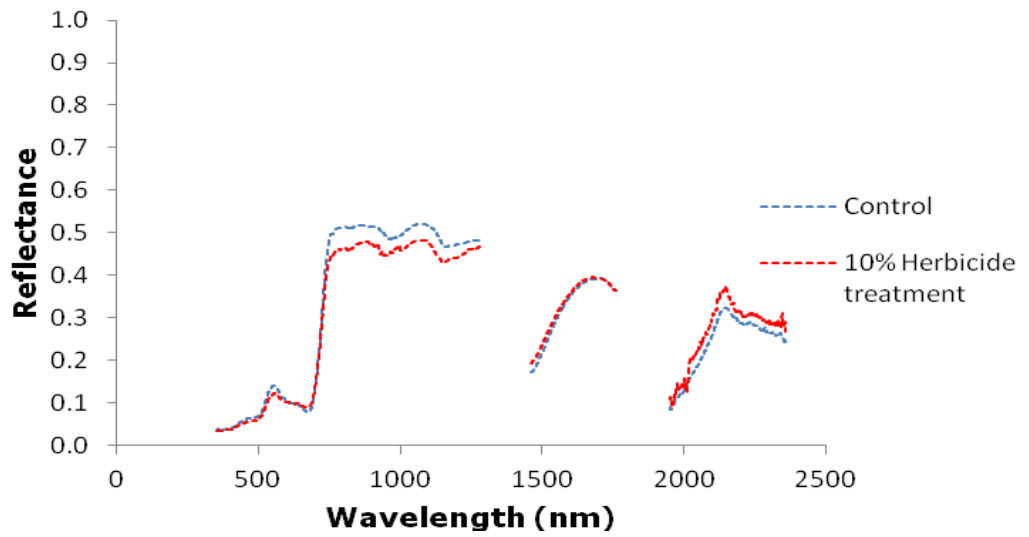
Figure 4-19. A-G: Reflectance difference between control, high and low gas region for maize grown in gassed plots on the respective dates.

4.2.5.2 Canopy reflectance differences between herbicide treatment and control.

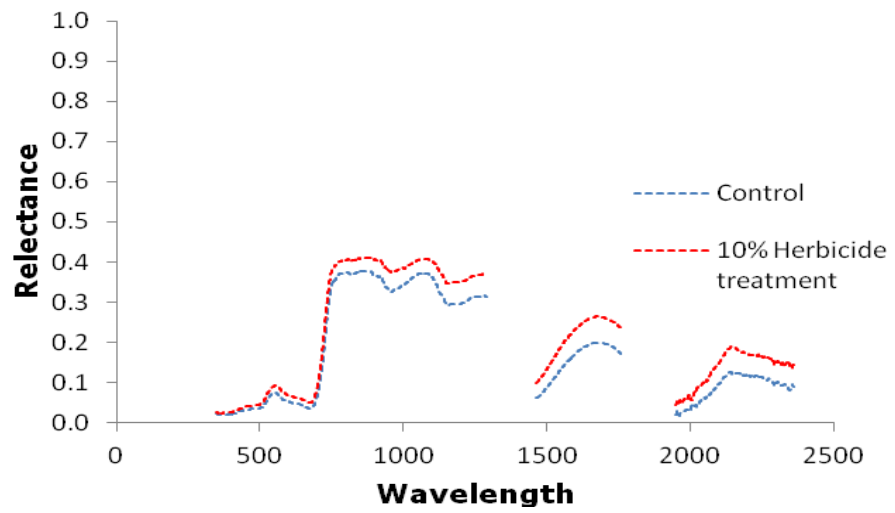
The temporal reflectance difference between herbicide treatment and control are shown in Figures 14-20a-g. Throughout the experiment compared to control plots there was no significant difference in the visible region of the spectrum when tested using ANOVA (Refer to appendix 12 for details. Except for Figure 14-20b, the NIR and SWIR depicts significant difference relative to control reflectance at 1120, 1206, 1518, 1605 nm, 2150 and 2365 nm using ANOVA (Refer to appendix 11 for details). The herbicide treatment had just one dose (10%) of treatment unlike the gas treatment as such the difference between treatment is therefore not relevant in this situation.



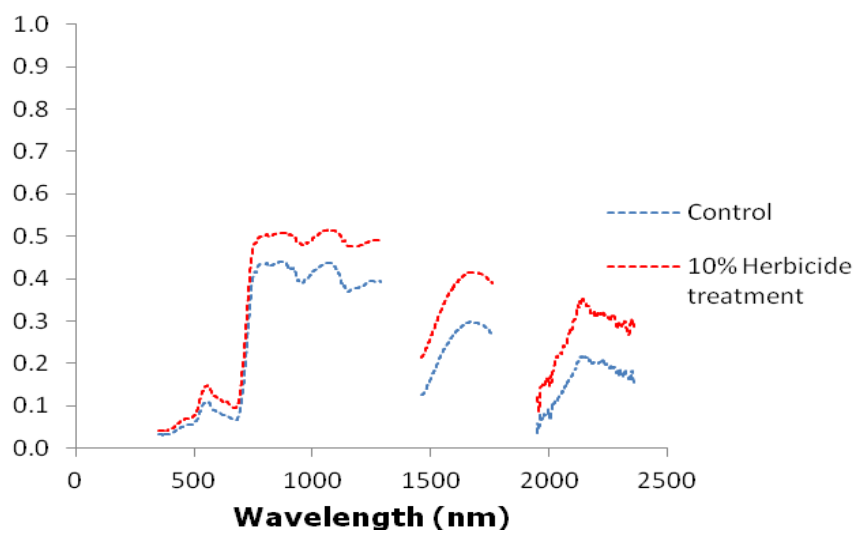
A. (16/07/2009).



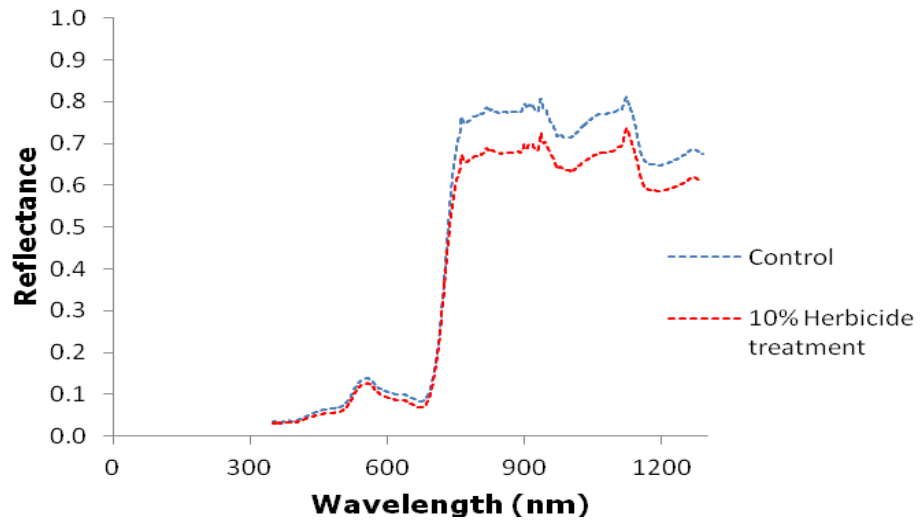
B. (23/07/2009).



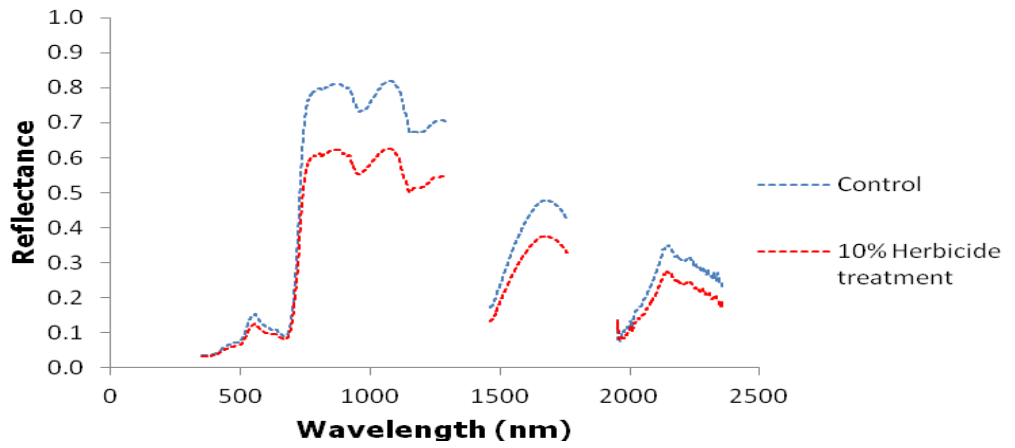
C. (13/08/2009).



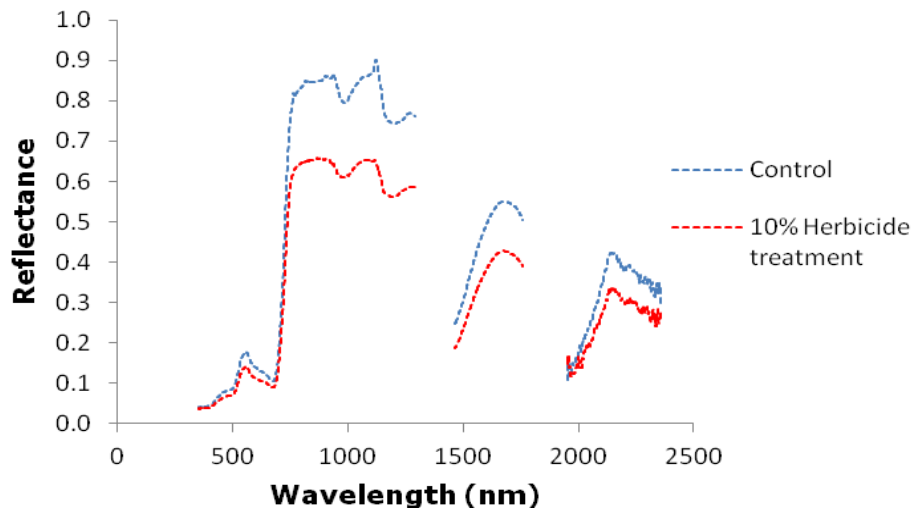
D. (18/08/2009).



E. (24/08/2009).



F. (08/09/2009).



G. (10/09/2009).

Figure 4-20. A-G: Reflectance difference between control and herbicide treated maize on dates above.

4.2.6 Derivative analysis.

Derivative analysis was carried out in order to detect stress caused by elevated concentration of soil CO₂ and sub-lethal dose (10%) of herbicide application on maize crop. The derivative of reflectance in the red-edge region was used in this study to determine the position and height of the red-edge peaks and other peaks that may indicate stress in plants (Smith *et. al.*, 2004a). For detailed information on the first derivative refer to section 3.10.2.

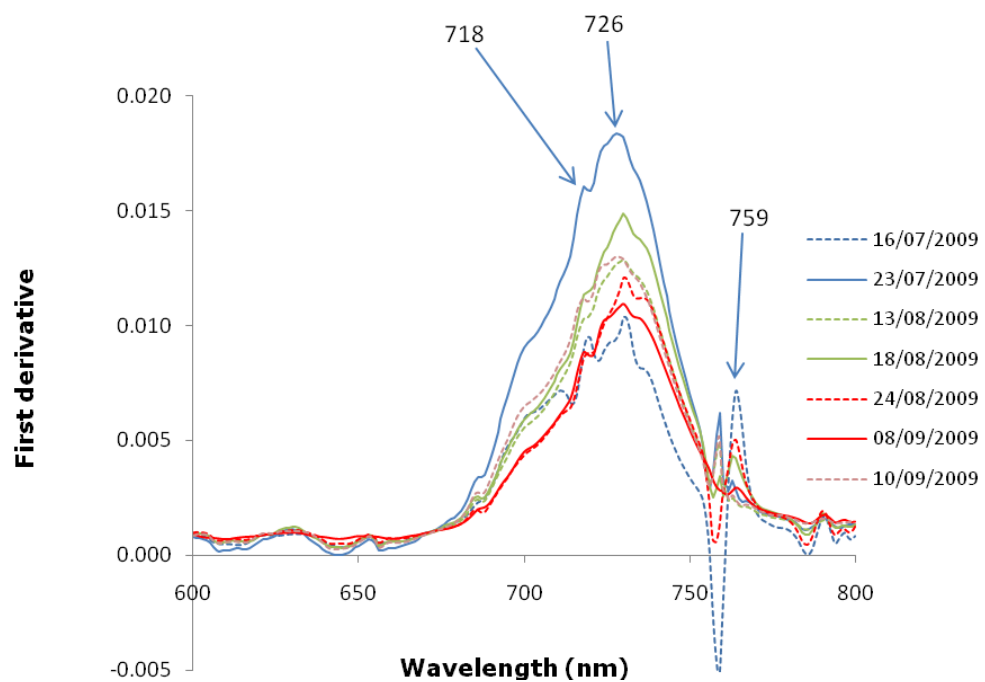


Figure 4-21. First derivative reflectance peaks of maize grown on CO₂ control plots.

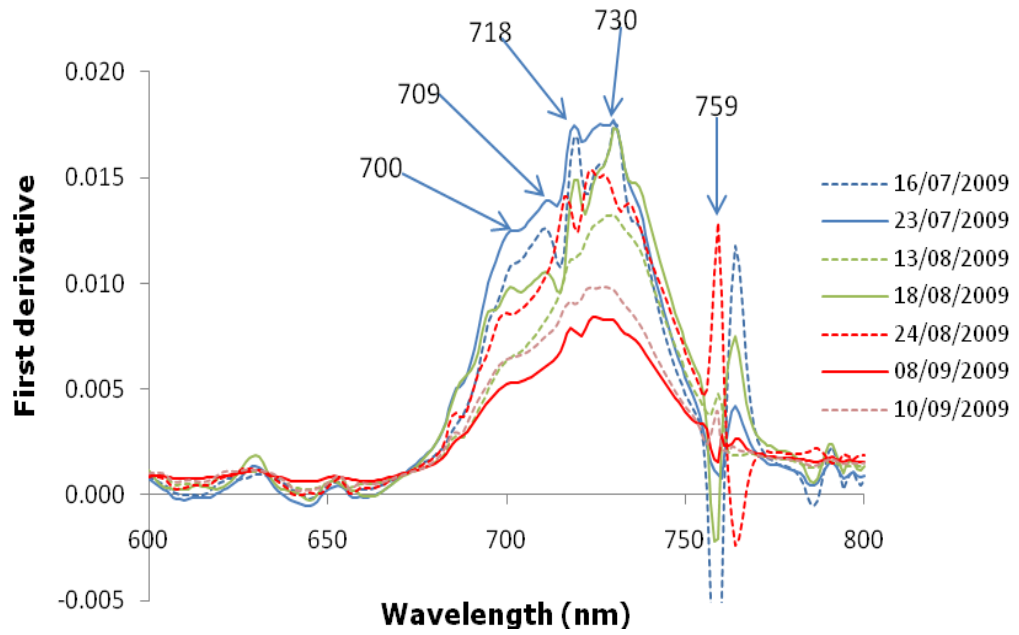


Figure 4-22. First derivative reflectance peaks of maize grown on low CO₂ zone.

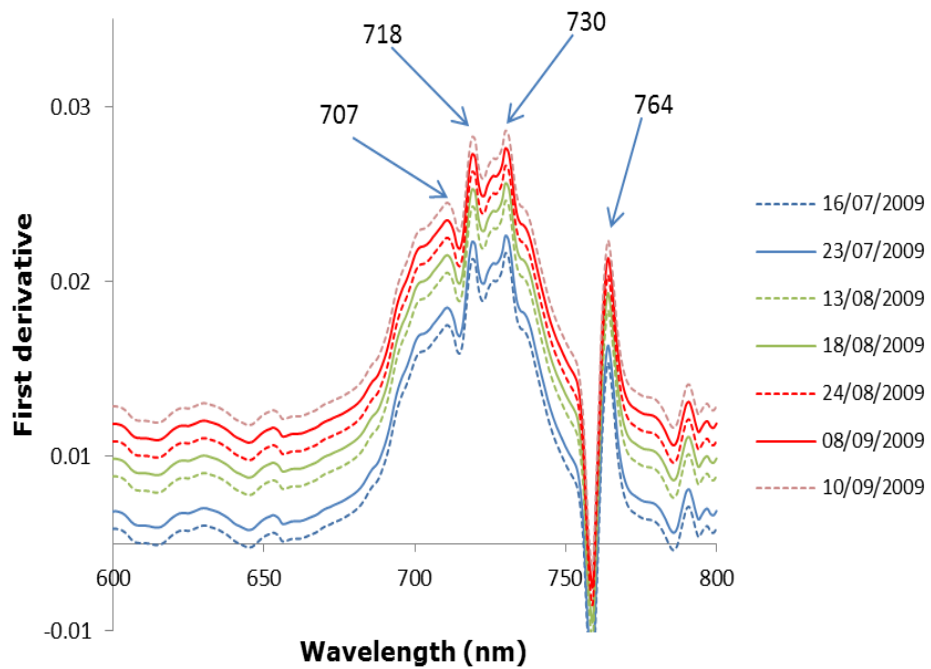


Figure 4-23. First derivative reflectance peak of maize grown on high CO₂ zone. Curves for different dates are offset vertically for clarity.

The first derivative spectra in the soil CO₂ experiment showing the red-edge peaks are depicted in Figures 4-21 to 4-23. Throughout the experiment, the CO₂ control plots were composed of a single maximum

peak at 726nm with smaller peaks or shoulders at 718 and 759nm, while the gased plots had double peaks at 718 and 730nm, with several secondary peaks or shoulders found between 707 and 794nm. These features were used to detect differences between control and CO₂ stressed maize.

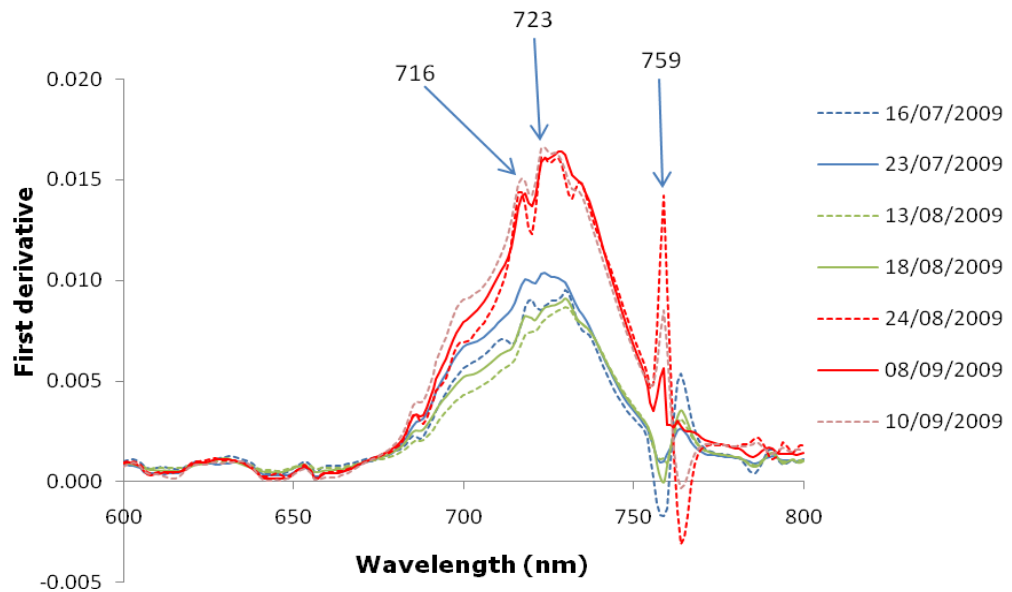


Figure 4-24. First derivative reflectance peaks of maize grown on herbicide control plots.

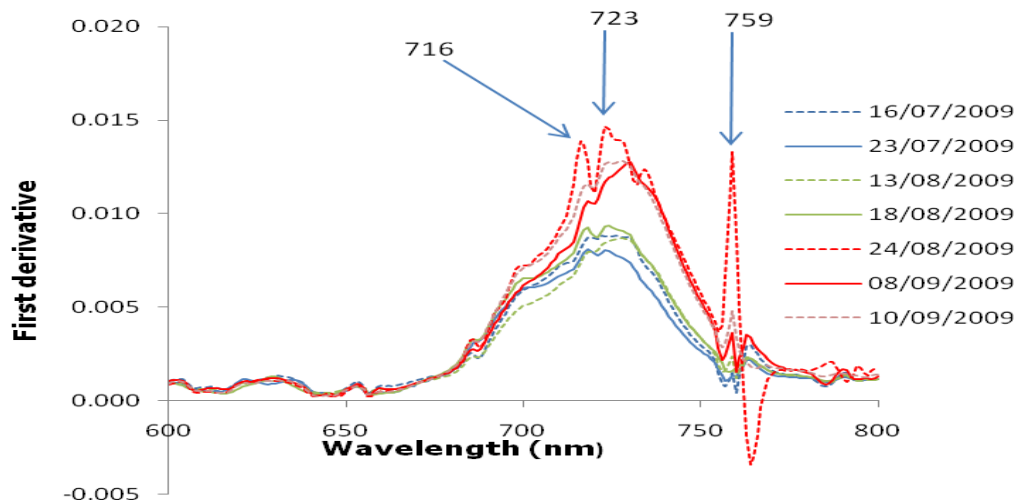


Figure 4-25. First derivative reflectance peaks of maize grown on herbicide treated plots.

The first derivative peaks for the herbicide control was composed of a single peak at 723nm with small shoulders or peaks at 759 and 716nm as shown in Figure 4-24. As the experiment progressed and the herbicide stress began to manifest, there was shift and change in the derivative peaks, the maximum peak became double at 716 and 723nm, the shoulder remained at 759nm (Figure 4-25).

By the late treatment period between 18th August and 10th September, 2009 most of the maize crops had turned yellow in all the treated plots, the peaks had decreased further in magnitude with a shift of the red edge position to shorter wavelengths; the major peaks were still between 716 and 723nm the shoulder remained at 759nm.

4.2.7 Red Edge Position.

Red edge position (REP) was calculated using the method proposed by Cho and Skidmore (2006); this was plotted over time for both experiments to determine any variations throughout the experiment. For more details on REP refer to section 3.10.2

In the soil CO₂ experiment as shown in Figure 4-26 there was an increase in the red edge position (REP) from t=2 until t=28, a drop in the position at t=33, then between t=39 and t=56, the REP continued to decrease until the last date, 10th September, 2009 when the crops were fully matured and ready for harvest. The REPs for gased maize showed larger differences between the treatments when compared with controls. There was significant difference from t=2 until t=56 (ANOVA (*p*-value 0.016; 0.025; 0.000; 0.010; 0.015; 0.001; 0.000; 0.000 ($\sigma \leq 0.05$); n=8) between the REPs of maize grown on high CO₂ concentration zone compared to the control plots during much of the experiment (Refer to appendix 13 for details) with no significant difference in low CO₂ zone at t=28 (ANOVA (*p*-value 0.413 ($\sigma \leq 0.05$); n=8). The difference between the REPs of low and

high CO₂ from t=2 until t=56 were statistically significant at all dates (ANOVA (*p*-value 0.038; 0.022; 0.003; 0.017; 0.000; 0.000; 0.000; 0.000) ($\sigma \leq 0.05$); n=8). Details can be found in appendix 13.

The maize herbicide experiment showed that there was significant difference (ANOVA (*p*-value 0.036; 0.005; 0.040; 0.048; 0.039; 0.000; ($\sigma \leq 0.05$); n=8) between the REP positions of the herbicide treated plots compared to controls during the experiment except at t=54 and 56, (ANOVA (*p*-value 0.486; 0.925). This could be attributed to the death of the plants at this stage. Refer to appendix 13 for details of the ANOVA results.

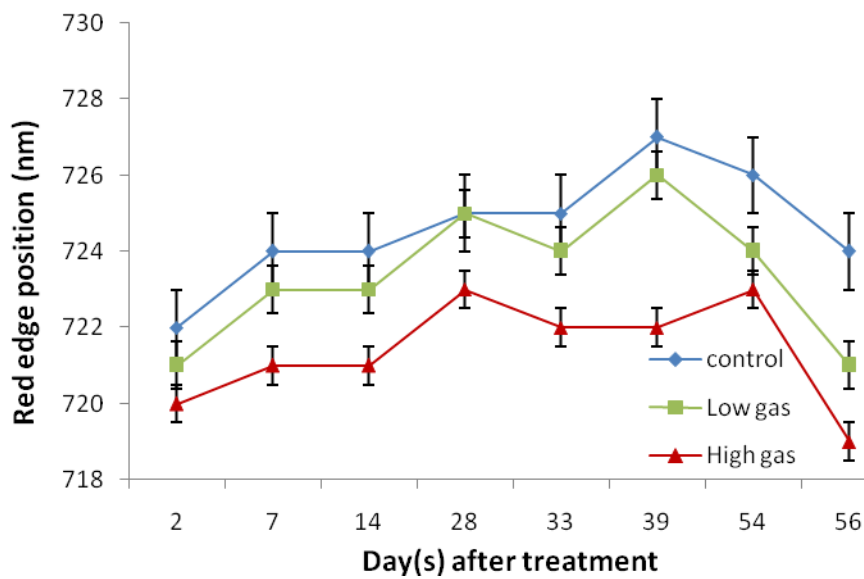


Figure 4-26. Temporal change in red edge position over time for maize control and CO₂ treatments.

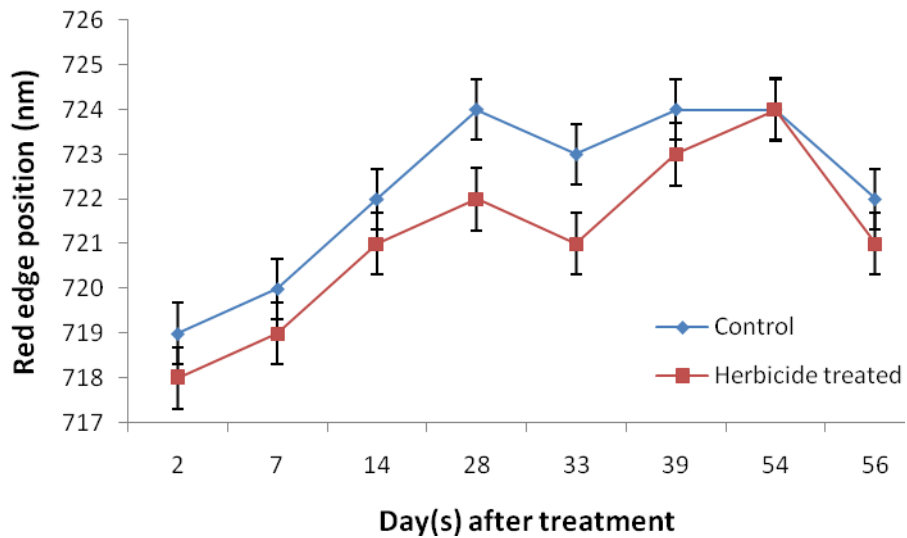


Figure 4-27. Temporal change in red edge position over time for maize control and herbicide treatment.

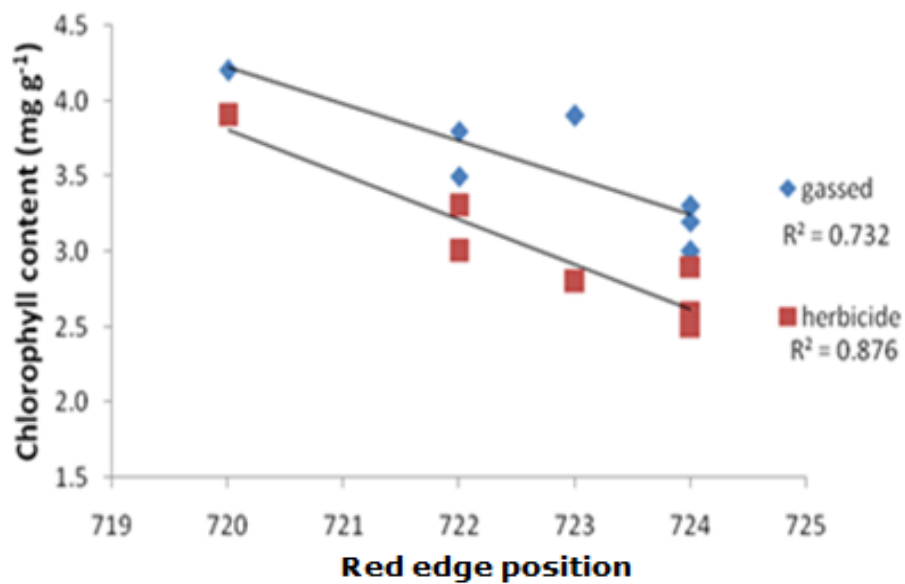


Figure 4-28. Relationship between red edge position and chlorophyll content.

The relationship between red edge position and chlorophyll content is shown in Figure 4-28. In this study strong correlation ($R^2=0.876$ for herbicide, 0.733 for gassed, p -value 0.028 ($\sigma \leq 0.05$); $n=7$) was found between red edge position and chlorophyll content of maize leaves, suggesting that the red edge position was due to the decreasing chlorophyll

content caused by each of the stresses. The R^2 value for the herbicide treatment was higher than the CO_2 , which may be a result of the fact that the herbicide treated maize grew slowly at the start of the season and the stress effect on the crop was gradual. This was evident by the slow response of herbicide stress as shown by the visual stress symptoms. More interesting is the fact that although the lines have similar slope, they clearly have different intercepts, so that the relationship of red-edge to chlorophyll depends on the nature of stress and possibly the experiment. A similar relationship between red edge position and chlorophyll content has been reported by Miller *et al.* (1990).

4.2.8 Spectral vegetation Indices.

In this study four indices were selected in order to determine the change in chlorophyll content over time as a result of stress due to CO_2 and herbicide on maize. These indices were chosen based on their sensitivity to stress. They have also been applied in stress studies by Lakarraju *et al.* (2010). The indices are: Chlorophyll Normalized Difference Index (*Chl NDI*), Pigment Specific Simple Ratios for chlorophyll *a* (*PSSRa*) and chlorophyll *b* (*PSSRb*), Physiological Reflectance Index (*PRI*). For additional information, refer to sections 3.10.3

4.2.8.1 Chlorophyll Normalized Difference Index (*Chl NDI*).

The temporal variation in Chlorophyll Normalised Difference Index (*Chl NDI*) for CO_2 and herbicide treatment compared to their corresponding controls are shown in Figures 4-29 and 4-30. In the control plots and low gas concentration zone there was an initial increase in total chlorophyll content, from $t=2$ to $t=33$, while high gas concentration compared to control showed little increase on the same dates. The average differences between the high CO_2 and control treatments at $t=2$ to 39 were statistically

significant when tested with ANOVA (*p-value* 0.011; 0.025; 0.000; 0.039; 0.005 ($\sigma \leq 0.05$); n=8). Refer to appendix 14 for the ANOVA details. Between t=45 to t=56, the high CO₂ zone compared to the control showed no significant difference (ANOVA (*p-value* 0.316; 0.089; 0.489 ($\sigma \leq 0.05$); n=8); at this stage maize had turned yellow and was ready for harvest. There was no significant difference (ANOVA (*p-value* 0.419; 0.878; 0.657; 0.998; 0.911; 0.791; 0.987; 3.847 ($\sigma \leq 0.05$); n=8) between control and low gas concentration throughout the duration of the experiment. The difference between high and low CO₂ zone was only significant at t=14 and 33 (ANOVA (*p-value* 0.011; 0.017 ($\sigma \leq 0.05$); n=8) as shown in Figure 4-29.

Chl NDI in the herbicide treated maize initially showed little variation between the treatments and control. The difference was not statistically significant with ANOVA (*p-value* 1.003; 0.123; 0.675 ($\sigma \leq 0.05$); n=8) between t=2 and t=14, this may be associated with slow response of maize to herbicide stress. Between t=33 and t=56 there was a significant difference (ANOVA (*p-value* 0.016; 0.024; 0.004; 0.039; 0.023 ($\sigma \leq 0.05$); n=8) in total chlorophyll content between the maize crop growing on herbicide treated plots and those on the control. At this point the visible signs of stress had manifested. Refer to appendix 14 for ANOVA details.

These results indicate that there was a decrease in chlorophyll content as measured by the index. This is associated with high CO₂ concentration compared to the corresponding control and low CO₂ concentration, as well as with the herbicide treatment.

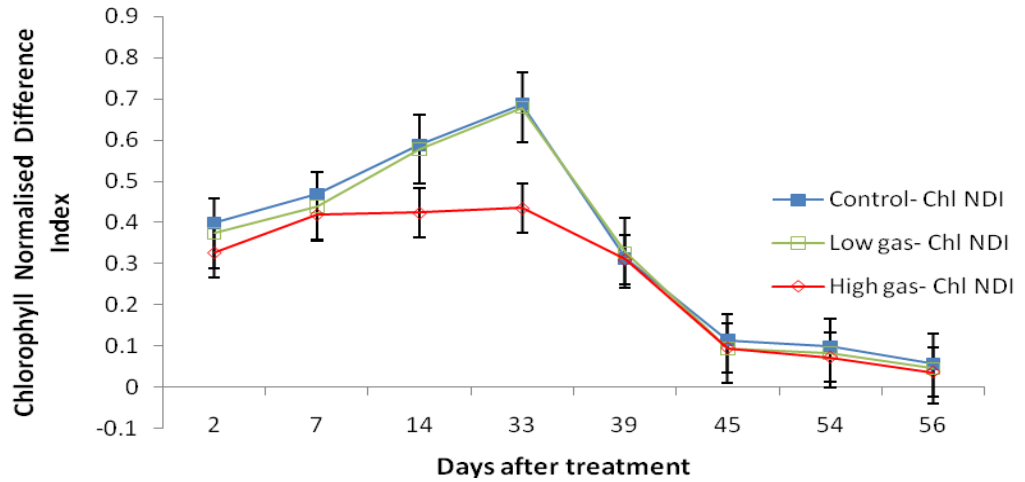


Figure 4-29. Temporal change in Chlorophyll Normalised Difference Index (Chl NDI) for maize crop treated with CO₂ and corresponding control plots.

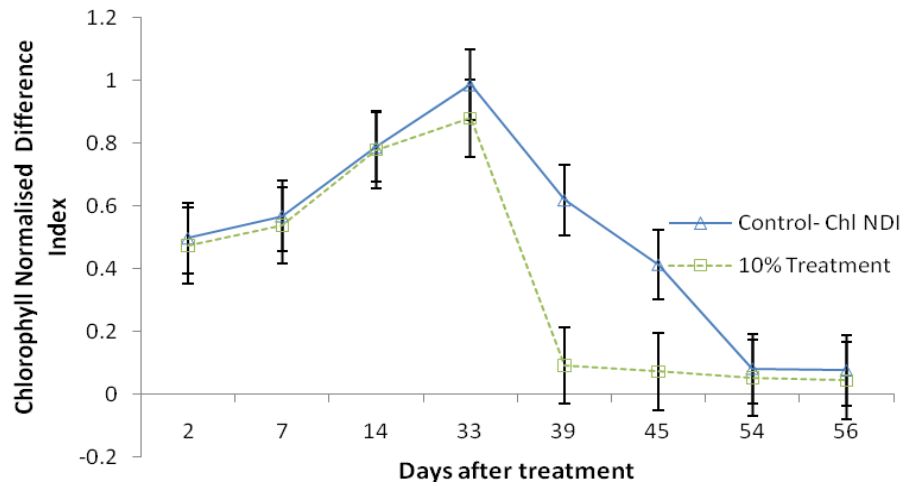


Figure 4-30. Temporal change in Chlorophyll Normalised Difference Index (Chl NDI) for crop treated with herbicide and corresponding control plots.

4.2.8.2 Pigment Specific Simple Ratios (*PSSRa* and *PSSRb*).

The patterns of change over time in pigment specific simple ratio for chlorophyll a and b indices for maize are shown in Figures 4-31 to 4-34. There was a significant difference (ANOVA (*p*-value 0.041; 0.005; 0.034; 0.047; 0.000; 0.016; 0.029; 0.049 ($\sigma \leq 0.05$); $n=8$) throughout the experiment duration between low and high CO₂ zones compared to corresponding control chlorophyll a. The pigment specific simple ratio for chlorophyll b in the high gas zone was significant (ANOVA (*p*-value 0.023; 0.018; 0.037; 0.008; 0.011; 0.000; 0.007; 0.021 ($\sigma \leq 0.05$); $n=8$) from

t=2 to t=56, while in the low CO₂ zone there was no significant difference at t=2 to t=14, (ANOVA (*p*-value 0.622; 1.413; 0.675 ($\sigma \leq 0.05$); n=8) from t=33 to t=56 the difference became significant (ANOVA (*p*-value 0.034; 0.024; 0.004; 0.039; 0.023 ($\sigma \leq 0.05$); n=8). For the herbicide treatment the *PSSRa* became significant from t=33 until t=56 (ANOVA (*p*-value 0.007; 0.0018; 0.002; 0.000; 0.000 ($\sigma \leq 0.05$); n=8). While *PSSRb* was not significant at t=2 to t=39, (ANOVA (*p*-value 1.836; 0.992; 0.544; 1.934 ($\sigma \leq 0.05$); n=8) the difference with control became significant from t=45 until t=54, (ANOVA (*p*-value 0.049; 0.033; 0.026 ($\sigma \leq 0.05$); n=8). Details of the ANOVA can be found in appendix 15.

The results show sensitivity of *PSSRa* to both low and high CO₂ at any point during the experiment, while *PSSRb* was responsive to high CO₂ earlier than low CO₂. Both *PSSRa* and *PSSRb* were only sensitive at much later date(s) between t=39 and t=54 in the herbicide experiment.

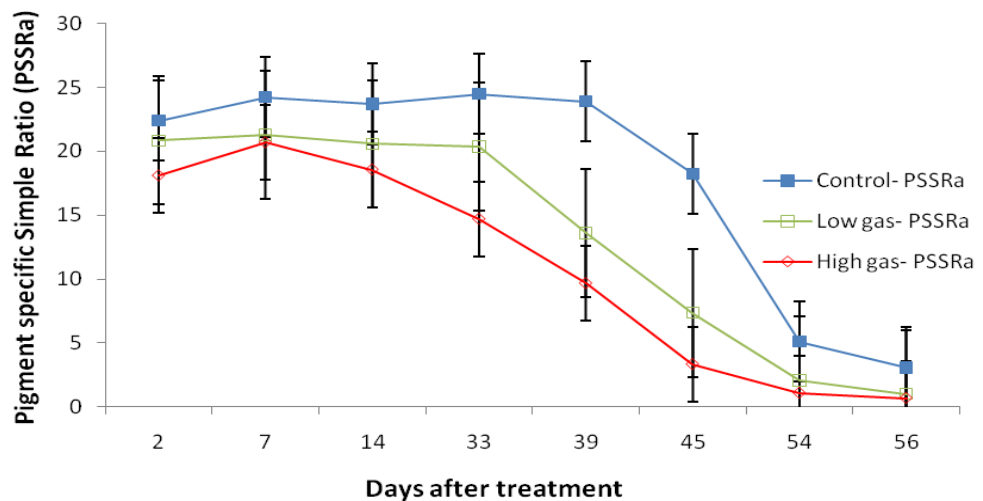


Figure 4-31. Temporal change in Pigment Specific Simple Ratio for chlorophyll a (*PSSRa*) in CO₂ and corresponding control plots.

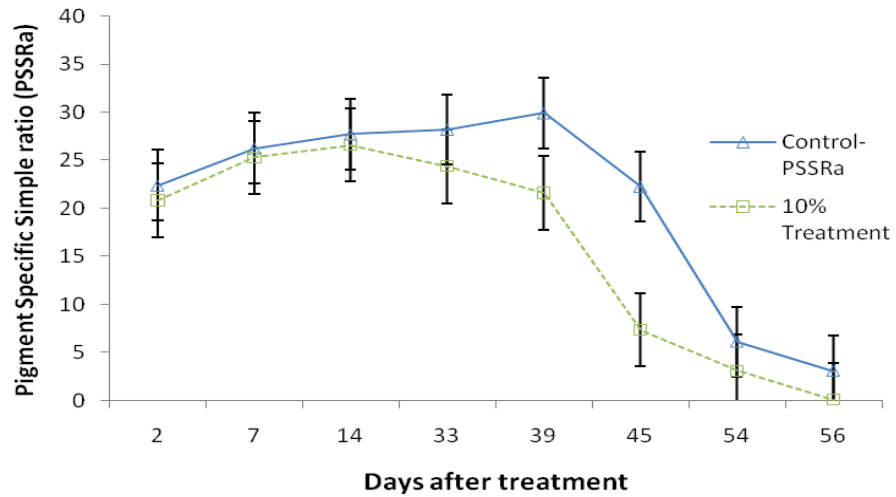


Figure 4-32. Temporal change in Pigment Specific Simple Ratio for chlorophyll a (PSSRa) in herbicide treated and corresponding control plots.

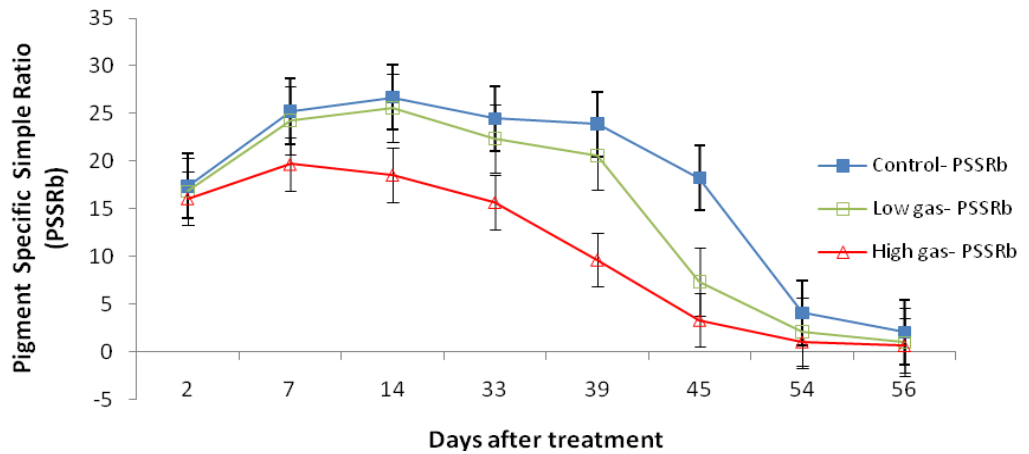


Figure 4-33. Temporal change in Pigment Specific Simple Ratio for chlorophyll b (PSSRb) in CO₂ and corresponding control plots.

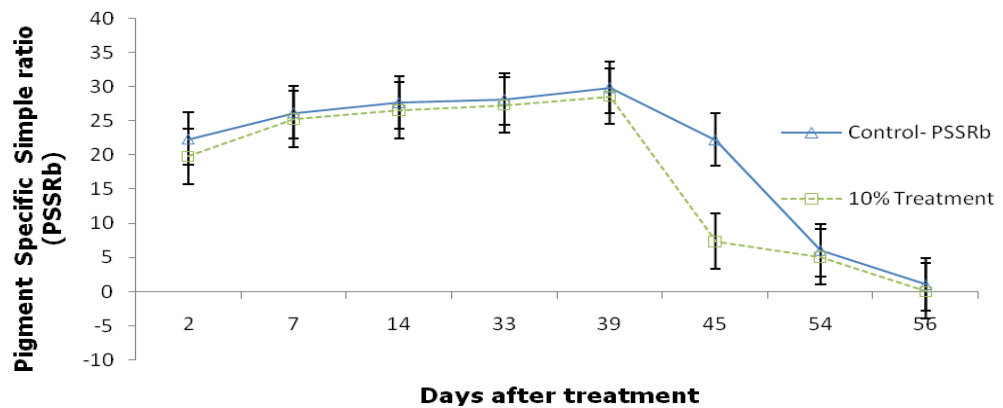


Figure 4-34. Temporal change in Pigment Specific Simple Ratio for chlorophyll b (PSSRb) in herbicide treated and corresponding control plots.

4.2.8.3 Physiological Reflectance Index (PRI)

The temporal changes in physiological reflectance index for CO₂ and herbicide treatment experiment with their respective controls are shown in Figures 4-35 and 4-36. The *PRI* of maize crop growing on high CO₂ concentration zone was higher than the *PRI* of those growing on low CO₂ concentration zone; control plots had the lowest *PRI*. At t=33, the *PRI* of the CO₂ treated plots increased. The difference between low and high CO₂ zone compared to the corresponding control treatments was statistically significant from t=33 until t=56 (ANOVA (*p-value* 0.022; 0.043; 0.008; 0.005; 0.000 ($\sigma \leq 0.05$); n=8). As the experiment progressed *PRI* continued to increase, which implies a reduction in photosynthetic activity (and chlorophyll content). Maracci *et al.* (1991) found that the photosynthetic efficiency of maize crop reduced over time with dehydration.

In maize treated with herbicide, the lowest *PRI* was observed in the control plots. The *PRI* of maize grown on herbicide treated plots and the control showed no significant difference (ANOVA (*p-value* 0.661; 0.987; 1.222; 0.563; 0.726 ($\sigma \leq 0.05$); n=8) from t=2 to t=39, however, from t=45 until t=56 the differences were statistically significant (ANOVA (*p-value* 0.033; 0.046; 0.049 ($\sigma \leq 0.05$); n=8). Refer to appendix 15 for ANOVA results.

From the *PRI* results shown, it implies that the index was not sensitive at earlier stage of both experiments; it was responsive much earlier in the CO₂ experiment when compared with the herbicide experiment.

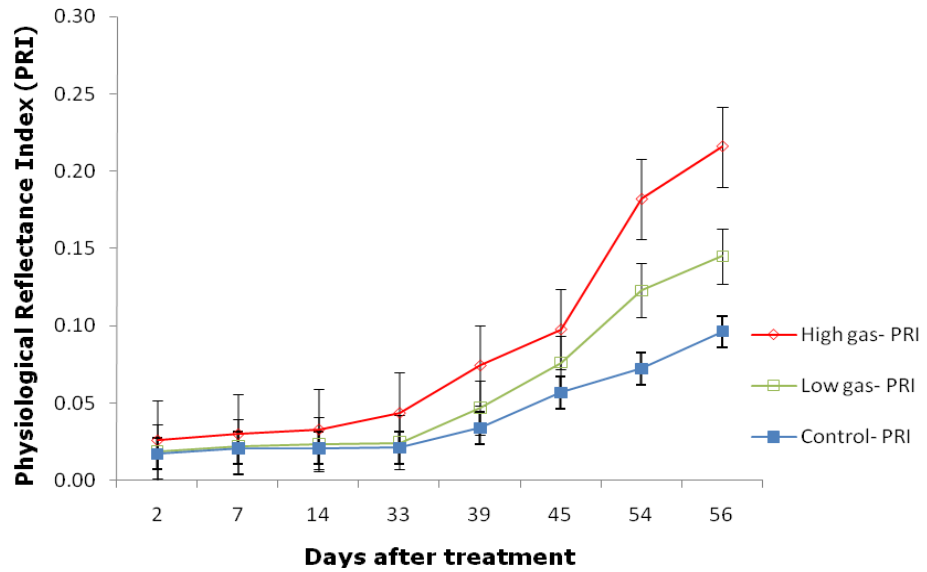


Figure 4-35. Temporal change in Physiological Reflectance Index (PRI) in CO₂ plots and control plots.

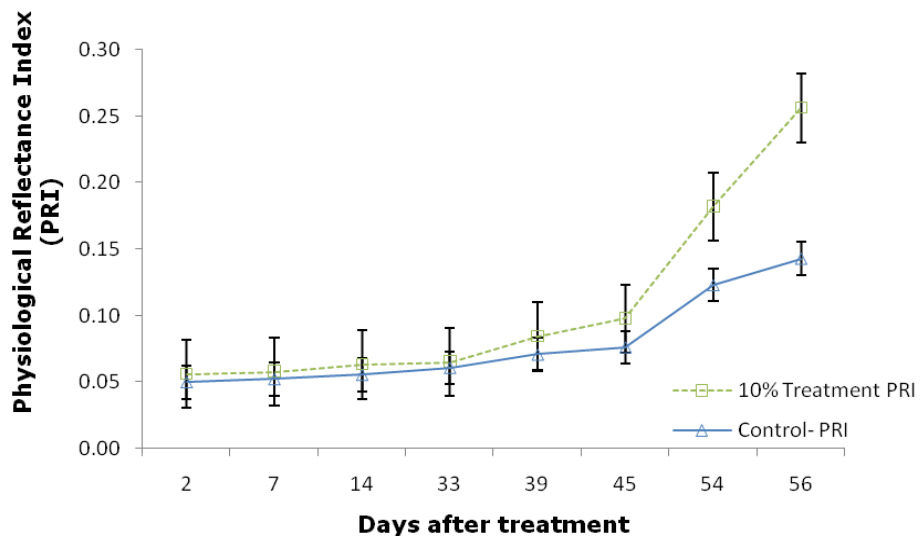


Figure 4-36. Temporal change in Physiological Reflectance Index (PRI) in herbicide treatment and control plots.

4.2.9 Continuum removal analysis.

In this study continuum removal analysis was carried out using the canopy reflectance in the blue (400–550nm) and red (550nm–750nm). (Refer to section 3.10.4 for details). These wavelength regions were selected because of their sensitivity to changes in chlorophyll content (Filella and

Penuelas, 1994, Lichtenthaler *et al.*, 1996). The regions have proved to be important in vegetation condition studies and are not usually affected by water absorption in green plants (Mutanga *et al.*, 2003). The data used in the analysis were not affected by any form of noise. Reflectance at those particular wavelengths that showed significant difference between control and CO₂ or herbicide treatment were analysed to determine the region that showed differences in band depth.

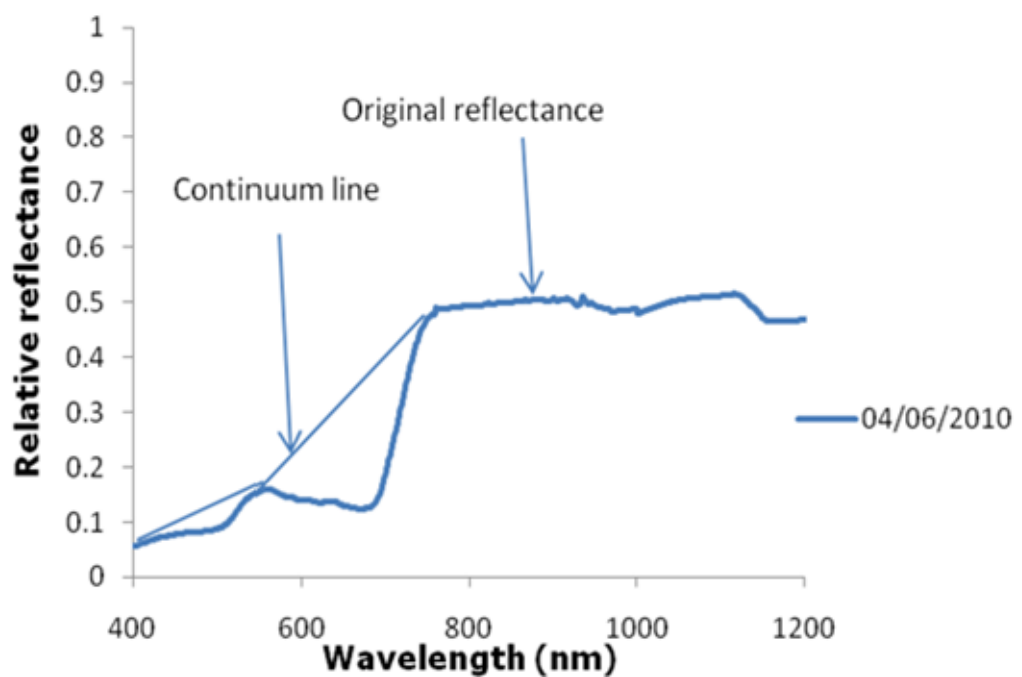
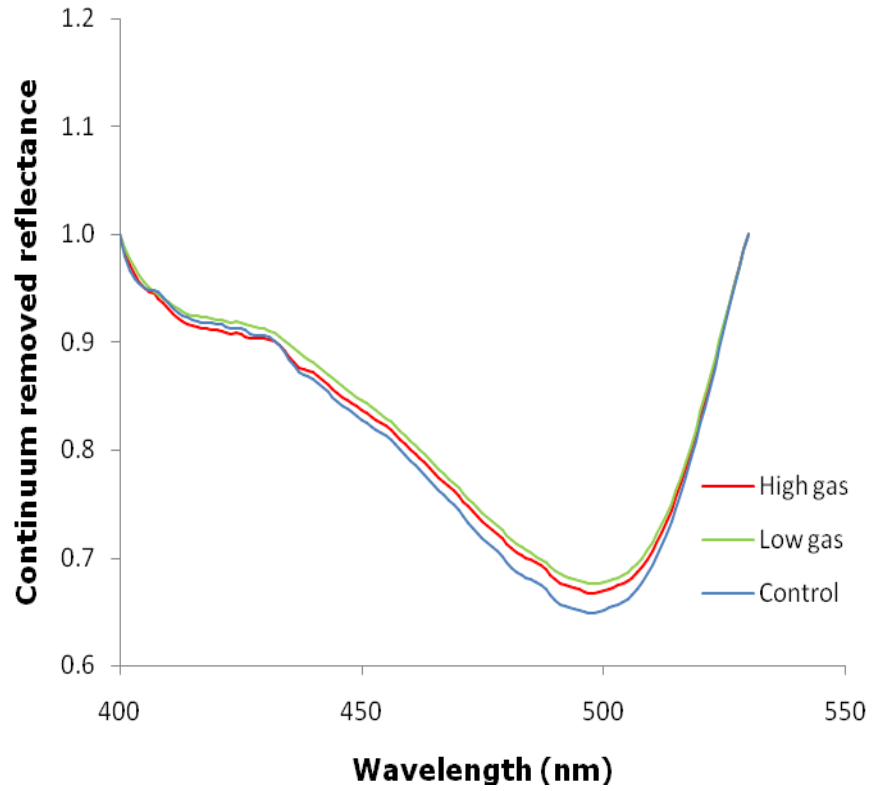
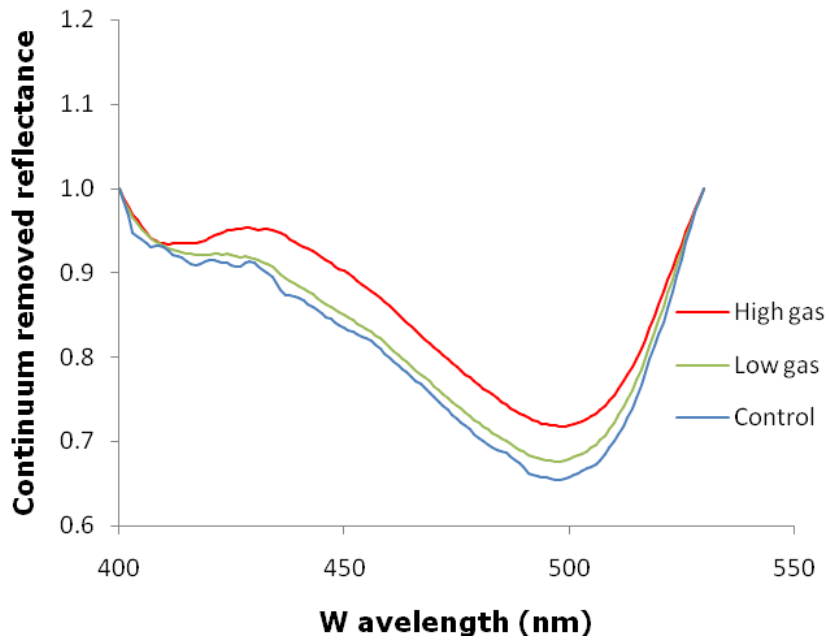


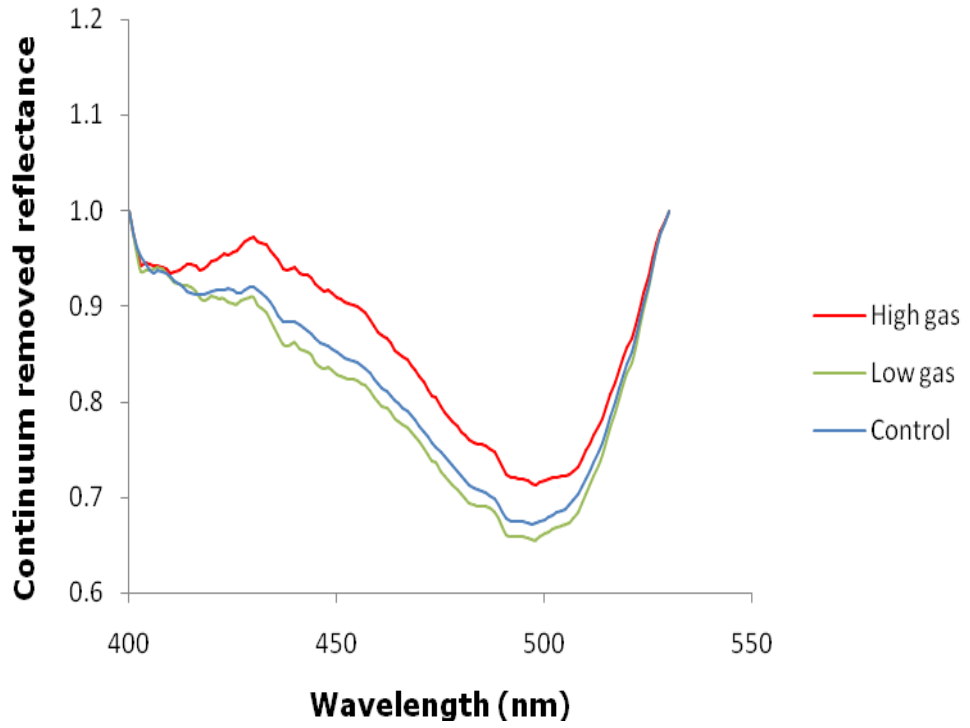
Figure 4-37. Diagram showing original reflectance measured on 4th June 2010 and the spectral regions (continuum line) where continuum removal was applied.



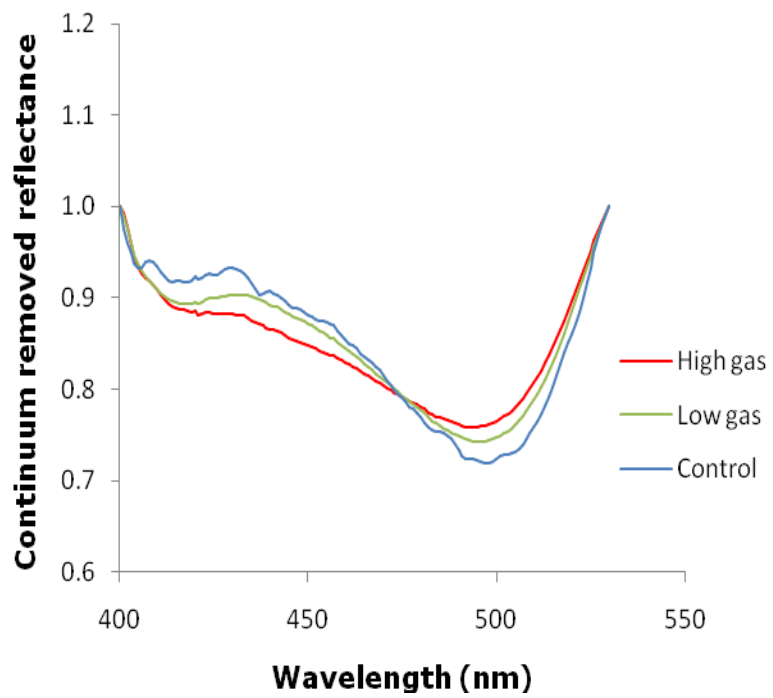
A. (16/07/2009).



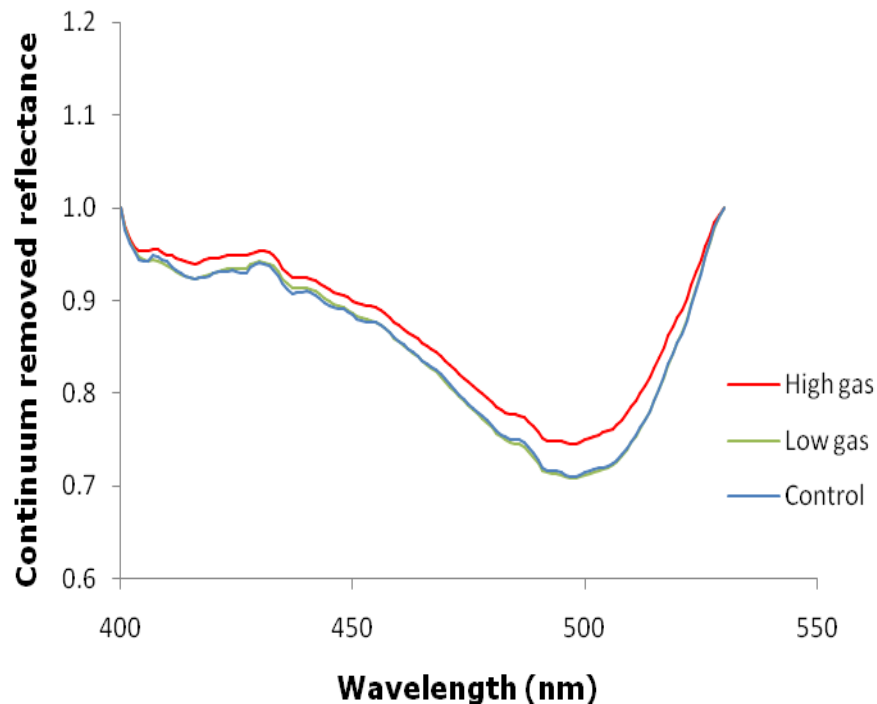
B. (23/07/2009).



C. (13/08/2009).



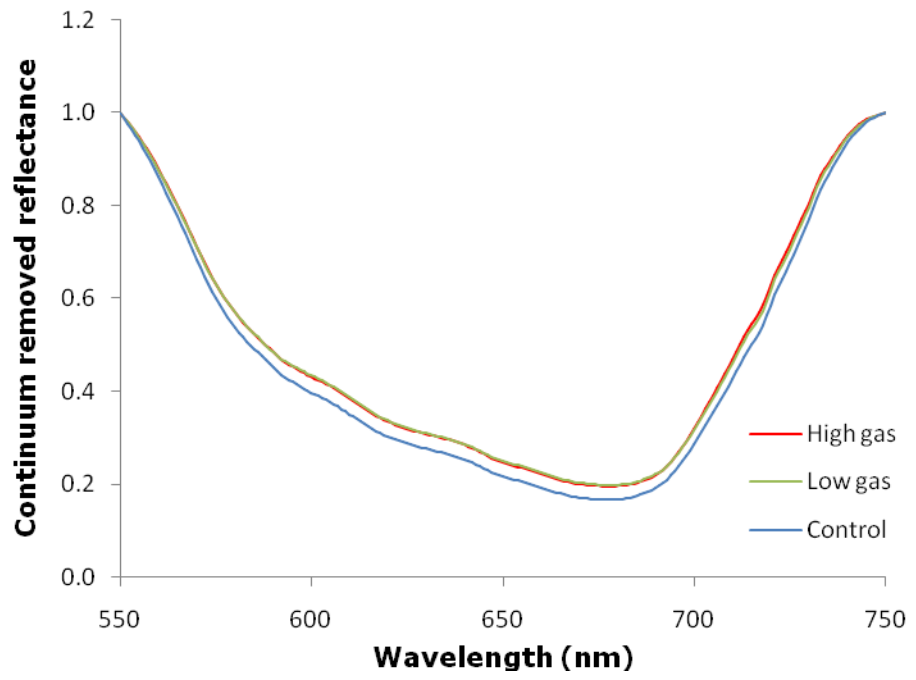
D. (24/08/2009).



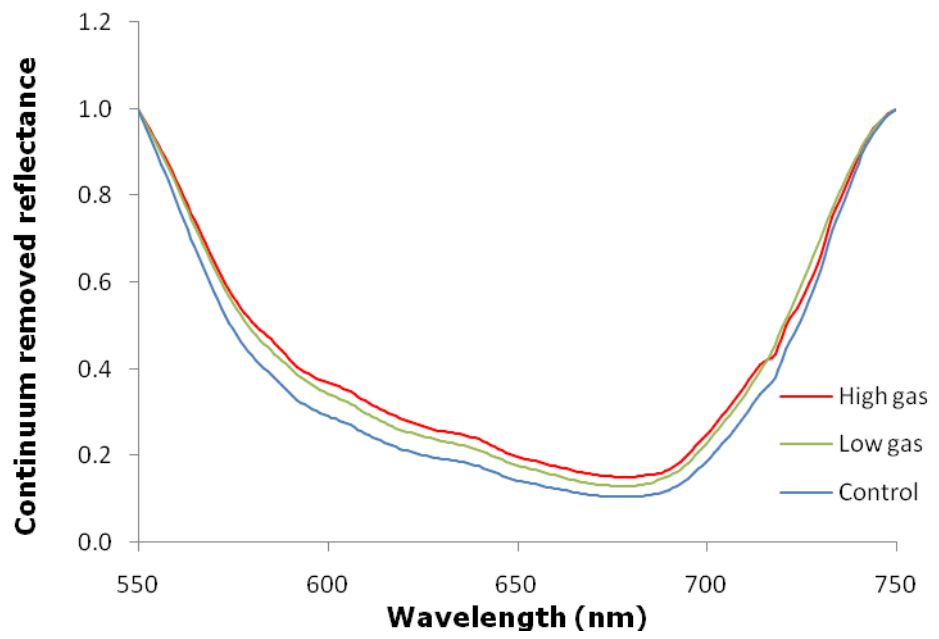
E. (10/09/2009).

Figure 4-38. A-E: Mean continuum removed reflectance at 400 - 550nm for maize crop growing on CO₂ control, low and high gassed zone measured during the respective dates.

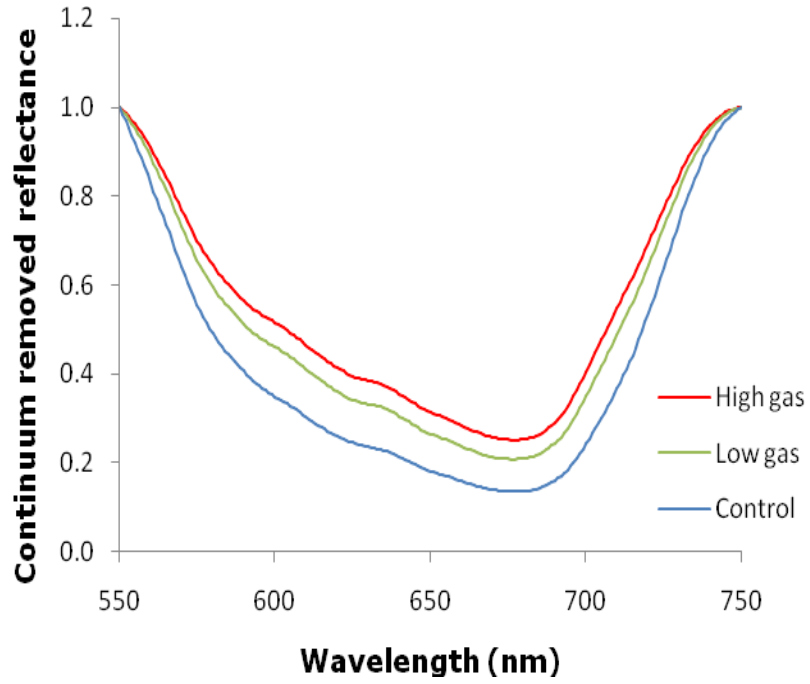
The average band depths at 473 and 488 nm for high CO₂, and at 500 and 509 nm for the low CO₂ region showed significant differences (Refer to appendix 16 for details of ANOVA) compared to the control band depths during the period of the experiment. However, comparing the average temporal difference between the treatment levels at various dates shows that there was significant difference at 410 and 512nm on 23/07/2009, 13/08/2009, 24/08/2009 and 10/09/2009. (Refer to appendix 16 for details). For the CO₂ treatment the absorption pit in the visible region was deepest in the control plots, followed by low and high CO₂ as shown in Figures 4-38a-e.



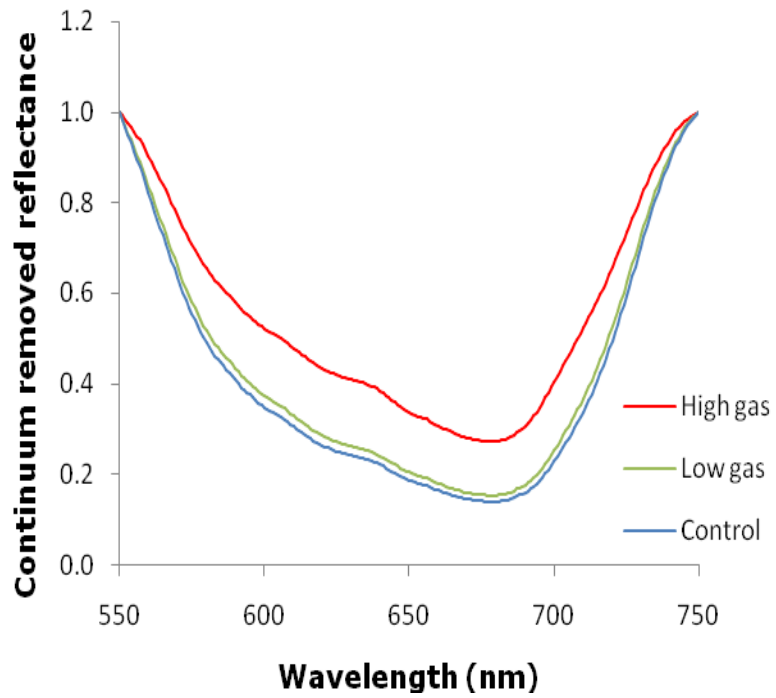
A. (16/07/2009).



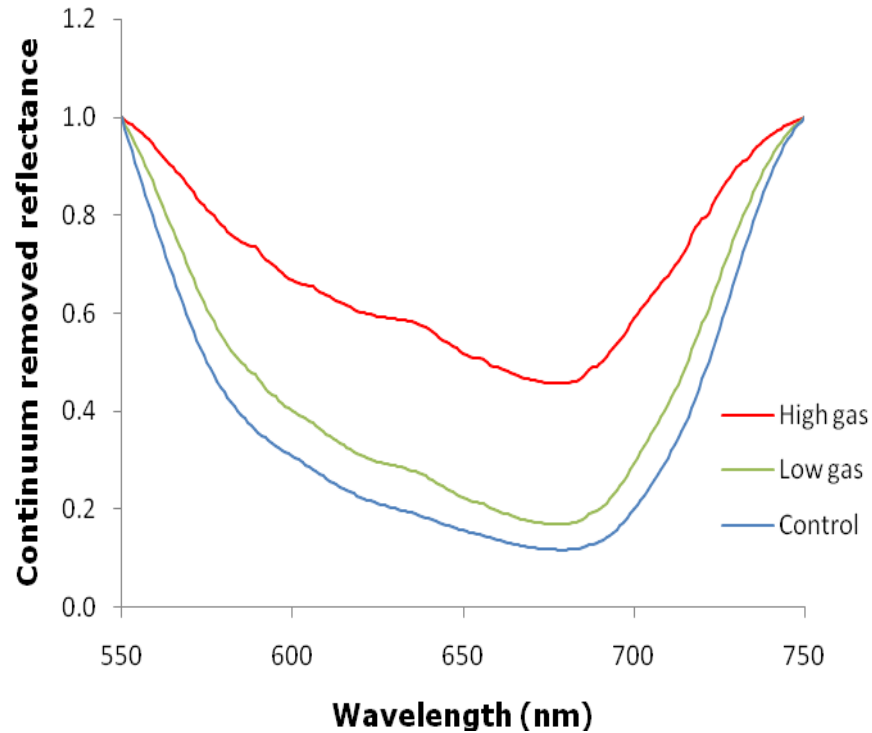
B. (23/07/2009).



C. (13/08/2009).



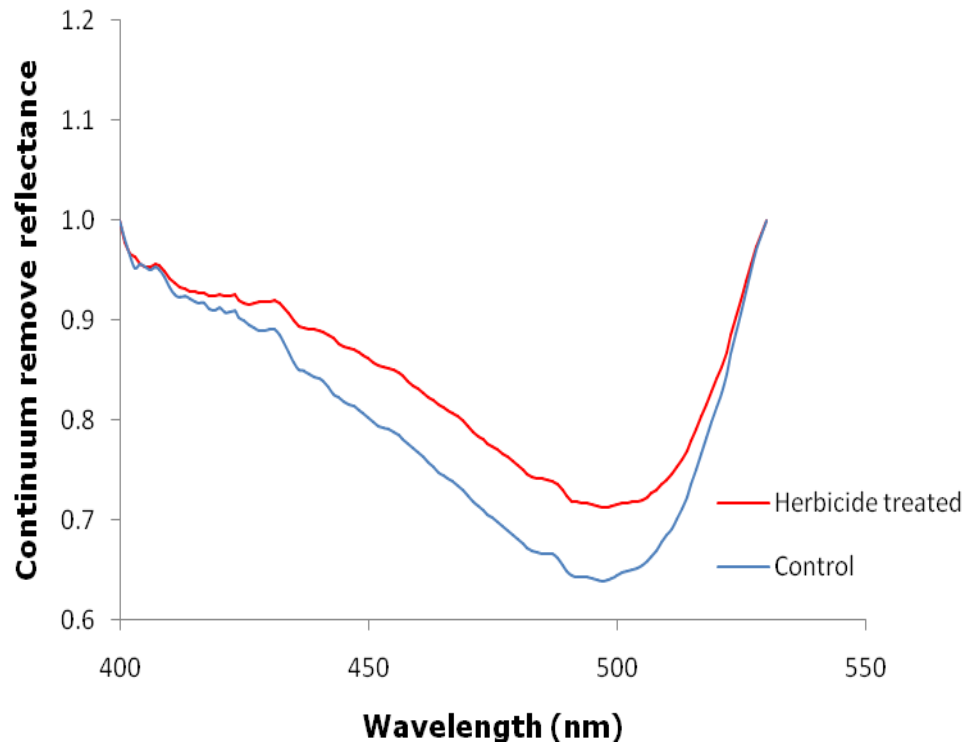
D. (24/08/2009).



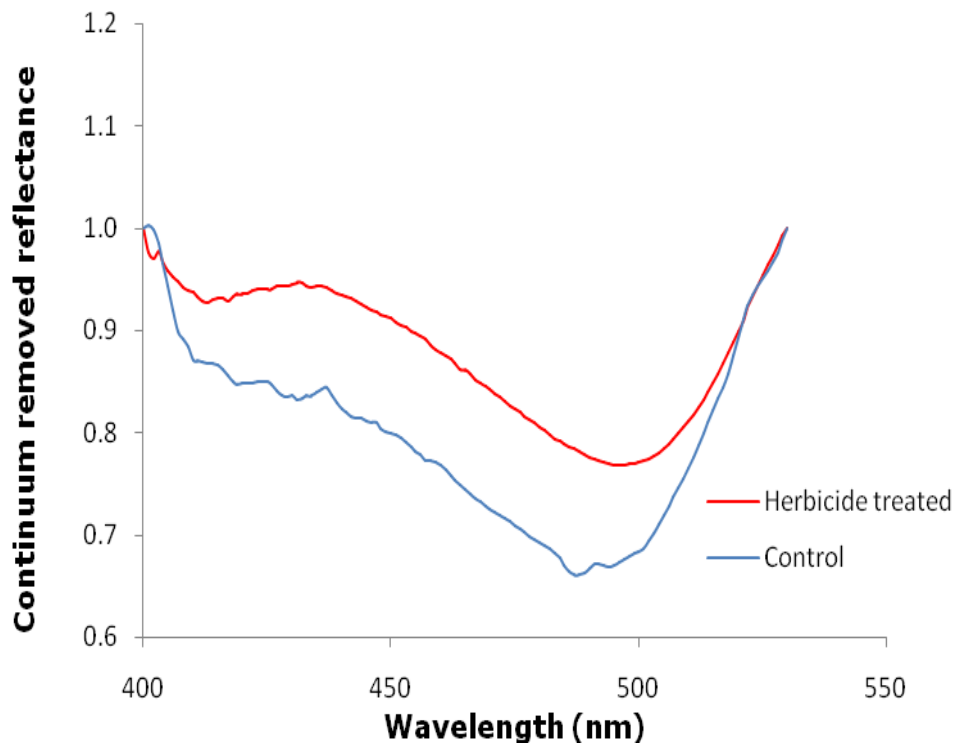
E. (10/09/2009).

Figure 4-39. A-E: Mean continuum removed reflectance at 550 - 750nm for maize crop growing on CO₂ control, low and high gased zone measured on the respective dates.

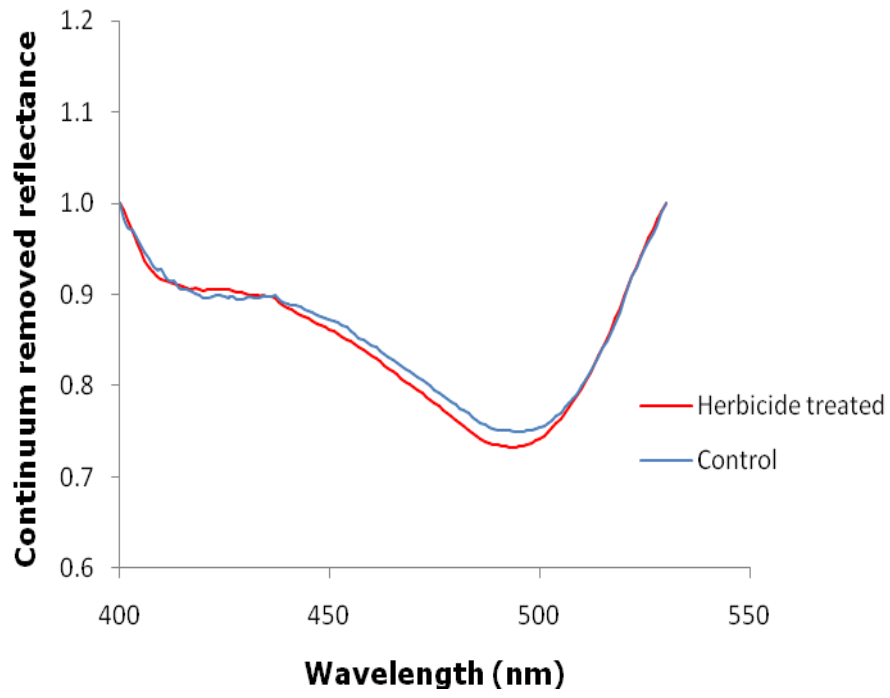
During the first week of the experiment (16-23/07/2009) the continuum removed reflectance for high and low CO₂ zone compared to the control at 550-750nm wavelength region showed no significant difference statistically when tested with ANOVA (Refer to appendix 17 for details) as depicted in Figures 4-39a and b, but at the fifth week (13/08/2009) after gassing the difference became significant. The wavelengths between 563 and 735nm were the much affected during the period 13/08/2009 until 10/09/2009 which is the last date of spectral reflectance, this is also the period which resulted in difference between the treatment levels, this could be attributed to the changes in chlorophyll absorption as the experiment progressed (Mutanga, 2003).



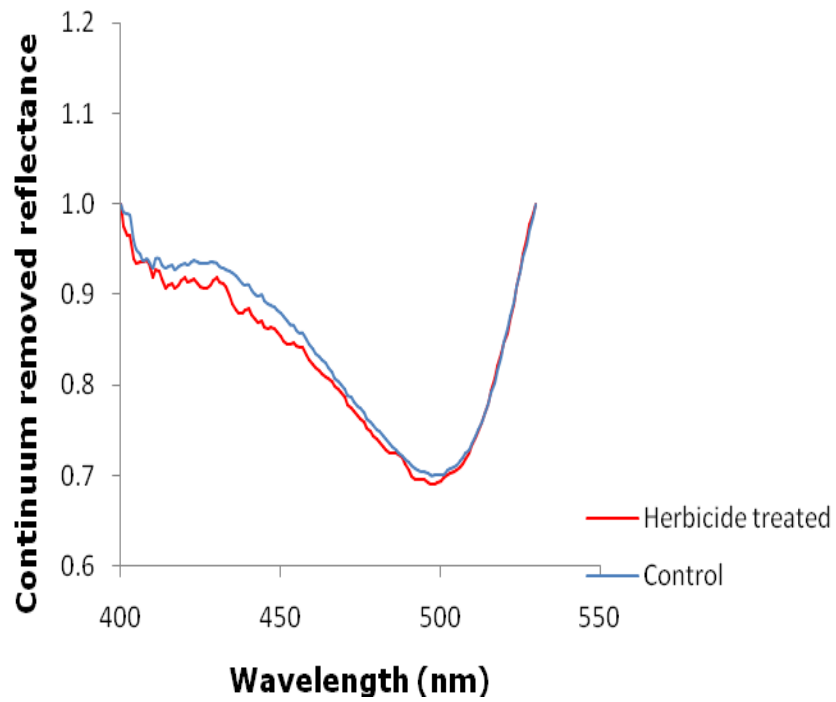
A. (16/07/2009).



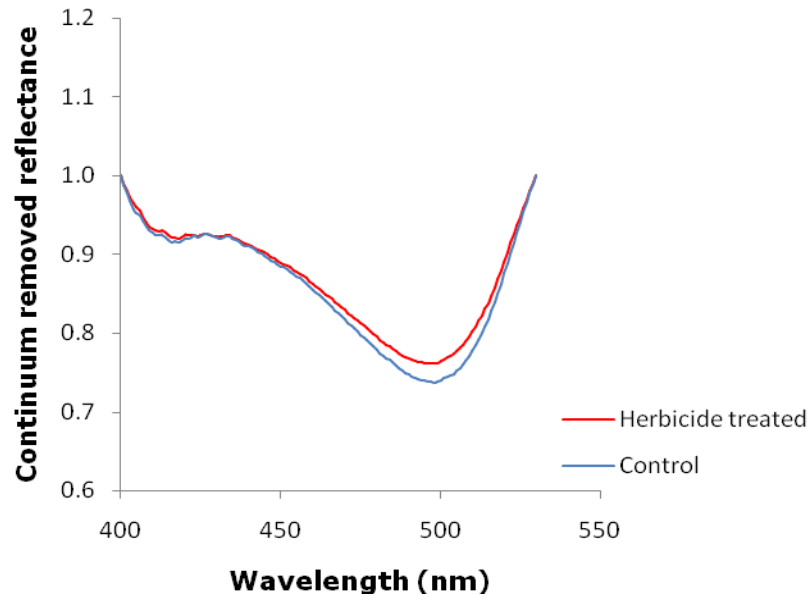
B. (23/07/2009).



C. (13/08/2009)3



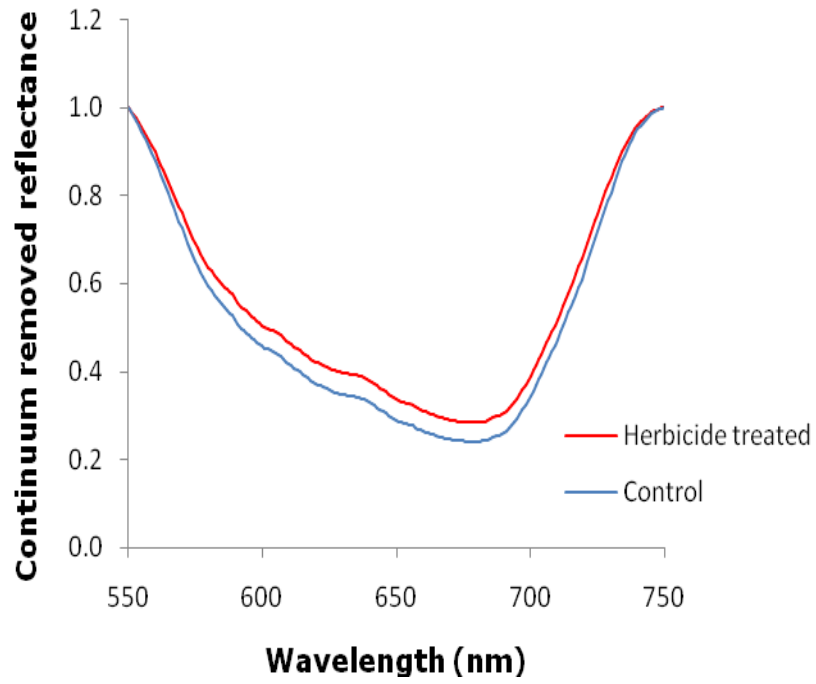
D. (24/08/2009).



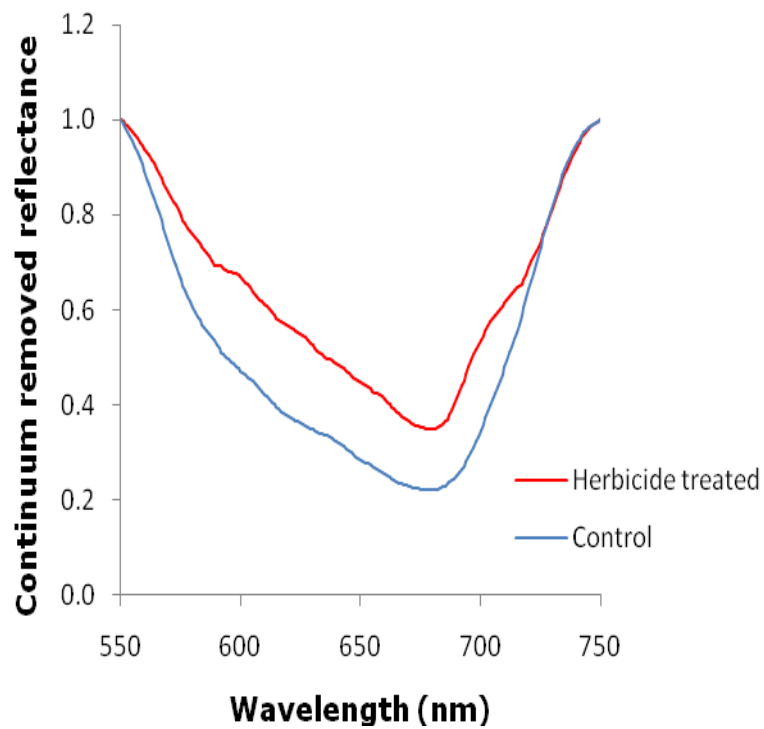
E. (10/09/2009).

Figure 4-40. A-E: Mean continuum removed reflectance at 400-550nm for herbicide treated maize crop and its control measured on the respective dates.

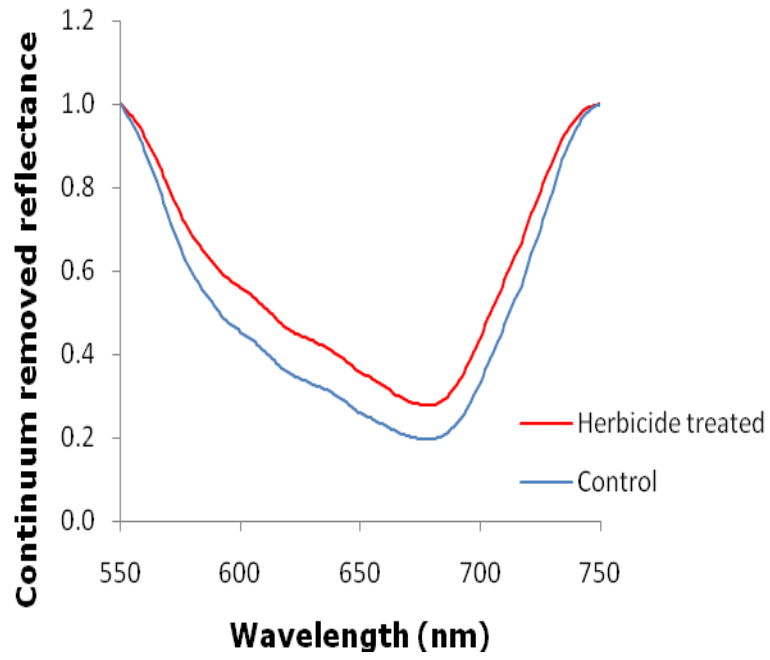
In order to test if there was any significant difference in continuum removed reflectance between the weeks in herbicide treated maize and control at 450-550nm region ANOVA was used. During the first and second week after treatment the wavelengths between 406-515nm showed significant difference (Refer to appendix 18 for ANOVA details) as depicted in Figures 4-40a and b. The band depths for the control experiment were also deeper compared to the treated plots, the deepness increased with date, i.e. the second week was deeper than the first week. From the third until fifth week of spectral measurements there was no significant difference (Refer to appendix 18 for ANOVA details) between control and herbicide treatment as shown in Figures 4-40c-e.



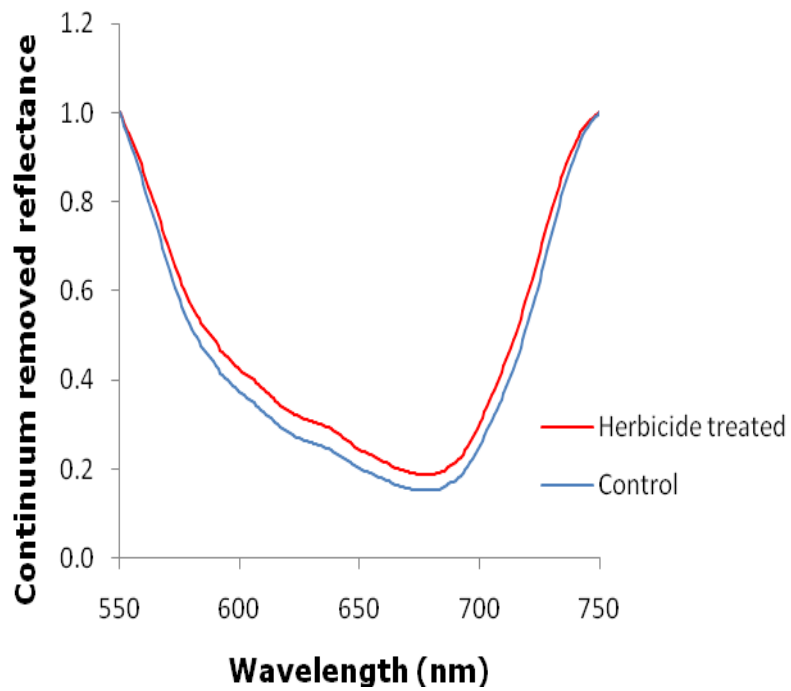
A. (16/07/2009).



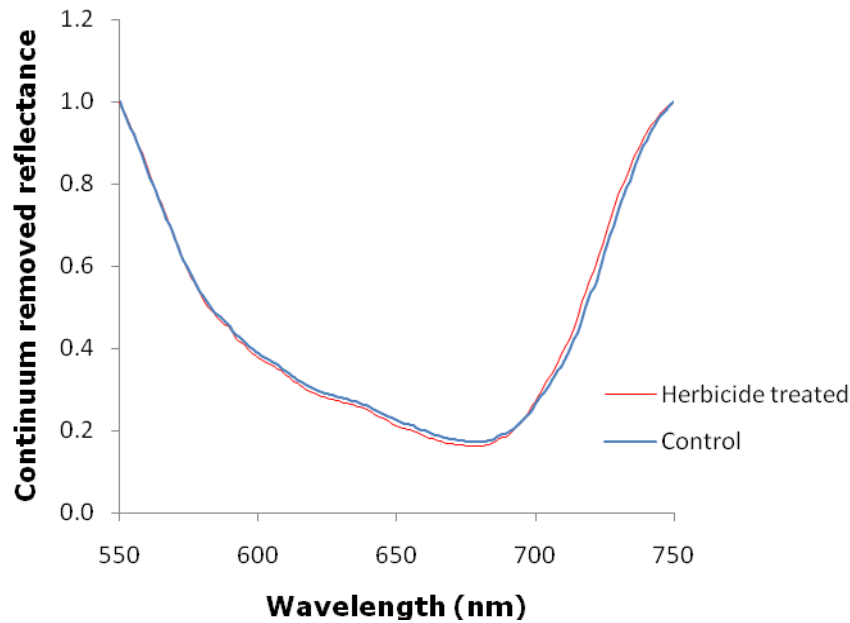
B. (23/07/2009).



C. (13/08/2009).



D. (24/08/2009).



E. (10/09/2009).

Figure 4-41. A-E: Mean continuum removed reflectance at 550-750nm for herbicide treated maize crop and its control measured on the respective dates.

The continuum removed reflectance in the red region (550-750 nm) for the herbicide experiment and its control were analysed to determine the wavelengths that showed significant difference. During the first to third week of spectral measurements the wavelengths between 589-717nm showed significant difference (Refer to appendix 19 for ANOVA details), however this was more pronounced in the second week of treatment as shown in Figure 4-41b. The fourth and fifth week showed no significant difference (Refer to appendix 19 for ANOVA details). The absorption trough of the control was wider and deepest in the second week of treatment followed by the herbicide treatment.

4.3 Conclusions.

This study has demonstrated the potential of hyperspectral remote sensing techniques in the detection and monitoring of vegetation stress in the field

due to underground CO₂ leaks using spectral signatures and other biophysical responses. Canopy reflectance could be used to discriminate maize plants stressed with elevated soil CO₂ and a contrasting stress (herbicide).

The result of the present study indicates that spectral reflectances of the treated plants were sensitive to both soil CO₂ and herbicide induced stress. Several studies have shown that stress generally increases reflectance in the visible region due to a decrease in the dominant absorption features such as the photosynthetic pigments (Horler *et al.*, 1983, Milton *et al.*, 1989, Carter, 1993, Carter and Miller, 1994, Smith *et al.*, 2004, Noomen *et al.*, 2008, Lakarraju *et al.*, 2010). Thus, radiation 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, Kochubey *et al.*, 2007). The nature and response of plants to stress differs in a variety of ways due to: the type of plant species, the stress type, time and length at which the stress occurred (Huang *et al.*, 1997a, 1997b, Zaidi *et al.*, 2003). This could be used to assess and quantify stress over time.

In this study, signs of visible stress symptoms were noticed 16 days after onset of CO₂ injection and 25 days after application of herbicide treatment, this was in the form of yellowing of leaves, which was more severe in the CO₂ experiment together with a reduction in maize growth (height, leaves, cobs and tillers) compared to the control plants, especially in the centre of gassed plots where the concentration of CO₂ was high. As the experiment progressed the leaves of the CO₂ treated maize became pale, curvy, dry and wilted. Maize stressed with herbicide responded slowly, this could be attributed to delay in the growth of the plants, there was change in colour from green to yellow, and the spread of the yellowing was gradual,

eventually covering the whole leaf. Even after harvest the maize cobs showed different responses, the CO₂ stressed maize cobs were distorted and shrivelled, while the herbicide stress cobs formed incomplete grain and immature cobs.

The position and height of the inflection point of the red edge for the two treatments also differed. The gassed plots had double peaks at 718 and 730nm, with secondary peaks or shoulders found between 707 and 764nm. These features were used to detect differences between control and CO₂ stressed maize. Throughout the experiment, the CO₂ control plots were composed of a single maximum peak at 726nm and several smaller peaks or shoulder between 718 and 759nm. The first derivative peak for the herbicide control was composed of a single peak at 723nm with small shoulders or peak at 716 and 759nm. As the experiment progressed and the herbicide stress begins to manifest, there was shift and change in the derivative peaks, the maximum peak became double at 716 and 723nm, the shoulder was still at 759nm but the magnitude had decreased.

The continuum removal analysis also showed that the treatments could be detected and distinguished using the band-depths; the absorption pits were deeper in both controls, as the severity of the treatment increased so did the deepness. The wavelengths sensitive to CO₂ stress in the blue region were found to be around 473, 488, 500 and 509nm, and between 563 and 735nm for the red region. While those for the herbicide stress were 406-515nm and 589-717nm respectively.

Vegetation indices used in this study were promising indicators of stress, though they were dependent on the stress types and stress severity, they could be applied in stress detection over time.

One limiting factor in this study is the uncertainty that the stress acting on the vegetation at that particular time was caused by the stressors applied. This was mitigated by the fact that the control experiment provided the opportunity for comparison with the treated plots which were tested statistically to establish any significant difference.

The study conducted has shown the potential of hyperspectral remote sensing technique to detect, monitor and discriminate between causes and/or level of stress agent(s). There is however, the need to investigate other causes of stress with varying concentration using different plant species.

CHAPTER FIVE

Remote sensing of barley (*Hordeum vulgare v Concerto*) stressed with CO₂ and herbicide.

5.1 Introduction.

Studies have shown that similar spectral responses may result from different stress effects which make it difficult to discriminate between the causative factors. Smith *et al.* (2005b) found that there was no significant difference between the spectral reflectance patterns of oilseed rape (*Brassica napus*) stressed with elevated concentration of natural gas and herbicide application.

In the present study barley was stressed with elevated concentration of CO₂ and different levels of herbicide treatments. Both stressors were primarily expected to affect the roots of the crop.

Barley was chosen as the model crop for this study. It is a well known crop used as source of food in different processed forms, thereby making a large contribution towards feeding the world's populace and its livestock (USDA, 2011). It is also classed as a C3 crop in terms of its photosynthetic activity and response to atmospheric carbon dioxide (Refer to section 2.3.1 for details).

Barley was planted on 8th April, 2010 (Refer to section 3.3.3.2 for details) at the ASGARD site (Refer to section 3.2 for details on the site) and in an open area about 250m further away. CO₂ injection into the soil and herbicide treatments were applied on 7th June, 2010, and 9th June, 2010 respectively; the little delay in the herbicide application was due to slow development of the crop in the field plots (Refer to section 3.3.3.2 for

details). The treatments were applied when the crops were fully established.

To enable comparison of the spectral reflectance response of the treatment types and levels at different periods during the experiment, spectral measurements were acquired on an approximately weekly basis from 3rd June until 10th August, 2010 (For details refer to section 3.5). At this stage the crops were matured and ready for harvest. Soil gas (CO₂ and O₂) measurements were also done thrice a week to determine any variations as the experiment progressed (Details in section 3.3.1). Soil pH analysis was also carried out before and after termination of CO₂ injection on 13th April and 31st August, 2010 respectively (Refer to section 3.7 for details). Chlorophyll analysis was done on 16th July, 2010 (43 days after gassing) (details are given in section 3.8). Following harvest on 10th August, 2010 the plants were taken to the laboratory for measurements of the length of tillers, total number of barley plants, total number of tillers, total number of grains and fresh weight of barley ears and stems. Some were oven dried for further analysis (For details refer to section 3.6). This was done to compare with the control plots in order to find out if there were changes after the termination of the experiment.

5.2 Data Analysis.

Data analysis were carried out using several methods to find out whether there were any significant difference between the treatment types and levels when compared with the control experiments. The position of the red edge peaks among the different treatment types and levels where compared, and the capability of the visible absorption region to discriminate between the treatment types and levels after applying continuum removal was tested. These regions (R₄₅₀₋₅₅₀ and R₅₅₀₋₇₅₀) have been used as stress indicators in vegetation studies as mentioned in

section 4.2.9. The vegetation indices used in this study (Chlorophyll Normalized Difference Index (*Chl NDI*)), Pigment Specific Simple Ratios for chlorophyll *a* (*PSSRa*) and chlorophyll *b* (*PSSRb*) and Physiological Reflectance Index (*PRI*) were also tested for their potential to determine and discriminate stress.

The statistical analysis used is analysis of variance (ANOVA) at 0.05 significant levels. This was carried out for low (1.6%) and high CO₂ (13.8%) concentration levels and for the different levels of herbicide treatments. Each treatment was compared with the respective controls (no treatment).

5.3 Results.

5.3.1 Visible stress symptoms.



Figure 5-1. Vertical view of barley showing control plot (left) and stress in the plot centre (right).

Figure 5-1 on the left shows the CO₂ control plots without any visible stress sign while to the right the visible sign of stress is shown in the plot centre where the concentration of CO₂ was high. The photographs were taken on 21st, June 2010 (10 days after CO₂ injection).



Figure 5-2. 40% herbicide treatment (left) and 20% herbicide treatment (right).



Figure 5-3. 10% herbicide treatment (left) and 5% herbicide treatment (right).

Figures 5-2 and 5-3 shows the various levels of herbicide treatment, the visible signs of stress were greater in the 40 and 20% treatment levels. These photographs were taken on 4th July, 2010 (20 days after treatment), at which point barley growing on 40 and 20% treatment plots had virtually turned yellow; the severity was greatest on the 40% treatment followed by 20, 10 and 5% respectively.

5.3.2 Biomass analysis.

At the end of the experiment on 10th August, 2010 the barley crops were harvested and transported in polythene bags to the laboratory for the measurement of the length of tillers, total number of barley plants, total number of tillers, total number of grains, fresh weight of barley ears and stems. Some of these materials were oven dried in at 100°C for four days

and reweighed to determine the dry weight (refer to section 3.6 for details).

It is essential to mention that the sampling technique adopted for the measurements of barley from ASGARD and that from the field (the herbicide plots) were different. To enable easy replication in the CO₂ plots at ASGARD a sampling frame which was 2.5 x 2.5 m and divided into twenty five 0.5 x 0.5 m squares was used. For more information on the sampling technique refer to section 3.4. The squares harvested were along the same transect as the ones used for spectral scanning. The two middle squares along the transect represent the high CO₂ concentration zone, while the two edge ones represent the low CO₂ concentration zone. For the control plots all four squares along the measurement transect were used for the analysis.

For the herbicide treated plots and control due to the several replicate number of plots (20 plots) 25% of each plot which is equivalent to 1 m² was used for the biomass analysis, this was adopted to reduce the time, resources and energy for the measurements.

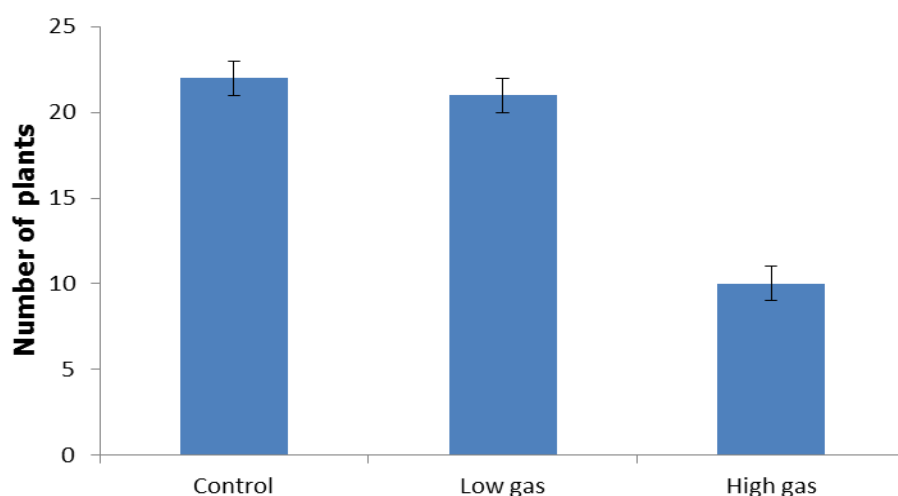


Figure 5-4. Mean number of barley plants in the control and CO₂ gassed plots. The error bars in this figure and subsequent ones represents standard error.

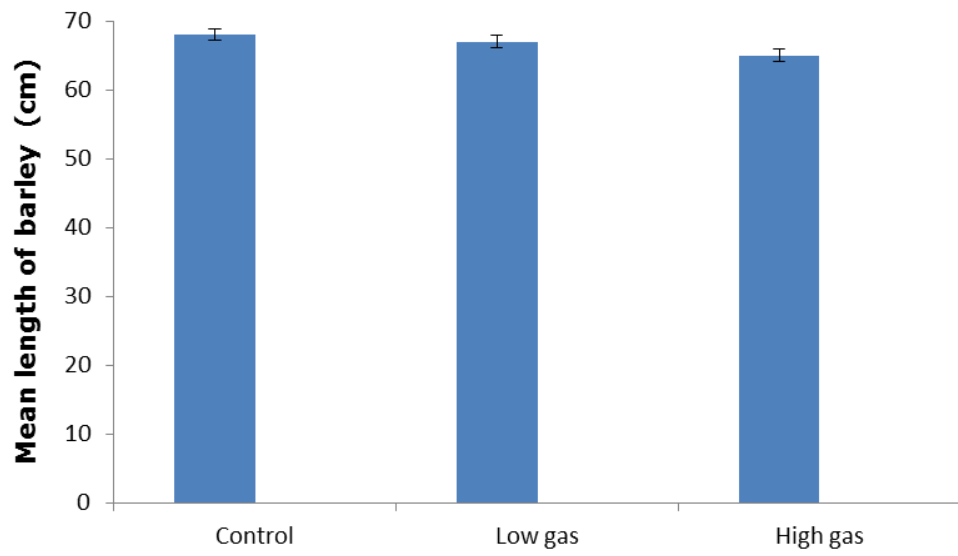


Figure 5-5. Mean length of barley plants in the control and CO₂ gassed plots.

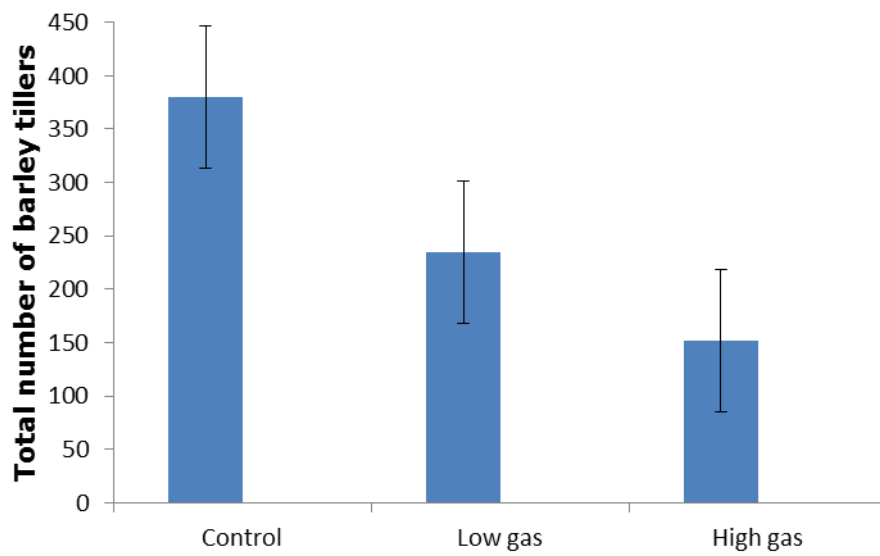


Figure 5-6. Total number of barley tillers in the control and CO₂ gassed plots.

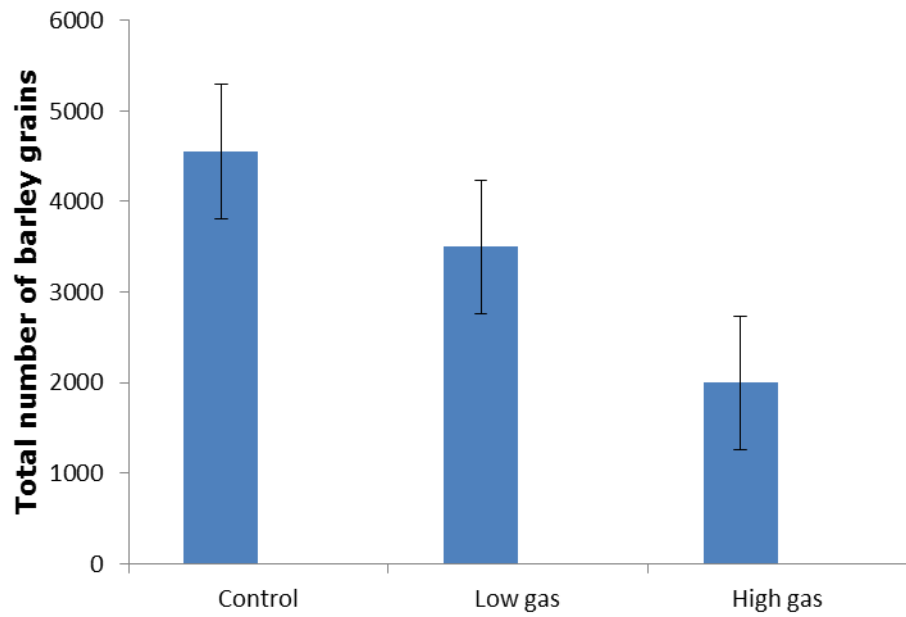


Figure 5-7. Total number of barley grains in the control and CO₂ gassed plots.

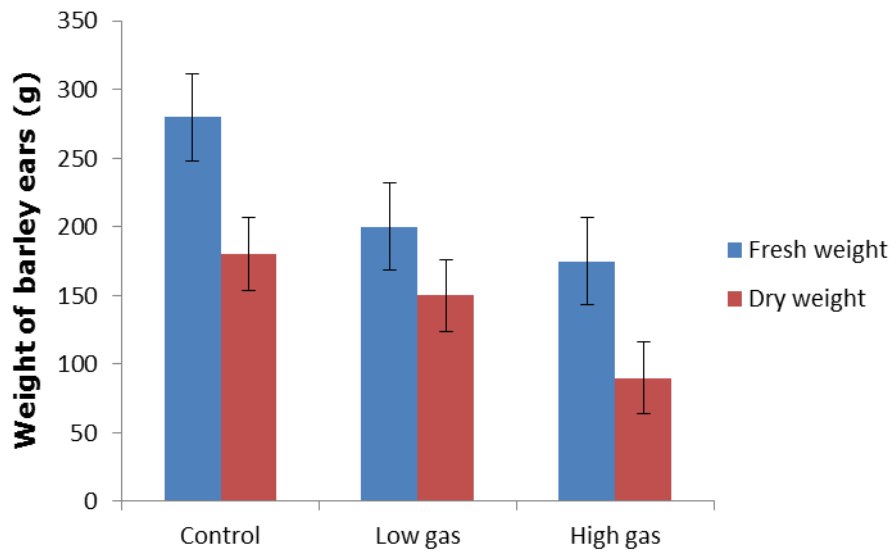


Figure 5-8. Fresh and dry weight of barley ears in the control and CO₂ gassed plots.

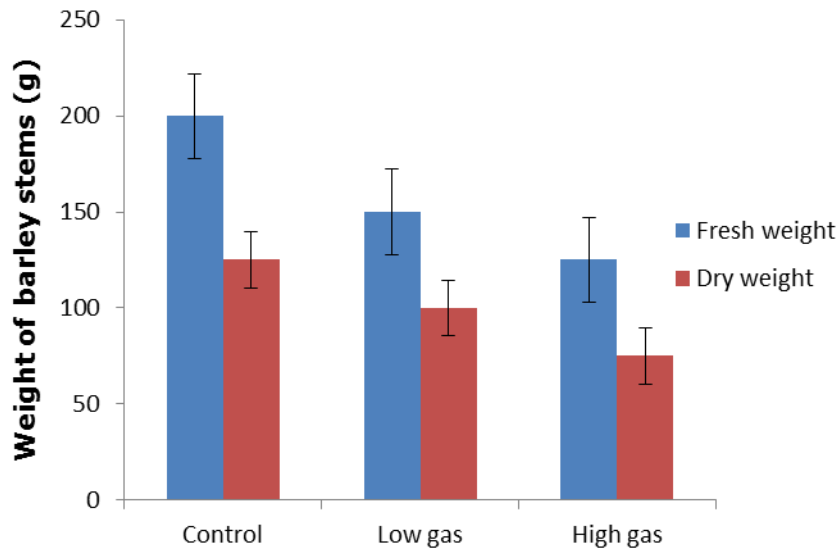


Figure 5-9. Fresh and dry weight of barley stems in the control and CO₂ gassed plots.

Compared to the control, the mean number of barley plants in the zone of high CO₂ concentration showed a difference of 50%, which was statistically significant when tested with ANOVA (*p-value* 0.041 ($\sigma \leq 0.05$); $n=16$) as depicted in Figure 5-4. There was no significant difference (*p-value* 0.123 ($\sigma \leq 0.05$); $n=16$) in the zone of low CO₂ concentration. This significant difference in the high CO₂ zone could be attributed to the severe effects of of the CO₂ in the middle of the plots which resulted in the death of barley.

However, Figure 5-5 showed no significant difference ANOVA (*p-value* 0.082 ($\sigma \leq 0.05$); $n=16$) (ANOVA, in mean lengths of barley plants in control, high and low CO₂ concentration zone. In Figure 5-6 the total number of tillers in barley plants and the total number of barley grains (Figure 5-7) in both low and high CO₂ zones showed significant differences from the control ANOVA (*p-value* 0.002; 0.017; 0.045; 0.012 ($\sigma \leq 0.05$); $n=16$). There were also significant differences ANOVA (*p-value* 0.00.018; 0.012; 0.032; 0.013 ($\sigma \leq 0.05$); $n=16$) between the fresh weight and dry weights of barley ears and stems as depicted by Figures 5-8 and 5-9 respectively. Refer to appendix 20 for details of ANOVA.

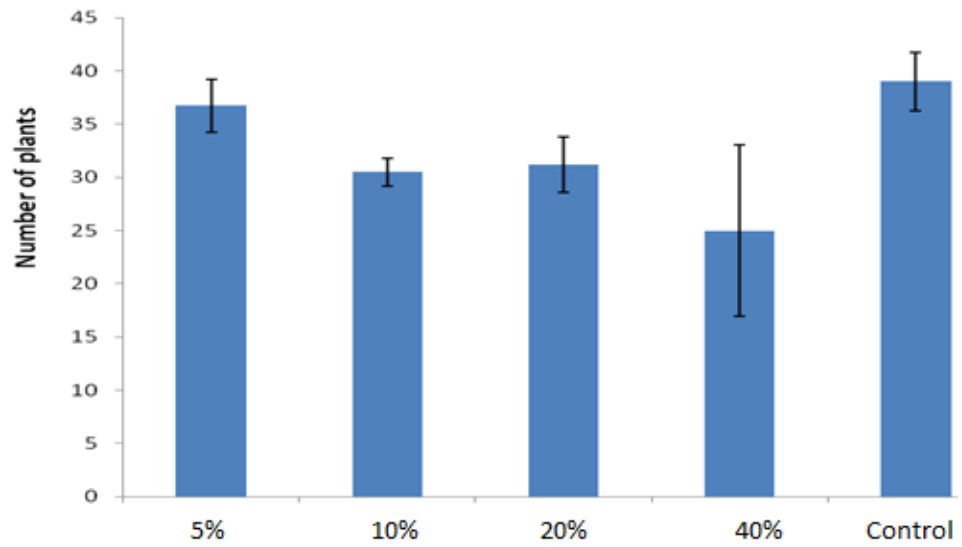


Figure 5-10. Mean number of barley plants with herbicide dose rate.

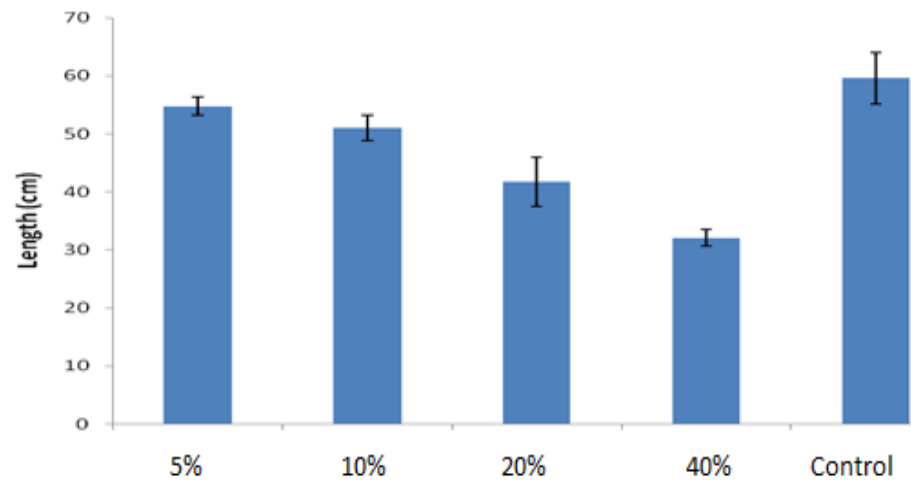


Figure 5-11. Mean length of tillers with herbicide dose rate.

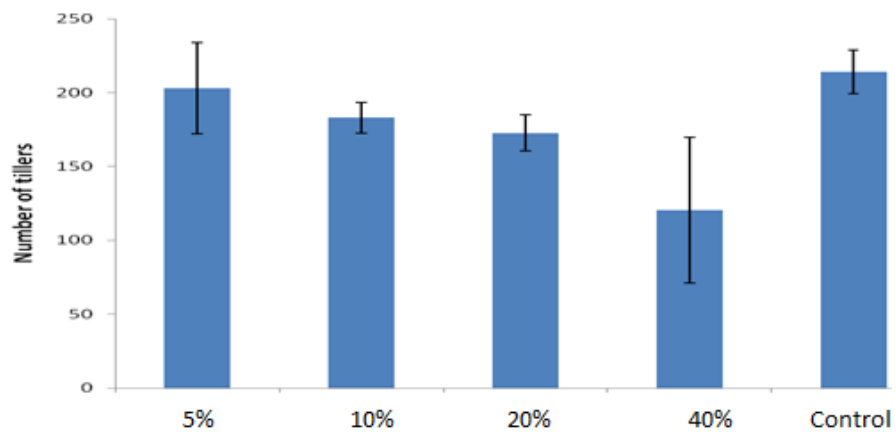


Figure 5-12. Total number of tillers with herbicide dose rate.

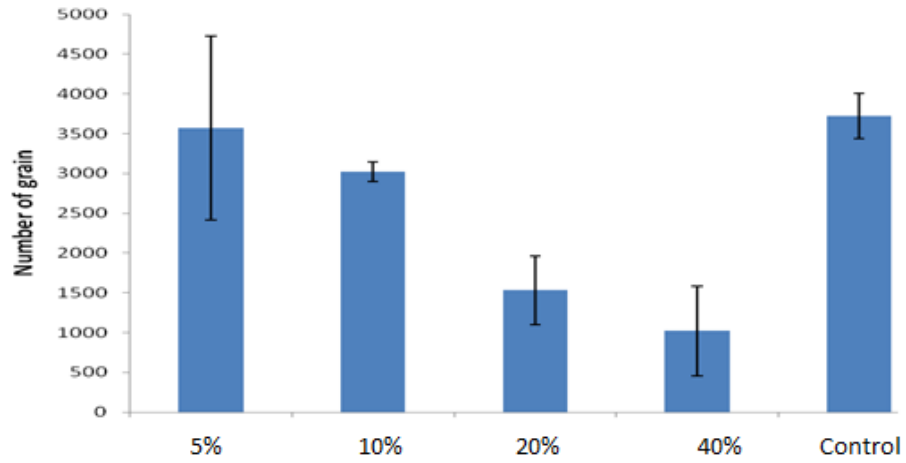


Figure 5-13. Number of barley grains with herbicide dose rate.

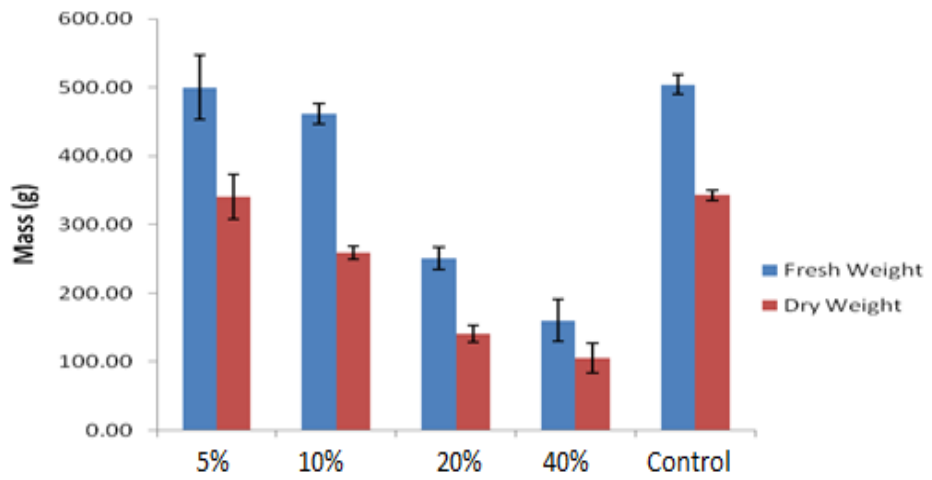


Figure 5-14. Fresh and dry weight of barley ears with herbicide dose rate.

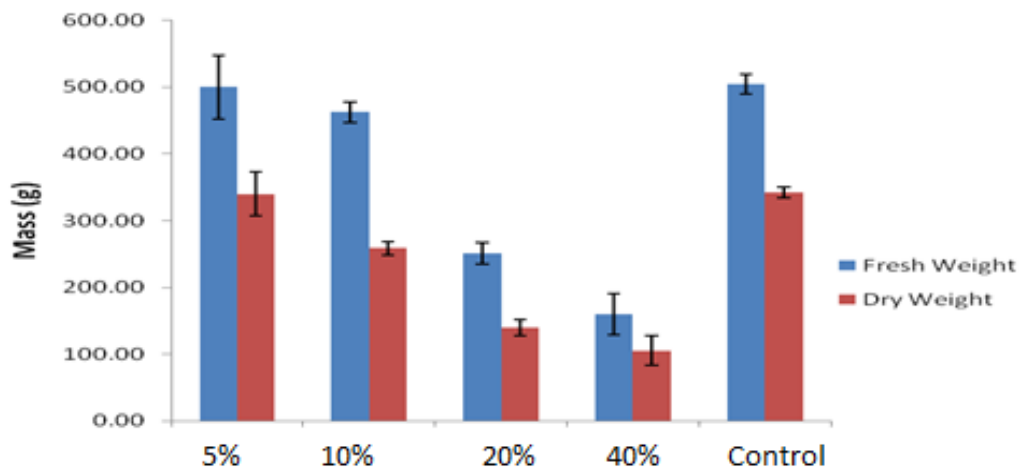


Figure 5-15. Fresh and dry weight of barley stems with herbicide dose rate.

The biomass analysis shows that the mean number of plants in the 5, 10 and 20% herbicide treatments showed no significant difference ANOVA (*p-value* 0.068; 0.55; 0.073 ($\sigma \leq 0.05$); n=16) compared to control plots while the 40% treatment level showed a significant difference ANOVA (*p-value* 0.041 ($\sigma \leq 0.05$); n=16) as depicted in Figure 5-10. While Figure 5-11 shows that there was significant difference ANOVA (*p-value* 0.012; 0.010 ($\sigma \leq 0.05$); n=16) in the length of tillers for the 20 and 40% herbicide treatment level compared to the control plots, there was no significant difference ANOVA (*p-value* 0.122; 0.068 ($\sigma \leq 0.05$); n=16) for the other treatment levels. The total number of tillers and grains in the 10, 20, and 40% treatment levels were statistically significant ANOVA (*p-value* 0.042 0.003; 0.040; 0.042; 0.016; 0.023 ($\sigma \leq 0.05$); n=16) compared to control while at the 5% level there was no significant difference ANOVA (*p-value* 0.091; 0.159 ($\sigma \leq 0.05$); n=16) as shown in Figures 5-12 and 13 respectively. There was a significant difference ANOVA (*p-value* 0.022; 0.031; 0.044; 0.011; 0.022; 0.036 ($\sigma \leq 0.05$); n=16) in the fresh weight and dry weight of barley ears and stems in the 10, 20, and 40% treatment levels as shown by Figures 5-14 and 5-15 respectively. Details of ANOVA result can be found in appendix 21.

5.3.3 Soil pH analysis.

Soil samples were taken in the CO₂ injection experimental plots on 13th April, 2010 before gas injection and on 31st August, 2010 after harvest. The samples were taken at 15-30cm and 45-60cm depths for the controls and gassed plots.

Figure 5-16 shows the various soil pH levels. The average pH for the control plots at the depth of 15-30cm was 6.05 before injection and 6.60 after, a difference of 0.55. At the depth of 45-60cm, the pre-injection pH was 6.45 while at post-injection it was 6.75, a difference of 0.3.

However, the difference between pre and post-injection for the gassed plot at 15-30 cm depth was 0.21 while at 45-60cm depth the difference was 0.5. The difference between post injection soil pH compared to control at 45-60cm was statistically significant (ANOVA, (*p-value 0.016* ($\sigma \leq 0.05$); $n=16$) $p \leq 0.05$) and may have been caused by the sinking of CO₂ down the soil profile during the experiment.

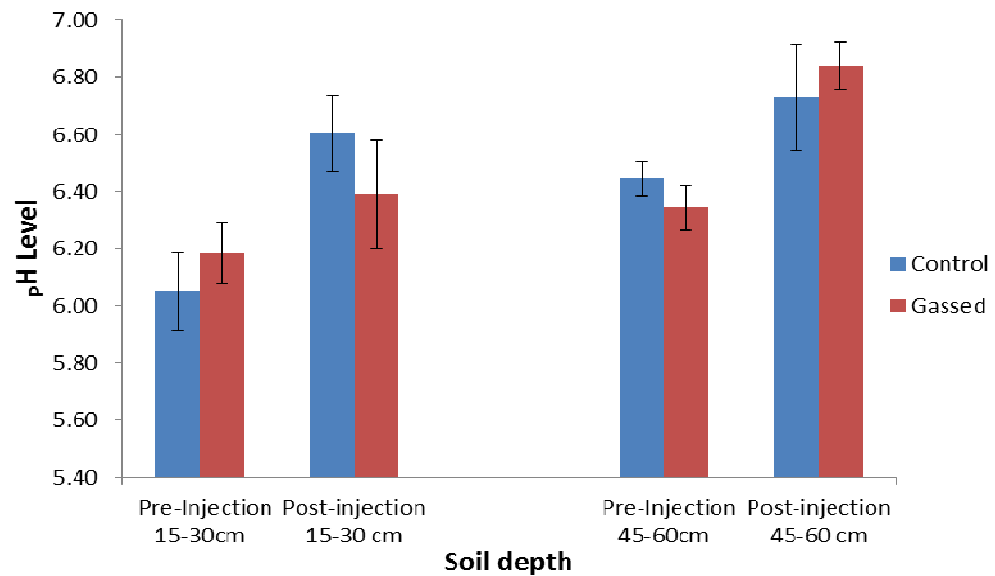


Figure 5-16. Change in soil pH following CO₂ injection in barley crop. The error bars in this Figure and subsequent ones represents standard error.

5.3.4 Chlorophyll analysis.

Chlorophyll content in plants is considered a vital element in determining the capacity of photosynthesis, stress indication and nutritional state (Xingang *et al.*, 2011). According to Carter (1993) reduction in chlorophyll can be associated to stress.

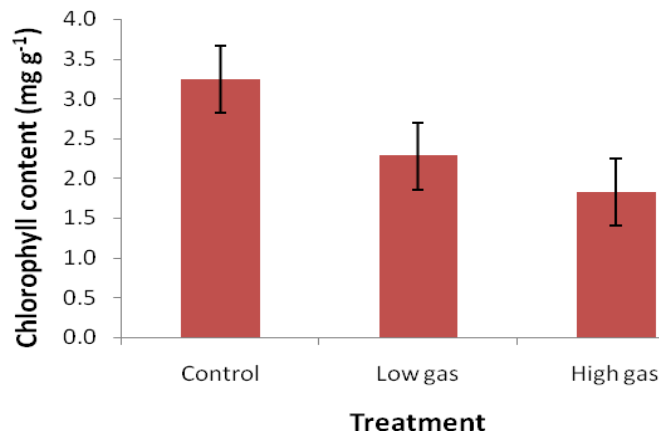


Figure 5-17. Average chlorophyll content for control, low and high CO₂ zone.

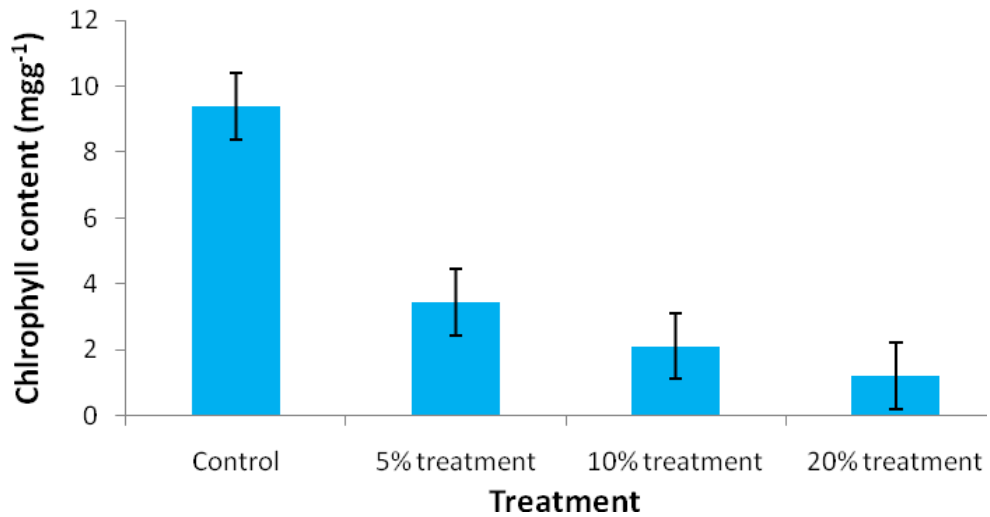


Figure 5-18. Average chlorophyll content for control and herbicide treated plots.

Chlorophyll content in the gassed plots decreased with about 35% in the low CO₂ zone and 40% in the high CO₂ zone when compared to control plots, while for the herbicide experiment it ranged between 70-90% for 5, 10 and 20% treatments. Compared to the barley crop grown on control plots, chlorophyll content decreased in both treatments, with a greater decrease in the herbicide treatment which is a function of the level of herbicide concentration as shown in Figures 5-17 and 5-18.

5.3.5 Canopy reflectance measurement.

Canopy reflectance measurements made throughout the duration of the experiments are shown in Figures 5-19 to 5-26. These measurements show that barley exposed to elevated concentrations of CO₂ or herbicide had increased reflectance in the visible region and decreased reflectance in the infrared. However, the average reflectance difference compared to control plots of the CO₂ experiment in the visible region of the spectrum showed no significant difference with ANOV (Refer to appendix 22 for details) as depicted in Figures 5-27a-d. The SWIR region likewise displayed no significant difference between the controls and low or high CO₂ regions, except on the last date (19/07/2010) at wavelengths 1482, 1718 nm and 1990, 2405 nm which showed significant difference using ANOVA (Refer to appendix 23).

In the herbicide experiment, the average temporal reflectance difference in the visible region of the spectrum showed significant difference with ANOVA (*p-value 0.012* ($\sigma \leq 0.05$); $n=16$) relative to control in the levels of herbicide treatment with some variations in the measurement date which may be attributed to difference in measured canopies structure and their associated shadow effect at nadir position (Sandmeier *et al.*, 1998), as shown in Figures 5-28d to 5-28f. Figures 5-28c-f showed significant difference at wavelengths 1500, 1680 nm and 2050, 2450 nm with larger differences occurring at the last date when tested with ANOVA (*p-value 0.032* ($\sigma \leq 0.05$); $n=16$) in the reflectance in SWIR region.

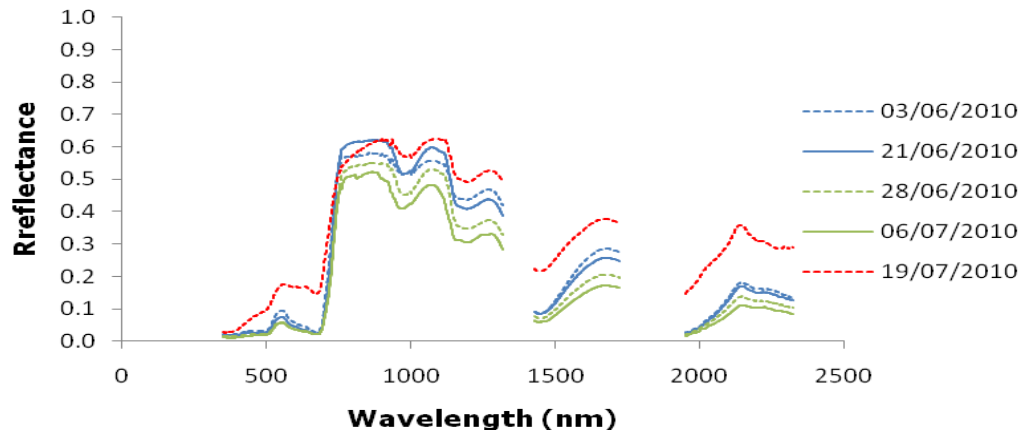


Figure 5-19. Reflectance spectra of barley grown on CO₂ control plots. The gaps in the spectrum in the Figure and subsequent ones are noisy regions affected by atmospheric water vapour.

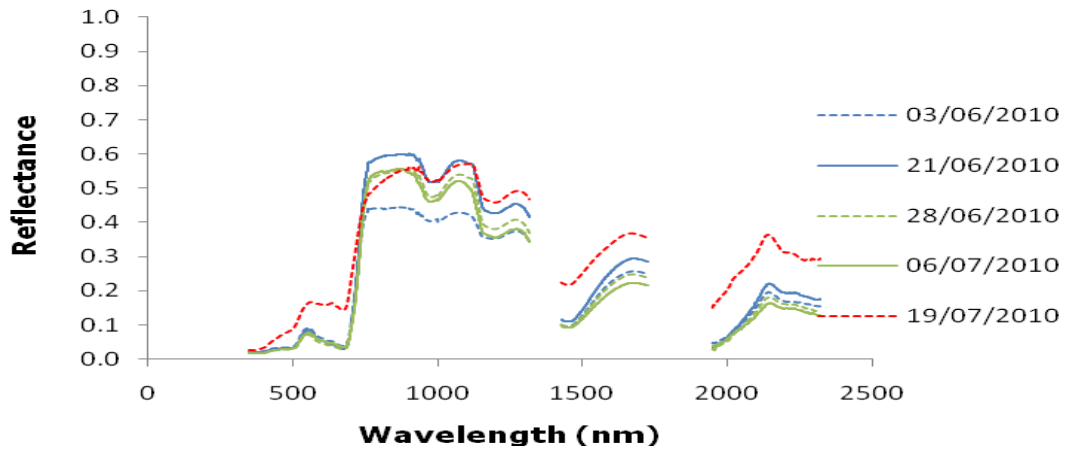


Figure 5-20. Reflectance spectra of barley grown on low CO₂ zones.

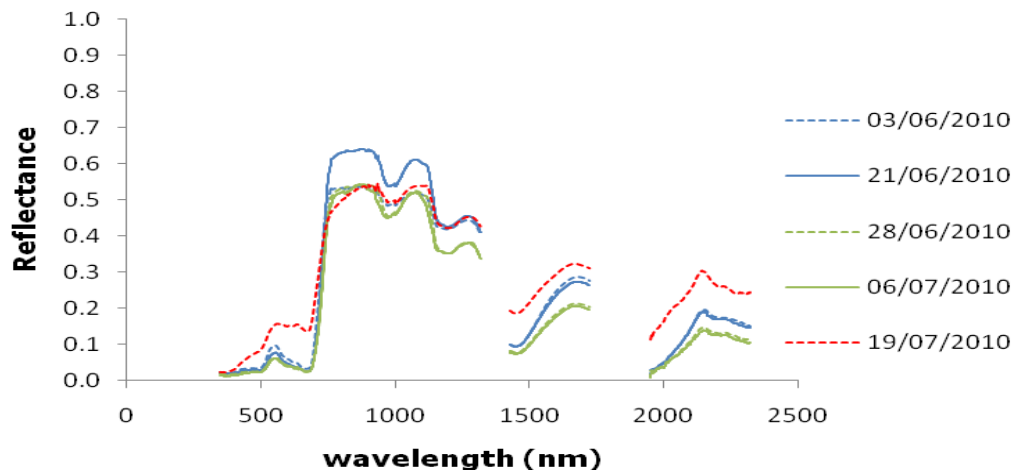


Figure 5-21. Reflectance spectra of barley grown on high CO₂ zones.

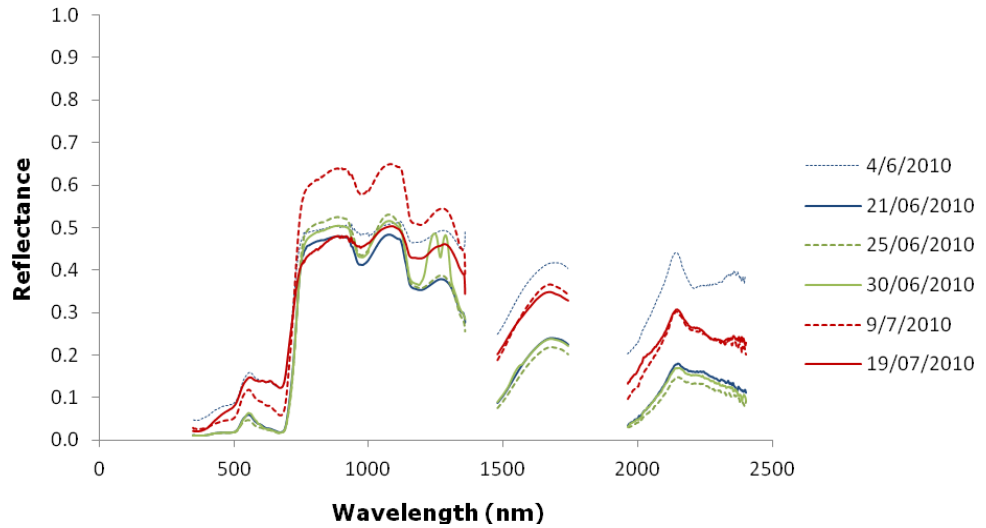


Figure 5-22. Reflectance spectra of barley grown on herbicide control plots.

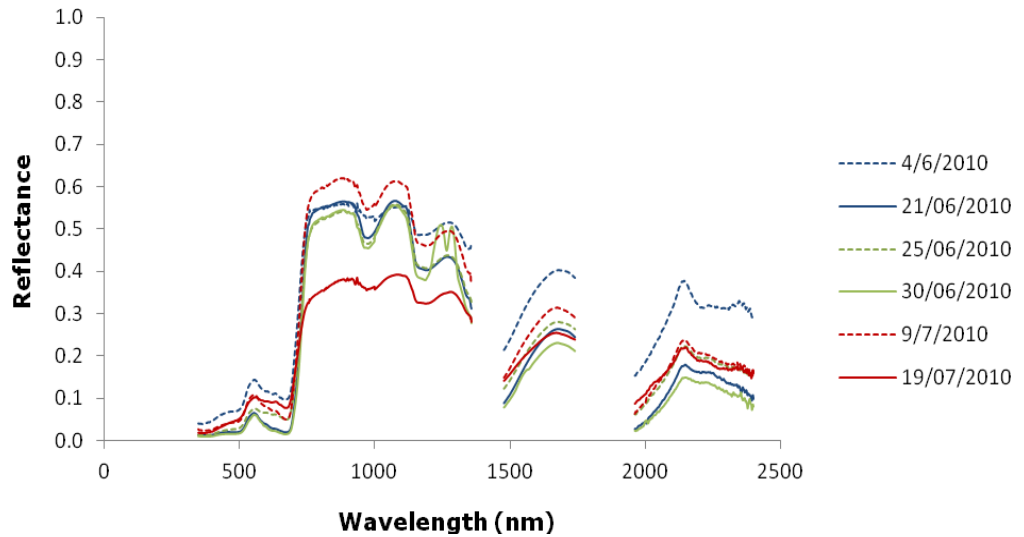


Figure 5-23. Reflectance spectra of barley grown on 5% herbicide treated plots.

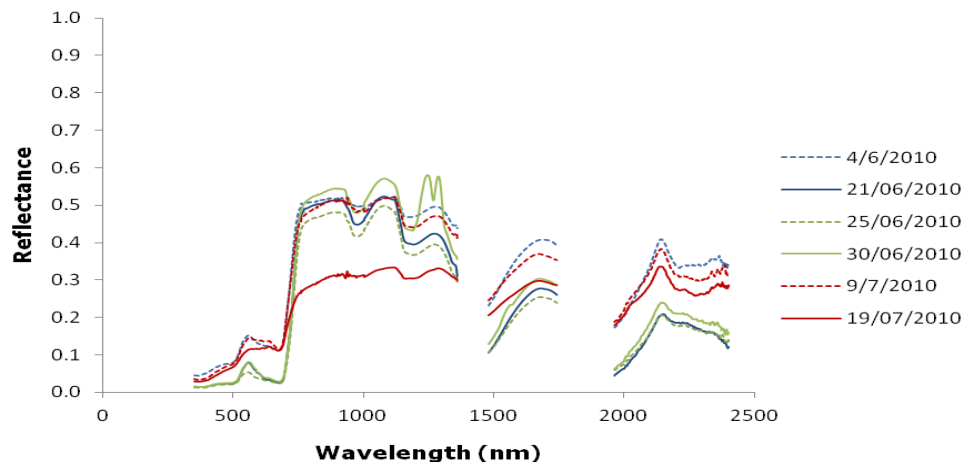


Figure 5-24. Reflectance spectra of barley grown on 10% herbicide treated plots.

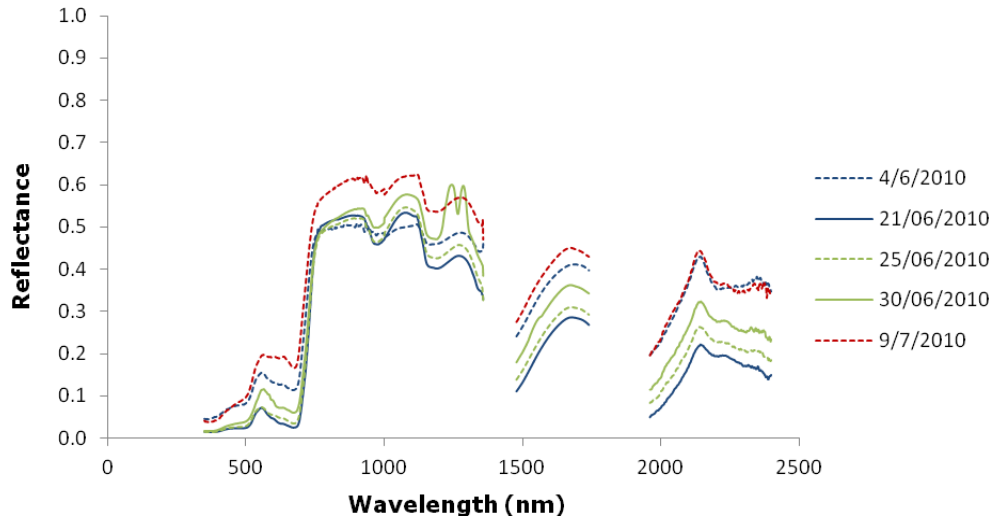


Figure 5-25. Reflectance spectra of barley grown on 20% herbicide treated plots.

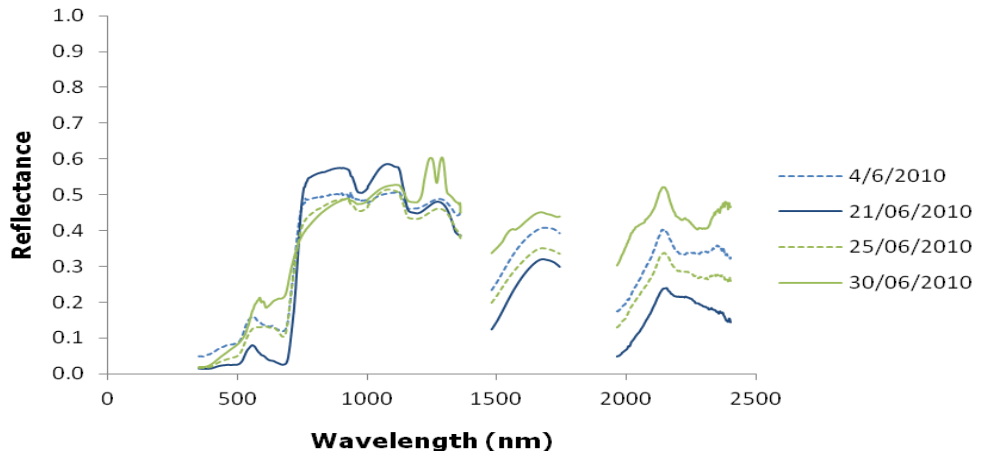
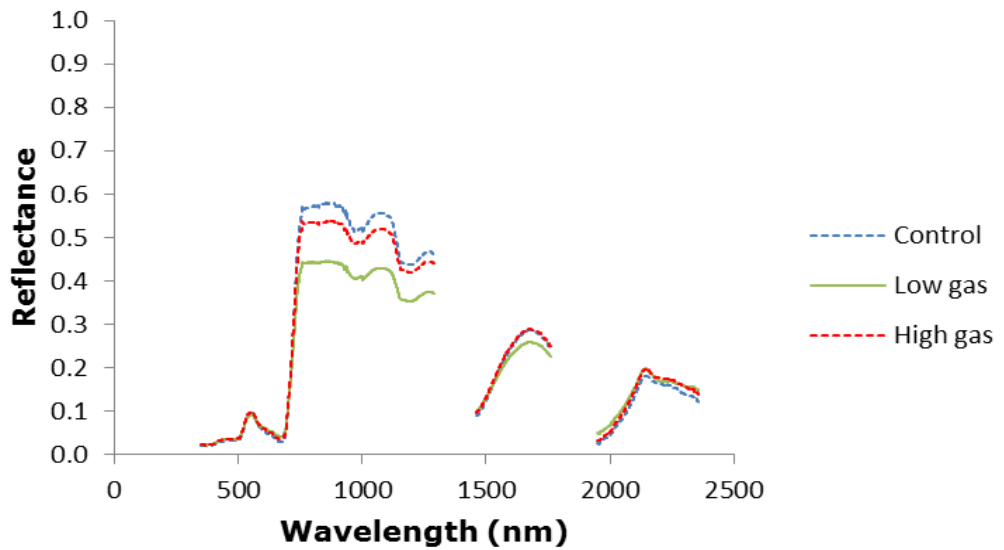
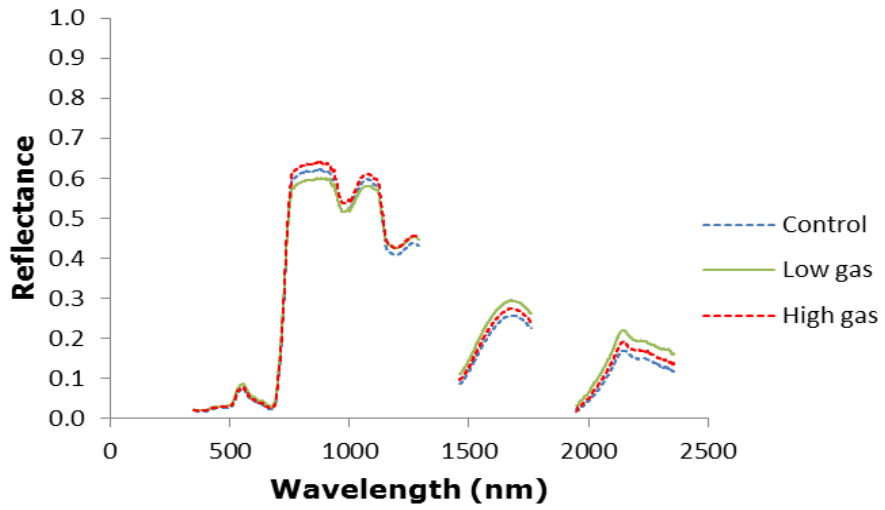


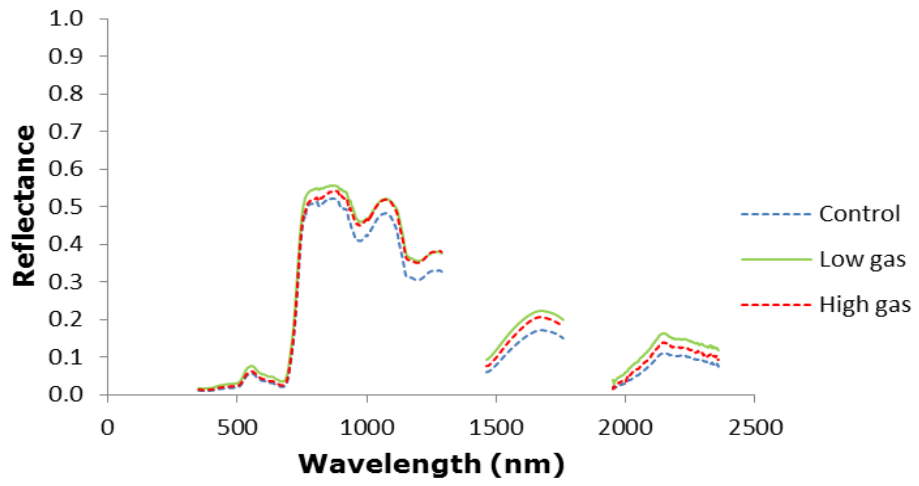
Figure 5-26. Reflectance spectra of barley grown on 40% herbicide treated plots.



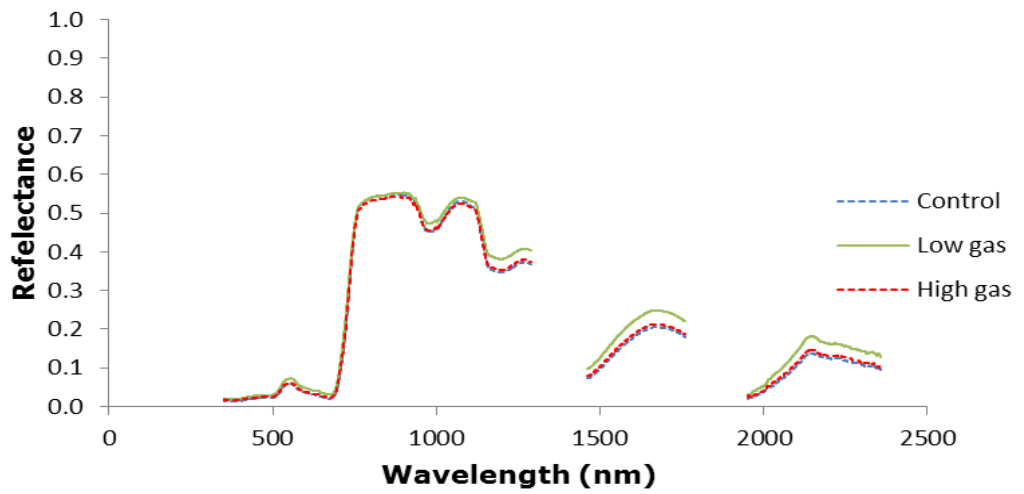
A. (03/06/2010).



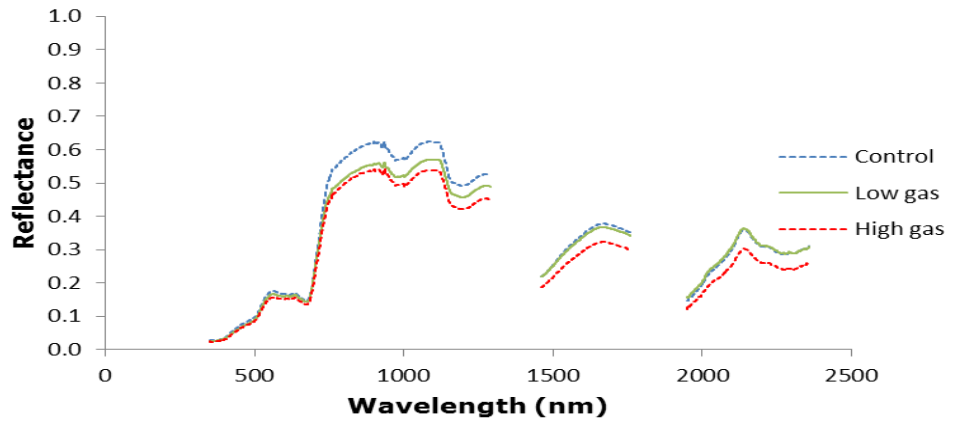
B. (21/06/2010).



C. (28/06/2010).

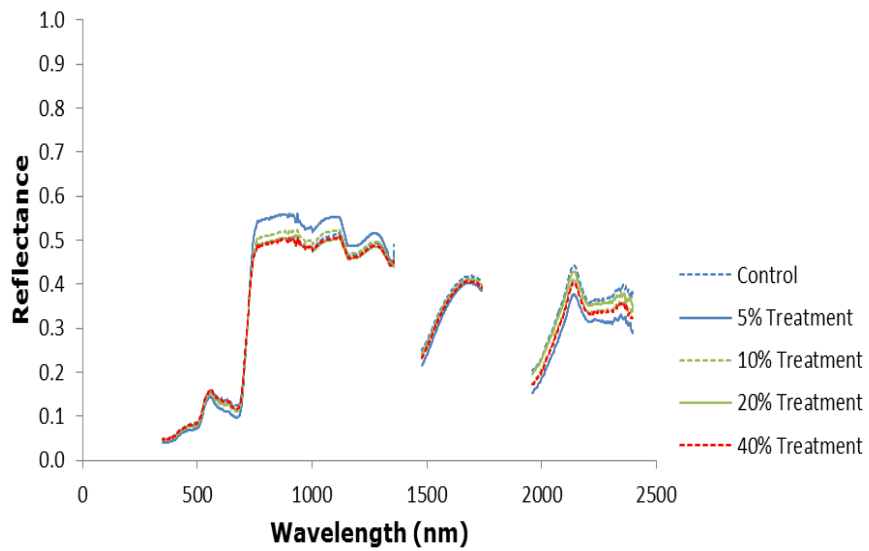


D. (06/07/2010).

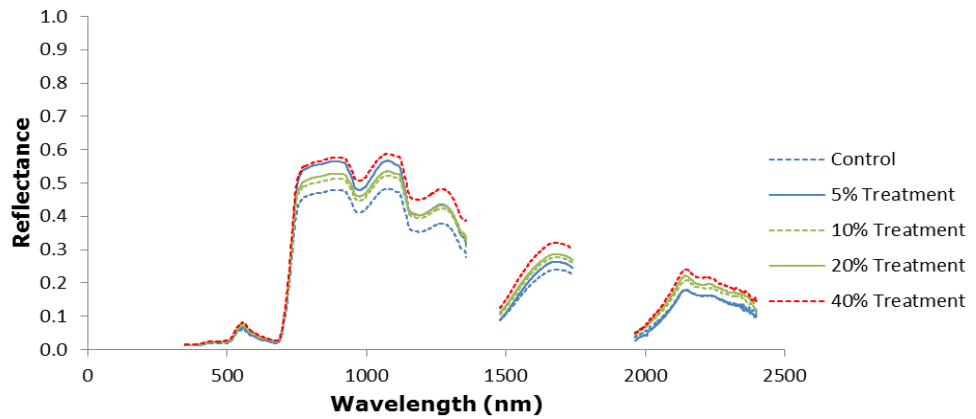


E. (19/07/2010)

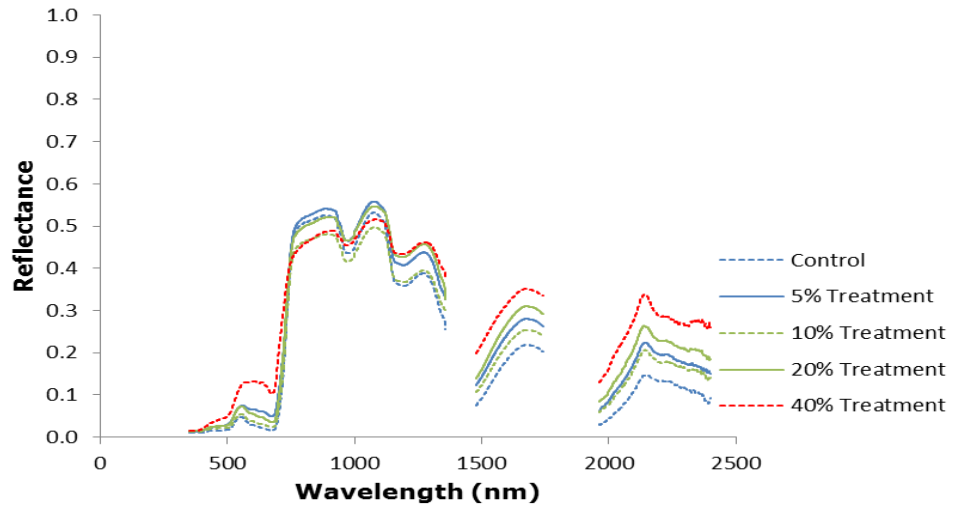
Figure 5-27. A-E: Reflectance differences between controls, high and low gas regions for barley grown in gassed plots on the given dates.



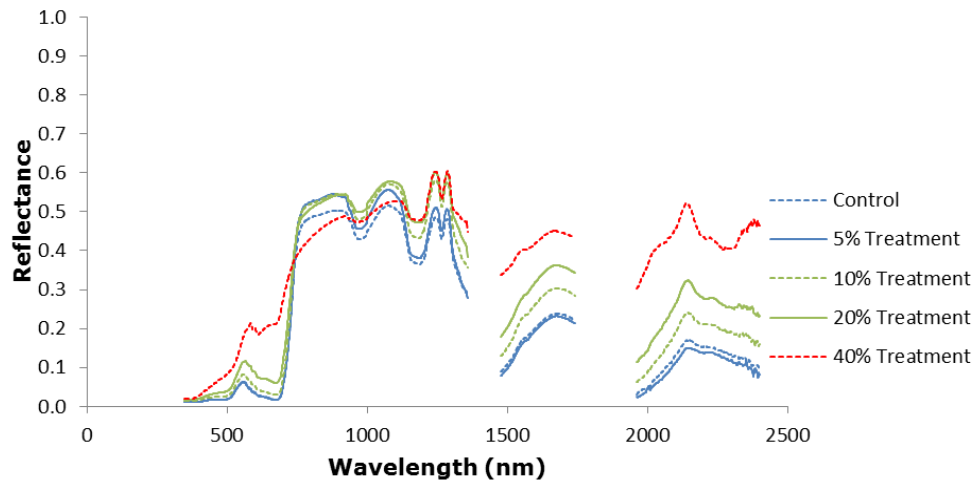
A. (04/06/2010).



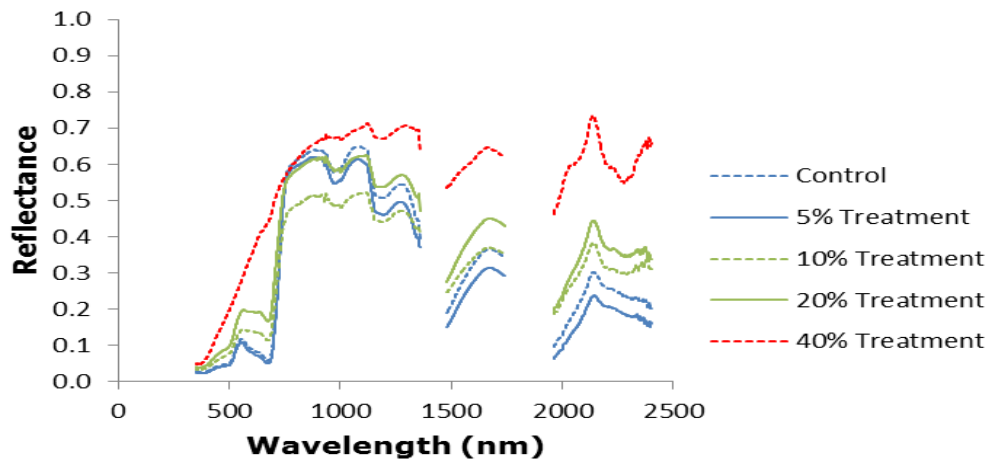
B. (21/06/2010).



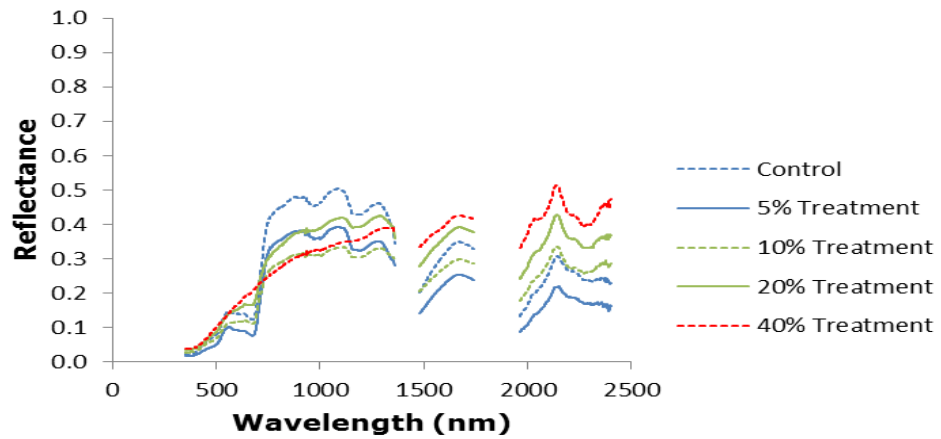
C. (25/06/2010).



D. (30/06/2010).



E. (09/07/2010).



F. 19/07/2010).

Figure 5-28. A-F: Reflectance differences between control and the different levels of herbicide treatment.

5.3.6 First derivative reflectance peaks.

There were differences in the derivative peaks for the different treatment types as well as in the concentration levels.

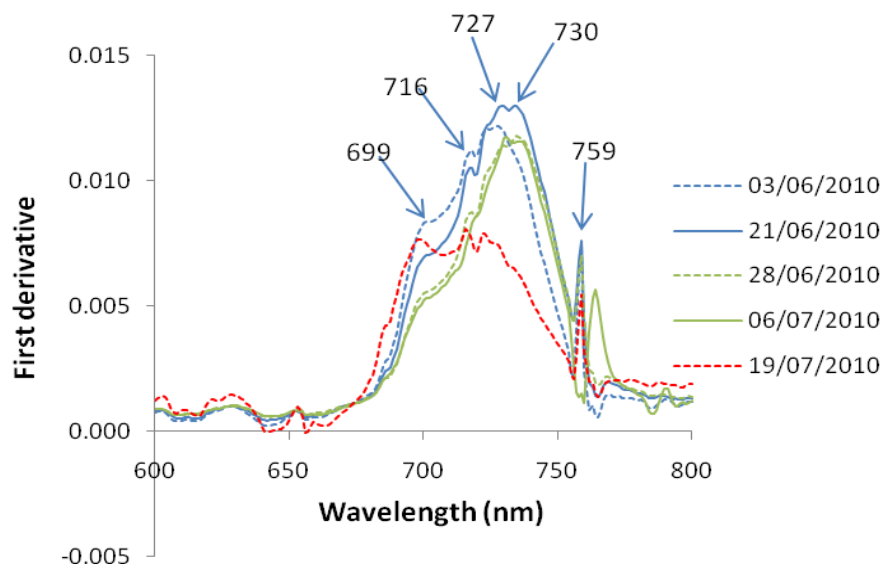


Figure 5-29. First derivative peaks of barley grown on control plots.

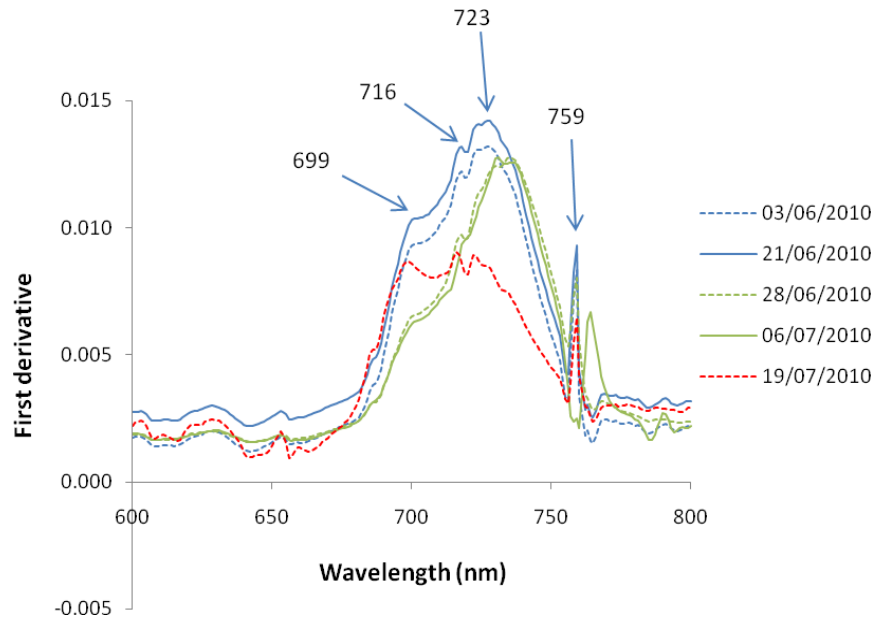


Figure 5-30. First derivative peaks of barley grown on low CO₂ zone.

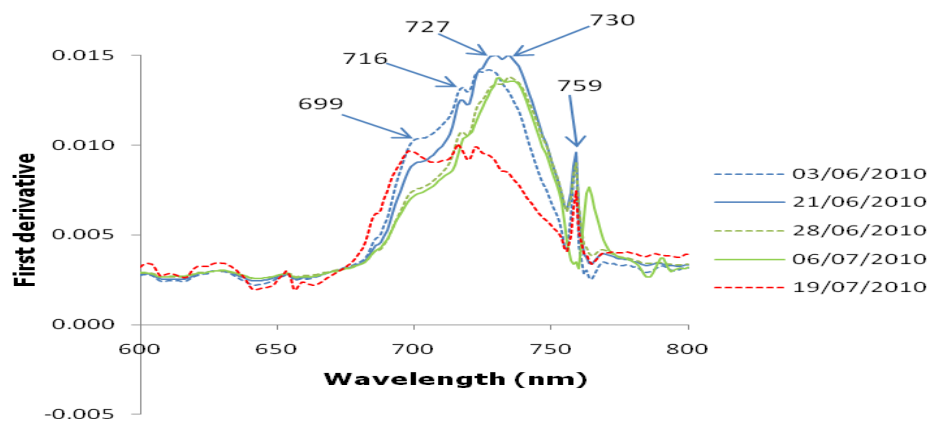


Figure 5-31. First derivative peaks of barley grown on high CO₂ zone.

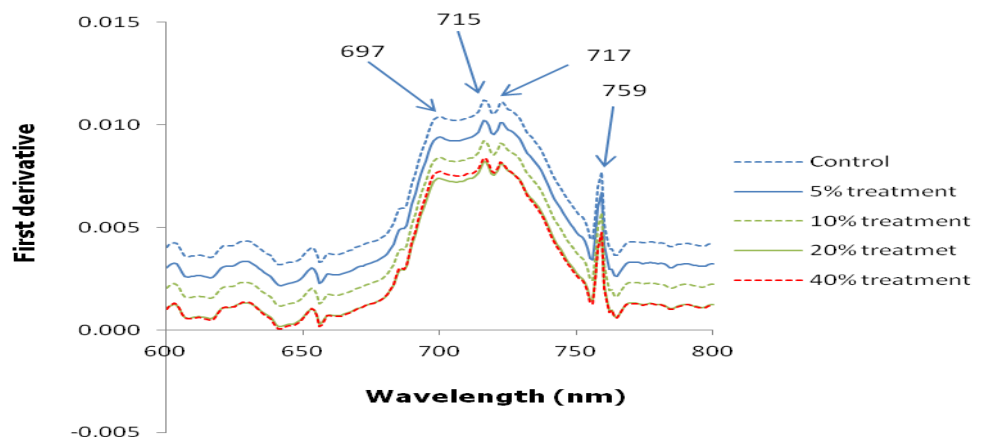


Figure 5-32. First derivative peaks of barley grown on herbicide experimental plots measured on 10th of June 2010, a day after application of herbicide.

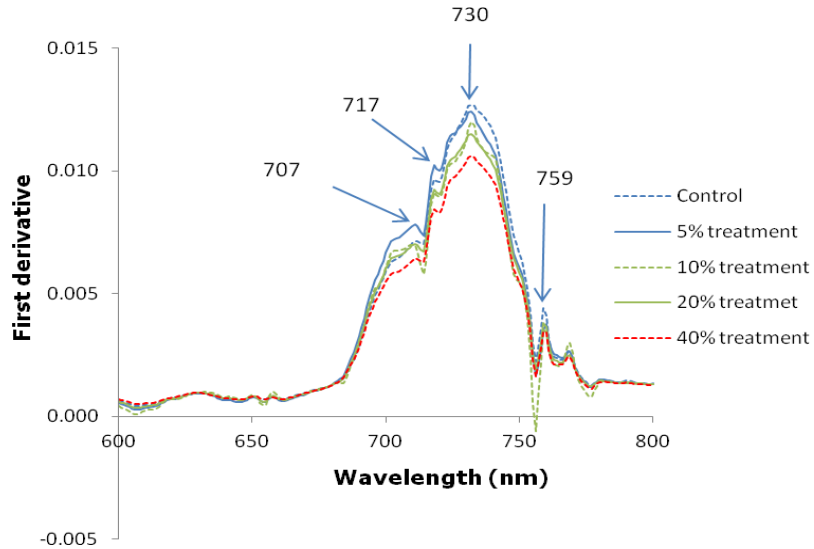


Figure 5-33. First derivative peaks of barley grown on the herbicide experimental plots measured on 21st of June 2010 (13 days after application of herbicide)

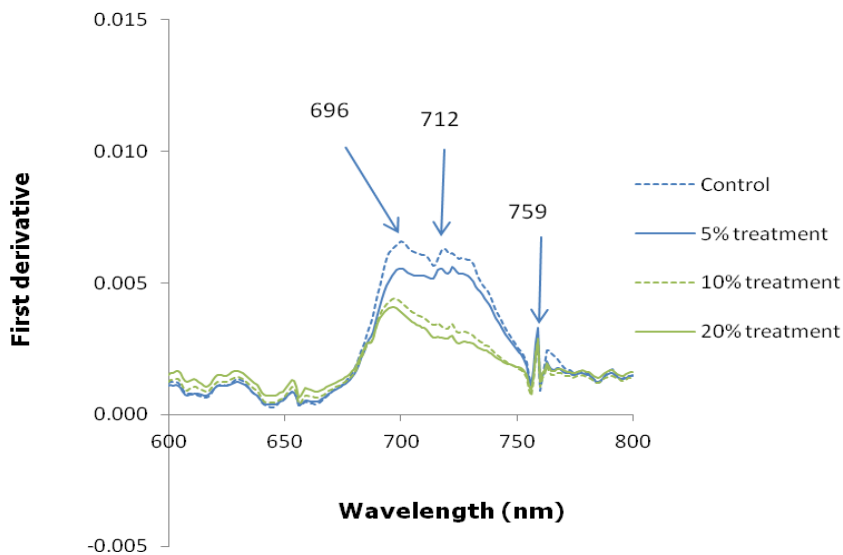


Figure 5-34. First derivative peaks of barley grown on herbicide experimental plots measured on 9th of July 2010 (30 days after application of herbicide). 40% herbicide treated barley had died off at this stage.

Differences in the first derivative red-edge peaks were apparent. Figures 5-29 to 5-34 show the first derivative spectra and the red-edge peaks for CO₂ and herbicide treatments with their respective controls. The CO₂ control had double peaks at 727 and 730nm with several smaller peaks or shoulders between 699 and 759nm. The region of low CO₂ concentration had a single peak at 723nm. While the region of high CO₂ concentration

had similar peaks to control. The major difference was that the magnitude of these peaks had increased with stress level with the dominant change occurring between 716 and 730nm.

Figures 5-32 to 5-34 shows the first derivative spectra of the red-edge peak for the herbicide treated plots at the early treatment period on 10th June, 2010 (a day after the application of herbicide), the middle period on 21st of June, 2010 (13 days after treatment) and late treatment period on 9th July, 2010 (30 days after treatment) respectively.

The first derivative peak for the early treatment period was composed of peaks at 697, 715 and 717 nm with small shoulders at 707, 717 and 759 nm. At the mid period the peak became single at 730 nm, the shoulder was still at 759 nm but the magnitude had decreased. By the late treatment period barley had turned yellow in all the treatments, the peaks had decreased further in magnitude. The major peaks were at 696 and 712 nm respectively with a further decrease in the magnitude of the shoulder which remained at 759 nm.

5.3.7 Temporal change in red-edge position.

The temporal change in the red-edge position was also analysed for the different treatment types.

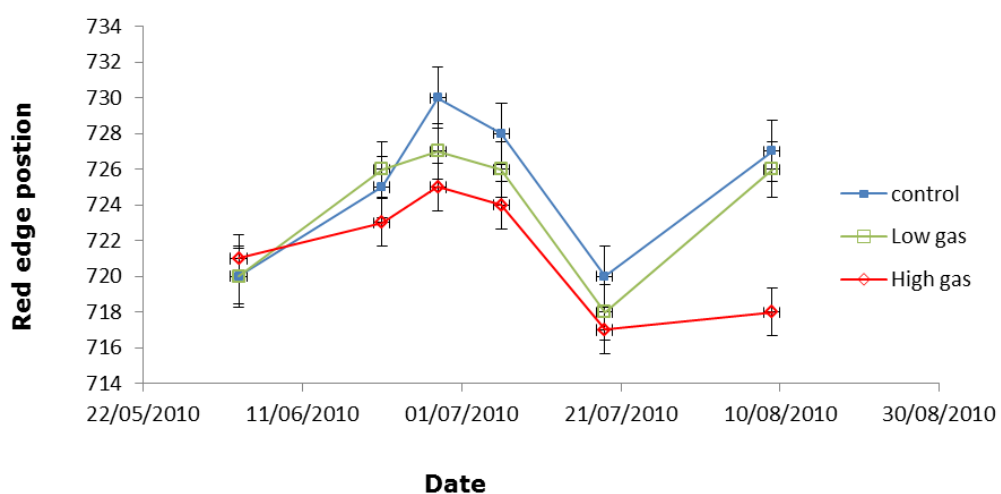


Figure 5-35. Temporal change in red edge position for gassed plots.

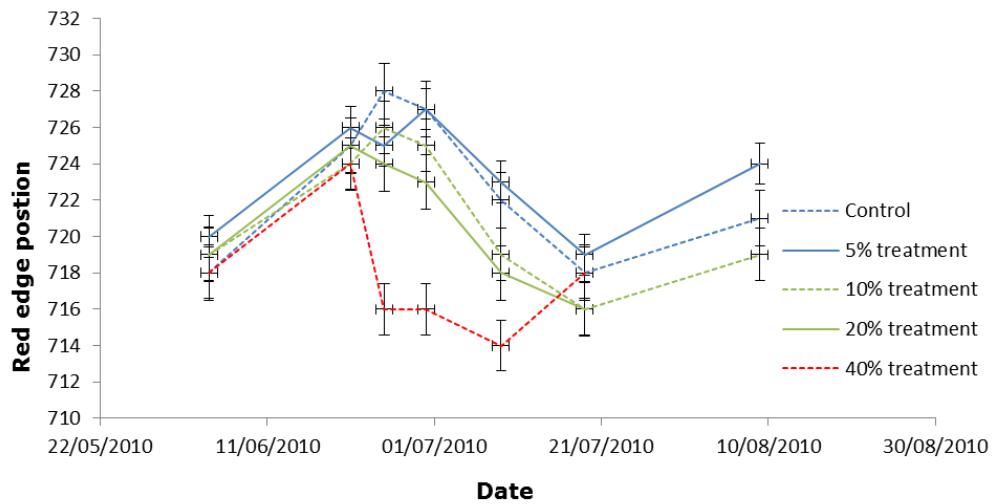


Figure 5-36. Temporal change in red edge position for herbicide treatment.

From the start of the CO₂ experiment the red edge position for both the control, low and high gas zone were between 720-721nm. But as the experiment progressed there was a rise to as high as 730nm in the control, followed by 727nm in low and 725nm in high CO₂ zones with a drop on 19/07/2010. At maturity on 09/08/2010 the red edge position rose slightly to 718nm in high CO₂, 726nm low and 727nm in the control. During the period 03/06/2010 until 09/08/2010, the red edge position was displaced towards longer wavelengths for both the control and low CO₂ zone where the gas concentration was low, which caused an 8nm shift in the position of red edge from 720 to 728nm, while the control from 720 to 727nm a shift of 7nm, the inner transect with higher gas concentration was displaced towards shorter wavelength 721 to 718nm a shift of 3nm.

However, for the herbicide treated plots at the beginning of June there was a steady rise in the red edge position for all levels of treatment as a result of slow response to the stress at the beginning of the experiment, but by mid-July the red edge position began to shift to shorter wavelength for

20% treatment, while the control and 5% treatments shifted to longer wavelength. At the end of the experiment, the control shifted from 718 to 721nm (3nm shift), the 5% treatment shifted from 720 to 724nm (4nm), the 20% treatment 719nm to 716nm, while 10% and 40% did not show any sign of shift at end of the experiment, at this stage the crop had turned yellow and was ready for harvest.

5.3.8 Vegetation indices.

The visible region has been used to determine the rate of changes in chlorophyll content over time (Lakkaraju *et. al.*, 2010), this region is characterised by the high absorption of radiation energy by the leaf pigments; which constitutes majorly the chlorophylls and carotenoids (Knipling, 1970). It is therefore possible to trail changes in chlorophyll by calculating vegetation indices using the visible region of the spectrum. *Chl NDI* is an indicator of total chlorophyll content and *PSSRa* and *PSSRb* are indicators of chlorophyll a and chlorophyll b respectively. Therefore these indices were chosen to estimate the changes over time in concentrations of total chlorophyll, chlorophyll a and chlorophyll b.

The vegetation indices (Refer to section 3.10.4 for details) applied in this study were tested to determine their sensitivity to the stresses.

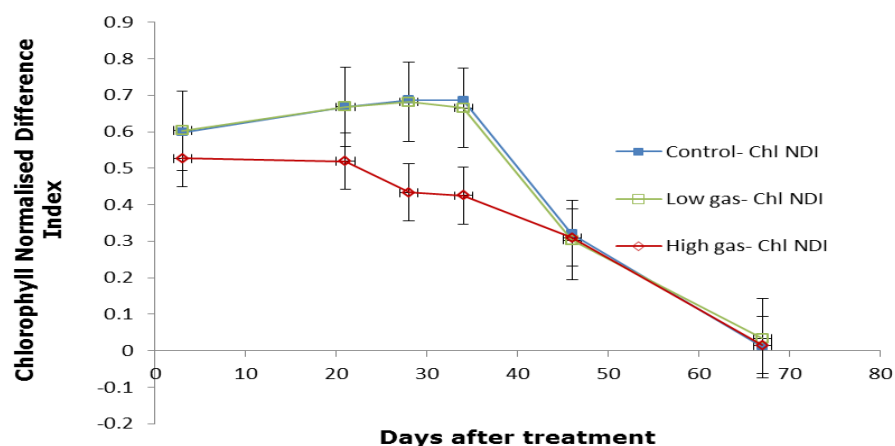


Figure 5-37. Temporal change in Chlorophyll Normalised Difference Index (Chl NDI) for CO₂ and control plots.

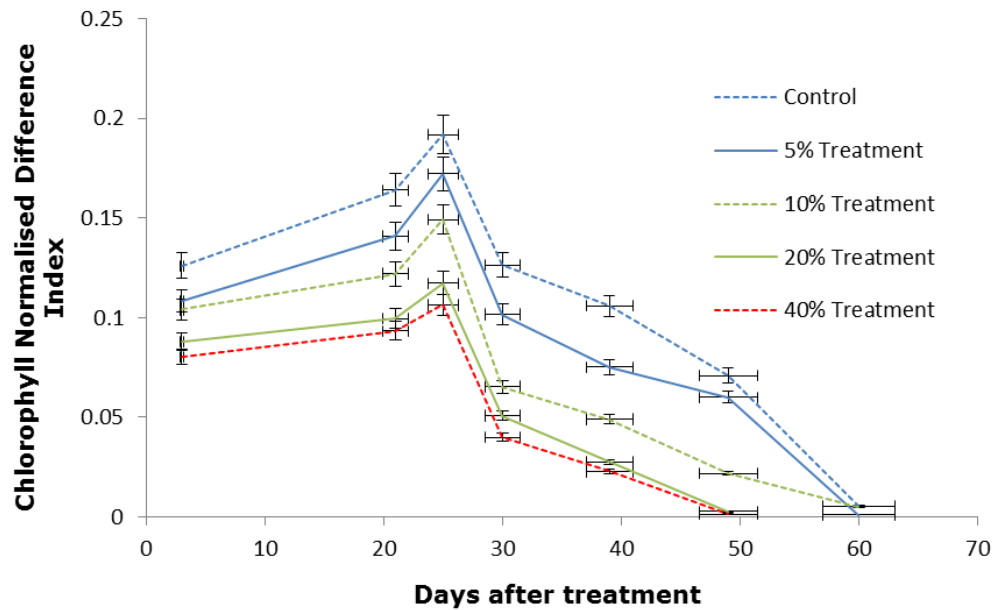


Figure 5-38. Temporal change in Chlorophyll Normalised Difference Index (Chl NDI) for herbicide treatment and control plots. The bars in these figures and subsequent ones represent standard error.

The temporal variation in Chlorophyll Normalised Difference Index (*Chl NDI*) for CO₂ and herbicide treated plots compared to their controls are shown in Figures 5-37 and 5-38.

The control plots and low gas concentration showed an initial increase in total chlorophyll content, from t=3 to t=34, while high gas concentration showed a decrease from day(t)=3 to t=67. The average difference between the high CO₂ and control treatments at t=3, 21, 28 and 34 were significantly different statistically (ANOVA (*p-value* 0.021; 0.035; 0.000; 0.019 ($\sigma \leq 0.05$); n=12). There was no significant difference (ANOVA (*p-value* 0.114; 0.597; 0.811; 0.617; 0.270; 0.708 ($\sigma \leq 0.05$); n=12) between control and low gas concentration throughout the duration of the experiment.

The difference between high and low CO₂ zone was only significant at t=21, 28 and 34 (ANOVA (*p-value* 0.041; 0.009; 0.007 ($\sigma \leq 0.05$); n=12) as shown in Figure 5-37.

The herbicide treated barley showed significant difference, (ANOVA (*p*-value 0.010; 0.038; 0.007; 0.008; 0.001; 0.003; 0.021 ($\sigma \leq 0.05$); $n=14$) from $t=3$ to $t=67$ between the 5% treatment level and control. However for other treatment levels (10, 20 and 40%) compared with the control, from $t=3$ until $t=49$ except for the final day ($t=67$) for 5% treatment, there were statistically significant differences (Refer to appendix 24 for ANOVA result).

Between $t=3$ and $t=25$ there was a gradual increase in chlorophyll contents in all the treatment levels. From $t=25$ there was decrease in chlorophyll content as at this point the visible signs of stress had started manifesting.

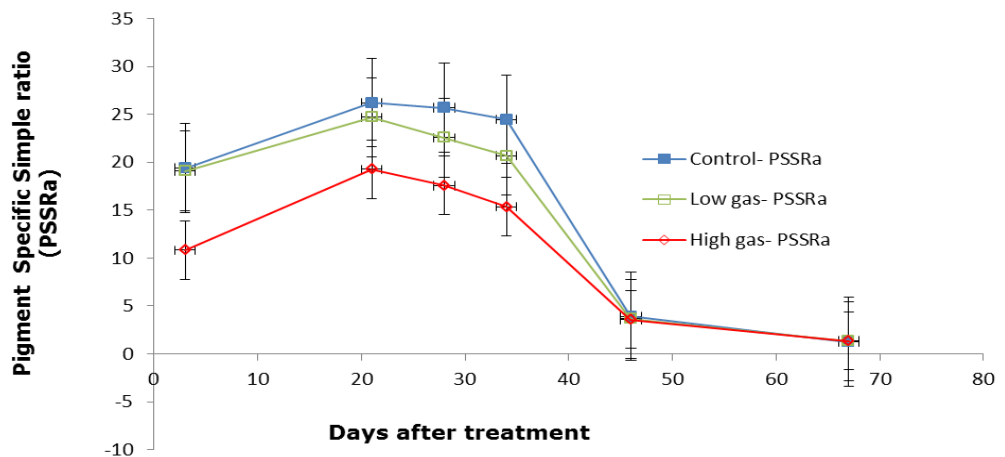


Figure 5-39. Temporal change in Pigment Specific Simple Ratio for chlorophyll a (PSSRa) in CO₂ and control plots.

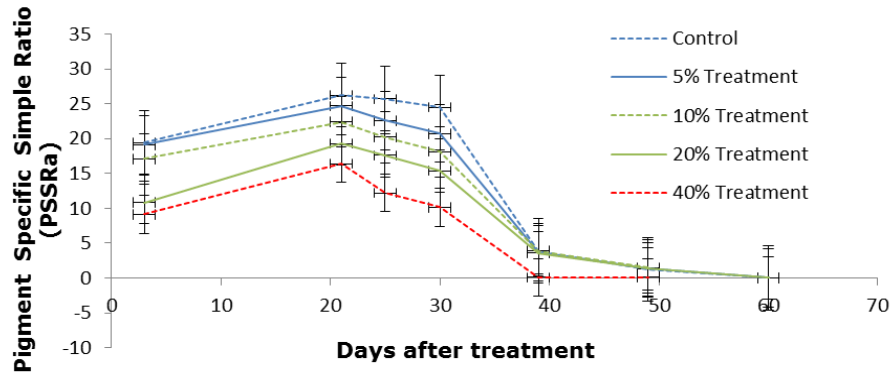


Figure 5-40. Temporal change in Pigment Specific Simple Ratio for chlorophyll a (PSSRa) in herbicide treated and control plots.

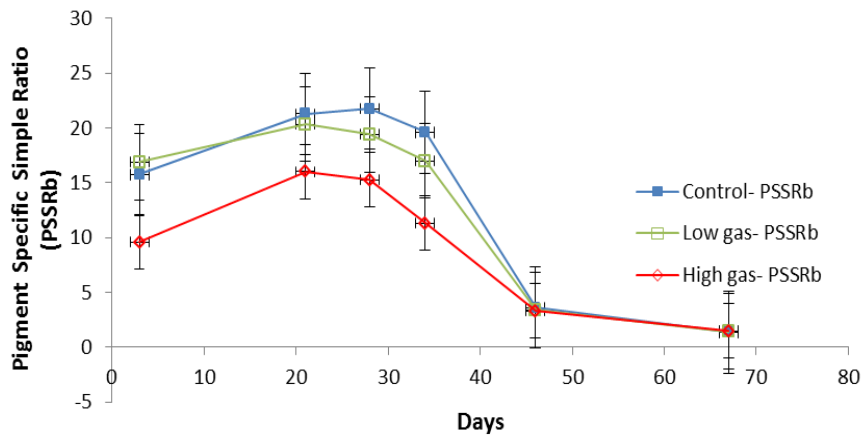


Figure 5-41. Temporal change in Pigment Specific Simple Ratio for chlorophyll b (PSSRb) in CO₂ and control plots.

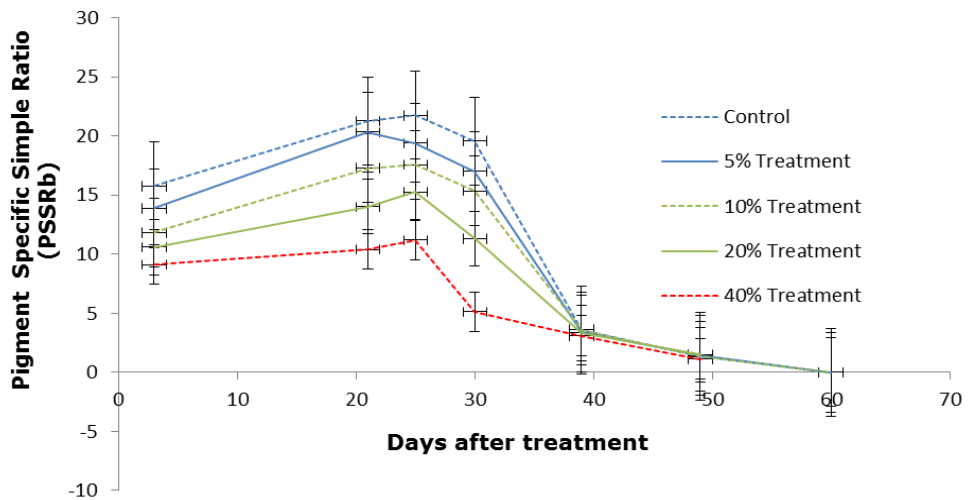


Figure 5-42. Temporal change in Pigment Specific Simple Ratio for chlorophyll b (PSSRb) in herbicide treated and control plots.

Figures 5-39 to 5-42 depict the pattern of change in *PSSRa* and *PSSRb* for the CO₂ and herbicide treatments. In barley treated with low CO₂ there was a statistically significant difference in *PSSRa* (ANOVA (*p*-value 0.027; 0.000; 0.003 ($\sigma \leq 0.05$); *n*=12) at *t*=21 until *t*=34, while for high CO₂ the change in the index was significant (ANOVA (*p*-value 0.019; 0.001; 0.028; 0.007 ($\sigma \leq 0.05$); *n*=12) from *t*=3 until *t*=34, showing that this index could be used for early detection at this level unlike the low CO₂ which became only sensitive at the fourth week after treatment. In the herbicide study the 10, 20 and 40% treatments were statistically significant (Refer to appendix 25 for details of ANOVA results) from *t*=3 until *t*=30 while at 5% it became significant (ANOVA (*p*-value 0.037; 0.027 ($\sigma \leq 0.05$); *n*=14) at *t*=25 and *t*=30 respectively. *PSSRb* was sensitive to all the herbicide treatment levels and high CO₂ from *t*=3 to *t*=30 (Refer to appendix 26 for details of ANOVA results). For the remaining part of the experiment the index was not sensitive to any of the herbicide treatment level. From *t*=39 until *t*=67 the crops were almost dead and the values of both chlorophyll a and b were almost the same across the treatments types and levels.

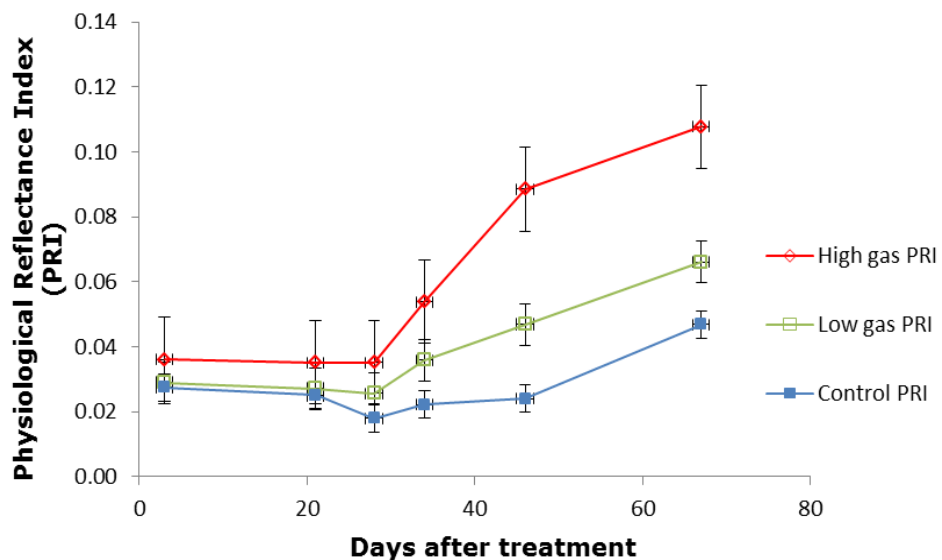


Figure 5-43. Temporal change in Physiological Reflectance Index (PRI) in CO₂ plot and control plots.

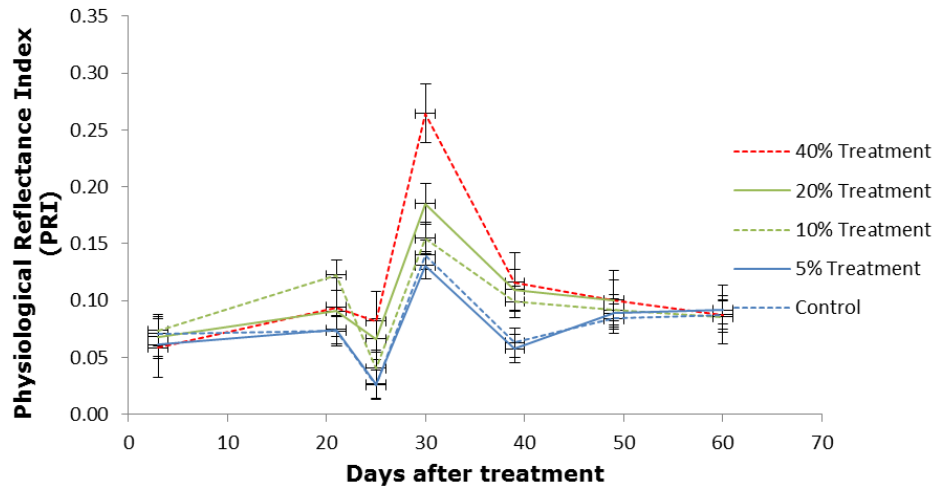


Figure 5-44. Temporal change in Physiological Reflectance Index (PRI) in herbicide treatment and control plots.

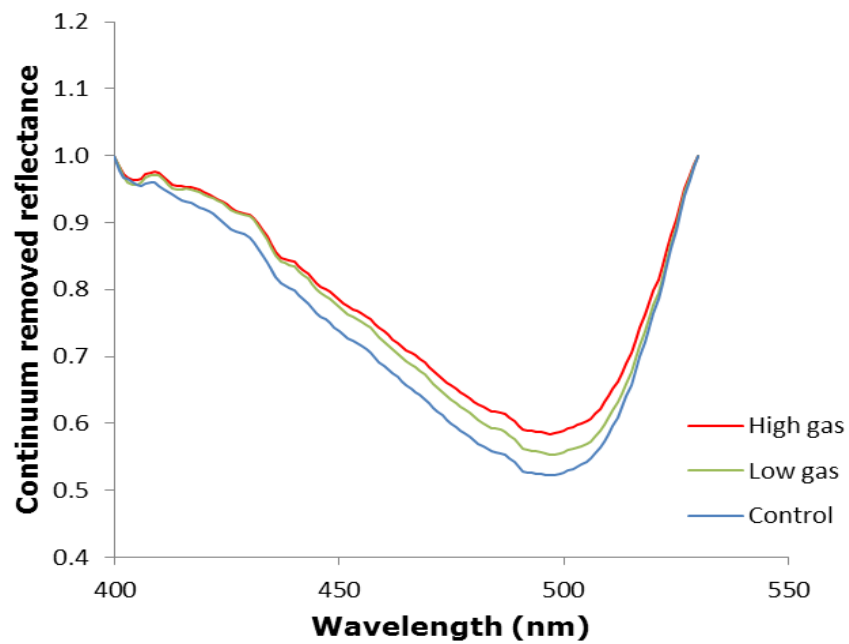
The *PRI* of barley crops growing on CO₂ and herbicide treated plots with controls are shown in Figures 5-43 and 5-44. The maximum difference between control *PRI* and CO₂ *PRI* occurred at t=46 and was 0.09 (high CO₂) 0.04 (low CO₂). The difference between the high CO₂ and control treatments from t=3 until t=67 was statistically significant (ANOVA (*p*-value 0.042; 0.013; 0.000; 0.000; 0.023; 0.011 ($\sigma \leq 0.05$); n=12). While for low CO₂ it became significant (ANOVA (*p*-value 0.028; 0.000; 0.000; 0.001; 0.050 ($\sigma \leq 0.05$); n=12) from t=21 until t=67.

The herbicide treatment recorded lowest *PRI* in the control plots and the 5% herbicide treatment at t=25 which was 0.3. At t=25 there was sudden drop in all the *PRI*'s for the different treatment levels as depicted in Figure 5-44. The *PRI*s were 0.04 (10% treatment), 0.06 (20% treatment) and 0.08 (40% treatment) which was attributed to a period of dry weather in late June 2010. There was statistically significant difference in the *PRI* of barley treated with 10, 20 and 40% herbicide from t=21 until t=49 levels (Refer to appendix 27 for details of ANOVA result). However, there was no significant difference (ANOVA (*p*-value 0.085; 0.196; 1.081; 0.619; 0.345; 0.614; 0.697 ($\sigma \leq 0.05$); n=14) for the 5% treatment and control

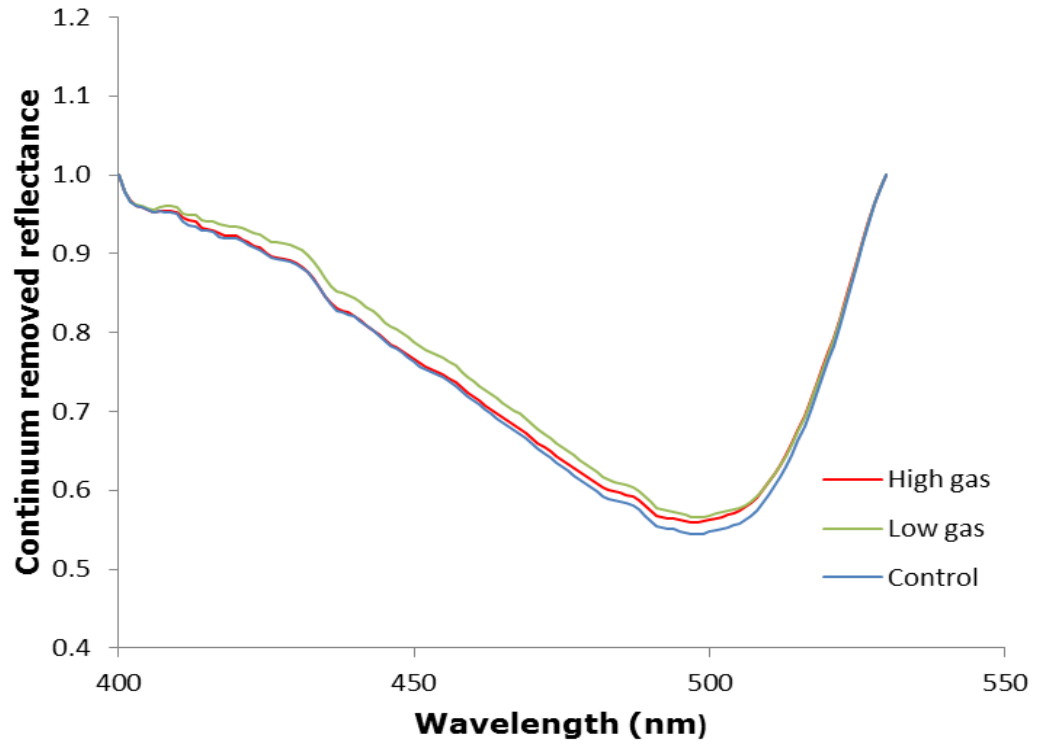
throughout the duration of the experiment. Details on the ANOVA for PRI can be found in appendix 27.

5.3.9 Continuum removal analysis.

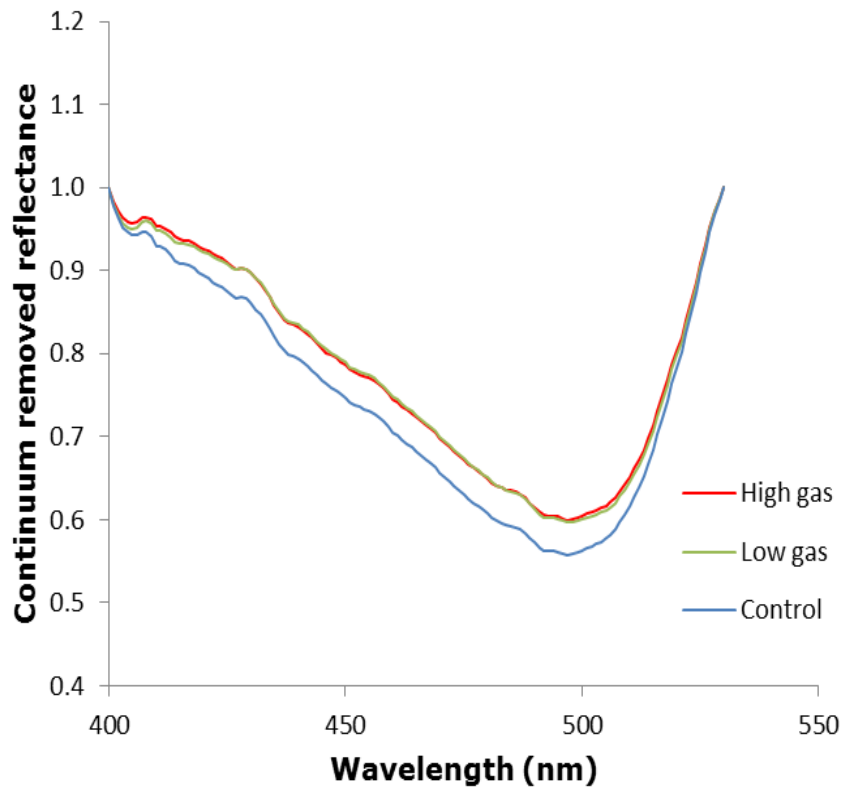
Applying continuum removal to individual absorption features of the reflectance spectra enables comparison to be carried out from a common baseline (Kokaly, 2001).



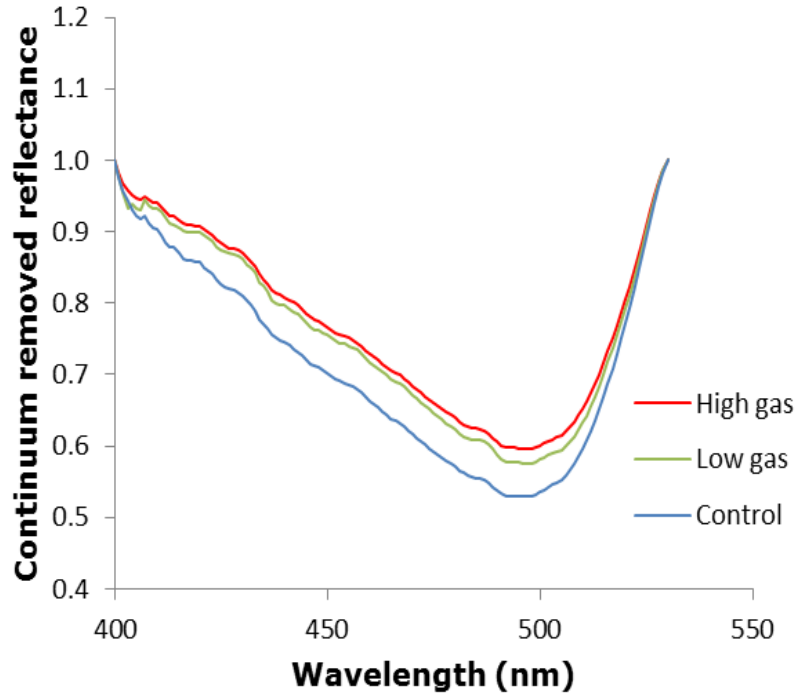
A. (03/06/2010).



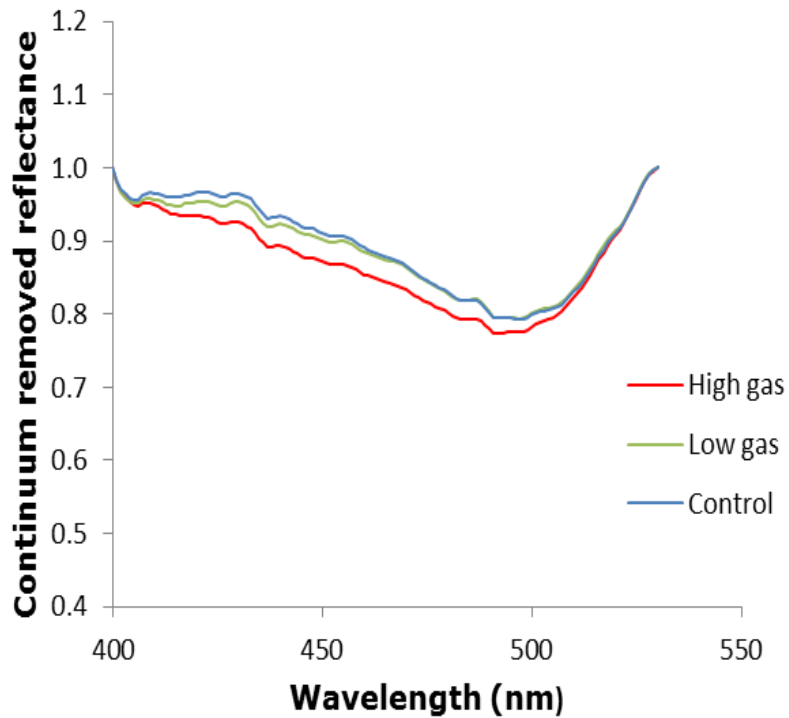
B. (21/06/2010).



C. (28/06/2010).

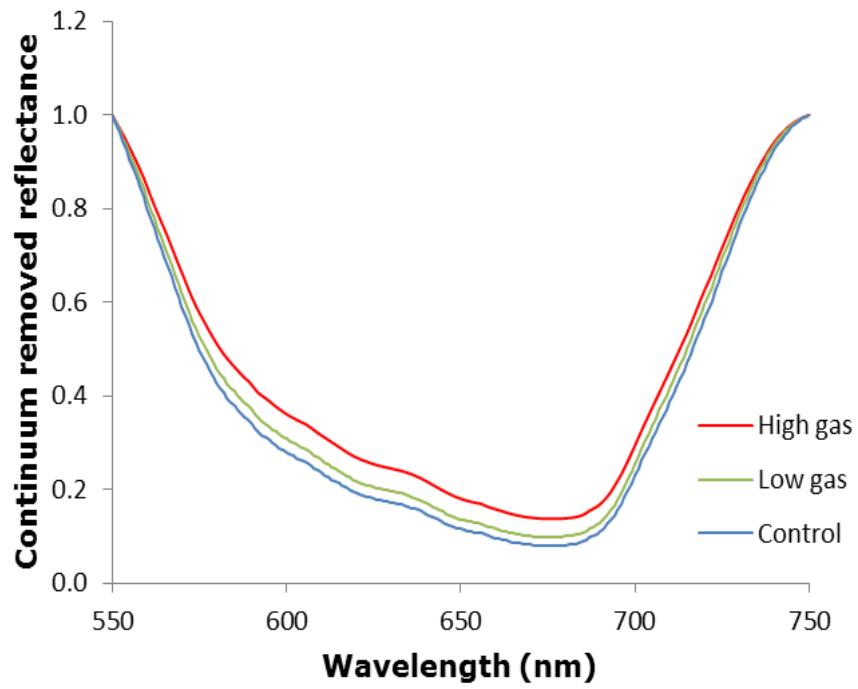


D.(06/07/2010).

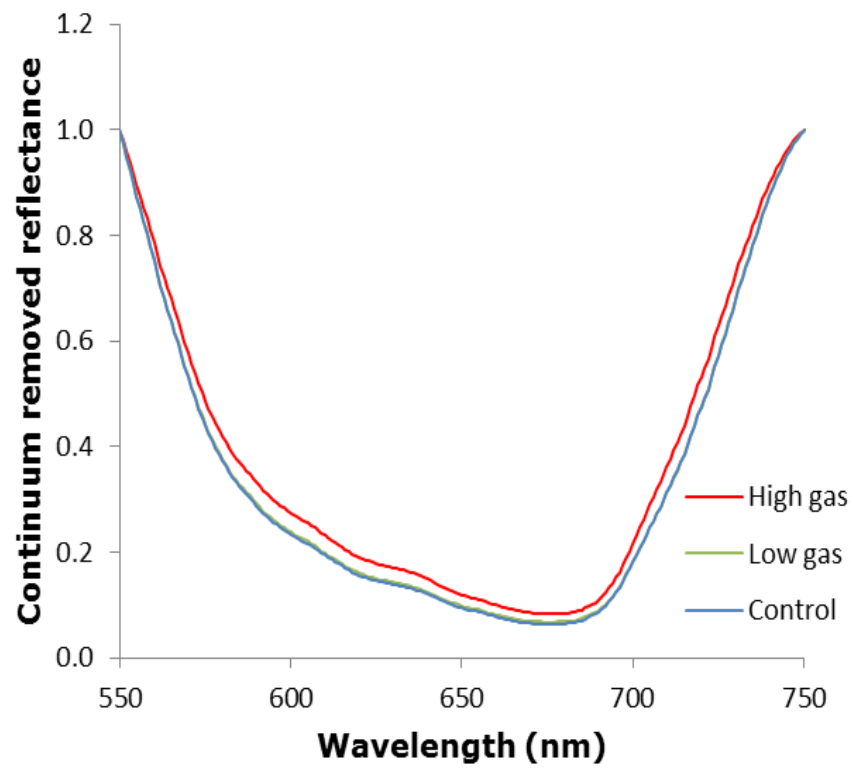


E.(19/07/2010)

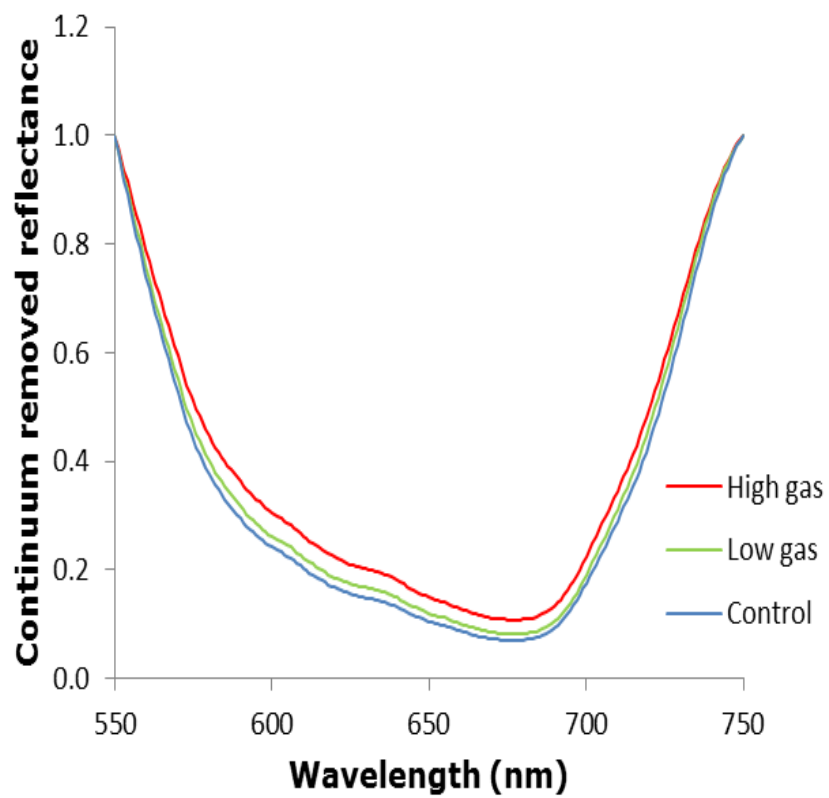
Figure 5-45. A-E: Continuum removed mean reflectance at 400 - 550nm for barley crop growing on CO₂ control, low and high gassed zone measured during the experiment.



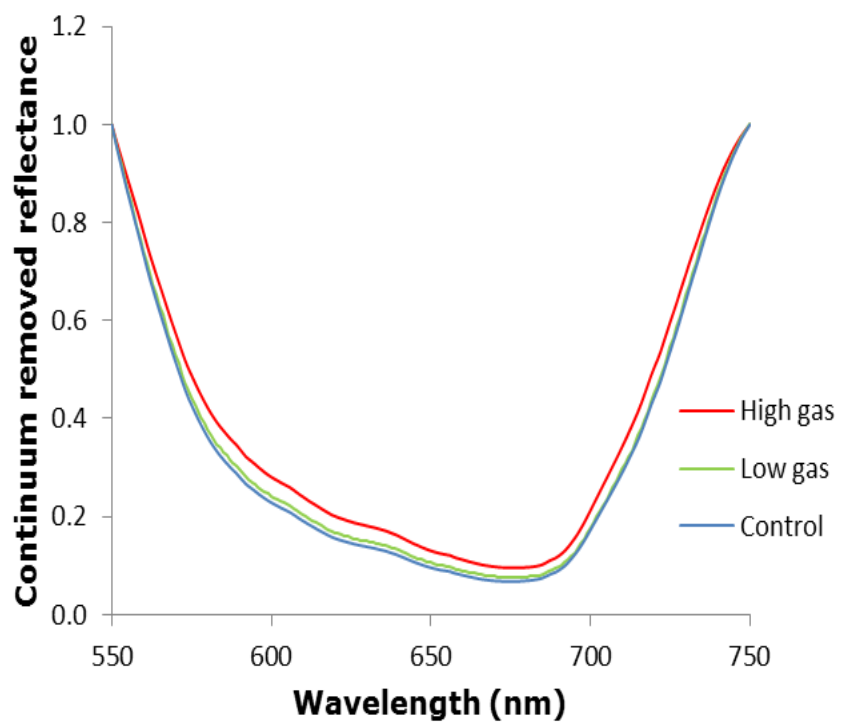
A. (03/06/2010).



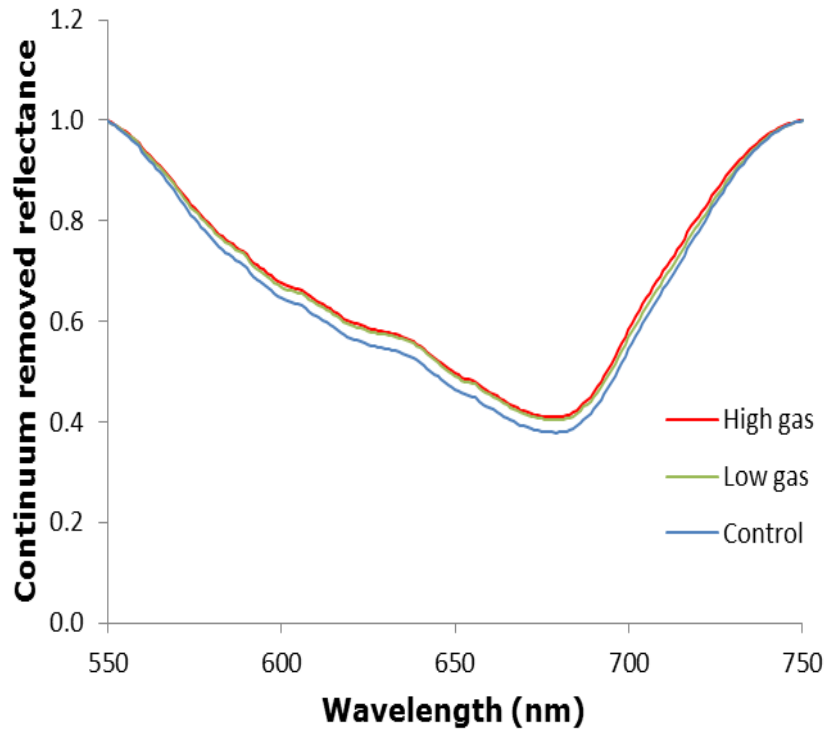
B. (21/06/2010).



C. (28/06/2010).

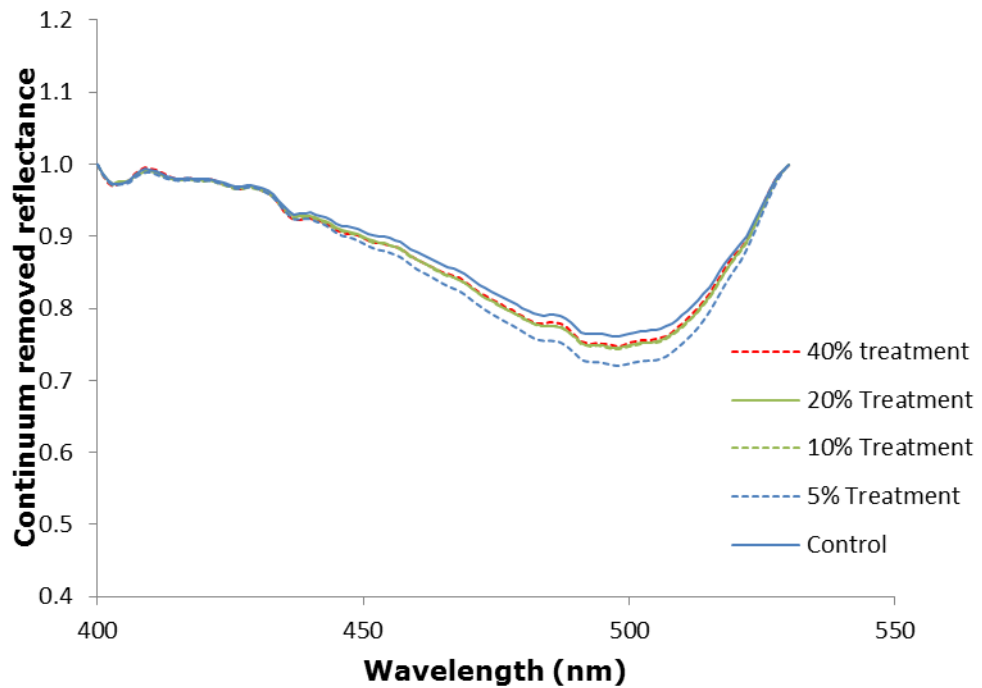


D. (06/07/2010).

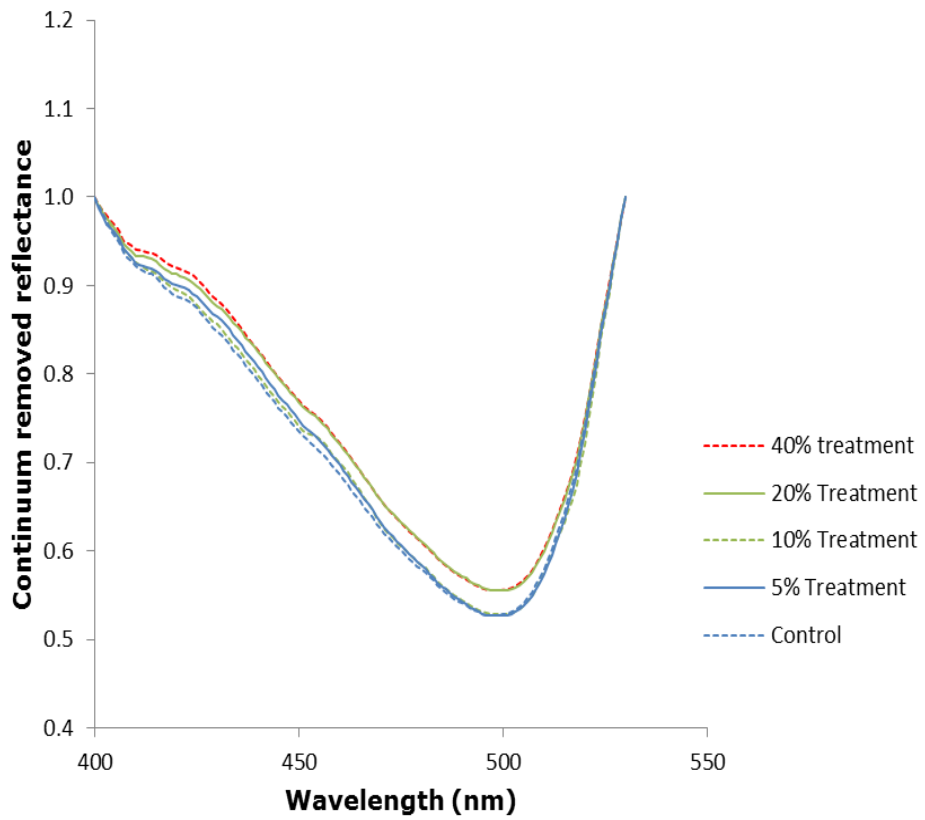


E. (19/07/2010)

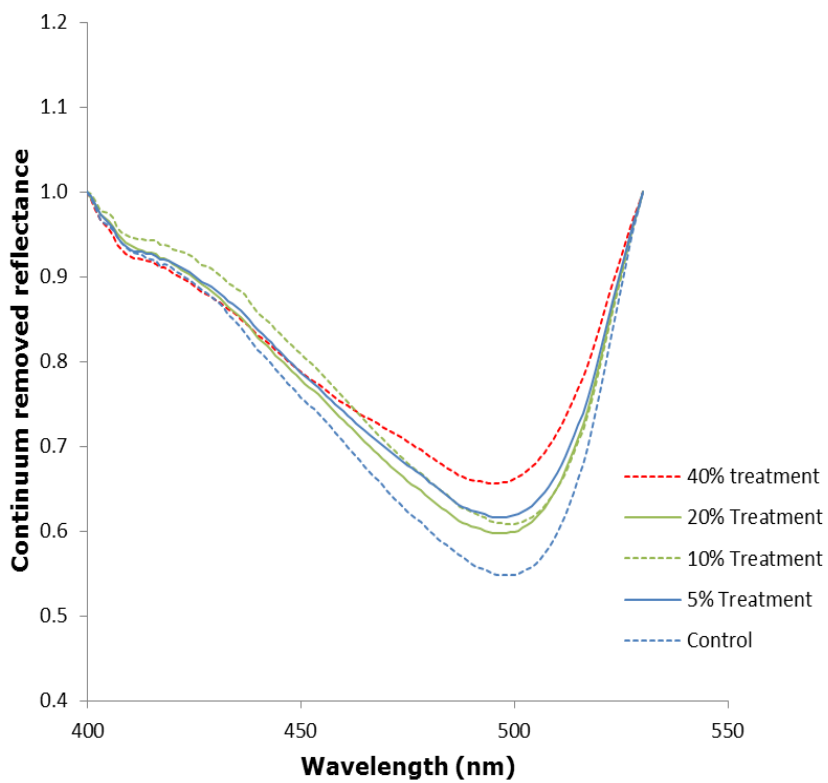
Figure 5-46. A-E: Mean Continuum removed reflectance at 550 - 750nm for barley crop growing on CO₂ control, low and high gassed zone measured during the experiment.



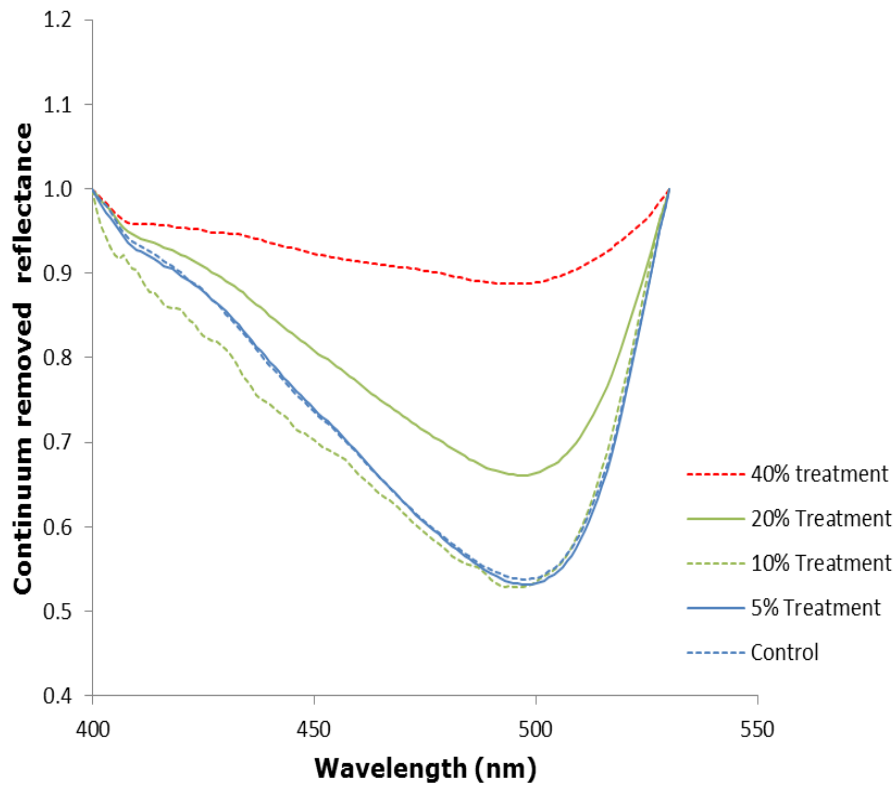
A. (04/06/2010).



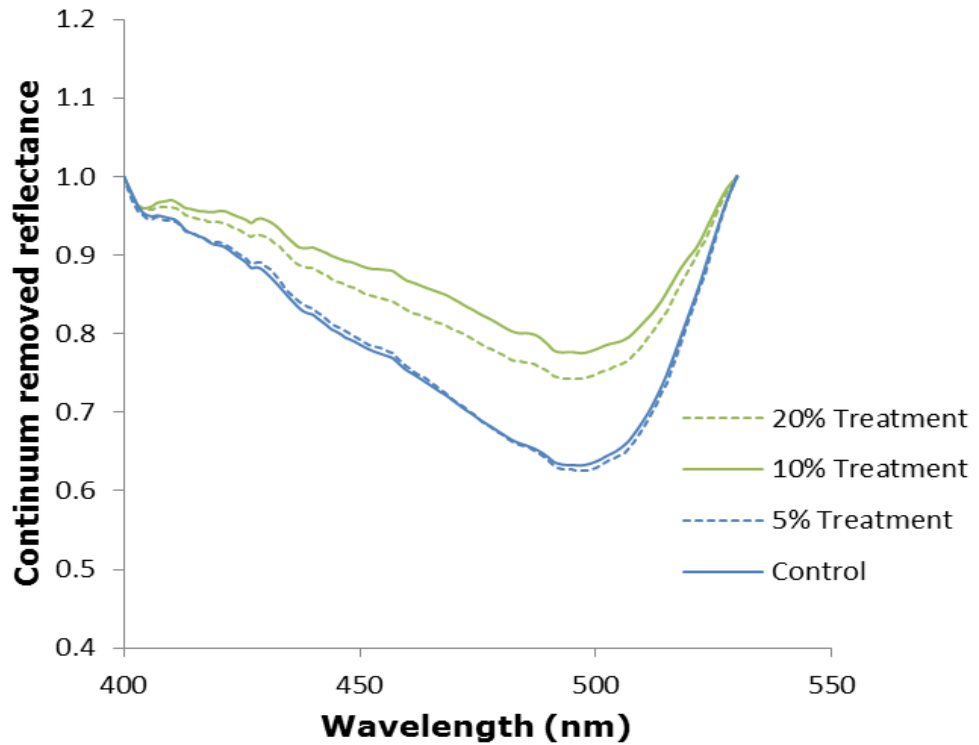
B. (21/06/2010).



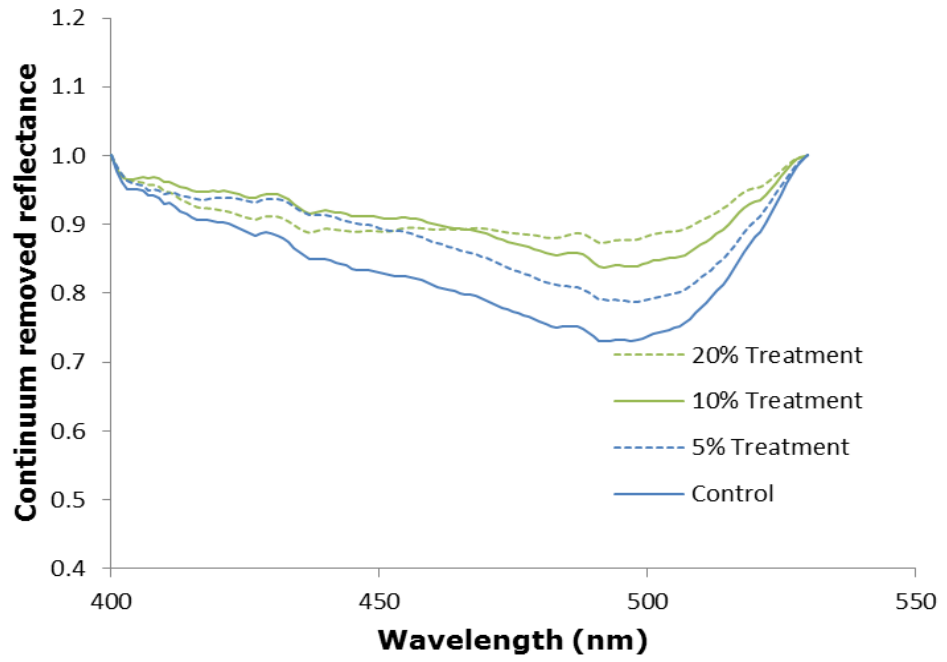
C. (25/06/2010).



D. (30/06/2010).

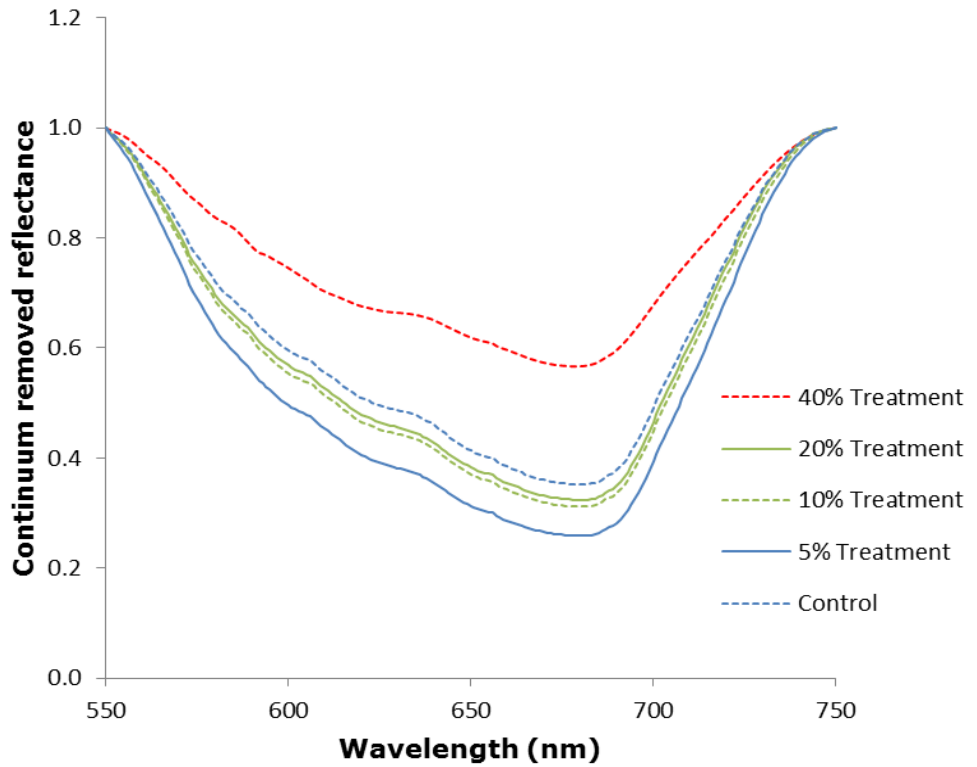


E. (09/07/2010).

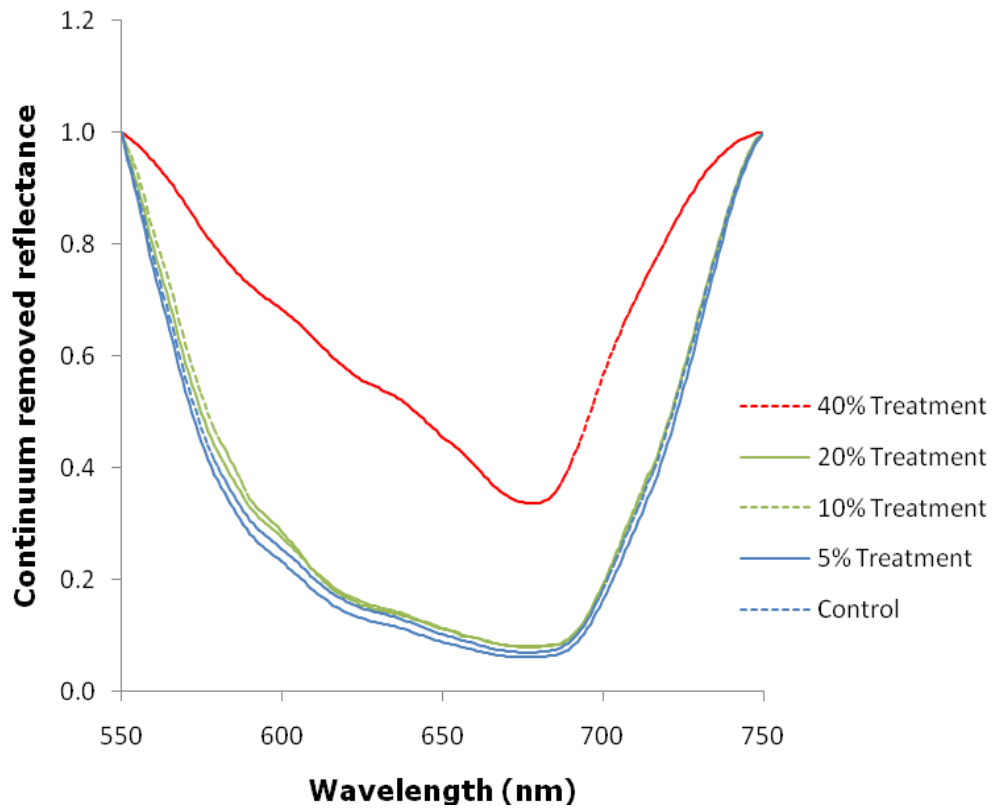


F.(19/07/2010)

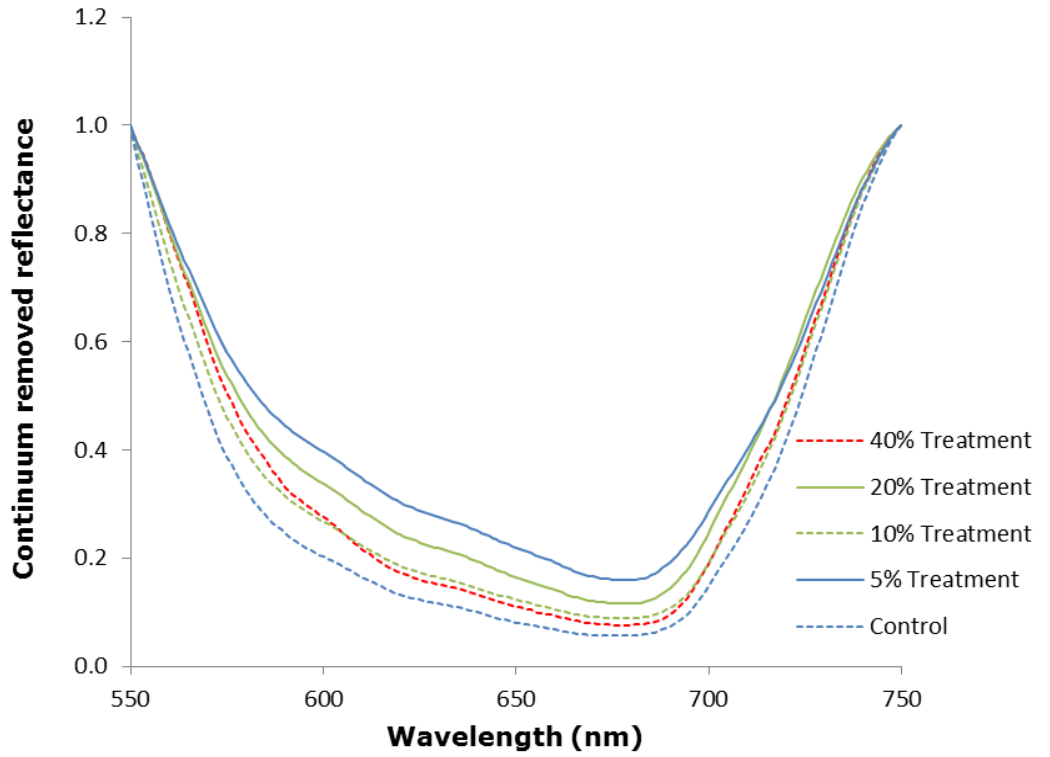
Figure 5-47. A-F: Mean Continuum removed reflectance at 400 - 550nm for barley crop growing on control and different levels of herbicide treatment measured during the experiment.



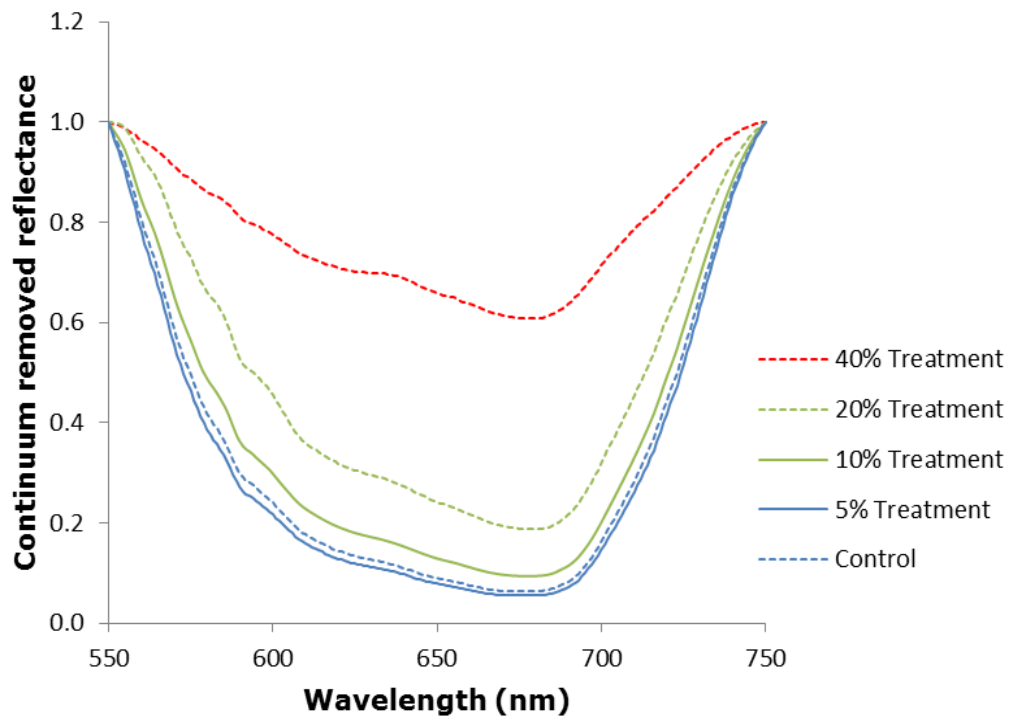
A.(04/06/2010).



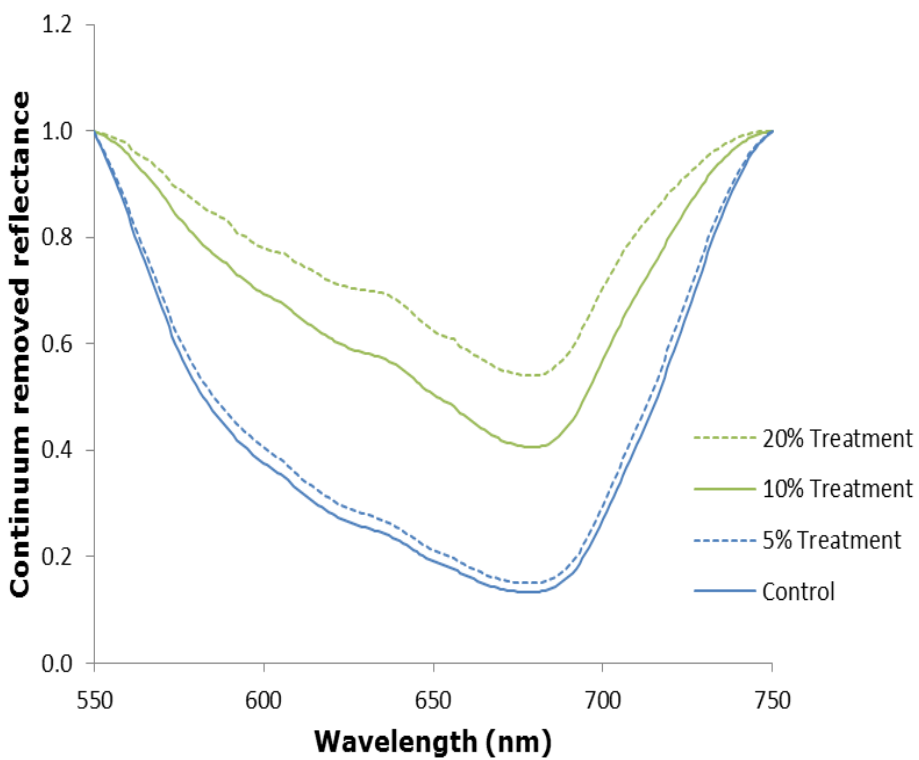
B.(21/06/2010).



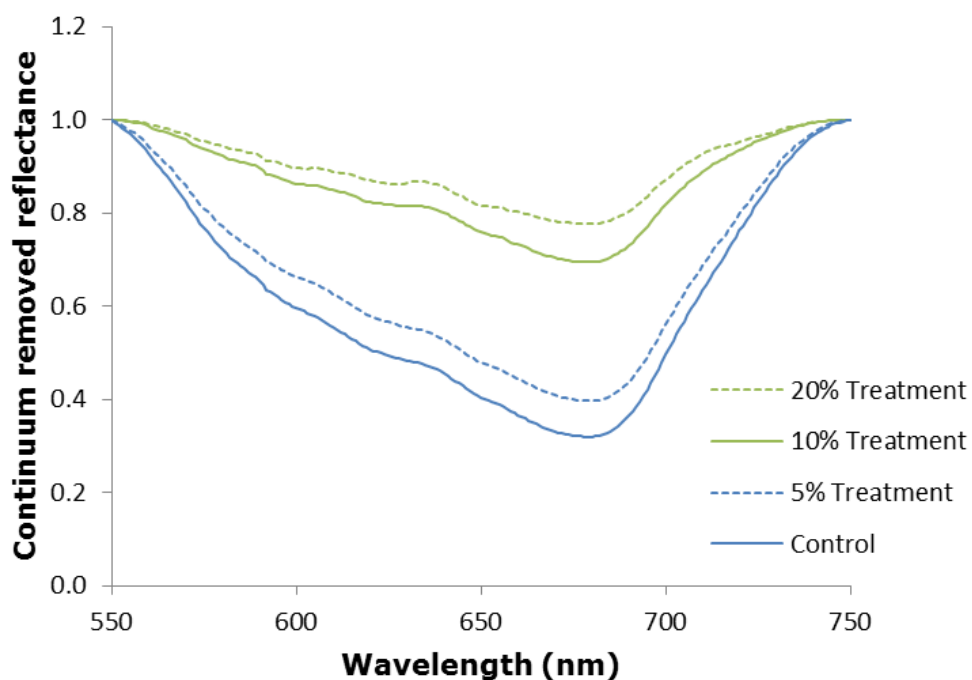
C.(25/06/2010).



D.(30/06/2010).



E.(09/07/2010).



F.(19/07/2010).

Figure 5-48. A-F: Mean Continuum removed reflectance at 400 - 550nm for barley crop growing on control and different levels of herbicide treatment measured during the experiment.

In determining the wavelength that best discriminates the different treatments, the reflectance at those particular wavelengths that showed significant difference between control and CO₂ treatments were analysed over time. The continuum removed reflectance (1-band depth) were analysed. ANOVA was used to determine the wavelength(s) that caused significant differences in band depth. The wavelengths in which CO₂ caused a statistically significant difference (ANOVA (*p*-value 0.033; 0.025; 0.004; 0.000 ($\sigma \leq 0.05$); n=200) in band depth are 405, 515 nm, 575 and 699nm which were also the best for discriminating between the different levels of CO₂ concentration.

There was no significant difference (ANOVA (*p*-value 0.163 ($\sigma \leq 0.05$); n=200) in band depth between low and high CO₂ levels during much of the experiment duration except for the first week after gassing as shown in

Figure 5-45a. At this point the effect of elevated concentration of CO₂ was not noticeable. One interesting observation made in this study is that the continuum removed reflectance of the control was the lowest for almost all the dates, followed by low and high CO₂ concentration zones respectively as depicted in Figures 5-45a-d. This result show that the values of continuum removed reflectance could be associated with the health status of plant, the lower the value, the healthier the vegetation status, while as stress increases the values get higher.

Figures 5-47a-f and 5-48a-f shows the pattern of band depths of the different levels of herbicide treatments in the visible region. In the blue region (400-550nm) the wavelengths that showed significant difference (ANOVA (*p-value* 0.008; 0.006 ($\sigma \leq 0.05$); n=150) compared to control were 440 and 514nm for the 10 and 20% herbicide treatment levels, 406 and 524nm (ANOVA (*p-value* 0.025; 0.036 ($\sigma \leq 0.05$); n=150) for the 40% level while none was significant (ANOVA (*p-value* 0.073 ($\sigma \leq 0.05$); n=150) for the 5%. This was more pronounced in the third week of measurement. At the red region (550-750nm) wavelengths 572 and 710nm were statistically significant (ANOVA (*p-value* 0.044; 0.000 ($\sigma \leq 0.05$); n=200) for 5 and 10% treatment levels, while 573 and 653nm (ANOVA (*p-value* 0.046; 0.001 ($\sigma \leq 0.05$); n=150) were for 20% treatment. In the first, second and fourth week the average difference between 40% treatment level with control shown difference at 550-750nm wavelength region.

However, at the fifth week, control and 5% herbicide level were not distinguishable as shown in Figure 5-48e, but at sixth week control, 5, 10 and 20% levels were significantly different (ANOVA (*p-value* 0.033; 0.000 ($\sigma \leq 0.05$); n=200) at wavelengths 599 and 697nm (Figure 5-48f).

It was also observed that the band depths of barley plants growing on the control plots were deeper on virtually all dates than all the treated plots at 550-750nm region, followed by 5, 10, 20 and 40% treatments level respectively. At 400-550nm region the absorption pit for control and 5% herbicide concentration were deeper, followed by 10, 20 and 40%. The 40% treatment had the least depth in absorption pit indicating that stress manifested earlier than other levels of treatment. The above results show that the depth of the absorption pit was a function of severity of the stress or level of concentration of the treatment.

5.4 Comparison of stress responses in maize (C4) and barley (C3) using hyperspectral remote sensing

In the present study two plant species (maize - C4 and barley - C3) were selected for investigation due to differences based on their physiological response to atmospheric CO₂ (section 2.3.1 for further details). The effects of stresses on these species could vary, and it could be expected that there might be differences between C3 and C4 plants in their response to soil CO₂. For instance, Boru *et al.*, 2003 found severe effects on shoot and growth of soybean (C3), with change in leaf colour occurring 2 days after treatment with 50% soil CO₂; this also resulted in 25% plant mortality. However, rice (C4) plant growth was not affected but a difference in root length was apparent. The work by Al-Traboulsi *et al.*, 2012a observed that in spring field bean (*Vicia faba L.* - C3) treated with soil CO₂ above 10%, the mean values for vegetative (shoot, stem and leaf dry weight per plant, leaf area per plant) and reproductive variables (pod and seed number per plant and seed dry weight per plant) were reduced by 36–65% compared to control plants.

C3 photosynthesis is limited by present day atmospheric CO₂ concentration while C4 photosynthesis is nearly CO₂ saturated (Flexas and Medrano, 2002). The presence of aerenchyma in maize (C4) which provides a pathway for gas transport from the stems to the root (Carmon-Silva *et al.*, 2007) is also a major difference from the barley (C3).

Several mechanisms are involved in the response of plants to soil CO₂, such as decrease in nutrient and water uptake (Matocha and Mostaghimi, 1988), and decrease in the cytoplasmic pH of root cells (Bunnell *et al.*, 2002). However, Ehlinger and Monson (1993) pointed out a number of methodological issues and concluded that constant soil CO₂ addition was required to show consistent effects.

The comparison of the time scale of stress responses of these plants could be limited by the fact that the experiments were conducted in two different years, which may result in some variations due to differences in climatic conditions. However, analysis of the climatic conditions of the two seasons shows that they were quite similar. Accumulated temperatures were slightly higher in 2009, although most of the difference was in January/February before the crops were sown. 2009 was also about 10% wetter. Solar radiation was virtually identical (refer to section 3.9 and appendix 7 for further details). Therefore, it should still be possible to compare the general trend of when these stresses were first detectable in the plants.

5.4.1 Visible stress symptoms

The visible stress symptoms such as chlorosis (change in leaf colour) and growth retardation in maize treated with elevated concentration of soil CO₂ was first noticed 16 days after the onset of soil CO₂ injection as compared to barley which was 10 days (for more details refer to sections 4.2.1 and

5.3.1), suggesting that barley was more sensitive to soil CO₂ treatment. This can be explained by the presence of aerenchyma in maize which is a gaseous transport route from the stem to the root that supplies oxygen when in need (El-Beltagy and Hall 1974, Walter *et al.*, 2004). This is in contrast to the herbicide treatment where stress symptoms were observed visually 20 and 25 days since the onset of herbicide application in barley and maize respectively.

Table 5-1: Summary of vegetation indices used in the study showing the first detectable day of stress in maize and barley. In the maize experiment there was only one level of herbicide treatment (10%), the dash (-) in the table signifies that stress was never detectable.

Indices	Treatment type	First detectable day after onset of CO ₂ and herbicide treatment	
		Maize	Barley
Chl NDI	Low CO ₂	-	-
	High CO ₂	14	3
	Herbicide (5%)		-
	Herbicide (10%)	33	3
	Herbicide (20%)		3
	Herbicide (40%)		3
PSSRa	Low CO ₂	2	21
	High CO ₂	2	3
	Herbicide (5%)		3
	Herbicide (10%)	33	3
	Herbicide (20%)		3
	Herbicide (40%)		3
PSSRb	Low CO ₂	33	21
	High CO ₂	2	3
	Herbicide (5%)		3
	Herbicide (10%)	-	3
	Herbicide (20%)		3
	Herbicide (40%)		3
PRI	Low CO ₂	39	21
	High CO ₂	2	3
	Herbicide (5%)		-
	Herbicide (10%)	45	21
	Herbicide (20%)		21
	Herbicide (40%)		21

The sensitivity of selected vegetation indices for the early detection of elevated soil CO₂ concentrations and sub-lethal herbicide treatments in maize and barley was explored; the aim was to find out if they could be used for early (pre-visual) detection of stresses. Some of these indices could detect stress as early as 2-3 days after treatment, well before any visible symptoms. Chl NDI was sensitive to low CO₂ stress in barley and 10 - 40% herbicide treatments 3 days after onset of application while PSSRa and PSSRb could detect low and high CO₂ in maize 2 days after treatment, while stress in barley treated with herbicide was detectable 3 days after. PRI was sensitive to high CO₂ in both maize and barley 2 and 3 days after respectively.

In terms of the sensitivity of the indices used in this study for early stress detection, *PSSRa* and *PSSRb* were consistently the most sensitive to stress (within 2-3 days) after soil CO₂ injection and herbicide treatments. *PSSRa* was able to detect both low and high soil CO₂ concentrations in maize 2 days after injection (table 5-1) as well as high CO₂ and all the levels of herbicide treatments in barley 3 days after application as shown in table 5-1. This suggests that the response of barley could be immediate regardless of the stress intensities as it relates to this study, although the degree of the response could vary. *PSSRa* could detect low CO₂ in maize earlier (2 days after injection) compared to barley (33 days). It may be that maize showed mild stress symptoms early on, but does not suffer such severe effects as in barley as the experiment progressed because of the aerenchyma that provides a protective mechanism that limits the level of damage. *PSSRb* was sensitive to high soil CO₂ in maize 2 days after injection (table 5-1) and 3 days after injection for barley as well as all the different levels of herbicide concentrations (table 5-1), suggesting that

these indices could be used for early detection of both stresses in maize and barley.

The implication of the findings of this investigation is that barley was more susceptible to both stresses as shown by the early visual symptoms and indices, however to confirm this claim, there is need for further studies involving the two plants in the same year in order to overcome the uncertainties that may arise as result of variations in climatic conditions.

5.5 Conclusion.

Based on the results of this study the following conclusions can be drawn:

The red edge first derivative peaks of CO₂ and herbicide treated barley with their respective controls differ and therefore can be used to discriminate between the treatment types and levels. The shift of the red edge position was to shorter wavelength for high CO₂ treatment while the control and low CO₂ shifted to longer wavelengths. In the herbicide treatment only the 20% treatment shifted to shorter wavelength, the control and 5% treatment shifted to longer wavelength. Early shift of the REP to shorter wavelengths after stress indicates its potential for early stress detection. Leaf developmental stage is likely to be a suitable argument in case of the shift of the REP in control to longer wavelength given variation in plant age and increase in chlorophyll during the period of spectral measurements.

Continuum removal of the blue and red region of the visible portion of the spectrum has potential for stress discrimination. The band depth analysis has demonstrated that with increase in concentration of CO₂ the absorption pit becomes wider and deeper. The wavelengths at which CO₂ caused a statistically significant difference compared to control using ANOVA were 405, 515, 575, and 699nm. The continuum removed reflectance of the

control was always deeper and wider than the low and high CO₂ treatments.

In the herbicide study, the wavelengths that showed significant difference in the blue region were 440 and 514nm for 10 and 20% treatments, 406 and 524nm for 40% while there were none for 5%. In the red region the wavelengths were 572 and 710nm for 5 and 10% treatments, 573 and 653 nm for 20% treatment. The depth of the absorption trough was dependent on the level of concentration, with control being deeper followed by the order of concentration.

The vegetation indices tested in this study have potential for stress detection and discrimination. *Chl NDI* was sensitive to high CO₂ treatment, 10, 20 and 40% herbicide treatment, *PSSRa* to high CO₂ at early stage of the treatment while *PSSRb* was sensitive to high CO₂ and all the herbicide treatment levels.

The results of this study have shown that hyperspectral remote sensing using canopy reflectance in the field taking into consideration the environmental conditions of the location at the time of the study can provide useful information on stress acting on the terrestrial ecosystem.

CHAPTER SIX

SUMMARY, DISCUSSION AND CONCLUSION

6.1 Introduction.

This chapter summarises the main research findings, provides a discussion of the contribution in the context of the existing literature, and outlines the limitations of the study as well as possible directions for future research.

The consensus among many researchers is that remote sensing is a useful tool for vegetation stress detection, but that it may not be possible to distinguish between different stressors using spectral reflectance alone (Carter, 1993, Masoni *et al.*, 1996, Smith *et al.*, 2005a).

The majority of previous studies on the effects of elevated soil CO₂ concentration on plants growth and reflectance have been at the leaf reflectance level in the laboratory (Curran, 1995, Datt, 1998, Carter, 2001, Kokaly, 2001, Smith *et al.*, 2004a, 2005b, Blackburn, 2007, Moorthy *et al.*, 2008, Noomen *et al.*, 2009). However, few investigations have been conducted at the canopy level in the field. It is well known that different relationships may be observed in leaf and canopy scale studies (Yuhong and Amy, 2010).

The research presented in this thesis investigated the effects of stress on hyperspectral features of the reflectance spectrum. It also tested the null hypothesis that vegetation stress effects cannot be discriminated from one another, with specific reference to CO₂ and herbicide (Glyphosate) stress which are two stresses that both affect the plant roots.

The main aim of the research was to find out whether hyperspectral remote sensing can detect and discriminate stress, using the examples of sub-surface CO₂ and herbicide stress. The research consists of four main objectives (Refer to section 1.7 for details).

To fully understand the effects of CO₂ and herbicide stress on vegetation reflectance, two major experiments were conducted on maize and barley in 2009 and 2010 respectively. These experiments investigated the effects of high and low CO₂ concentration on vegetation growth and canopy reflectance as well as of different levels of herbicide treatment using hyperspectral remote sensing technique.

6.2 Summary and discussion.

This section focuses on the summary of the research findings of the two experiments and discusses them in the context of earlier studies.

6.2.1 Spectral and physiological responses of maize (*Zea mays*) to elevated soil CO₂ and herbicide stress.

This study investigated the spectral and physiological responses of maize subjected to elevated soil CO₂ and herbicide at canopy level in the field. The research sought to evaluate the potential of remote sensing to detect and discriminate between two stresses that both impact on plant roots thereby affecting their growth and development.

In this study, differences in the shape of the first derivative spectra were observed. In the canopy spectra it was observed that maize growing on elevated CO₂ concentrations had a double peak at 718 and 730nm in the first derivative that identifies the red edge. The CO₂ control plots had single peak at 726nm with smaller peaks or shoulders at 718 and 759nm (Refer to Figure 4-21), while the gassed plots had double peaks at 718 and 730nm, with several secondary peaks or shoulders found between 707 and 794nm (Refer to Figures 4-22 and 4-23).

The maize treated with herbicide had a single peak at 723nm with shoulders at 716 and 759nm (Refer to Figure 4-24). As the experiment progressed and the herbicide stress began to manifest, the derivatives

doubled at 716 and 723nm, the shoulder was still at 759nm (Refer to Figure 4-25). The major peak at 730nm was prominent in the gassed plots which did not occur in the herbicide experiment.

Lakarraju *et al.* (2010) found a double peak in plants subjected to elevated CO₂ concentration, the first peak observed was between 720 and 723nm while the second peak was positioned between 730 and 733nm. Horler *et al.* (1983) also identified two peaks in the derivative spectra, the first at around 700nm was attributed to the chlorophyll content in the plant leaves and the second at around 725nm was linked to leaf scattering.

A previous study by Pysek and Pysek (1989) observed discolouring of leaves, a change in the shape of reflectance curves and a decrease in the near-infrared reflectance in plants growing near an artificial gas leak. In this study maize growing on plots with elevated CO₂ concentration and herbicide treatment showed an increase in reflectance in the visible region with greater increase found in the plants at the centre of the plots where the gas concentration was higher.

In the present study, patches of decreased growth were noticed in the maize growing on the middle of the field plots where the elevated CO₂ concentration was high thereby resulting in 60% decrease in growth compared to the controls, and about 10% at the plot edge (Refer to table 4-1). This shows that growth was inversely related to soil CO₂ concentration, as the effect of CO₂ in the plot centre was greater than at the edges and the control.

The numbers of leaves and tillers were significantly lower at the plot centre compared to the edges, while the numbers of immature maize cobs were likewise higher at the plot centre (Refer to table 4-1 for more information). For the maize growing on herbicide plots, there was no significant difference in the plant height, number of tillers, and the number of leaves

while the number of primary cobs in the control was 50% higher than the treated plots. The number of immature cobs in the herbicide treated maize plots was also 50% higher than the control plots (Refer to table 4-2). The number of maize cobs and tillers on gassed plots were significantly lower than the control plots due to the effects of CO₂ (Refer to Figure 4-6 and 4-7); this was not the same in the herbicide plots that showed no significant difference. Both CO₂ and herbicide treatments showed significant difference in the fresh and dry weights of maize leaves and stems (Refer to Figures 4-9 and 4-10).

These results can be compared to the work of Boru *et al.* (2003) who showed that the effect of elevated soil CO₂ on soybean (*Glycine max*) was a reduction in shoot growth and leaf greenness. Huang *et al.* (1997) found that 10% CO₂ in combination with 5% O₂ in the soil led to decrease in shoot growth of wheat (*Triticum aestivium*). In the present study both the below and above the ground biomass were measured for both treatment types and level as a basis for comparison with the control experiments.

Chlorophyll content in both CO₂ and herbicide treated plots showed a decrease in the range of 40-50% compared to control plots; the reduction was more in the centre of the plots where soil CO₂ concentration was higher (Figure 4-13 shows the details)).

The continuum removal analysis also showed that both treatments could be detected and distinguished using the band-depths; the band depths at 473 and 488nm for high CO₂ zone showed significant difference relative to control and 500 and 509nm for low CO₂ (Refer to Figures 4-38a to 4-38e) . The absorption pits were deeper in both controls, as the stress treatments increased the absorption pit became shallower. For herbicide treated maize only the wavelengths at 406, 515, (Refer to Figures 4-40a and 4-40b) 589

and 717nm showed significant difference compared to control (Refer to Figure 4-41b).

Vegetation indices used in this study were promising indicators of stress. Although they were dependent on the stress type and stress severity, they could be applied in stress detection and discrimination over time. The results show that *PSSRa* was sensitive to both low and high CO₂ at any point during the experiment (Figure 4-31), while *PSSRb* was responsive to high CO₂ earlier than low CO₂ (Figure 4-33). Both *PSSRa* and *PSSRb* were only sensitive at much later dates in the herbicide experiment (Figures 4-32 and 4-34). From the *PRI* results, the index was not sensitive at an early stage in either experiment, although it was responsive much earlier in the CO₂ experiment when compared with the herbicide experiment (Figures 4-35 and 4-36). *Chl NDI* was sensitive to high CO₂ in the second week followed by herbicide treatment (For details refer to Figures 4-29 and 4-30).

6.2.2 Remote sensing of barley (*Hordeum vulgare* v *Concerto*) stressed with CO₂ and herbicide.

Further investigation was conducted on barley in 2010. This research showed that the canopy reflectance of barley crops exposed to elevated concentrations of CO₂ or herbicide increased in the visible region and decreased reflectance in the infrared. This result can be compared with observations of Lakarraju *et al.* (2010) who studied the effects of elevated soil CO₂ on three different plant species: Dandelion (*Taraxacum officinale*), Orchard grass (*Dactylis glomerata*), and Kentucky blue grass (*Poa pratensis*) and found that the station with elevated CO₂ showed an increase reflectance in visible region and a decrease in near-infrared reflectance when compared with the control where there was no treatment. This is further confirmed by the work of Pysek and Pysek (1989) who also found

an increase in reflectance at red wavelengths and a decrease in near-infrared region in a study of the effects of natural gas leakage on vegetation. Smith *et al.* (2004a) found that soil oxygen displacement by waterlogging caused a significant increase in reflectance in the visible region between 508 and 654nm and in the red-edge region (REP) between 692 - 742nm with little change in the NIR in bean.

However, the present study extended the scope of the investigation to the SWIR region, unlike the above mentioned ones that concentrated on only the visible region to draw their conclusions. It was found that the SWIR region of the crops grown on herbicide treated plots showed significant differences compared to the control; (Refer to Figures 5-28c-f) this was in contrast to the CO₂ treated plots, which showed no significant difference in the SWIR region when compared with the control (Refer to Figures 5-27a d).

In this study, both elevated concentration of CO₂ and herbicide treatments showed changes in the first derivative of reflectance with movements in the position of the red-edge. In barley it was generally found that the position of the red-edge shifted to shorter wavelengths for stressed plants. Noomen *et al.* (2009) also found that the reflectance of stressed plants often shows a shift of the 'red edge' position towards shorter wavelengths. Smith *et al.* (2004a) observed that the REP of waterlogged bean shifted towards shorter wavelengths compared to the controls. Horler (1983) in a study of the phenological crop development of winter wheat and spring barley showed an initial shift of the red-edge position towards longer wavelengths as chlorophyll concentration increased with crop maturity followed by a shift to shorter wavelengths as senescence began. A similar result was reported by Miller (1991) in a study of four tree varieties, the reflectance

from the leaves showed a shift in the REP to longer wavelengths as the leaves matured followed by a shift to shorter wavelengths in senescence.

Changes were observed in the magnitude of the first derivative and other wavelengths. Barley treated with elevated soil CO₂ had maximum peaks between 716 and 730nm and several smaller peaks or shoulders between 699 and 759nm (Refer to Figures 5-30 and 5-31). These features were used to detect differences between control and CO₂ stressed barley. The magnitude of these peaks decreased with stress, with the dominant change occurring between 716 and 730nm.

According to Boochs *et al.* (1990) the derivative peaks of winter wheat ranged between 725 and 740nm with a shoulder at 703nm through the growing season. Railyan and Korobov (1993) found peaks at 705 and 720nm for *triticales* in the vegetative stage. 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.

In a study conducted by Smith *et al.*, (2004a) they found that soil oxygen displacement was found to be related to an inconsistent change in the magnitude of the first derivative at the position of the red edge in bean and barley, which either increased or decreased relative to the control. 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.

In this study the first derivative peak for the early herbicide treatment period was composed of peaks at 697, 715 and 717nm with a small shoulder or peak at 759nm (Refer to Figure 5-32). As the experiment progressed and the herbicide stress began to manifest, there was a change in the derivative peaks; the maximum peak became single at 730nm with

the smaller peak at 717nm, and shoulders at 707 and 759nm but the magnitude had decreased (Refer to Figure 5-33).

By the late treatment period barley had turned yellow in all the treatments, the peaks had decreased further in magnitude; the major peaks were between 696 and 712nm respectively with a further decrease in the magnitude of the shoulder which remained at 759nm (Refer to Figure 5-34). The magnitudes of the peaks decreased with both CO₂ and herbicide stress.

Continuum removal analysis showed that the absorption pits for the controls in both treatments were deeper followed in order by the different levels of concentration of the treatment types. For elevated CO₂, the control was deeper followed by the low then high CO₂ concentration zone (Refer to Figures 5-45a-d and 5-46a-e). In the herbicide treatment, the depth was also dependent on the treatment level with control being deeper, then the 5, 10, 20 and 40% treatment levels respectively (Refer to Figure 5-47f).

The band depth was used to determine the best wavelengths to discriminate between the treatment types and levels of concentration. In the visible region (blue), the wavelengths between 405-507nm were promising wavelengths to distinguish between low and high CO₂ concentration, the wavelengths 575 and 699nm were the most appropriate in the red region.

However, in the herbicide treatment there were no wavelengths in the blue region suitable for discriminating 5% herbicide level and control. At 10 and 20% herbicide levels the best wavelength was found to be 440 and 514nm, and 406 and 524nm for 40% herbicide concentration. The wavelengths at 572 and 710nm were able to distinguish 5 and 10% treatments, and 573

and 653nm for 20% treatment, while none was appropriate for 40% treatment level.

This result can be compared with the work of Noomen *et al.* (2006). In a study carried out using continuum removed band analysis for detecting the effects of natural gas, methane and ethane on maize reflectance, they found that the wavelengths in the red region between 560-700nm were the best for discriminating ethane from the other gases. In addition they also found some water absorption wavelengths features at 1420-1448nm were the best for discriminating all the gases, 1456- 1480nm for natural gas and ethane, while 1909- 2052nm was for only ethane.

The difference between the current study and the one mentioned above is that the latter used natural gas, methane and ethane on one crop (maize-C4) while this study used two different stress factors (CO₂ and herbicide) with same mode of action on two crops (Barley and maize) which are C3 and C4 respectively.

Chlorophyll content in plants is considered a major indicator of stress severity and photosynthetic activity, (Carter, 1993 and Xingang *et al.*, 2011). Kochubey and Kazantsev (2007) found that leaf chlorophyll content plays a major role in assessing plant health status. The chlorophyll content of barley treated with CO₂ decreased in the range of 40-50% compared to the control (Refer to Figure 5-17), while for the herbicide treatment the decrease was between 70-90%, as a function of the level of concentration of the treatment (Refer to Figure 5-18).

The results of this study indicate that there was a decrease in chlorophyll content associated with high CO₂ concentration compared to control and low CO₂ concentration, as well as the different levels of herbicide treatment.

In this study four vegetation indices were used to determine their suitability for stress detection and discrimination. *Chl NDI* was sensitive to high CO₂ as well as to the different levels of herbicide treated barley. *PSSRa* and *PSSRb* and *PRI* indices could only detect high CO₂ and the whole herbicide treatment levels early compared to control. Strachan *et al.* (2002) observed that an increasing *PRI* correlates with decreasing photosynthetic efficiency which is associated with stressed vegetation as shown by this study.

Lakaraju *et al.* (2010) found that the Chlorophyll Normalized Difference Index (*Chl NDI*) decreased, suggesting a decrease in chlorophyll content with time. Pigment Specific Simple Ratios (both *PSSRa* and *PSSRb*) also decreased for stressed vegetation compared to that at the control site, indicating a reduction in both chlorophyll a and chlorophyll b.

The biomass analysis carried out on barley after harvest showed that there was significant difference in the fresh and dry weight of barley ears, stems, (Refer to Figures 5-8 and 5-9) total number of barley tillers (refer to Figure 5-6) as well as total number of barley grains (Refer to Figure 5-7) and mean number of plants in high CO₂ concentration zone (Refer to Figure 5-4) in both plots treated with elevated CO₂ concentration as well as herbicide, except for small variations in the 5% herbicide treatment and occasionally in the 10 and 20%.

6.3 Discussion.

The storage of CO₂ underground has the potential to leak. Such leaks could occur at any stage of the storage process and might range from abrupt leakage following sudden failure of the geological storage cap rock to more gradual leakage through fractures and geological faults (IPCC 2005, Steven *et al.*, 2010, Al-Traboulsiet *al.*, 2012b). Minor seeps of gas may diffuse through the storage media up to the surface causing an increase in soil CO₂

concentration which, depending on the size of the leak could affect the vegetation growing in that soil (Klusman, 2003).

In the context of leakage from CCS systems, there are three scenarios, leakage from above-ground pipelines, buried pipelines or deep stores. The present study is not relevant to leakage from above-ground pipelines as such leaks would not affect soil CO₂, although Mazzoldi *et al.* (2008) found that such leaks could form patches of frozen CO₂, creating a secondary hazard as they sublime.

A well aerated soil should have a CO₂ concentration close to that of the atmosphere. The CO₂ may be between 0.15 and 2.5% in the surface layers of the soil (Stolwijk and Thinman 1957, Russel, 1973) but occasionally figures of between 10 and 12% have been recorded (Stolwijk and Thinman 1957, Russel 1973). The CO₂ concentration increases with depth and moisture content of the soil and is higher in cropped soils than in fallow land (Russel, 1973). The concentration of CO₂ in soil rapidly increases after rain because its diffusion through soil is restricted by water saturation (Yoshioka *et al.*, 1998).

The potential impact of leakage on the flora and fauna in the biosphere above a CO₂ reservoir needs to be taken into consideration before selecting CCS storage sites. One measure of doing this is the geological characterisation of the storage site and surrounding areas, simulation of CO₂ injection into the site, and studies of the long-term fate of the stored CO₂. These studies should be undertaken before commencing injection (Holloway, 2005), with a view to assuring the public of its safety; this will enable the populace to gain confidence in its application in combating/minimising climate change related problems.

Vertical migration of leaking CO₂ will lead to dissolution into shallower ground waters and production of carbonic acid which would reduce pH

(Klusman, 2003, Al-Traboulsi *et al.*, 2012a). A reduction in pH may lead to mobilization of toxic metals, leaching of biological nutrients and modification of proton gradients across biological membranes (Bruant *et al.*, 2002). Moreover, other possibilities are that large amounts of CO₂ could change the pH and redox potential of soil or alter natural microbial environments (Noomen *et al.*, 2008). When stressed over long periods, vegetation can be stunted in growth, have reduced water content, or decreased leaf chlorophyll concentrations (Smith *et al.* 2004a, 2005b).

However, there is little information with regards to the potential impact of CO₂ leakage from CCS facilities on the gaseous soil environment (Al-Traboulsi *et al.*, 2012a). Studies have shown that prolonged below-ground release of CO₂ caused plant mortality in autumn and spring sown field bean crops and increasingly reduced the vegetative and reproductive growth of surviving plants (Al-Traboulsi *et al.*, 2012a, b), it also reduces above and below ground growth in turf composed of mixed grass species (Pierce and Sjögersten, 2009). The most likely cause of stress resulting from leakage from CO₂ storage is that CO₂ gas displaces oxygen to the roots of the plant, which occurs in natural gas leaks.

Whilst elevated CO₂ in the atmosphere can stimulate plant growth, elevated soil CO₂ will usually be detrimental to plant (IPCC, 2006). Plants have different sensitivity to lack of soil CO₂. Plants with *aerenchyma*, such as rice, have a gas transport pathway from the stems to the roots, which allows them to withstand flooding and the same mechanism can mitigate the effects of high CO₂ in soil by supplying the roots with oxygen (Kozlowski, 1984, Crawford, 1992). Hypoxia caused by depletion of O₂ is common in flooded or waterlogged soils and landfill sites and presents an unfavourable environment for most plant species (Parent *et al.*, 2008) which may adversely affect their growth and productivity (Pociecha *et al.*,

2008). Several studies have shown that a lack of sufficient O₂ to support respiration causes damage and root death in plants exposed to hypoxia (Henshaw *et al.*, 2007, Shi *et al.*, 2007, Horchani *et al.*, 2009). The effect of flooding has been examined in *Vicia faba major L.* (Balakhnina and Bennicelli, 2010) and numerous other species including *Fraxinus pennsylvanica* (Sena-Gomes and Kozlowski, 1980). Low soil O₂ associated with hypoxic conditions induced by flooding may also reduce root permeability (Clarkson *et al.*, 2000) and leaf area, contributing to the inhibition of photosynthesis and assimilate production during the later stages of growth (Sena-Gomes and Kozlowski, 1980).

Compared to laboratory studies fewer investigations have been conducted in field conditions, but studies of leakage of natural gas (which is mainly methane) indicate that oxygen deprivation in the soil causes severe stress symptoms in plants (Smith *et al.*, 2005a). Emissions from natural sources, such as volcanic springs, or from landfill, are the closest analogues to leakage from a carbon dioxide storage site (Chan *et al.*, 1991, Zhang *et al.*, 1995, Sorey *et al.*, 2000) and show similar patterns of stress effects, but their interpretation is complicated by the presence of other toxic gases and the possibility that the local vegetation may have adapted over time (Vodnik *et al.*, 2002, 2006).

There is also the possibility of direct effects of soil CO₂ on plants. In the laboratory studies of plant responses to soil CO₂ reviewed by Steven *et al.* (2010), there is evidence that soil CO₂ above 10% may cause damage to root systems independently of the effects of oxygen deprivation and that different species may be more or less sensitive. Uptake of water and nutrients may also be affected by soil CO₂ (Zhang *et al.*, 1995). Soil fauna may also be severely affected by CO₂, with Sustr and Simek (1996) finding behavioural responses at levels ranging, according to species, from 2 -

39% CO₂, paralysis from 10 - 59% CO₂ and death in some species at levels as low as 11%.

6.3.1 Detection of CO₂ leaks by remote sensing.

Leak detection is critical for the viability of CCS schemes in terms of accounting, safety and public acceptance (Pollak *et al.*, 2011, De Best-Waldhober *et al.*, 2011). The responses of terrestrial vegetation could be used as a proxy to identify leaks from underground CCS systems (Steven *et al.*, 2010). Remote sensing systems cover large areas and would, in principle, circumvent the spatial sampling issues associated with ground-based monitoring.

Hyperspectral remote sensing is a useful technique for monitoring spectral change in vegetation. Plant stress can occur when the environmental conditions are not favourable for suitable plant growth which could be caused by many factors such as drought, extreme heat or cold, insect infestation, water logging, bacterial diseases, oxygen depletion, nutrient deficiencies, or acidic soil (Lichtenthaler, 1996, 1998, Male *et al.*, 2010).

Remote sensing techniques focusing on the stress responses of terrestrial vegetation have been shown to be effective in detecting leaks from natural gas pipelines (Smith, 2002), and there is evidence that this approach may also be feasible for CO₂ (Steven *et al.*, 2010). Plants subjected to soil gassing exhibit classic symptoms of stress such as chlorosis, either (as in the case of natural gas leakage) due to deprivation of oxygen at the roots, or due to direct physiological effects of CO₂. Hyperspectral remote sensing techniques can identify the signs of such stress at sub-visual levels (Smith *et al.*, 2004b, Noomen *et al.*, 2008).

One significant potential application of hyperspectral imaging for monitoring CO₂ leakage is the detection of changes in plant health and communities related to elevated soil CO₂ concentration (Martini *et al.*,

2002). Changes in spectral reflectance and the use of spectra vegetation indices as well as some specific wavelength regions such as red edge position have also been deployed for the detection of stress in several plant species (Sims and Gamon, 2002, Smith *et al.*, 2005b, Noomen, 2007). Decrease in chlorophyll production capacity and other related biochemical components have also been associated with stress in plants (Morthy *et al.*, 2008).

Spectral reflectance changes caused by stress in plants can vary with date and time of data acquisition. The implication of this in remote sensing is that there is need for specific calibration of responses in relation to sampling period because of changes in physiological status of vegetation (Mutanga *et al.*, 2003). Invariably, this can play a very important role when acquiring remote sensing data in the field as any change can affect the validity and/or authenticity of the information desired.

6.3.2 Synthesis of experimental findings.

In chapter 4 the effects of elevated soil CO₂ and herbicide stress on maize was studied, while chapter 5 dealt with the same stressors but with a more sophisticated herbicide trial that had a range of concentration levels and using barley as the model species. This section synthesises the results of the two experiments with regards to their implications for the remote detection and discrimination of these stressors.

The effects of these stresses are species dependent, and it was expected that there might be differences between C3 and C4 plants in their response to soil CO₂ as their physiological response to atmospheric CO₂ are also very different. The presence of *aerenchyma* in maize (C4) which provides a pathway for gas transport from the stems to the root (Carmon-Silva *et al.*, 2007) is also a major difference from the barley (C3) used in this study. C3 photosynthesis is limited by present day atmospheric CO₂ concentration

while C4 photosynthesis is nearly CO₂ saturated (Flexas and Medrano, 2002).

In this study maize was more tolerant to soil CO₂ than barley as depicted by the slower response to stress. Signs of visible stress symptoms on maize were noticed sixteen days after soil CO₂ injection and twenty days after herbicide treatment (Refer to section 4.2.1 for details). Patches of decreased growth in the middle of the field plots where the elevated soil CO₂ concentration was high resulted in 60% decrease in growth compared to the controls and about 10% at the plot edge were noticed (Refer to section 4.2.2 for details). In contrast the visible signs were noticed on barley ten days after gassing and twenty five days after herbicide application (Refer to section 5.3.1 for details).

The greater sensitivity of barley is likely to originate from the absence of appropriate physiological and anatomical adaptations to survive hypoxic conditions (El-Beltagy and Hall 1974, Walter *et al.*, 2004). By contrast, production of adventitious roots and formation of *aerenchyma* are common responses of maize to hypoxia (Jackson *et al.*, 1985, Atwell *et al.*, 1988, He *et al.*, 1994, Gunawardena *et al.*, 2001, Mano *et al.*, 2006). Roots experiencing the highest soil CO₂ and roots produced during the injection period enabled aerobic metabolism to continue (Colmer and Greenway 2011, Postma and Lynch, 2011). This may provide an explanation for the much greater resistance of *Zea mays* to simulated leakage from CCS systems compared to less tolerant species such as *Vicia faba* (Al-Traboulsi *et al.*, 2012b). This contrast suggests that such leakage may induce variable, potentially severe but spatially contained damage to terrestrial vegetation depending on the species involved, providing a potentially important tool for assessing the integrity of CCS systems.

The severity of the adverse effects of elevated CO₂ on shoot growth and yield of maize at harvest was less than observed in barley. The number of maize leaves per plant in the plot centre was 50% compared to the control and plot edge. While the number of tillers per- plant and maize primary cobs were about 60% and 75% of the control plots respectively. There was an increase of 50% in the number of immature cobs in the plot centre (Table 4-1). The numbers of tillers plant- per, barley grains, fresh and dry weight of barley ears and stems in the plot centre where there was high soil CO₂ concentration was 30, 35, 25 and 30% of control (See Figures 5-6, 5-7, 5-8 and 5-9 respectively). Al-Traboulsi *et al.* (2012b) also observed that field bean (*Vicia faba*) was more susceptible compared to maize, using the same exposure facility and CO₂ injection rate. Pierce and Sjögersten (2009) reported that exposure of turf containing a mixture of *Lolium perenne*, *Festuca rubra* and *Phleum pratense* to elevated soil CO₂ using the ASGARD facility reduced above and below-ground biomass by 21 and 12%, respectively after 10 weeks of exposure.

By contrast, *Vicia faba* showed much greater plant mortality (75%) than maize near the centre of the gassed plots and greater decreases in above-ground vegetative (49%) and reproductive growth in terms of pod and seed number plant⁻¹, which were reduced by 42 and 46% respectively (Al-Traboulsi *et al.*, 2012b). This contrast suggests that susceptibility to elevated soil CO₂ and severely depleted O₂ may vary greatly between species. Legumes such as *Vicia faba* may be more susceptible because they have nitrogen fixing nodules, which are known to have high demand for oxygen (Pociecha *et al.*, 2008).

It is therefore suggested that hypoxic conditions induced by CO₂ injection into the soil in the present study rapidly induced chlorosis, reduced plant

growth and induced premature senescence, ultimately causing plant death in the areas of lowest soil O₂.

The result from this study has furthered the understanding of stress detection for stresses caused by a variety of factors. Plant stress arising from elevated soil CO₂ is also detectable using remote sensing. In particular, analysis of hyperspectral data using continuum removal (Refer to sections 4.2.9 and 5.3.9) showed that both treatments elevated soil CO₂ and herbicide treatments could be detected and discriminated using the band-depths; the absorption pits were deeper in both controls compared to the stressed plants, which were dependent on the stress and crop type. This analysis was able to identify some specific wavelength ranges suitable for the discrimination of the different types of stresses applied in the present study. Continuum removal analysis emphasises absorption troughs and is not affected by variations in albedo (Schmidt and Skidmore, 2001). This could be used as basis for the developments of algorithms that will aid in the analysis of the shape, depth and slope of major absorption features in the visible region of the spectrum.

The vegetation indices used in this study were promising indicators of early stress detection. Some indices performed better than others in terms of how early these stresses can be detected depending on the stress type, species and duration of stress. *ChINDI* was sensitive to high soil CO₂ concentration in maize and barley, sub-lethal herbicide treatment at 10% - 40% level in barley and was insensitive to both low CO₂ in the barley and maize as well as 10% herbicide treatment in maize. *PSSRa* was a good indicator of early CO₂ stress in maize and high CO₂ in barley as well as 10-40% herbicide treatments. *PSSRb* could detect high CO₂ level in maize and barley and all levels (5-40%) of herbicide treatments. *PRI* was insensitive

to 5% herbicide treatment in barley but sensitive to high CO₂ in maize at early stage of the experiment.

6.4 Limitations of the study.

While this study has shown evidence about the possibility to detect and discriminate between plant stresses caused by elevated concentration of soil CO₂ and herbicide under controlled environment, there may be problems associated with their real world application. The study was based on high and low CO₂ treatment zones. The region of high soil CO₂ concentration ranged between 4.0% - 28.0%, while that of low was 1.5% - 13.0% which may not be considered truly representative of conditions that plant would encounter during leaks.

Thus, it may be difficult to translate the general responses of plants to CO₂ stress, as stress conditions may occur at varying intensity and duration in field situations. However, the ASGARD set up does generate similar environmental variability. It is much more realistic in this regard compared to a laboratory based experiment. Additionally, other stress factors such as nutrient deficiency and soil water deficit may also be affecting plants growing in the field at the same time.

In this study one of the most important factors was the varying weather conditions, during the experiment between July and September of 2009 and 2010 respectively there were some periods of dry weather. Any changes between seasons could be responsible for some of the differences in the experiments. However, the two seasons were actually quite similar (refer to appendix 7 for details). Accumulated temperatures were slightly higher in 2009, although most of the differences was in January/February before the crops were sown, 2009 was also about 10% wetter. Solar radiation was virtually identical. The main period of interest is about day

100 (early April) to day 200 (early July). This is one of the characteristics of a realistic environment that the experimenter does not control.

Furthermore, this study used just two crop species and thus, their responses to stress may not have adequately represented the type of stress responses that other plants may show under field conditions. Measurements on a wider range of species would not have been practical within the scope of this study.

The field experiments were conducted in a natural plant growth environment with some degree of control which provides a realism that is difficult to achieve in the laboratory. However, the weather conditions in the field cannot be controlled.

6.5 Conclusion.

The main aim of this study is to assess the potential of remote sensing to detect CO₂ leakage from CCS repositories. Further to this, the capability of remote sensing to discriminate between stresses with similar mode of action is explored.

The investigations carried out in this thesis have shown that remote sensing can accomplish these tasks. This main conclusion was reached from the following observations made in this thesis:

Further to this, the objectives of the study as stated in section 1.7 have been achieved as follows:

1. The study has furthered the understanding of the impacts of elevated soil CO₂ on the growth and development of crops with specific reference to maize and barley crop. Stressed crops are susceptible to growth retardation, reduction in yield, chlorosis, early senescence and eventually death due to inability to cope with stresses.

2. The stress responses of the species of crops used in this study were different. Maize (C4) was found to be more resistant to elevated soil CO₂, than barley (C3). This as stated above could be as a result of the presence of *aerenchyma* in the stem of maize that supplies oxygen to the roots.

3. This research has also shown that CO₂ or herbicide stress in plants can be detected and discriminated using hyperspectral remote sensing techniques. This is in contrast to earlier studies which concluded that remote sensing alone cannot detect as well as discriminate stresses arising from a variety of factors. Continuum removal analysis was able to identify some specific wavelengths that could be used to detect and discriminate between the different stresses in the crops used in this study. It has also furthered our knowledge of the canopy spectral reflectance characteristics of barley and maize stressed with CO₂ and herbicide.

4. An evaluation of the effectiveness of a range of hyperspectral analysis techniques has demonstrated their capability in stress studies; some were able to detect stresses at early stage of the plant growth (e.g. PSSRa and PSSRb) while others could only be used at later date (PRI and ChINDI). A more pertinent implication of the findings of this study is the potential application of the responses of terrestrial vegetation to identify leaks from underground CCS systems. Vegetation indices applied in this thesis, as shown in the previous chapters have also played a prominent role in stress studies; some were sensitive to a particular stress and concentration while others were less sensitive.

The results of these investigations have demonstrated that hyperspectral remote sensing techniques have the potential to detect and discriminate between different stressors using their spectral response; this depends on the stress type and concentrations. This is an area that has not been

widely studied at canopy level under field conditions, although there are studies at leaf reflectance level. However, further investigations will be required to be carried out under different field to upscale and generalise the results (Refer to section 6.6 for details).

The research findings of this study could be put into practical use in remote sensing for leak detection. CCS facilities are usually confined to specific locations/ sites, thereby making it possible for remote detection of anomalies in vegetation spectra in areas within/ around CCS site (Male *et al.*, 2010). The abnormalities could be used as indicator(s) of stress caused by leaking CO₂ from CCS site. The spatial distribution of stressed vegetation will provide information about the path ways of CO₂ migration during leakage. Hyperspectral plant signature could be used as basis to certify that the purpose of CCS is not compromised through leakage. The results of this study suggest that some vegetation indices used were capable of detecting stress. The continuum removal analysis was able to identify some specific wavelengths sensitive to stress caused by elevated soil CO₂. When this is confirmed, personnel's could be deployed to the sites to test for the presence of CO₂ in the soil. This would need to be monitored every week as the plant develops to ascertain the level(s) of soil CO₂. The procedure is similar to the current one which is used for the monitoring natural gas pipelines, where helicopters are flown intermittently (usually every two or more weeks) along the length of pipelines looking for anomalies of various kinds, including gas leaks. This technique is expensive and time consuming due to the resources involved (Tedesco, 1995), therefore, remote sensing could be an alternative approach for detection of leakage (Van Persie *et al.*, 2004). Operationally, airborne systems (using aircrafts) might be the most convenient in short term as space borne systems do not currently have the required high spectral resolution for

continuum removal. But as CCS industry grows, there is the possibility that in the future dedicated satellite system might become a realistic option.

6.6 Future research directions.

This research has provided a basis for the study of plant stress caused by CO₂ and herbicide. It has also shown that CO₂ or herbicide stress in plants can be detected, and discriminated using hyperspectral remote sensing.

Based on the limitations of this study and the findings in this thesis, the following proposals are made for future research:

1. There is a need to test this approach under different field conditions, such as wet years, under drought and on different soil types and different plant species, since the results of this study were obtained under a limited range of conditions. This will help to establish whether subtle spectral features are consistently detectable in spectra of leaves and plant canopies in the field situation. Such studies would investigate if the same treatment dose of stresses will have the same effect on plants growing in different field condition.
2. While two 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, where responses may be affected by competition. Hence, the use of a mixture of plant species at different growth stages as well as inter-cropping, where two or more different crops and/or species are grown together to closely mimic natural vegetation communities is proposed for future investigation.
3. Further to this, there is need for independent study of other crops and/or species using a variety of stress factors to determine which ones can be distinguished from each other.

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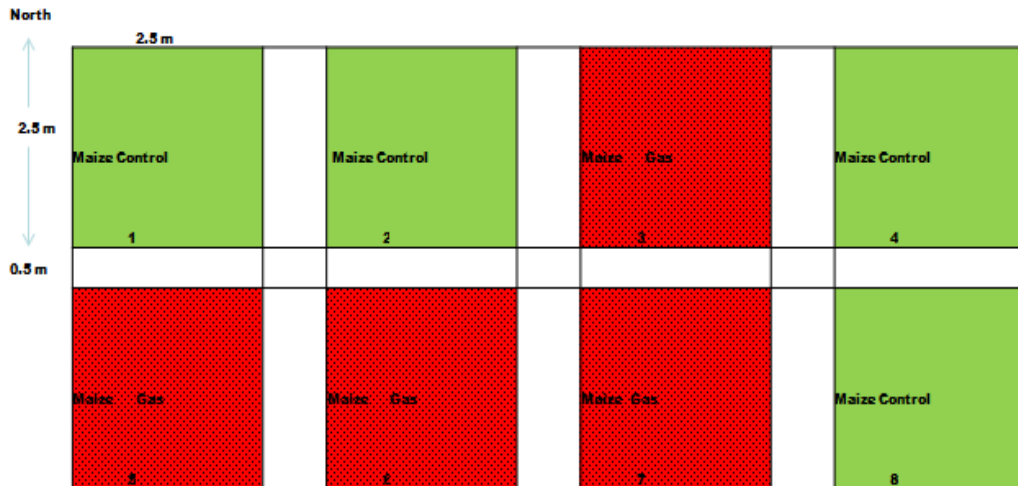
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APPENDICES

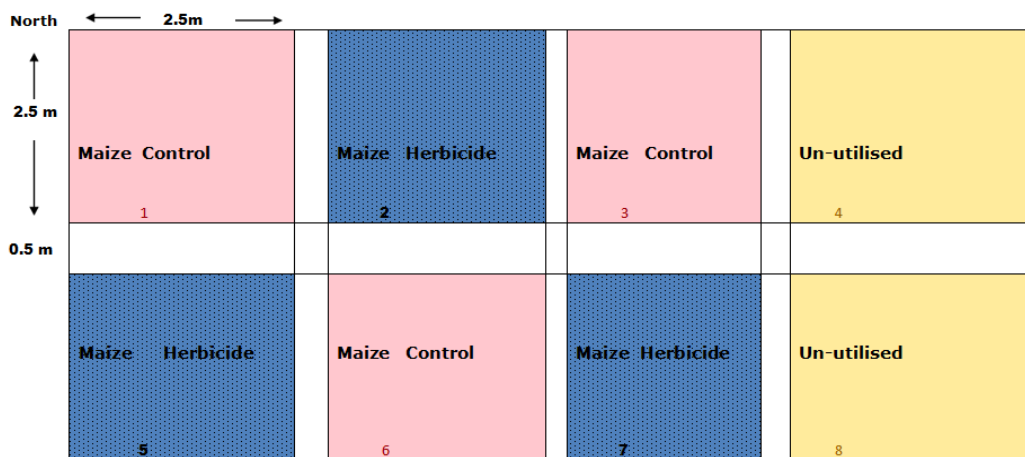
Appendix 1.

Schematic representation of plots showing gassed and control plots for maize and herbicide treated experiment for 2009.

CO₂ experiment 2009.



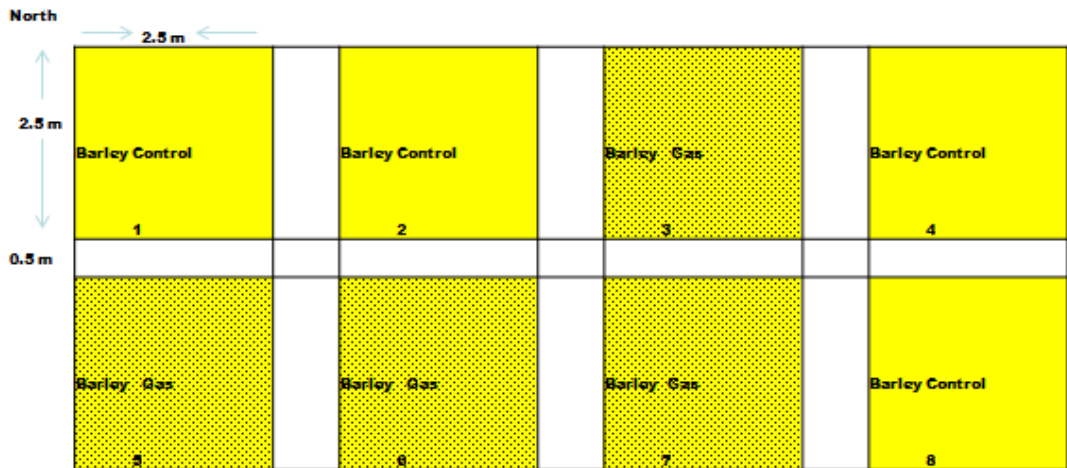
Maize herbicide experiment 2009.



Appendix 2.

Diagrams showing ASgard plots identifying both gassed and control barley experiment for 2010 as well as herbicide field experiment.

CO₂ experiment 2010.



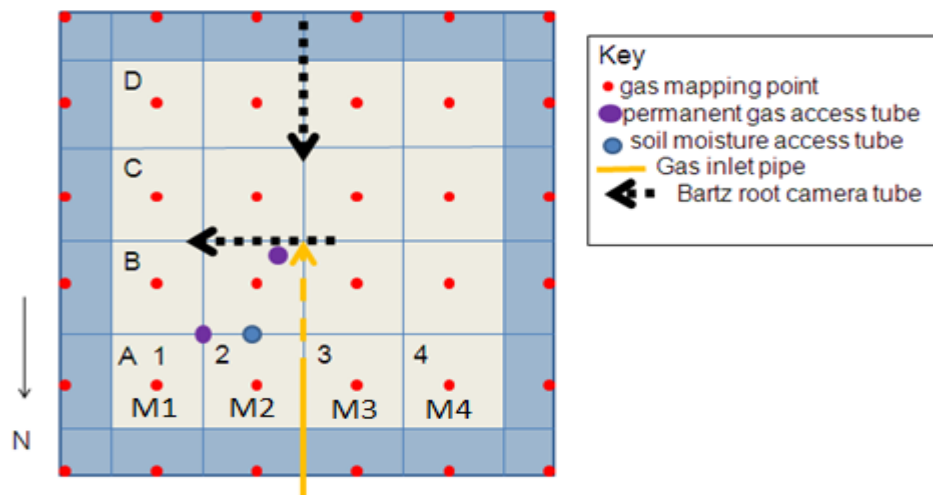
Maize herbicide experiment 2010.



Appendix 3.

Gas concentration measurements.

The diagram below shows a map of an individual gassed plot with the infrastructure therein. The red dots represent the gas mapping points where barholing measurements were made at the end of the experiment. The permanent gas access tubes were used to derive a seasonal average at the end of the season in order to account for the spatial variation across the measurements points represented by M1 to M4. Sub-plots are represented by A1-A4, B1-B4, C1-C4 and D1-D4. The sub-plots of interest in this study are A1-A4 (Sub-plots where measurements were made in both gassed and control plots), and B1 and B2 (Sub plots where permanent gas access tubes were located). In calculating the seasonal average CO₂ concentration distribution, the barholing data are used to represent the spatial distribution of CO₂ across the plots and the values are then scaled up according to the seasonal average of daily measurements made in the fixed tube. The main assumption is that the spatial pattern does not vary even though the concentration of CO₂ on any given day may vary with location.



Maize (22nd September, 2009) barholing measurements for gassed plots.

Note: The figures in blue are the barholing CO₂ measurements while the ones in black are the O₂. The data corresponds to the red points on the map above. The red area is the point of interest in this study, which coincides with the spectral measurement and gas measurement locations.

Gassed plot 1.

12.5	22.9	0.8	0.5	5.7	2
17	15.9	20.4	19.6	19.1	17
4.1	7.4	12.8	12.6	10.9	2.4
19.9	19.5	18.3	17.8	17.7	19.2
3.6	2.6	32.9	27.7	11.2	3
19.7	19.8	13.4	14.3	18.4	19.9
2.4	3.5	38.5	40.1	6	4.1
20.1	19.9	12.7	10.3	19.3	19.7
1.4	2.4	14.4	13.8	4.5	2
20.2	20.1	18	17.2	20	20.3
0.6	1.1	2.5	3.1	1.5	5.2
20.5	20.4	20.3	20.2	20.4	20.2

Gassed plot 2.

4.3	4.1	4.7	4.6	1.9	1.6
20	20.2	20	20	20.4	20.5
6	10.5	14.1	12.5	6.1	4.7
19.5	18.8	17.8	18.3	19.7	19.9
7.3	13.2	33.2	29.1	11.7	6.7
19.3	17.8	14	14.8	18.3	19.4
18	17.1	48.3	36.2	13.4	4.8
17	16.5	10.1	12.8	17.6	19.7
10.6	16.2	27.1	16.1	9.2	4.6
18.7	17.1	15.4	17	18.9	20.1
5.2	6	8.8	7.3	5.3	3.1
19.5	19.3	19	19.2	19.5	19.4

Gassed plot 3.

1.8	2.1	3.8	6	5.9	4
20.5	20.1	20	19.7	19.8	20
1.5	3.2	9.1	16.3	5.8	6.4
20.7	20	18.4	18.5	19.4	19.3
1.4	8.2	22.2	24.2	22	12
20.5	18.3	15.3	13.4	16.3	18.2
2.5	12.6	13.8	53.4	19.2	13.6
20.2	18.7	11.4	9.4	15.5	17.8
3	7.1	10	16.9	14.2	7.3
20.1	19.4	18.5	16.8	18.3	19.2
1.5	2	4.4	4.3	3.7	2.9
20.6	20.4	19.7	19.6	19.9	20.3

Gassed plot 4.

1.1	1.4	2.6	2.1	1.2	2.5
20.5	20.4	20.2	20.2	20.5	20.2
1.1	1.5	2.2	2.4	1.5	2.2
20.7	20.6	20.4	20.5	20.6	20.6
2.1	1.5	4.2	3.3	1.7	2.4
20.3	20.4	20.1	20.3	20.5	20.3
2.8	1.7	10.3	27	1.8	3.9
20.1	20.4	18.4	13.1	20.2	20
1.7	1.5	9.2	15.1	4.5	2
20.7	20.1	18.5	17.3	20.1	20.4
1.3	1.8	2.5	3.8	1.9	1.2
20.6	20.5	20.1	20.1	20.5	20.6

Barley (17th August, 2010) barholing measurements for gassed plots.

Gassed plot 1

1	0.6	0.8	1.8	1.7	2.4
20.1	20.3	20.4	20.3	20.1	20.1
1.4	0.9	1	1.4	1.9	2.3
20.4	20.4	20.4	20.3	20.2	20.2
3.7	2.3	3.8	10.6	1.9	2.2
19.8	20.1	20.1	18.8	20.3	20.2
3.8	3.5	24.9	16.2	1.7	2.1
19.5	19.9	15.8	17.5	20.3	20.3
4.8	3	3.7	7	2.8	2.1
19.6	20.1	20	19.2	20	20.1
3.5	2.7	4.3	3.7	2.3	2.2
19.8	20	19.8	19.9	19.9	20.2

Gassed plot 2

1.6	1.9	3.7	2.7	3.5	3.5
20	20.1	19.8	20	19.9	19.8
1.9	2.5	3.6	5.8	6.5	4.5
20	20	19.6	19.3	19.5	19.7
1	3.4	24.9	41.1	5.9	5.3
20.4	20.1	16.1	14.1	19.4	19.5
1.6	1.1	13.6	44.7	5.5	3.9
20.2	20.4	17.8	12.1	19.5	19.8
1.6	1.4	3.2	3.9	2.4	2.6
20.3	20.3	20	19.9	20.2	20.1
1.7	2.1	3	2.4	2.7	1.4
20.2	20.2	19.9	20	20	20.1

Gassed plot 3

2.1	3.1	2.2	3.3	2.4	1.5
20.1	20.1	20.1	19.9	20.1	20.3
1.2	4.8	2	2.3	3.2	0.9
20.3	19.5	19.8	20	20	20.3
1.5	4.4	2.4	15.3	3.7	1.4
20.1	19.8	20.1	17.9	19.8	20.1
0.8	1.3	7.4	64.9	3.3	0.9
20.2	20.2	18.9	9.8	20	20.1
0.4	0.9	1.5	10	1.7	0.9
20.3	20.3	20.3	19	20.1	20.2
0.7	0.7	1.5	1.6	1.3	0.7
20.2	20.3	20.1	20.2	20.3	20.2

Gassed plot 4

1.4	1	1.3	1.7	0.8	1.2
19.9	20	20	20	20.1	20.1
1.2	1	1.9	2.4	1.4	1.2
20	20.1	19.8	20	20	20
1.2	1.7	9.9	22.4	2.7	2.3
19.9	19.9	18.8	16.9	19.8	19.8
1.8	2.4	6.7	43.6	3.2	1.7
19.8	19.9	18.9	14.8	19.9	19.9
1.6	1.4	1.5	2.8	1.4	1.3
19.9	20	20	19.7	20	20.1
0.8	0.7	1.1	1.8	1.3	1
20.1	20	20	19.9	19.9	19.8

Barholing data.

Barholing point	M1	M2	M3	M4		
Sub-plot number	A1	A2	A3	A4	B1	B2
CO2 measurement (Gassed Plot 1)	2.4	14.4	13.8	4.5	3.5	8.5
CO2 measurement (Gassed Plot 2)	16.2	27.1	18.1	9.2	17.3	45.3
CO2 measurement (Gassed plot 3)	7.1	10	16.9	14.2	12.6	33.8
CO2 measurement (Gassed plot 4)	3.5	9.2	15.1	4.5	1.7	10.3
Total	29.2	60.7	63.9	32.4	35.1	97.9
Average	7.3	15.18	15.98	8.1	8.78	24.48

CO₂ seasonal average for Maize plots (2009).

Seasonal average at 70cm from the plot centre= 24.28

$$M1 = (CO_2)_{A1} \times \text{CO}_2 \text{ Seasonal average at 70cm}$$

$$\text{Average CO}_2 \{A1+A2+B1+B2\}$$

Where A1=7.3, A2=15.18, B1=8.78, B2=24.48

$$M1 = 7.3 \times \underline{24.48}$$

13.9

$$M1 = 7.3 \times 1.74 = 12.70$$

$$M2 = 15.18 \times 1.74 = 26.41$$

$$M3 = 15.98 \times 1.74 = 27.81$$

$$M4 = 8.1 \times 1.74 = 14.09$$

$$\text{High gas zone} = M2 + M3 / 2 = (26.41 + 27.81) / 2$$

$$= 27.11$$

$$\text{Low gas zone} = M1 + M4 / 2 = (12.70 + 14.09) / 2$$

$$= 13.40$$

Barholing data for barley (2010)

Barholing point	M1	M2	M3	M4		
Sub-plot number	A1	A2	A3	A4	B1	B2
CO2 measurement (Gassed Plot 1)	3	3.7	7	2.8	3.5	24.9
CO2 measurement (Gassed Plot 2)	1.4	3.2	3.9	2.4	1.1	13.6
CO2 measurement (Gassed plot 3)	0.9	1.5	10	1.7	1.3	7.4
CO2 measurement (Gassed plot 4)	1.4	1.5	2.8	1.4	2.4	6.7
Total	6.7	9.9	23.7	8.3	8.3	52.6
Average	1.68	2.48	5.93	2.08	2.08	13.5

CO₂ seasonal average for Barley plots (2010).

Seasonal average at 70cm from the plot centre= 4.11

$M1 = (CO_2)_{A1} \times CO_2 \text{ Seasonal average at 70cm}$

Average CO₂ {A1+A2+B1+B2}

Where A1=1.68, A2=2.48, B1=2.08, B2=13.15

$M1 = 1.68 \times 0.85 = 1.43$

$M2 = 2.48 \times 0.85 = 2.11$

$M3 = 5.93 \times 0.85 = 5.04$

$M4 = 2.08 \times 0.85 = 1.77$

High gas= $(2.11+5.04)/2$

= 3.58

Low gas= $(1.43+1.77)/2$

= 1.6

Appendix 4.

Sample of field spectra data converted to ASCII file before importation to excel work sheet, note that the wavelengths end at 2500 nm.

Text conversions of header file \USERS\sani\field\field2.002

The instrument number was 6279/2

New ASD spectrum file: Program version = 3.01 file version = 4.03

Spectrum saved: 06/25/2010 at 11:01:46

VNIR integration time: 34

VNIR channel 1 wavelength = 350 wavelength step = 1

There were 50 samples per data value

Xmin = 350 xmax= 2500

Ymin= 0 ymax= 1.25

The instrument digitizes spectral values to 16 bits

SWIR1 gain was 41 offset was 2058

SWIR2 gain was 16 offset was 2069

Join between VNIR and SWIR1 was 1000 nm

Join between SWIR1 and SWIR2 was 1800 nm

VNIR dark signal subtracted

50 dark measurements taken Fri Jun 25 10:58:09 2010

DCC value was 0

Data is compared to a white reference:

50 white reference measurements taken Fri Jun 25 10:58:14 2010

There was a remote cosine receptor attached

Spectrum file is reflectance data

GPS-Latitude is S0

GPS-Longitude is E0

GPS-Altitude is 0

GPS-UTC is Fri Jun 25 00:00:00 2010

Wavelength field2.002

350	1.32087906822562E-02
351	1.26789016649127E-02
352	1.22017739340663E-02
353	1.24643677845597E-02
354	0.012551979161799
355	1.23405400663614E-02
356	1.18502313271165E-02
357	1.16530507802963E-02
358	1.17588127031922E-02
359	1.19471903890371E-02
360	1.17803039029241E-02
361	1.17365168407559E-02
362	0.01179856993258
363	1.19820041581988E-02
364	1.18162594735622E-02
365	1.16676557809114E-02
366	1.15708410739899E-02
367	1.16352587938309E-02
368	1.14965057000518E-02
369	1.13765019923449E-02
370	1.16033516824245E-02
371	1.15254409611225E-02
372	0.011432908475399
373	1.14122405648232E-02
374	1.12504884600639E-02
375	1.13132037222385E-02
376	1.15151545032859E-02
377	1.14473272114992E-02

378 1.14251086488366E-02
379 1.14184143021703E-02
380 1.14036127924919E-02
381 1.12640392035246E-02
382 1.11930184066296E-02
383 1.12372543662786E-02
384 1.13043040037155E-02
385 1.14197079092264E-02
386 1.15494932979345E-02
387 1.16018578410149E-02
388 1.16446539759636E-02
389 0.011657421477139

Appendix 5.

Determination of soil pH.

This is a standard procedure accredited by United Kingdom accreditation service (UKAS) and Environmental agency's monitoring certificate scheme (MCERTS).

Instrumentation.

The pH measurements were made using a bench pH meter and a solid body combination pH electrode. Calibration of the pH electrodes was performed using commercial pH buffers.

Sample Preparation.

Samples were dried at $40 \pm 4^\circ\text{C}$ and sieved to either <2 mm or <250 μm , depending on requirements.

Reagents.

1.0 M calcium chloride stock solution – Dissolve 73.5 ± 0.05 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (analytical grade or equivalent) in deionised water in a beaker and made up to volume in a 500 ml volumetric flask . Alternatively, 147.0 ± 0.1 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ is made up to 1000 ml depending on the volume required. The dissolution of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ in water is exothermic and care was taken to cool the beaker during dissolution.

0.01 M calcium chloride solution – Prepared by 100-fold dilution of 1.0 M CaCl_2 stock solution with deionised water. Pipette 10 ml of 1 M CaCl_2 solution into a 1000 ml volumetric flask and made up to volume with deionised water. Other volumes may be used. This solution has an expiry of seven days from the date of preparation.

Preparation of Soil Suspensions.

The representative test portion of the soil sample was put in a beaker containing a stirrer bar and 0.01 M CaCl_2 solution was added to give a final solid to solution

ratio of 1:2.5. Typically 10.0 or 5.0 ± 0.1 g of soil is mixed with 25 or 12.5 ± 1 ml respectively of 0.01 M CaCl_2 solution. Where insufficient sample is available, smaller masses may be taken as long as there is sufficient CaCl_2 slurry to cover the pH electrode. The mass of the sample was taken and its particle size on the Soil pH Method Form. The beakers were placed on a magnetic stirrer and stirred for at least 5 minutes. The suspension was allowed to settle for at least 15 minutes

Measuring pH.

The pH electrode was immersed in the soil suspension and stirred periodically until the meter indicates that the pH is stable. The value was the recorded on the Soil pH Method Form. Between samples, the electrode was rinsed with deionised water and then dried by gently wiping with a clean tissue. The pH 7 buffer was measured immediately before and after each batch of no more than 20 samples to check for drift. If the pH value obtained differs by more than ± 0.05 pH units from the temperature corrected nominal value, the instrument was recalibrated and the samples reanalysed.

Appendix 6.

Chlorophyll analysis.

Chlorophyll extraction and analysis were carried out using the standard method of Bruinsma (1963). Chlorophyll samples were extracted from the leaves of known area (1cm^2) by grinding with pestle using a mortar and adding 5ml of extraction solvent (80% propanone; 15% methanol: 5% distilled water) using a pipette. A small amount of purified sand was added to facilitate grinding. The suspension was transferred to a clean 15ml centrifuge tube and the procedure repeated until the volume of extract reached 10ml. The tube was capped and placed in the centrifuge, checked for balance and then centrifuged at 3000 rpm for 5 minutes.

The absorbance of the chlorophyll solution was measured in a spectrometer (UNICAM Helios, Cambridge, UK) at wavelengths 663 nm and 645 nm, using 80% propanone solution as a standard.

Chlorophyll concentration of chlorophyll a and b in mg l^{-1} was calculated as:

$$\text{Chlorophyll a (mg l}^{-1}\text{)} = 12.7 A_{663} - 2.7 A_{645}$$

$$\text{Chlorophyll b (mg l}^{-1}\text{)} = 22.9 A_{645} - 4.7 A_{663}$$

Where A_{645} and A_{663} are the absorbance at 645 and 663nm respectively.

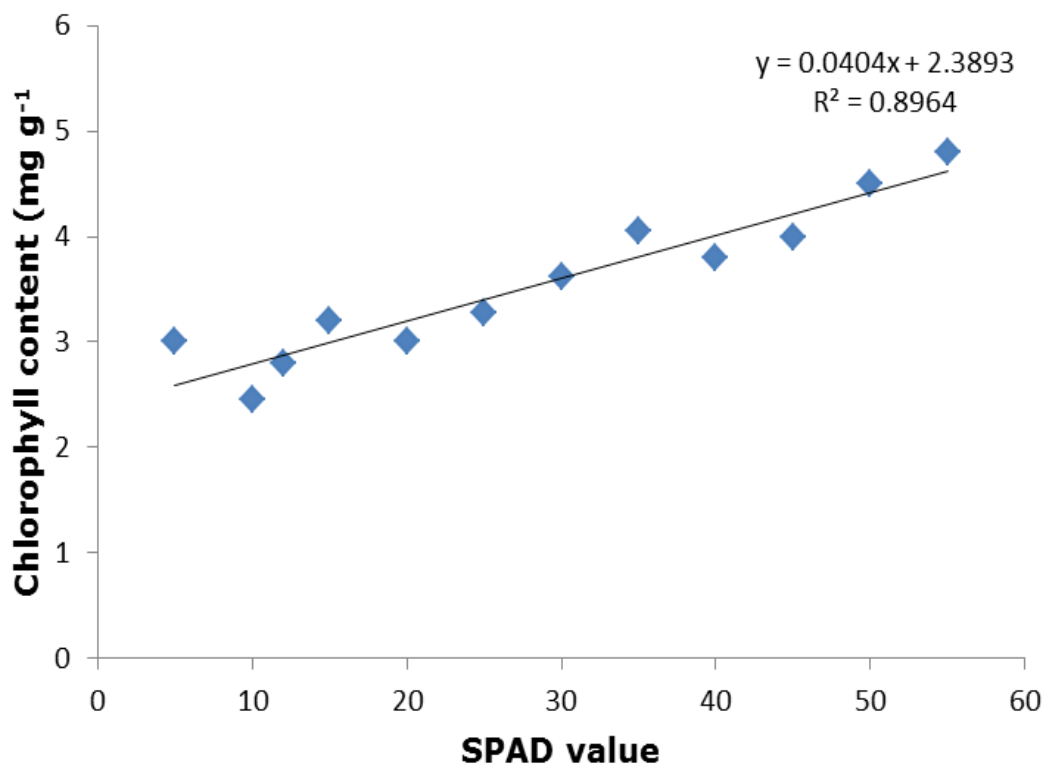
The concentration of chlorophyll in the leaf, expressed on an area basis, is found from:

$$\text{Chlorophyll (mg cm}^{-2}\text{)} = (V/A) \times \text{Chl}_x \text{ (mg l}^{-1}\text{)}$$

Where V = Volume of 80% propanone used in dilution, A = total area of the sample and Chl_x = Chlorophyll a or b concentration of the solution in mg l^{-1} .

SPAD Calibration

In 2009 a SPAD meter (Minolta SPAD, UK) was used to take six readings per maize leaf of four upper leaves in the same measurements sub-plots as used for spectral scanning which was then averaged. A calibration of the SPAD values to determine chlorophyll content of maize leaves were carried out in the laboratory using the above equation.



Appendix 7.

Climatic conditions.

The climatic conditions (mean daily as well as cumulative air temperature, total rainfall and solar irradiance) of the study area for the period 2009 and 2010 were taken from Sutton Bonnington meteorological station which is about 400m away from the ASGARD site. These were compared to determine possible variations during the study.

2009 data				2010 data			
Day	Air tem-C	Total rain-mm	Solar-Kjm-2d-1	Day	Air tem-C	Total rain-mm	Solar-Kjm-2d-1
1	-1.06354	0.4	411.679	1	-0.23233	0.2	3172.372
2	1.58666	0	2610.56	2	0.8205	2.4	972.611
3	0.182	0	1082.96	3	-1.12863	0	3554.022
4	-0.47454	0	786.412	4	-2.03967	0.2	5450.697
5	1.111	2	2263.862	5	-0.37967	0	858.856
6	-1.75896	0	1991.489	6	-0.81046	1	2877.696
7	1.69125	0.4	1051.291	7	-3.02758	0.4	4539.227
8	2.55621	1.8	1485.879	8	-1.22717	0	3629.31
9	0.56479	0	1572.749	9	-1.16058	0.2	2832.265
10	-0.72338	0	1634.702	10	1.11408	0.2	701.218
11	6.96296	0	1601.53	11	0.50988	1.4	683.612
12	9.42954	12.2	661.431	12	0.79871	0	871.696
13	5.91158	0.2	2605.907	13	-0.13968	1	1434.867
14	1.01166	0.2	3215.445	14	0.50342	0.6	1611.442
15	6.35646	0.4	1129.763	15	3.50821	2.4	936.33
16	7.39495	0.4	1643.674	16	5.53754	7	835.993
17	7.76554	7.6	3861.695	17	4.77996	0.2	3440.24
18	4.44138	2	4572.257	18	6.30925	0	3053.659
19	3.87546	6.8	2706.149	19	4.78038	0	1080.751
20	3.65925	0	4145.06	20	3.43983	0	717.931
21	3.88617	1.2	4094.072	21	3.53963	0	2919.236
22	6.58429	5	2530.506	22	6.81133	11.8	634.816
23	3.95792	3.8	2460.203	23	4.08121	0	3229.771
24	2.90242	0.2	3608.994	24	4.01892	1.8	2581.524
25	6.56958	1.6	2764.536	25	2.61488	0.2	689.638
26	5.16788	0	2541.63	26	1.29008	0	3059.748
27	3.60946	0.4	3278.908	27	4.49329	0	2310.581
28	6.19442	7.8	1852.276	28	5.16208	0	4348.305
29	3.53283	0.2	1661.646	29	2.49354	1	3942.774
30	3.24642	0	2848.88	30	0.05333	0	5950.699
31	3.01588	0	4641.753	31	0.32383	0	4381.967
32	0.08438	0	2103.707	32	1.47492	0.2	4776.606
33	-0.71233	3.2	2413.976	33	3.97783	4.2	2215.649
34	-0.50117	1.6	6022.716	34	0.96029	3.4	2584.44
35	0.31596	0.2	6408.539	35	4.75792	0.2	1645.197
36	0.35038	3.8	2249.688	36	5.49271	3.8	4671.397

37	0.19979	1	3789.805	37	3.02913	0.2	1391.509
38	-0.05125	0.4	7972.602	38	3.39879	0.2	2216.444
39	0.19529	1.6	4521.285	39	1.81583	0.2	1493.446
40	0.76592	9	2812.66	40	2.58488	0.2	4704.489
41	2.49	2.6	6245.439	41	0.99675	0.2	5563.263
42	1.7067	0.2	2904.328	42	1.51083	0.2	6559.263
43	0.5881	3.6	2948.472	43	3.43967	0	4133.273
44	2.84208	0.4	6289.331	44	2.72725	1.4	3985.53
45	1.63092	0	4559.922	45	3.18929	0	2939.338
46	6.2647	0	2513.775	46	3.25079	3.2	2193.954
47	7.71358	0	5719.162	47	3.26971	0.6	8171.53
48	8.83892	0	3917.905	48	1.5965	0.8	1934.445
49	7.73463	5.6	811.923	49	1.52658	2.4	3670.072
50	7.15579	0.6	5810.804	50	1.35988	2	5908.801
51	5.77729	0	5469.294	51	0.826173	0.2	8058.265
52	7.22017	0	9533.168	52	0.92825	3.2	6692.568
53	8.28479	0	3895.75	53	1.05283	0	4418.246
54	8.77467	0	5991.91	54	1.46496	2.2	4922.463
55	8.96113	0	2793.41	55	5.74104	0.4	3968.017
56	8.62557	0	4735.528	56	6.50075	3	3859.858
57	7.35446	0	1595.804	57	4.3859	7	3217.984
58	9.40779	0	3556.563	58	3.85308	1.6	3044.209
59	7.70096	0	4081.238	59	4.04421	0	3417.15
60	6.92329	0	7767.83	60	3.41767	0	11139.811
61	6.40346	0	8725.221	61	2.12408	0.2	10803.909
62	7.33817	10.2	3636.833	62	1.92967	0	7113.762
63	3.73888	0	10613.625	63	1.57879	0.2	9501.344
64	2.75996	0	10145.144	64	3.42983	0.2	10593.25
65	4.71021	0	11567.808	65	2.357	0	5756.462
66	8.69763	0.2	8348.617	66	-1.04263	0	14445.5
67	5.26763	6.4	7801.366	67	0.94804	0.2	11575.784
68	6.58863	0	10293.816	68	5.01138	0	8661.47
69	6.81504	6.2	5194.596	69	3.55254	0	5162.416
70	7.61179	0.2	8657.947	70	3.14804	0.2	7944.269
71	10.09146	1.2	8102.722	71	5.67146	1.6	4237.961
72	8.17113	0	5734.146	72	5.76146	0	10684.903
73	9.64375	0	13537.745	73	7.20158	0	11186.606
74	8.45238	0	11312.766	74	6.84963	0	8533.761
75	8.91067	0	21215.985	75	7.04417	0	10608.889
76	5.88775	0	3342.658	76	9.84575	0	9401.247
77	4.85879	0.2	6998.164	77	11.40675	0	5560.552
78	4.85646	0.2	7492.319	78	10.61042	5.6	9973.024
79	7.41483	0	14381.417	79	9.80679	2.8	4671.554
80	7.10108	0	14871.683	80	7.77504	0.2	12247.275
81	8.53575	0	10484.694	81	8.20654	0.2	6550.934
82	8.14525	0.2	7179.379	82	7.94708	0.8	9603.509
83	6.45592	0.6	12330.403	83	10.94667	4	6757.725
84	8.40233	2.8	11321.238	84	11.23958	7	10419.936
85	8.13938	0.6	9719.471	85	9.30492	0.6	11496.573
86	6.03508	3	9906.2	86	8.66675	0	13083.97
87	5.59892	0.2	8799.131	87	7.84663	0	12137.185
88	4.94279	0	17333.963	88	8.37313	7.4	3890.577
89	8.38792	0	8794.763	89	7.81375	4	6079.689
90	10.99325	0	14044.314	90	3.50304	0.4	5671.457

91	11.60692	0	16180.987	91	4.70604	5.4	1271.986
92	7.28825	0	5907.94	92	6.48808	2	7749.828
93	8.158083	0	13597.74	93	7.17692	6.6	11950.996
94	9.739042	0	16452.714	94	6.59267	0.2	14757.641
95	8.45308	0	17836.128	95	9.62317	0.2	10403.068
96	10.68908	0	10253.691	96	11.16683	0	10496.161
97	9.81242	1.4	15263.303	97	8.05521	0.8	6117.796
98	10.31975	1	19711.041	98	9.17829	0	18495.05
99	11.64529	1.8	6859.808	99	10.09083	0	15729.399
100	10.75917	4.4	4395.012	100	10.8385	0	17390.397
101	9.19	0.4	7145.088	101	7.96117	0	14489.34
102	9.32783	0	7548.823	102	7.42333	0	9146.984
103	10.89979	0	13390.535	103	7.723	0.2	16812.662
104	10.72504	0	12083.473	104	8.05	0	14102.86
105	10.74183	6.6	9225.339	105	6.58725	0	5450.19
106	8.81229	2.4	2358.902	106	7.37713	0	1684.782
107	9.42554	0	9406.033	107	10.22417	0	22069.248
108	8.25279	0	17238.392	108	9.74108	0	17655.531
109	8.60371	0	20585.285	109	7.0045	0	6591.594
110	9.68754	0	22897.422	110	6.68171	0	21220.665
111	10.36371	0	21157.577	111	5.731	0	22419.913
112	10.50642	0	22070.018	112	7.29821	0	22550.69
113	12.87029	0	16574.677	113	10.38354	0	21900.548
114	13.45292	0	20679.214	114	12.83613	0	19927.228
115	10.56608	0.4	16490.123	115	13.045	3	15147.069
116	10.20808	0	18212.033	116	12.13796	0	18845.747
117	7.64292	10.8	7413.07	117	13.59058	0	19369.675
118	8.64842	0.2	14013.608	118	15.77458	0	11359.535
119	10.33704	0	21466.722	119	12.59104	5	9057.394
120	12.73625	0	10971.399	120	10.32838	3.2	13370.199
121	13.179167	0	16772.672	121	10.35079	0.6	19290.803
122	11.51629	0.2	18030.764	122	6.97417	0	8987.715
123	9.19225	1	21560.833	123	6.09233	1.6	17190.082
124	9.08742	1.4	7480.456	124	8.82721	0.2	19728.468
125	12.96458	0	9842.552	125	10.76796	0	90220.093
126	14.115	0.2	15719.642	126	8.934	0.2	9068.757
127	12.46458	0.2	24352.132	127	8.398	1.8	15246.013
128	10.87754	0.4	22139.16	128	7.52208	2.2	7411.499
129	10.49533	0	19821.74	129	7.32121	0	19280.266
130	10.55929	0	19017.638	130	5.28396	0	9480.616
131	9.69783	0	26090.094	131	6.67333	0	19649.575
132	10.802	0	27311.512	132	5.94375	1.4	17406.607
133	10.52788	1.6	6094.093	133	8.12933	0	18590.294
134	10.78333	6.6	3306.615	134	9.91658	0	17571.592
135	11.51667	12.6	7526.496	135	10.808	0	22997.981
136	9.95588	1.6	15411.341	136	10.73358	0	22677.454
137	10.11979	1.6	14452.628	137	11.13396	0	20683.566
138	11.97833	0.2	15666.839	138	12.26321	0	23388.739
139	11.17458	4.2	15982.936	139	15.71667	0	16211.288
140	11.89208	0	1816.43	140	18.23542	0	18788.677
141	12.034083	0	21622.359	141	17.38542	0	23624.116
142	12.26504	0	16085.205	142	17.32625	0	28302.387
143	14.98958	0	22857.986	143	20.27	0	28430.278
144	14.05375	0.2	29136.528	144	16.81333	0	25667.14

145	14.48917	1.4	10294.472	145	11.99375	0	13252.434
146	11.73792	0.6	19847.312	146	9.8425	2	7789.081
147	13.15958	0	15838.095	147	10.50492	0.2	24831.081
148	17.27583	0	18189.795	148	12.2	0	27901.855
149	17.6625	0	25622.059	149	12.40958	4.8	6980.466
150	14.91279	0	29641.648	150	130.03	0	23947.442
151	16.19125	0	29202.939	151	12.5125	0	10857.021
152	16.68933	0	29769.029	152	11.44417	2.8	6930.058
153	17.64304	0	27486.571	153	14.07246	0	2298.953
154	12.27963	0	8146.487	154	15.982923	0	28647.862
155	11.33358	0	22282.25	155	17.97288	0	27567.637
156	11.82763	11	20492.386	156	19.89958	8.6	21644.209
157	10.40542	16.2	9652.037	157	15.44333	15.2	11291.301
158	9.62742	17.2	5853.419	158	14.66	1.8	13632.116
159	11.87721	0	18314.283	159	13.53667	6.4	8147.42
160	12.52958	0	12992.433	160	14.22625	18.4	11069.969
161	12.60958	3.8	15064.412	161	13.19917	0.4	5249.473
162	12.31704	0.4	26912.556	162	14.0175	0	21789.728
163	13.64421	0	27275.817	163	14.36125	0	23164.161
164	17.14	0	22085.93	164	13.70292	10.8	17899.649
165	16.717	0	26867.917	165	12.44292	0.6	13350.153
166	14.63417	7.4	18984.935	166	11.78633	0	20175.216
167	15.81	0	26902.247	167	12.65333	0	31226.841
168	14.24417	1.6	9865.293	168	13.9005	0	29930.808
169	13.49667	0.2	19683.893	169	11.845	1.4	8901.492
170	13.17875	0	22613.988	170	10.65663	0	18044.907
171	13.455	1.4	18530.937	171	13.72983	0	26272.343
172	14.69042	0	22367.362	172	18.14708	0	28714.83
173	16.52542	0	13060.702	173	19.08833	0	30024.968
174	18.40625	0	24738.695	174	18.55167	0	27198.931
175	15.8575	0	29962.244	175	16.970417	0	18422.99
176	16.97083	0	20060.818	176	16.69129	0	27467.538
177	17.91833	0.2	15178.008	177	19.69417	0	23515.105
178	17.93958	0	9486.108	178	20.6125	0	26469.52
179	18.73292	0	16978.286	179	19.44125	0.2	27039.707
180	20.195	0	21632.776	180	18.615	2.6	24433.152
181	22.13333	0	20294.99	181	19.06708	0	23111.51
182	23.36375	0.8	25685.242	182	19.79458	0	16552.421
183	20.0125	0	27894.674	183	19.05125	0.2	18020.796
184	17.47458	10.8	10553.712	184	17.10833	0	23693.365
185	18.85958	0.2	22515.129	185	17.59583	0	22339.287
186	18.48125	0	21051.671	186	15.15258	0	19505.654
187	15.57208	1.2	17481.813	187	17.13088	0	20763.218
188	13.92792	11.2	9864.99	188	19.61792	0	17137.864
189	14.81833	3.8	16238.55	189	17.9975	0	20841.291
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191	15.07833	0	24846.012	191	21.72958	0	19447.926
192	10.89412	2.6	11268.834	192	18.13875	0	24898.605
193	17.06625	1.2	19501.642	193	13.565	5.6	4207.151
194	16.2825	2.2	18308.629	194	14.54458	6.2	6706.971
195	16.09042	1.8	16684.153	195	16.88583	11.4	13613.958
196	17.20375	2.8	17809.656	196	17.09333	3.8	15668.151
197	16.3225	5.8	16754.314	197	15.82458	3	17619.764
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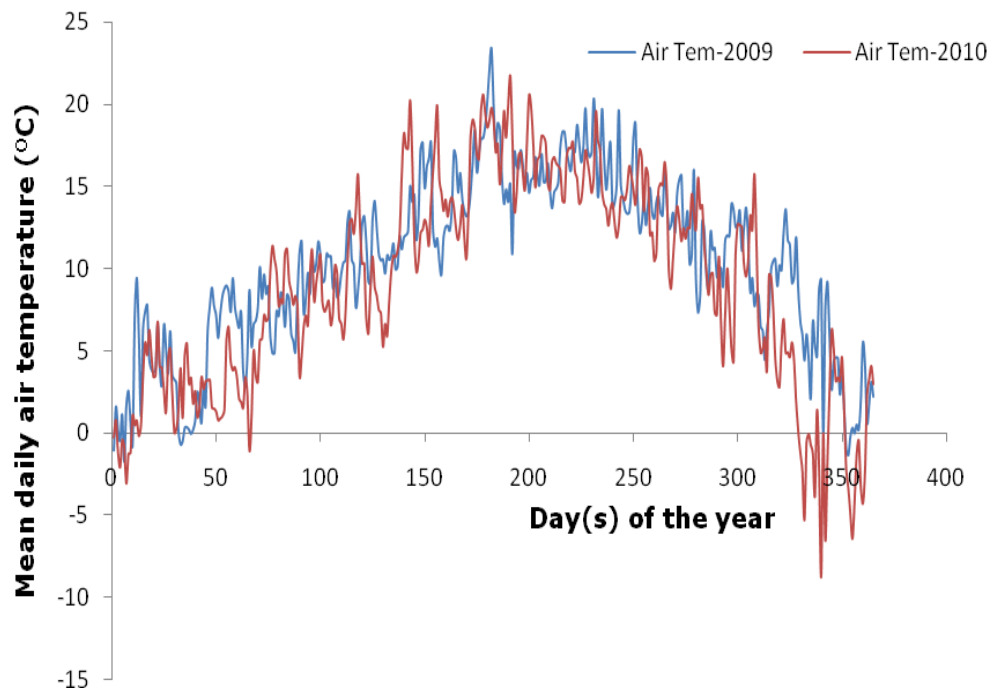
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201	15.38375	0.4	23856.886	201	19.81292	0.8	14188.047
202	15.57917	0.8	7240.126	202	17.69958	0	21238.576
203	16.77797	2.6	15380.292	203	14.97917	2.8	14235.666
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206	16.97042	1.4	21974.263	206	18.09208	0	14506.543
207	15.27125	0.2	8921.015	207	18.04792	0.8	8228.361
208	15.29583	3.8	18150.223	208	17.63958	0.2	11095.351
209	16.40875	0.2	18467.239	209	15.18708	0.6	10415.955
210	14.76792	25.2	4079.211	210	14.80583	0	11248.484
211	13.67292	1.2	18418.439	211	16.519167	3.2	10196.843
212	14.62663	0.8	15931.816	212	16.81	0.2	11010.61
213	14.8525	9.6	5224.672	213	16.44125	0	13596.235
214	15.26208	0.2	21824.318	214	16.2675	0	13108.764
215	17.44083	0	17604.699	215	16.00625	0	13711.156
216	18.31167	5.4	5501.754	216	14.17833	5.8	10527.463
217	18.30208	10	10577.526	217	14.08375	0	15225.985
218	17.16458	12.4	14735.161	218	16.81667	0.2	8792.965
219	16.46667	6.6	16969.242	219	17.35292	0	13635.841
220	15.94458	0	23379.5	220	17.34292	0	19107.917
221	17.27083	0	22297.201	221	17.72167	6.1	17643.573
222	17.09792	0.4	8737.75	222	15.92167	0	20469.809
223	18.75	0	18769.805	223	15.50875	0	18148.237
224	17.64417	3	10669.915	224	13.99917	7.8	9063.971
225	16.46667	0	16185.45	225	14.19375	5	7645.637
226	17.34875	0	14100.113	226	15.46	0.8	12163.151
227	19.74708	0	15998.44	227	17.20542	0	15061.032
228	17.32958	0	18715.233	228	16.55083	0.4	19535.927
229	16.78333	0	12276.205	229	15.67417	0.6	9903.255
230	17.06375	0	13548.548	230	14.63542	0	13232.197
231	20.33625	0	20888.357	231	15.63625	8.2	13107.979
232	17.98125	0	13414.282	232	19.56208	4.4	8822.699
233	14.37292	0.8	14567.106	233	17.8175	11	8180.772
234	16.27083	0	21689.419	234	17.25	4	17185.592
235	19.71917	0	17145.151	235	14.74167	16.2	9859.273
236	16.67292	0	9514.956	236	13.94208	0.8	14491.735
237	14.795	1.8	14999.344	237	13.645	5	8632.845
238	16.33042	3	6822.534	238	12.6425	10.8	3267.76
239	17.63042	0	13884.289	239	13.665	0	16831.72
240	13.6975	4.2	15223.322	240	13.99458	1	16063.698
241	13.99875	0	16667.096	241	12.82417	0	13869.623
242	15.10667	0.8	8232.776	242	11.91255	0.2	17669.9
243	19.63667	0	9719.689	243	12.65371	0	17871.033
244	15.05875	2.6	13189.122	244	14.37683	0	17963.968
245	14.28625	9.2	8334.349	245	14.37875	0	16539.975
246	13.57983	3	11376.27	246	14.21167	0	15613.332
247	13.34167	0	15031.537	247	14.92525	0	11903.621
248	13.40708	0	9904.225	248	16.25583	0	10025.507
249	14.85542	0.4	10034.592	249	15.70542	0	9525.414
250	17.92667	0.2	14072.935	250	14.84375	0.2	13922.796
251	18.81042	0.4	11419.837	251	13.9175	0	12202.703
252	14.20467	0	18936.12	252	15.67667	1.6	13106.861

253	12.18733	0	18074.78	253	17.28958	5.8	7684.146
254	12.70213	0	16750.957	254	16.77667	1	12059.081
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256	12.64583	0	9738.859	256	16.13708	0	5633.528
257	13.95458	0	9525.548	257	15.68292	2.8	6968.042
258	14.89958	0	9535.781	258	12.06375	1.4	6387.45
259	13.40458	0	11226.985	259	12.23208	0	11928.843
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261	14.05792	0	11569.936	261	10.97513	0.8	11156.67
262	14.43125	0	12289.315	262	14.79667	0.4	8166.393
263	13.43263	0	15425.28	263	15.21917	0	11162.219
264	13.23433	0	7222.07	264	15.11017	0	10907.05
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266	14.11083	0	8330.634	266	14.95383	1.2	7626.935
267	12.42083	0	10908.104	267	9.95	1.2	4791.443
268	12.61254	0	8222.759	268	8.80354	0	10842.369
269	13.39471	0	7105.731	269	10.63238	0	4552.234
270	12.20254	0	7870.344	270	12.63208	0.4	1411.491
271	14.60583	0.2	5112.85	271	13.82542	0.2	4056.419
272	15.41583	0	9619.122	272	12.96375	5.2	2114.043
273	15.66875	0	6603.461	273	11.71079	0.2	10733.827
274	12.26583	0	9416.487	274	12.485	11.2	1792.678
275	12.47333	0	4559.944	275	11.85183	3.2	10048.266
276	13.43375	0.6	7151.903	276	12.51958	13.6	2675.503
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278	10.83988	0	8701.716	278	14.57375	0.2	7504.388
279	16.01833	14.4	4642.688	279	13.3875	0.8	1034.844
280	9.3035	5.6	7103.821	280	12.43229	0.4	9033.106
281	7.317	0.2	9335.723	281	15.55292	0.2	2674.28
282	8.39192	1.4	4529.623	282	13.68625	0	2542.932
283	12.8775	0	9323.878	283	13.87583	0	9803.456
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285	9.53725	0	10747.122	285	9.86083	0	3559.261
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287	12.22083	0	6359.662	287	9.67454	0	1906.586
288	11.13208	2.4	2023.218	288	9.74683	1.6	2886.026
289	11.24708	0	7463.761	289	7.34475	0.2	6338.327
290	9.4745	0	5975.332	290	7.21371	0.2	10247.771
291	9.61583	0	7829.626	291	10.75563	2	3296.985
292	10.02692	0	5921.585	292	6.72521	5.8	3124.067
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294	11.72292	0.8	3615.64	294	7.23038	0	6682.42
295	12.0325	0	3854.679	295	10.03813	0.8	6925.27
296	11.96875	0	6000.413	296	7.68958	5.2	3189.713
297	13.93375	1	2841.788	297	5.12654	0.2	7669.052
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299	12.52417	0	3104.362	299	10.91258	3.2	1373.994
300	12.70333	1.6	2776.311	300	12.73083	0.4	6332.323
301	13.51125	0	6132.126	301	12.67083	0	3877.902
302	11.09438	0	3956.764	302	12.54458	0	2656.02
303	12.46917	0	3507.365	303	10.30667	0	5819.465
304	13.71	1.6	4528.758	304	9.55754	0.4	1821.413
305	11.82375	1.5	2888.642	305	10.51342	0.2	2657.309
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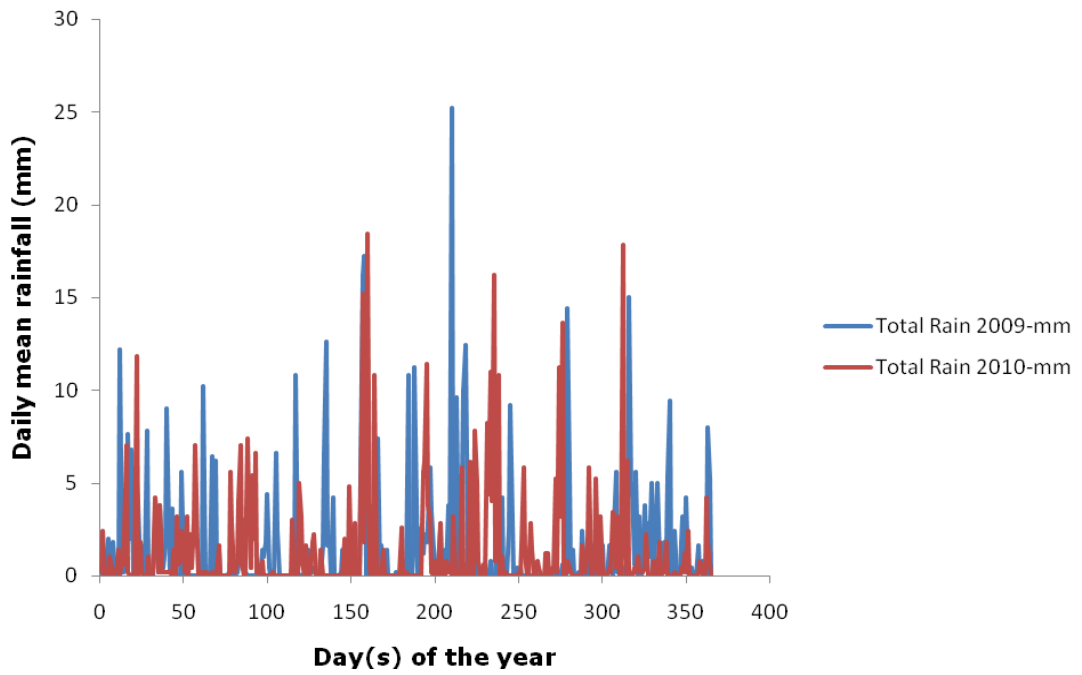
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311	6.46808	0.4	6438.406	311	4.91858	0	6548.966
312	6.20808	0	5072.37	312	5.15375	17.8	622.586
313	4.43221	0.2	6600.844	313	5.83083	1.8	2757.927
314	6.30008	0.8	1749.154	314	3.84242	0	4497.947
315	7.378	1.2	1701.981	315	9.67742	6.2	2376.831
316	9.94267	15	3000.796	316	9.53496	0	2524.439
317	10.45042	4.4	2044.708	317	7.66033	0	5672.256
318	10.58121	4.6	3202.038	318	4.57467	0	2131.089
319	8.92863	0.6	4909.296	319	3.18271	0.4	5581.41
320	10.19021	5.6	3923.18	320	2.84783	0.2	5019.68
321	9.90083	0	5197.992	321	5.51254	1	865.585
322	12.20833	3.2	895.253	322	6.96408	0.2	1430.273
323	13.60625	0	3619.177	323	4.90129	0.2	1955.209
324	11.739	1.4	3197.112	324	4.94867	0.2	765.594
325	11.43225	3.8	1248.557	325	4.66421	0.4	2500.854
326	9.12854	1.6	2320.349	326	5.549	2.2	2448.93
327	9.48	2.8	2793.142	327	4.44075	0	3116.687
328	11.905	1	1389.457	328	1.92588	0	3474.739
329	8.77608	5	3553.8	329	0.14779	0	1984.43
330	6.78854	0.2	3997.462	330	-1.15479	0.2	5130.761
331	5.95454	0	4152.063	331	-2.31771	0.8	4545.565
332	4.41404	3.8	1276.968	332	-5.2395	0	5285.742
333	6.00054	5	2863.561	333	-0.53196	0.2	2658.897
334	4.46542	0.8	3205.353	334	-0.01421	1.8	1399.81
335	2.08904	1.4	3499.421	335	-0.67983	0	2427.512
336	6.75417	0.4	1992.963	336	-1.20154	0.2	1479.621
337	5.28788	1.8	1052.602	337	-3.80708	0.2	4434.308
338	4.68375	1.6	3274.807	338	1.44038	1.8	2237.715
339	8.77596	5.4	3135.652	339	-2.22421	0	4712.446
340	9.314	9.4	2874.446	340	-8.70885	0	4870.316
341	0	0	0	341	0	0	0
342	7.58013	0.8	2324.254	342	-6.50521	0	3627.003
343	9.19713	2.4	1962.25	343	-1.74025	0.2	3806.069
344	5.96867	0	3361.797	344	2.52458	0	3508.795
345	2.64217	0.4	993.692	345	6.27617	0	876.627
346	4.39054	0.2	2931.957	346	5.15388	0	3243.531
347	4.59708	1.8	2447.254	347	3.20575	0.4	1785.795
348	4.53558	3.2	941.639	348	3.40758	0.2	1197.527
349	2.34358	0.4	537.719	349	3.1925	1.2	301.995
350	3.47913	4.2	781.77	350	4.6325	0.2	991.816
351	1.50667	0.2	2623.93	351	1.45683	2.4	445.86
352	-0.99475	0	1796.189	352	-2.14504	0	3230.38
353	-1.35625	0.4	4156.459	353	-3.33254	0	1022.927
354	-0.21188	0	3315.128	354	-4.93025	0	3428.598
355	0.30629	0.2	2807.149	355	-6.382	0	1002.285
356	-0.0345	0	3330.186	356	-4.12379	0	641.064
357	0.50329	1.6	2535.542	357	-1.28621	0.12	704.291
358	0.19092	0.2	1601.376	358	-0.41488	0.8	1734.943
359	1.97067	0.6	2541.069	359	-3.25646	0.4	2293.796
360	5.49904	0.8	3291.791	360	-4.24533	0	4497.496

361	4.15171	0.4	1576.621	361	-2.32629	0.2	2353.471
362	0.60813	0	2628.729	362	2.37483	4.2	789.89
363	1.77146	8	591.162	363	3.31954	1.8	1080.803
364	3.09638	5.2	443.566	364	4.10888	0.6	1230.307
365	2.208	0	2050.05	365	3.01629	0	953.437

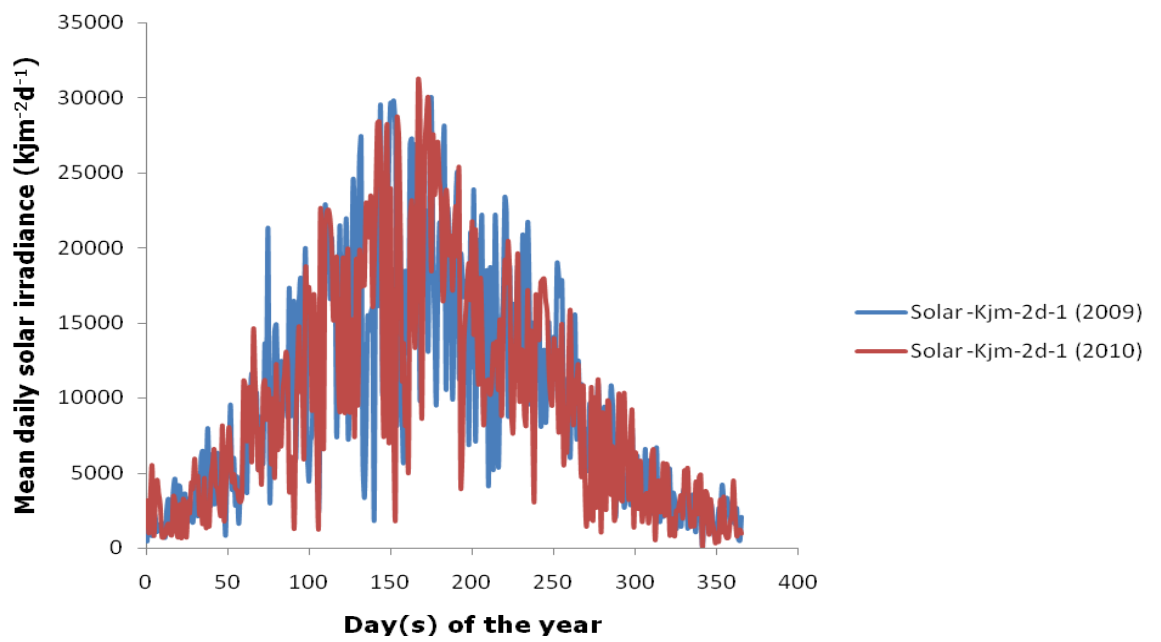
Mean daily air temperature (°C).



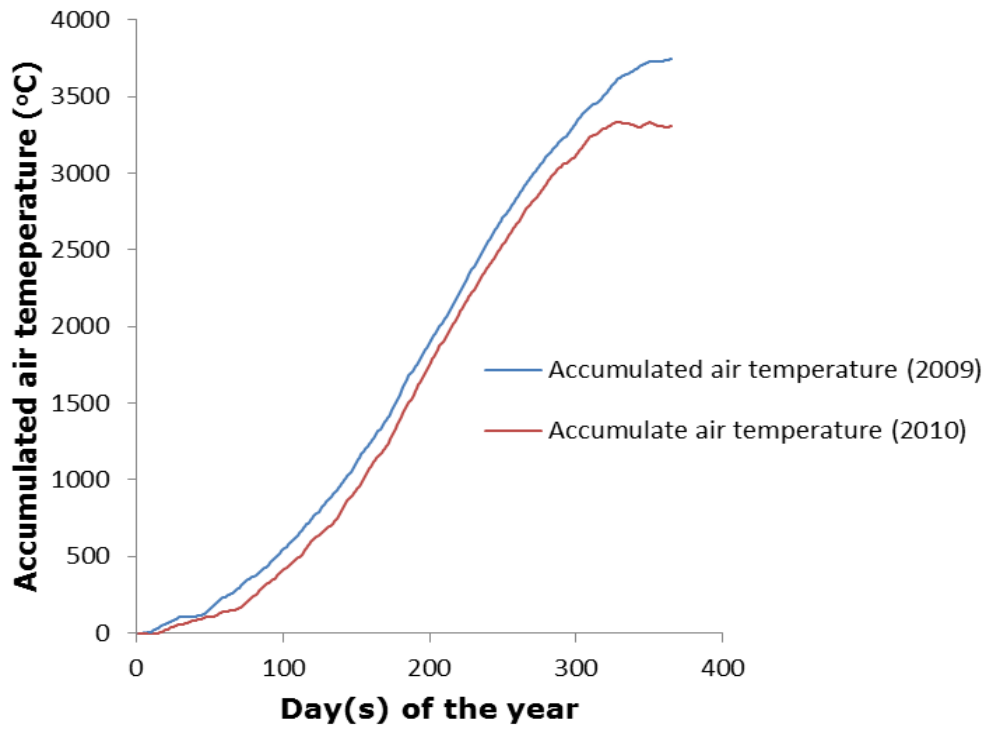
Daily mean rainfall (mm).



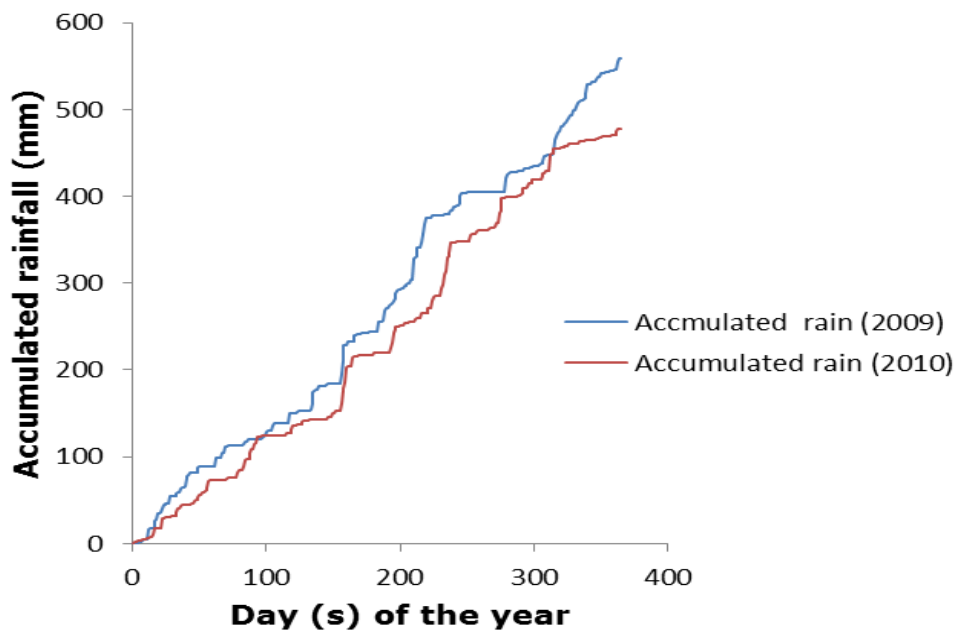
Mean daily solar irradiance ($\text{kJm}^{-2}\text{d}^{-1}$).



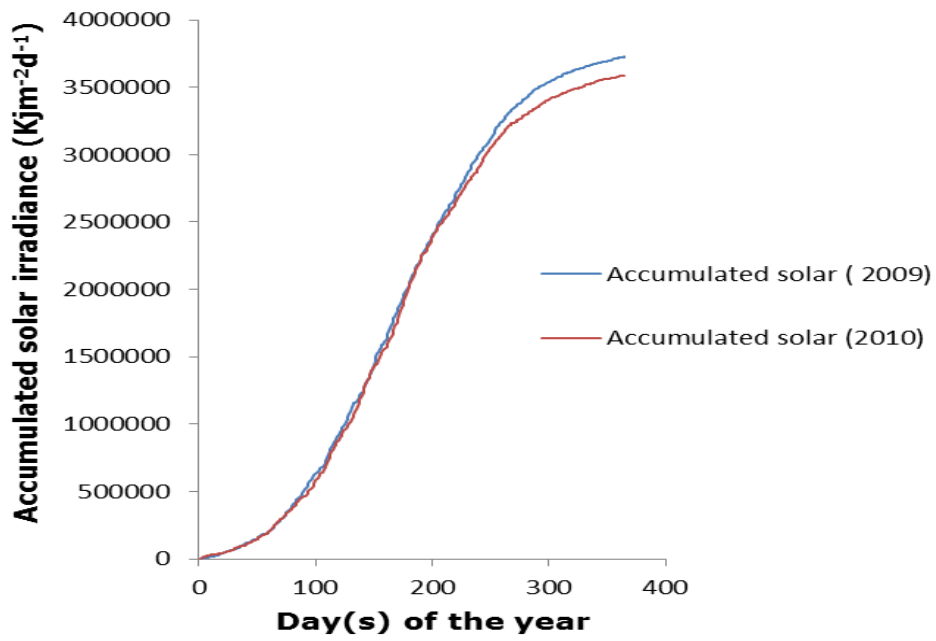
Accumulated air temperature (°C).



Accumulated rainfall (mm).



Accumulated solar irradiance ($\text{kJm}^{-2}\text{d}^{-1}$).



Appendix 8

Results of One-way ANOVA showing wavelengths in the visible region where reflectance differences between the three treatments (Control, low and high CO₂) were significant. Sample size 300.

Wavelength	F-ratio	P-value
450	0.074056	0.022566
451	0.074268	0.02261
452	0.074546	0.022703
453	0.074882	0.022811
454	0.075293	0.022919
455	0.075619	0.022999
456	0.075923	0.023064
457	0.0762	0.02311
458	0.076284	0.023103
459	0.076327	0.023122
460	0.076388	0.02315
461	0.076579	0.023165
462	0.076763	0.023162
463	0.076921	0.023154
464	0.077043	0.023143
465	0.077174	0.023115
466	0.07735	0.023088
467	0.077563	0.023062
468	0.077762	0.023024
469	0.077828	0.02298
470	0.077824	0.022941
471	0.07779	0.022924
472	0.077792	0.022919

473	0.077819	0.022926
474	0.07787	0.022944
475	0.077977	0.022955
476	0.078053	0.022951
477	0.078118	0.022944
478	0.078231	0.022974
479	0.078292	0.022996
480	0.078338	0.023011
481	0.078391	0.023016
482	0.078483	0.023021
483	0.07868	0.023006
484	0.078999	0.022969
485	0.079449	0.022971
486	0.079829	0.022982
487	0.080112	0.022994
488	0.080179	0.022993
489	0.080066	0.023042
490	0.079877	0.023105
491	0.079643	0.023167
492	0.079773	0.023181
493	0.080092	0.023225
494	0.080555	0.023297
495	0.08094	0.023353
496	0.081234	0.023437
497	0.081515	0.02355
498	0.081874	0.023704
499	0.08246	0.023851

500	0.083088	0.024008
501	0.083714	0.024179
502	0.084326	0.024373
503	0.08486	0.024612
504	0.085334	0.024893
505	0.085827	0.025223
506	0.086497	0.025579
507	0.087319	0.02598
508	0.088344	0.026467
509	0.08944	0.026998
510	0.09058	0.027565
511	0.091767	0.028172
512	0.092996	0.02886
513	0.094404	0.029591
514	0.095944	0.030349
515	0.097597	0.031121
516	0.099424	0.03193
517	0.101328	0.032839
518	0.103234	0.033903
519	0.105035	0.035122
520	0.106739	0.03643
521	0.108385	0.037822
522	0.110409	0.039374
523	0.112699	0.040976
524	0.115125	0.042551
525	0.117659	0.044006
526	0.120171	0.04547

527	0.12256	0.046971
528	0.124753	0.048517
529	0.126602	0.049016
530	0.128287	0.049097
531	0.129935	0.049901
532	0.132157	0.049031
533	0.134248	0.049061
534	0.136113	0.049084
535	0.137587	0.049074

Appendix 9

Results of One-way ANOVA showing wavelengths where reflectance differences between the three treatments (Control, low and high CO₂) are significant. Sample size 130.

Wavelegth	F-ratio	P-value
574	0.144068	0.049644
575	0.143415	0.048749
576	0.142697	0.047807
577	0.141888	0.046877
578	0.14104	0.045984
579	0.140275	0.045223
580	0.139518	0.044563
581	0.138826	0.04398
582	0.138249	0.043484
583	0.137858	0.042981
584	0.137556	0.042435
585	0.137318	0.041841
586	0.137029	0.041202
587	0.136742	0.040447
588	0.136513	0.049635
589	0.136475	0.048835
590	0.135734	0.048253
591	0.134781	0.047834
592	0.133754	0.047589
593	0.133636	0.047422
594	0.133495	0.047204
595	0.133282	0.04695
596	0.132836	0.046732
597	0.132461	0.046538
598	0.132145	0.046335
599	0.131896	0.046089
600	0.131753	0.04574
601	0.131756	0.045336
602	0.131922	0.04488
603	0.132027	0.044415
604	0.132227	0.043904

605	0.132448	0.043347
606	0.132472	0.042725
607	0.132077	0.042058
608	0.131552	0.04138
609	0.131049	0.040715
610	0.130661	0.040113
611	0.130248	0.039521
612	0.129805	0.038937
613	0.129435	0.03838
614	0.129022	0.037818
615	0.128591	0.037272
616	0.128198	0.036789
617	0.127658	0.036378
618	0.127141	0.036019
619	0.126718	0.035717
620	0.126531	0.03544
621	0.126478	0.03516
622	0.126531	0.034879
623	0.126559	0.034618
624	0.12662	0.034398
625	0.126677	0.034212
626	0.126689	0.034068
627	0.126906	0.033959
628	0.127163	0.033883
629	0.127427	0.033845
630	0.127692	0.033816
631	0.127971	0.033781
632	0.128227	0.033742
633	0.128327	0.033703
634	0.128341	0.033646
635	0.128282	0.033565
636	0.12813	0.033444
637	0.127814	0.0333
638	0.127472	0.033102
639	0.127108	0.032849
640	0.12655	0.032568

641	0.125793	0.032296
642	0.124944	0.032034
643	0.12414	0.031787
644	0.123403	0.031498
645	0.1227	0.031177
646	0.122018	0.030819
647	0.121298	0.030456
648	0.120667	0.030121
649	0.120115	0.029824
650	0.119536	0.029629
651	0.119168	0.029455
652	0.118913	0.029286
653	0.118694	0.029105
654	0.118771	0.02875
655	0.118859	0.028286
656	0.11888	0.027711
657	0.117683	0.02754
658	0.116982	0.027432
659	0.116667	0.027345
660	0.116441	0.027065
661	0.116119	0.026756
662	0.115779	0.026424
663	0.115474	0.026061
664	0.115114	0.025763
665	0.11479	0.025489
666	0.114522	0.025234
667	0.114364	0.025031
668	0.114221	0.024878
669	0.114118	0.024757
670	0.114129	0.024639
671	0.114182	0.024561
672	0.114245	0.024526
673	0.114301	0.024551
674	0.114551	0.024594
675	0.114797	0.024654
676	0.115031	0.024737

677	0.115347	0.024882
678	0.115702	0.025036
679	0.116104	0.02521
680	0.11659	0.025437
681	0.117148	0.025734
682	0.117826	0.026075
683	0.118667	0.026461
684	0.11968	0.026938
685	0.121172	0.027365
686	0.123192	0.027872
687	0.125288	0.028984
688	0.12704	0.030214
689	0.128903	0.031541
690	0.131573	0.033108
691	0.134748	0.03521
692	0.138356	0.037527
693	0.142534	0.040075
694	0.147319	0.043215
695	0.152358	0.046819
696	0.157575	0.040719
697	0.163411	0.044877
698	0.169596	0.049125
699	0.176042	0.043584
700	0.18264	0.048317

Appendix 10

Results of ANOVA showing wavelengths in NIR where reflectance differences between the three treatments (Control, low and high CO₂) are significant. Sample size 300.

Date	16/07/2009		23/07/2009		13/08/2009		18/08/2009		24/08/2009		08/09/2009		10/09/2009	
Wavelength	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
701	0.048131439	0.01272815	0.011368375	0.0114705	0.028618556	0.022207392	0.03401062	0.0147353	0.016596378	0.012504771	0.027589559	0.0227136	0.039537537	0.011532661
702	0.047990843	0.01246919	0.011012933	0.0113268	0.027858426	0.022203825	0.03354763	0.0148996	0.016814386	0.012646665	0.027985895	0.0230741	0.040210863	0.011413357
703	0.047749067	0.01228006	0.010806947	0.0112307	0.027008095	0.022167851	0.03333939	0.0150271	0.017050586	0.012800551	0.028443489	0.0233045	0.040741926	0.011337687
704	0.047275137	0.01230419	0.011217549	0.011302	0.026477632	0.02214668	0.03348449	0.0152532	0.017329727	0.012982285	0.028838686	0.0237752	0.041218372	0.011404647
705	0.047647142	0.01227682	0.01121195	0.0113321	0.026528999	0.0216857	0.03325265	0.0154758	0.017637609	0.013151452	0.029118577	0.0240917	0.041661895	0.011429252
706	0.047786254	0.0123602	0.011039039	0.0113314	0.026748714	0.021548078	0.03352182	0.0156916	0.017950285	0.013310738	0.029349878	0.0243295	0.042123226	0.0114265
707	0.047580119	0.01255551	0.010752304	0.0113004	0.027036952	0.021795868	0.03430461	0.0158907	0.018195746	0.013467547	0.029737344	0.0247054	0.042799439	0.011396623
708	0.04773058	0.01247571	0.010600173	0.0111979	0.026641509	0.02172463	0.03361402	0.0160941	0.018489738	0.013613827	0.030084384	0.0249862	0.043458923	0.011274449
709	0.047782666	0.01224313	0.010610297	0.0111812	0.026448394	0.021695018	0.03345692	0.0162779	0.018763912	0.01376696	0.030431174	0.0252927	0.04414696	0.011220835
710	0.047833703	0.01196088	0.010662172	0.0111866	0.026387721	0.021693002	0.03367049	0.0164213	0.01896664	0.013945761	0.030814858	0.0257298	0.044922091	0.011192174
711	0.048108443	0.01181688	0.010511007	0.011083	0.026381395	0.0217042	0.03413102	0.0165943	0.019274722	0.014106914	0.031116229	0.0261549	0.045569622	0.011103289
712	0.048010446	0.01179642	0.010433648	0.0110787	0.026378324	0.021619767	0.03427974	0.0167844	0.019558193	0.014255516	0.031488121	0.026559	0.046202016	0.011080206
713	0.047815278	0.01181112	0.010417477	0.0110902	0.026203421	0.021589495	0.03405262	0.0169899	0.019803319	0.014393488	0.031942528	0.0269449	0.046842259	0.011073587
714	0.047626729	0.01182764	0.010469103	0.0110825	0.02573724	0.02169234	0.03333957	0.017207	0.020061327	0.014535962	0.032332374	0.0273492	0.047606586	0.011063769
715	0.047456617	0.01199714	0.010460956	0.0109563	0.026141854	0.021758665	0.03311978	0.0173985	0.020302342	0.014679063	0.032665561	0.027724	0.048328106	0.010916356

Date	16/07/2009		23/07/2009		13/08/2009		18/08/2009		24/08/2009		08/09/2009		10/09/2009	
Wavelength	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
716	0.047260753	0.01196451	0.010394309	0.0108894	0.026242281	0.021639677	0.0328935	0.0175724	0.020532818	0.014821533	0.032950426	0.0280658	0.049042689	0.010835055
717	0.047058388	0.01175224	0.010296744	0.0108807	0.025980264	0.021377511	0.0326362	0.01774	0.020765601	0.014962968	0.033182881	0.0283545	0.049844063	0.010820066
718	0.04705904	0.0116916	0.010406559	0.0108391	0.025331466	0.021340303	0.03245958	0.0178986	0.020990149	0.015090904	0.033481938	0.0286649	0.030481229	0.01078136
719	0.047144193	0.01173343	0.010355154	0.0108692	0.025066253	0.021251772	0.03276422	0.0180464	0.02120515	0.015205236	0.033762063	0.0289829	0.030193483	0.010791078
720	0.047313678	0.01177775	0.010293758	0.0109037	0.025041813	0.021200566	0.0332305	0.0181803	0.021408686	0.01530295	0.033980717	0.0293082	0.029941599	0.010804124
721	0.047620401	0.01168242	0.010434171	0.0108478	0.025186501	0.021334552	0.03350658	0.0182902	0.021628696	0.015423887	0.034471234	0.0298484	0.02969239	0.010757279
722	0.048002639	0.01172196	0.01031129	0.0108051	0.025649708	0.021547801	0.03322736	0.0184216	0.021843436	0.015541	0.034847056	0.030301	0.029447757	0.010724064
723	0.048412789	0.01173124	0.010254546	0.0107811	0.025839491	0.021693794	0.0330788	0.018573	0.02205145	0.015650542	0.035098779	0.0306535	0.029234316	0.010700795
724	0.048844357	0.01165422	0.010375893	0.0107801	0.025525571	0.021716257	0.0332955	0.0187181	0.022257727	0.015737722	0.035347867	0.0309842	0.029046235	0.01068775
725	0.049289344	0.01163188	0.010104744	0.0107617	0.025995641	0.021988004	0.03365171	0.0188414	0.022421469	0.015824647	0.035521316	0.0312624	0.02888158	0.010687602
726	0.049679572	0.01157764	0.01006179	0.0107635	0.026171149	0.022068825	0.03394734	0.0189411	0.022585091	0.015906741	0.035642833	0.0314805	0.028749914	0.010681948
727	0.05000507	0.01151619	0.010195959	0.0107814	0.026109434	0.022024179	0.03411372	0.019007	0.022811212	0.015978468	0.035722952	0.0316071	0.028642817	0.010670733
728	0.041712022	0.0138631	0.013750944	0.0115327	0.026990067	0.017412111	0.03305286	0.0191013	0.023000397	0.016040092	0.03584331	0.0317895	0.028566149	0.010653579
729	0.041551472	0.01352313	0.013375099	0.0114134	0.02598323	0.017300424	0.03238116	0.0191789	0.023168249	0.016098856	0.03597655	0.0319904	0.02852474	0.010618668
730	0.041282555	0.01327904	0.01316359	0.0113377	0.024892263	0.017179152	0.03206075	0.0192253	0.023316309	0.016156388	0.036117123	0.0322028	0.028463326	0.01059207
731	0.040787424	0.01333317	0.013553965	0.0114046	0.024412602	0.017094347	0.03242715	0.0193092	0.023435063	0.016238273	0.036324249	0.0326208	0.028414182	0.010606192
732	0.040904378	0.01329051	0.013592561	0.0114293	0.024639208	0.017002438	0.03211328	0.0193917	0.023542192	0.016291825	0.036602848	0.0330467	0.028369997	0.010635719
733	0.040939854	0.01336722	0.013435155	0.0114265	0.02482642	0.01710989	0.03230425	0.0194704	0.023645876	0.016328484	0.036913525	0.0334536	0.028307993	0.010676395

Date	16/07/2009		23/07/2009		13/08/2009		18/08/2009		24/08/2009		08/09/2009		10/09/2009	
Wavelength	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
734	0.040813082	0.01356683	0.013122736	0.0113966	0.024864699	0.017395951	0.03304664	0.0195381	0.023779084	0.016388062	0.037123339	0.0337386	0.028233879	0.010726438
735	0.040855325	0.01339294	0.012895544	0.0112744	0.02463166	0.016947534	0.03214797	0.0195841	0.023923803	0.016435334	0.037316421	0.0340444	0.028136379	0.010712215
736	0.040957529	0.01315337	0.012897301	0.0112208	0.024548146	0.016937195	0.03185131	0.0196241	0.02405419	0.016481429	0.037494263	0.0343522	0.027997297	0.010694034
737	0.041109756	0.01292011	0.012978897	0.0111922	0.024531226	0.017110042	0.03200717	0.0196698	0.02414448	0.016536504	0.037655163	0.0346539	0.027836228	0.010685926
738	0.041329714	0.01284874	0.012856551	0.0111033	0.024433525	0.017012878	0.03257057	0.0197149	0.024286094	0.016591778	0.037884929	0.0349015	0.02763017	0.010738835
739	0.041087832	0.01277235	0.012782517	0.0110802	0.024365222	0.016918317	0.03273416	0.0197773	0.024412414	0.016649127	0.038098056	0.0352136	0.02737672	0.010724746
740	0.040823907	0.01268214	0.012765509	0.0110736	0.024118736	0.016882083	0.03252039	0.0198579	0.024513076	0.016708423	0.038282589	0.0356073	0.027099729	0.010725517
741	0.04073171	0.01256898	0.012821204	0.0110638	0.023556691	0.0169404	0.03185894	0.0198889	0.024642998	0.01676121	0.038526841	0.0359474	0.026816009	0.010802426
742	0.040504365	0.01275599	0.012807193	0.0109164	0.023920625	0.016980132	0.03141625	0.019932	0.024748979	0.016807641	0.038746928	0.0362754	0.026531056	0.01089989
743	0.040282138	0.01277391	0.012779248	0.0108351	0.023990599	0.016893912	0.0311385	0.0199794	0.024846041	0.016848687	0.038950132	0.0365933	0.026251871	0.010986367
744	0.040087469	0.01262746	0.012751977	0.0108201	0.023707448	0.016693827	0.0309909	0.0200123	0.024967935	0.016882734	0.039144114	0.0369003	0.025946593	0.011052865
745	0.040056549	0.01257305	0.012824054	0.0107814	0.023079966	0.016467741	0.03082334	0.0200869	0.025089134	0.016935142	0.039367892	0.0372214	0.025618094	0.011043787
746	0.040106395	0.01259611	0.01277522	0.0107911	0.022762132	0.01640901	0.03112687	0.0201771	0.025201235	0.016990609	0.039611631	0.0375768	0.025258815	0.01107826
747	0.040223712	0.01261212	0.012719234	0.0108041	0.022703666	0.016457035	0.03158332	0.0202726	0.025297231	0.017041402	0.039877454	0.0379914	0.024920477	0.011153656
748	0.040430533	0.01249525	0.012815341	0.0107573	0.022976817	0.016586309	0.03183763	0.0203293	0.025387562	0.01707305	0.040156845	0.0383527	0.024609184	0.011291299
749	0.040652245	0.01250516	0.012683111	0.0107241	0.023482883	0.016783983	0.03142951	0.0203747	0.025481847	0.017103662	0.04042341	0.0386754	0.024328463	0.011376722
750	0.040941962	0.01251313	0.012613246	0.0107008	0.023589227	0.01683261	0.03125407	0.0204091	0.025578937	0.017134232	0.040662562	0.0389644	0.024113555	0.011456913
751	0.041331295	0.01247894	0.012704966	0.0106878	0.023030723	0.016638481	0.03162788	0.0204024	0.025647728	0.017153959	0.040703798	0.0392312	0.023931456	0.011549355

Date	16/07/2009		23/07/2009		13/08/2009		18/08/2009		24/08/2009		08/09/2009		10/09/2009	
Wavelength	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
752	0.041662749	0.0124434	0.012486198	0.0106876	0.023611491	0.01699517	0.03194411	0.0204186	0.025749519	0.017172364	0.040743615	0.0394569	0.023761284	0.011668109
753	0.041975846	0.01238779	0.012480636	0.0106819	0.023836025	0.017094925	0.0321842	0.0204406	0.025859569	0.017187044	0.040817235	0.0396756	0.023577872	0.011777514
754	0.042237135	0.01232519	0.012635199	0.0106707	0.023769437	0.017004591	0.03231259	0.0204407	0.025937911	0.017191732	0.04100626	0.0399475	0.023309829	0.011881007
755	0.042232548	0.01232299	0.012693783	0.0106536	0.023804688	0.017082112	0.03208257	0.0204278	0.02601	0.017198632	0.0411298	0.0402433	0.022963008	0.012015948
756	0.042369084	0.01237288	0.012687872	0.0106187	0.023562886	0.017237897	0.03203239	0.0204144	0.026084695	0.017202533	0.041248135	0.0405287	0.022525558	0.012142642
757	0.042576418	0.01241713	0.012655556	0.0105921	0.023378449	0.017386511	0.03213911	0.0204052	0.026166161	0.017200905	0.041382818	0.0407894	0.02233022	0.012261707
758	0.042815608	0.01239065	0.012623542	0.0106062	0.023636478	0.017432313	0.03246205	0.0203775	0.026250865	0.017201103	0.041493515	0.0410323	0.022179374	0.012374153
759	0.042851164	0.01242837	0.01269924	0.0106357	0.023579738	0.017565658	0.03245941	0.020357	0.026320185	0.017198245	0.041601684	0.0413152	0.02204692	0.012504771
760	0.043034904	0.01244742	0.012761584	0.0106764	0.023619826	0.017678043	0.03246029	0.0203404	0.026380831	0.017193685	0.041711919	0.0416298	0.021791136	0.012646665
761	0.043451781	0.01242904	0.01278479	0.0107264	0.023842248	0.017743779	0.03253886	0.0203012	0.026476883	0.017196434	0.041847513	0.0419188	0.021507101	0.012800551
762	0.043980146	0.01242075	0.01286637	0.0107122	0.023756316	0.017747132	0.03287832	0.0202533	0.026556859	0.017186286	0.041921925	0.0421477	0.021208643	0.012982285
763	0.044332916	0.01236553	0.01297676	0.010694	0.023976661	0.018111126	0.03310099	0.0202098	0.026633878	0.01717763	0.041975653	0.042344	0.020900107	0.013151452
764	0.044562015	0.01232125	0.01308208	0.0106859	0.024352504	0.018598303	0.03327495	0.0201872	0.026720501	0.017187502	0.042044022	0.0425222	0.020636993	0.013310738
765	0.044760679	0.01247669	0.013076199	0.0107388	0.024524312	0.018541604	0.03358557	0.0201734	0.026802567	0.017176037	0.042104644	0.042704	0.020405942	0.013467547
766	0.044713157	0.01253471	0.013164108	0.0107247	0.024346525	0.018536518	0.03376634	0.0201653	0.02688367	0.017169569	0.042169661	0.0428949	0.02020652	0.013613827
767	0.04459302	0.01261433	0.013288915	0.0107255	0.024151899	0.018630957	0.03388811	0.0201627	0.026964308	0.017174819	0.042241691	0.0430972	0.020033794	0.01376696
768	0.044492585	0.01280351	0.013422072	0.0108024	0.024158334	0.018889855	0.03397559	0.0201665	0.027029153	0.017182786	0.042253687	0.0433184	0.01991005	0.013945761
769	0.04453487	0.01282367	0.013499654	0.0108999	0.024018705	0.018802425	0.03365218	0.0201524	0.027101699	0.017184631	0.042319382	0.0435385	0.019817758	0.014106914

Date	16/07/2009		23/07/2009		13/08/2009		18/08/2009		24/08/2009		08/09/2009		10/09/2009	
Wavelength	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
770	0.044722261	0.01287493	0.013663309	0.0109864	0.024095153	0.018862019	0.03378809	0.0201331	0.027178123	0.017183586	0.042414512	0.0437561	0.019730303	0.014255516
771	0.045068366	0.01296358	0.013897114	0.0110529	0.024384933	0.019111616	0.03439253	0.0201488	0.027248507	0.017189134	0.042473738	0.0439682	0.019683014	0.014393488
772	0.045879376	0.01290546	0.013852309	0.0110438	0.024462805	0.019524028	0.03461225	0.0201565	0.027284438	0.017181331	0.042528057	0.0441762	0.019675797	0.014535962
773	0.046339508	0.01301848	0.014017346	0.0110783	0.024708805	0.019842854	0.03500298	0.0201605	0.027320145	0.01717157	0.042583112	0.0443508	0.019720622	0.026990067
774	0.046592533	0.01320499	0.014265623	0.0111537	0.025016676	0.020048755	0.0354084	0.0201627	0.027381001	0.017167565	0.042643501	0.0444582	0.019782532	0.02598323
775	0.046786362	0.01334686	0.01443892	0.0112913	0.025255544	0.02006081	0.0355906	0.0201574	0.02743559	0.01718965	0.042704218	0.044648	0.019859822	0.024892263
776	0.047025781	0.01338697	0.014572903	0.0113767	0.025411134	0.020230419	0.03579996	0.020135	0.027497827	0.017205389	0.042812039	0.0449286	0.019957325	0.024412602
777	0.047131794	0.01346549	0.014743797	0.0114569	0.025451971	0.020335851	0.03584227	0.020097	0.027567388	0.0172113	0.042975128	0.0453035	0.020121961	0.024639208
778	0.047034517	0.01363723	0.014987029	0.0115494	0.02535689	0.020299731	0.0356381	0.0200953	0.02759895	0.017220887	0.043172524	0.0456944	0.020280142	0.02482642
779	0.046913511	0.01372095	0.015231416	0.0116681	0.025428008	0.020310848	0.03604056	0.0201095	0.027630642	0.017229259	0.043335693	0.0460603	0.020451218	0.024864699
780	0.046990703	0.01382872	0.015413465	0.0117775	0.02548255	0.020438502	0.03650222	0.0201258	0.027676443	0.017238076	0.043466991	0.046373	0.020673563	0.02463166
781	0.047263153	0.01396483	0.015555436	0.011881	0.025553773	0.02067752	0.03698082	0.0201136	0.027775371	0.017251369	0.043555672	0.0465405	0.020969235	0.024548146
782	0.047860726	0.01415974	0.015786787	0.0120159	0.025901393	0.021105526	0.03745148	0.0201392	0.027882223	0.01726324	0.043558276	0.0465692	0.021307149	0.024531226
783	0.04843291	0.01434108	0.016031475	0.0121426	0.02625015	0.021562242	0.03787456	0.0201721	0.027999626	0.017282728	0.043509784	0.0465554	0.021685848	0.024433525
784	0.049078538	0.01448603	0.01625033	0.0122617	0.026653575	0.021977984	0.03830011	0.0202	0.028130398	0.017315735	0.043413978	0.0465326	0.02215268	0.024365222
785	0.049958351	0.01455966	0.016399088	0.0123742	0.027207465	0.022276601	0.03879489	0.0202116	0.028287677	0.017360979	0.043511723	0.0468157	0.022622464	0.024118736
786	0.046077313	0.01481752	0.013957376	0.0156596	0.035736877	0.028326774	0.03924139	0.0202529	0.028414841	0.017407157	0.043694767	0.0471921	0.023197216	0.023556691
787	0.046069173	0.01461203	0.013718227	0.0155555	0.034833005	0.028279836	0.03883562	0.0203187	0.028518718	0.017457393	0.043946728	0.0476287	0.024271767	0.023920625

Date	16/07/2009		23/07/2009		13/08/2009		18/08/2009		24/08/2009		08/09/2009		10/09/2009	
Wavelength	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
788	0.045902177	0.0144622	0.013558198	0.0154699	0.033900037	0.028158141	0.03865276	0.0203581	0.028669941	0.01754279	0.044214492	0.0479807	0.025392112	0.023990599
789	0.045332853	0.01441751	0.013852462	0.0155081	0.033379275	0.027878967	0.03883319	0.0204409	0.028861638	0.017627997	0.044459262	0.048306	0.026582313	0.023707448
790	0.045471651	0.01434378	0.013797528	0.0155127	0.033581571	0.027716141	0.03861945	0.0205514	0.029071565	0.01772314	0.044723771	0.048631	0.028010137	0.023079966
791	0.045550153	0.01437099	0.013587991	0.0154822	0.033886554	0.027772094	0.03904124	0.0206823	0.029277095	0.01784576	0.045073267	0.0489894	0.029932058	0.022762132
792	0.04547276	0.01450098	0.013271781	0.0154212	0.034182157	0.028025383	0.04009496	0.0208207	0.029511717	0.017976089	0.04547218	0.0495482	0.032087815	0.022703666
793	0.045493914	0.0144588	0.013157928	0.0154029	0.034027291	0.027889978	0.03909118	0.0137777	0.013086101	0.015254084	0.036030936	0.0298429	0.034504328	0.022976817
794	0.045657069	0.01429293	0.013228051	0.0153835	0.033971934	0.027895615	0.0389188	0.013887	0.013066911	0.015290068	0.036235823	0.0298835	0.017412111	0.033052856
795	0.045881158	0.0140807	0.0133405	0.015353	0.033974612	0.027958249	0.03929	0.0139665	0.01310475	0.01534556	0.03612434	0.0297855	0.017300424	0.032381163
796	0.046031828	0.01395702	0.013206121	0.0152803	0.033978071	0.027926544	0.03991501	0.0140535	0.013185036	0.015411533	0.035937158	0.0297862	0.017179152	0.032060746
797	0.04581589	0.01398748	0.01308232	0.0152876	0.033900817	0.027899198	0.04008725	0.0141744	0.013311562	0.015484088	0.035810524	0.0300766	0.017094347	0.032427147
798	0.045586603	0.01397384	0.013043396	0.0152951	0.033573832	0.027871051	0.03990095	0.0142143	0.013380481	0.015628102	0.035755399	0.0298468	0.017002438	0.032113283
799	0.045500139	0.01381436	0.01314083	0.0152658	0.032861232	0.027838917	0.03932323	0.0142931	0.01346126	0.015739294	0.035975528	0.0298596	0.01710989	0.032304246
800	0.045159161	0.01398939	0.013120114	0.01513	0.033326438	0.027909745	0.03883133	0.0144129	0.013547607	0.015808637	0.036448944	0.0301762	0.017395951	0.033046643
801	0.044889225	0.01397757	0.013081239	0.015072	0.033510254	0.027813621	0.03862069	0.0144593	0.013477636	0.015886597	0.036774086	0.0306792	0.016947534	0.032147972
802	0.044713248	0.01379101	0.013041266	0.0150859	0.033328784	0.0275581	0.03862043	0.0145543	0.013589684	0.015976042	0.037219482	0.0310514	0.016937195	0.031851306
803	0.044698647	0.01378684	0.013089183	0.0150404	0.032716119	0.02726319	0.03824304	0.0146855	0.013784471	0.016092482	0.03767012	0.0313036	0.017110042	0.032007172
804	0.044768343	0.0138137	0.013007908	0.0150719	0.032427619	0.027193865	0.03852949	0.0148568	0.013947506	0.016281807	0.037957177	0.0314052	0.017012878	0.032570572
805	0.04492833	0.0138409	0.012948317	0.0151056	0.032381485	0.027267001	0.03901468	0.0149237	0.014023335	0.016401112	0.038168207	0.0315144	0.016918317	0.032734162

Date	16/07/2009		23/07/2009		13/08/2009		18/08/2009		24/08/2009		08/09/2009		10/09/2009	
Wavelength	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
806	0.04524317	0.01382501	0.013139067	0.0150352	0.032594938	0.027445719	0.0391596	0.0150162	0.014129223	0.016516899	0.038307561	0.0315545	0.016882083	0.032520387
807	0.045548007	0.01377753	0.013008638	0.0150247	0.033152593	0.027701942	0.03883195	0.0151843	0.014311963	0.016654693	0.03837285	0.0314945	0.0169404	0.031858935
808	0.04589482	0.01373405	0.012895457	0.0150259	0.033433343	0.027848609	0.03870858	0.0153415	0.014498797	0.016843039	0.038407157	0.0313743	0.016980132	0.031416246
809	0.046310422	0.01370583	0.012905993	0.0150256	0.033198009	0.027809311	0.03903364	0.0154948	0.014598469	0.017009762	0.038503861	0.0314878	0.016893912	0.031138499
810	0.046713005	0.01367765	0.012689581	0.0149863	0.033893023	0.027962661	0.03946971	0.0156446	0.014648435	0.017158197	0.038686472	0.0318065	0.016693827	0.030990905
811	0.047122474	0.01364396	0.01267744	0.0149862	0.034209339	0.028018217	0.03979557	0.0158072	0.014854047	0.01733079	0.039153827	0.0323143	0.016467741	0.030823339
812	0.047492666	0.01361646	0.01282455	0.0150127	0.034209177	0.0280031	0.03994445	0.0159597	0.015046855	0.017492069	0.039746544	0.0328418	0.01640901	0.031126871
813	0.047550057	0.01366055	0.012930096	0.0150048	0.034223945	0.028059075	0.03950345	0.0161017	0.015185063	0.017652832	0.040418248	0.0333396	0.016457035	0.031583321
814	0.047723143	0.01370781	0.012941178	0.0150061	0.03405224	0.028272653	0.03924901	0.0162328	0.015205685	0.017829186	0.041183021	0.0337607	0.016586309	0.031837634
815	0.047924415	0.01373522	0.012902655	0.015015	0.033956107	0.028484371	0.0391865	0.0164258	0.015335181	0.018005829	0.041827227	0.0342934	0.016783983	0.031429511
816	0.048073111	0.0137073	0.012836012	0.0150336	0.034253228	0.02852278	0.0394237	0.0166163	0.015496294	0.01820731	0.04245611	0.0347197	0.01683261	0.031254067
817	0.048194513	0.01372894	0.012849142	0.0150946	0.034240115	0.028610404	0.03937336	0.0167909	0.015674918	0.018443187	0.043097499	0.0349835	0.016638481	0.031627884
818	0.048457939	0.01373561	0.012860057	0.0151465	0.034331473	0.028715718	0.03937921	0.0170112	0.015857044	0.018637825	0.043576836	0.0354609	0.01699517	0.03194411
819	0.048908115	0.01371379	0.012852738	0.0151765	0.034619859	0.028833241	0.03951963	0.0172181	0.01605846	0.01885292	0.043991617	0.0358171	0.017094925	0.032184198
820	0.049423248	0.01374894	0.012917407	0.0151684	0.034903723	0.028965889	0.03980175	0.0174254	0.016266243	0.019079873	0.044387811	0.0361119	0.017004591	0.032312592
821	0.049875011	0.01376122	0.013004993	0.0152003	0.035430727	0.029363601	0.04003587	0.0176857	0.016444757	0.019301441	0.044922498	0.0365219	0.017082112	0.032082567
822	0.014875747	0.01555405	0.016964258	0.0419117	0.035988518	0.047927799	0.01532559	0.0160349	0.019118147	0.328657106	0.222090531	0.211856	0.017237897	0.032032388
823	0.014592063	0.0151538	0.016893827	0.041144	0.035873284	0.047958499	0.01508543	0.0157488	0.019086718	0.334765129	0.224116702	0.2127062	0.017386511	0.032139112

Date	16/07/2009		23/07/2009		13/08/2009		18/08/2009		24/08/2009		08/09/2009		10/09/2009	
Wavelength	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
824	0.014435362	0.01492816	0.01685505	0.040308	0.035714445	0.047931518	0.01496747	0.0156102	0.019070179	0.34071501	0.226127114	0.21365	0.017432313	0.032462054
825	0.01458154	0.01533391	0.016851463	0.0397209	0.035371921	0.04737385	0.01507595	0.0159907	0.019067727	0.346718054	0.22836215	0.2147978	0.017565658	0.032459405
826	0.01455496	0.01535193	0.016853217	0.0397741	0.035230479	0.047632537	0.01503549	0.0159709	0.01903279	0.352754798	0.230529286	0.2158441	0.017678043	0.03246029
827	0.014589774	0.01518075	0.016893107	0.0398574	0.035226346	0.047744498	0.01505347	0.0157633	0.019072692	0.35872652	0.232643899	0.2169089	0.017743779	0.032538861
828	0.014704522	0.01486982	0.016965433	0.0398781	0.035329382	0.047597257	0.01514449	0.0154208	0.019185981	0.364540305	0.234691374	0.2180038	0.017747132	0.03287832
829	0.014624309	0.01471736	0.016874949	0.0397287	0.035205072	0.047610426	0.01503048	0.0152774	0.019067773	0.370309848	0.23686653	0.2192589	0.01811126	0.033100994
830	0.014379798	0.01478364	0.016845202	0.039652	0.035280041	0.047700527	0.01479493	0.015327	0.019091677	0.375787996	0.238859817	0.220515	0.018598303	0.03327495
831	0.014096455	0.01491238	0.016823175	0.039612	0.03544135	0.04784429	0.01452991	0.0154351	0.019145259	0.380957335	0.240700312	0.2216761	0.018541604	0.033585568
832	0.014020133	0.01479487	0.016691547	0.0395474	0.035496298	0.048030629	0.01441204	0.0153396	0.018993855	0.386096731	0.24252506	0.2226195	0.018536518	0.033766344
833	0.014003179	0.01471843	0.016709117	0.0395176	0.03535768	0.047870636	0.01444468	0.0153112	0.01908609	0.391333245	0.244383059	0.223564	0.018630957	0.033888112
834	0.013978859	0.01465885	0.01676621	0.0393204	0.035241962	0.04767039	0.01444969	0.015301	0.019194473	0.396416843	0.246126063	0.2244951	0.018889855	0.033975586
835	0.013914041	0.01460706	0.016821333	0.0388244	0.035250537	0.047559317	0.0143386	0.0152899	0.019218906	0.401094757	0.247614753	0.225382	0.018802425	0.033652176
836	0.014118735	0.01456677	0.016649234	0.0390297	0.035167619	0.047171312	0.0144894	0.0152554	0.019094663	0.40583802	0.249338843	0.2265079	0.018862019	0.033788088
837	0.014133155	0.01454242	0.01656335	0.0390255	0.034938352	0.046921949	0.01446929	0.0152585	0.019059366	0.410348915	0.250928324	0.227524	0.019111616	0.034392532
838	0.013968823	0.0145399	0.016571083	0.0387603	0.034611683	0.04682395	0.01429026	0.0152964	0.019116706	0.414613552	0.252376635	0.2284279	0.019524028	0.034612247
839	0.013927953	0.01462914	0.016591056	0.0381774	0.034598481	0.04683734	0.01425676	0.0153241	0.019200761	0.41876968	0.253871601	0.2293996	0.019842854	0.035002978
840	0.013928947	0.0146458	0.016650314	0.0378546	0.034587434	0.046905415	0.01431052	0.0153138	0.019319903	0.4225787	0.255102906	0.2301455	0.020048755	0.035408402
841	0.013938727	0.01466545	0.016693402	0.0378142	0.034614615	0.047051811	0.01436481	0.0153148	0.01943045	0.426143192	0.256231871	0.23082	0.02006081	0.035590597

Date	16/07/2009		23/07/2009		13/08/2009		18/08/2009		24/08/2009		08/09/2009		10/09/2009	
Wavelength	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
842	0.013914478	0.01479988	0.016632463	0.0382474	0.034753912	0.047359243	0.0142959	0.0154047	0.019467835	0.429573856	0.257473648	0.2316295	0.020230419	0.035799956
843	0.01389908	0.0146749	0.016595064	0.0387952	0.035033607	0.047701105	0.01430874	0.0153263	0.019484474	0.432731628	0.258520875	0.232305	0.020335851	0.03584227
844	0.013881758	0.01455719	0.016607746	0.0390368	0.035204766	0.048111274	0.01432084	0.0153026	0.01953118	0.435757689	0.259504821	0.2329687	0.020299731	0.035638104
845	0.013858675	0.01452943	0.016686748	0.0387905	0.035171975	0.048616143	0.01430657	0.0154109	0.019628882	0.438689068	0.2604599	0.2336578	0.020310848	0.036040557
846	0.013863221	0.01436531	0.016675364	0.0394075	0.035629307	0.049039666	0.01425925	0.0152563	0.019706985	0.441391498	0.261409935	0.2342628	0.020438502	0.036502223
847	0.013797482	0.01435809	0.016690686	0.0397072	0.035737379	0.049481886	0.01421319	0.015309	0.019827766	0.443915963	0.262285411	0.2349403	0.02067752	0.03698082
848	0.013699545	0.01448036	0.016726199	0.039755	0.035622786	0.049893476	0.01417904	0.0155162	0.019986234	0.446278818	0.263096638	0.2356528	0.021105526	0.037451483
849	0.01376235	0.01462484	0.016741827	0.0399163	0.035932047	0.049976994	0.01421439	0.0156274	0.020165534	0.448500305	0.263875496	0.2362186	0.021562242	0.037874565
850	0.01384712	0.01466393	0.016772451	0.039796	0.035991884	0.488249823	0.46267005	0.4103366	0.462578461	0.450480662	0.264605019	0.2367808	0.021977984	0.038300106
851	0.013908003	0.01465551	0.016815357	0.0397201	0.036000195	0.489999808	0.46676412	0.4143043	0.466785163	0.452334613	0.265303332	0.2373547	0.022276601	0.038794888
852	0.013882651	0.01463584	0.016873864	0.0400572	0.036136321	0.491626434	0.47013573	0.4179432	0.470681883	0.454124138	0.265978228	0.2379629	0.022713612	0.039537537
853	0.013918355	0.0146865	0.016932067	0.0401409	0.036290174	0.493050843	0.47241972	0.4212563	0.474218592	0.455793045	0.266626552	0.2385288	0.023074094	0.040210863
854	0.013912985	0.01471913	0.01702194	0.0403344	0.03641333	0.49371966	0.47148921	0.4245119	0.477676928	0.456766501	0.266673457	0.2387343	0.023304536	0.040741926
855	0.013844617	0.01471507	0.017149467	0.0407142	0.036498655	0.493475787	0.46700696	0.4278465	0.481213592	0.456875123	0.265944324	0.2384705	0.023775182	0.041218372
856	0.013897717	0.01478449	0.017172028	0.0408634	0.036846072	0.493103825	0.46699803	0.4299815	0.483715534	0.455914922	0.264506165	0.2372937	0.024091688	0.041661895
857	0.013889995	0.01488644	0.01722058	0.0413178	0.037356616	0.497219339	0.46851201	0.4318701	0.485460415	0.458831936	0.266695987	0.2380093	0.024329482	0.042123226
858	0.01388238	0.01500382	0.017298847	0.04192	0.037834331	0.028884355	0.024825728	0.014014	0.027099	0.029335	0.031527	0.026828	0.024705392	0.042799439
859	0.014066998	0.01509776	0.017442308	0.0422907	0.037646741	0.029309459	0.025258698	0.014194	0.027499	0.029783	0.032030	0.027229	0.024986176	0.043458923

Date	16/07/2009		23/07/2009		13/08/2009		18/08/2009		24/08/2009		08/09/2009		10/09/2009	
Wavelength	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
860	0.014172098	0.01520646	0.01753433	0.042356	0.037520854	0.029645676	0.025738606	0.014459	0.027847	0.030223	0.032549	0.027558	0.025292687	0.04414696
861	0.014275152	0.01531721	0.017640964	0.0423336	0.037494281	0.029989913	0.025962412	0.014463	0.028106	0.030452	0.032852	0.027552	0.025729802	0.044922091
862	0.01442591	0.01541891	0.017810313	0.0423379	0.037626585	0.030208187	0.026144393	0.014526	0.028337	0.030753	0.033198	0.027652	0.026154878	0.045569622
863	0.01450268	0.01549932	0.018008575	0.0423565	0.037649707	0.030397613	0.026350271	0.014633	0.028558	0.031089	0.033560	0.027842	0.026558952	0.046202016
864	0.014559374	0.01564662	0.018184911	0.0425963	0.037794623	0.030878108	0.026825594	0.014764	0.028832	0.031365	0.033863	0.028127	0.026944865	0.046842259
865	0.014600859	0.01584653	0.018331227	0.0430464	0.038082939	0.031217068	0.027131917	0.014934	0.029162	0.031740	0.034289	0.028570	0.027349236	0.047606586
866	0.014623415	0.01584061	0.018458868	0.0434265	0.038570188	0.031519677	0.027422648	0.015111	0.029491	0.032140	0.034746	0.029026	0.037318	0.049704686
867	0.014745046	0.01598417	0.018641486	0.0440064	0.038906962	0.031856533	0.027811347	0.015277	0.029782	0.032530	0.035191	0.029402	0.037708	0.049576409
868	0.014918806	0.01620282	0.018851882	0.0446097	0.039132701	0.032234877	0.028098974	0.015352	0.030106	0.032833	0.035573	0.029821	0.038065	0.049494572
869	0.015100442	0.01642101	0.019067951	0.0449807	0.039267021	0.032596748	0.028443489	0.015480	0.030453	0.033182	0.035996	0.030005	0.038246	0.049429599
870	0.01519342	0.01656271	0.019289364	0.0453614	0.039300023	0.032937560	0.028855016	0.015668	0.030813	0.033585	0.036468	0.029948	0.038257	0.049280401
871	0.015315353	0.01674404	0.019521031	0.0456331	0.03926692	0.033281043	0.029123573	0.015711	0.031031	0.033900	0.036886	0.030154	0.038496	0.049146194
872	0.015513337	0.01701466	0.019772426	0.045752	0.03917212	0.033625070	0.029417485	0.015769	0.031229	0.034209	0.037188	0.030461	0.038796	0.049037192
873	0.015633204	0.01720223	0.020032281	0.0459504	0.039149144	0.033932675	0.029699136	0.015842	0.031411	0.034498	0.037423	0.030827	0.039139	0.049011368
874	0.015779867	0.01734974	0.020291807	0.0461898	0.039282678	0.034105323	0.029882506	0.015941	0.031575	0.034727	0.037670	0.031197	0.039515	0.048980758
875	0.015949097	0.0174801	0.02054773	0.0464997	0.039559938	0.034238156	0.030056184	0.016023	0.031794	0.034943	0.037938	0.031454	0.039799	0.048952285
876	0.016124414	0.0177117	0.02081194	0.0471428	0.040048461	0.034428608	0.030281356	0.016096	0.032002	0.035162	0.038192	0.031636	0.040020	0.048933040
877	0.0162959	0.01793024	0.021041948	0.0478789	0.040576419	0.034740679	0.030603768	0.016164	0.032170	0.035397	0.038412	0.031741	0.040182	0.048888244

Date	16/07/2009		23/07/2009		13/08/2009		18/08/2009		24/08/2009		08/09/2009		10/09/2009	
Wavelength	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
878	0.016443769	0.01811958	0.021268299	0.0486973	0.041088425	0.035150494	0.030916831	0.016251	0.032479	0.035771	0.038817	0.031906	0.040428	0.048780378
879	0.016542268	0.01825643	0.021527848	0.049656	0.041538342	0.035537433	0.031217072	0.016382	0.032776	0.036143	0.039243	0.032118	0.040719	0.048597109
880	0.016733016	0.01843917	0.021798058	0.0505043	0.042095978	0.035970664	0.031488735	0.016535	0.033034	0.036479	0.039645	0.032360	0.041038	0.048396431
881	0.01691385	0.0186449	0.02209361	0.0513215	0.042545965	0.035982493	0.031623378	0.016529	0.033145	0.036580	0.039739	0.032534	0.041280	0.048139319
882	0.017062242	0.0188722	0.022422978	0.0521294	0.042833748	0.035986982	0.031632148	0.016527	0.033186	0.036652	0.039794	0.032740	0.041498	0.047848132
883	0.01732763	0.01911581	0.022731102	0.0528504	0.043326881	0.035952184	0.031603150	0.016499	0.033161	0.036677	0.039796	0.032878	0.041627	0.047576640
884	0.017577937	0.01936859	0.023065296	0.0534614	0.043685329	0.035947375	0.031632993	0.016393	0.033043	0.036605	0.039704	0.032788	0.041542	0.047337115
885	0.017814143	0.01962163	0.023411099	0.0540244	0.043973297	0.035858311	0.031597596	0.016358	0.032923	0.036544	0.039611	0.032590	0.041361	0.047084883
886	0.018037657	0.01985073	0.023733576	0.0547379	0.044375949	0.035799351	0.031542491	0.016310	0.032837	0.036523	0.039557	0.032441	0.041264	0.046790585
887	0.018240647	0.02013847	0.024036137	0.0554413	0.044742632	0.035804294	0.031477600	0.016226	0.032798	0.036555	0.039558	0.032388	0.041311	0.046488333
888	0.018424249	0.02040485	0.024341271	0.0561461	0.045130908	0.036134835	0.031730991	0.016338	0.033052	0.036835	0.039827	0.032514	0.041575	0.046173036
889	0.018586423	0.02058878	0.024673515	0.0568763	0.045599487	0.036434926	0.031952664	0.016436	0.033318	0.037110	0.040123	0.032774	0.041919	0.045852002
890	0.018774756	0.02085006	0.02497942	0.057552	0.046153012	0.036681149	0.032128301	0.016493	0.033546	0.037351	0.040404	0.033097	0.042290	0.045607142
891	0.018984373	0.02108713	0.025282571	0.0582833	0.046669829	0.036768962	0.032195736	0.016382	0.033529	0.037441	0.040501	0.033228	0.042507	0.045375217
892	0.019212866	0.02128965	0.025585729	0.0590815	0.047139976	0.036878224	0.032276176	0.016405	0.033618	0.037509	0.040567	0.033360	0.042683	0.045150258
893	0.019427056	0.02150187	0.025874418	0.0598648	0.047633826	0.036981709	0.032356571	0.016445	0.033703	0.037566	0.040610	0.033429	0.042813	0.044929698
894	0.019636531	0.02171094	0.026168983	0.0605238	0.048092565	0.037055347	0.032427277	0.016414	0.033691	0.037619	0.040628	0.033349	0.042870	0.044757962
895	0.019837141	0.02191853	0.026463778	0.0611185	0.048519813	0.037143528	0.032452706	0.016384	0.033768	0.037691	0.040694	0.033312	0.042906	0.044569243

Date	16/07/2009		23/07/2009		13/08/2009		18/08/2009		24/08/2009		08/09/2009		10/09/2009	
Wavelength	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
896	0.020018176	0.02213159	0.026751915	0.0617589	0.048914402	0.037278276	0.032510336	0.016391	0.033885	0.037802	0.040796	0.033330	0.043024	0.044336934
897	0.020184936	0.02231622	0.027024898	0.0623437	0.049332194	0.037461895	0.032612927	0.016437	0.034032	0.037952	0.040935	0.033411	0.043241	0.044108391
898	0.020340773	0.02249558	0.027275576	0.0629109	0.049767792	0.037567906	0.032727204	0.016447	0.034173	0.038108	0.041150	0.033628	0.043490	0.043937948
899	0.014735339	0.01659638	0.012504771	0.0275896	0.022713612	0.037653573	0.032773808	0.016443	0.034246	0.038183	0.041221	0.033757	0.043660	0.043819055
900	0.014899641	0.01681439	0.012646665	0.0279859	0.023074094	0.037731074	0.032788958	0.016438	0.034283	0.038210	0.041214	0.033827	0.043782	0.043721721
901	0.015027138	0.01705059	0.012800551	0.0284435	0.023304536	0.037827957	0.032848142	0.016465	0.034344	0.038242	0.041256	0.033876	0.043906	0.043663546
902	0.015253154	0.01732973	0.012982285	0.0288387	0.023775182	0.037937868	0.032900795	0.016471	0.034460	0.038368	0.041378	0.033921	0.044055	0.043627292
903	0.015475781	0.01763761	0.013151452	0.0291186	0.024091688	0.038093261	0.032983560	0.016492	0.034616	0.038544	0.041558	0.033977	0.044231	0.043604057
904	0.015691618	0.01795029	0.013310738	0.0293499	0.024329482	0.038328689	0.033128135	0.016551	0.034816	0.038762	0.041803	0.034056	0.044445	0.0495011
905	0.015890673	0.01819575	0.013467547	0.0297373	0.024705392	0.038469411	0.033237554	0.016564	0.034969	0.038848	0.041912	0.034196	0.044681	0.0492608
906	0.016094113	0.01848974	0.013613827	0.0300844	0.024986176	0.038608700	0.033346366	0.016571	0.035096	0.038914	0.041995	0.034301	0.044873	0.0490218
907	0.016277944	0.01876391	0.01376696	0.0304312	0.025292687	0.038742092	0.033445265	0.016571	0.035189	0.038970	0.042062	0.034365	0.045017	0.0487648
908	0.016421345	0.01896664	0.013945761	0.0308149	0.025729802	0.038697790	0.033372153	0.016480	0.035129	0.039030	0.042085	0.034412	0.045118	0.0484752
909	0.016594283	0.01927472	0.014106914	0.0311162	0.026154878	0.038670935	0.033307642	0.016460	0.035128	0.039002	0.042032	0.034445	0.045249	0.0481890
910	0.016784381	0.01955819	0.014255516	0.0314881	0.026558952	0.038672224	0.033268202	0.016461	0.035154	0.038961	0.041980	0.034456	0.045369	0.0478674
911	0.016989904	0.01980332	0.014393488	0.0319425	0.026944865	0.038735740	0.033296175	0.016403	0.035161	0.039034	0.042063	0.034420	0.045401	0.0475165
912	0.017206992	0.02006133	0.014535962	0.0323324	0.027349236	0.038774375	0.033310786	0.016349	0.035184	0.039086	0.042100	0.034327	0.045406	0.0472501

Date	16/07/2009		23/07/2009		13/08/2009		18/08/2009		24/08/2009		08/09/2009		10/09/2009	
Wavelength	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
913	0.017398468	0.02030234	0.014679063	0.0326656	0.027724031	0.038809501	0.033314615	0.016317	0.035199	0.039100	0.042104	0.034265	0.045439	0.0469788
914	0.017572383	0.02053282	0.014821533	0.0329504	0.028065784	0.038848333	0.033306357	0.016321	0.035195	0.039062	0.042074	0.034273	0.045525	0.0467053
915	0.017739951	0.0207656	0.014962968	0.0331829	0.0283545	0.038825389	0.033236738	0.016326	0.035237	0.039074	0.042077	0.034352	0.045673	0.0464394
916	0.015999	0.036939	0.026764	0.028066	0.024457	0.038873997	0.033233013	0.016314	0.035296	0.039107	0.042109	0.034429	0.045856	0.0462297
917	0.016015	0.036729	0.026781	0.028164	0.024296	0.038981073	0.033285648	0.016291	0.035366	0.039157	0.042160	0.034504	0.046064	0.0460175
918	0.016173	0.036562	0.026797	0.028228	0.024229	0.039048459	0.033319127	0.016295	0.035406	0.039205	0.042190	0.034598	0.046256	0.0457819
919	0.015965	0.036253	0.026202	0.027691	0.024225	0.039056182	0.033305682	0.016216	0.035367	0.039197	0.042150	0.034493	0.046290	0.0455436
920	0.016463	0.036683	0.026946	0.028237	0.024303	0.039021119	0.033250410	0.016118	0.035300	0.039161	0.042092	0.034326	0.046258	0.0453746
921	0.016583	0.037002	0.027229	0.028540	0.024127	0.038942665	0.033140030	0.016065	0.035251	0.039121	0.042067	0.034227	0.046232	0.0452635
922	0.016198	0.037063	0.026837	0.028418	0.023722	0.038845357	0.033024449	0.016065	0.035214	0.039066	0.042009	0.034181	0.046292	0.0451469
923	0.015597	0.036165	0.026358	0.027784	0.024460	0.038791746	0.032937776	0.016031	0.035175	0.039005	0.041929	0.034137	0.046349	0.0450666
924	0.015552	0.035999	0.026238	0.027642	0.024512	0.038800009	0.032890577	0.015940	0.035127	0.038941	0.041827	0.034086	0.046384	0.0450180
925	0.015786	0.036203	0.026297	0.027763	0.024246	0.038772941	0.032843750	0.015865	0.035083	0.038971	0.041820	0.034129	0.046560	0.0450113
926	0.015863	0.036189	0.026239	0.027787	0.024284	0.038741697	0.032807939	0.015822	0.035071	0.038945	0.041780	0.034130	0.046689	0.0449724
927	0.016331	0.036606	0.026532	0.027895	0.024353	0.038723823	0.032787532	0.015796	0.035080	0.038892	0.041734	0.034093	0.046781	0.0449350
928	0.016527	0.036806	0.026700	0.027947	0.024481	0.038784496	0.032804433	0.015752	0.035082	0.038892	0.041780	0.034012	0.046852	0.0449071
929	0.016097	0.036449	0.026502	0.027870	0.024697	0.038783859	0.032757644	0.015733	0.035091	0.038875	0.041759	0.033942	0.046902	0.0448739
930	0.015504	0.035960	0.025910	0.027442	0.024267	0.038761634	0.032699171	0.015709	0.035080	0.038858	0.041718	0.033880	0.046975	0.0448692

Date	16/07/2009		23/07/2009		13/08/2009		18/08/2009		24/08/2009		08/09/2009		10/09/2009	
Wavelength	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
931	0.015369	0.035800	0.025729	0.027291	0.023963	0.038739000	0.032662865	0.015658	0.035018	0.038851	0.041685	0.033828	0.047110	0.0448758
932	0.015623	0.035918	0.025901	0.027401	0.023826	0.038690232	0.032578789	0.015654	0.035044	0.038840	0.041657	0.033847	0.047292	0.0448425
933	0.015548	0.035746	0.025630	0.027405	0.023840	0.038721543	0.032566473	0.015634	0.035085	0.038839	0.041645	0.033919	0.047512	0.0447844
934	0.015636	0.035754	0.025537	0.027319	0.023797	0.038852632	0.032643892	0.015595	0.035144	0.038862	0.041662	0.034041	0.047781	0.0446953
935	0.015825	0.035905	0.025637	0.027313	0.023869	0.039107513	0.032798558	0.015621	0.035345	0.039059	0.041847	0.034192	0.048185	0.0445576
936	0.016087	0.036230	0.026067	0.027681	0.024371	0.039284740	0.032885812	0.015646	0.035506	0.039224	0.042052	0.034303	0.048518	0.0443369
937	0.016080	0.036297	0.026160	0.027671	0.024573	0.039378524	0.032900702	0.015666	0.035622	0.039341	0.042216	0.034358	0.048779	0.0441214
938	0.016045	0.036364	0.026229	0.027671	0.024842	0.039335486	0.032797307	0.015666	0.035658	0.039351	0.042177	0.034292	0.048928	0.0439197
939	0.016054	0.036515	0.026369	0.027804	0.025306	0.039203942	0.032692872	0.015569	0.035495	0.039157	0.041944	0.034119	0.048899	0.03208094
940	0.016115	0.036678	0.026397	0.027854	0.025447	0.039027825	0.032557618	0.015457	0.035320	0.038960	0.041704	0.033970	0.048906	0.03288826
941	0.016248	0.036975	0.026513	0.028057	0.025561	0.038817298	0.032371677	0.015362	0.035217	0.038847	0.041541	0.033910	0.049057	0.03362767
942	0.016447	0.037362	0.026726	0.028388	0.025676	0.038888972	0.032395564	0.015423	0.035399	0.038934	0.041668	0.034021	0.049405	0.03430136
943	0.016727	0.037647	0.027115	0.028746	0.025898	0.039053798	0.032478068	0.015489	0.035616	0.039095	0.041862	0.034173	0.049809	0.03488187
944	0.017050	0.037813	0.027319	0.028825	0.025989	0.038670935	0.033307642	0.016460	0.035128	0.039002	0.042032	0.034445	0.045249	0.03542204
945	0.017209	0.037931	0.027439	0.028867	0.026131	0.038672224	0.033268202	0.016461	0.035154	0.038961	0.041980	0.034456	0.045369	0.03618051
946	0.016893	0.038051	0.027534	0.029108	0.026540	0.038735740	0.033296175	0.016403	0.035161	0.039034	0.042063	0.034420	0.045401	0.03691069
947	0.016758	0.038083	0.027664	0.029240	0.026721	0.038774375	0.033310786	0.016349	0.035184	0.039086	0.042100	0.034327	0.045406	0.03755726
948	0.016712	0.038185	0.027756	0.029314	0.026877	0.038809501	0.033314615	0.016317	0.035199	0.039100	0.042104	0.034265	0.045439	0.03803585

Date	16/07/2009		23/07/2009		13/08/2009		18/08/2009		24/08/2009		08/09/2009		10/09/2009	
Wavelength	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
949	0.016755	0.038405	0.027808	0.029346	0.027052	0.038848333	0.033306357	0.016321	0.035195	0.039062	0.042074	0.034273	0.045525	0.03839761
950	0.017413	0.039190	0.028639	0.029916	0.027561	0.038825389	0.033236738	0.016326	0.035237	0.039074	0.042077	0.034352	0.045673	0.03875707
951	0.017787	0.039836	0.029135	0.030385	0.028041	0.038873997	0.033233013	0.016314	0.035296	0.039107	0.042109	0.034429	0.045856	0.03913767
952	0.017916	0.040262	0.029362	0.030704	0.028479	0.038981073	0.033285648	0.016291	0.035366	0.039157	0.042160	0.034504	0.046064	0.03951759
953	0.017775	0.040074	0.029355	0.030620	0.028823	0.039048459	0.033319127	0.016295	0.035406	0.039205	0.042190	0.034598	0.046256	0.03982440
954	0.017972	0.040315	0.029643	0.030986	0.028891	0.039056182	0.033305682	0.016216	0.035367	0.039197	0.042150	0.034493	0.046290	0.04007478
955	0.017984	0.040440	0.029807	0.031191	0.028865	0.039021119	0.033250410	0.016118	0.035300	0.039161	0.042092	0.034326	0.046258	0.04029063
956	0.017401	0.040043	0.029524	0.030771	0.028836	0.038942665	0.033140030	0.016065	0.035251	0.039121	0.042067	0.034227	0.046232	0.04040501
957	0.017491	0.040275	0.029661	0.030894	0.029019	0.038845357	0.033024449	0.016065	0.035214	0.039066	0.042009	0.034181	0.046292	0.04052285
958	0.017774	0.040697	0.029969	0.031215	0.029257	0.038791746	0.032937776	0.016031	0.035175	0.039005	0.041929	0.034137	0.046349	0.04071400
959	0.018161	0.041246	0.030406	0.031672	0.029560	0.038800009	0.032890577	0.015940	0.035127	0.038941	0.041827	0.034086	0.046384	0.04090432
960	0.018262	0.041819	0.030883	0.032019	0.030382	0.038772941	0.032843750	0.015865	0.035083	0.038971	0.041820	0.034129	0.046560	0.04109924
961	0.018300	0.042449	0.031394	0.032346	0.031049	0.038741697	0.032807939	0.015822	0.035071	0.038945	0.041780	0.034130	0.046689	0.04129326
962	0.018433	0.043108	0.031937	0.032722	0.031577	0.038723823	0.032787532	0.015796	0.035080	0.038892	0.041734	0.034093	0.046781	0.04141153
963	0.018985	0.043796	0.032550	0.033313	0.031945	0.038784496	0.032804433	0.015752	0.035082	0.038892	0.041780	0.034012	0.046852	0.04150454
964	0.019012	0.044136	0.032689	0.033508	0.032222	0.038783859	0.032757644	0.015733	0.035091	0.038875	0.041759	0.033942	0.046902	0.04160133
965	0.018972	0.044446	0.032827	0.033669	0.032434	0.038761634	0.032699171	0.015709	0.035080	0.038858	0.041718	0.033880	0.046975	0.04176091
966	0.019007	0.044833	0.033122	0.033915	0.032590	0.038739000	0.032662865	0.015658	0.035018	0.038851	0.041685	0.033828	0.047110	0.04179411

Date	16/07/2009		23/07/2009		13/08/2009		18/08/2009		24/08/2009		08/09/2009		10/09/2009	
Wavelength	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
967	0.018927	0.045089	0.033366	0.034079	0.032820	0.038690232	0.032578789	0.015654	0.035044	0.038840	0.041657	0.033847	0.047292	0.04178499
968	0.018987	0.045520	0.033734	0.034392	0.033182	0.038721543	0.032566473	0.015634	0.035085	0.038839	0.041645	0.033919	0.047512	0.04175794
969	0.019178	0.046108	0.034209	0.034816	0.033651	0.038852632	0.032643892	0.015595	0.035144	0.038862	0.041662	0.034041	0.047781	0.04169824
970	0.019519	0.046849	0.034768	0.035214	0.034188	0.039107513	0.032798558	0.015621	0.035345	0.039059	0.041847	0.034192	0.048185	0.04161371
971	0.019808	0.047702	0.035393	0.035695	0.034874	0.039284740	0.032885812	0.015646	0.035506	0.039224	0.042052	0.034303	0.048518	0.04149437
972	0.020163	0.048697	0.036084	0.036277	0.035696	0.039378524	0.032900702	0.015666	0.035622	0.039341	0.042216	0.034358	0.048779	0.04126561
973	0.020750	0.049972	0.036897	0.037046	0.036733	0.039335486	0.032797307	0.015666	0.035658	0.039351	0.042177	0.034292	0.048928	0.04092026
974	0.021261	0.051087	0.037766	0.037773	0.037590	0.039203942	0.032692872	0.015569	0.035495	0.039157	0.041944	0.034119	0.048899	0.04052892
975	0.021676	0.052137	0.038556	0.038455	0.038324	0.039027825	0.032557618	0.015457	0.035320	0.038960	0.041704	0.033970	0.048906	0.04014883
976	0.024480859	0.022784259	0.012881	0.022192	0.024893	0.038817298	0.032371677	0.015362	0.035217	0.038847	0.041541	0.033910	0.049057	0.04270692
977	0.024252519	0.022670645	0.012867	0.022132	0.025213	0.038888972	0.032395564	0.015423	0.035399	0.038934	0.041668	0.034021	0.049405	0.04247854
978	0.024190867	0.022652231	0.013023	0.022193	0.025373	0.039053798	0.032478068	0.015489	0.035616	0.039095	0.041862	0.034173	0.049809	0.04212664
979	0.024221748	0.022378085	0.012876	0.021758	0.024363	0.023600990	0.021170015	0.012190	0.021518	0.023810	0.025083	0.022909	0.029454	0.04172586
980	0.024369305	0.022900926	0.013049	0.021993	0.024355	0.023498822	0.021140734	0.011901	0.021354	0.024113	0.025282	0.022910	0.029427	0.04133677
981	0.024224803	0.022956489	0.013026	0.022103	0.024538	0.023518868	0.021049557	0.011683	0.021092	0.023745	0.025062	0.022345	0.028978	0.04094678
982	0.023767816	0.022421569	0.012780	0.021994	0.024769	0.023625884	0.021221908	0.011730	0.021173	0.023643	0.024942	0.021928	0.028748	0.04054880
983	0.023573749	0.022119416	0.013149	0.021955	0.024630	0.023720162	0.021327978	0.011866	0.021357	0.023678	0.024965	0.021748	0.028650	0.04013566
984	0.023409620	0.021671403	0.012997	0.021919	0.024285	0.023687361	0.020979181	0.011951	0.021447	0.023788	0.025254	0.022024	0.028665	0.04122135

Date	16/07/2009		23/07/2009		13/08/2009		18/08/2009		24/08/2009		08/09/2009		10/09/2009	
Wavelength	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
985	0.023289496	0.021262031	0.012604	0.021850	0.023880	0.023743289	0.020997468	0.011996	0.021689	0.023708	0.025209	0.022191	0.028997	0.04147539
986	0.023267450	0.021316487	0.012436	0.021641	0.023694	0.023824036	0.021129135	0.012015	0.021901	0.023845	0.025319	0.022459	0.029483	0.04160550
987	0.023417132	0.021760764	0.012409	0.021393	0.023762	0.023923581	0.021325042	0.012009	0.022043	0.024295	0.025701	0.022878	0.030090	0.04173910
988	0.023460701	0.021978194	0.012267	0.021089	0.023791	0.024320023	0.021373494	0.012149	0.022260	0.024579	0.026114	0.023290	0.030348	0.04196066
989	0.023249935	0.021644605	0.011866	0.020707	0.023639	0.024583869	0.021507973	0.012172	0.022500	0.024863	0.026441	0.023459	0.030663	0.04215049
990	0.023129735	0.021317024	0.011932	0.020834	0.023539	0.024794707	0.021703895	0.012195	0.022745	0.025077	0.026654	0.023393	0.030846	0.04234282
991	0.022832187	0.020880111	0.011943	0.020715	0.023531	0.025174368	0.021923523	0.012590	0.022955	0.024955	0.026598	0.022955	0.030210	0.04253558
992	0.022427002	0.020405700	0.011904	0.020411	0.023569	0.025050601	0.021823715	0.012562	0.022807	0.025060	0.026656	0.022901	0.030267	0.04265857
993	0.022658143	0.020616338	0.012154	0.020837	0.023241	0.024752997	0.021596417	0.012331	0.022542	0.025089	0.026628	0.022938	0.030416	0.04274815
994	0.022555627	0.020643365	0.011994	0.020676	0.023177	0.024443658	0.021327555	0.011975	0.022283	0.024814	0.026350	0.022900	0.030285	0.04283869
995	0.022372087	0.020585626	0.011760	0.020384	0.023211	0.024577610	0.021383064	0.012085	0.022495	0.025032	0.026602	0.023257	0.030750	0.04299773
996	0.022424759	0.020536536	0.011891	0.020535	0.023162	0.024683259	0.021445964	0.012232	0.022811	0.025223	0.026821	0.023524	0.031119	0.04304564
997	0.022748070	0.020640031	0.011696	0.020636	0.023261	0.024748364	0.021506879	0.012369	0.023180	0.025349	0.026968	0.023671	0.031336	0.04304316
998	0.023012444	0.020799022	0.011722	0.020842	0.023517	0.025283588	0.021907747	0.012536	0.023555	0.025636	0.027341	0.023976	0.031672	0.04300394
999	0.023104580	0.020993449	0.012182	0.021221	0.023960	0.025720129	0.022204349	0.012716	0.024042	0.026062	0.027762	0.023949	0.031784	0.04294116
1000	0.023492837	0.021124294	0.012306	0.021485	0.023706	0.021978194	0.012266926	0.021089	0.023791	0.024320	0.021373	0.012149	0.022260	0.04284450

Appendix 11

Results of ANOVA showing wavelengths in SWIR (1500, 1605, 1680, 1760, 2064, 220, 2315, 2320)) where reflectance differences between the three treatments (Control, low and high CO₂) are significant. Sample size 1000 (1000-2500nm).

Date	16/07/2009		23/07/2009		13/08/2009		18/08/2009		24/08/2009		08/09/2009		10/09/2009	
Wavelegth	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
1500	0.0175281	0.0223862	0.019449	0.0257235	0.0215927	0.0295317	0.0229963	0.0324814	0.02388	0.0349298	0.0245429	0.0369637	0.025313	0.038896
1605	0.017632	0.0226548	0.0197007	0.0262025	0.0218289	0.0299534	0.0231488	0.032817	0.023983	0.0351647	0.0246181	0.0372258	0.0253925	0.0391177
1680	0.0178797	0.0230267	0.0199298	0.026651	0.022012	0.0303177	0.0232822	0.0331495	0.024085	0.0354388	0.0247012	0.0374589	0.0254519	0.0393436
1760	0.0181274	0.0234257	0.0201416	0.027022	0.0221805	0.0306652	0.0233894	0.0335063	0.024152	0.0357212	0.0247841	0.0376792	0.0254567	0.0395569
2064	0.0183441	0.0238406	0.0203635	0.0275087	0.0223501	0.031013	0.0235076	0.0338118	0.0242115	0.0359851	0.0248447	0.0379196	0.0254834	0.0398085
2200	0.0186223	0.0243003	0.0206024	0.0279414	0.0224794	0.0313896	0.0236092	0.0341054	0.0242853	0.0362052	0.0249563	0.0381788	0.0255196	0.0400709
2315	0.018894	0.024785	0.0208581	0.0283066	0.0226391	0.0317635	0.0236845	0.0343962	0.0243629	0.0364747	0.0250845	0.0384349	0.0255485	0.040301
2320	0.0191631	0.0252758	0.0211056	0.0287378	0.0228266	0.0321301	0.0237798	0.0346784	0.0244488	0.036731	0.0252144	0.0386762	0.0255463	0.0405315

Appendix 12

Results of ANOVA showing wavelengths in SWIR region where reflectance differences were significant in herbicide treated plots and control . Sample size 1500.

Date	16/07/2009		23/07/2009		13/08/2009		18/08/2009		24/08/2009		08/09/2009		10/09/2009	
Wavelegth	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
1120	0.0255363	0.0407603	0.0253726	0.0421749	0.0253515	0.0434825	0.0254346	0.0444835	0.0254596	0.0454361	0.0257472	0.0470134	0.0199794	0.024846
1206	0.0255255	0.0409876	0.0253375	0.0424049	0.0253517	0.0436705	0.0254489	0.0446311	0.0254764	0.0456596	0.0258281	0.0472921	0.0200123	0.0249679
1518	0.0254941	0.0412198	0.0253128	0.0426361	0.0253493	0.0438598	0.0254398	0.0447869	0.0255272	0.0458871	0.0259051	0.0476244	0.0200869	0.0250891
1605	0.0254741	0.0414539	0.0253079	0.0428613	0.0253844	0.0440472	0.0254065	0.0449535	0.0255884	0.0461244	0.0260191	0.0480069	0.0201771	0.0252012
2150	0.0254603	0.0416906	0.0253159	0.043082	0.0254115	0.0441891	0.0254165	0.0451022	0.0256512	0.0463744	0.0261629	0.0484237	0.0202726	0.0252972
2360	0.025414	0.04194	0.0253365	0.0432978	0.0254295	0.0443253	0.025436	0.04526	0.0256877	0.046709	0.0263402	0.0488661	0.0203293	0.0253876

Appendix 13

Results of ANOVA showing the red edge position of maize grown on herbicide plots and control at a distance of 50, 100, 150 and 200cm. Sample size 128.

Variable	Measurement (day after treatment)	F- ratio	P- value (Sig)
Red edge position	2	3.055	0.036
	7	2.532	0.005
	14	4.144	0.040
	28	6.551	0.048
	33	2.645	0.039
	39	3.047	0.000
	54	0.609	0.486
	56	0.744	0.925

From t=2 until 39 there was statistically significant difference ($p \leq 0.05$ level) while t= 54 and 56 were not significant

Appendix 14

ANOVA result for temporal change in Chlorophyll Normalised Difference Index (**ChINDI**) for maize grown on gassed and herbicide treated plots at four levels of distance, 50, 100, 150 and 200 cm along the transect of measurement around the sampling tube which is installed 15 cm from the plot centre . Sample size is 128 observations.

Variable	Measurement (day after treatment)	F- ratio	P- value (Sig)
ChINDI (Control vs high CO ₂)	2	8.54	0.011
	7	46.12	0.025
	14	22.36	0.000
	33	60.51	0.039
	39	17.79	0.005
	45	0.653	0.316
	54	0.967	0.089
	56	0.104	0.489
Low vs high CO ₂	2	0.888	0.718
	7	0.675	1.302
	14	10.01	0.011
	33	20.78	0.017
	39	4.235	0.710
	45	0.987	0.654
	54	0.132	0.310
	56	0.709	0.872
Control vs Low	2	0.682	0.419
	7	0.118	0.878
	14	0.702	0.657
	33	0.731	0.998
	39	0.876	0.911
	45	0.631	0.791
	54	0.522	0.987
	56	0.877	3.847
Herbicide treatment	2	0.847	1.003
	7	2.556	0.123
	14	1.566	0.675
	33	18.145	0.016
	39	16.785	0.024
	45	14.002	0.004
	54	12.987	0.039
56	8.953	0.023	

Appendix 15

ANOVA result for temporal change in Pigment Specific Simple Ratios for chlorophyll a and b (**PSSRa and PSSRb**) and Physiological Reflectance Index (**PRI**) for maize grown on gassed and herbicide treated plots at four levels of distance, 50, 100, 150 and 200 cm along the transect of measurement around the sampling tube which is installed 15 cm from the plot centre - Sample size is 128 observations.

Variable	Measurement (day after treatment)	F- ratio	P- value (Sig)
PSSRa (Low vs high CO ₂)	2	14.14	0.041
	7	25.24	0.005
	14	12.17	0.034
	33	10.66	0.047
	39	7.09	0.000
	45	8.54	0.016
	54	16.22	0.029
	56	23.04	0.049
PSSRb (Control vs high CO ₂)	2	11.11	0.023
	7	10.75	0.018
	14	11.01	0.037
	33	18.08	0.008
	39	14.25	0.011
	45	7.17	0.000
	54	8.12	0.007
	56	10.09	0.021
PSSRb(Control vs Low)	2	0.272	0.622
	7	0.625	1.413
	14	0.912	0.675
	33	10.31	0.034
	39	10.87	0.024
	45	16.31	0.004
	54	20.52	0.039
	56	10.97	0.023
PSSRa-Herbicide treatment	2	0.747	1.555
	7	0.166	0.767
	14	0.546	2.666
	33	18.45	0.007
	39	26.95	0.018
	45	16.06	0.002
	54	13.72	0.000
	56	18.93	0.000
PSSRb-Herbicide treatment	2	0.914	1.836
	7	0.887	0.992
	14	0.656	0.544
	33	0.976	1.934
	39	0.754	0.671
	45	12.07	0.049
	54	11.01	0.033
	56	9.45	0.026
PRI (Low vs high CO ₂)	2	0.457	0.276
	7	0.993	1.222
	14	0.678	0.543
	33	16.02	0.022
	39	19.15	0.043
	45	23.23	0.008
	54	25.07	0.005
	56	27.88	0.000
PRI-Herbicide treatment	2	0.881	0.661
	7	1.234	0.987

Variable	Measurement (day after treatment)	F- ratio	P- value (Sig)
	14	3.243	1.222
	33	0.777	0.563
	39	0.959	0.726
	45	12.92	0.033
	54	14.01	0.046
	56	17.78	0.049

Appendix 16

ANOVA results showing the band-depth (410, 473, 488, 500, 509 and 512nm) at which there was significant difference in the different treatment (Control, low and high gas) for maize grown on gassed plots at four levels of distance, 50, 100, 150 and 200 cm along the transect of measurement around the sampling tube which is installed 15 cm from the plot centre - Sample size is 150

Date	16/07/2009		23/07/2009		13/08/2009		24/08/2009		10/09/2009	
	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
410-Low/High gas	0.019624	0.000651	0.022096	0.019326	0.025131	0.043977	0.028213	0.018755	0.031127	0.028813
473-High gas	0.019920	0.001252	0.022424	0.031197	0.025371	0.043335	0.028508	0.02952	0.031298	0.031963
488-High gas	0.020234	0.001246	0.022826	0.03697	0.025685	0.042935	0.028761	0.033983	0.031501	0.042522
500-Low gas	0.020565	0.00128	0.023209	0.037964	0.026027	0.043448	0.028994	0.034373	0.031711	0.045094
509-Low gas	0.020882	0.00135	0.023559	0.040034	0.026374	0.048939	0.029255	0.03766	0.031909	0.044147
512-High/Low gas	0.021190	0.001443	0.023847	0.042796	0.026634	0.049787	0.029481	0.041591	0.032087	0.041795

Appendix 17

ANOVA results showing the band-depth which there was significant difference in the different treatments (Control, low and high gas) for maize grown on gassed plots at four levels of distance, 50, 100, 150 and 200 cm along the transect of measurement around the sampling tube which is installed 15 cm from the plot centre -
 Sample size is 200. Note that the wavelength between 550-750nm were not significance between 16-23/09/09 while on 13/08/09 they became significant.

Date	16/07/2009		23/07/2009		13/08/2009	
Band-depth	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
550	0.014951	0.078477	0.0060164	0.5824495	0.0071775	0.0271527
551	0.150118	0.076347	0.0062733	0.5978772	0.0074719	0.0274454
552	0.15085	0.077093	0.0064369	0.5987093	0.0076228	0.0276041
553	0.151481	0.07779	0.0059765	0.5997128	0.0069664	0.0271647
554	0.151991	0.07836	0.0060804	0.6007841	0.0072483	0.0273110
555	0.152353	0.078747	0.0062792	0.6019614	0.0075298	0.0274293
556	0.152685	0.07883	0.0064845	0.6032028	0.0076625	0.0274362
557	0.152959	0.078724	0.0063090	0.6044148	0.0073131	0.0273896
558	0.153146	0.078444	0.0062126	0.6056601	0.0072377	0.0273007
559	0.153011	0.07802	0.0061815	0.6070026	0.0073191	0.0272197
560	0.152572	0.077672	0.0062242	0.6082419	0.0073967	0.0272606
561	0.152001	0.07737	0.0061442	0.6094020	0.0073094	0.0271296
562	0.151435	0.077093	0.0060313	0.6105999	0.0072142	0.0269955
563	0.151028	0.076811	0.0059178	0.6118307	0.0071802	0.0269327
564	0.150649	0.076363	0.0058925	0.6131018	0.0071117	0.0270071
565	0.150268	0.075686	0.0059411	0.6143455	0.0071225	0.0271680
566	0.149669	0.07488	0.0060222	0.6154818	0.0072039	0.0273743
567	0.148917	0.073916	0.0057856	0.6165826	0.0072492	0.0273072
568	0.148033	0.072814	0.0059079	0.6179177	0.0072629	0.0273662
569	0.147045	0.071603	0.0061236	0.6193671	0.0072913	0.0274488
570	0.146132	0.070364	0.0061069	0.6206158	0.0074101	0.0274221
571	0.145109	0.069169	0.0061462	0.6217332	0.0073448	0.0273085

572	0.143796	0.068097	0.0061357	0.6228909	0.0072584	0.0270969
573	0.142603	0.067082	0.0060348	0.6240419	0.0071973	0.0267662
574	0.141698	0.066099	0.0061729	0.6251790	0.0072380	0.0267854
575	0.14109	0.065138	0.0062054	0.6264465	0.0072683	0.0267480
576	0.140387	0.064059	0.0061341	0.6277741	0.0072738	0.0266554
577	0.139586	0.062939	0.0059598	0.6290844	0.0071826	0.0265460
578	0.138741	0.061848	0.0059698	0.6303197	0.0072343	0.0266816
579	0.137975	0.060983	0.0060520	0.6314991	0.0073075	0.0268923
580	0.137214	0.060242	0.0061252	0.6326160	0.0072840	0.0270358
581	0.136526	0.059615	0.0060844	0.6337542	0.0073137	0.0268967
582	0.135962	0.05913	0.0060137	0.6349684	0.0072726	0.0267334
583	0.13558	0.058605	0.0059287	0.6361538	0.0071304	0.0265977
584	0.135295	0.057972	0.0058126	0.6372246	0.0069968	0.0263450
585	0.135078	0.05723	0.0058648	0.6381877	0.0070480	0.0262605
586	0.134785	0.056391	0.0060022	0.6392089	0.0071954	0.0263066
587	0.134502	0.055299	0.0060272	0.6402948	0.0072330	0.0264580
588	0.134304	0.054086	0.0058518	0.6414047	0.0070442	0.0264936
589	0.134371	0.05289	0.0057193	0.6424943	0.0069409	0.0266123
590	0.133571	0.05201	0.0058166	0.6436259	0.0071724	0.0269781
591	0.132525	0.051399	0.0056943	0.6447734	0.0071740	0.0270560
592	0.131404	0.051085	0.0056925	0.6458023	0.0072334	0.0270826
593	0.131323	0.051098	0.0058286	0.6467049	0.0073624	0.0270812
594	0.131196	0.051386	0.0058918	0.6478220	0.0073379	0.0270137
595	0.13097	0.051649	0.0057939	0.6490243	0.0072400	0.0268489
596	0.130467	0.051821	0.0057076	0.6499265	0.0071400	0.0267012
597	0.130024	0.052074	0.0059282	0.6507758	0.0071534	0.0267669
598	0.129645	0.052626	0.0059074	0.6517476	0.0072598	0.0270244
599	0.129359	0.053011	0.0058260	0.6527690	0.0073466	0.0273518
600	0.129191	0.053568	0.0057359	0.6537933	0.0073753	0.0277251
601	0.129201	0.054371	0.0057029	0.6547419	0.0073812	0.0279647
602	0.129413	0.054953	0.0057259	0.6555609	0.0074420	0.0280889
603	0.129548	0.055933	0.0057952	0.6562964	0.0075407	0.0281324

604	0.129817	0.057072	0.0059022	0.6571105	0.0076026	0.0282202
605	0.130132	0.057754	0.0059989	0.6579430	0.0076684	0.0282446
606	0.130244	0.05817	0.0060400	0.6586196	0.0077062	0.0282218
607	0.129895	0.058513	0.0059553	0.6594368	0.0076721	0.0281454
608	0.129396	0.05888	0.0060756	0.6604406	0.0078422	0.0282651
609	0.12891	0.059282	0.0061473	0.6613697	0.0079648	0.0284066
610	0.128513	0.060005	0.0061087	0.6622210	0.0079793	0.0285320
611	0.128094	0.061035	0.0061894	0.6629161	0.0081053	0.0288295
612	0.127654	0.062071	0.0062264	0.6634971	0.0082143	0.0291046
613	0.127328	0.063217	0.0062391	0.6640899	0.0083138	0.0293621
614	0.12693	0.064384	0.0062792	0.6646679	0.0084258	0.0296165
615	0.126491	0.065491	0.0063252	0.6652105	0.0085570	0.0299285
616	0.126057	0.066348	0.0063925	0.6657411	0.0086904	0.0302120
617	0.125444	0.067029	0.0065012	0.6662986	0.0088169	0.0303998
618	0.124859	0.067559	0.0065703	0.6668995	0.0088763	0.0306293
619	0.124387	0.068093	0.0066403	0.6674210	0.0089996	0.0309358
620	0.124219	0.06879	0.0067152	0.6677104	0.0091940	0.0313146
621	0.124205	0.069658	0.0067684	0.6680259	0.0092896	0.0316092
622	0.124306	0.070908	0.0068135	0.6685511	0.0093885	0.0319454
623	0.124333	0.072261	0.0068435	0.6689527	0.0094922	0.0322992
624	0.124381	0.073741	0.0068333	0.6692327	0.0096094	0.0326341
625	0.124429	0.075489	0.0068737	0.6695338	0.0097161	0.0329951
626	0.124455	0.07706	0.0069445	0.6698095	0.0098233	0.0333465
627	0.124703	0.078584	0.0070446	0.6700470	0.0099372	0.0336717
628	0.125006	0.080099	0.0070571	0.6702616	0.0100258	0.0339145
629	0.125335	0.081525	0.0071263	0.6704609	0.0101195	0.0341638
630	0.125655	0.085314	0.0072409	0.6706253	0.0102176	0.0344317
631	0.126002	0.086729	0.0072430	0.6707619	0.0103016	0.0347901
632	0.126336	0.088144	0.0072663	0.6708579	0.0103933	0.0351672
633	0.126518	0.089581	0.0072994	0.6707895	0.0104876	0.0355684
634	0.126599	0.090991	0.0073333	0.6705651	0.0105819	0.0360274
635	0.126593	0.092472	0.0073730	0.6705003	0.0107026	0.0365394

636	0.126473	0.09403	0.0074049	0.6703621	0.0108029	0.0369805
637	0.12617	0.095495	0.0074242	0.6701716	0.0108670	0.0373077
638	0.125847	0.096887	0.0074532	0.6700219	0.0108980	0.0373971
639	0.12551	0.098221	0.0074687	0.6698045	0.0108965	0.0375296
640	0.124938	0.099533	0.0074828	0.6695318	0.0108909	0.0377154
641	0.12413	0.100825	0.0075469	0.6691398	0.0109942	0.0380200
642	0.123206	0.102106	0.0075915	0.6686936	0.0110547	0.0383030
643	0.122313	0.103394	0.0076173	0.6683530	0.0110992	0.0385752
644	0.121488	0.105178	0.0076165	0.6681113	0.0111440	0.0388474
645	0.120722	0.106822	0.0076535	0.6677901	0.0112275	0.0391110
646	0.120019	0.108286	0.0076985	0.6673062	0.0112973	0.0393357
647	0.119238	0.109642	0.0077461	0.6665788	0.0113449	0.0395173
648	0.118544	0.110888	0.0077888	0.6658030	0.0113929	0.0397171
649	0.117927	0.112009	0.0078148	0.6650981	0.0114469	0.0399394
650	0.117281	0.112963	0.0078325	0.6642680	0.0115051	0.0401801
651	0.116897	0.113876	0.0078586	0.6634545	0.0115663	0.0404410
652	0.11665	0.114832	0.0078703	0.6627223	0.0115968	0.0406030
653	0.116446	0.115861	0.0078906	0.6618695	0.0116197	0.0407206
654	0.116555	0.117327	0.0079356	0.6611733	0.0116465	0.0408130
655	0.11671	0.118901	0.0079483	0.6606824	0.0116800	0.0409429
656	0.116846	0.120492	0.0079627	0.6602051	0.0117159	0.0410855
657	0.115396	0.121796	0.0079820	0.6595526	0.0117534	0.0412425
658	0.114615	0.123096	0.0080072	0.6589386	0.0117847	0.0414521
659	0.114348	0.124347	0.0080145	0.6584131	0.0118248	0.0416339
660	0.114187	0.125511	0.0080211	0.6576493	0.0118624	0.0417854
661	0.113864	0.126745	0.0080576	0.6566669	0.0118754	0.0418866
662	0.113517	0.128033	0.0080611	0.6557893	0.0118893	0.0419878
663	0.113249	0.129374	0.0080629	0.6549364	0.0119045	0.0420936
664	0.112883	0.130805	0.0080743	0.6541556	0.0119216	0.0422069
665	0.112566	0.132112	0.0080669	0.6530709	0.0119067	0.0422768
666	0.112325	0.133348	0.0080727	0.6514723	0.0119162	0.0423440
667	0.112153	0.134604	0.0080879	0.6498993	0.0119445	0.0424118

668	0.111986	0.13597	0.0080827	0.6479917	0.0119495	0.0424901
669	0.111861	0.137423	0.0080950	0.6459317	0.0119676	0.0426027
670	0.11189	0.138987	0.0081055	0.6437856	0.0119854	0.0427211
671	0.111957	0.140493	0.0080919	0.6421664	0.0119873	0.0428148
672	0.112026	0.141974	0.0080773	0.6403624	0.0120009	0.0429388
673	0.112082	0.143421	0.0080520	0.6392075	0.0119978	0.0430397
674	0.112343	0.144584	0.0080133	0.6379855	0.0119704	0.0431030
675	0.112572	0.145736	0.0080340	0.6348145	0.0119860	0.0432078
676	0.11277	0.14694	0.0080295	0.6320992	0.0119910	0.0432783
677	0.113109	0.14834	0.0080180	0.6305073	0.0119942	0.0433326
678	0.113483	0.149717	0.0080565	0.6287106	0.0120254	0.0434244
679	0.113894	0.151074	0.0080428	0.6268474	0.0120309	0.0435061
680	0.114364	0.152411	0.0080199	0.6241989	0.0120330	0.0435865
681	0.114884	0.153758	0.0080159	0.6211226	0.0120469	0.0436721
682	0.115534	0.15514	0.0080102	0.6182963	0.0120481	0.0437278
683	0.11637	0.156552	0.0080066	0.6159533	0.0120530	0.0437687
684	0.117346	0.157974	0.0080072	0.6131372	0.0120633	0.0437923
685	0.118873	0.15926	0.0080333	0.6100718	0.0120712	0.0437354
686	0.120983	0.160468	0.0080628	0.6067051	0.0120838	0.0437039
687	0.123072	0.161615	0.0080863	0.6039391	0.0121024	0.0437182
688	0.124658	0.162739	0.0080790	0.6014729	0.0121331	0.0438479
689	0.126362	0.163896	0.0080762	0.5985412	0.0121744	0.0440979
690	0.129025	0.165104	0.0080585	0.5949508	0.0122087	0.0443626
691	0.132191	0.166256	0.0080131	0.5911469	0.0122259	0.0445948
692	0.135804	0.167455	0.0080474	0.5873442	0.0122811	0.0447205
693	0.139999	0.168683	0.0080772	0.5835388	0.0123301	0.0448216
694	0.144757	0.169907	0.0081026	0.5794564	0.0123768	0.0449187
695	0.149744	0.065053	0.0081585	0.5750951	0.0124695	0.0451331
696	0.154911	0.065759	0.0081722	0.5707347	0.0125399	0.0453735
697	0.160783	0.066397	0.0081771	0.5661415	0.0126040	0.0456100
698	0.167011	0.059431	0.0082131	0.5619640	0.0126790	0.0457974
699	0.173495	0.063759	0.0082587	0.5592510	0.0127773	0.0460175

700	0.180119	0.068373	0.0083222	0.5572179	0.0128927	0.0462718
701	0.186824	0.073468	0.0084092	0.5553685	0.0130271	0.0465678
702	0.193472	0.078954	0.0084904	0.5533572	0.0131654	0.0469354
703	0.199966	0.084759	0.0085425	0.5513050	0.0133026	0.0473166
704	0.206563	0.090591	0.0085795	0.5500645	0.0134472	0.0477280
705	0.213125	0.096284	0.0086571	0.5495135	0.0136420	0.0482638
706	0.219665	0.101958	0.0087462	0.5489560	0.0138383	0.0487917
707	0.226126	0.107787	0.0088402	0.5483072	0.0140467	0.0493096
708	0.232686	0.113589	0.0089359	0.5478030	0.0142820	0.0498129
709	0.239274	0.119479	0.0090900	0.5473856	0.0145474	0.0202564
710	0.245864	0.125518	0.0092582	0.5470600	0.0148495	0.0204679
711	0.252673	0.131499	0.0094319	0.5468486	0.0151884	0.0206860
712	0.259459	0.137535	0.0095524	0.5466734	0.0154690	0.0209025
713	0.266231	0.143566	0.0097252	0.5464754	0.0158069	0.0211254
714	0.273074	0.14913	0.0099176	0.5461870	0.0161795	0.0213882
715	0.279926	0.152835	0.0100619	0.5459293	0.0165575	0.0216757
716	0.287208	0.156931	0.0103161	0.5456912	0.0170082	0.0218596
717	0.295206	0.164539	0.0105917	0.5454507	0.0174904	0.0220500
718	0.302924	0.172579	0.0108397	0.5452838	0.0179809	0.0222488
719	0.3107	0.181075	0.0111123	0.5451481	0.0185547	0.0224699
720	0.317759	0.189033	0.0113538	0.5449473	0.0191367	0.0226835
721	0.324417	0.196795	0.0115725	0.5448065	0.0197232	0.0228904
722	0.331648	0.204465	0.0118877	0.5447537	0.0203505	0.0230899
723	0.339863	0.213012	0.0121912	0.5447062	0.0209442	0.0233043
724	0.34764	0.222419	0.0124953	0.5446436	0.0215228	0.0235088
725	0.355119	0.231805	0.0128330	0.5446190	0.0221347	0.0237139
726	0.362412	0.241026	0.0131554	0.5445848	0.0226937	0.0239705
727	0.369865	0.250346	0.0134710	0.5444697	0.0232731	0.0242017
728	0.377231	0.259962	0.0137788	0.5443497	0.0238942	0.0244197
729	0.384516	0.269678	0.0140412	0.5440351	0.0245041	0.0246344
730	0.391622	0.279352	0.0142975	0.5437014	0.0250746	0.0248685
731	0.398618	0.289181	0.0145543	0.5436464	0.0256053	0.0251012

732	0.405204	0.3002	0.0148421	0.5436756	0.0260828	0.0253292
733	0.411422	0.31164	0.0151031	0.5436951	0.0265286	0.0255829
734	0.417614	0.322833	0.0153283	0.5436779	0.0269457	0.0258232
735	0.423765	0.332743	0.0154965	0.5435160	0.0273299	0.0260606
736	0.429721	0.342598	0.0156684	0.5432747	0.0276837	0.0263211
737	0.435278	0.352911	0.0158293	0.5431200	0.0280034	0.0265443
738	0.440805	0.362501	0.0159756	0.5430158	0.0282853	0.0267732
739	0.445998	0.372171	0.0161297	0.5430002	0.0285282	0.0161138
740	0.450841	0.381913	0.0162489	0.5432210	0.0287402	0.0162326
741	0.455475	0.391484	0.0163409	0.5433950	0.0289295	0.0163298
742	0.459666	0.401272	0.0164133	0.5433965	0.0291082	0.0164094
743	0.463571	0.410691	0.0164801	0.5434191	0.0293069	0.0164817
744	0.467367	0.418794	0.0165498	0.5435073	0.0294924	0.0165532
745	0.470733	0.426382	0.0166290	0.5435982	0.0296402	0.0166280
746	0.473855	0.433401	0.0167010	0.5438610	0.0297798	0.0167045
747	0.476766	0.439766	0.0167823	0.5441480	0.0299257	0.0167785
748	0.479438	0.444573	0.0168687	0.5443613	0.0300742	0.0168448
749	0.481943	0.449162	0.0168872	0.5445731	0.0301570	0.0151935
750	0.484276	0.453615	0.0169334	0.5448772	0.0302658	0.0118377

Appendix 18

ANOVA results showing the band-depth which there was significant difference in the herbicide treatment and control for maize grown at four levels of distance, 50, 100, 150 and 200 cm along the transect of measurement -. Sample size is 109. Note that the wavelength between 406-515nm were significant between 16-23/09/09 while from 13/08/09 until 10/09/09 they were not significant.

Date	16/07/2009		23/07/2009		13/08/2009		24/08/2009		10/09/2009	
	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
406	0.0210609	0.0180931	0.0339574	0.03214186	0.1039371	0.1214027	0.0576086	0.0692867	0.1551276	0.1962679
407	0.0213559	0.0109083	0.0345405	0.03259291	0.1092745	0.1474919	0.0600552	0.0720183	0.1555202	0.1966217
408	0.0216500	0.0110826	0.0350867	0.03308980	0.1150562	0.1515651	0.0627031	0.0752879	0.1558984	0.1971893
409	0.0220121	0.0112811	0.0356862	0.03362038	0.1204246	0.1552433	0.0651593	0.0786109	0.1562406	0.1978405
410	0.0224423	0.0114957	0.0363459	0.03417779	0.1256547	0.1586611	0.0675139	0.0819586	0.1565065	0.1984538
411	0.0227835	0.0116707	0.0369645	0.03465933	0.1307175	0.1622254	0.0697785	0.0846976	0.1568220	0.1989621
412	0.0231296	0.0118234	0.0375662	0.03512257	0.1365426	0.1664619	0.0723499	0.0875150	0.1571239	0.1994220
413	0.0234723	0.0119722	0.0381624	0.03558870	0.1430764	0.1709572	0.0751036	0.0908053	0.1573919	0.1998461
414	0.0237976	0.0121602	0.0387880	0.03611723	0.1497051	0.1753626	0.0778566	0.0942922	0.1575953	0.2002837
415	0.0241212	0.0123002	0.0394393	0.03666237	0.1562282	0.1796349	0.0805627	0.0977849	0.1578568	0.2007076

416	0.0244573	0.0124565	0.0400716	0.03719242	0.1628410	0.1839525	0.0833307	0.1013407	0.1581650	0.2011209
417	0.0248185	0.0126649	0.0406635	0.03769244	0.1696236	0.1883639	0.0861036	0.1049483	0.1584635	0.2015229
418	0.0251663	0.0128727	0.0411916	0.03813492	0.1765155	0.1927994	0.0888788	0.1085580	0.1587599	0.2019715
419	0.0255299	0.0130902	0.0417992	0.03865885	0.1834481	0.1972046	0.0916407	0.1121610	0.1590149	0.2024004
420	0.0259036	0.0133095	0.0424736	0.03925356	0.1907525	0.2017065	0.0946901	0.1164499	0.1591696	0.2027320
421	0.0262440	0.0134685	0.0430779	0.03979966	0.1978361	0.2059991	0.0975738	0.1206618	0.1592301	0.2030332
422	0.0265647	0.0136265	0.0437034	0.04032229	0.2045490	0.2100500	0.1002497	0.1245708	0.1593177	0.2032411
423	0.0268744	0.0137944	0.0443511	0.04083811	0.2112605	0.2141786	0.1028575	0.1280747	0.1594585	0.2033336
424	0.0271910	0.0139982	0.0450568	0.04138475	0.2180349	0.2183553	0.1054317	0.1314823	0.1596078	0.2032689
425	0.0275144	0.0142097	0.0458062	0.04194454	0.2248101	0.2225153	0.1079894	0.1349541	0.1597167	0.2035104
426	0.0278447	0.0144143	0.0465071	0.04250773	0.2313729	0.2265099	0.1104651	0.1384922	0.1598158	0.2039028
427	0.0281827	0.0146071	0.0471276	0.04307158	0.2378335	0.2304057	0.1129345	0.1419153	0.1599979	0.2039880
428	0.0284558	0.0147673	0.0475053	0.04347359	0.2441582	0.2342069	0.1153234	0.1452930	0.1603584	0.2038640
429	0.0287027	0.0149128	0.0478714	0.04385177	0.2503014	0.2378900	0.1176158	0.1485928	0.1607021	0.2038641
430	0.0289403	0.0150609	0.0482555	0.04423284	0.2562037	0.2414318	0.1198198	0.1516836	0.1608890	0.2042397
431	0.0292335	0.0152856	0.0487833	0.04472670	0.2618214	0.2447781	0.1218643	0.1546184	0.1608651	0.2045925
432	0.0295234	0.0154703	0.0492689	0.04518367	0.2671656	0.2479628	0.1237697	0.1573720	0.1609565	0.2049040

433	0.0297970	0.0156436	0.0497502	0.04562319	0.2722695	0.2510452	0.1255490	0.1598960	0.1611866	0.2051770
434	0.0300423	0.0158293	0.0502702	0.04606479	0.2769981	0.2539078	0.1272195	0.1622607	0.1614006	0.2053670
435	0.0303410	0.0160299	0.0508249	0.04659615	0.2814948	0.2566508	0.1287997	0.1644990	0.1616228	0.2056022
436	0.0306244	0.0162201	0.0513111	0.04708257	0.2857896	0.2592997	0.1302895	0.1666134	0.1617808	0.2058483
437	0.0308810	0.0163959	0.0517125	0.04750182	0.2897065	0.2617516	0.1316715	0.1685365	0.1617021	0.2060457
438	0.0311529	0.0165820	0.0521333	0.04794282	0.2934529	0.2640915	0.1329766	0.1703579	0.1617049	0.2062304
439	0.0314127	0.0167663	0.0525919	0.04837045	0.2970199	0.2663305	0.1342073	0.1720798	0.1618274	0.2063059
440	0.0316710	0.0169539	0.0530722	0.04879526	0.3002857	0.2684793	0.1353487	0.1736722	0.1621188	0.2061970
441	0.0319556	0.0171604	0.0535453	0.04924225	0.3032494	0.2704021	0.1363628	0.1751062	0.1623793	0.2062282
442	0.0321991	0.0173286	0.0539403	0.04967666	0.3060096	0.2721994	0.1372857	0.1764313	0.1625592	0.2064142
443	0.0324380	0.0174926	0.0542947	0.0081454	0.3085860	0.2739197	0.1381171	0.1776545	0.1626593	0.2067088
444	0.0326935	0.0176724	0.0546205	0.0076158	0.3109044	0.2754806	0.1389037	0.1787370	0.1627059	0.2068830
445	0.0329265	0.0178584	0.0549629	0.0071539	0.3130448	0.2769631	0.1395678	0.1798354	0.1628029	0.2070339
446	0.0331634	0.0180392	0.0553068	0.0071142	0.3150484	0.2784121	0.1400899	0.1809901	0.1629005	0.2071480
447	0.0334052	0.0182166	0.0556589	0.0067252	0.3162492	0.2795801	0.1400626	0.1812192	0.1629351	0.2071854
448	0.0336198	0.0184084	0.0560916	0.0068812	0.3175845	0.2812667	0.1405538	0.1800971	0.1630074	0.2072614
449	0.0338344	0.0185941	0.0565169	0.0076262	0.3194080	0.2839528	0.1416082	0.1773827	0.1631245	0.2073390

450	0.0340512	0.0187758	0.0569144	0.0076769	0.3204781	0.2850556	0.1410231	0.1782348	0.1632926	0.2074092
451	0.0342762	0.0189564	0.0572344	0.0075495	0.3214722	0.2858535	0.1414395	0.1785726	0.1633847	0.2075052
452	0.0344660	0.0191245	0.0575058	0.0072941	0.3226319	0.2865701	0.1421170	0.1793278	0.1634495	0.2076004
453	0.0346494	0.0192945	0.0577837	0.0069277	0.3240421	0.2872789	0.1427674	0.1808727	0.1635085	0.2076753
454	0.0348364	0.0194727	0.0580912	0.0067982	0.3256380	0.2880555	0.1435926	0.1830643	0.1636439	0.2076439
455	0.0350198	0.0196367	0.0583952	0.0067574	0.3270378	0.2887902	0.1443073	0.1848440	0.1637076	0.2076631
456	0.0352092	0.0198057	0.0586683	0.0067541	0.3283311	0.2895311	0.1449504	0.1863295	0.1637349	0.2076956
457	0.0354017	0.0199756	0.0589179	0.0070818	0.3294424	0.2903771	0.1454360	0.1873148	0.1637382	0.2077173
458	0.0355805	0.0201189	0.0591925	0.0073310	0.3304140	0.2911403	0.1458767	0.1880654	0.1638487	0.2078025
459	0.0357665	0.0202953	0.0595108	0.0074271	0.3312835	0.2918757	0.1462752	0.1886674	0.1639741	0.2078721
460	0.0359483	0.0204781	0.0598474	0.0068323	0.3320063	0.2926273	0.1465915	0.1891256	0.1641046	0.2079135
461	0.0361092	0.0206377	0.0601784	0.0066456	0.3327419	0.2933693	0.1468942	0.1895841	0.1642628	0.2079233
462	0.0362897	0.0207968	0.0605032	0.0066786	0.3334548	0.2940997	0.1472037	0.1900116	0.1643018	0.2079183
463	0.0364475	0.0209442	0.0608058	0.0067813	0.3341299	0.2948174	0.1475299	0.1903915	0.1642139	0.2078880
464	0.0365708	0.0210765	0.0610803	0.0066984	0.3347519	0.2955465	0.1477501	0.1907659	0.1639159	0.2077950
465	0.0367548	0.0212591	0.0613744	0.0067146	0.3353346	0.2962442	0.1479715	0.1910663	0.1636205	0.2075674
466	0.0369195	0.0214075	0.0616411	0.0069339	0.3358861	0.2969179	0.1482035	0.1913124	0.1633254	0.2072237

467	0.0370728	0.0215423	0.0618926	0.0070713	0.3364156	0.2975822	0.1484795	0.1915952	0.1630305	0.2067259
468	0.0372390	0.0217289	0.0621674	0.0071147	0.3369054	0.2982585	0.1487128	0.1918553	0.1625477	0.2058163
469	0.0373933	0.0218969	0.0624547	0.0070731	0.3373955	0.2989197	0.1489235	0.1921111	0.1621209	0.2047897
470	0.0375347	0.0220620	0.0627579	0.0069412	0.3379304	0.2995274	0.1491237	0.1923808	0.1617603	0.2036313
471	0.0376575	0.0222348	0.0630857	0.0068613	0.3383455	0.3001647	0.1493559	0.1925736	0.1613424	0.2022495
472	0.0378071	0.0223874	0.0633551	0.0067842	0.3387408	0.3008156	0.1495367	0.1927329	0.1609671	0.2009109
473	0.0379653	0.0225375	0.0635828	0.0066598	0.3391347	0.3014764	0.1496428	0.1928628	0.1607922	0.1997599
474	0.0381294	0.0226878	0.0637682	0.0068951	0.3395047	0.3021286	0.1496919	0.1928243	0.1612016	0.1992286
475	0.0382657	0.0228251	0.0638604	0.0071750	0.3397951	0.3026871	0.1496788	0.1926982	0.1615496	0.1991339
476	0.0384440	0.0229745	0.0640461	0.0074270	0.3400164	0.3032006	0.1496368	0.1925068	0.1618363	0.1994804
477	0.0386408	0.0231345	0.0643101	0.0073442	0.3401642	0.3037972	0.1496764	0.1923143	0.1620548	0.2003054
478	0.0388112	0.0233066	0.0646562	0.0071716	0.3404912	0.3044337	0.1498547	0.1924106	0.1618633	0.2013022
479	0.0390169	0.0234913	0.0651324	0.0069751	0.3409313	0.3050730	0.1501119	0.1926953	0.1615455	0.2019652
480	0.0392252	0.0236679	0.0656491	0.0069405	0.3414731	0.3056829	0.1504306	0.1931605	0.1611747	0.2022432
481	0.0394198	0.0238235	0.0661709	0.0069691	0.3421074	0.3063067	0.1507996	0.1937233	0.1610752	0.2015964
482	0.0396862	0.0240215	0.0665585	0.0070193	0.3426073	0.3069062	0.1510388	0.1941245	0.1611410	0.2005682
483	0.0399529	0.0241926	0.0669171	0.0070695	0.3429403	0.3074812	0.1511292	0.1943051	0.1612214	0.1995066

484	0.0402203	0.0243441	0.0672724	0.0071572	0.3430901	0.3080669	0.1511861	0.1939436	0.1611524	0.1988681
485	0.0405251	0.0245552	0.0677790	0.0071650	0.3433599	0.3086510	0.1512751	0.1939673	0.1608908	0.1986935
486	0.0408364	0.0247465	0.0683205	0.0070810	0.3437134	0.3092417	0.1513937	0.1942278	0.1606572	0.1985562
487	0.0411615	0.0249247	0.0688754	0.0068773	0.3441316	0.3098784	0.1515560	0.1945506	0.1604983	0.1983739
488	0.0415130	0.0250893	0.0694153	0.0067446	0.3444270	0.3104086	0.1516839	0.1945538	0.1604049	0.1978763
489	0.0419040	0.0252647	0.0699337	0.0067582	0.3447141	0.3109341	0.1518125	0.1945207	0.1604643	0.1974186
490	0.0423193	0.0254572	0.0704957	0.0071258	0.3450386	0.3114900	0.1519559	0.1945757	0.1606742	0.1970938
491	0.0427604	0.0256724	0.0711289	0.0073470	0.3454907	0.3121263	0.1522107	0.1948374	0.1610868	0.1972269
492	0.0432650	0.0258928	0.0719247	0.0074194	0.3459422	0.3127254	0.1525040	0.1952196	0.1615446	0.1979087
493	0.0437735	0.0261067	0.0727527	0.0073259	0.3463867	0.3132811	0.1528145	0.1957059	0.1618831	0.1987289
494	0.0442935	0.0263176	0.0736257	0.0072178	0.3468571	0.3138225	0.1530508	0.1962708	0.1619927	0.1995217
495	0.0448766	0.0265444	0.0746441	0.0070314	0.3472340	0.3143727	0.1531987	0.1965499	0.1618757	0.2004039
496	0.0455248	0.0267885	0.0756610	0.0068296	0.3474991	0.3149208	0.1532724	0.1966020	0.1616727	0.2008793
497	0.0462023	0.0270326	0.0766806	0.0069517	0.3475563	0.3154395	0.1532467	0.1963749	0.1613983	0.2009151
498	0.0468946	0.0272629	0.0777103	0.0071607	0.3475488	0.3159411	0.1530650	0.1959557	0.1610891	0.2004722
499	0.0476948	0.0275264	0.0788526	0.0073807	0.3474716	0.3163980	0.1527799	0.1954166	0.1606230	0.1999695
500	0.0485506	0.0278242	0.0800832	0.0075479	0.3473058	0.3167970	0.1523796	0.1947451	0.1599497	0.1993656

501	0.0494571	0.0281555	0.0814064	0.0075597	0.3469119	0.3171371	0.1519960	0.1937710	0.1589032	0.1985761
502	0.0503366	0.0284271	0.0827831	0.0075457	0.3465072	0.3174937	0.1516288	0.1926807	0.1580145	0.1976805
503	0.0512974	0.0287124	0.0842241	0.0075345	0.3461423	0.3178836	0.1513268	0.1915543	0.1571636	0.1965647
504	0.0523163	0.0290054	0.0857118	0.0075094	0.3461113	0.3183517	0.1513558	0.1909450	0.1563320	0.1950678
505	0.0533904	0.0293013	0.0872594	0.0074630	0.3463553	0.3189666	0.1516386	0.1909437	0.1547697	0.1915717
506	0.0545521	0.0296227	0.0888564	0.0074530	0.3468644	0.3196456	0.1521374	0.1914009	0.1532780	0.1879497
507	0.0557804	0.0299716	0.0905929	0.0076993	0.3476942	0.3203311	0.1528722	0.1923053	0.1519300	0.1842968
508	0.0570590	0.0303508	0.0925293	0.0078621	0.3485050	0.3209646	0.1530531	0.1936329	0.1504157	0.1804475
509	0.0584632	0.0307375	0.0946773	0.0079421	0.3489102	0.3214065	0.1530419	0.1943993	0.1499561	0.1770227
510	0.0598597	0.0311091	0.0969446	0.0079121	0.3488714	0.3216716	0.1529153	0.1944549	0.1505578	0.1748669
511	0.0612502	0.0314698	0.0992915	0.0079975	0.3488804	0.3222210	0.1536046	0.1933959	0.1522078	0.1754477
512	0.0627803	0.0318811	0.1014924	0.0080454	0.3492962	0.3228372	0.1539754	0.1937854	0.1514341	0.1753973
513	0.0642644	0.0322393	0.1035494	0.0080468	0.3499034	0.3234146	0.1541249	0.1949598	0.1504662	0.1757361
514	0.0657109	0.0325775	0.1055129	0.0081925	0.3504095	0.3238465	0.1542590	0.1958899	0.1496962	0.1765961
515	0.0671815	0.0329510	0.1075023	0.0082830	0.3504750	0.3242452	0.1543580	0.1958328	0.1483310	0.1764707

Appendix 19

ANOVA results showing the band-depth which there was significant difference in the herbicide treatment and control for maize grown at four levels of distance, 50, 100, 150 and 200 cm along the transect of measurement - Sample size is 128. Note that the wavelength between 589-717nm were significant between 16/09/09-13/08/09 while from 24/08/09 until 10/09/09 they were not significant.

Date	16/07/2009		23/07/2009		13/08/2009		4/08/2009		10/09/2009	
Wavelength	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
589	0.0193311	0.029155	0.0047739	0.0131502	0.0060745	0.0176618	0.0972256	0.08627893	0.0751917	0.0965575
590	0.0185781	0.0240041	0.0040396	0.0123990	0.0060595	0.0176637	0.0987164	0.0536383	0.0750173	0.0956243
591	0.0178370	0.0234994	0.0033490	0.0115958	0.0060519	0.0176645	0.1000435	0.0543520	0.0748604	0.0948555
592	0.0177793	0.0233921	0.0033692	0.0113297	0.0060930	0.0176871	0.1013451	0.0550549	0.0747165	0.0942480
593	0.0176264	0.0233074	0.0030658	0.0112374	0.0061364	0.0177428	0.1026106	0.0557385	0.0745752	0.0935532
594	0.0178982	0.0235678	0.0032421	0.0115400	0.0061760	0.0177896	0.1038305	0.0563951	0.0744598	0.0928107
595	0.0185971	0.0241568	0.0039465	0.0122077	0.0062076	0.0178240	0.1049965	0.0570204	0.0743550	0.0920493
596	0.0185752	0.0240425	0.0042067	0.0122887	0.0061802	0.0178762	0.1060887	0.0576143	0.0742339	0.0913433
597	0.0184566	0.0237437	0.0041127	0.0122249	0.0061709	0.0179417	0.1070956	0.0581720	0.0741545	0.0906625
598	0.0182270	0.0233360	0.0037303	0.0119673	0.0061803	0.0180078	0.1079865	0.0586122	0.0741066	0.0900522

599	0.0177698	0.0228979	0.0030012	0.0112650	0.0062185	0.0180454	0.1088752	0.0590528	0.0740937	0.0895545
600	0.0174628	0.0229349	0.0028612	0.0109261	0.0062336	0.0179553	0.1097511	0.0594853	0.0740904	0.0891313
601	0.0173713	0.0230828	0.0029447	0.0108656	0.0062570	0.0178919	0.1105722	0.0598787	0.0741046	0.0887938
602	0.0175679	0.0231995	0.0031304	0.0111003	0.0063048	0.0179331	0.1114235	0.0602898	0.0741381	0.0885277
603	0.0178332	0.0235959	0.0033810	0.0113001	0.0063643	0.0180348	0.1122507	0.0606971	0.0742082	0.0882567
604	0.0180161	0.0239491	0.0035696	0.0114063	0.0063954	0.0181125	0.1130151	0.0610873	0.0742792	0.0880218
605	0.0180733	0.0241877	0.0036554	0.0113942	0.0063999	0.0181620	0.1137648	0.0614118	0.0743504	0.0878505
606	0.0175975	0.0237673	0.0032589	0.0109754	0.0063985	0.0181775	0.1145023	0.0617589	0.0744167	0.0878360
607	0.0173446	0.0235294	0.0031021	0.0107790	0.0064240	0.0181943	0.1152243	0.0621294	0.0743854	0.0872153
608	0.0172850	0.0234495	0.0030510	0.0107245	0.0064789	0.0182480	0.1158703	0.0624170	0.0743884	0.0865169
609	0.0174804	0.0235768	0.0029683	0.0107715	0.0065812	0.0184045	0.1165400	0.0627357	0.0744724	0.0859137
610	0.0174179	0.0234352	0.0028855	0.0107335	0.0066921	0.0185731	0.1171902	0.0630445	0.0745751	0.0857857
611	0.0175108	0.0233127	0.0029319	0.0107884	0.0068100	0.0187437	0.1177335	0.0632615	0.0746799	0.0856570
612	0.0179125	0.0233001	0.0031686	0.0110062	0.0069361	0.0189144	0.1182422	0.0634705	0.0747834	0.0854846
613	0.0180270	0.0233573	0.0033534	0.0111601	0.0070238	0.0190684	0.1187072	0.0636593	0.0748646	0.0851011
614	0.0180123	0.0233678	0.0034098	0.0111561	0.0070842	0.0191732	0.1191205	0.0638207	0.0749896	0.0847993
615	0.0179058	0.0233411	0.0033422	0.0110335	0.0071402	0.0192511	0.1193486	0.0638525	0.0751167	0.0845377

616	0.0178174	0.0233208	0.0031083	0.0109362	0.0072868	0.0193887	0.1195099	0.0638449	0.0752094	0.0842940
617	0.0178235	0.0235122	0.0030924	0.0108237	0.0074659	0.0196065	0.1196107	0.0638030	0.0753316	0.0841490
618	0.0178188	0.0236567	0.0031261	0.0107224	0.0076732	0.0198716	0.1196388	0.0637261	0.0754561	0.0840881
619	0.0176858	0.0234525	0.0030568	0.0106674	0.0079188	0.0201787	0.1196357	0.0636356	0.0755795	0.0841125
620	0.0179535	0.0236199	0.0032157	0.0108589	0.0081240	0.0204961	0.1195912	0.0635230	0.0757450	0.0840712
621	0.0182408	0.0237923	0.0034314	0.0111062	0.0083177	0.0207799	0.1194735	0.0633675	0.0759025	0.0840877
622	0.0184630	0.0238853	0.0036653	0.0113655	0.0085087	0.0210265	0.1192897	0.0631563	0.0760408	0.0841113
623	0.0185084	0.0236699	0.0036880	0.0113735	0.0087731	0.0213095	0.1190477	0.0629022	0.0761153	0.0839976
624	0.0184101	0.0233974	0.0034677	0.0112249	0.0090666	0.0216825	0.1187395	0.0626002	0.0761591	0.0836231
625	0.0182672	0.0231803	0.0031702	0.0110164	0.0093916	0.0221191	0.1183546	0.0622509	0.0761880	0.0831615
626	0.0183567	0.0234054	0.0032564	0.0110040	0.0097811	0.0226019	0.1179525	0.0618957	0.0762064	0.0827058
627	0.0184607	0.0237145	0.0033583	0.0109542	0.0101984	0.0231262	0.1175234	0.0615259	0.0762080	0.0822711
628	0.0185018	0.0240326	0.0034088	0.0109073	0.0106242	0.0236710	0.1170265	0.0611084	0.0761998	0.0818224
629	0.0184021	0.0243032	0.0033413	0.0108935	0.0110408	0.0242189	0.1164542	0.0606578	0.0761851	0.0813668
630	0.0185715	0.0244660	0.0035362	0.0110103	0.0115153	0.0247382	0.1158628	0.0602019	0.0761965	0.0810176
631	0.0186269	0.0244538	0.0035555	0.0110127	0.0120082	0.0252282	0.1152937	0.0597640	0.0761563	0.0806021
632	0.0185407	0.0242690	0.0033695	0.0108901	0.0125166	0.0257082	0.1147554	0.0593459	0.0760874	0.0801468

633	0.0184608	0.0239614	0.0031363	0.0108080	0.0130666	0.0263726	0.1142133	0.0589141	0.0760195	0.0796918
634	0.0183903	0.0237100	0.0030468	0.0107271	0.0136814	0.0271179	0.1136656	0.0584677	0.0759737	0.0792267
635	0.0184304	0.0236544	0.0030987	0.0107105	0.0143083	0.0278714	0.1131959	0.0581016	0.0759560	0.0788040
636	0.0188148	0.0241353	0.0033669	0.0108987	0.0149045	0.0285644	0.1127662	0.0577779	0.0759745	0.0784543
637	0.0190807	0.0246017	0.0035925	0.0110746	0.0155088	0.0293165	0.1123596	0.0574731	0.0760462	0.0782301
638	0.0192220	0.0250112	0.0037311	0.0111470	0.0161058	0.0300491	0.1119606	0.0571501	0.0761541	0.0780357
639	0.0192249	0.0253534	0.0037608	0.0110745	0.0166833	0.0307273	0.1116299	0.0569000	0.0762981	0.0778658
640	0.0191374	0.0255603	0.0036290	0.0109081	0.0172508	0.0313090	0.1113546	0.0566822	0.0764839	0.0777221
641	0.0190305	0.0255569	0.0034410	0.0106933	0.0178063	0.0318847	0.1111437	0.0564855	0.0766297	0.0776270
642	0.0189522	0.0254301	0.0032605	0.0104979	0.0183464	0.0324725	0.1109521	0.0562951	0.0767726	0.0775768
643	0.0191656	0.0255721	0.0033989	0.0106845	0.0188538	0.0331636	0.1107770	0.0561051	0.0769519	0.0775891
644	0.0194978	0.0257600	0.0035709	0.0109100	0.0193573	0.0337873	0.1106181	0.0559156	0.0771415	0.0777202
645	0.0198652	0.0259602	0.0037715	0.0111390	0.0198313	0.0343412	0.1104962	0.0557473	0.0773264	0.0778768
646	0.0202139	0.0261522	0.0040169	0.0113464	0.0202438	0.0347983	0.1103775	0.0555568	0.0775023	0.0780411
647	0.0204473	0.0263823	0.0040068	0.0114185	0.0205705	0.0351652	0.1102440	0.0553326	0.0777026	0.0782476
648	0.0206260	0.0266140	0.0039687	0.0114425	0.0208815	0.0355376	0.1100659	0.0550489	0.0778762	0.0784871
649	0.0207628	0.0268410	0.0039537	0.0114362	0.0211884	0.0359332	0.1100228	0.0549401	0.0780234	0.0787211

650	0.0209323	0.0271060	0.0039220	0.0113819	0.0214462	0.0362820	0.1100530	0.0549232	0.0781356	0.0788227
651	0.0211306	0.0274073	0.0038783	0.0113794	0.0216980	0.0366039	0.1101476	0.0549866	0.0782192	0.0788351
652	0.0213830	0.0277702	0.0038761	0.0114546	0.0219401	0.0368968	0.1101991	0.0549650	0.0782738	0.0787458
653	0.0218162	0.0283293	0.0041249	0.0117448	0.0221555	0.0371388	0.1102741	0.0549572	0.0782885	0.0785050
654	0.0222428	0.0288082	0.0042987	0.0119749	0.0223114	0.0373031	0.1103721	0.0549685	0.0782885	0.0781898
655	0.0225963	0.0292118	0.0043747	0.0121421	0.0224466	0.0374532	0.1104892	0.0550213	0.0782440	0.0777936
656	0.0228090	0.0295261	0.0043028	0.0122259	0.0225803	0.0376264	0.1106060	0.0550899	0.0781465	0.0773036
657	0.0231281	0.0298621	0.0043399	0.0123190	0.0227846	0.0378689	0.1107249	0.0551575	0.0779361	0.0765720
658	0.0234445	0.0302286	0.0043395	0.0124044	0.0229795	0.0381104	0.1108495	0.0552090	0.0777147	0.0757421
659	0.0237541	0.0306345	0.0042972	0.0124883	0.0231559	0.0383379	0.1109944	0.0552571	0.0774807	0.0748480
660	0.0242249	0.0312071	0.0044736	0.0127133	0.0232969	0.0384781	0.1111260	0.0552720	0.0772194	0.0739045
661	0.0246476	0.0317630	0.0045980	0.0129134	0.0234457	0.0386350	0.1112328	0.0552448	0.0769720	0.0730257
662	0.0250472	0.0323159	0.0046846	0.0130979	0.0235852	0.0387986	0.1111984	0.0551808	0.0767420	0.0721975
663	0.0254755	0.0329074	0.0047448	0.0132822	0.0236789	0.0389545	0.1111297	0.0550740	0.0765321	0.0714142
664	0.0258890	0.0334593	0.0048221	0.0134438	0.0237318	0.0390258	0.1110276	0.0549332	0.0763017	0.0706100
665	0.0262620	0.0339519	0.0048713	0.0135742	0.0237543	0.0390485	0.1108795	0.0547708	0.0760585	0.0697862
666	0.0265753	0.0343651	0.0048640	0.0136623	0.0237447	0.0390269	0.1107341	0.0546525	0.0758243	0.0689787

667	0.0268790	0.0346918	0.0048431	0.0138072	0.0237635	0.0390472	0.1105859	0.0545274	0.0757425	0.0683988
668	0.0272294	0.0350298	0.0048733	0.0140132	0.0237269	0.0390220	0.1104330	0.0543617	0.0757158	0.0679418
669	0.0276179	0.0353898	0.0049504	0.0142625	0.0236297	0.0389394	0.1101988	0.0541538	0.0757351	0.0675702
670	0.0279747	0.0358338	0.0050210	0.0144293	0.0234457	0.0387318	0.1099538	0.0539353	0.0758121	0.0672749
671	0.0283981	0.0364174	0.0051140	0.0146183	0.0232442	0.0384422	0.1097121	0.0537157	0.0759006	0.0671082
672	0.0288134	0.0370471	0.0051959	0.0147813	0.0230129	0.0381127	0.1095461	0.0535623	0.0759217	0.0668767
673	0.0291317	0.0376439	0.0052195	0.0148471	0.0227218	0.0377724	0.1094011	0.0533937	0.0758385	0.0665078
674	0.0294426	0.0382068	0.0052083	0.0148346	0.0224303	0.0374478	0.1092690	0.0532195	0.0757181	0.0656255
675	0.0297156	0.0386548	0.0051813	0.0148430	0.0221349	0.0371262	0.1091438	0.0530585	0.0756261	0.0649431
676	0.0299372	0.0389516	0.0051419	0.0149019	0.0218312	0.0368011	0.1090541	0.0529281	0.0755440	0.0644105
677	0.0301987	0.0391679	0.0051804	0.0151244	0.0214561	0.0364114	0.1089341	0.0527909	0.0753880	0.0637964
678	0.0304847	0.0393938	0.0052328	0.0153105	0.0210731	0.0359752	0.1087541	0.0526328	0.0752108	0.0631230
679	0.0307871	0.0396461	0.0052943	0.0154643	0.0206703	0.0354967	0.1086270	0.0525161	0.0750133	0.0624333
680	0.0310928	0.0400116	0.0053597	0.0155971	0.0202011	0.0349815	0.1084869	0.0523848	0.0747856	0.0617496
681	0.0313651	0.0403741	0.0053939	0.0156851	0.0197309	0.0344667	0.1083404	0.0522506	0.0746322	0.0610890
682	0.0316168	0.0407177	0.0054057	0.0157438	0.0192565	0.0339278	0.1082465	0.0522025	0.0744652	0.0604533
683	0.0318552	0.0410285	0.0053924	0.0157739	0.0187785	0.0333400	0.1082360	0.0521532	0.0742744	0.0598484

684	0.0320871	0.0412954	0.0053984	0.0158579	0.0183582	0.0328312	0.1082968	0.0521198	0.0741788	0.0593534
685	0.0323273	0.0415365	0.0054203	0.0159692	0.0179759	0.0323957	0.1084486	0.0521295	0.0741153	0.0589230
686	0.0325781	0.0417569	0.0054582	0.0161024	0.0176257	0.0320273	0.1086888	0.0521171	0.0740815	0.0585619
687	0.0328536	0.0420484	0.0055313	0.0162358	0.0172489	0.0316060	0.1089467	0.0521190	0.0740880	0.0583144
688	0.0331096	0.0423816	0.0055818	0.0163119	0.0168951	0.0311830	0.1092089	0.0521459	0.0741780	0.0581438
689	0.0333572	0.0427286	0.0056181	0.0163563	0.0165617	0.0307715	0.1096017	0.0522397	0.0742968	0.0580114
690	0.0336191	0.0430227	0.0056522	0.0164143	0.0162641	0.0304195	0.1099836	0.0523183	0.0744214	0.0579083
691	0.0337864	0.0431676	0.0056363	0.0164877	0.0159832	0.0300854	0.1103328	0.0523750	0.0745769	0.0579143
692	0.0339450	0.0432686	0.0056324	0.0165844	0.0157296	0.0297760	0.1105686	0.0523784	0.0747853	0.0579970
693	0.0341435	0.0433749	0.0056804	0.0167168	0.0155167	0.0295017	0.1107316	0.0523772	0.0750428	0.0581469
694	0.0343467	0.0435454	0.0057306	0.0168163	0.0153570	0.0292928	0.1108057	0.0523381	0.0753056	0.0583112
695	0.0345475	0.0437461	0.0057747	0.0169110	0.0151813	0.0290914	0.1107448	0.0522233	0.0756519	0.0585833
696	0.0347503	0.0439770	0.0058157	0.0170034	0.0149870	0.0288907	0.1106163	0.0521412	0.0760560	0.0589489
697	0.0349959	0.0442683	0.0058949	0.0170819	0.0148041	0.0286849	0.1104177	0.0520305	0.0765068	0.0594346
698	0.0351533	0.0444578	0.0059204	0.0171284	0.0146405	0.0285043	0.1101471	0.0518827	0.0770436	0.0600297
699	0.0352523	0.0445772	0.0058971	0.0171418	0.0144942	0.0283631	0.1097979	0.0516960	0.0776854	0.0607966
700	0.0353173	0.0446478	0.0058081	0.0171031	0.0143804	0.0283164	0.1094499	0.0515282	0.0784610	0.0617906

701	0.0354882	0.0448502	0.0058698	0.0171808	0.0141851	0.0280429	0.1090788	0.0513578	0.0794636	0.0631194
702	0.0356532	0.0450431	0.0059226	0.0172498	0.0140053	0.0277546	0.1086134	0.0511363	0.0806237	0.0648230
703	0.0357708	0.0451736	0.0059062	0.0172630	0.0138787	0.0275243	0.1082019	0.3601332	0.0820291	0.0670205
704	0.0359067	0.0452804	0.0059591	0.0173553	0.0137894	0.0274575	0.1078046	0.3651913	0.0836914	0.0696363
705	0.0360438	0.0453749	0.0060133	0.0174547	0.0137038	0.0273772	0.1074013	0.3698118	0.0854777	0.0723118
706	0.0361738	0.0454586	0.0060555	0.0175412	0.0136150	0.0272698	0.1069725	0.3741322	0.0873035	0.0750672
707	0.0362497	0.0455365	0.0060196	0.0175087	0.0134971	0.0270827	0.1065643	0.3781756	0.0893247	0.0782922
708	0.0363256	0.0456182	0.0059843	0.0175110	0.0134371	0.0269627	0.1062026	0.3818308	0.0916073	0.0820597
709	0.0364166	0.0457198	0.0059671	0.0175313	0.0134020	0.0268743	0.1060463	0.3847846	0.0940379	0.0861743
710	0.0365454	0.0458675	0.0059967	0.0175592	0.0133717	0.0267979	0.1058837	0.3869666	0.0967116	0.0908057
711	0.0366805	0.0460376	0.0060198	0.0175924	0.0133388	0.0267327	0.1057568	0.3878390	0.0996714	0.0959006
712	0.0368078	0.0462127	0.0060452	0.0176188	0.0132937	0.0266738	0.1057370	0.3918849	0.1027552	0.1011874
713	0.0369229	0.0463887	0.0060753	0.0176350	0.0132327	0.0266197	0.1056613	0.3999542	0.1058218	0.1064637
714	0.0370460	0.0465150	0.0060963	0.0176575	0.0131375	0.0265374	0.1055143	0.3988105	0.1090330	0.1121320
715	0.0371167	0.0465932	0.0060778	0.0176477	0.0130487	0.0265005	0.1052737	0.4009354	0.1122646	0.1180010
716	0.0371650	0.0466493	0.0060450	0.0176271	0.0129646	0.0264854	0.1049762	0.4035635	0.1156375	0.1241252
717	0.0372820	0.0467602	0.0060745	0.0176618	0.0128844	0.0264349	0.1046695	0.4055958	0.1191179	0.1303331

Appendix 20

Summary of ANOVA to determine the effect of elevated soil CO₂ on
barley plant after harvest. n=16

Variable	F	P
Mean number of barley plants-		
High gas	11.90	0.041
Low gas	0.272	0.123
Mean length of barley		
High gas	10.741	0.082
Low gas	1.106	0.210
Total number of tillers		
High gas	13.023	0.002
Low gas	20.594	0.017
Total number of grains		
High gas	7.816	0.045
Low gas	8.199	0.012
Fresh and dry weight of barley ears		
High gas	6.139	0.018
Low gas	6.922	0.012
Fresh and dry weight of barley stems		
High gas	11.326	0.032
Low gas	11.045	0.013

Appendix 21

Summary of ANOVA to determine the effect of different levels of herbicide concentration on barley plant after harvest. n=16

Variable	F	P
Mean number of barley plants- Herbicide treatment		
5%	0.870	0.068
10%	0.772	0.055
20%	0.991	0.073
40%	11.901	0.041
Mean length of barley tillers		
5%	0.714	0.122
10%	0.841	0.068
20%	10.232	0.012
40%	13.083	0.010
Total number of tillers		
5%	1.998	0.091
10%	5.794	0.042
20%	8.008	0.003
40%	9.457	0.040
Total number of grains		
5%	0.276	0.159
10%	13.19	0.042
20%	15.07	0.016
40%	18.21	0.023
Fresh and dry weight of barley ears		
5%	0.908	1.108
10%	12.87	0.022
20%	14.21	0.031
40%	16.03	0.044
Fresh and dry weight of barley stems		
5%	0.685	0.596
10%	9.03	0.011
20%	10.36	0.022
40%	11.05	0.036

Appendix 22

Results of one way ANOVA showing the average reflectance difference compared to control plots of the CO₂ experiment in the visible region of the spectrum, at all the wavelengths there was no significant difference ($p \leq 0.05$). Sample size=300.

Wavelength	F-ratio	P-value
400	0.2135121	0.22302865
401	0.2204003	0.21220495
402	0.2273471	0.21784003
403	0.2343845	0.22360255
404	0.2414817	0.22940139
405	0.2486663	0.23530844
406	0.2559655	0.24137382
407	0.2633850	0.24752221
408	0.2709405	0.25379488
409	0.2786776	0.26022646
410	0.2867328	0.26690412
411	0.2945138	0.27328619
412	0.3026600	0.27993780
413	0.3111999	0.28692779
414	0.3207645	0.29487097
415	0.3310505	0.30355877
416	0.3404287	0.31162706
417	0.3502102	0.32005900
418	0.3579729	0.32678419
419	0.3658420	0.33361253
420	0.3757161	0.34208044
421	0.3856254	0.35068396

422	0.3950233	0.35892749
423	0.4038753	0.36679232
424	0.4126757	0.37467775
425	0.4212149	0.38237211
426	0.4293896	0.38978601
427	0.4378732	0.39763075
428	0.4461054	0.40526065
429	0.4537448	0.41235101
430	0.4607570	0.41886267
431	0.4675530	0.42524880
432	0.4744670	0.43175799
433	0.4815168	0.43837804
434	0.4875753	0.44415721
435	0.4935969	0.44991437
436	0.4995250	0.45560312
437	0.5046883	0.46069521
438	0.5099360	0.46578518
439	0.5149788	0.47065923
440	0.5194402	0.47503820
441	0.5238113	0.47933304
442	0.5279835	0.48344406
443	0.5319151	0.48733428
444	0.5352863	0.49074331
445	0.5385504	0.49403176
446	0.5417108	0.49720168
447	0.5447211	0.50019914
448	0.5474750	0.50298852

449	0.5500397	0.50563180
450	0.5524103	0.50813830
451	0.5547144	0.51049447
452	0.5582504	0.51378155
453	0.5634643	0.51835591
454	0.5669528	0.52082109
455	0.5626281	0.51716024
456	0.5512456	0.50817299
457	0.5647008	0.51983517
458	0.5645468	0.52028745
459	0.5631295	0.51978898
460	0.5652981	0.52237332
461	0.5685724	0.52573889
462	0.5713708	0.52855390
463	0.5737857	0.53096873
464	0.5750469	0.53242749
465	0.5762858	0.53381205
466	0.5774925	0.53514755
467	0.5785906	0.53639603
468	0.5798662	0.53779876
469	0.5809933	0.53905308
470	0.5818242	0.54002047
471	0.5830992	0.54139751
472	0.5841244	0.54256094
473	0.5849592	0.54356039
474	0.5859566	0.54468572
475	0.5868441	0.54573482

476	0.5877831	0.54682726
477	0.5889776	0.54811656
478	0.5897593	0.54902005
479	0.5906979	0.55004096
480	0.5919264	0.55129427
481	0.5930914	0.55246294
482	0.5941281	0.55355817
483	0.5950233	0.55455947
484	0.5956155	0.55529356
485	0.5964202	0.55623853
486	0.5973080	0.55726147
487	0.5982069	0.55828404
488	0.5993268	0.55958796
489	0.6005916	0.56095427
490	0.6019100	0.56228507
491	0.6018876	0.56240058
492	0.6029079	0.56349689
493	0.6044102	0.56504679
494	0.6055391	0.56625038
495	0.6060613	0.56697124
496	0.6066256	0.56769836
497	0.6075393	0.56864887
498	0.6081472	0.56940413
499	0.6088921	0.57032478
500	0.6098507	0.57145810
501	0.6114805	0.57310390
502	0.6127591	0.57435733

503	0.6138237	0.57538581
504	0.6147191	0.57627100
505	0.6159846	0.57741064
506	0.6175326	0.57875937
507	0.6194205	0.58036810
508	0.6198221	0.58069599
509	0.6197027	0.58068061
510	0.6191128	0.58037287
511	0.6185861	0.58020675
512	0.6183834	0.58043927
513	0.6184936	0.58096558
514	0.6189862	0.58176929
515	0.6241354	0.58621734
516	0.6269749	0.58875209
517	0.6264152	0.58851886
518	0.6205768	0.58411163
519	0.6221426	0.58583707
520	0.6274714	0.59057659
521	0.6304053	0.59303677
522	0.6299152	0.59279042
523	0.6288136	0.59212029
524	0.6283940	0.59209698
525	0.6299411	0.59364009
526	0.6309059	0.59471619
527	0.6311412	0.59520787
528	0.6310720	0.59546095
529	0.6319599	0.59652269

530	0.6332634	0.59792632
531	0.6344636	0.59922445
532	0.6352621	0.60018241
533	0.6360977	0.60113603
534	0.6371210	0.60219693
535	0.6381308	0.60328776
536	0.6390773	0.60431802
537	0.6399479	0.60529220
538	0.6406099	0.60617799
539	0.6413687	0.60706466
540	0.6423706	0.60812747
541	0.6438729	0.60962290
542	0.6453322	0.61096048
543	0.6462519	0.61187702
544	0.6464958	0.61229372
545	0.6467059	0.61279881
546	0.6482239	0.61424482
547	0.6501737	0.61599028
548	0.6497880	0.61599046
549	0.6482970	0.61510080
550	0.6477575	0.61497426
551	0.6497963	0.61690897
552	0.6521782	0.61914343
553	0.6535836	0.62050349
554	0.6540006	0.62098122
555	0.6544785	0.62151206
556	0.6552590	0.62238324

557	0.6564443	0.62359965
558	0.6584467	0.62535638
559	0.6593530	0.62625110
560	0.6592458	0.62640548
561	0.6578755	0.62566042
562	0.6578043	0.62580776
563	0.6589902	0.62702984
564	0.6610841	0.62901795
565	0.6623684	0.63021046
566	0.6632850	0.63112849
567	0.6640306	0.63193512
568	0.6647404	0.63275510
569	0.6652193	0.63330728
570	0.6657314	0.63387948
571	0.6663464	0.63454521
572	0.6672218	0.63544101
573	0.6679764	0.63626474
574	0.6685860	0.63698924
575	0.6688920	0.63745987
576	0.6692975	0.63797891
577	0.6699848	0.63869798
578	0.6712020	0.63980836
579	0.6721097	0.64070624
580	0.6727196	0.64142019
581	0.6730286	0.64194977
582	0.6734310	0.64250523
583	0.6743755	0.64341837

584	0.6757789	0.64465654
585	0.6776719	0.64629948
586	0.6793374	0.64762896
587	0.6809267	0.64891124
588	0.6824814	0.65026307
589	0.6830079	0.65062875
590	0.6830662	0.65057141
591	0.6826010	0.65005636
592	0.6813329	0.64893383
593	0.6798324	0.64784014
594	0.6779505	0.64663219
595	0.6754016	0.64510316
596	0.6741140	0.64448678
597	0.6751375	0.64555573
598	0.6789870	0.64868623
599	0.6844444	0.65312493
600	0.6884643	0.65644985
601	0.6907475	0.65840977
602	0.6885313	0.65674669
603	0.6856401	0.65447813
604	0.6834478	0.65278077
605	0.6840358	0.65340644
606	0.6857324	0.65480441
607	0.6872095	0.65604150
608	0.6881362	0.65688217
609	0.6872240	0.65644968
610	0.6852080	0.65510094

611	0.6829107	0.65351140
612	0.6825736	0.65351290
613	0.6836295	0.65461755
614	0.6856561	0.65646785
615	0.6887102	0.65909529
616	0.6917459	0.66177464
617	0.6936327	0.66347677
618	0.6944005	0.66419989
619	0.6949573	0.66446942
620	0.6967139	0.66570103
621	0.6992609	0.66755086
622	0.7020143	0.66952997
623	0.7042198	0.67112541
624	0.7050139	0.67146921
625	0.7030318	0.66930521
626	0.6975218	0.66449809
627	0.6912301	0.65887243
628	0.6837591	0.65210277
629	0.6733827	0.64360678
630	0.6615625	0.63443917
631	0.6523998	0.62761962
632	0.6529145	0.62832224
633	0.6602067	0.63406456
634	0.6682154	0.64046067
635	0.6758533	0.64670962
636	0.6829470	0.65213430
637	0.6859037	0.65438515

638	0.6851842	0.65373796
639	0.6791909	0.64865524
640	0.6737450	0.64457625
641	0.6696308	0.64168030
642	0.6684309	0.64090884
643	0.6696481	0.64136124
644	0.6705294	0.64186758
645	0.6707430	0.64225411
646	0.6706219	0.64213306
647	0.6693818	0.64106518
648	0.6675065	0.63957983
649	0.6655306	0.63843089
650	0.6647694	0.63754016
651	0.6639715	0.63667643
652	0.6626183	0.63576561
653	0.6613581	0.63493508
654	0.6601034	0.63408178
655	0.6590271	0.63335168
656	0.6592708	0.63371104
657	0.6593266	0.63391566
658	0.6590590	0.63390034
659	0.6581531	0.63348627
660	0.6592003	0.63447195
661	0.6602578	0.63555557
662	0.6609335	0.63647932
663	0.6640029	0.63937324
664	0.6674910	0.64223200

665	0.6712568	0.64510453
666	0.6749297	0.64809346
667	0.6766750	0.64975679
668	0.6771162	0.65034354
669	0.6762074	0.64961332
670	0.6744355	0.64830524
671	0.6727774	0.64716983
672	0.6714239	0.64635217
673	0.6709812	0.64612722
674	0.6711912	0.64654136
675	0.6720276	0.64748102
676	0.6737174	0.64891386
677	0.6753368	0.65034324
678	0.6768798	0.65180564
679	0.6783448	0.65334606
680	0.6795169	0.65464050
681	0.6807581	0.65598696
682	0.6820707	0.65735745
683	0.6832220	0.65828305
684	0.6843844	0.65941501
685	0.6856343	0.66066253
686	0.6871230	0.66194278
687	0.6880385	0.66282958
688	0.6888299	0.66372275
689	0.6896274	0.66476285
690	0.6907845	0.66568649
691	0.6922361	0.66703171

692	0.6938420	0.66863251
693	0.6949099	0.66963106
694	0.6959322	0.67078519
695	0.6969421	0.67189491
696	0.6979478	0.67269915
697	0.7098585	0.67878133
698	0.7108936	0.67975336
699	0.7119130	0.68071747
700	0.7129397	0.68176544

Appendix 23

Wavelengths at SWIR region which shows significant difference on the last date (19/07/2010) of spectral measurement.

Date	19/07/2010	
Wavelegth	F-ratio	P-value
1482	0.0147581	0.0071558
1718	0.0240671	0.0090263
1990	0.0141584	0.0068171
2405	0.0156586	0.0076747

Appendix 24

ANOVA result for temporal change in Chlorophyll Normalised Difference Index (**ChINDI**) for barley grown on gassed and herbicide treated plots at four levels of distance, 50, 100, 150 and 200 cm along the transect of measurement.

Variable	Measurement (day after treatment)	F- ratio	P- value (Sig)
ChINDI (Control vs high CO ₂)	3	11.44	0.021
	21	16.32	0.035
	28	24.61	0.000
	34	40.23	0.019
	47	0.668	0.423
	67	0.957	0.634
Low vs high CO ₂	3	0.878	0.625
	21	14.09	0.041
	28	12.12	0.009
	34	10.75	0.007
	47	0.909	0.070
	67	0.816	0.063
Control vs Low	3	0.718	0.114
	21	0.755	0.597
	28	1.101	0.811
	34	0.878	0.617
	47	0.375	0.270
	67	0.909	0.708
Herbicide treatment 5%	3	10.82	0.010
	21	23.18	0.038
	25	12.02	0.007
	30	10.31	0.008
	39	9.87	0.001
	49	8.12	0.003
	67	6.99	0.021
10%	3	22.20	0.011
	21	6.09	0.005
	25	10.40	0.045
	30	3.96	0.022
	39	21.67	0.010
	49	10.09	0.049
	67	0.911	0.105
20%	3	14.01	0.036
	21	15.23	0.044
	25	17.90	0.065
	30	29.11	0.000
	39	43.96	0.001
	49	65.39	0.000
	67	0.756	0.552

40%	3	16.87	0.038
	21	18.13	0.005
	25	22.33	0.012
	30	26.18	0.015
	39	45.35	0.000
	49	53.22	0.000
	67	0.962	1.002

Appendix 25

ANOVA result for temporal change in Pigment Specific Simple Ratio (**PSSRa**) for barley grown on gassed and herbicide treated plots at four levels of distance, 50, 100, 150 and 200 cm along the transect of measurement.

Variable	Measurement (day after treatment)	F- ratio	P- value (Sig)	
PSSRa (Control vs low CO ₂)	3	0.742	0.121	
	21	26.02	0.027	
	28	33.51	0.000	
	34	52.23	0.003	
	47	0.897	0.513	
	67	0.907	0.702	
	PSSRa (Control vs High CO ₂)	3	10.18	0.019
21		12.09	0.001	
28		18.22	0.028	
34		21.55	0.007	
47		0.991	0.076	
67		0.834	0.063	
PSSRa-Herbicide treatment		3	0.888	0.624
	21	0.755	0.597	
	25	11.21	0.037	
	30	18.22	0.027	
	39	0.317	0.261	
	49	0.816	0.611	
	67	0.777	0.419	
	10%	3	20.02	0.010
		21	43.14	0.044
		25	26.02	0.008
		30	10.21	0.023
		39	0.872	0.601
		49	0.817	0.059
		67	0.995	1.021
20%	3	22.10	0.011	
	21	7.09	0.005	
	25	11.40	0.045	
	30	13.96	0.022	
	39	0.679	0.090	
	49	0.809	0.089	
	67	0.911	0.105	
40%	3	34.11	0.041	
	21	15.63	0.024	
	25	17.90	0.065	
	30	29.11	0.000	
	39	0.960	1.001	
	49	0.639	0.056	
	67	0.816	0.612	

Appendix 26

ANOVA result for temporal change in Pigment Specific Simple Ratio (**PSSRb**) for barley grown on gassed and herbicide treated plots at four levels of distance, 50, 100, 150 and 200 cm along the transect of measurement.

Variable	Measurement (day after treatment)	F- ratio	P- value (Sig)
PSSRb(Control vs High CO ₂)	3	10.14	0.071
	21	25.21	0.033
	28	32.71	0.000
	34	42.73	0.000
	47	0.917	0.623
	67	1.805	0.902
	PSSRb-Herbicide treatment	3	10.58
21		10.55	0.026
25		11.21	0.011
30		18.22	0.019
39		0.317	0.565
49		0.816	0.634
67		0.777	0.097
5%		3	19.72
	21	33.54	0.038
	25	26.02	0.008
	30	10.21	0.003
	39	0.812	0.601
	49	0.887	0.079
	67	0.975	1.081
10%	3	32.60	0.001
	21	17.11	0.004
	25	14.20	0.056
	30	13.17	0.032
	39	0.679	0.089
	49	0.809	0.091
	67	0.911	0.117
20%	3	33.71	0.041
	21	15.09	0.024
	25	17.23	0.065
	30	29.34	0.000
	39	0.945	1.001
	49	0.658	0.056
	67	0.987	0.612
40%	3	33.71	0.041
	21	15.09	0.024
	25	17.23	0.065
	30	29.34	0.000
	39	0.945	1.001
	49	0.658	0.056
	67	0.987	0.612

Appendix 27

ANOVA result for temporal change in Physiological Reflectance Index(**PRI**) for barley grown on gassed and herbicide treated plots at four levels of distance, 50, 100, 150 and 200 cm along the transect of measurement.

Variable	Measurement (day after treatment)	F- ratio	P- value (Sig)	
PRI(Control vs High CO ₂)	3	20.89	0.042	
	21	27.33	0.013	
	28	37.82	0.000	
	34	44.73	0.000	
	47	60.75	0.023	
	67	73.52	0.011	
PRI(Control vs Low CO ₂)	3	0.999	1.023	
	21	25.09	0.028	
	28	45.11	0.000	
	34	52.06	0.000	
	49	45.66	0.001	
	67	0.786	0.050	
PRI-Herbicide treatment	5%	3	0.558	0.085
		21	0.659	0.196
		25	1.219	1.081
		30	0.822	0.619
		39	0.437	0.345
		49	0.723	0.614
		67	0.787	0.697
	10%	3	0.885	0.076
		21	23.14	0.047
		25	16.02	0.028
		30	14.21	0.033
		39	10.12	0.025
		49	10.78	0.019
		67	0.975	1.221
20%	3	0.760	0.061	
	21	27.91	0.004	
	25	14.20	0.056	
	30	13.17	0.032	
	39	0.679	0.089	
	49	0.809	0.091	
	67	0.911	0.217	
40%	3	0.878	0.071	
	21	16.13	0.024	
	25	17.33	0.045	
	30	39.34	0.000	
	39	10.94	0.001	
	49	11.68	0.046	
	67	0.987	0.612	