



Early detection of crop injury from herbicide glyphosate by leaf biochemical parameter inversion

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ABSTRACT

Early detection of crop injury from herbicide glyphosate is of significant importance in crop management. In this paper, we attempt to detect glyphosate-induced crop injury by PROSPECT (leaf optical PROperty SPECTra model) inversion through leaf hyperspectral reflectance measurements for non-Glyphosate-Resistant (non-GR) soybean and non-GR cotton leaves. The PROSPECT model was inverted to retrieve chlorophyll content (C_{a+b}), equivalent water thickness (C_w), and leaf mass per area (C_m) from leaf hyperspectral reflectance spectra. The leaf stress conditions were then evaluated by examining the temporal variations of these biochemical constituents after glyphosate treatment. The approach was validated with greenhouse-measured datasets. Results indicated that the leaf injury caused by glyphosate treatments could be detected shortly after the spraying for both soybean and cotton by PROSPECT inversion, with C_{a+b} of the leaves treated with high dose solution decreasing more rapidly compared with leaves left untreated, whereas the C_w and C_m showed no obvious difference between treated and untreated leaves. For both non-GR soybean and non-GR cotton, the retrieved C_{a+b} values of the glyphosate treated plants from leaf hyperspectral data could be distinguished from that of the untreated plants within 48 h after the treatment, which could be employed as a useful indicator for glyphosate injury detection. These findings demonstrate the feasibility of applying the PROSPECT inversion technique for the early detection of leaf injury from glyphosate and its potential for agricultural plant status monitoring.

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Introduction

Glyphosate drift has been of particular concern recently because it can cause injury or mortality to off-target sensitive non-Glyphosate-Resistant (non-GR) crops (Ding et al., 2011). For the early detection of crop injury from off-target glyphosate drift, portions of the visible and near-infrared reflectance spectra are ideal indicators of stress because stress-induced changes of leaf interior structure and growth status could alter the spectrum from that of a healthy leaf (Huang et al., 2012).

Foliar biochemical properties represent the growth status of plants, and they are good indicators of glyphosate-induced leaf injury (Reddy et al., 2000, 2010; Koger et al., 2005). For the purpose of detecting the crop injury caused by glyphosate drift, traditional methods of directly measuring the leaf biochemical parameters in vivo are labor- and time-intensive and cannot meet

requirements for rapid and large-scale monitoring. Several studies have attempted to develop indirect approaches for detecting crop stress (e.g. water-stress and nitrogen-stress) with hyperspectral reflectance data (Barnes et al., 1992; Carter, 1994; Filella and Peñuelas, 1994). Recently, these indirect approaches have been introduced for detection of glyphosate-induced crop injury by the biological remote sensing community. For example, in an airborne remote sensing experiment, Huang et al. (2010) assessed damage to cotton caused by spray drift from aerially applied glyphosate by mapping the NDVI (Normalized Difference Vegetation Index) image of the experimental area. More recently, Huang et al. (2012) used hyperspectral reflectance data to distinguish the glyphosate injured soybean and cotton leaves from the healthy ones by calculating the NDVI, RVI (Ratio Vegetation Index), SAVI (Soil Adjusted Vegetation Index), and DVI (Difference Vegetation Index) of each leaf. In a greenhouse experiment, Yao et al. (2012) found that hyperspectral imaging of plant canopy was a useful tool for early detection of soybean injury due to glyphosate application, and that spectral derivative indices proved to be a good indicator for glyphosate injury. As these vegetation indices were not specifically designed

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for crop injury detection and therefore less effective, spectral feature extraction methods were introduced (Zhao et al., 2014).

Current efforts depend primarily on constructing vegetation indices or spectral features that potentially relate to glyphosate-induced crop stress. But these methods are not physically-based and may not be applied effectively over a wide range of species. Physically-based radiative transfer models that quantitatively relate foliar biochemical properties to reflectance spectra can inherently provide more consistent results over multiple species and have the potential of improving detection of glyphosate-induced crop injury.

In this study, we attempted to detect glyphosate-induced leaf injury through quantitative estimation of foliar biochemical contents from leaf hyperspectral reflectance measurements. This was accomplished by inversion of a physically based radiative transfer model, PROSPECT (leaf optical PROperty SPECTra model) (Jacquemoud and Baret, 1990; Fourty et al., 1996; Jacquemoud et al., 1996, 2000; Feret et al., 2008). To obtain a more accurate result, we applied an improved procedure for model inversion to improve the retrieval accuracies of the foliar biochemical parameters: chlorophyll content (C_{a+b} , chlorophyll a + b content, in unit of $\mu\text{g}/\text{cm}^2$), equivalent water thickness (C_w , mass of water per leaf area, in unit of g/cm^2), leaf mass per area (C_m , mass of dry matter per leaf area, in unit of g/cm^2), and leaf structural parameter (N , number of compact layers specifying the average number of air/cell walls interfaces within the mesophyll). In order to evaluate the effectiveness of the proposed inversion procedure, correlation scalograms of retrieved versus measured values were plotted for C_{a+b} , C_w , and C_m , respectively. Glyphosate-induced leaf injury was then analyzed by examining temporal variations of these retrieved biochemical parameters after leaf treatment at high-dose, low-dose and no glyphosate. Finally, advantages and potential of this proposed method were discussed.

Experiment

The experiment was conducted in a greenhouse located at the USDA-Agricultural Research Service, Crop Production Systems Research Unit, Stoneville, Mississippi on December 17–20, 2012, and repeated February 4–7, 2013. The crops were planted in pots using a Completely Randomized Design (CRD), and growing conditions for the plants set temperature to 23.9 °C in the daytime and 21.1 °C at night. Four weeks after planting, the plants were treated and the leaves of them were measured for spectral reflectance experiment. The four week schedule to spray glyphosate was determined by weed scientists to simulate the situation in field to effectively control weeds.

In each experiment, 36 pots of non-GR cotton (cultivar FM955LL) and 36 pots of non-GR soybean (cultivar SO80120LL) were used to obtain leaf reflectance spectra and foliar biochemical properties. For each crop, we divided the pots randomly into 3 treatment groups: 12 plants were sprayed with 0.433 kg ae/ha solution of glyphosate (0.5X group; $X = 0.866 \text{ kg ae/ha}$, which is the label rate of glyphosate); another 12 plants were sprayed with half of the 0.5X dose (0.25X group); the remaining 12 plants were used as controls with no glyphosate treatment (CTRL group). Glyphosate solutions were prepared using a commercial formulation of the potassium salt of glyphosate (Roundup WeatherMax, Monsanto Agricultural Co., St. Louis, MO), and applied using a CO_2 -pressurized backpack sprayer that delivered 140 L/ha of spray solution at 193 kPa. After the glyphosate spraying, leaf reflectance and biochemical parameters (C_{a+b} , C_w , and C_m) of three plants for each group were measured at 6, 24, 48, 72 Hours After the Treatment (HAT) to study plant response to glyphosate.

Leaf reflectance measurements were acquired by using an ASD integrating sphere apparatus coupled with the ASD FieldSpec 3 Hi-Res spectroradiometer (ASD Inc., Boulder, CO., USA), yielding a 1-nm spectral resolution in the visible to near-infrared range (400–2500 nm). Connected with the integrating sphere, Spare Lamps (Qty 2, Osram #64225, 6V, 10W) provides a collimated beam as the light source, which illuminates the sample or the Reference Standard.

The reflectance of leaf sample was measured following the procedure described in the manual of ASD integrating sphere (ASD Inc., 2008) in which three measurements are required: sample measurement (I_s), stray light measurement (I_d), and Reference Standard measurement (I_r). These spectra were collected in raw DN (Digital Number) mode. An integration time of 544 ms was used for all the measurements. With the known reflectance of the Reference Standard, R_r , the reflectance of the sample for a given center wavelength and spectral bandpass, R_s , is calculated as follows:

$$R_s = \frac{(I_s - I_d)R_r}{I_r - I_d} \quad (1)$$

One of the lowermost trifoliate leaves for soybean and twin leaves for cotton was selected for the measurements of the reflectance. These leaves were identified before the glyphosate treatment to make sure leaves at the same position of each plant were used for all four days. The leaves were large enough to cover the port of the integrating sphere. The location of the leaf sample changed three times during the measurement (avoiding main veins of the leaf in the port) to acquire the mean spectrum of the leaf.

After the leaf reflectance measurement, the leaf sample's area was immediately measured using a LI-COR 3100 Area Meter (LI-COR, Inc., Lincoln, NE, USA). The sample was then dropped into a vial with DiMethyl Sulfoxide (DMSO) and covered with aluminum foil. After 24 h in the dark environment, the solution was used for chlorophyll analysis using a Shimadzu UV160U Spectrophotometer (Shimadzu Corp., Kyoto, Japan). In order to calculate C_w and C_m , the remaining leaves of the plants were scanned to determine the leaf area and weighed to measure their fresh weights. Then they were oven-dried at 45–50 °C for 48 h, and reweighed to determine the dry weights. The mean values and ranges of C_{a+b} , C_w , and C_m over these two experiments are summarized in Table 1.

Methods

An improved approach for PROSPECT inversion was implemented for enhanced retrieval accuracy of leaf biochemical parameters. The PROSPECT model was first used to generate an artificial dataset, which would be used in sensitivity analysis; in this case a sensitive wavelength region was selected for each input parameter of PROSPECT. Based on the sensitivity analysis result, each parameter was assigned a specific merit function on its sensitive wavelength region, and a global optimization algorithm was used to retrieve these parameters. Finally, the accuracy of the inversion process was evaluated by comparing the retrieved and measured values. After the leaf biochemical parameters were retrieved by model inversion, glyphosate-induced leaf injury was analyzed by examining the temporal variations of these retrieved values. The schematic representation of the injury detection process is shown in Fig. 1.

Artificial data generation

When N , C_{a+b} , C_w , and C_m are determined, leaf hemispherical reflectance spectra in the wavelength band of 400–2500 nm can be simulated by PROSPECT. The model was first calibrated using the method given by Feret et al. (2008) and Li and Wang (2011) with the data of CTRL groups. For

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