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Remote sensing of the effect of the herbicide glyphosate on the leaf spectral reflectance of pea plants (*pisum sativum l.*)

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ABSTRACT: Results from a remote sensing study on leaf spectral reflectance changes of pea plants due to herbicide glyphosate action applied at three concentrations (0.1 μM, 1 μM and 10 μM), low as compared to the herbicide field dose used in the agricultural practice are presented and discussed. The glyphosate is one of the most frequently used herbicides in Bulgaria, mainly in the common agriculture regions. Leaf spectral reflectance data were obtained by a multichannel spectrometer designed in STIL-BAS. The data were registered in the visible and near infrared spectral ranges (480 ÷ 810 nm) in 128 channels with 2.6 nm spectral resolution (halfwidth) and 2 mm^2 spatial resolution. The spectrometric measurements were performed on fresh, immediately picked off pea leaves in two leaf node on the 14th day after treating with the herbicide. To assess the statistical significance of the differences between leaf spectral reflectance characteristics of control and treated with the three glyphosate concentrations plants we applied an approach based on discriminant analysis and other statistical methods. The fresh weight of the plants was used as the biometric parameter to assess the changes in the plant physiological status. Statistically significant differences at $p < 0.05$ between the spectral reflectance characteristics of control and treated plants were established in the four most informative for plants spectral ranges: green (520 ÷ 580 nm), maximal chlorophyll absorption (630 ÷ 680 nm), red (690 ÷ 730 nm) and near infrared (740 ÷ 810 nm).

1 INTRODUCTION

In the precision agriculture many studies have been devoted to the use of biotic information to diagnose plant responses to changing environment. To obtain the adequate information a special emphasis has been laid on the application of non-destructive and non-invasive measuring methods. In this respect remote sensing technologies provide an important tool to aid site-specific management of crops. Remote sensing has the potential to provide real-time analysis of the attributes of a growing crop. The results of such analyses form the basis for making timely management decisions that affect the outcome of the current crop. The information on plant response to growth conditions that is supplied by remote sensors such as spectrometers, radiometers etc. is used to achieve the optimization of the controlled system, increase in plant production and sustainable agriculture. However, like other precision agriculture technologies, the information from remote sensing is more meaningful when combined with other available data.

Almost all of the applications of remote sensing in agriculture to date have been based on observing crops in distinct ranges of the electromagnetic spectrum. Agricultural remote sensing is commonly carried out in the visible, near-infrared and thermal infrared portions of the spectrum; however, new applications in the microwave area are under development.

Optical remote sensing provides a powerful tool for monitoring changes in the crop canopy over the season of growing. Spectrometric measurements of the reflected solar radiation contain important information pertinent to the vegetation status. They have been used to estimate yield, monitor plant health and plant stress, and to characterize properties of vegetation such as plant biomass and leaf area index. In the discipline of Weed Science, most applications of optical remote sensing methods have been focused on detecting the presence of weeds within a crop canopy and to reveal herbicide injury of plants. Remotely sensed measurements of plant multi-spectral reflectance can provide a quick, inexpensive, and non-destructive method of a real time assessment of biophysical variable estimation of the whole field. Current methods of crop assessment can be tedious, laborious, and costly and time consuming.

In recent years there has been a tendency to replace the uniform, early season weed control choices with alternative approaches that rely on applying post-emergence herbicides in case of a need. Inappropriate herbicide applications can have unintended side effects on crop performance and the environment. Therefore, there is increased interest in using remote sensing methods to detect and define accidental herbicide damages to a crop. This provides the possibility the crop to be targeted with the appropriate amount of herbicide for ground and aerial spray treatment. Such approaches avoid applications to weed-free areas and help in reducing herbicide usage and potential contamination of ground waters without compromising weed control.

Glyphosate [(*N*-phosphonomethyl)glycine] is one of the most frequently used herbicides in Bulgaria, mainly in the common agriculture regions. It is a highly effective broad-spectrum, non-selective, post-emergence herbicide which is used extensively worldwide. The target site of glyphosate action is the enzyme 5-enoylpyruvylshikimate-3-phosphate synthase (Schünbrunn *et al.* 2001).

The inhibition of this enzyme causes an accumulation of shikimic acid and a consecutive diminish in the biosynthesis of aromatic amino acids, auxins, vitamins, as well as a number of key metabolites produced via the shikimate pathway, all this leads to a suspended plant growth, and in turn to plant death (Cobb 1992). The glyphosate degradation appears to be very slow or is not taking place in higher plants (Ashton & Crafts 1981).

The present study is aimed to identify through changes in leaf spectral reflectance stress impact on pea plants due to glyphosate application. Recent studies show that a glyphosate tolerance can be successfully achieved by genetic engineering manipulations in some crops such as maize plants (Hetherongton *et al.* 1999) as well as by appropriate compounds which are able to diminish the glyphosate toxicity (Sergiev 2006). Nevertheless, to assess the activity of glyphosate is continuing to be a problem of current interest. The action of glyphosate was investigated when applied at three concentrations

(0.1 μM, 1 μM and 10 μM) which are lower than the field dose of this herbicide commonly used in the agricultural practice.

2 METHODS

2.1 *Plant material*

The experiments were carried out with pea plants (Pisum sativum, L., cv. Skinado) produced by the local market. Seeds were surface-sterilized with $KMnO_4$ and soaked in water with an air pump, for a period of 4 hours. After germination in dark thermostatic chamber for 3 days, the plants were transferred into a growth chamber (12/12 h photoperiod, photon flux density 70 μmol $m^{-2}sec^{-1}$, and temperature $25 \pm 1°C$) and were grown as water cultures (Hoagland-Arnon nutrient medium). Three-day-old seedlings were subjected to herbicide treatment. Glyphosate (Roundup®, produced by Monsanto Co.) was added to the nutrient solution in 0.1 μM, 1 μM and 10 μM concentrations, corresponding to 0.069 μg/l, 0.69 μg/l and 6.9 μg/l. The concentration applied on the roots was selected on the basis of our preliminary experiments. Nutrient solutions were renewed twice a week. All measurements were made on the 14th day after beginning the experiment. The changes in the physiological status of the model pea plants treated with the herbicide were assessed with fresh weight of the aboveground part of the plants.

2.2 *Spectral remote sensing method*

The spectral data for the radiation reflected by the plants studied was obtained from spectrometric measurements yielding the spectral reflectance in the visible and near-infrared spectral ranges.

The spectral reflectance is defined as the part of incident energy which is reflected within a given spectral interval of the electromagnetic spectrum from the object of interest. Most of the energy given off by the Sun is in the visible and near-infrared wavelength ranges. As a result, most remote sensing of vegetation uses those wavelengths. However, the most useful information is provided by differences in the reflectance within the various portions of the electromagnetic spectrum. These differences in reflectance along the entire electromagnetic spectrum can be used to distinguish healthy vegetation from necrotic or stressed vegetation (Carter 1993). Various factors, including drought, nutritional deficiency, disease, nematodes and herbicide injury can reduce or alter the chlorophyll content and other substances that affect the reflectance of the vegetation. In general, the spectral reflectance of plant leaves is a function of their structure, water content and concentration of biochemical substances. It is a reliable indicator for changes in the plant physiology arising under the impact of different stress factors and different concentrations (Yanev *et al*. 2000).

The leaf spectral reflectance data were obtained with the help of a multichannel spectrometer 'Spectrum 256' designed in STIL-BAS (Mishev *et al.* 1999). The data were registered in the visible and near infrared spectral ranges ($480 \div 810$ nm) in 128 channels with 2.6 nm spectral resolution (halfwidth) and 2 mm^2 spatial resolution.

The present spectrometric measurements were carried out in laboratory. A specialized measuring set up was built up. It consists of the spectrometer 'Spectrum 256', an optical bench, an optical table, a standard white screen with coverage of barium sulfate, a movable platform, a light source – a special holder with three halogen lamps arranged under 120° on circumference, and three regulated power sources of high stability for energizing the lamps. The optical table to which the multichannel spectrometer is fixed has two degrees of freedom. It can move both in vertical and perpendicular directions with respect to the optical axis. This way the planar scanning of leaf specimens of the plants under study is enabled. The movable platform (black in color) upon which the pea plant leaves are arranged one adjacent to the other and fixed, is located at a distance of 2.5 m from the spectrometer objective and is set perpendicular to its optical axis.

The spectrometric measurements were performed on fresh, immediately picked off pea leaves of 15 plants (untreated – control, and treated with every one of the glyphosate concentrations) in two leaf node. The leaf spectral reflectance for on average 25 areas (pixels) was registered on the 14th day after treating the plants with the herbicide. Further the value in a given wavelength of each spectral reflectance characteristic (SRC), obtained from one area will be denoted as "case".

The spectral data were transformed in relative units, i.e. in spectral reflectance coefficients. For this purpose every spectrometric measurement was attended with registration of the reflected from the standard screen radiation (100 spectra on average) and 150 records of the dark current for all channels of the spectrometer.

2.3 *Statistical methods*

To assess statistically significant changes in the arising differences in the leaf SRCs between control and treated plants a novel approach (Krezhova *et al.* 2005) was developed. The basic statistical method herein used is the discriminant analysis that allows the researcher to detect variables by means of which to discriminate between different groups of objects assigning posterior probabilities to the classified objects. The t-criterion of Student was applied for 8 wavelengths chosen to be disposed uniformly over the range including the green, red and the near infrared bands ($\lambda_1 = 524.2$ nm, $\lambda_2 = 539.8$ nm, $\lambda_3 = 552.8$ nm, $\lambda_4 = 667.2$ nm, $\lambda_5 = 703.6$ nm, $\lambda_6 = 719.2$ nm, $\lambda_7 = 724.4$ nm, and $\lambda_8 = 758.2$ nm). The results obtained through the t-criterion enable to choose which combination of wavelengths to be used in the discriminant analysis. Different combinations of wavelengths may allow better classification results to be achieved.

3 RESULTS AND DISCUSSION

3.1 *Fresh weight of aboveground part of pea plants*

The results for the effect of long-term action of the different concentrations of herbicide on the fresh weight of pea plants are presented in Table 1. The 14 days' treatment with 10 µM glyphosate caused a reduction of the fresh weight of shoot by 12.2% in comparison with the control. However, it is seen from the data that the lower herbicide concentrations (1 and 0.1 µM) did not provoke a significantly smaller reduction of this biotic parameter. The similar tendency was observed in relation to the dry matter of aboveground part (data not shown). Comparing with the findings for other herbicides such as paraquat, atrazine etc., which were objects of our previous experiments in the

Table 1. Effect of different concentrations of glyphosate on the fresh weight of aboveground part of pea plants. SD = standard deviation.

Variant	Fresh weight [mg/plant ± SD]	% to the control
Control	457.4 ± 19.6	100.0
0.1 μM	481.3 ± 24.8	105.2
1 μM	436.1 ± 29.5	95.3
10 μM	401.7 ± 22.4	87.8

same model system, e.g. (Krezhova *et al.* 2005a, b) the inhibitory effect of glyphosate on the plant growth is weakly expressed. For instance, the injury of plants treated for the same duration of 14 days with paraquat at the same concentrations is far more strongly pronounced and the weight reduction of shoot is reaching 60 % for herbicide concentrations of 10 μM.

3.2 *Leaf spectral reflectance*

The averaged SRC (for leaves of control and treated plants) calculated from SRC obtained from all 25 areas according to paragraph 2.2 are shown in Figure 1. The differences between SRC of leaves of control and treated plant are most explicitly manifested in the spectral ranges: green (520 ÷ 580 nm), maximal chlorophyll absorption (630 ÷ 680 nm) red (690 ÷ 730 nm) and near infrared (740 ÷ 810 nm).

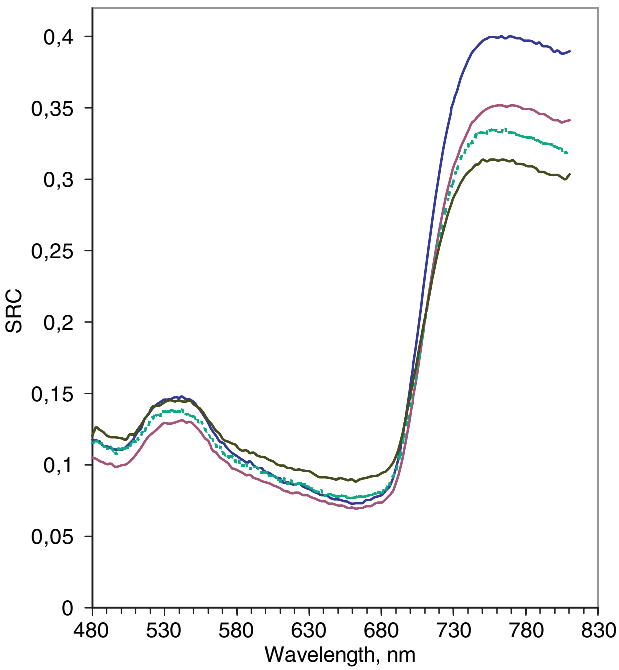

Figure 1. Average spectral reflectance characteristics of control (thick full line) and glyphosate treated leaves at concentration: 0.1 μM – (dashed line), 1μM – (circle), and 10 μM – (thin line).

Table 2. p-values of the t-criterion for pairs of the control and treated plants.

Glyphosate. concentration [μM]	λ [nm]							
	524.4	539.8	552.8	667.2	703.6	719.2	724.4	758.2
0.1	0.522	0.617	0.006	<0.001	0.054	<0.001	<0.001	<0.001
1.0	<0.001	0.074	0.049	<0.001	0.105	<0.001	<0.001	<0.001
10	0.008	0.003	0.007	<0.001	<0.001	<0.001	<0.001	<0.001

The t-criterion of Student was applied to estimate the statistical significance of the differences between SRC in the eight wavelengths above discussed. In all the wavelengths examined and in the cases of glyphosate concentration of 10 μM the values of SRC of treated and control plants differ statistically significantly at $p < 0.05$ from those of glyphosate concentration of 1 μM. Only at the wavelengths 539.8 nm and 703.6 nm the values of SRC of treated and control plants do not differ statistically significantly at $p < 0.05$. Finally, in the case of the lowest applied glyphosate concentration of 0.1 μM only in the wavelength 524.4 nm and 539.8 nm the values of SRC of treated and control plants do not differ statistically significantly at $p < 0.05$. So SRC may be reliably used as a tool for recognition of the glyphosate impact on pea plants especially in the ranges of maximal chlorophyll absorption (630 ÷ 680 nm) and the near infrared band (720 ÷ 810 nm).

This result was conclusively confirmed by the application of the linear discriminant analysis (following the algorithm proposed in Krezhova *et al.* 2005) in the three dimensional space defined by $\lambda = 539.8$, 719.2 and 794.6 nm.

The grouping variable was defined by the preliminary information for belonging of the different cases to control or treated plants. Almost all cases (SRC of leaves of controls and treated plants) were correctly (90 ÷ 95%) classified, i.e. the a priori introduced belonging to the groups of control and treated plants were confirmed. A high linear correlation coefficient ($R > 0.99$) was established to exist between SRC values in the wavelengths 719.2 nm and 758.2 nm (for example, see Figure 2 and 3) for all glyphosate concentrations examined.

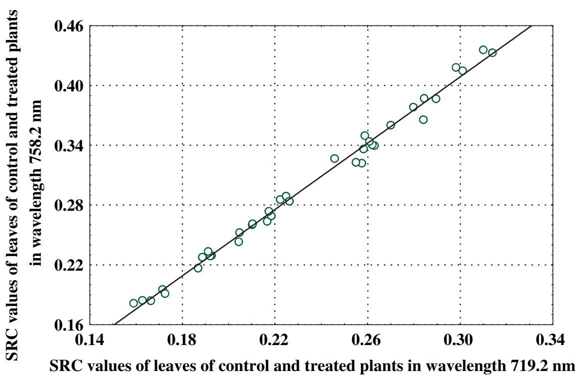

Figure 2. Scatter plot and linear regression of SRC values of control and treated plants regressed together for gliphosate concentration 0.1 μM.

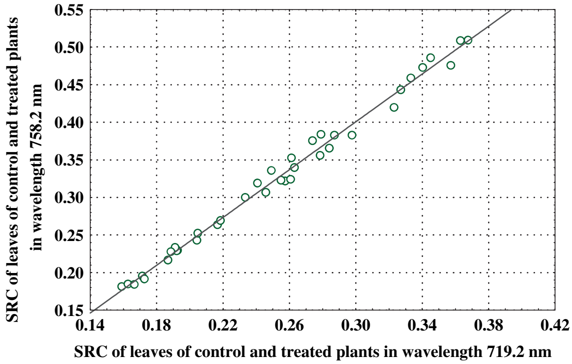

Figure 3. Scatter plot and linear regression of SRC values of control and treated plants regressed together with gliphosate concentration 10 μM.

4 CONCLUSIONS

Differences between the spectral reflectance characteristics of leaves of control and fourteen days' long treated with glyphosate pea plants were established. The findings come out directly from the remote sensing data but gain in affirmativeness by treating them with statistical methods. Regardless of the low concentration of the applied herbicide, the changes in reflectance were safely distinguished in all the three most informative for plants spectral ranges: green (520 ÷ 580 nm), red (690 ÷ 730 nm) and near infrared (740 ÷ 810 nm). The extremely high linear correlation above discussed indicates that one of the wavelengths examined is not necessary for further research. Herbicide injury was manifested with in the green and red ranges – reduction of SRC of treated plants in comparison with the control plants, while in the near infrared range on the opposite – an elevation of SRC of treated plants was observed. In contrast, the fresh weight of the plants when used as the direct biometric parameter to assess the influence of glyphosate application did not provide a convincing indication of early changes in the plant physiological status at the low doses studied. It is believed that this study will help in assessments of herbicide injury problems and in defining the optimum rate of glyphosate application.

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