

REMOTE SENSING OF BARLEY STRESSED WITH CO₂ AND HERBICIDE

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ABSTRACT

Stress cause crops to grow at less than their full potential and can cause reduction in yield; this is as a result of threats emanating from the causative factor(s) known as stressors. Field experiments were conducted in 2010 to compare the effects of two stress-inducing agents on the spectral reflectance of barley: (1) High concentration of Carbon dioxide in soil and (2) four different levels of concentration of herbicide application. Carbon dioxide concentrations up to 80% in soil were applied to experimental plots as part of a study of the potential effects of leakage from carbon capture and storage. In a separate set of plots, glyphogan herbicide (Makhteshim Agan, UK) containing 360g l⁻¹ of glyphosate was applied at four different levels of concentration, 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% of the usual lethal dose for barley crop, diluted to give the normal rate of spray coverage. Thus 0.1, 0.2, 0.4, and 0.8 ml Glophogan in 80 ml of water was sprayed evenly over each of the four plots treatment levels. This was designed to provide a range of levels of stress to the barley crop. Plant stress effects were detected by spectral scanning between 350 and 2500 nm with an ASD Fieldspec FR spectroradiometer (ASD, Boulder, USA). Canopy reflectance spectra were used to locate the position and height of the inflection point of the red edge by derivative analysis and to investigate other peaks that may indicate stress in plants. Measurements of soil gas concentration, and chlorophyll content were carried out at various stages of the crop development to determine any variations as the experiments progressed.

KEYWORDS: Hyperspectral; Red-edge; Stress; Canopy reflectance, carbon-dioxide, herbicide

INTRODUCTION

With the effects of climate change on the rise, reduction of the amount of CO₂ released into the atmosphere is crucial (Erin J.M et al., 2010). Concentrations of atmospheric CO₂ has risen from 280 parts per million (ppm) prior to the industrial revolution to 390 ppm today (IPCC, 2007). The rate at which atmospheric CO₂ concentrations are rising is accelerating and is projected to drive changes in climate ecosystem services, crop yields, and human health worldwide in the coming decades (Intergovernmental Panel on Climate Change, 2007).

One strategy to mitigate the rise in atmospheric concentration is to capture the considerable quantities of waste CO₂ gas generated by power plants, ethanol manufacturing, and coal gasification facilities and inject it into subsurface reservoirs for long-term isolation, i.e., geologic sequestration (Christopher S, et al., 2010). Oil reservoirs and natural gas fields have demonstrated the capability of geologic formations to entrap fluids for millions of years. Nevertheless, because the potential exists for some leakage to the atmosphere as sequestered CO₂ gas migrates in a formation (Benson, 2005), monitoring is required. Furthermore, sequestered gas may migrate laterally within a formation to escape to the surface through a borehole, fracture, or fault. Multiyear remote sensing surveillance could identify potential leakage in areas not easily monitored by fixed point methods such as along pipelines and areas peripheral to injection sites.

Detection and characterisation of potential CO₂ leakage will be challenging due to the large spatial and temporal variation in background CO₂ fluxes within which a leakage signal may exist (Cortis et al., 2008). Remote sensing can also provide a secondary method for leak detection where supplemental information is desired. Indirect detection of leakage could be achieved by identifying higher than average vegetation canopy temperatures that resulted from plant exposure to elevated CO₂ concentrations (Cortis et al., 2008).

Injection of CO₂ into deep underground formations, known as geologic carbon sequestration, can be an effective method of mitigation by preventing the CO₂ injected into underground sequestration formations from entering the atmosphere. In order for geologic sequestration to be successful, methods of verification are important to maintain the sequestered CO₂ and to assure the public that the sequestration operation is safe. It is therefore important to develop techniques for long-term CO₂ leak detection at the surfaces of the CO₂ sequestration fields (Pickles and Cover 2005).

Analyzing hyperspectral plant signatures over CO₂ sequestration fields can confirm that the sequestration fields have not been compromised. If a leak were to occur, the excess amount of CO₂ in the top layers of soil near the surface would stress the vegetation above the sequestration field, which can be seen as changes in their spectral signatures.

CO₂-induced stress has been recognized in the spectral signature of plants over volcanic CO₂ vents at the Latera (Bateson et al. 2008) and Long Valley (De Jong 1996; Hausback et al. 1998; Martini et al. 2000) calderas, as well as in laboratory experiments (Noomen and Skidmore 2009).

Conversely, if vegetation over a sequestration field has healthy spectral signatures, it would indicate that CO₂ is being sequestered effectively. Because the basic requirement of this technique is just the presence of healthy vegetation over the sequestration field, hyperspectral plant signatures are particularly useful tool for monitoring sequestration fields for years to centuries (Pickles and Cover 2005).

In this study barley crop was exposed to elevated concentration of soil CO₂ as the primary stressor to investigate its effects on the spectral reflectance, in another experiment carried out in parallel, herbicide was applied to same crop at different levels of concentration, the aim was to find out if this could be distinguished from the CO₂ stress. Both stressors would primarily affect the roots of barley crop.

MATERIALS AND METHODS

Site

An Artificial Soil Gassing and Response Detection facility (ASGARD) has been developed at the Sutton Bonington Campus of University of Nottingham, United Kingdom (52.2°N, 1.2°W) where CO₂ is injected into soil plots to investigate effects on the soil ecology and on the growth and development of plants. The gas is released from a diffusive point source 0.6 m below the centre of each 2.5 x 2.5 m plot. Eight plots (each 2.5 x 2.5 m) were laid out within the experimental area to enable gas to be delivered to different crops. To allow spectral reflectance measurements of vegetation to be made on the plots, the facility was positioned so that it was not influenced by shade from trees or fencing.

To avoid obstructing the measurement area, and to enable overhead spectral measurements to be taken without the gas pipe being in the field of view, the tubing was inserted into augured holes at an angle of 30 degrees to the vertical, such that the gas was delivered into the soil 0.6 meters below the centre of the plot. Gas flow to each plot can be individually regulated.

Plants and Treatments

Two experiments were carried out on barley in 2010 to assess whether there are changes in canopy reflectance as a response from these stresses and to investigate if these stressors can be distinguished from one another.

For the carbon dioxide experiment barley (*Hordeum vulgare* v *Concerto*) was sown at a density of 250 seeds m⁻², (Thousand Grain weight (TGW) 50g) in 20 rows at a spacing of 12.5 cm into eight plots (each 2.5 x 2.5 m) on April 8, 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 plant germinated on April 21, 2010. Netting was placed above the seedlings to avoid damage by rodents, birds and other animals. An electric fence was erected round the experimental plots to further prevent any intrusion. Following germination and crop development, experimental treatments were applied to four plots as described below, while four plots were left as control plots and thus received no experimental treatments.

Gas Delivery and Instrumentation

In the CO₂ experiment gas was delivered continually from 7 June 2010, (62 days after sowing) to four plots at a rate of 1 litre per minute, with four plots as controls. Soil gas concentration was measured three times a week. Gas injection to the four plots was terminated on 27 August, 2010 by which point the crop had fully matured and was ready for harvest, the visible damage to the crops was obvious in the treated plots at this stage.

Carbon dioxide is stored in 2 x 200 L cryogenic vessels (BOC, Derby, UK) that are refilled as required from a road tanker. The CO₂ is delivered via a single inlet mass flow controller (Alicat, Tucson, USA) to 16 individual mass flow controllers (Alicat, 0.1-10 L 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).

The CO₂ gas is delivered to the experimental plots via 16 lengths of copper pipe from the instrument shed and supported on uni-fit railing which is concreted into the ground. Each length of copper pipe is connected to plastic piping (15-mm internal diameter) which runs along the North edge of the plots and delivers the gas to each treatment plot.

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

Vertical plastic sampling tubes (100 mm 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 30 cm. The tubing is sealed at the bottom but 14 equally spaced holes (4.5-mm diameter) drilled in the lower 15 cm enables 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. Two tubes are installed at 15 and 70 cm 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 15 cm from the centre of each control plot.

Soil Gas Concentration Measurement

Gas concentration in the soil was measured by means of vertical plastic tubes perforated at the bottom and installed at a distance of 15cm and 70cm from the centre of each plot at a depth of 60cm. Soil CO₂ and O₂ concentrations were measured three times weekly from June 7, 2010 using a GA2000 Landfill Gas Analyser (Geotechnical Instruments, Warwickshire, UK). The analyser measures CO₂ concentration in the range 0-100% and extracts a sample at a flow rate of 300 ml min⁻¹ and O₂ concentration in the range of 0-25% by internal electro-chemical cell. For taking the 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 gases was 30 seconds.

Herbicide Treatment

The herbicide experiment started on same day as the CO₂ experiment. A separate block of twenty plots (1.6 x 2.5 m) was set up in an open field in the same campus of the University of Nottingham, United Kingdom where crops are cultivated on a larger scale for commercial purposes. These plots were machine drilled with barley at a rate of 250 seeds m⁻². The same quantity of fertiliser was applied at same times; sixteen randomly selected plots were treated with herbicide while four plots were left as control as such no treatment were effected on them. Glyphogan (Makhteshim-Agan UK Ltd) containing 360g l⁻¹ of glyphosate was applied on June 3, 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 200l ha⁻¹ of water. These rates are equivalent to 5, 10, 20 and 40% of the usual lethal dose for barley crop, diluted to give the normal rate of spray coverage. Thus 0.1, 0.2, 0.4, and 0.8 ml Glyphogan in 80 ml of water was sprayed evenly over each of the four treatment levels. This was designed to provide a range of levels of stress to the barley crop for the investigation of the spectral responses.

Spectral Measurements

Plant stress effects were detected by spectral scanning weekly, when the weather permitted from 3 June, 2010 to 15 September. The scans were made between 350 and 2500 nm with an ASD Fieldspec FR spectroradiometer (ASD, Boulder, USA) fitted with a fibre optic probe having a 23 degrees field of view. The sampling interval over the 350–1050 nm range is 1.4 nm with a resolution of 3 nm (band width at half maximum). Over the 1050–2500 nm range the sampling interval is about 2nm and the spectral resolution is between 10 and 12 nm. Vegetation canopy reflectance was measured in a transect of four measurements at 50 cm intervals across each plot for the CO₂ experiment, care was taken to measure the spectral reflectance on the same spot using tiny pegs flag at each measurement point as the permanent marker to ensure that spectral measurements were done at exactly same spot every time. In the field plots, one measurement was taken at the middle of each plot for the four replica of herbicide concentration. Measurements were made at twelve different occasions throughout the experiment duration.

The results are then interpolated by the ASD software to produce readings at every 1 nm. Measurements were taken between 10:30 am and 1:30 pm. Conditions varied from cloud free to overcast skies but care was taken to

avoid scans when clouds were passing overhead. Scans were taken from a height of 1.23 meters looking towards the nadir position. Scans were optimised and referenced against a halon panel prior to scanning the vegetation.

Spectral Analysis

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

The first derivative of the canopy reflectance spectrum was analysed to detect the effects of CO₂ and herbicide induced stress on barley. Prior to analysis, the first derivative was smoothed using the 5 point weighted moving average as defined by Smith et al. (2004). This technique was primarily used to locate the position of red edge, which is the position of maximum slope of the reflectance between 670 nm and 800 nm. A simple operation of dividing the difference between successive spectral values by the wavelength interval separating them was carried out.

The position of red edge (REP)-which is the inflection point on the slope between the red absorption and near infrared reflectance, is often used to correlate chlorophyll content with reflectance (Horler et al., 1983; Curran et al., 1995).

Chlorophyll Analysis

Chlorophyll analysis was carried out at day 43, at this point both stress were at their maximum, this was done by removing, using a pair of scissors, 1cm² area from the leaves of the plant that had been used for spectral scanning, for the gassed plots four upper leaves were removed along the transect from the edge of each plot at 50,100,150 and 200 cm. While for the herbicide treated plots four leaves were removed from each treatments including control plots. The leaves were stored in polythene bags at 4°C in the dark to prevent chlorophyll break down, until chlorophyll extraction is carried out. The extraction and analysis is usually done 24-48 hours after the sampling.

Chlorophyll extraction and analysis were carried out using the method of Bruinsma (1963). Chlorophyll samples were extracted from the leaves of known area by grinding with pestle using a mortar and adding 5 ml of extraction solvent (80% acetone; 15% methanol; 5% distilled water) using pipette. A small amount of purified sand was added to facilitate grinding. The suspension was transferred to a clean 15 ml centrifuge tube, until the volume of extract reaches 10 ml. The tube was capped and placed in the centrifuge, 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% acetone solution as a standard.

RESULTS AND DISCUSSIONS

Spectra Data

Canopy reflectance measurements showed that barley crops exposed to the highest concentration of CO₂ and herbicide had increased reflectance in the visible region and decrease reflectance in the infrared. Figures 1a-c illustrates this temporal response with reflectance measurements for control, low and high gassed plots.

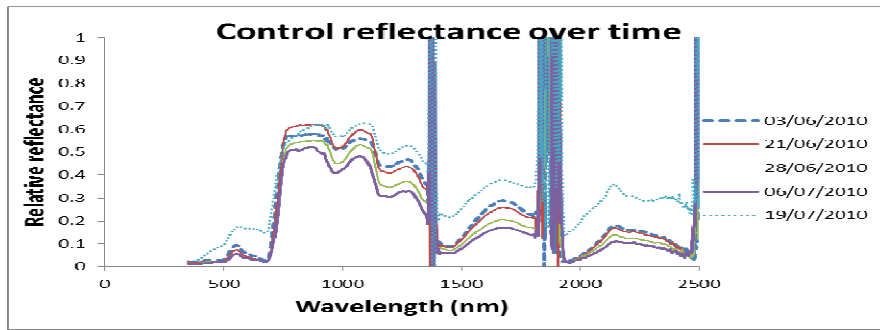


Figure 1a. Control reflectance for CO₂ plot.

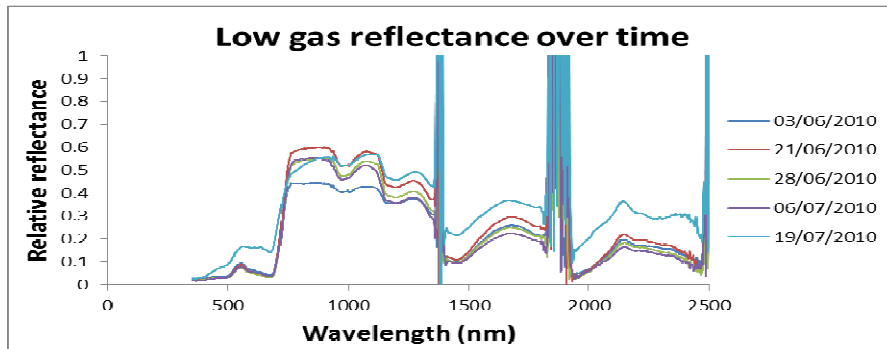


Figure 1b. Low gas reflectance for CO₂ plots.

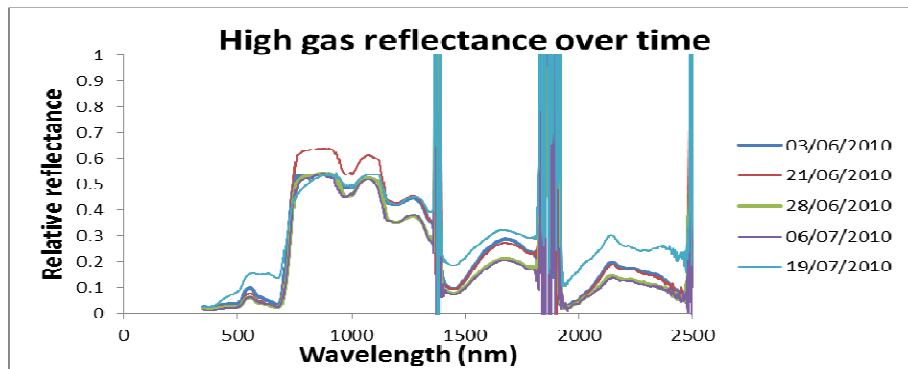


Figure 1c. High gas reflectance for CO₂ plots.

Reflectance of barley was measured throughout the experiment in 2010, each line for the control represents an average of sixteen spectral measurements (four plots) measured from the barley at 50, 100, 150 and 200 cm along the transect. The low gas represents an average of eight spectral measurements at 50 and 200 cm from the transect, while the high gas represents eight spectral measurements at 100 and 150 cm along the transect. The gaps in the spectra are noisy regions affected by atmospheric water vapour. Derivative spectra were calculated to provide a more sensitive analysis.

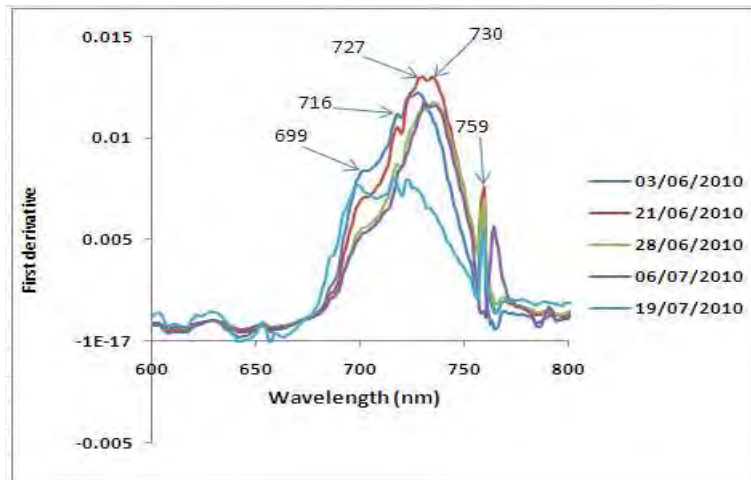


Figure2a. First derivative of reflectance in control plots for barley.

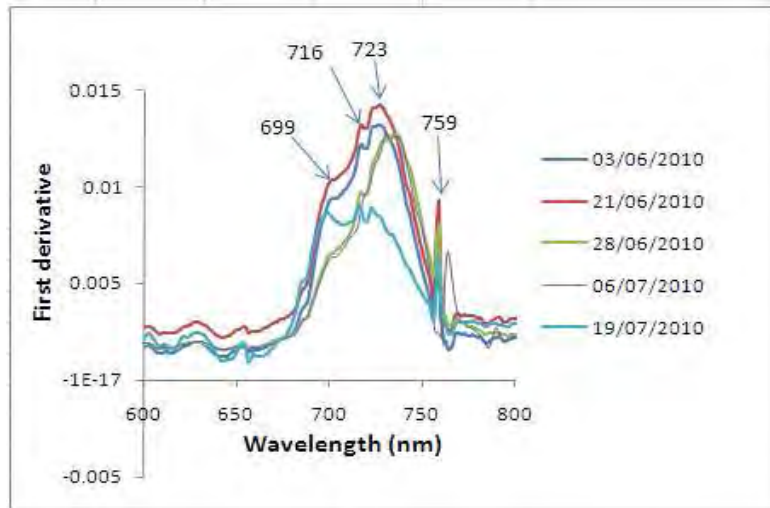


Figure2b. First derivative of reflectance in low CO₂ zone for barley.

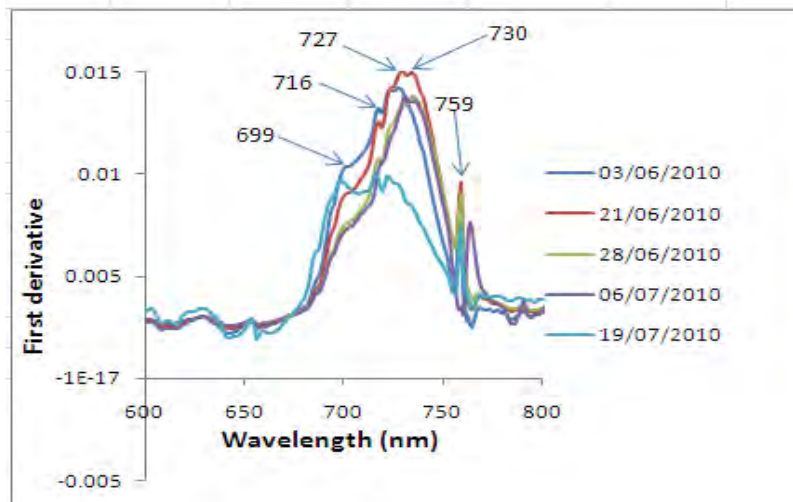


Figure2c. First derivative of reflectance in high CO₂ zone for barley.

Figures 2a-c shows the first derivative peaks throughout the experiment, this is composed of a maximum peak between 716 and 730 nm and smaller peaks or shoulder at 699 and 759 nm. These features were used to detect differences between control and gassed stressed barley. The magnitude of these peaks decreases with stress, depicting a shift towards shorter wavelength. This is in agreement with study by Noomen *et al.*, 2003 who found out that the reflectance of stressed plants often shows a shift of the 'red edge' position towards shorter wavelengths.

Several researchers have identified similar features in derivative spectra. According to Boochs *et al.*, (1990) the derivative peaks of winter wheat ranged between 725 and 740 nm with a shoulder at 703 nm though the growing season. Railyan and Korobov (1993) found peaks at 705 and 720 nm for *triticale* 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.

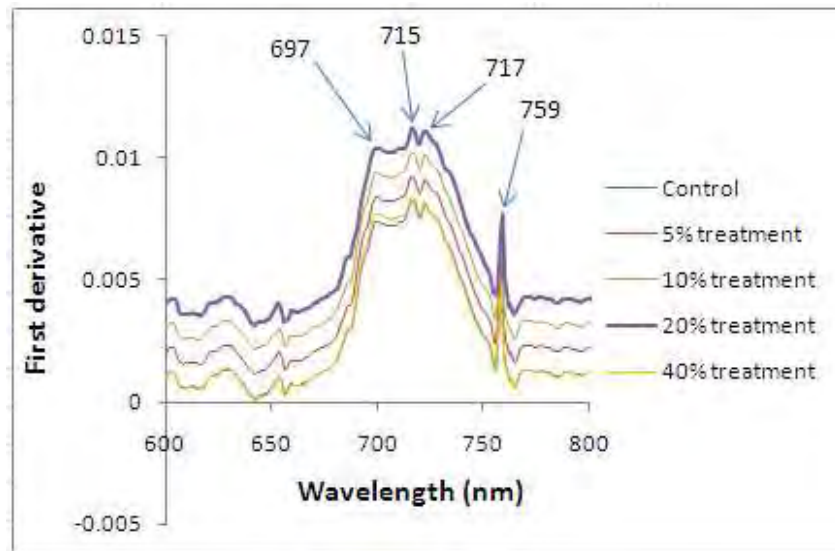


Figure 3a. First derivative spectra of the red edge peak for the herbicide treated plots for early treatment period measured on 4th of June, 2010, a day after application of herbicide.

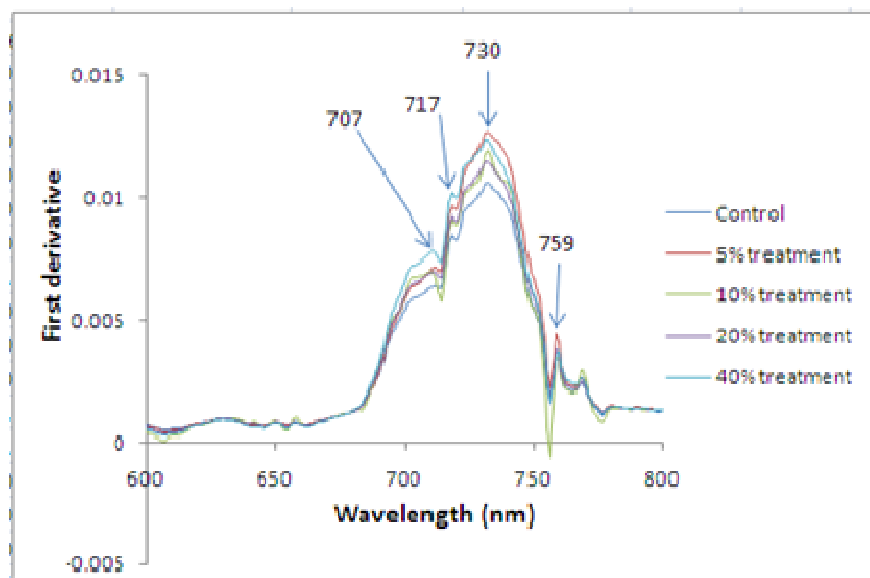


Figure 3b. First derivative spectra of the red edge peak for the herbicide treated plots for middle treatment period measured on 21st of June, 2010, (16 days after application of herbicide).

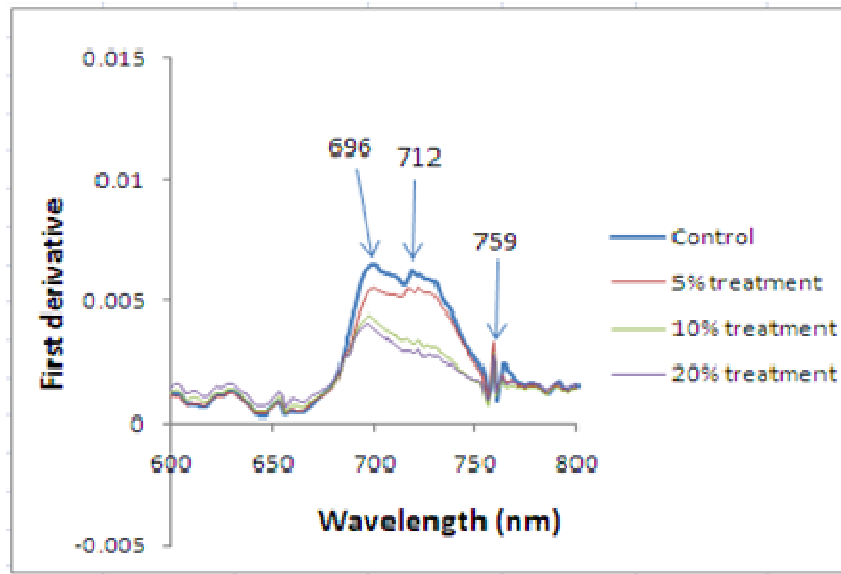


Figure 3c. First derivative spectra of the red edge peak for the herbicide treated plots for late treatment period measured on 9th of July, 2010 (35 days after application of herbicide).

The first derivative peak for the early treatment period was composed of peaks at 697, 715 and 717 nm with small shoulder or peak at 759 nm. As the experiment progressed and the herbicide stress begins to manifest, there was a shift and change in the derivative peaks, the maximum peak was between 707 and 730 nm with the major peak 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 with a shift of the red edge position to shorter wavelengths, the major peaks were between 696 and 712 nm respectively with a further decrease in the magnitude of the shoulder which remained at 759 nm.

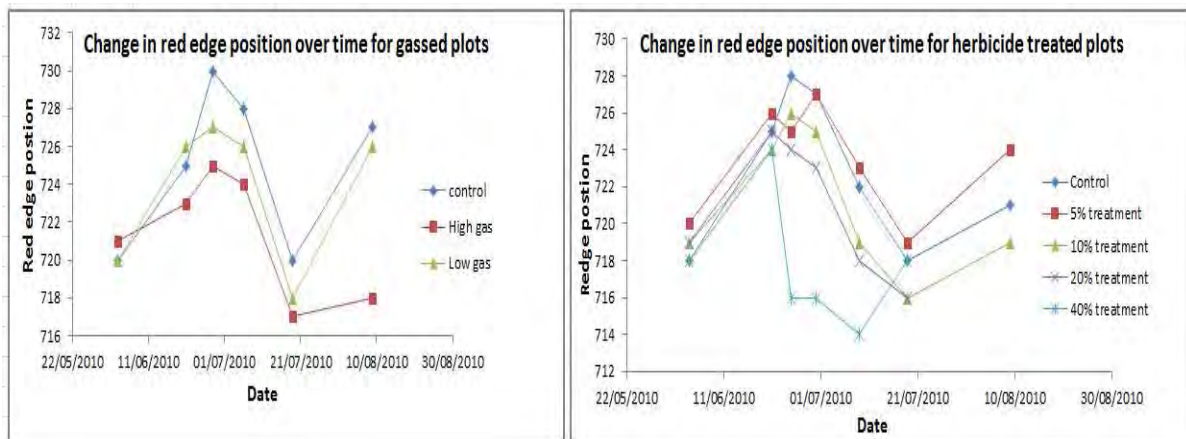


Figure 4. Temporal change in red edge position for CO₂ and herbicide treatment.

The red edge position was displaced rapidly towards longer wavelength for both the control and outer transect where the gas concentration was low, the outer transect caused an 8 nm shift in the position of red edge from 720 nm to 728 nm, while the control 720 nm to 727 nm a shift of 7 nm, the inner transect with higher gas concentration was displaced towards shorter wavelength 721 nm to 718 nm a shift of 3 nm.

However for the herbicide treated plots at the beginning of June there was a steady increase in the red edge position for all levels of treatment, but by mid-July the red edge position began to shift to shorter wavelength, at the end of the experiment, the control shifted from 718 nm to 721 nm (3 nm shift), 5% treatment shifted from 720 nm to

724 nm (4 nm), 20% treatment 719 nm to 716 nm, 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.

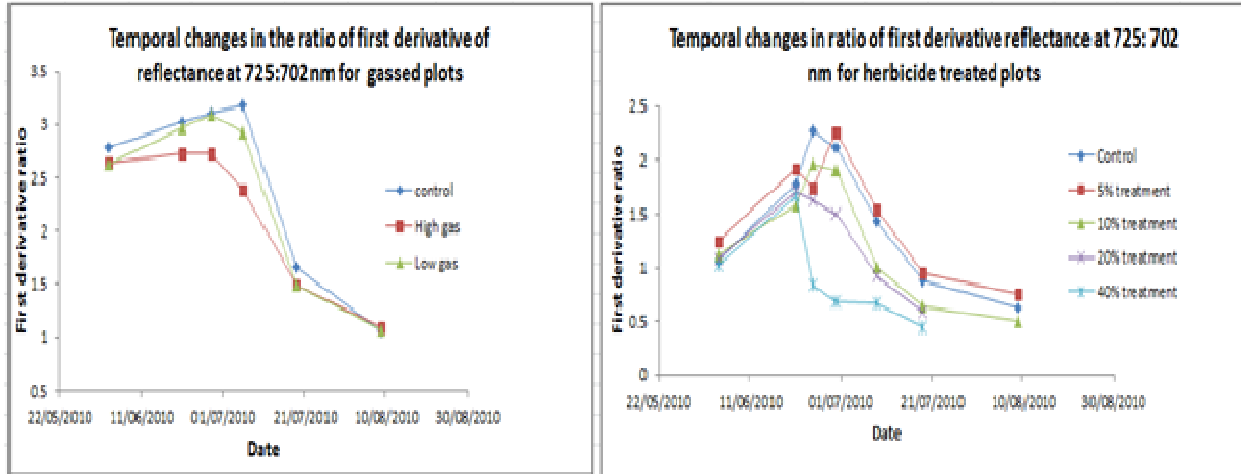


Figure 5. Temporal change ratio in the of first derivative of reflectance at 725:702 nm.

Figure:5 shows the temporal changes in the ratio of first derivative at 725:702 that occurred throughout the duration of the experiment. This index has been identified as a stress sensitive index by Smith et al., 2004. Between early June and late June there was little difference in the ratio between the control and the outer transect where the concentration of gas was minimal, while the difference in ratio between the control and inner transect was higher due to higher gas concentration in the centre of the plots. However in early July, the ratio measured in the gassed plots decreased further and the difference between the control and both the inner and the outer transect became apparent. The ratio in all the plots decreased throughout the remaining part of the experiment i.e. mid July till early August, at this particular time the barley crop had fully matured, leaves turned yellow and ready for harvest.

The barley crop with several levels of herbicide treatment on barley crop reacted differently in response to stress, between early June and late June when the effect of the herbicide applied was minimal, the difference in the ratio with control was little especially with the lowest concentration of 5%. From late June to mid July the ratio increased progressively, at this stage the ratio between control and 40 and 20% treatment are the highest, by the end of July the 40 and 20% treated field barley had turn yellow and died off, leaving just the control, 5 and 10% herbicide treated plots, in August the crop had turned yellow and ready was for harvest, the ratio between control and 10% and 5% had decreased as well but minimal at 5%.

Chlorophyll Content

Compared to barley crops in the control plots, chlorophyll content decreased by more than 75% in each treatment type ($p \leq 0.05$). For the low CO_2 zone the decrease was about 40% and 50% for high CO_2 zone, while for herbicide treatment it ranges between 70-90% for 5, 10 and 20%. Compared to the plants in control, chlorophyll content decreased in both treatments with herbicides decrease more than the gassed plots as shown in figures 6 and 7.

The concentration of chlorophyll in leaves and canopies can be expected to be a key indicator of such measures of physiological status, as photosynthetic capacity, developmental stage, productivity and stress (Whittaker and Marks, 1975; Danks et al., 1983). However; the distribution of chlorophyll within canopy varies in time and space.

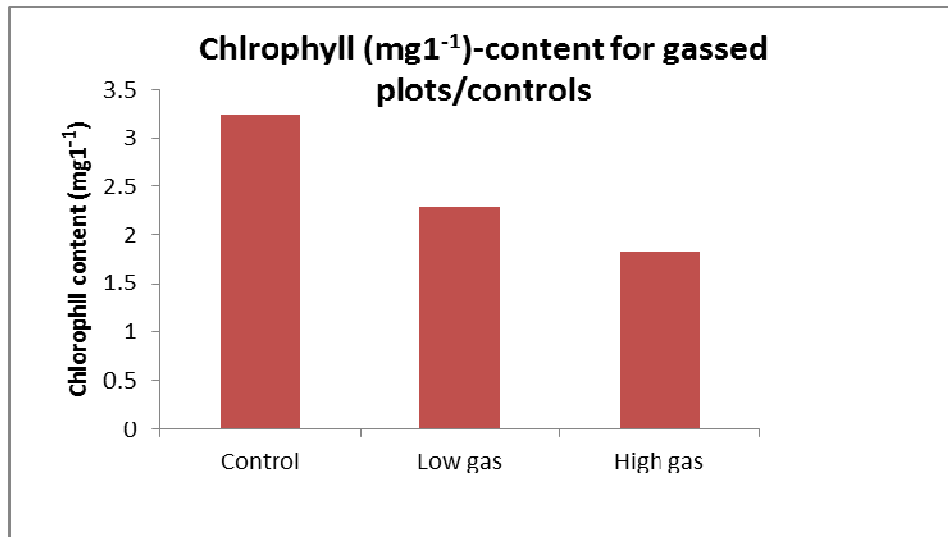


Figure 6. Average chlorophyll content for control, low and high CO₂ plots.

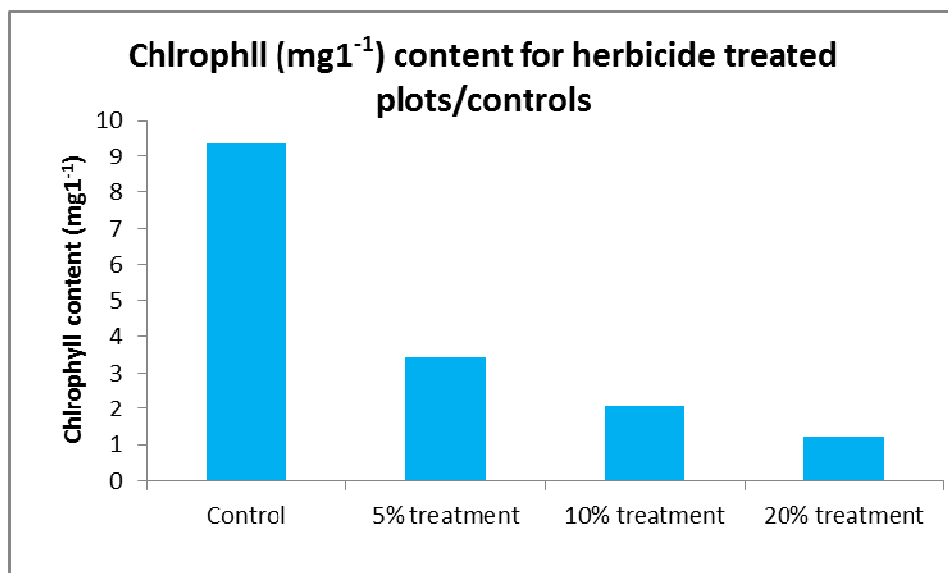


Figure 7. Average chlorophyll content for control and herbicide treated plots.

Visible Stress Symptoms

Yellowing of leaves, more severe in the CO₂ treatment, was observed in both experiments, and reduction in growth around the area of gas injection, the herbicide induced stress showed similar response but the magnitude was dependent on the concentration. In the CO₂ experiment, these symptoms of stress were visible 7 days after gas injection started. The leaves became more yellowish compared to the control. This shows a quick response to stress when compared to similar studies by Smith et al., (2004) where visible stress symptoms were noticed 14-21 days after treatment of barley and bean in pot which resulted in reduction in oxygen available for root respiration. The work of Pysek and Pysek (1989) found that visible stress symptoms occurred 15-30 days due to elevated concentrations of natural gas in the soil.

This result is consistent with the work by Venkata et al., 2010 where visible stress symptoms in the form of purple discoloration of leaves of dandelion (*T.officinale*) were noticed seven days after the beginning of CO₂ injection.

The leaves of barley treated with 40 and 20 % herbicide (Glyphosate) start to show visible stress symptoms in form of yellowing of leaves 21 days after treatment, 10% treatment exhibited stress symptoms 36 days after treatment, while 5% treatment showed visible stress symptoms 46 days after treatment.

Glyphosate is a highly mobile herbicide that is transported largely in the symplastic system after being absorbed by the leaves (Adcock et al 1990) causing stress throughout the plant, including the roots. The herbicide inhibits the aromatic amino acid biosynthesis pathway, reducing the production of protein and thus inhibiting plant growth (Ashton and Crafts 1981). In this study, leaves first started to inhibit yellow patches and progressed gradually and eventually covering a larger portion of the leaves. These results can be compared to the work of Ketel (1996) who found out that some yellowing of leaves of common lambs quarters (*Chenopodium album*) occurred one week after being sprayed with 25% of glyphosate.

As shown by this study and other studies in the literature the first evidence of stress symptoms is dependent on specie type, concentration level of the stressor and at what point the stress inducing agent (stressor) is applied.



Figure 8a. Barley CO₂ control plot .

Figure 8b. Stress barley crop in the plot middle.



Figure 9a. 40% herbicide treatment.

Figure 9b. 20% herbicide treatment.



Figure 9c. 10% herbicide treatment.

Figure 9d. herbicide: 5% treatment.

Figures 9a-d shows the various levels of herbicide treatment, the 40 and 20 % treatment levels were more visible in the photographs, these were taken 25 days after treatment, at this point barley growing on 40 and 20% treatment levels had virtually turned yellow but the severity was more at the 40% treatment followed by 20, 10 and 5 % respectively.

CONCLUSION

Elevated concentrations of CO₂ in the soil and dilute herbicide application caused stress in barley. Stress was detected by visual symptoms and changes in spectral reflectance of the vegetation. These visible symptoms manifested as chlorosis of the leaves and decrease in chlorophyll content. Stress causes crops to grow at less than their full potential and can cause reductions in yield. Differences in spectral features around the red edge are also evident. Remote sensing has previously been used to detect stress in plants before visible symptoms are observed and double peaks in the red-edge region have been linked to chlorophyll fluorescence. The differences observed between the stresses may be due to source of the stress; however the changes in the red edge position are consistent with the degree of chlorophyll loss. Although, the presence of elevated levels of soil gas was detected, the symptoms are believed to be a generic response to soil oxygen depletion.

The first stress symptom in barley was observed at t=7, this is relatively soon compared to similar studies on the effects of gas on plant growth. Pysek and Pysek (1989) observed a decrease in plant height and vegetation cover 15 to 30 days after the start of their experiment in which they gassed several species with natural gas, while studies by Smith et al (2004) showed that the first visible symptoms in grass and dwarf bean (*phaseolus vulgaris*) gassed with natural gas occurred respectively 44 days and 2 to 3 weeks after gassing started. As shown by these studies the first evidence of stress symptoms is dependent on species type and at what point the stress inducing agent (stressor) was applied.

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