



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



Feng Zhao<sup>a,\*</sup>, Yiqing Guo<sup>a</sup>, Yanbo Huang<sup>b</sup>, Krishna N. Reddy<sup>b</sup>, Matthew A. Lee<sup>b</sup>, Reginald S. Fletcher<sup>b</sup>, Steven J. Thomson<sup>b</sup>

<sup>a</sup> School of Instrumentation Science and Opto-electronics Engineering, Beihang University, Beijing 100191, PR China

<sup>b</sup> USDA-Agricultural Research Service, Crop Production Systems Research Unit, 141 Experiment Station Road, Stoneville, MS 38776, USA

### ARTICLE INFO

#### Article history:

Received 23 December 2013

Accepted 12 March 2014

#### Keywords:

Crop injury

Glyphosate

Foliar biochemistry

Sensitivity analysis

Model inversion

Hyperspectral

### 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.

© 2014 Elsevier B.V. All rights reserved.

### 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

\* Corresponding author. Tel.: +86 10 82315884.

E-mail address: zhaofeng@buaa.edu.cn (F. Zhao).

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  $\text{g}/\text{cm}^2$ ), leaf mass per area ( $C_m$ , mass of dry matter per leaf area, in unit of  $\text{g}/\text{cm}^2$ ), 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  $\text{CO}_2$ -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

**Table 1**  
Leaf biochemical data from greenhouse-measured datasets (December 2012 and February 2013). The maximum, minimum, and mean values of leaf chlorophyll content ( $C_{a+b}$ ), water content ( $C_w$ ), and dry matter content ( $C_m$ ) of soybean and cotton acquired in these experiments are shown in the table.

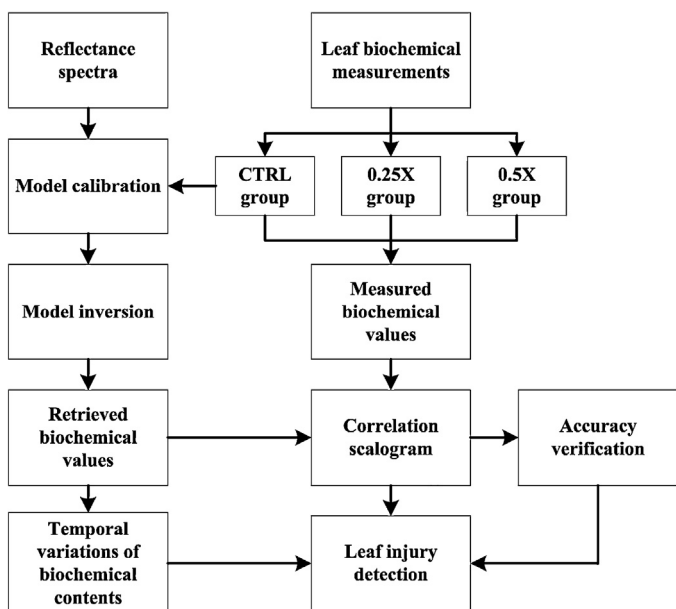
Species	Soybean			Cotton		
	CTRL <sup>a</sup>	0.25X <sup>a</sup>	0.5X <sup>a</sup>	CTRL <sup>a</sup>	0.25X <sup>a</sup>	0.5X <sup>a</sup>
$C_{a+b}$ ( $\mu\text{g}/\text{cm}^2$ )						
Min.	8.1146	6.4691	4.8688	5.3578	4.1407	2.8086
Max.	19.094	18.127	18.432	14.227	13.591	19.106
Mean	13.259	11.456	10.965	10.163	9.1355	8.1611
$C_w$ ( $\text{g}/\text{cm}^2$ )						
Min.	0.0104	0.0103	0.0098	0.0148	0.0135	0.0135
Max.	0.0135	0.0136	0.0147	0.0212	0.0267	0.0246
Mean	0.0120	0.0122	0.0123	0.0175	0.0175	0.0177
$C_m$ ( $\text{g}/\text{cm}^2$ )						
Min.	0.0020	0.0018	0.0019	0.0027	0.0023	0.0024
Max.	0.0027	0.0030	0.0030	0.0038	0.0045	0.0042
Mean	0.0023	0.0024	0.0024	0.0030	0.0030	0.0030

<sup>a</sup> CTRL group contains leaves with no glyphosate treatment; 0.25X group contains leaves treated with 0.217 kg ae/ha solution of glyphosate; 0.5X group contains leaves treated with 0.433 kg ae/ha solution of glyphosate.

**Table 2**  
Sensitive wavelength regions for PROSPECT input parameters.

Parameter	$N$	$C_{a+b}$	$C_w$	$C_m$
Sensitive wavelength region	760–1300 nm	400–760 nm	1900–2100 nm	2100–2300 nm

artificial data generation, ranges of  $C_{a+b}$ ,  $C_w$ , and  $C_m$  were defined as 2.8086–19.106  $\mu\text{g}/\text{cm}^2$ , 0.0098–0.0267  $\text{g}/\text{cm}^2$ , and 0.0018–0.0045  $\text{g}/\text{cm}^2$ , respectively, since they could cover all the greenhouse-measured values presented in Table 1.  $N$  was assigned a reasonable range of 1–4, which could describe a wide range of mesophyll structures of different leaf species (Jacquemoud and Baret, 1990). One thousand combinations of the parameters were randomly selected from these ranges as the inputs and 1000 reflectance spectra were produced by model simulation. All combinations of parameters with the corresponding reflectance spectra formed our artificial dataset, which would be used in sensitivity analysis of PROSPECT.



**Fig. 1.** Schematic representation of the leaf injury detection process.

### Sensitivity analysis of PROSPECT

The method of EFAST (Extended Fourier Amplitude Sensitivity Test), which was proposed by Saltelli et al. (1999), was used for sensitivity analysis of PROSPECT in our study. The artificial dataset previously simulated by PROSPECT was used as input data. EFAST allows the simultaneous computation of the first order and the total sensitivity indices for a given input variable. The first order sensitivity index gives the independent effect of the corresponding parameters, while the total sensitivity index contains both independent effect of each parameter and the interaction effects with the others (Saltelli et al., 2008).

The result of PROSPECT sensitivity analysis showed that each parameter had its own comparatively sensitive spectral band. Within wavelengths between 760 and 1300 nm,  $N$  was the crucial parameter that contributed more than 90% uncertainty of the outputs. For shorter wavelengths in the visible band of 400–760 nm,  $C_{a+b}$  had the greatest influence. Compared with  $N$  and  $C_{a+b}$ ,  $C_w$  and  $C_m$  were more sensitive in the short-wave infrared band. It could be seen that  $C_w$  is the most sensitive parameter in 1900–2100 nm, while  $C_m$  was relatively more sensitive in 2100–2300 nm compared with others wavelengths. There was little difference between the first order and total sensitivity indices in most cases (<5%), suggesting that interaction effects among different parameters were small. The sensitive bands for these parameters are summarized in Table 2. More details about the PROSPECT sensitivity analysis with the method of EFAST could be found in Zhao et al. (2014).

### Model inversion approach

Many approaches have been used in previous research for PROSPECT inversion, almost all of which retrieve input parameters by minimizing a single merit function defining on the entire optical domain from 400 to 2500 nm with a classical optimization algorithm (most of them are local optimization algorithms like the downhill simplex) (Fourty et al., 1996; Jacquemoud et al., 1996; Feret et al., 2008; Romero et al., 2012). With these traditional approaches, the algorithms always choose downhill direction in each step, in order to reach a nearby solution as quickly as possible.

This mode leads to a local, but not necessarily a global minimum in the inversion process (Nocedal and Wright, 2006).

In order to construct an optimization algorithm with high computational efficiency as well as the ability to reach the global minimum of the merit function, the method of simulated annealing (Metropolis et al., 1953) is imposed on the classical downhill simplex algorithm (Nelder and Mead, 1965) in our study, resulting in an efficient global optimization algorithm. Compared with classical optimization algorithms, the proposed algorithm should be more capable of finding the global minimum instead of the local one. Instead of choose downhill direction in every step, an uphill step is accepted in this algorithm. The probability of the uphill steps ( $P_{up}$ ) is determined by  $T$ , a controllable factor analogous to the temperature used in the simulated annealing algorithm. When  $T$  reduces to 0, the algorithm naturally converges to the classical downhill simplex algorithm.

The accuracy of PROSPECT inversion could be further improved through assigning a specific merit function for each retrieved parameter, by which the interaction effects between different parameters could be alleviated (Li and Wang, 2011). Therefore, instead of minimizing the single merit function to determine  $N$ ,  $C_{a+b}$ ,  $C_w$ , and  $C_m$  simultaneously from the spectral bands of 400–2500 nm, a specific merit function was assigned for each parameter defined on its own sensitive spectral band determined by the sensitivity analysis result, and then the foliar biochemical parameters were retrieved in steps by minimizing their own specific merit functions.

A detailed description of the inversion approach is as follows:

- (1) Merit functions definition. For a parameter  $x$  of  $N$ ,  $C_{a+b}$ ,  $C_w$  or  $C_m$  with its sensitive wavelengths set  $W_x$ , which contains all wavelengths in its sensitive wavelength region previously shown in Table 2 with a step of 1 nm, its specific merit function is defined as

$$J(x) = \sum_{\lambda \in W_x} (R_{\text{meas}}(\lambda) - R_{\text{mod}}(\lambda))^2 \quad (2)$$

where  $R_{\text{meas}}(\lambda)$  is the measured reflectance at the wavelength of  $\lambda$ , and  $R_{\text{mod}}(\lambda)$  is the modeled one.

- (2) Initial parameter value selection. Select initial guesses ( $N^{\text{guess}}$ ,  $C_{a+b}^{\text{guess}}$ ,  $C_w^{\text{guess}}$ , and  $C_m^{\text{guess}}$ ) for  $N$ ,  $C_{a+b}$ ,  $C_w$  and  $C_m$ : these values could be determined from a priori information provided that such information exists, or randomly selected from their ranges we have defined for artificial data simulation. As the optimization algorithm we used is a global one, the method of selecting initial values would not affect the retrieval result.
- (3) Initial  $T$  ( $T^0$ ) selection. Select  $T^0$  for the simulated annealing algorithm, which should not be too high since it will cost too much computational time, and should not be too low so that the algorithm could have enough time to find the global minimum.  $T^0$  was assigned 6 in our study.
- (4) Parameter retrieval. Retrieve foliar biochemical parameters one by one, by minimizing their specific merit functions defined in step (1). The retrieval order was determined by their total sensitivities over all the wavelength bands of 400–2500 nm determined by sensitivity analysis results presented previously, with the most sensitive parameter retrieved first. This step consists of four sub-steps:
  - (4.1)  $N$  determination. Determine the parameter  $N$  by minimizing its specific merit function, while keeping the other parameters at their initial values.
  - (4.2)  $C_{a+b}$  determination. Determine the parameter  $C_{a+b}$  by minimizing its specific merit function, while  $N$  is kept at its computed value, which has been determined in step (4.1), and the remaining parameters are kept at their initial values.

- (4.3)  $C_w$  determination. Determine the parameter  $C_w$  by minimizing its specific merit function, while  $N$  and  $C_{a+b}$  are kept at their computed value and  $C_m$  is kept at their initial values.

- (4.4)  $C_m$  determination. Determine the parameter  $C_m$  by minimizing its specific merit function, while the other parameters are all kept at their computed values.

- (5)  $T$  adjustment. Reduce current value of  $T$  by  $\delta$ , and repeat step (4) aiming to find a more accurate estimation of the parameters, with use of the computed values of  $N$ ,  $C_{a+b}$ ,  $C_w$ , and  $C_m$  as their initial guesses. In our study,  $T$  was reduced by 2 each time, until  $T$  reached zero.

- (6) Program termination. Stop the search process if the threshold value  $\varepsilon$  set for ending the program is achieved (i.e. relative change in modeled reflectance spectra between the current and the last round is small enough), or  $T$  reaches or is less than zero. The current values of  $N$ ,  $C_{a+b}$ ,  $C_w$ , and  $C_m$  are the best guesses ( $N^*$ ,  $C_{a+b}^*$ ,  $C_w^*$ , and  $C_m^*$ ). The threshold value  $\varepsilon$  was assigned  $10^{-6}$  in our study.

Compared with traditional approaches, this proposed approach could potentially improve the accuracy of PROSPECT inversion through separating the single merit function into different ones according to the sensitivity analysis results, and using an efficient global optimization algorithm instead of a classical one.

#### Statistical analysis

The correlation scalograms of retrieved versus measured values were plotted for  $C_{a+b}$ ,  $C_w$ , and  $C_m$ , respectively. The coefficient of determination ( $R^2$ ) and Root-Mean-Square Error (RMSE) were calculated to evaluate the accuracy of the inversion process. Then the mean values of six leaves of the biochemical contents ( $C_{a+b}$ ,  $C_w$ , and  $C_m$ ) for each CTRL, 0.25X, and 0.5X group at 6, 24, 48, and 72 HAT respectively were calculated. Glyphosate-induced leaf injury was analyzed by examining the separability of these mean values. SPSS 19 Statistics (SPSS Inc., Chicago, IL, USA) was used for the separation analysis. A one-way ANalysis Of VAriance (ANOVA) with Duncan's multiple range test with a  $p$ -value of 0.05 (0.05 confidence probability) was applied to differentiate these mean values.

## Results

#### Retrieval results of leaf biochemical parameters

Fig. 2 presents retrieved versus measured values of  $C_{a+b}$ ,  $C_w$ , and  $C_m$  for all tested leaves of soybean and cotton. The results showed that all the retrieved values agree well with the corresponding measured values, especially for  $C_{a+b}$  (Fig. 2a) and  $C_w$  (Fig. 2b). For both soybean and cotton,  $R^2$  of  $C_{a+b}$  was 0.8654 for soybean and 0.8903 for cotton, respectively, and the  $R^2$  of  $C_w$  was 0.7643 for soybean and 0.7138 for cotton, respectively. Compared with  $C_{a+b}$  and  $C_w$ , the estimation accuracy of  $C_m$  was relatively low (Fig. 2c), with the  $R^2$  being 0.5845 for soybean and 0.5099 for cotton, respectively, which is probably due to the limited range and the low sensitivity of  $C_m$ . Moreover, the RMSE values of  $C_{a+b}$ ,  $C_w$ , and  $C_m$  were all low: 1.2278  $\mu\text{g}/\text{cm}^2$ , 0.0005  $\text{g}/\text{cm}^2$ , 0.0042  $\text{g}/\text{cm}^2$  for soybean and 0.9144  $\mu\text{g}/\text{cm}^2$ , 0.0124  $\text{g}/\text{cm}^2$ , 0.0003  $\text{g}/\text{cm}^2$  for cotton, respectively.

#### Temporal variations of leaf biochemical contents

Fig. 3 shows the temporal variations of retrieved  $C_{a+b}$ ,  $C_w$ , and  $C_m$  for soybean leaves after the glyphosate treatment.  $C_{a+b}$  of the higher dose solution treated leaves was seen to decrease more rapidly compared with the  $C_{a+b}$  of the other groups. The differences became

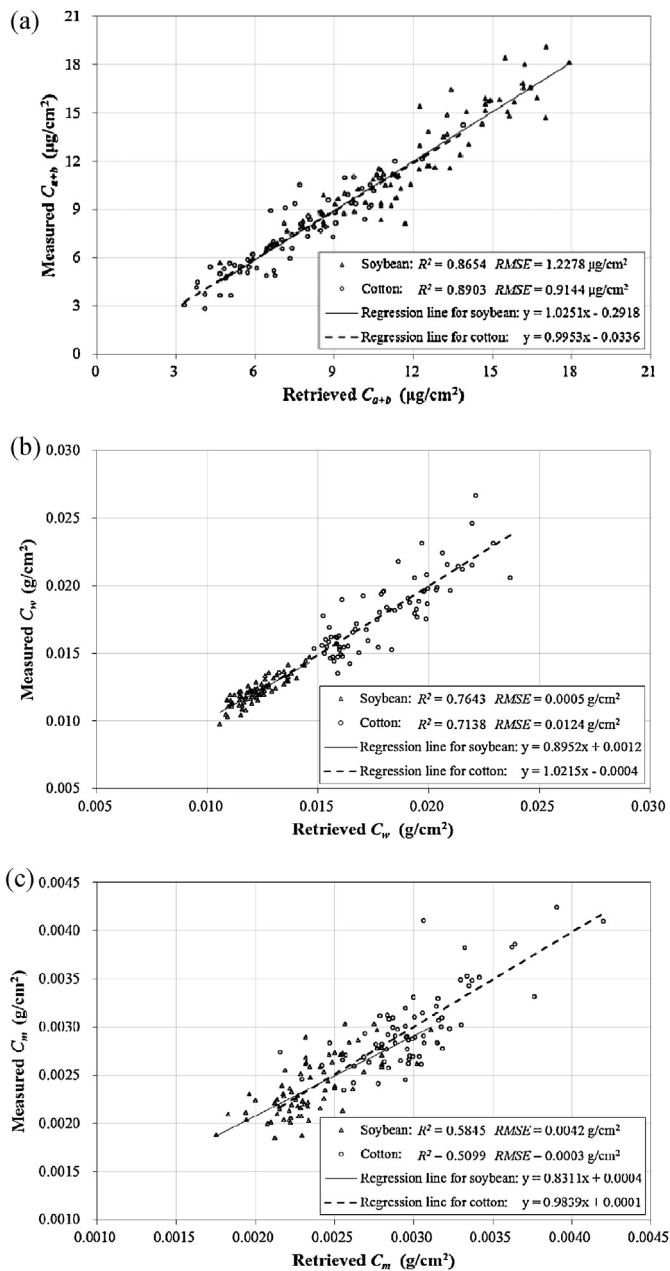


Fig. 2. Scatter diagram of retrieved versus measured (a) chlorophyll content ( $C_{a+b}$ ), (b) equivalent water thickness ( $C_w$ ), and (c) leaf mass per area ( $C_m$ ).

more apparent at 72 HAT. In contrast,  $C_w$  and  $C_m$  indicated no obvious differences among the three groups from 6 HAT to 72 HAT.  $C_w$  of all three groups maintained a relatively stable value with a slight increase with time, while the  $C_m$  indicated a slight decrease.

Results for cotton were illustrated in Fig. 4 for  $C_{a+b}$ ,  $C_w$ , and  $C_m$ . Similar to results for soybean,  $C_{a+b}$  of CTRL group were quite stable with time, while  $C_{a+b}$  of the 0.25X group indicated a decreasing trend.  $C_{a+b}$  of the 0.5X group decreased even more rapidly.  $C_w$  and  $C_m$  of the three groups all indicated an increasing trend with time, but there are no consistent differences among different groups.

Then separability analysis of the three groups at 6, 24, 48, and 72 HAT for the measured and retrieved biochemical contents of soybean and cotton were conducted. Duncan's multiple range tests were used in this analysis to examine the separability of the mean biochemical values of six leaf samples of the same group.

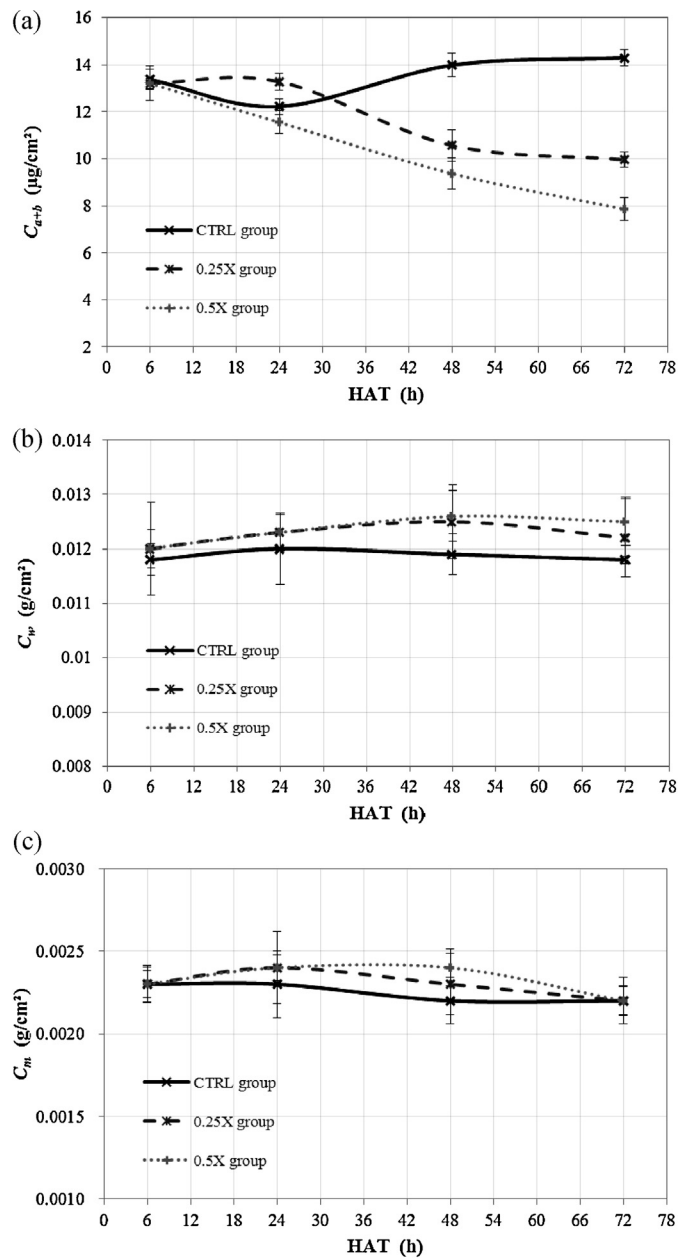
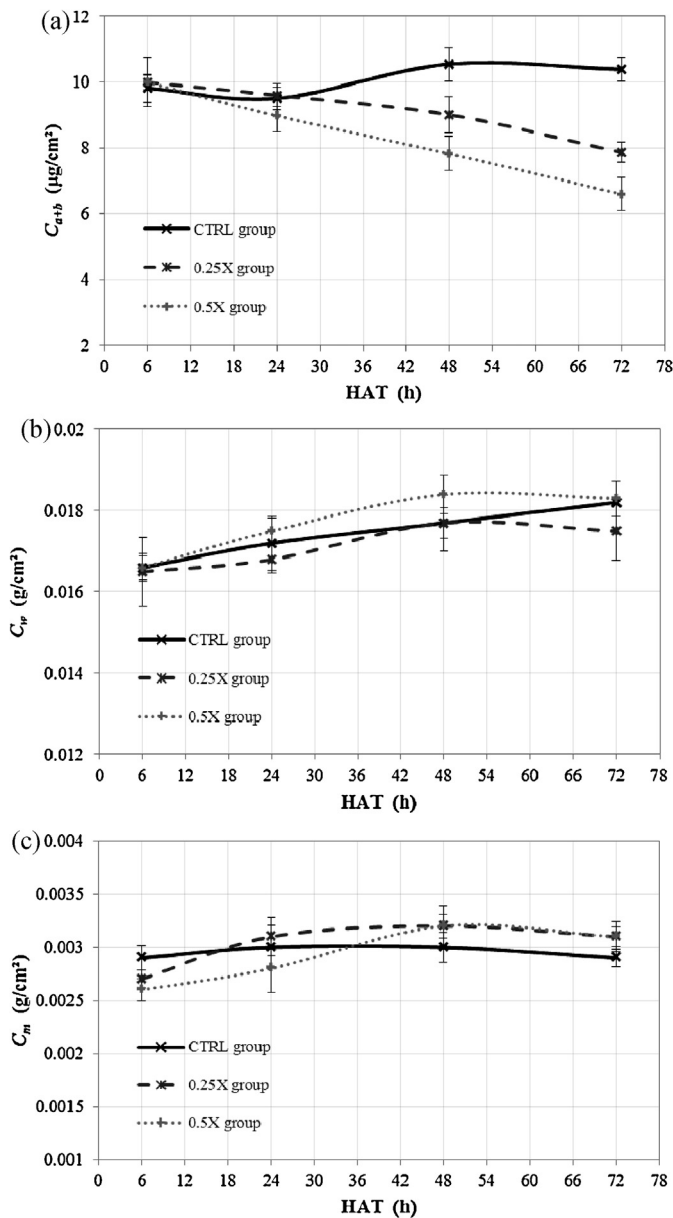


Fig. 3. Temporal variations of retrieved (a) chlorophyll content ( $C_{a+b}$ ), (b) equivalent water thickness ( $C_w$ ), and (c) leaf mass per area ( $C_m$ ) after the treatment for all groups of soybean. Each point is a mean value of six leaves growing under the same conditions. Error bars indicate the standard deviation of each point.

Table 3 shows the mean values of the retrieved  $C_{a+b}$ ,  $C_w$ , and  $C_m$  of the three groups at 6, 24, 48, and 72 HAT for soybean.  $C_{a+b}$  for the 0.25X and 0.5X groups were significantly different from that of the CTRL group at 48 HAT but not significantly different from each other.  $C_{a+b}$  among three groups was significantly different at 72 HAT. For  $C_w$  and  $C_m$ , the differences among the three groups were not significant from 6 HAT to 72 HAT. The statistical results for mean measured values were identical to those of the correspondingly retrieved ones.

Table 4 summarizes the mean values of the retrieved  $C_{a+b}$ ,  $C_w$ , and  $C_m$  of the three groups 6, 24, 48, and 72 HAT for cotton.  $C_{a+b}$  of the three groups can be significantly distinguished at and beyond 48 HAT.  $C_w$  and  $C_m$  of the three groups show no significant difference from 6 HAT to 72 HAT. For the mean measured values of cotton, the same separation results were acquired.



**Fig. 4.** Temporal variations of retrieved (a) chlorophyll content ( $C_{a+b}$ ), (b) equivalent water thickness ( $C_w$ ), and (c) leaf mass per area ( $C_m$ ) after the treatment for all groups of cotton. Each point is a mean value of six leaves growing under the same conditions. Error bars indicate the standard deviation of each point.

**Discussion**

*Performance of the inversion procedure*

Foliar biochemistry is a potentially advantageous indicator for the early detection of crop stress caused by glyphosate injury. As an important but relatively uninvestigated problem, detecting crop injury through retrieval of foliar biochemical parameters by inverting a leaf radiative transfer model, PROSPECT, from reflectance spectra has been presented. To precisely estimate foliar biochemical parameters, several improved inversion approaches have been proposed to improve the retrieval accuracy. For example, Li and Wang (2011) separated the single merit function into different ones according to sensitivity analysis result performed by Sobel method, and retrieve foliar biochemical parameters by minimizing these merit functions with genetic algorithm. Here we applied a similar

**Table 3**

Retrieved foliar biochemical contents for experimental soybean leaves of the three groups at 6, 24, 48, 72 HAT. Each value is the mean of six leaves for the same group. The statistics were analyzed using Duncan's multiple range tests.\*

Group	CTRL	0.25X	0.5X
<b>6 HAT</b>			
$C_{a+b}$ ( $\mu\text{g}/\text{cm}^2$ )	13.3671 <sup>a</sup>	13.2046 <sup>a</sup>	13.1985 <sup>a</sup>
$C_w$ ( $\text{g}/\text{cm}^2$ )	0.0118 <sup>a</sup>	0.0120 <sup>a</sup>	0.0120 <sup>a</sup>
$C_m$ ( $\text{g}/\text{cm}^2$ )	0.0023 <sup>a</sup>	0.0023 <sup>a</sup>	0.0023 <sup>a</sup>
<b>24 HAT</b>			
$C_{a+b}$ ( $\mu\text{g}/\text{cm}^2$ )	12.2172 <sup>a</sup>	13.2699 <sup>a</sup>	11.5416 <sup>a</sup>
$C_w$ ( $\text{g}/\text{cm}^2$ )	0.0120 <sup>a</sup>	0.0123 <sup>a</sup>	0.0123 <sup>a</sup>
$C_m$ ( $\text{g}/\text{cm}^2$ )	0.0023 <sup>a</sup>	0.0024 <sup>a</sup>	0.0024 <sup>a</sup>
<b>48 HAT</b>			
$C_{a+b}$ ( $\mu\text{g}/\text{cm}^2$ )	13.9737 <sup>a</sup>	10.5587 <sup>b</sup>	9.3648 <sup>b</sup>
$C_w$ ( $\text{g}/\text{cm}^2$ )	0.0119 <sup>a</sup>	0.0125 <sup>a</sup>	0.0126 <sup>a</sup>
$C_m$ ( $\text{g}/\text{cm}^2$ )	0.0022 <sup>a</sup>	0.0023 <sup>a</sup>	0.0024 <sup>a</sup>
<b>72 HAT</b>			
$C_{a+b}$ ( $\mu\text{g}/\text{cm}^2$ )	14.2865 <sup>a</sup>	9.9559 <sup>b</sup>	7.8522 <sup>c</sup>
$C_w$ ( $\text{g}/\text{cm}^2$ )	0.0118 <sup>a</sup>	0.0122 <sup>a</sup>	0.0125 <sup>a</sup>
$C_m$ ( $\text{g}/\text{cm}^2$ )	0.0022 <sup>a</sup>	0.0022 <sup>a</sup>	0.0022 <sup>a</sup>

\* Mean values are not significantly different with the same letter in each row at 0.05 level.

inversion approach with the EFAST method for sensitivity analysis and simulated annealing algorithm coupled with downhill simplex method to minimize merit functions.

For the greenhouse-measured data, high retrieval accuracies were achieved as illustrated in Fig. 2. Compared with previous studies (Fourty et al., 1996; Li and Wang, 2011; Romero et al., 2012), in which the RMSE values of  $C_{a+b}$ ,  $C_w$ , and  $C_m$  were between ranges of 5.17–32.35  $\mu\text{g}/\text{cm}^2$ , 0.0011–0.0057  $\text{g}/\text{cm}^2$ , and 0.0006–0.0049  $\text{g}/\text{cm}^2$ , respectively, we determined RMSE values to be quite low in our study. RMSE values in our study were 1.2278  $\mu\text{g}/\text{cm}^2$ , 0.0005  $\text{g}/\text{cm}^2$ , and 0.0042  $\text{g}/\text{cm}^2$  for soybean and 0.9144  $\mu\text{g}/\text{cm}^2$ , 0.0124  $\text{g}/\text{cm}^2$ , and 0.0003  $\text{g}/\text{cm}^2$  for cotton, respectively. These results indicate that the retrieval accuracies of PROSPECT parameters have been improved by using the method proposed here. We should also note that the measured values of  $C_{a+b}$ ,  $C_w$ , and  $C_m$  by the above mentioned studies had wider ranges than those in our experiment, since the leaves used in this study were sampled from plants of early growing stages. Further evaluation of our proposed inversion approach is still needed for more datasets. However, results herein indicate high retrieval accuracy in

**Table 4**

Retrieved foliar biochemical contents for experimental cotton leaves of the three groups at 6, 24, 48, 72 HAT. Each value is the mean of six leaves for the same group. The statistics were analyzed using Duncan's multiple range tests.\*

Group	CTRL	0.25X	0.5X
<b>6 HAT</b>			
$C_{a+b}$ ( $\mu\text{g}/\text{cm}^2$ )	9.8102 <sup>a</sup>	9.9867 <sup>a</sup>	9.9867 <sup>a</sup>
$C_w$ ( $\text{g}/\text{cm}^2$ )	0.0166 <sup>a</sup>	0.0165 <sup>a</sup>	0.0166 <sup>a</sup>
$C_m$ ( $\text{g}/\text{cm}^2$ )	0.0029 <sup>a</sup>	0.0027 <sup>a</sup>	0.0026 <sup>a</sup>
<b>24 HAT</b>			
$C_{a+b}$ ( $\mu\text{g}/\text{cm}^2$ )	9.5036 <sup>a</sup>	9.5951 <sup>a</sup>	8.9698 <sup>a</sup>
$C_w$ ( $\text{g}/\text{cm}^2$ )	0.0172 <sup>a</sup>	0.0168 <sup>a</sup>	0.0175 <sup>a</sup>
$C_m$ ( $\text{g}/\text{cm}^2$ )	0.0030 <sup>a</sup>	0.0031 <sup>a</sup>	0.0028 <sup>a</sup>
<b>48 HAT</b>			
$C_{a+b}$ ( $\mu\text{g}/\text{cm}^2$ )	10.5367 <sup>a</sup>	9.0023 <sup>b</sup>	7.8207 <sup>c</sup>
$C_w$ ( $\text{g}/\text{cm}^2$ )	0.0177 <sup>a</sup>	0.0177 <sup>a</sup>	0.0184 <sup>a</sup>
$C_m$ ( $\text{g}/\text{cm}^2$ )	0.0030 <sup>a</sup>	0.0032 <sup>a</sup>	0.0032 <sup>a</sup>
<b>72 HAT</b>			
$C_{a+b}$ ( $\mu\text{g}/\text{cm}^2$ )	10.3765 <sup>a</sup>	7.8610 <sup>b</sup>	6.5937 <sup>c</sup>
$C_w$ ( $\text{g}/\text{cm}^2$ )	0.0182 <sup>a</sup>	0.0175 <sup>a</sup>	0.0183 <sup>a</sup>
$C_m$ ( $\text{g}/\text{cm}^2$ )	0.0029 <sup>a</sup>	0.0031 <sup>a</sup>	0.0031 <sup>a</sup>

\* Mean values are not significantly different with the same letter in each row at 0.05 level.

the inversion process as an encouraging result. This approach helps better estimate temporal variations of leaf biochemical parameters after the glyphosate treatment.

#### *Biochemical basis of crop injury from glyphosate*

When glyphosate is applied to non-GR crops, it will be metabolized by plants. The main metabolite of glyphosate, aminomethylphosphonic acid, will be found in the leaves of these glyphosate treated plants (Sammons and Tran, 2008). This exhibits a half-life of 25–75 days and is known to cause a continuing injury or even mortality to non-GR plants (Mamy et al., 2005).

Several studies have certified that glyphosate-induced leaf injury is always accompanied with the reduction of chlorophyll content (Reddy et al., 2000, 2010; Koger et al., 2005). Therefore, the onset of injury could be detected early if we can observe a reduction in  $C_{a+b}$ . Compared with traditional methods of directly measuring  $C_{a+b}$  in vivo, quantitatively estimating its content non-destructively with leaf hyperspectral reflectance measurements is a better choice for glyphosate injury detection.

It should be noted that this study was conducted under controlled growing conditions to focus on the stress induced by glyphosate. The other variables were controlled not to be additional sources of stress in the experiments. Therefore, in this study, the variations in the biochemical contents of leaves should be caused by glyphosate treatment. But under the field conditions, other stress factors (e.g. water-, temperature- and pest-stress) may also induce  $C_{a+b}$  decline. Therefore, a priori knowledge (e.g. farming management data) that the stress is largely caused by glyphosate should be helpful to effectively use the method proposed in this paper. This work is the first step in showing that the glyphosate injured plants can be distinguished from the healthy ones by PROSPECT inversion. For  $C_{a+b}$  of both soybean and cotton, retrieved values agreed well with the measured values, as shown in Fig. 2a. This indicates the proposed method can reflect the variations of  $C_{a+b}$  caused by glyphosate treatment. Therefore, by examining temporal variation of retrieved  $C_{a+b}$  from leaf hyperspectral reflectance measurements after treatment, glyphosate-induced leaf injury could be detected early. As shown in Table 3 for soybean, retrieved  $C_{a+b}$  values of the 0.25X and 0.5X groups could be separated from that of the CTRL group at 48 HAT, and the three groups could be totally separated at 72 HAT. For cotton shown in Table 4, the three groups could be separated from each other at and beyond 48 HAT.

#### *Potential of applying PROSPECT inversion to glyphosate-induced crop injury detection*

Although we have successfully detected the glyphosate-induced crop injury by leaf biochemical parameter inversion, the results could be further improved. Firstly, new-born leaves at the top of the plants after the glyphosate treatment are more sensitive to the decline of  $C_{a+b}$ . But instead of selecting these leaves as samples, we selected the lower-most trifoliolate leaves for soybean and twin leaves for cotton leaves. These leaves were present before glyphosate treatment to make sure leaves at the same position of each plant were used for all four days. So the decrease of  $C_{a+b}$  was less apparent. Secondly, since glyphosate is phytotoxic to crops by an unknown mechanism,  $C_{a+b}$  may not be the most direct indicator of crop injury from glyphosate. In order to improve glyphosate injury detection result, future study would be useful to fully explore the biochemical basis of the relationship between glyphosate injury and leaf reflectance spectrum.

Compared with traditionally used vegetation indices, the proposed model inversion method indicates some potential advantages for glyphosate injury detection. This method is more physically-based and thus can be more easily extended to the

canopy scale by coupling with a canopy radiative transfer model such as SAIL (Scattering by Arbitrarily Inclined Leaves) (Verhoef, 1984) for full canopy, or row canopy radiative transfer model (Zhao et al., 2010) for typical row crops. Different from leaf spectra, canopy spectral features are strongly affected by confounding factors such as crop architecture, sun and viewing geometry, canopy shadowing, and the contribution of soil background. This may present difficulties when using vegetation indices. By coupling with canopy radiative transfer models, the method proposed in this study has the potential to be up-scaled to the canopy level, which should be suitable for large region applications. Our further studies will focus on monitoring glyphosate injury at the canopy scale.

#### **Conclusions**

Early detection of crop injury from glyphosate is of significant importance in crop management. With leaf hyperspectral reflectance measurements, we have proposed a new method for detection of glyphosate-induced leaf injury by PROSPECT inversion. To obtain accurate estimation of leaf biochemical contents, we have applied an improved PROSPECT inversion procedure by assigning a specific merit function for each biochemical parameter based on the sensitivity analysis results of PROSPECT and employing an efficient global optimization algorithm. The performance of the improved inversion procedure was validated by greenhouse-measured datasets. Results showed good agreement between foliar biochemical parameters retrieved from reflectance spectra and greenhouse-measured values. Glyphosate-induced leaf injury could be detected in a timely manner by examining temporal variation of the retrieved value of  $C_{a+b}$  from leaf hyperspectral reflectance measurements after treatment. The glyphosate treated groups could be differentiated from CTRL groups 48 HAT for both soybean and cotton, with the  $C_{a+b}$  of higher dose treated leaves decreasing more rapidly, whereas  $C_w$  and  $C_m$  showed no consistent differences among different groups. Moreover, this newly proposed procedure is more physically based, and could thus be up-scaled to the canopy level and used for large-scale agricultural plant status monitoring by airborne or space-borne observations. Results presented herein demonstrate that PROSPECT inversion has promising potential for detecting the onset of glyphosate-induced leaf injury and could be further developed for practical use.

#### **Disclaimer**

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

#### **Acknowledgements**

This work was supported by Chinese Natural Science Foundation under Project 41371325 and 40901156, the Civil Aerospace Technology Pre-research Project of China (Grant No. D040201-03), and the State Key Laboratory of Remote Sensing Science under Project 2009kfjj022. We thank Efren Ford and Paige Goodlett for technical assistance.

#### **References**

- ASD Inc., 2008. ASD Document 600660 Rev. B, Integrating Sphere User Manual.
- Barnes, J.D., Balaguer, L., Manrique, E., Elvira, S., Davison, A.W., 1992. A reappraisal of the use of DMSO for the extraction and determination of chlorophylls a and b in lichens and higher plants. *Environ. Exp. Bot.* 32, 85–100.
- Carter, G.A., 1994. Ratios of leaf reflectances in narrow wavebands as indicators of plant stress. *Int. J. Remote Sens.* 15, 697–703.

- Ding, W., Reddy, K.N., Krutz, L.J., Thomson, S.J., Huang, Y., Zablutowicz, R.M., 2011. Biological response of soybean and cotton to aerial glyphosate drift. *J. Crop Improv.* 25, 291–302.
- Feret, J.B., François, C., Asner, G.P., Gitelson, A.A., Martin, R.E., Bidet, L.P., Ustin, S.L., Le Maire, G., Jacquemoud, S., 2008. PROSPECT-4 and 5: advances in the leaf optical properties model separating photosynthetic pigments. *Remote Sens. Environ.* 112, 3030–3043.
- Filella, I., Peñuelas, J., 1994. The red edge position and shape as indicators of plant chlorophyll content, biomass and hydric status. *Int. J. Remote Sens.* 15, 1459–1470.
- Fourty, T., Baret, F., Jacquemoud, S., Schmuck, G., Verdebout, J., 1996. Leaf optical properties with explicit description of its biochemical composition: direct and inverse problems. *Remote Sens. Environ.* 56, 104–117.
- Huang, Y., Thomson, S.J., Ortiz, B.V., Reddy, K.N., Ding, W., Zablutowicz, R.M., Bright, J.R., 2010. Airborne remote sensing assessment of the damage to cotton caused by spray drift from aerially applied glyphosate through spray deposition measurements. *Biosyst. Eng.* 107, 212–220.
- Huang, Y., Thomson, S.J., Molin, W.T., Reddy, K.N., Yao, H., 2012. Early detection of soybean plant injury from glyphosate by measuring chlorophyll reflectance and fluorescence. *J. Agric. Sci.* 4, 117–124.
- Jacquemoud, S., Baret, F., 1990. PROSPECT: a model of leaf optical properties spectra. *Remote Sens. Environ.* 34, 75–91.
- Jacquemoud, S., Ustin, S.L., Verdebout, J., Schmuck, G., Andreoli, G., Hosgood, B., 1996. Estimating leaf biochemistry using the PROSPECT leaf optical properties model. *Remote Sens. Environ.* 56, 194–202.
- Jacquemoud, S., Bacour, C., Poilve, H., Frangi, J.P., 2000. Comparison of four radiative transfer models to simulate plant canopies reflectance: direct and inverse mode. *Remote Sens. Environ.* 74, 471–481.
- Koger, C.H., Shaner, D.L., Krutz, L.J., Walker, T.W., Buehring, N., Henry, W.B., Thomas, W.E., Wilcut, J.W., 2005. Rice (*Oryza sativa*) response to drift rates of glyphosate. *Pest Manage. Sci.* 61, 1161–1167.
- Li, P., Wang, Q., 2011. Retrieval of leaf biochemical parameters using PROSPECT inversion: a new approach for alleviating ill-posed problems. *IEEE Trans. Geosci. Remote Sens.* 49, 2499–2506.
- Mamy, L., Barriuso, E., Gabrielle, B., 2005. Environmental fate of herbicides trifluralin, metazachlor, metamitron and sulcotrione compared with that of glyphosate, a substitute broad spectrum herbicide for different glyphosate-resistant crops. *Pest Manage. Sci.* 61, 905–916.
- Metropolis, N., Rosenbluth, A.W., Rosenbluth, M.N., Teller, A.H., Teller, E., 1953. Equation of state calculations by fast computing machines. *J. Chem. Phys.* 21, 1087–1092.
- Nelder, J.A., Mead, R., 1965. A simplex method for function minimization. *Comput. J.* 7, 308–313.
- Nocedal, J., Wright, S.J., 2006. Numerical Optimization, second edition. Springer Science+Business Media, LLC, New York.
- Reddy, K.N., Hoagland, R.E., Zablutowicz, R.M., 2000. Effect of glyphosate on growth, chlorophyll, and nodulation in glyphosate-resistant and susceptible soybean (*Glycine max*) varieties. *J. New Seeds* 2, 37–52.
- Reddy, K.N., Ding, W., Zablutowicz, R.M., Thomson, S.J., Huang, Y., Krutz, L.J., 2010. Biological responses to glyphosate drift from aerial application in non-glyphosate-resistant corn. *Pest Manage. Sci.* 66, 1148–1154.
- Romero, A., Aguado, I., Yebra, M., 2012. Estimation of dry matter content in leaves using normalized indexes and PROSPECT model inversion. *Int. J. Remote Sens.* 33, 396–414.
- Saltelli, A., Tarantola, S., Chan, K., 1999. A quantitative, model independent method for global sensitivity analysis of model output. *Technometrics* 41, 39–56.
- Saltelli, A., Ratto, M., Andres, T., Campolongo, F., Cariboni, J., Gatelli, D., Saisana, M., Tarantola, S., 2008. Global Sensitivity Analysis. The Primer. John Wiley & Sons Ltd., New York.
- Sammons, D.R., Tran, M., 2008. Examining yellow flash in Roundup ready soybean. In: North Central Weed Science Society Proceedings, 63, p. 120.
- Verhoef, W., 1984. Light scattering by leaf layers with application to canopy reflectance modeling: the SAIL model. *Remote Sens. Environ.* 6, 125–184.
- Yao, H., Huang, Y., Hruska, Z., Tomson, S.J., Reddy, K.N., 2012. Using vegetation index and modified derivative for early detection of soybean plant injury from glyphosate. *Comput. Electron. Agric.* 89, 145–157.
- Zhao, F., Gu, X., Verhoef, W., Wang, Q., Yu, T., Liu, Q., Huang, H., Qin, W., Chen, L., Zhao, H., 2010. A spectral directional reflectance model of row crops. *Remote Sens. Environ.* 114, 265–285.
- Zhao, F., Huang, Y., Guo, Y., Reddy, K.N., Lee, M.A., Fletcher, R.S., Thomson, S.J., Zhao, H., 2014. Early detection of crop injury from glyphosate on soybean and cotton using plant leaf hyperspectral data. *Remote Sens.* – Basel 6, 1538–1563.